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Christoph A. Weber; Christoph Zechner

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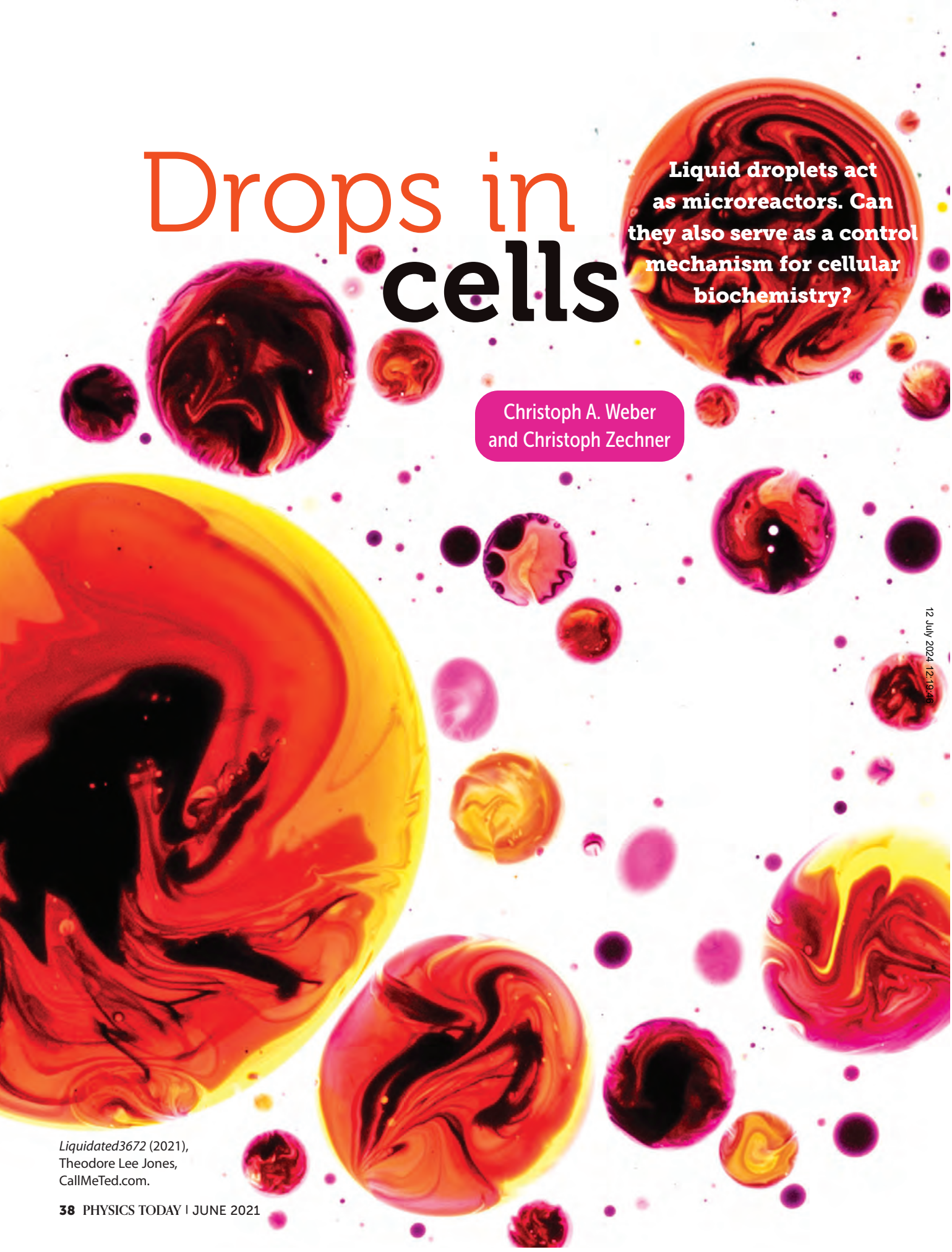

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Christoph Weber is affiliated with the Max Planck Institute for the Physics of Complex Systems in Dresden, Germany, and the Center for Systems Biology Dresden (CSBD).
Christoph Zechner is affiliated with the CSBD and the Max Planck Institute of Molecular Cell Biology and Genetics, also in Dresden (photo © MPI–CBG).

A major challenge in cell biology remains unraveling how cells control their biochemical reaction cycles. For instance, how do they regulate gene expression in response to stress? How does their metabolism change when resources are scarce? Control theory has proven useful in understanding how networks of chemical reactions can robustly tackle those and other tasks.[1] The essential ingredients in such approaches are chemical feedback loops that create control mechanisms similar to the circuits that regulate, for example, the temperature of a heating system, the humidity of an archive, or the pH of a fermentation tank.

Theories for the control of biochemical reactions have largely focused on homogeneous, well-stirred environments. However, macromolecules inside cells are often highly organized in space by specialized subunits called organelles. Some organelles, such as the cell nucleus, are bound by a membrane. By contrast, another class of organelles—biomolecular condensates—show the hallmark physical properties of liquid-like droplets, and they provide chemically distinct environments for biochemical reactions.[2–4]

Such droplets can act as microreactors for biochemical reactions in a living cell (see figure 1). Their liquid nature sustains the fast diffusion of reactants while their specific composition gives rise to the partitioning of reactants in or out of the droplets. In general, the concentrations of reactants inside condensates differ from the concentrations outside. Those differences modify reaction fluxes, which, in turn, can dramatically affect reaction yield and other properties of chemical reactions. Just how such modified fluxes govern the biochemistry inside cells remains poorly understood.

In this article, we use the physics of phase separation and control theory to speculate on how liquid condensates could realize feedback control strategies in living cells.

Controlling well-mixed systems

Biochemical reactions serve as biology's basic building blocks for processing and controlling cellular information. They regulate cellular metabolism and the sensing of environmental cues through signal transduction. They also regulate the expression of genes, whose information, stored in DNA, is transcribed and translated into functional proteins.

Networks of chemical reactions provide a useful mathematical description of such processes. In that formalism, molecules are described as particles, which diffuse randomly inside a reaction volume. When two (or more) molecules of the right type encounter one another, they can undergo a chemical reaction. Once the reaction occurs, the original reactant molecules are converted into a set of product molecules. In the bimolecular reaction, $A + B \rightleftharpoons C + D$, molecule types A and B are converted into C and D. Intracellular processes typically comprise a multitude of reaction cycles, in which the products of one reaction serve as the reactants of another reaction and so forth. That interdependence results in intricate networks of chemical reactions, which can exhibit rich and complex dynamical behaviors, such as oscillations or multistability.

The dynamics of chemical reaction networks can be described using the theory of chemical kinetics, which captures how the concentrations of different chemically interacting molecules change with time. In its conventional form, chemical kinetics applies to well-mixed systems—that is, to reactions that run much more slowly than the molecules diffuse inside the reaction volume. In other words, the size of the reaction volume is much smaller than the corresponding reaction–diffusion length scale. Local changes in concentration that arise from a reaction will therefore be instantaneously homogenized by fast diffusion. In such a well-mixed system, the rate at which a reaction takes place depends only on the number of molecules present in the system; the exact spatial positions of those molecules become irrelevant. Well-mixed chemical systems can be fully characterized in terms of the temporal dynamics of the reactants' concentration levels.

A hallmark of biochemical networks inside cells is their remarkable robustness against random fluctuations in their molecular constituents, changes in their surroundings, or other potential disturbances. Previous studies have demonstrated that negative feedback mechanisms play a pivotal role in stabilizing various cellular processes against such disturbances.[5] Autoregulatory gene networks, in which a protein inhibits its own expression, are archetypical examples. If the protein level falls below a certain set point, the protein's inhibitory effect on transcription weakens and the protein level begins to rise. As the set point is approached (or exceeded), the negative feedback becomes more pronounced, which in turn reduces production of more of the protein. In that way, protein levels can be maintained within narrow ranges, despite potential disturbances that may affect the system.

Although that simple example illustrates the core idea behind negative feedback regulation, biological systems are often substantially more complex, involving many reaction cycles, concurrent feedback loops, and strong nonlinearities. Extracting simple principles that shed light on the functioning of such systems becomes challenging.

Control theory provides a rich framework for abstracting

and analyzing dynamical systems subject to feedback. Beyond its traditional applications in engineering, control theory has proven a powerful way to study and reverse-engineer biological systems. In fact, by casting a given biological system within the formalism of control theory, a large repertoire of mathematical tools and concepts becomes applicable. Those tools and concepts make it possible to assess the essential properties of the system under consideration, even when some of its details are unknown.

Control theory basics

In a classical control problem, one considers an arbitrary physical process with input x and output y. For example, y could correspond to the temperature of a room, whereas x could be the power of a heating device in that room. The goal is to control the output y with respect to a certain reference value u, such as a desired ambient temperature, through a negative feedback loop. That loop contains a controller, which measures the mismatch between y and u and calculates an appropriate control input x to reduce the mismatch.

The function that maps y and u to x is referred to as a control law. In engineering applications, it is chosen based on performance and available resources. One of the simplest control laws is called proportional control. Here, the controller adjusts x purely based on the instantaneous mismatch between y and u. More precisely, $x = G(u - y) = eG$, where G is the feedback gain, which determines how strongly the controller reacts to a mismatch e. When G is sufficiently high, the closed-loop dynamics will be predominantly governed by $G(u - y)$, which in turn will effectively reduce the mismatch between u and y. Control is exerted even in the presence of a potential disturbance d; that disturbance could be a physical perturbation to the system or additional and possibly unknown fluxes and fluctuations.

More effective controllers can be achieved by extending the control law and adding terms that are proportional to the integral of the mismatch e, its derivative, or both. So-called proportional-integral-derivative controllers exhibit improved dynamical properties compared with pure proportional ones, such as zero steady-state error or improved convergence toward the desired reference value u.

To demonstrate more concretely how biochemical feedback systems can be studied using control theory, let's consider a simple toy model of protein expression.[6] In that system, proteins are produced and degraded with rate constants x and γ (see figure 2a). Dynamics are affected by a perturbation d, which for simplicity we consider constant. In a steady state, the protein level is given by $y = (x + d)/\gamma$. That is, protein level y is sensitive to the perturbation d and scales linearly with it.

Now consider a modification to the network: The protein negatively regulates the expression of additional protein by binding to its own promoter (figure 2b). In that case, the protein production rate can be described using a Hill-type function such that $x(y) = \lambda K^n/(K^n + y^n)$; n, K, and λ are positive parameters. For small y, the production rate will be close to λ, whereas for large y, it approaches zero. Linearizing the function around the steady-state value of y reveals a simple controller structure, in which the protein production rate is approximately given by the mismatch between some reference u and the protein level y, multiplied by a constant gain G (see figure 2b). Both u and G are functions of the kinetic parameters K, n, and λ; explicit dependences are omitted here for compactness.

That simple analysis shows that our genetic feedback circuit acts, to first order, like a proportional-feedback controller in that it tries to maintain the protein level y at some target value u. Thus if G is sufficiently large, y attains values close to u, even in the presence of the perturbation d. That can be seen when comparing the protein levels in time subject to a constant perturbation for the open-loop and closed-loop genetic circuits (figure 2c). Although the open-loop circuit is sensitive to perturbations, they are largely suppressed in the corresponding closed-loop system. References 6–8 provide further information on how control theory can elucidate biological and other physical systems.

Phase separation and chemical reactions

Liquid condensates can form in a multicomponent mixture via phase separation and stably coexist within an environment of lower concentration.[9] The thermodynamic behavior of such a mixture is governed by the minimization of the free energy, which accounts for the competition between the interaction energy and entropy. For phase separation to occur, molecules in a solvent need to attract each other or repel the solvent molecules such that the gain in interaction energy of a coexistence state outcompetes the corresponding disadvantage of forming an interface.

That phase coexistence is affected by various physicochemical control parameters such as concentrations of molecules and salt, pH, and temperature. The parameters define the phase diagram, which depicts the equilibrium parameters of the coexisting phases—that is, the condensate and its environment. Minimization of the free energy at a fixed average concentration of molecules implies that coexisting phases have the same chemical potential at thermodynamic equilibrium. The chemical potential corresponds to the slope of the free-energy density. Most importantly, the coexisting phases might differ not only in their respective concentrations but also in salt concentrations, pH, and other control parameters.

A spatially heterogeneous chemical potential leads to molecular fluxes, whose existence indicates that the system is not at thermodynamic equilibrium. An emulsion composed of many droplets provides one example. To see why an emulsion is not at equilibrium, we need to consider the droplet interfaces. The

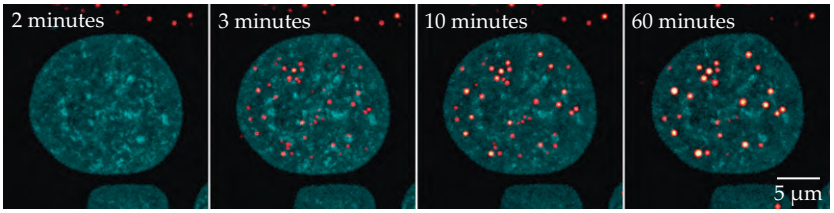

FIGURE 1. LIQUID DROPLETS could conceivably act as feedback controllers to regulate biochemical processes in cells. In this microscope image, fluorescently labeled proteins form liquid droplets inside the nucleus of a HeLa cell after the ambient temperature is lowered. (Image © Adam Klosin.)

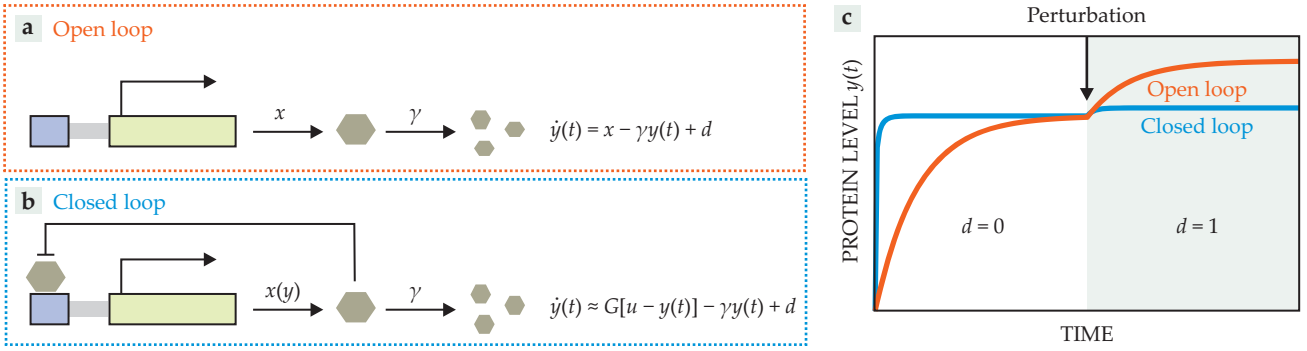

FIGURE 2. THE EXPRESSION OF A GENE (bent arrow on green rectangle) is initiated in this toy model by the activation of a promoter (blue rectangle) to yield the corresponding protein (gray hexagons) at a concentration that changes at the rate $y(t)$. **(a)** In the absence of feedback (open loop), protein molecules are produced and degraded with the rate constants x and γ, respectively. **(b)** In the presence of negative feedback (closed loop), the protein inhibits its own expression rate, which depends on the protein concentration y. **(c)** Open-loop expression is less robust against a constant perturbation d than closed-loop expression.

total interfacial area is lowest—and therefore the free energy is minimized—in the case of a single droplet. An emulsion of many droplets thus evolves with time toward a single drop corresponding to thermodynamic equilibrium.

Chemical reactions can affect the coexistence of phases. At thermodynamic equilibrium, a chemical reaction imposes a further constraint on the system: The sum of reactants' chemical potentials on each side of the reaction scheme (weighted by the stochiometric factors) must balance.

In general, in a mixture with M independent components and s chemical reactions at thermodynamic equilibrium, there are only $(M - s)$ independent concentrations.[10] The remaining concentrations are typically associated with the conserved quantities of the reactions, such as the total mass or the total molecular volumes of the reactants. For example, an incompressible ternary mixture ($M = 2$) with a reversible chemical reaction $A \leftrightharpoons B$ ($s = 1$) gives one independent concentration that is equal to the conserved quantity of the chemical reaction. In general, the phase diagram exhibits a lowered dimension compared with the same system without chemical reactions, and the axes of the corresponding phase diagram of the reaction—the maximal number of coexisting phases—reduce by one for each chemical constraint. Thus, according to the Gibbs phase rule, chemical reactions at thermodynamic equilibrium suppress the coexistence of multiple phases. That reduction to a smaller set of conserved variables and the suppression of multiphase coexistence do not necessarily prevail away from thermodynamic equilibrium.

An interesting case is a chemical reaction maintained away from equilibrium through the supply of fuel molecules. Those molecules get irreversibly degraded in a reaction step that breaks the reaction rates' detailed balance and leads to phenomena that are impossible at thermodynamic equilibrium.[9] A paradigm of such reactions is the division of liquid condensates, which represents the inverse kinetics of thermodynamic systems becoming more heterogeneous via fusion.

Coexisting phases also affect chemical reactions. To see why, consider a simple mixture of two types of molecules: scaffolds and clients. Scaffold molecules make up the condensates, whereas clients undergo chemical reactions.[11] If the clients are dilute, their impact on the scaffold-rich condensates can be neglected. However, because of the presence of condensates, the reacting clients experience a spatially heterogeneous environment. Such an environment has essentially two key effects on chemical reactions. First, the reacting clients are partitioned in or out of the condensates, where partitioning arises from the interactions between clients and scaffold molecules. Second, inside each phase, clients follow a reaction–diffusion type of kinetics with reaction rates specific to each phase. The rates emerge because each phase differs in its concentration and in its reaction rate constants. The two effects are instrumental in liquid condensates' regulation of chemical reaction, and they embody the condensates' potential as control strategies for biochemical reactions.

Control of chemical reactions in demixed systems

To gain a better understanding of how phase-separating systems could control reactions, it is useful to analyze their dynamical properties from a control theory perspective. To that end, we consider a simple phase-separating system that demixes into two phases: one rich in scaffold molecules; the other, poor (figure 3a). Kinetic equations for the scaffold concentrations can be derived from thermodynamic considerations in the limit where diffusion through the droplet is fast compared with the first-order reaction rate constant.[12] In that limit, concentrations in each phase are approximately homogeneous, which means the change in the dilute-phase scaffold concentration $c^{\mathrm{II}}(t)$ can be described by an ordinary differential equation:

$$\frac{\mathrm{d}}{\mathrm{d}t} c^{\mathrm{II}}(t) = \frac{kV}{V - V^{\mathrm{I}}(t)} [c^{\mathrm{II}}_{\mathrm{eq}} - c^{\mathrm{II}}(t)] + s[c^{\mathrm{II}}(t), V^{\mathrm{I}}(t)], \qquad (1)$$

where k is a relaxation rate toward equilibrium that depends on diffusivity, for instance. The term $c^{\mathrm{II}}_{\mathrm{eq}}$ is the equilibrium dilute-phase concentration, and V and $V^{\mathrm{I}}(t)$ are the total and droplet volume, respectively. The equation captures the partitioning of scaffold molecules between the dilute and dense phases. The kinetics of $c^{\mathrm{II}}(t)$ are affected by the reaction flux s, possibly through the production and turnover of scaffold molecules. That reaction flux drives the system away from equilibrium.

We can now recast this phase-separating system using the notion of feedback control. In particular, if we consider the dilute-phase scaffold concentration as the system's output $y(t)$, then the sum of the two exchange fluxes on the right-hand side implement what can be thought of as an error calculation between the current concentration—that is, the output $y(t) = c^{\mathrm{II}}(t)$—and the corresponding equilibrium concentration, or the reference value $u = c^{\mathrm{II}}_{\mathrm{eq}}$. The resulting error is multiplied by a time-dependent gain $G = kV/[V - V^{\mathrm{I}}(t)]$.

The droplet therefore acts like a proportional controller to maintain dilute-phase scaffold concentrations at a certain set point, despite additional fluxes and perturbations (see figure 3b). The effectiveness of the controller depends on the feedback gain G, which emerges from the physical properties of the mixture, such as interaction strengths among scaffold molecules and their diffusivity.

A similar feedback control structure can also be identified for dilute molecules that partition into liquid condensates. However, in contrast to the scaffold molecules, the controller no longer regulates absolute concentrations, but instead adjusts the ratio between inside and outside concentrations. We call that ratio the effective partition coefficient $p(t) = c^{\mathrm{I}}(t)/c^{\mathrm{II}}(t)$. How $p(t)$ changes can be expressed by the equation

$$\frac{\mathrm{d}}{\mathrm{d}t}\,p(t) = kV\left(\frac{1}{V^{\mathrm{I}}} + \frac{p(t)}{V^{\mathrm{II}}}\right)[p_{\mathrm{eq}} - p(t)] + s[c^{\mathrm{I}}(t), c^{\mathrm{II}}(t)]. \qquad (2)$$

Here, p_{eq} (defined as $c^{\mathrm{I}}_{\mathrm{eq}}/c^{\mathrm{II}}_{\mathrm{eq}}$) is the partition coefficient at equilibrium, and V^{I} and V^{II} are the volumes, respectively, of the dilute and dense phases. The expression suggests that for client molecules, the droplet resembles a proportional controller with output $y(t) = p(t)$, reference $u = p_{\mathrm{eq}}$, and feedback gain (see figure 4a). Thus, whereas client concentrations in each phase are sensitive to additional fluxes and perturbations, the partition coefficient can be robustly maintained through the feedback control loop (see figure 4b). The feedback, in turn, provides interesting ways to control the concentrations of downstream chemical processes such as enzymatic reactions.

How plausible is the idea that biomolecular condensates serve as feedback controllers inside cells? Although the field is still young, several recent studies point toward biological systems in which droplet-mediated feedback control may indeed be relevant. One example is the suppression of concentration fluctuations.[12–14] Because the concentration of scaffold molecules inside and outside the condensate is thermodynamically constrained, the condensate is expected to respond to fluctuating concentrations by changing its size. The concentrations within each phase are much less affected. In line with the scaffold control scheme shown in figures 3b and 3c, the condensate can thus be understood as a feedback controller, which tries to minimize the mismatch between the concentrations in each phase and their reference equilibrium value. The effectiveness of the controller (as reflected by the feedback gain G) depends on the underlying physical parameters and interactions. Earlier studies have used control-theoretical concepts to identify hard lower bounds on the suppression of noise in homogeneous biochemical feedback circuits.[15] Understanding how spatial compartmentalization affects those results is an important open problem.

A related and more complex negative feedback circuit has been recently proposed in the context of transcriptional condensates.[16] They are thought to enhance the transcription of messenger RNA (mRNA) by concentrating the required transcriptional machinery, such as transcription factors, cofactors, and polymerases. Interestingly, the study found that as soon as the newly transcribed mRNA exceeds a certain set point, it can promote the dissolution of the condensate and thereby arrest transcription. This gives rise to a negative feedback circuit, which could reliably control the duration and output of transcription despite potential disturbances.

Evidence is growing that condensates can control aggregation processes, including formation of physiological filaments such as actins and microtubules, and of disease-related fibrils such as amyloids. Although the biological roles of aggregates are diverse, their formation shares some common physical principles. Initial aggregates form via primary nucleation and grow mostly at their ends, and secondary nucleation allows small aggregates to form near the surface of existing aggregates.[17]

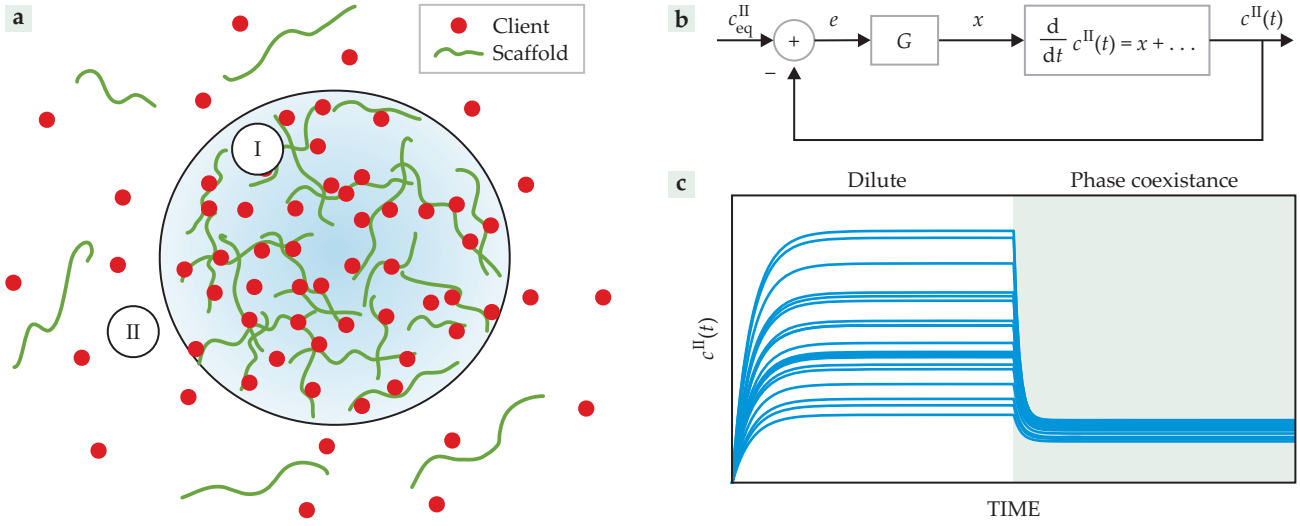

FIGURE 3. FEEDBACK CONTROL in a phase-separating system. This schematic illustration of the system **(a)** features scaffold (green) and client (red) molecules both inside (I) and outside (II) a phase-separated droplet. **(b)** Feedback control maintains the concentration of scaffold molecules outside the droplet, $c^{\mathrm{II}}(t)$, at its equilibrium value $c^{\mathrm{II}}_{\mathrm{eq}}$ through a proportional controller whose gain G depends on various quantities. Among them are the droplet volume and relaxation rate toward equilibrium. **(c)** Example trajectories demonstrating scaffold-based control for the case when the production rate of scaffold molecules varies randomly. As soon as phase separation is activated and droplets form (gray shaded area), dilute-phase concentrations are tightly regulated through feedback control.

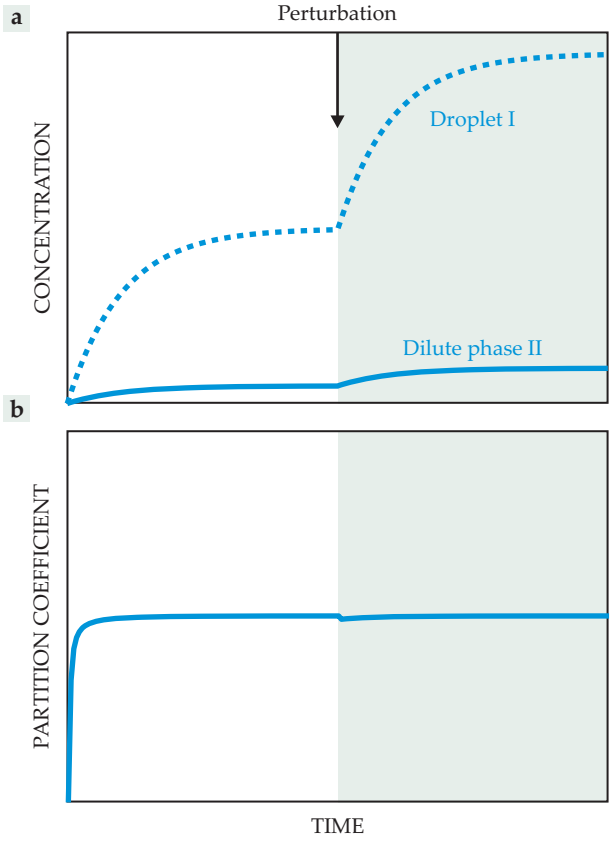

FIGURE 4. FEEDBACK CONTROL of clients. The concentration of a client **(a)** is sensitive to the onset of a perturbation (gray area), but the partition coefficient **(b)**—that is, the ratio of concentrations in the droplet and dilute phase—is robustly maintained by the droplet-mediated proportional controller.

Interestingly, condensates can mediate a feedback mechanism for aggregates already forming solely via primary nucleation. As monomers partition into the condensate and aggregate within it, their concentration inside the condensate should decrease. However, a fraction of the aggregated monomers is replenished by the partitioning flux, which tries to maintain the partitioning of monomers inside and outside the condensates. As a result, the growth of aggregates inside the condensates is favored, whereas aggregation outside is suppressed. For this simple aggregation process, condensates provide a mechanism that is reminiscent of a control circuit. The scaffold-rich condensate serves as a controller that adjusts the partitioning of aggregation-prone monomers and thus regulates the nucleation kinetics. That feedback is similar to the client example depicted in figure 4 and equation 2.

Researchers have recently found a similar but slightly more intricate feedback mechanism that is mediated by condensates. It occurs in systems of fibrils that undergo secondary nucleation and grow at their ends.[18] Interestingly, the mechanism controls the number and size of aggregates by changing the condensate's characteristic physical parameters, such as the monomer partitioning and condensate size. The findings support the potential relevance of condensates in regulating physiological assemblies or disease-related amyloid fibrils.

Final thoughts

The ideas outlined in this article illustrate how condensates in living cells could act as biomolecular controllers, akin to a thermostat regulating the temperature of a room. The similarities rely on the ability of condensates to mediate negative feedback and stabilize the properties of chemical reactions in dynamically changing conditions. The feedback in phase-separating systems originates from condensates coexisting with an outer phase of different concentration. That situation can give rise to molecule fluxes through the condensate interface that maintain specific concentration levels inside and outside or maintain functions of the concentrations.

We limited our biomolecular examples to proportional control, which is one of the simplest feedback control architectures. However, more robust and effective control strategies, such as integral feedback control,[1,8] could be realized when phase separation is coupled to additional reaction cycles. That possibility suggests a fruitful avenue for further research.

But why is the view that condensates can mediate feedback control helpful in the first place? First, consider a different question: Is it useful to describe a modern computer by modeling the physics of electron transport in each individual transistor? For many physicists, the answer to this question is no—because such an approach would be exceedingly complex and could fail to capture the system's emergent properties.

Cells host an extremely and analogously complex network of biochemical processes, many of which we are only beginning to understand. Analyzing such systems through control theory provides a universal strategy to extract the relevant structural and dynamical features of biological processes—without requiring a detailed physical description of the system. Understanding the interplay between spatial compartmentalization and biochemical reactions through control theory may open up new avenues to comprehending and even controlling functionality in living cells.

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