Phase separation

https://doi.org/10.1038/s44286-026-00369-0

Out of equilibrium and into structure

Ece Büber & Job Boekhoven

Check for updates

Condensates are often treated as equilibrium droplets, yet they can be remodeled by transient environmental cues. Now, a study shows how the rate of a pH change determines whether droplets stay uniform or develop vacuoles.

Biomolecular condensates are liquids inside another liquid, yet they are far from being simple oil-in-water droplets. Although condensates are typically viewed as homogeneous liquids formed by liquid–liquid phase separation, they are made of dynamic materials that can rapidly reorganize as a response to environmental stimuli such as pH, ionic strength and temperature. This can give rise to internal organization, from core–shell architectures to gradients in density, viscosity or charge. Internal organization is common, from the multilayered nucleolus and core–shell stress granules to spatially inhomogeneous or network-like condensate interiors observed in recent studies[1,2]. Such internal features can strongly affect how molecules partition or react[3]. How these structures arise and how external stimuli can reshape them are emerging questions in the field. A recent community perspective reframed biomolecular condensates as active, out-of-equilibrium materials shaped not only by thermodynamics but also by kinetics[4]. This shift has drawn attention to how kinetic effects shape condensate structure.

One example comes from work reported in *Nature Chemical Engineering* by Modi and colleagues[5], who show that the rate of a pH change can control whether a condensate remains uniform or hollows into a vacuole. The authors revisited a pH-sensitive model system based on glucose oxidase and diethylaminoethyl (DEAE)-dextran to probe how the condensates made of these components change under pH variations. An elegant capillary-based assay coupled to microscopy allowed direct visualization of how different pH change rates drive condensates out of equilibrium. By quantifying local pH with a colorimetric indicator and tracking hundreds of droplets, the study linked rates of pH change to droplet size and internal reorganization.

Across capillary experiments, condensates that experienced a faster pH drop, or those larger in size, were more likely to develop vacuoles, whereas slower pH changes or smaller condensates remained uniform (Fig. 1). The difference lies in timing. When the external pH change is faster than the diffusion of molecules, the exchange of material between the droplet and its surroundings becomes diffusion limited, driving the condensate out of equilibrium. A composition gradient arises because material at the droplet interface responds faster than the interior during the pH change. Unable to redistribute quickly enough, the droplet transiently separates into dense and dilute regions, forming vacuoles that appear as transient cavities inside the condensate. When the pH change is slow, diffusion has time to keep up, letting the droplet adjust smoothly and preserve its uniformity.

To quantify these observations, the authors developed a minimal theoretical model linking pH-dependent molecular interactions with phase-separation kinetics. According to the model, when the pH change is too quick for diffusion to keep up, the phase boundary moves abruptly, pushing the condensate composition into the unstable spinodal region. In this regime, small fluctuations in local composition are no longer suppressed but instead amplify spontaneously.

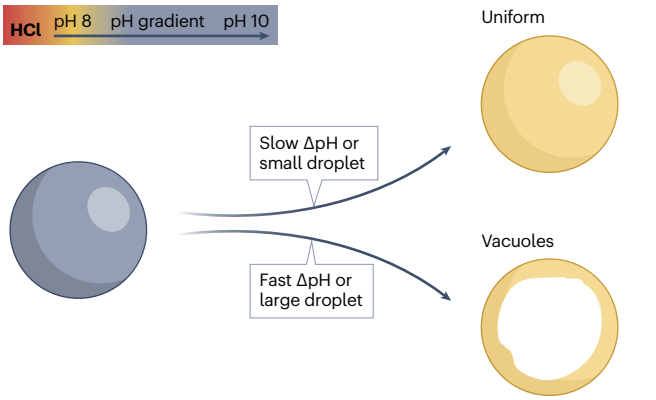

Fig. 1 | pH-driven non-equilibrium structuring of condensates. A capillary-based pH gradient creates different rates of acidification across condensates. When the external pH changes faster than internal molecular diffusion, condensates fall out of equilibrium and undergo spinodal decomposition to form vacuoles, whereas slow pH changes maintain uniform interiors.

Such instabilities push the formation of vacuoles before the system relaxes back to its equilibrium. Simulations verify the experimentally observed boundary between droplet size and pH change rate, confirming that larger droplets, with slower internal diffusion, are more prone to spinodal decomposition and form vacuoles. Together, the results show that condensate properties stem from both its molecular composition and the timescale of environmental change and internal diffusion.

Beyond this enzyme–polymer pair, the study exposes a broader principle for soft and biological matter: condensates are not only governed by equilibrium thermodynamics but also can be reshaped by transient, out-of-equilibrium dynamics. The work shows how even a simple perturbation, such as a pH change, can drive a condensate through regimes where diffusion and reaction kinetics determine structure.

Real cellular condensates are far more complex, with ongoing biochemical activity that may continuously push them away from equilibrium. Integrating these active processes will prove important for connecting minimal models to living systems. Still, this work offers a clear message for both biology and materials design: structure can arise not solely from what a system is made of but also from how far and how fast it is driven from equilibrium.

Exploring how controlled perturbations such as chemical fueling, confined enzymatic activity or time-dependent environmental changes affect condensate structure can help to determine which non-equilibrium pathways are most relevant in cells, where condensates interact continuously with active processes. The ability to tune condensate

structure through controlled kinetic driving could inspire new classes of soft materials with programmable architectures, temporal responses or self-organizing behaviors, properties that are difficult to achieve under equilibrium conditions alone.

Ece Büber ✉ **& Job Boekhoven** ✉
Department of Bioscience, School of Natural Sciences, Technical University of Munich, Garching, Germany.
✉e-mail: ece.bueber@tum.de; job.boekhoven@tum.de

Published online: 16 March 2026

References

1. Farag, M. et al. *Nat. Commun.* **13**, 7722 (2022).
2. Dar, F. et al. *Nat. Commun.* **15**, 3413 (2024).
3. Villegas, J. A., Heidenreich, M. & Levy, E. D. *Nat. Chem. Biol.* **18**, 1319–1329 (2022).
4. Alberti, S. et al. *Nat. Commun.* **16**, 7730 (2025).
5. Modi, N. et al. *Nat. Chem. Eng.* https://doi.org/10.1038/s44286-025-00322-7 (2026).

Competing interests

The authors declare no competing interests.