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How membranes influence intracellular phase separation

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Liquid–liquid phase separation (LLPS) within cells is a captivating phenomenon known to aid the organization of cellular components; however, its complex kinetics have remained a puzzle. Now, a new study elucidates the crosstalk between the phase state of an encapsulating membrane and LLPS dynamics.

When binary fluids undergo phase separation, they transition from a uniform, homogeneous phase to a far-from-equilibrium state. In this state, the droplet phase experiences growth, increasing its volume fraction through material transport mechanisms, while coarsening leads to changes in droplet morphology and size. These kinetic processes, involving material transport through diffusion and flow, apply universally across various material systems, including metals, polymers, colloids and liquid crystals. However, when phase separation occurs within a confined system, the container boundaries and surface wetting effects (resulting from the contacts between the boundary and the confined droplets) introduce new constraints that influence kinetics and morphology. This phenomenon, known as surface-directed spinodal decomposition, has been extensively explored in studies of polymer mixtures undergoing liquid–liquid phase separation in contact with solid surfaces[1].

The biological cell, as the fundamental unit of life, also represents a confined container, but its wall (the membrane) is soft and flexible. Several studies[2,3] have recreated the crowded cellular environment within artificial cells. They use giant unilamellar vesicles (GUVs) of cell-size dimensions, encapsulating solutions of two chemically dissimilar and electrically neutral polymers: polyethylene glycol (PEG) and dextran. When phase separation occurs, the soft vesicle membrane that defines the container boundaries can exhibit preferential wetting by one of the emerging phases. This gives rise to a multitude of intriguing phenomena related to the

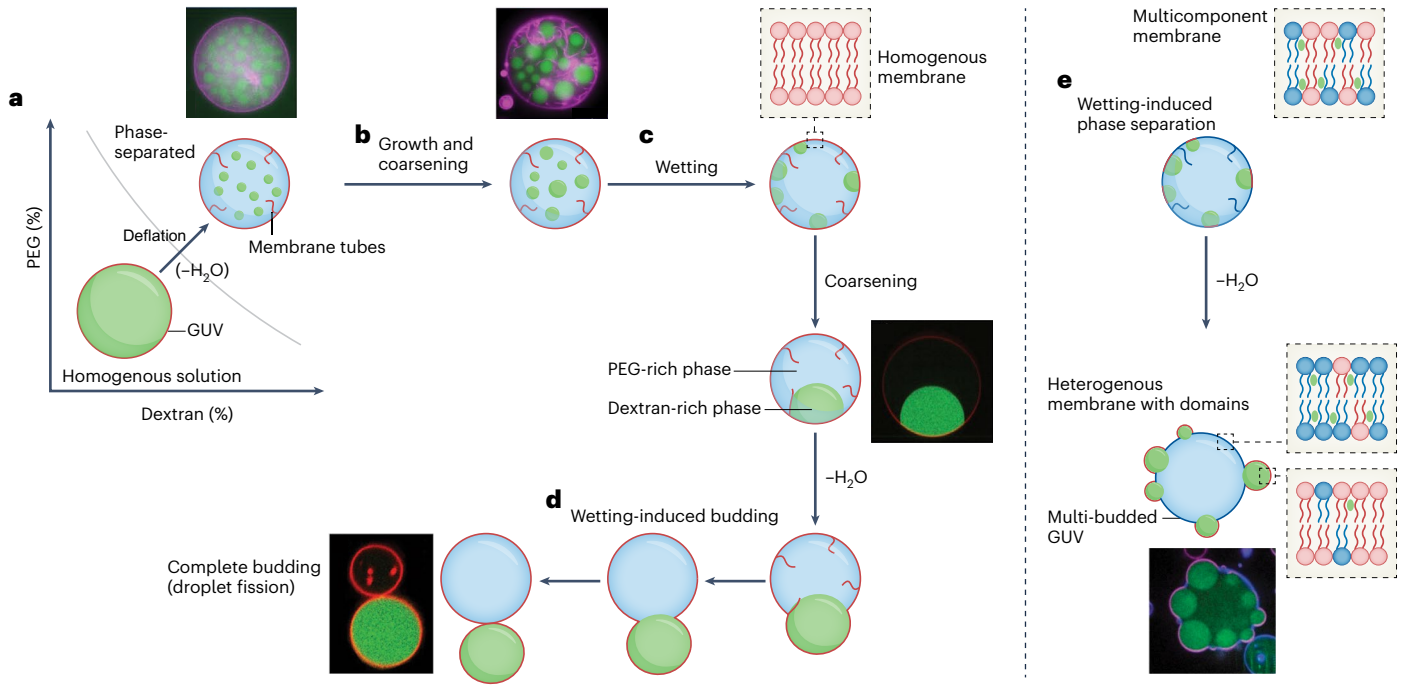

Fig. 1 | Response of vesicles loaded with PEG and dextran when exposed to osmotic deflation. a, Upon deflation, the homogeneous PEG–dextran solutions within the vesicle undergo phase separation crossing the binodal (grey line) in the phase diagram. If deflation produces excess membrane area, it can be stored in membrane nanotubes or buds. **b**, The formed dextran-rich droplets (green) grow and coarsen over time. **c**, The droplets can undergo a wetting transition by forming contacts with the membrane. **d**, In homogeneous (here represented by single-component) membranes, further deflation can result in wetting-induced budding, resembling exocytosis. **e**, Multicomponent membranes can undergo lateral phase separation coupled to the droplet phase wetting them. Example confocal images are reproduced from ref. 3 (panels c and d), Wiley; and ref. 4 (panels a, b and e), Springer Nature Ltd.

flexible nature of the membrane, including vesicle budding reminiscent of exo- or endocytosis, wetting transitions, vesicle division and the formation of membrane nanotubes[3] (see Fig. 1a–d). Beyond these processes, the vesicle membrane, as a soft supporting confinement, introduces new constraints that influence the phase separation kinetics and morphological evolution.

A new study[4] conducted by the research teams of Atul Parikh and Christine Keating now sheds light on the intricate interplay between membranes and the dynamic process of phase separation, showing the fascinating interactions between PEG- and dextran-rich droplets in GUVs. The researchers applied hypertonic stress to the vesicles to induce real-time phase separation and observed a series of distinct stages. Initially, tubular protrusions formed within the GUVs. Subsequently, dextran-rich droplets exhibited growth and coarsening (see Fig. 1a,b). These droplets then accumulated on the surface of the vesicle membrane (Fig. 1c), resulting in a unique partial "emulsification". The study unveiled that the growth of membrane-bound droplets is driven by a combination of evaporation–condensation and Brownian coagulation mechanisms.

An intriguing twist emerged as the droplets interacted with the vesicle membrane. The wetting of the membrane by the droplets led to the development of outwardly protruding buds, effectively dividing the GUV membrane into segments with different curvatures and mechanical tensions. This budding process gave rise to a remarkable metastable multi-droplet organization (such as the multi-budded GUV shown in Fig. 1e).

To mimic the complexity of cellular membranes, the authors explored GUVs made of a multicomponent homogeneous lipid mixture. Such membranes are not only deformable but can also undergo lateral phase separation and exhibit preferential affinity to one of the phases. The wetting by the droplets led to a lateral redistribution of the lipids that matched the droplet-induced budding pattern. The researchers observed lateral phase separation into membrane domains (Fig. 1e), driven by mechanical and chemical factors influenced by droplet wetting.

The intriguing phenomenon of LLPS within real cells, forming condensates often referred to as “membraneless organelles”, plays a crucial role in organizing cellular components. Because cellular LLPS strongly depends on the concentration of the involved molecules, salt, cosolutes and crowding conditions[5], it may be tempting to overlook the potential contributions of the confining membrane. This tendency is further influenced by the term "membraneless", which misleadingly suggests the exclusion of membranes. However, it is important to note that LLPS can indeed occur at the membrane, involving various membrane clusters in crucial cellular processes, such as signaling pathways and the recruitment of cytoskeletal elements, endocytosis and nuclear transport.

The discoveries made by Parikh, Keating and colleagues[4] carry profound implications for comprehending the non-equilibrium behaviors of LLPS within cell-like compartments. These findings underscore how confinement within small volumes and interactions with flexible, deformable and multicomponent membranes, susceptible to phase separation, can profoundly impact LLPS. This, in turn, provides valuable insights into the functional arrangement of membraneless organelles in both living cells and artificial cell models.

Studies in GUVs as artificial cells and in vitro reconstituted micron-sized macromolecular condensates showcase the potential to microscopically magnify processes of LLPS that take place in the confined and crowded volumes of cells. Research employing GUVs helps unveil membrane responses that are typically challenging to resolve, as exemplified by the coupling of LLPS and membrane phases[4,6], wetting transitions and complex membrane remodeling[7], and wetting-induced packing in the membrane[8].

It remains to be demonstrated whether the dynamic features elucidated by Parikh, Keating and co-workers[4] in the context of simple-component membranes encapsulating PEG–dextran solutions can be extrapolated to the intricate cellular environment with more complex membranes. However, it is conceivable that features and processes such as the stability, formation and localization of nanoscopic membrane rafts — which remain subjects of debate in the literature — might undergo substantial alterations due to wetting and interactions with protein condensates, as exemplified in the GUV model system featuring micron-sized domains. Despite the use of less biologically relevant molecules in this study, investigations involving in vitro reconstituted protein condensates offer compelling evidence of the interplay between protein-mediated LLPS and lateral phase separation in the membrane[6,9].

The extent to which the dominant kinetic pathways discussed by Parikh, Keating and colleagues[4] would retain their significance within the complex intracellular environment, characterized by a multitude of active processes, remains uncertain. Nevertheless, it is evident that fundamental physical laws can be applied to conceptualize cellular processes, thereby fostering a deeper understanding and better predictive capabilities in biology. The research from the groups of Parikh and Keating[4] provide valuable insights into the intricate dynamics of phase separation within confined spaces, illuminating the complex droplet interactions between bulk (three-dimensional) LLPS within vesicles and lateral (two-dimensional) lipid–lipid phase separation on the vesicle membrane. The observed dynamic coupling of phase separation with membrane morphology and composition reveals a new dimension of sophistication in cellular organization.

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Competing interests

The author declares no competing interests.