BIOPHYSICS

The glassiness of hardening protein droplets

Protein condensates can age to form glasses that increase in viscosity but retain elasticity

By **Huaiying Zhang**

In addition to dissolving in the watery cytosol, proteins can self-assemble into materials with different mechanical properties, such as solid filaments or soft gels, to facilitate various cellular functions. In the past decade, proteins have been shown to undergo liquid-liquid phase separation (LLPS) to form liquid droplets in which proteins are highly concentrated but are still dynamic and fluid (*1*, *2*). Many cellular compartments, such as nucleoli and stress granules, are phase-separated droplets. Although some droplets remain liquid for function, others harden into a less dynamic state over time (*3*, *4*). On page 1317 of this issue, Jawerth *et al.* (*5*) report that the hardening protein droplets are Maxwell glasses. These are Maxwell fluids that age like glasses in that viscosity increases with age, whereas the elasticity changes little over time (see the figure).

There are many distinctive features of LLPS that can be functionally relevant, one of which is the liquid property of the resulting droplets. Droplet fluidity is vital for ensuring proper chemical reactions occurring within the compartment. Disrupting the liquid properties of the nucleolus, for example, alters ribosomal RNA biogenesis (*6*). In addition, droplet fusion can be used for force generation to organize cellular space, such as clustering genomic loci (*7*, *8*) or bundling cytoskeleton filaments (*9*).

However, condensed phases formed with LLPS are not simple liquids but have diverse and changing material properties and are collectively called biomolecular condensates (*1*). The dependence of droplet fluidity on environmental factors (such as salt concentration) and condensate composition (such as RNA-to-protein ratio) has been revealed in vitro, with active rheology measured with optical tweezers and passive rheology measured with microbeads (*10*–*12*). Reconstituted droplets also undergo aging and maturation; that is, their material properties change with time.

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Some condensates harden into a less dynamic state in which they do not fuse but stick together after collision, and the proteins rearrange less within them (*3*, *4*). Hardened condensates might be functional in that they can inhibit some chemical reactions or provide structural rigidity. The hardening process can also be vital for creating different material properties needed in a multistep cellular process. For example, in centrosome condensate formation with the protein spindle-defective protein 5 (SPD-5), the initial dynamic liquid may allow rapid protein incorporation early in the cell cycle, whereas the hardened condensate may provide the centrosome with the rigidity required to resist microtubule-pulling forces during mitosis (*4*).

In addition to hardening, some aged droplets even nucleate protein aggregates, including amyloids that are linked to various diseases (*10*, *13*, *14*). Thus, organizing biochemistry through LLPS may come at the cost of promoting pathological protein aggregation. However, the ability to nucleate solids is also exploited to seed cytoskeleton filaments, including actin and microtubules, from liquid condensates that concentrate monomers (*9*). Not only do condensate material properties change with environment, composition, and time, different material properties also coexist in subcompartments of a single condensate such as the nucleolus (*15*). These examples highlight the numerous ways that various condensate material properties can be combined to achieve complex functionality.

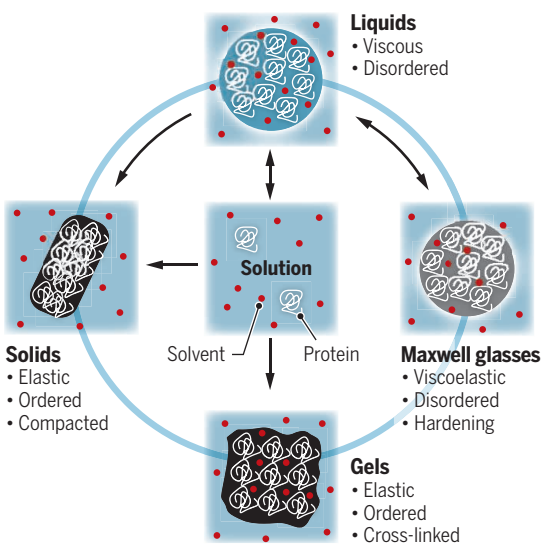
Jawerth *et al.* characterized the material properties of reconstituted protein condensates that harden over time by combining optical tweezer manipulation and microrheology. These hardening condensates behave like Maxwell fluids that have both viscous and elastic components present at all times. The elasticity of the Maxwell fluid changes little with age, indicating that hardening is not a gelation process in which molecules become cross-linked. However, the viscosity strongly increases with age, suggesting that the molecular dynamics are hindered by protein jamming within the liquid.

Because this material exhibits the behavior of Maxwell fluid but ages like glass, the authors call it Maxwell glass. Agreeing with the rheological results, no substantial structural changes within hardening condensates were observed with cryo–electron microscopy. Condensate-size shrinkage and increased protein density within the condensate were observed with fluorescent microscopy. The origin of protein jamming and how it links to protein chemistry await to be determined but will be needed to understand why some condensates harden over time and others do not.

Jawerth *et al.* followed the hardening of five different proteins [*Caenorhabditis elegans* protein PGL-3 (guanyl-specific ribonuclease pgl-3) and mammalian proteins FUS (RNA-binding protein FUS), EWSR1 (RNA-binding protein EWS), DAZAP1 (DAZ-associated protein 1), and TAF15 (TATA-binding protein–associated factor 2N)] with fluorescence recovery after photobleaching and studied the rheology of PGL-3 and FUS with optical tweezer and microrheology methods. Future work could expand the rheological studies to other condensates. For example, porous meshwork in hardened *Saccharomyces cerevisiae* and

Phases that proteins form

Proteins dissolved in solution (middle square) can self-assemble into liquids, solids, and gels. Jawerth *et al.* report that proteins can also harden into a new phase, a Maxwell glass. Protein liquids and Maxwell glasses are easier to reverse (double arrows) than protein gels and solids (single arrows).



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Schizosaccharomyces pombe Sup35 condensates was observed with cryo–electron microscopy, which suggests that they could be gels (*3*). Rheology studies on hardening Sup35 condensates would help to determine whether and how they age differently from condensates reported in this study. Nevertheless, it can no longer be assumed that all nondynamic condensates are gels because they may be Maxwell glasses.

Distinguishing glass-like and gel-like responses of hardened condensates is not only conceptually but functionally essential. Both gels and glasses can be structurally stable. Gel stiffness can be actively regulated by the degree of cross-linking and can be tailored to sustain different magnitudes of forces. A glass can act as a mechanical sensor, just like liquid droplets (*7*), because it can flow under stress. A jammed glass only allows small molecules to pass, whereas the larger pores of a gel permit diffusion of macromolecules such as proteins. However, glasses are more easily fluidized. A gel would be more desirable for a condensate where structure rigidity and chemical reactions are both needed, such as for centrosomes (*4*). A glass is suitable for slowing down all macromolecule movement through jamming and allowing small molecules to pass through and quickly fluidize the content when needed, such as for stress-sensing condensates (*3*).

It will be exciting to see which nondynamic condensates in cells are gels and which ones are glasses, and how cells exploit their material properties for functions. However, unlike the in vitro results reported by Jawerth *et al*., directly probing rheological properties of endogenous condensates remains technically challenging. Tools for forming de novo condensates in live cells in a controlled manner may be useful for engineering condensates suitable for optical tweezer manipulation or embedding microbeads to follow condensate rheology (*7*, *8*). ■

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CORONAVIRUS

Remembering seasonal coronaviruses

Antibodies against seasonal coronaviruses react with SARS-CoV-2

By **Jenna J. Guthmiller**[1] and **Patrick C. Wilson**[1,2]

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) has differential effects according to age, with symptomatic and severe infections mostly occurring in older adults. One possible explanation for this variation is that children and younger adults have more preexisting immunity against seasonal human coronaviruses (HCoVs) that cross-react with SARS-CoV-2, providing protection from severe and even symptomatic SARS-CoV-2 infection. Consistently, SARS-CoV-2 cross-reactive memory $CD4^+$ and $CD8^+$ T cells against the spike protein, the major surface protein of coronaviruses, have been reported in unexposed individuals (*1*, *2*). Whether humoral immunity (antibodies and memory B cells) against SARS-CoV-2 cross-reacts with seasonal HCoVs is now emerging. On page 1339 of this issue, Ng *et al*. (*3*) and Shrock *et al*. (*4*) reveal that individuals exposed and unexposed to SARS-CoV-2 have cross-reactive serum antibodies against the spike protein of SARS-CoV-2 and seasonal HCoVs.

Four seasonal HCoV strains cause cold symptoms in humans: 229E, NL63, OC43, and HKU1. Despite using different host receptors for cellular entry, all HCoVs express the spike protein on their surface. The spike protein is composed of two subunits: S1 contains the receptor-binding domain (RBD), which is responsible for binding to host cell receptors, and S2 is critical for mediating viral and host cell membrane fusion and cell entry. The fusion peptide of the S2 subunit is highly conserved among seasonal HCoVs and zoonotic coronaviruses, including SARS-CoV-2 (*3*), whereas S1 is more variable.

Shrock *et al*. found that people unexposed to SARS-CoV-2 have cross-reactive antibody responses against an array of coronaviruses, including the recently emerged SARS-CoV-2. However, most of this preexisting immunity targets a few epitopes on the spike protein, nucleocapsid protein, and the nonstructural proteins that are encoded by open reading frame 1. Using a sensitive flow cytometric assay to detect cross-reactive serum antibodies, Ng *et al*. found that individuals unexposed to SARS-CoV-2 possessed neutralizing antibodies against the S2 protein. Cross-reactive antibodies were class-switched to the mature antibody isotypes immunoglobulin G (IgG) and IgA, suggesting that B cells producing these cross-reactive antibodies were induced by a previous immune response against infection with seasonal HCoVs.

Upon SARS-CoV-2 infection, individuals showed increased production of antibodies that cross-react with the spike proteins of SARS-CoV-2 and seasonal HCoVs, called back-boosting (see the figure). Back-boosting is a common phenomenon observed after influenza virus infection and vaccination that results in the recall of antibodies targeting conserved epitopes of past circulating influenza viruses (*5*). Back-boosting can be directed to nonprotective epitopes, such as those on the unexposed nucleocapsid protein, which is referred to as original antigenic sin. However, back-boosting can lead to the recall of broadly neutralizing antibodies, as observed with the 2009 pandemic H1N1 influenza virus (*6*). Shrock *et al*. and Ng *et al*. both identified that SARS-CoV-2 infection boosted antibody responses against several conserved epitopes, including the fusion peptide of the S2 subunit. Together, these studies solidify the existence of cross-reactive humoral immunity against HCoVs and SARS-CoV-2.

Many of the cross-reactive antibodies are specific for epitopes on the fusion peptide or nearby epitopes on the S2 subunit. These antibodies likely neutralize coronaviruses by blocking viral membrane fusion and host cell entry (*7*). Although Ng *et al*. showed that cross-reactive antibodies neutralized SARS-CoV-2 infection in vitro, it is unknown whether cross-reactive antibodies specific for the fusion peptide prevent SARS-CoV-2 infection or limit COVID-19 severity in vivo. Nonetheless, because the fusion peptide is highly conserved across coronaviruses (*8*), it is an attractive target for a universal coronavirus vaccine that could generate broadly neutralizing antibodies and thereby protect against seasonal HCoVs, SARS-CoV-2, and future zoonotic coronavirus spillovers.

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