PHASE SEPARATION

Insulating proteins drop by drop

Ulrich Krauss

Liquid-liquid phase separation, yielding membraneless organelles, allows for the sequestration and functional insulation of cellular proteins. A modularly-built, synthetic membraneless organelle platform enables efficient control over endogenous cellular activities by knockdown of protein function or controlled protein release.

Intracellular compartments or organelles play an essential role in separating and orchestrating cellular reaction pathways. Apart from membrane-separated organelles such as the nucleus or mitochondria there are also membraneless organelles, also known as biomolecular condensates, which can sequester biomolecules by liquid-liquid phase separation (LLPS). These condensates usually form from highly concentrated and enriched biomolecules such as proteins and nucleic acids, yielding micron-sized liquid-like droplets within the cytoplasm[1] that play various roles in a multitude of cellular processes[2]. In recent years, LLPS-based membraneless organelles have been engineered for various applications ranging from cell biology to metabolic engineering[2-3]. In this issue, Garabedian *et al.*[4] present a synthetic membraneless organelle platform which allows for the efficient control of endogenous cellular activities by sequestering native proteins to synthetic organelles or *vice versa* releasing native proteins from preformed organelles, thereby enabling modular control of cellular decision making.

A key factor for LLPS is multivalency, a property of certain biomolecules to undergo low-affinity interactions and form a dynamically rearranging network that partitions into a separate phase from the surrounding solution. In nature, this is either achieved by interaction of multiple folded interaction domains or via self-association of intrinsically disordered (protein) regions (IDRs), which promote LLPS via multivalent weak interactions mediated by 'sticky' patches of residues along their disordered polymer chain[2-3]. Proteins capable of LLPS can act as scaffolds to which other cargo biomolecules (nucleic acids, proteins, substrates) can be sequestered.

While multiple synthetic membraneless organelle systems[2-3], as well as strategies not relying on LLPS[5] have been described for the sequestration of protein cargo and/or the functional insulation of endogenous proteins, existing systems possess a number of limitations. Tagging the endogenous cargo with large IDRs may alter protein function and hence compromise generalizability of the strategy. Related strategies that rely on inducibly anchoring endogenous targets to cellular structures such as the plasma membrane[5], might interfere with function. Likewise, it remains largely unclear how efficient such IDR-tagging-based cargo recruitment strategies are and how much of the target cargo can be recruited to the synthetic organelles. With the exception of light-based systems such as optoDroplets and optoClusters[2] many LLPS-based cargo protein sequestration strategies are difficult to reverse.

To address these issues, Garabedian *et al.*[4] utilized the arginine/glycine-rich (RGG) domain of the *Caenorhabditis elegans* P granule protein LAF1 as an IDR tag, which was previously shown to enable LLPS[6]. To allow for efficient scaffold formation and cargo recruitment, the authors screened constructs consisting of 2 or 3 RGG repeats and tested different cognate interaction motifs such as different coiled-coil domain pairs and the FK506 binding protein (FKBP)/FKBP−rapamycin binding protein (FRB) pair for scaffold formation and cargo recruitment. Their best-performing scaffold construct, consisting of the

thermally reversible coiled-coil domain TsCC(A) fused with three RGG domains and GFP as a reporter, localized over 95% of the total fusion protein in condensates, with over 90% of the corresponding TsCC(B)-tagged cargo recruited to the scaffold condensate. This construct was therefore selected for further experiments to sequester native enzymes to allow control of cellular decision-making.

To demonstrate the utility of this synthetic membraneless organelle system for functional insulation of native proteins, the authors sequestered the guanine nucleotide exchange factor (GEF) Cdc24 and the cell-cycle serine/threonine kinase Cdc5, thus controlling cell growth and division. Sequestering a formin to synthetic organelles enabled regulation of the spatial organization of the actin cytoskeleton. The authors also generated systems for the controlled release of cargo from condensates, relying either on the thermally responsive nature of the TsCC(A)/TsCC(B) coiled-coil interaction, which allows for cargo release above a critical temperature, or by employing the photocleavable protein PhoCl, which is cleaved upon illumination with violet light[7].

The method presented by Garabedian *et al.* represents a promising alternative for the functional (and reversible) knockdown of endogenous protein functions, only limited by the ability to produce the scaffold protein in a form that yields membraneless-organelle droplets and to genetically tag the cellular target for recruitment. By opting for cargo recruitment via cognate interaction domains, which are small in size, the functional impact on the cellular target remains limited, although it is most likely not nil. While efficient sequestration (above 90% of the cargo) was achieved in some cases, lower efficiencies were observed in other cases, indicating the need for further optimization. As is often the case for new methods, general applicability to various cellular targets (cytoplasmic or membrane bound) and/or model organisms (beyond yeasts and mammalian cells), needs to be seen. Similarly, the utility of the method for other purposes, such as sequestering multiple metabolic enzymes to increase reaction rates[8], or allowing for dynamic rewiring or optimization of metabolic pathways[9], appears to be a promising extension of the method beyond the field of cell biology. Last but not least, the expansion of the method to prokaryotes, which likely requires much more tinkering (or the use of alternative IDRs[10]), would be a welcome addition. Our understanding of the molecular principles governing LLPS has matured to a degree that finally allows harnessing the underlying physical principles for cellular engineering and biotechnology – the future of synthetic LLPS organelles is certainly bright.

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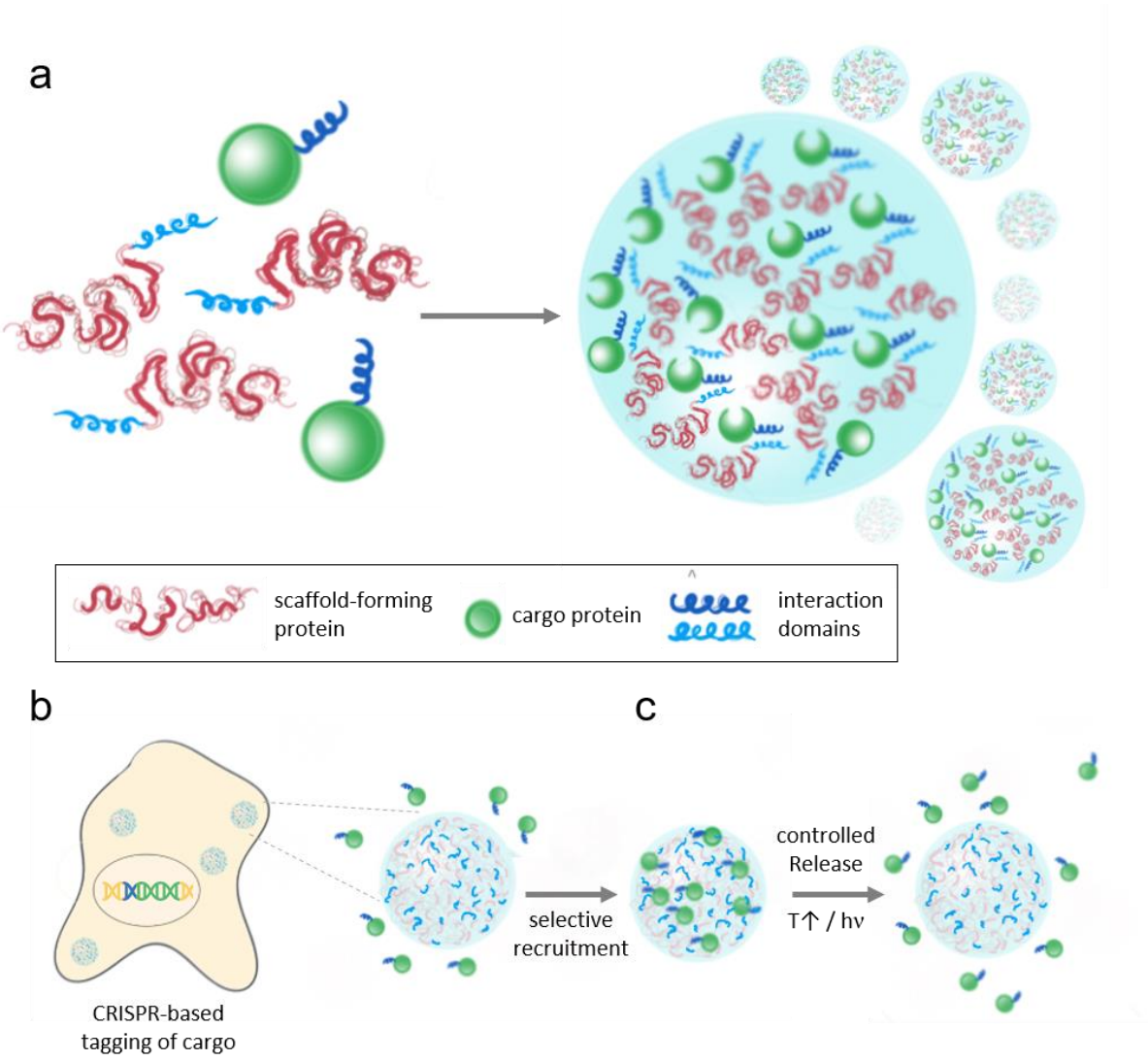

Figure 1. LLPS-based insulation of endogenous enzymes. a, Native cargo proteins (green spheres) tagged with a cognate interaction domain (dark blue) can be recruited to LLPS-based membraneless organelle droplets formed by a correspondingly tagged (light blue) scaffold-forming protein, containing multiple repeats of a disorded protein region (IDR; red). **b,** Insulation of the cargo within the droplets results in knockdown of protein functions. **c,** Reversal of the cargo-scaffold interaction, triggered by a temperature shift or by a light pulse, allows cargo release from the droplets.

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The author declares no competing interests.

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