Bell and colleagues recorded the spectrum again. Each atmospheric component absorbs stellar light at a distinct frequency, like a unique fingerprint, so by comparing the two spectra acquired and analysing which frequencies were missing during WASP-80b's transit (known as the primary eclipse) the researchers could infer the atmospheric composition at the planet's terminator.

Bell *et al*. also measured the light emitted by the planet's dayside. To achieve this, JWST observed the WASP-80 system during the secondary eclipse, when the planet disappeared behind the star. Through the comparison of the star's spectrum alone (collected during the eclipse) with the combined spectrum of both the star and planet (obtained just before and after the secondary eclipse), the researchers were able to separate the light coming from the planet's dayside and hence determine its atmospheric composition.

The team's measurements show evidence of methane, along with water, in the planet's atmosphere at both the terminator and the dayside. The methane concentration seems to be the same in these two regions, indicating a well-mixed atmosphere. The findings suggest that the composition of WASP-80b's atmosphere differs from that of the Sun in terms of both the carbon-to-oxygen ratio and the concentration of heavy metals. However, the measurements were not precise enough to make definitive speculations about the planet's formation, and this emphasizes the need to access a broader wavelength range than is possible with the particular JWST instrument used by Bell and colleagues.

Nonetheless, the limitations of JWST's predecessors are implicit in the absence of methane in previous studies of transiting planets made with space telescopes. These limitations are attributed to the small wavelength coverage of the instruments, warranting a re-evaluation of planets previously observed with space-based instruments. This is especially pertinent for warm exoplanets, for which chemical models predict methane to be the main carbon-bearing species.

Bell and colleagues found convincing evidence for methane using just one JWST instrument, offering limited wavelength coverage. Their success in this protracted game of hide and seek suggests that using the full spectral capabilities of JWST could offer valuable insights into exoplanet formation, migration and, in turn, comparisons with planets in our Solar System. These efforts might also provide crucial insights into the potential habitability of exoplanets, particularly those that have the perfect combination of characteristics for hosting life — another game worth playing.

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Biophysics

Protein condensation regulates water in cells

J. Pedro de Souza & Howard A. Stone

Proteins can condense to form membraneless organelles, which act as vessels for biochemical reactions in cells. An investigation shows that protein condensation is also a cellular mechanism for controlling water availability. **See p.842**

Cells maintain an internal pool of water that is essential for biological processes. Moreover, they need to regulate this water supply quickly in response to environmental challenges that affect water availability — such as high temperatures or imbalances of dissolved molecules — to prevent damage and maintain their normal functions. On page 842, Watson *et al*.[1] show that subcellular-scale phase transitions involving the condensation of proteins and the redistribution of water molecules[2] can enable such rapid responses.

One way in which cells control their internal chemical environment is by allowing the entry or exit of water across the cell membrane through osmosis. Osmosis is the passive transport of water across a semipermeable membrane, and depends on the relative concentrations of dissolved molecules (solutes). That is, if the solution outside the cell is hypo-osmotic (the solute concentration is lower than that in the cell), then water is driven into the cell. The opposite is true in hyperosmotic conditions (in which the solute concentration is higher outside than in the cell). However, the known cellular regulatory mechanisms that combat osmotic fluctuations operate on timescales of minutes to hours[1,3], and might not be fast enough to protect cells when rapid environmental changes occur.

"Biomolecular condensates act as a buffer against environmental changes that could have catastrophic consequences for cells."

Moreover, molecules in aqueous environments, such as the inside of a cell, are hydrated. In other words, they are surrounded by a layer (or sometimes a few layers) of ordered water molecules around the dissolved molecule. In cells, this 'bound' water is distinct from the disordered, 'free' water that constitutes the rest of the aqueous cytoplasm. Water in cells helps to determine the conformation of macromolecules such as proteins, thereby affecting the functions of these molecules. And because chemical groups exposed on the surface of macromolecules interact with water, those molecules can reciprocally affect the fractions of water that are bound or free[4].

Besides their hydration by intracellular water, proteins and other macromolecules can also associate with each other. These interactions can reorganize the macromolecules in a way that causes them to form distinct phases in cells[5,6], a process known as biomolecular phase separation. The resulting phases are called biomolecular condensates, and can concentrate and segregate macromolecules into membraneless organelles. These organelles thus act as self-assembled containers for biochemical reactions in cells.

Condensates contain water interspersed between neighbouring macromolecules, hydrating the surfaces of those molecules and facilitating their interactions. Some evidence points to a connection between water-mediated interactions and biomolecular phase separation, such that the association of proteins in condensates expels some bound water from protein surfaces[2,7]. This prompted Watson *et al*. to study how biomolecular phase separation affects the equilibrium between bound and free water in cells.

The authors first showed that the chemical responses of cells to changes in environmental

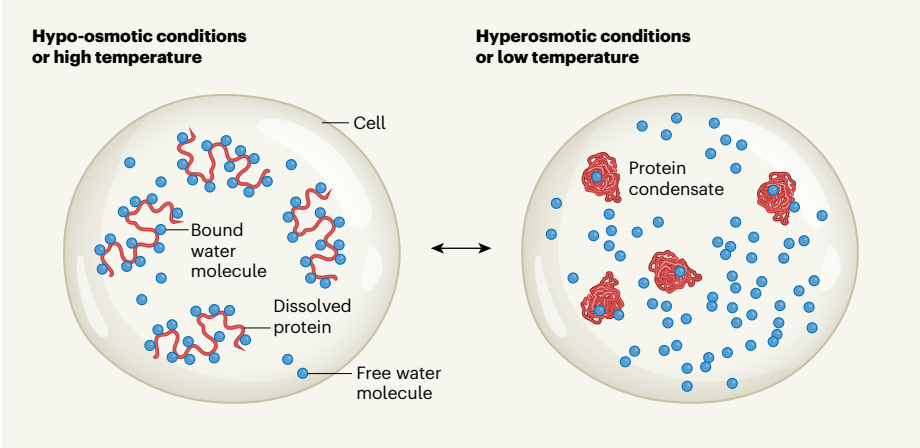

Figure 1 | Biomolecular condensates buffer cells against osmotic or thermal fluctuations. In cells, dissolved macromolecules such as proteins bind to one or more layers of water molecules, which restricts the movement of these water molecules in the cytoplasm and limits their availability for biological processes. However, proteins can also condense to form membraneless droplets, thereby releasing some of the bound water and producing free water molecules. Watson *et al.*[1] report that protein condensation can buffer the cell's response to environmental changes that alter the internal availability of water. In hypo-osmotic conditions (lower solute concentrations outside the cell than inside) or at high temperatures – both of which cause an increase in free water molecules in the cytoplasm – proteins dissolve and capture some of the free water. By contrast, in hyperosmotic conditions (higher solute concentrations outside the cell than inside) or at low temperatures – both of which reduce the amount of free water – proteins condense, releasing bound water molecules.

temperature are approximately equivalent to those produced by independent changes to the environment's osmotic strength. More specifically, the response to temperature increases is similar to that induced by hypo-osmotic conditions, whereas temperature decreases have a similar effect to hyperosmotic conditions. These observations are consistent with the idea that bound water is released to become free water with increasing temperature, and that free water becomes bound with decreasing temperature. The observed behaviour goes beyond what would be expected for an 'ideal solution' – the model of chemical solutions that is typically used for mathematical descriptions of osmosis, in which osmotic changes in a solution are linear with respect to temperature and chemical concentration.

On the basis of their observations, Watson *et al.* proposed that proteins influence the availability of water in cells. To verify this, they analysed cultured cells to identify proteins that increase in abundance both with temperature and when the cells are in hypo-osmotic environments. The authors found that many of these proteins are known to undergo biomolecular phase separation. Moreover, they observed that condensate formation occurred rapidly (within seconds) in response to environmental perturbations. They also showed that intrinsically disordered regions (IDRs) of proteins are central to the organization of water in cells, again using experiments in which the temperature or the osmotic strength of the cell environment was changed.

As a further step, Watson *et al.* showed that biomolecular condensates act as a buffer against environmental changes that could have catastrophic consequences for cells. In conditions that cause an increase in free water molecules in the cell (a hypo-osmotic environment and/or a high temperature), proteins dissolve into the cytoplasm, thus exposing IDRs and reducing the number of free water molecules (Fig. 1a). Alternatively, in conditions that cause a decrease in free water molecules in the cell (hyperosmotic environment and/or low temperature), proteins condense, thus removing IDRs from the cytoplasm and releasing water molecules (Fig. 1b). The transition between different states of the proteins enables the cell to maintain an equilibrium between bound and free water to mitigate environmental perturbations. In the language of thermodynamics, the phase behaviour of the biomolecular condensates adjusts the chemical potential of the water to maintain equilibrium.

Watson and colleagues' multifaceted investigation weaves together several lines of experimental evidence, including studies of heat-sensitive yeast cells, calcium signalling in cartilage cells from mice, the condensation of proteins such as bovine serum albumin, and osmotic measurements of extracts from frog eggs, among many others. Together, this evidence reveals how general principles of chemical thermodynamics influence a wide variety of cell types when cells are exposed to environmental challenges or shocks.

The idea that phase transitions can act as buffers is not unexpected. In everyday life, for example, we use a solid–liquid phase transition – the melting of ice into liquid water – to buffer the temperature of cold beverages. Nevertheless, the connection between protein condensation and water-regulation feedback in cells has not been demonstrated before, although the importance of water in these phase transitions has been recognized[2]. Whether biomolecular condensation has a functional role in certain specific cellular processes has been debated[8], but the non-specific regulation of free water in cells could certainly have provided an evolutionary impetus for condensation to occur. The osmotic feedback of phase separation presents yet another example of biomolecular condensation influencing cellular activity[5].

The role of water thermodynamics in biomolecular phase separation should now be examined in more detail. First, models based on the physical processes that underpin condensation should be developed to quantify the buffering capacity and concentration range of biomolecular condensates during thermal or osmotic challenges. These predictions could then be tested experimentally in stressed cells, and the osmotic regulation could be quantitatively linked to the IDR composition (how much of each protein consists of IDRs). Perturbations to the osmotic potential of water might exhibit a complex relationship with the density of the electrical charges of macromolecules in the crowded interior of the cell[9], depending on the ionic composition of the cytosol.

These veins of research would further define the mechanism by which free water is buffered by condensates, and would help to explain the delicate biochemical and osmotic balances that keep living organisms alive even when stressed. The next steps promise to be an exciting phase for work in this field.

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