

# Synthetic protein condensates for cellular and metabolic engineering

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Protein condensates are distinct structures assembled in living cells that concentrate molecules via phase separation in a confined subcellular compartment. In the past decade, remarkable advances have been made to discover the fundamental roles of the condensates in spatiotemporal control of cellular metabolism and physiology and to reveal the molecular principles, components and driving forces that underlie their formation. Here we review the unique properties of the condensates, the promise and hurdles for harnessing them toward purposeful design and manipulation of biological functions in living cells. In particular, we highlight recent advances in mining and understanding the proteinaceous components for creating designer condensates, along with the engineering approaches to manipulate their material properties and biological functions. With these advances, a greater variety of complex organelle-like structures can be built for diverse applications, with unprecedented effects on synthetic biology.

A wide variety of cellular activities in cells are highly coordinated and spatiotemporally organized by numerous organelles<sup>1</sup>. Classical organelles are surrounded by lipid bilayer membranes that separate their interior contents from the exterior environment. However, many organelles such as the nucleoli, Cajal bodies and ribonucleoprotein (RNP) granules are not membrane-enclosed, which are termed membraneless organelles or biomolecular condensates. Because of the lack of membranes, biomolecules within these condensates can easily exchange with their counterparts in the exterior milieu. In addition, the formation and dissolution of the condensates can occur reversibly and rapidly within a few seconds<sup>2</sup>. Owing to their unique dynamics, the condensates have been proposed as key players in many cellular processes, such as gene regulation, higher-order chromatin organization, cellular signaling and stress responses<sup>3,4</sup>.

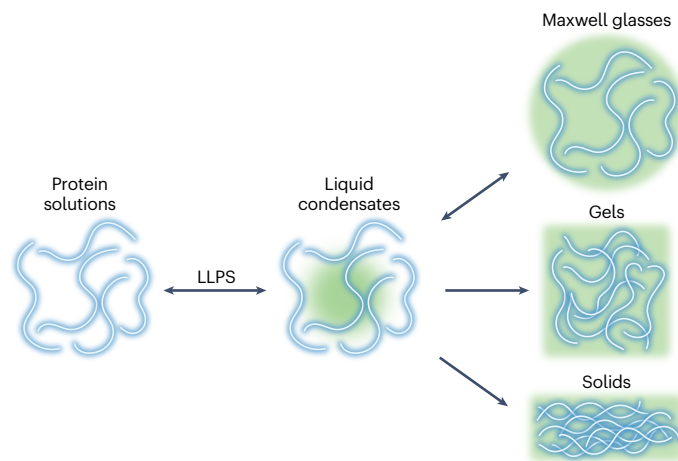
Increasing evidence has shown that liquid–liquid phase separation (LLPS) of proteins (sometimes mixtures with nucleic acids) underlies the formation of condensates<sup>4</sup>. LLPS is a spontaneous assembly process that separates a homogeneous solution into dense and coexisting dilute phases. In general, the constituent proteins of the condensates are highly disordered in solution structures and phase separate via weak multivalent interactions between the protein molecules. Research over the past decade has greatly increased our understanding of intracellular phase separations, their underlying biophysical principles and

the regulation and roles of the condensate states in biological function and dysfunction. However, it remains challenging to create on-demand functional condensates from scratch in living cells<sup>5</sup>.

Synthetic biology aims to create biological devices and systems with sophisticated functions from the basic molecular parts. This has inspired scientists to study the formation and functions of the existing cellular systems for purposeful modification and redesign<sup>6</sup>. Indeed, the growing understanding of natural protein condensates has sparked many efforts to develop genetic tools for constructing synthetic condensates from scratch. Here, we review the development and use of these advanced technologies for creating synthetic protein condensates. First, we briefly introduce the existing, natural protein condensates and their physical properties, and then highlight the building blocks, toolbox and strategies for the design and construction of artificial condensates with on-demand functions and responsive properties. Finally, we show how these synthetic condensates can be harnessed for cellular and metabolic engineering and discuss the current challenges and opportunities in engineering synthetic protein condensates for reprogramming living organisms.

## Diversity of protein condensates

Protein condensates are relatively new types of subcellular structure and can adopt various material forms<sup>7</sup>, such as liquid condensates<sup>8</sup>,



**Fig. 1 | Different types of protein condensate.** Protein solutions undergo LLPS into liquid condensates, which can further age into Maxwell glasses, gels and solids. The liquid condensates and Maxwell glasses are easier to reverse (double arrows) than the protein gels and solids (single arrows).

Maxwell glasses<sup>9</sup>, gels<sup>10</sup> and solids<sup>11</sup> as long as their formation involves LLPS (Fig. 1). In general, the formation of liquid condensates through LLPS of homogenous protein solutions is reversible. However, the liquid condensates can further transition to gels or solid aggregates, which often form irreversibly.

There are three ways identified so far in which a protein liquid can age. One way is gelation, which introduces physical cross-links by noncovalent interactions between the condensate components. When the density of the time-dependent cross-links reaches a threshold, a highly connected protein network also called a physical gel is formed. Several amyotrophic lateral sclerosis (ALS)-related proteins including fused in sarcoma (FUS), heterogeneous nuclear RNP A1 and other ALS-associated RNP body proteins form gels *in vitro* at high protein concentrations and/or upon ageing<sup>8</sup>. It is now clear that these protein solutions initially undergo LLPS, but the resulting droplets are more gel-like over time.

Another way is the time-dependent hardening of liquid condensates into a less dynamic state called Maxwell glasses. This state can be identified with characterization methods such as optical tweezers, single-particle tracking or rheological approaches *in vitro*<sup>9</sup>. The elasticity of a Maxwell fluid changes little over time, suggesting that this process is not gelation in which protein molecules become cross-linked. However, the viscosity of the Maxwell fluid markedly increases with time, which indicates that the molecular dynamics are hampered within the aging liquid. One typical example of Maxwell glasses is the cytosol of nonreplicating, dormant organisms<sup>12</sup>. In biological systems, the glassy state is important for the control of protein dynamics. For example, diffusion of misfolding-prone proteins can be slowed down in the glassy state, which also slows down their aggregation and provides a protective mechanism<sup>13</sup>.

Alternatively, liquid protein condensates can age to form aggregates, such as pathological amyloids that are usually associated with diseases<sup>8</sup>. Therefore, care should be taken that organizing cellular activities through LLPS may lead to protein aggregation. However, the ability to nucleate solids could also be used to seed the formation of functional cytoskeleton filaments<sup>14</sup>. Although increasing evidence has emerged to correlate the physical states of condensates and their functions, how the diverse physical states regulate the functional outcomes remains an open question.

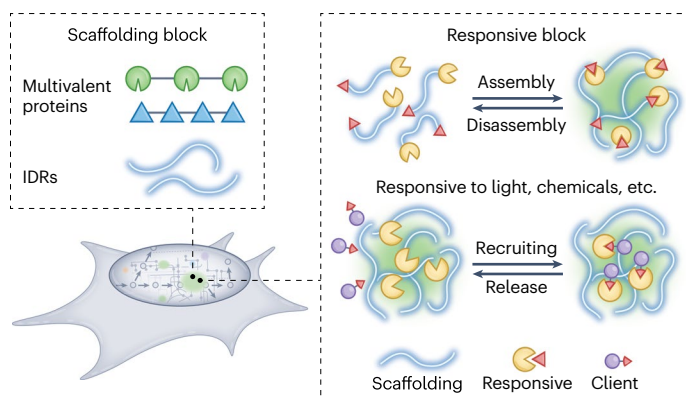
## Building blocks of protein condensate

Protein condensates are composed of at least two classes of components. The first class consists of molecules called scaffolding proteins that are necessary for condensate assembly, whereas the second class is dispensable for condensate formation and termed clients that often localize to the condensates to endow biological functions. Obviously, it is possible to combine a scaffold and a client as a genetically encoded fusion for synthetic condensate formation<sup>15,16</sup>. However, from a synthetic biology point of view, it is more flexible to fuse responsive interaction domains to the scaffold and client proteins and drive client colocalization at the posttranslational level. This would make highly dynamic condensates as the client proteins can be rapidly recruited in and released out and the condensates themselves can also form and dissociate rapidly. Therefore, the scaffold, client and responsive modules are the three major building blocks to construct synthetic protein condensates. As the clients could be any enzymes or signaling factors for functionalization of the condensates, we will not introduce them in detail here.

### Scaffolding block

A defining feature of the scaffolding blocks is their multivalency of adhesive domains and interacting motifs for driving phase transitions (Fig. 2). Multivalency can usually occur between folded domains that are connected by flexible linkers or intrinsically disordered regions (IDRs) as distinctive linear motifs that enable multivalent interactions. Of course, tunable multivalency can be achieved by arranging the above two forms of scaffolding blocks in a combinatorial manner<sup>17</sup>.

The number of scaffolding blocks reported forming liquid condensates has recently grown rapidly<sup>18–20</sup>. Well-known examples identified in eukaryotes comprise the RNP granule protein FUS<sup>21</sup>, stress granule-related protein TDP-43 (ref. 22) and P granule protein LAF-1 (ref. 23). In prokaryotes, many candidates have also been identified, including the DEAD-box RNA helicases<sup>24</sup>, polar organizing protein Z (PopZ)<sup>25,26</sup> and carboxysome protein CcmM<sup>27,28</sup>, although some of them need further investigations to determine whether their endogenous expression levels enable LLPS. More interestingly, artificial disordered proteins such as spider dragline silk<sup>15</sup>, elastin<sup>29</sup> and resilin-like proteins<sup>15,30</sup> are also reported to form liquid condensates. These proteins share three key features similar to the phase-separating intracellular



**Fig. 2 | Building blocks of synthetic protein condensates.** The scaffolding blocks can be multivalent proteins with folded domains or proteins with IDRs necessary for condensate formation. The responsive blocks, which are adapted or redesigned from the native protein condensates or complexes, enable controllable assembly/disassembly of the protein condensates and recruiting/release of the client proteins in response to environmental cues.

IDPs: low complexity, highly repetitive and rich in a limited set of disorder-promoting amino acids<sup>31</sup>.

To help researchers find the proteins capable of phase separation, several web-accessible databases have been built by collecting and integrating different public sources of data and information<sup>32,33</sup>. As the amino acid sequence of a protein is proposed to encode its phase-separating capacity, many efforts have been made to exploit the amino acid sequence to assess the likelihood of phase separation. The first-generation predictors are able to predict phase-separation propensities of proteins based on very limited interaction types and sequence features<sup>34</sup>. Most recently, several machine-learning models have been developed by defining more features from the amino acid sequences of the experimentally validated proteins and demonstrated to be superior to the first-generation predictors<sup>35,36</sup>.

### Responsive blocks

Responsive blocks provide intriguing opportunities for controllable assembly/disassembly of the protein condensates and recruiting/release of the client proteins (Fig. 2). This is made possible owing to the availability of a set of modular and orthogonal protein interaction domains. These domains can be fused to the scaffolding or client proteins at the amino- or carboxyl-terminus and the resulting fusions that bear complementary motifs form functional protein condensates via noncovalent interactions. Some commonly used interaction domains are adapted from natural protein–protein interactions (PPIs), such as GTPase-binding ligand and domain pair, and cohesin–dockerin pair<sup>37,38</sup>. In addition, a set of synthetic interaction motifs based on coiled coils have also emerged and provided a range of orthogonal, specific interaction partners with tunable binding affinities<sup>38</sup>.

Notably, many interaction domains can also act as switches in response to light, chemicals or other stimuli and, thus, have great potential to endow the synthetic condensates with flexible and dynamic features. The most commonly used light-induced switches include homodimerizable VVD<sup>39</sup>, heterodimerizable LOV2–PDZ domain pair<sup>40</sup>, cryptochrome 2 (Cry2)–CIB1 pair<sup>41</sup>, and oligomerizable Cry2<sup>42</sup>, which sense 440–480 nm light. To expand their applications in deep tissues<sup>43</sup>, sets of near-infrared light-responsive modules sensing 650–900 nm light, such as phytochrome B and PIF pair<sup>44</sup>, bacterial phytochrome BphP1–PpsR2 pair<sup>45</sup> are also developed. Compared with light, chemical trigger is simpler and cheaper yet limited by irreversibility and lack of spatial control within the cells. One of the most popular switches is the rapamycin-inducible heterodimerization of FK506-binding protein (FKBP) and the FKBP–rapamycin binding (FRB) domain. As an

alternative mechanism, temperature can control protein complexes with several advantages as it can be flexibly applied to biological samples, either globally using simple heat sources or locally targeted to deep tissues. The most commonly used thermal-induced switches are screened from a series of transcriptional regulators, which exhibit tunable thresholds within the physiologically relevant temperature range<sup>46</sup>. Furthermore, the landscape of protein switches can be greatly expanded by rational protein design and directed evolution for use in the construction of synthetic protein condensates<sup>47,48</sup>.

### Synthetic protein condensates with responsive properties

Dynamically responsive properties are the unique features of liquid protein condensates that can be formed and dissolved in response to various stimuli. Control of synthetic protein condensates has been mainly realized via three routes: (i) by using interaction domains that actively change the multivalency of the scaffolding blocks (through noncovalent modifications)<sup>49–51</sup>; (ii) by a chemical perturbation to cause a passive change in the multivalent interactions<sup>52,53</sup> and (iii) by enzymatic reactions to actively change the scaffolding valency (through covalent modifications)<sup>54</sup>. For control of the responsive properties of the synthetic condensates and biological functions, a number of tools have been developed to actuate the formation and dissolution of the protein condensates by light, chemicals or enzymes (Table 1).

#### Light-controlled protein condensates

A growing suite of light-induced PPIs domains is available to regulate the protein condensates in living cells. Earlier, an ‘optoDroplet’ system was developed by fusing light-responsive protein Cry2 to IDR proteins and using light to activate their phase transitions (Fig. 3a). By tuning the homo-interaction strength of Cry2, it has been possible to trigger the formation of liquid or gel-like protein condensates using light<sup>49</sup>. More recently, light-controlled Corelets and PixELLS condensates have been developed<sup>50</sup>. The two-component Corelets system consists of an assembled core of ferritin heavy chain (FTH1) protein subunits bound to iLID and an SspB-IDR fusion. The SspB-IDR component is dispersed in the dark and recruited by the light-activated iLID to the ferritin cores for assembly into synthetic protein condensates. This assembly is bidirectional as the SspB-IDR modules can redisperse in the dark<sup>50</sup>. In contrast to the undefined oligomerization of Cry2 interactions within the optoDroplets, ferritin has a well-defined oligomerization state that therefore makes Corelets more suitable for studying phase transitions of proteins. The PixELLS system is established by fusing PixE and PixD with IDRs, respectively, and the resulting protein condensates are formed by the association of PixE and PixD fusions in the dark and dissolved upon blue-light irradiation<sup>51</sup>. Furthermore, these light-responsive protein condensates can be tailored with on-demand functions to create synthetic membraneless organelles, which carry out specialized subcellular tasks under specific light<sup>49,55</sup>.

#### Chemo-controlled protein condensates

Introducing small molecules to regulate the protein condensates is simple and efficient<sup>56,57</sup>. An intriguing strategy is to use chemicals to induce the assembly of protein condensates in cells. The key of this strategy is to engineer a multimodule protein that contains scaffolding domains and chemical-binding domains such as hexahistidine (6His) tag (Fig. 3b). It has been demonstrated that the metal-6His coordination-mediated scaffold clustering increases scaffolding valency, which subsequently leads to condensate formation. The condensate properties such as droplet forming capability and droplet morphology can be modulated by adjusting the nature of scaffolding proteins or the metal ions used and by tuning the metal/protein ratios. This versatile approach not only provides access to dynamic protein condensates but also facilitates exploration of the molecular and structural features of condensing proteins<sup>52</sup>.

**Table 1 | Synthetic protein condensates in cells**

| Name                                  | Scaffolding blocks  | Responsive blocks   | Stimulus                             | Clients  | Hosts   | Applications   |
|---------------------------------------|---|---|--------------------------------------|--|---|--|
| Tools for engineering condensates     |   |   |                                      |  |   |  |
| optoDroplet                           | FUS <sub>N</sub> , DDX4 <sub>N</sub> , hnRNAPA1 <sub>C</sub>                                    | Cry2, Cry2olig  | Blue light                           | mCherry  | Mammalian cells                                       | Optogenetic control of condensate assembly <sup>49</sup>                                   |
| Corelets                              | FTH1, FUS <sub>N</sub> , HNRNPA1 <sub>C</sub> , TDP-43 <sub>C</sub> , DDX4 <sub>N</sub> , PGL-1 | iLID-SspB   | Blue light                           | eGFP, mCherry  | Mammalian cells, yeast, <i>Caenorhabditis elegans</i> | Mapping intracellular phase diagrams <sup>50</sup>   |
| PixELLS                               | FUS <sub>N</sub>  | PixE-PixD   | Blue light                           | FusionRed, Citrine   | Mammalian cells                                       | Optogenetic control of condensate disassembly <sup>51</sup>                                |
| -                                     | RGG fused with mussel-foot proteins   | Water-soluble chlorophyll proteins                                    | Red light                            | eGFP   | Mammalian cells                                       | Light-induced liquid-to-solid transition <sup>77</sup>                                     |
| -                                     | PRM-SH3   | Hexahistidine tag   | Zn <sup>2+</sup>                     | mCherry  | Mammalian cells                                       | Metal ion control of condensate assembly <sup>52</sup>                                     |
| -                                     | RGG domains   | TEV recognition site, SZ1-SZ2 pairs                                   | TEV protease                         | RFP, GFP   | Mammalian cells                                       | Enzyme control of condensate disassembly <sup>54</sup>                                     |
| DisCo                                 | FUS, Cry2   | FKBP-FRB, FKBP12-DHFR, CaM-CBP  | Rapamycin, Zapalag, Ca <sup>2+</sup> | C-block (FKBP, CBP)  | Mammalian cells                                       | Controlled disruption of condensates <sup>59</sup>   |
| -                                     | Im2-E9 synthetic protein scaffold   | Dimer and tetramer domain   | N/A                                  | RFP, YFP   | Yeast   | High-resolution phase diagrams mapping in vivo <sup>20</sup>                               |
| Condensates for cellular engineering  |   |   |                                      |  |   |  |
| CasDrop                               | BRD4 <sub>ΔN</sub> , FUS <sub>N</sub> , TAF15 <sub>N</sub>                                      | iLID-SspB   | Blue light                           | Transcriptional regulators   | Mammalian cells                                       | Chromatin restructuring <sup>61</sup>  |
| DropletTFs                            | FUS <sub>N</sub> , DDX4 <sub>N</sub> , hnRNAPA1 <sub>C</sub>                                    | Cry2-CIBn   | Blue light                           | Transactivating VP16 domain  | Mammalian cells                                       | Gene transcription enhancement <sup>62</sup>   |
| -                                     | PopZ  | SpmXΔC adaptors   | N/A                                  | Split T7 RNA polymerase  | <i>Escherichia coli</i>                               | Asymmetric division in <i>E. coli</i> <sup>64</sup>  |
| -                                     | RGG domains   | SZ1-SZ2 pairs, TsCC(A)-TsCC(B), FKBP-FRB, photocleavable domain PhoCl | Heat, rapamycin, blue light          | Cdc24, Cdc5, Rac1, ERK1, Par6  | Yeast, Mammalian cells                                | Sequester native factors for controlling cell behavior <sup>67</sup>                       |
| SPREC-In, SPREC-Out, optoSPREC        | PB1-AG, PB1-MR  | FKBP-FRB, Lov2-Zdk1   | Rapamycin, blue light                | Vav2, SOS, TEVp, Vav2  | Mammalian cells                                       | Sequestration and release of signaling proteins for regulating cell behavior <sup>68</sup> |
| Condensates for metabolic engineering |   |   |                                      |  |   |  |
| optoDroplet/<br>PixELLS               | FUS <sub>N</sub>  | Cry2, PixE-PixD   | Blue light                           | VioE and VioC  | Yeast   | Shunt metabolic flux <sup>16</sup>   |
| -                                     | Spider silk proteins  | N/A   | N/A                                  | Metallothionein, Dat and Ddc   | <i>E. coli</i>  | Compartmentalized biosynthesis <sup>15</sup>   |
| -                                     | Artificial IDPs   | N/A   | Heat                                 | LacZ ap  | <i>E. coli</i>  | Compartmentalized enzyme reaction <sup>30</sup>  |
| -                                     | RGG domain  | RIAD-RIDD   | N/A                                  | Idi and IspA   | <i>E. coli</i>  | Enhancement of α-farnesene production <sup>75</sup>  |
| PhASE                                 | FUSLCD, GCN4, SIM-SUMO  | Cry2-CIB1 pair  | Blue light                           | Rluc, Xyle   | <i>E. coli</i>  | Light-regulated metabolic reactions <sup>71</sup>  |
| Orthogonally translating organelles   | FUS-EWSR1, SPD5, KIF  | mRNA::ms2-MCP, tRNA <sup>Pyl</sup> -PylRS pair                        | N/A                                  | Nup153::GFP <sup>149TAG</sup> , VIM <sup>116TAG</sup> , INSR <sup>676TAG</sup> | Mammalian cells                                       | Specific and selective protein translation <sup>76</sup>                                   |

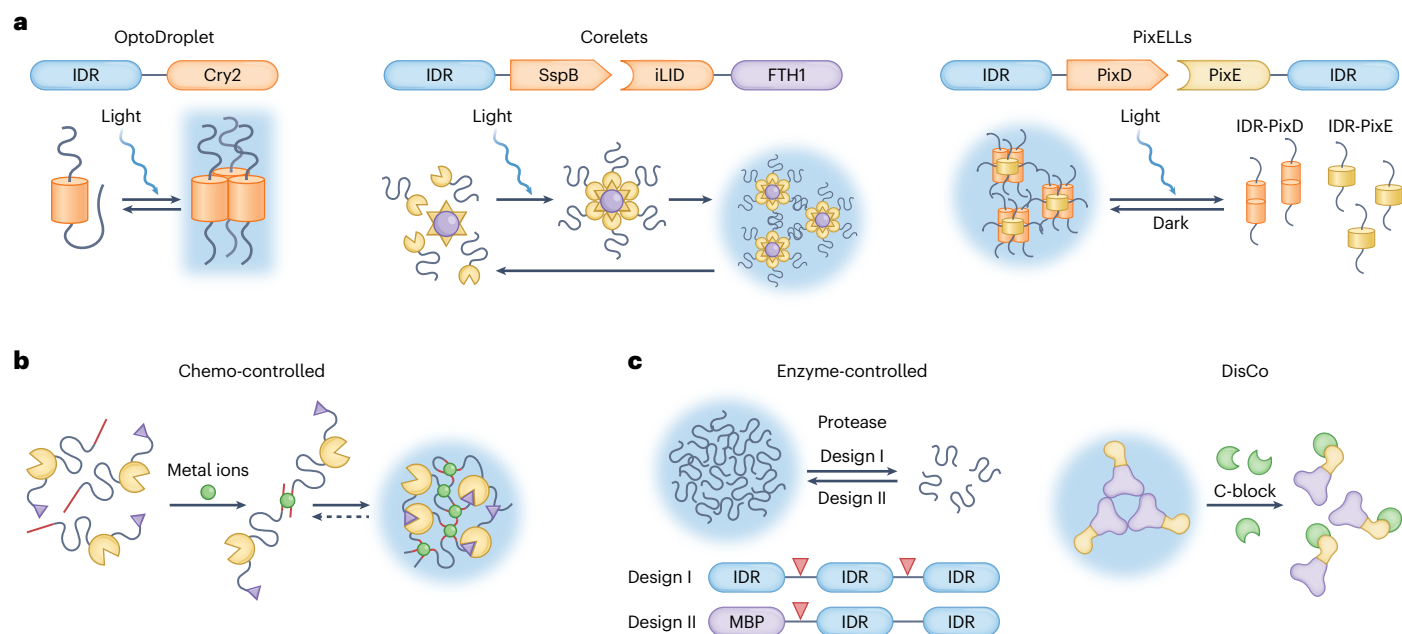
Alternatively, chemicals can be used to disrupt the driving forces that underlie the formation of the protein condensates, such as 1,6-hexanediol which interferes with weak hydrophobic interactions and urea which interferes with hydrogen-bonding interactions. However, a major drawback of this method is its low specificity and potential toxicity to the cells, and thus, care should be taken especially when the cells are treated with the disrupting chemicals at high concentrations for extended times<sup>58</sup>. Until now, many chemicals have been identified to dissolve protein condensates, but few are reported to be biphasic modulators. Interestingly, 4,4'-dianilino-1,1'-binaphthyl-5,5'-disulfonic acid (bis-ANS) which contains aromatic groups and negative charges

has been identified to act as a potent biphasic modulator<sup>53</sup>. At low concentrations, this small molecule strongly promotes LLPS of TDP-43, whereas it disrupts the liquid droplets at higher concentrations. This modulatory capacity of bis-ANS and similar compounds has also been extended to a number of other proteins except TDP-43. In addition, the identified chemical features crucial to modulation of phase transitions may provide a molecular foundation for the design of LLPS modulators.

### Enzyme-controlled protein condensates

Compared with light or chemicals, enzymes can be genetically encoded and facily expressed by the cells themselves to modulate the protein





**Fig. 3 | Tools for controlling synthetic protein condensates in cells. a**, OptoDroplet, a photoswitchable oligomerization Cry2 domain is fused to an IDR-rich protein to drive homotypic, light-induced condensation. Corelets, upon light illumination, up to 24 IDR modules are captured by each assembled core of human FTH1, which subsequently phase separate by multivalent IDR interactions in a reversible manner. PixELLS, PixD and PixE associate in the dark into large multisubunit complexes, which dissociate into dimers of PixD and monomers of PixE within seconds upon blue light stimulation. **b**, Chemo-controlled

(metal ion-induced) phase separation into protein condensates with a single scaffold construct (ligand–receptor–hexahistidine). **c**, Enzyme-controlled (protease-mediated) disassembly of condensates through reduction in valency, and assembly by removal of the solubility-enhancing maltose binding protein tag (left). Alternatively, in the nonenzymatic disassembly of condensate (DisCo) system, the small binding protein (C-block) binds to a ‘hook’ domain on the condensate-forming scaffold, resulting in condensate dissolution (right). The DisCo scheme is modified with permission from Hernandez-Candia et al.<sup>59</sup>.

condensates. For example, enzyme-controlled protein condensates have been generated by introducing enzyme cleavage sites between the intrinsically disordered, arginine- and glycine-rich RGG domains, or between a solubilizing tag and RGG domains (Fig. 3c). The formation and dissolution of the synthetic condensates are then enzymatically triggered by tuning the miscibility and RGG domain valency<sup>54</sup>. This controlling system can work well in cytoplasmic extracts and in mammalian cells but is short in the relatively longer response time than the light and chemical control systems.

To overcome the above obstacle, protein-binding domains have been used to tune the dynamics of synthetic protein condensates. A typical example is the disassembly of condensates (DisCo) platform in which a small binding protein (C-block) is inducibly recruited to the condensate-forming scaffold, thus triggering condensate dissociation in minutes<sup>59</sup>. So far, DisCo has been successfully used to disrupt condensates of FUS and polyglutamine-containing huntingtin condensates to potentially prevent the associated diseases. In addition, the combination of DisCo with the optogenetic Cry2olig tool has enabled bidirectional control of condensate assembly and disassembly with light and the chemical rapamycin.

### Designer protein condensates for diverse applications

With the necessary building blocks and genetic tools in hand, synthetic biologists have been able to design and construct synthetic protein condensates with responsive properties and on-demand functions. Indeed, many efforts have been devoted to create designer protein condensates whose intracellular activities can be manipulated by external cues such as light or small molecules. This has offered an intriguing opportunity for compartmentalizing biochemical reactions into spatially separated and temporally programmed ‘synthetic organelles’.

These synthetic compartments can serve as microbial cell factories for the production of complex chemicals and signal transduction hubs in artificial signaling circuits inside living cells. Obviously, synthetic protein condensates offer unique advantages in cellular and metabolic engineering of organisms<sup>60</sup>.

### Cellular engineering

Synthetic protein condensates that harbor specific functions have enabled diverse aspects of cellular engineering, from chromatin restructuring<sup>61</sup>, transcription factor clustering<sup>62,63</sup> to controlling cell divisions<sup>64–66</sup>. For example, a fascinating CRISPR–Cas9-based optogenetic technology, CasDrop has been developed for controlled liquid phase condensation of various nuclear proteins at specific genomic loci (Fig. 4a). These nuclear condensates can mechanically sense and restructure the genome, which causes distal targeted genomic elements to be pulled together, and mechanically exclude nonspecific components of the cell genome<sup>61</sup>. Also, transcription factors are designed to form DropletTFs, a series of synthetic coacervates that bind to and initiate transcription from targeted promoters (Fig. 4b). They have been demonstrated to trigger markedly increased gene transcription compared with their noncoacervate forming counterparts in mammalian cells and mice<sup>62</sup>. More recently, a synthetic condensate strategy has been developed by using the central polarity protein PopZ of *Caulobacter crescentus*, an  $\alpha$ -proteobacterium that reproduces by asymmetric division<sup>26</sup>. Introduction of this polarized scaffold protein and another oligomeric pole-targeting DivIVA has led to intracellular asymmetry and asymmetric division in *Escherichia coli*, a model bacterium that normally divides symmetrically<sup>64</sup>.

Until now, synthetic condensate systems amenable to sequestration of chromosome-encoded endogenous proteins are rare. To this end, a synthetic condensate platform has been established for

modulating cellular activities via condensation of the target endogenous proteins for controlling cellular behaviors (Fig. 4c). This is realized by genomic tagging of the endogenous clients with high-affinity dimerization motifs such as the coiled-coil TsCC(B) domain<sup>67</sup>. By recruiting over 83% of the native enzymes to the synthetic condensates, the polarization and division behaviors of the baker's yeast cells can be efficiently controlled via sequestration and functional isolation of the guanine nucleotide exchange factor Cdc24 and kinase Cdc5, respectively. More importantly, the cells could be switched between functional states through controlled cargo recruitment and release from the condensates in a repeatable manner by thermal treatment. Owing to these merits, the synthetic condensate platform has proven modular, robust and generalizable to control cell behaviors in a variety of model systems<sup>67</sup>. However, the recruitment efficiency for some client needs to be improved, and the general applicability to various proteins and organisms remains to be tested.

With the modular design principle, SPREC-In, SPREC-Out and opto-SPREC condensate systems have been developed for controlling cellular behaviors in living mammalian cells<sup>68</sup>. In SPREC-In, FKBP-tagged clients can be recruited from the cytosol to the FRB-containing condensates within minutes following rapamycin addition. This system allows the recruitment of the cytoplasmic catalytic domain of Vav2 (Vav2<sub>cat</sub>), a guanine nucleotide exchange factor that normally induces plasma membrane protrusion and ruffling, into condensates to inactivate the protein activity leading to substantial shrinkage of the HeLa cells. The combination of the SPREC-In scheme with a proximity-dependent protease has led to the SPREC-Out system, which triggers target protein release from the condensates to the cytoplasm upon rapamycin treatment. Furthermore, the LOVTRAP-based optoSPREC system has been developed to release and recruit clients in a reversible and repeatable manner, simply by switching the blue light on and off. As these condensate systems are applicable to control activities of a variety of endogenous signaling proteins and enzymes, a new platform has thus been established for chemogenetic and optogenetic control of cellular behaviors in mammalian cells<sup>68</sup>. Notably, as the PBI-AG scaffolding proteins used tend to form gel-like clusters with reduced fluidity, the efficiencies of client protein recruitment, release and activity modulation of these systems might be restricted and remain to be further explored.

### Metabolic engineering

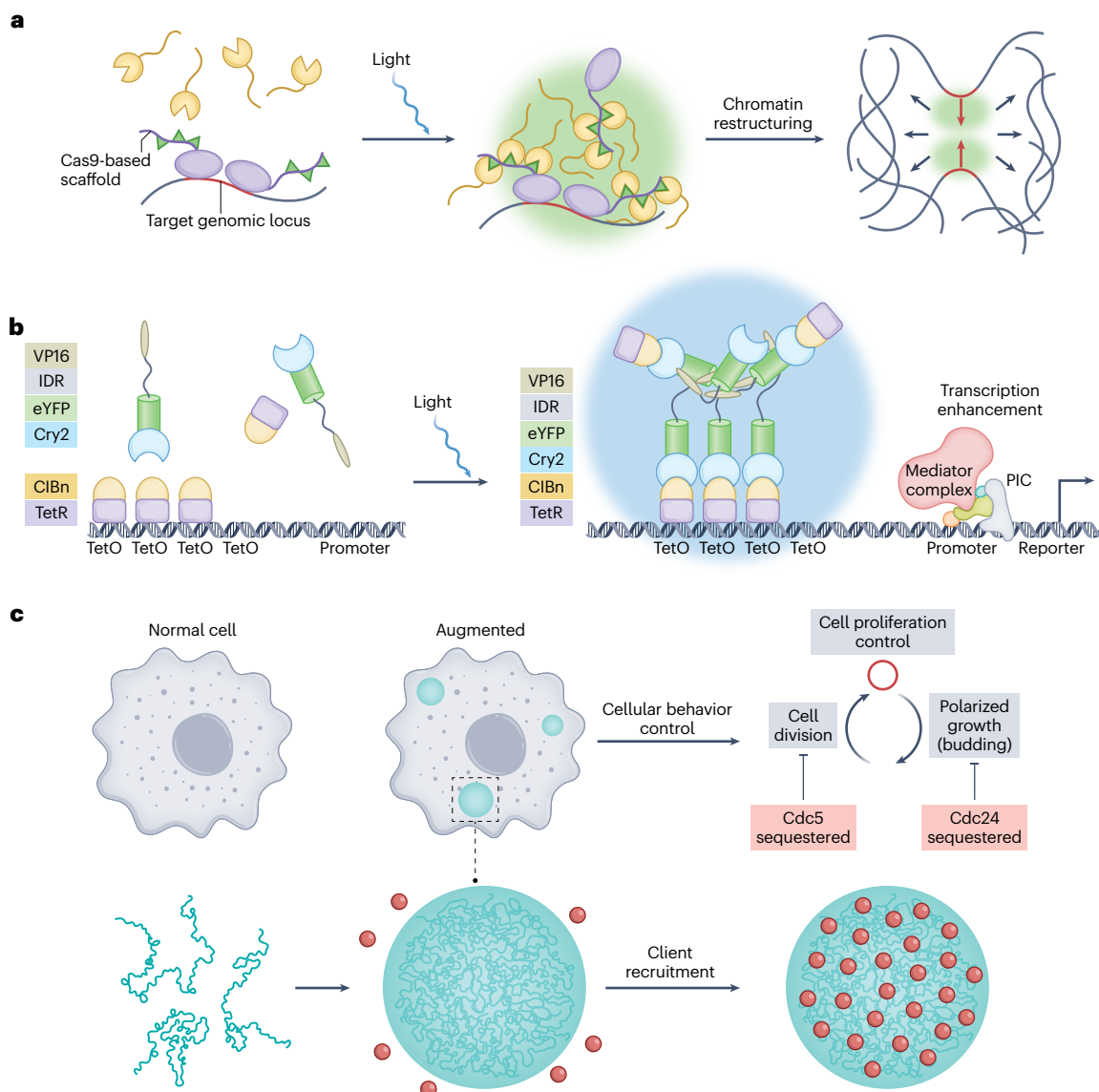
Metabolic engineering of organisms has great potential for the sustainable supply of various valuable products, including fine chemicals, fuels, pharmaceuticals and materials. However, it is rather challenging to make efficient cell factories for biosynthesis because the cells have evolved robust metabolic networks beneficial for their own growth. This challenge may be alleviated by synthetic metabolic organelles that cluster or exclude specific metabolic enzymes within the cells. As synthetic organelles are supposed to be independent of host cell physiology, they are unlikely to adversely affect cellular functions or to be regulated by endogenous mechanisms. Therefore, synthetic organelles have great potential in the metabolic engineering of organisms for improved biosynthesis of metabolites of interest<sup>69–71</sup>.

Synthetic condensates are hypothesized to increase enzymatic reaction rates by concentrating the enzymes and substrates (Fig. 5a). This hypothesis has recently been verified in an *in vitro* condensate system where an enzyme cascade is recruited into the synthetic condensates for SUMOylation<sup>72</sup>. The reaction rates can be increased up to 36-fold in the phase-separated droplets compared to the surrounding bulk, depending on the substrate Michaelis constant  $K_m$ . Furthermore, two mechanisms have been reported to accelerate the reactions in synthetic condensates. In addition to increased concentration, another mechanism is molecular organization that affords a scaffold-dependent decrease in the substrate  $K_m$ . Albeit revealed from a simplified *in vitro* model, these mechanisms may inspire efforts to create synthetic condensates for increasing enzymatic rate in cells<sup>72,73</sup>.

The sheer complexity of metabolic pathways, which may have multiple branches, makes it difficult to increase the production of a desirable compound through a particular biosynthetic pathway. Traditional approaches to overcome this challenge include permanent and complete knockout of the competing pathway genes and temporary gene knockdown at transcriptional and translation levels<sup>74</sup>. Unlike these methods, synthetic protein condensates provide a new means for metabolic engineering by compartmentalizing pathway enzymes at the post-translation level (Fig. 5b). For example, light-responsive condensate systems OptoDroplet and PixELLS have recently been used to trigger dynamic formation and dissolution of metabolically active enzyme clusters in yeast<sup>16</sup>. The light-switchable clustering can enhance target product formation sixfold and product specificity eighteenfold by lowering the concentration of intermediate metabolites and decreasing metabolic flux through the competing pathway. Notably, this proof-of-concept demonstration provides an intriguing opportunity to adopt synthetic condensates for metabolic engineering. However, shortcomings of this approach may arise and need to be addressed: fusion of enzymes to large scaffolds can reduce their activities and some enzyme fusions can lead to constitutive clustering that precludes remote light control. A promising way to tackle these shortcomings would be the engineering of synthetic condensates based on modularly designed scaffolding and light-responsive PPI domains for recruitment and release of metabolic enzymes.

Many metabolic pathways involve highly toxic intermediates to the cells, such as metabolites containing reactive groups (for example, formaldehyde, methylglyoxal and glutaconyl-CoA) and those acting as competitive analog against other key metabolites. In metabolic engineering of these pathways, it is highly desirable to prevent the overaccumulation of toxic intermediates which would otherwise be detrimental or lethal to the host cells. Compartmentalization of the enzymes responsible for the formation and utilization of the toxic intermediates into synthetic condensates is promising to overcome the toxicity issue (Fig. 5c). Recently, our group has developed such condensates by fusing the intrinsically disordered silk-like protein with the two enzymes responsible for the conversion of aspartate  $\beta$ -semialdehyde into 1,3-diaminopropane<sup>15</sup>. The formation of functional compartments inside the prokaryotic *E. coli* cells enabled *de novo* production of the platform three-carbon diamine. More recently, a similar condensate strategy was used for improving the production of a sesquiterpene,  $\alpha$ -farnesene in engineered *E. coli*. Overexpressed enzymes Idi and IspA were recruited into the condensates through the peptide-peptide interaction pair RIAD-RIDD, which alleviates the cytotoxicity of Idi-catalyzed products leading to an appreciable enhancement in  $\alpha$ -farnesene production<sup>75</sup>.

The ability of synthetic condensates to act as compartmentalized reactors is not limited to metabolic pathways. In fact, new functionalities can be engineered into the synthetic condensates within the cells. A fascinating example is orthogonally translating synthetic designer organelles within which selective and specific protein translation has been achieved (Fig. 5d). This organelle system offers a new technology to genetic code expansion that introduces noncanonical amino acids into proteins in a codon-specific and mRNA-selective manner<sup>76</sup>. Typically, genetic code expansion relies on an orthogonal tRNA-tRNA synthase pair to introduce noncanonical amino acids in place of a stop codon, and stop codon suppression can happen for each cytoplasmic mRNA that terminates on the stop codon, which inevitably leads to substantial off-target effects. This problem has been well mitigated in the orthogonally translating organelles by enriching selected mRNAs and the unnatural tRNA synthase into synthetic condensates. Furthermore, these orthogonally translating organelles have demonstrated utility and robustness in selectively decoding any of the three-stop codons for various proteins with different noncanonical amino acid functionalities and thus provide an avenue for orthogonal



**Fig. 4 | Synthetic protein condensates for cellular engineering.** **a**, Light-induced formation of the CasDrop condensates at specific genomic loci for chromatin structuring. This serves as mechanical chromatin filters by physically pulling in targeted loci while excluding nontargeted chromatin. **b**, Light-triggered formation of synthetic Droplet TFs. Owing to binding to CIBn-TetR and homo-oligomerization of Cry2, the condensed Droplet TFs at the tetO operator

sites increase the transactivating domain VP16 density and downstream reporter gene expression. **c**, Assembling condensates from disordered scaffold proteins to recruit clients for cell cycle control. Insulation of the native enzymes (Cdc5 and Cdc24) will block their normal cellular functions. The images in **a** and **c** are adapted with permission from Shin et al.<sup>61</sup> and Garabedian et al.<sup>67</sup>.

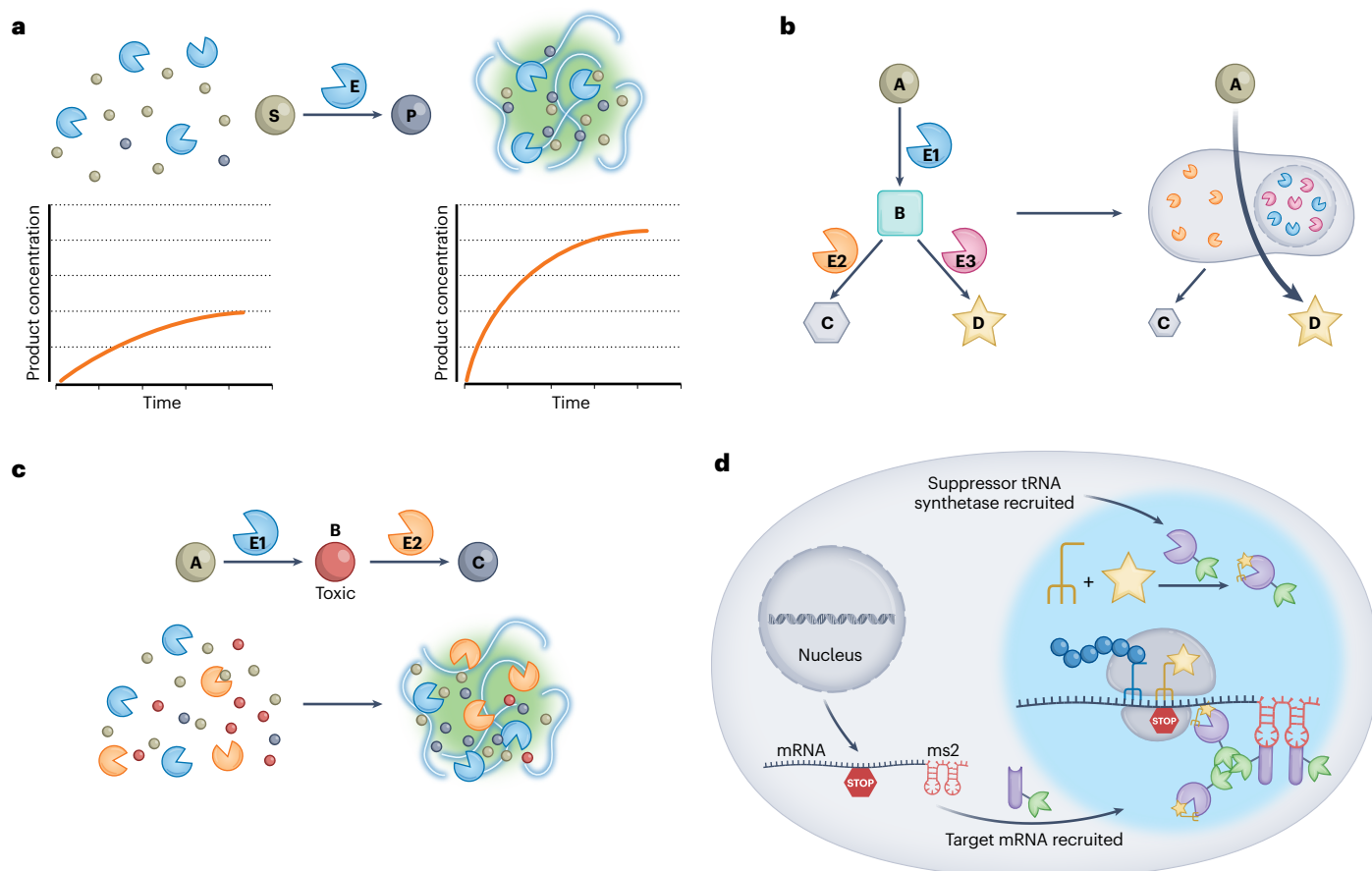
translation and protein engineering in eukaryotic cells and other organisms of interest.

It is increasingly recognized that the physical properties of condensates are important for their natural biological functions and dysfunctions<sup>8</sup>. This inspires the development of tools for tuning materials properties of synthetic protein condensates as a means to modulate their functions in engineered organisms<sup>77,78</sup>. By rationally designing variants of the scaffolding proteins, bioengineers have been able to control the material properties of synthetic condensates in vitro, such as fluid mobility<sup>78</sup>, viscoelasticity<sup>79</sup>, relaxation and fusion dynamics<sup>80,81</sup>. Yet it remains a challenge to explore the link between the emergent material properties of the synthetic condensates and their in vivo functions. To this end, reliable methods have to be first established for monitoring and controlling their material properties within living cells, which may dynamically change over time and upon environmental perturbations.

## Future perspectives

This Review has looked beyond protein condensates as purely natural structures within the cells and highlighted how the current progress and challenges serve as an inspiration for bioengineers. The combination of synthetic biology framework with design principles has shed light on this fast-developing area of synthetic protein condensates. Several pioneering and milestone studies have paved the way and demonstrated the great potentials of synthetic protein condensates. With the engineering tools in hand, we have been able to create new functional organelles with immediate implications in cellular and metabolic engineering of living cells<sup>82–84</sup>. Although predicting the translational potential of these condensates is difficult, we anticipate that a better understanding of them will offer new options for cellular and metabolic reprogramming in living organisms and help us generate advanced systems to address biomedical needs<sup>17,85</sup>.





**Fig. 5 | Synthetic condensates for metabolic engineering.** **a**, Condensation can increase the local concentration of enzymes and substrates, thus increasing rates of product formation. **b**, Selective co-compartmentalization of certain enzymes in a complex metabolic pathway can divert metabolites toward one branch pathway. **c**, Condensation of a metabolic pathway involving a toxic intermediate can increase the product formation rate by concentrating enzymes and

alleviating toxicity of the intermediate. **d**, An orthogonally translating organelle allows codon-specific and mRNA-selective introduction of noncanonical functionalities into proteins in cells. The organelle is specifically enriched with the mRNA tagged with ms2 loops, and the synthetase that aminoacylates the suppressor tRNA with its cognate noncanonical amino acid. The image in **b** was adapted with permission from Zhao et al.<sup>16</sup>.

The remarkable advances achieved in the past few years have provided intriguing opportunities in engineering synthetic protein condensates and have also revealed substantial challenges. First, the unique physical states of protein condensates and their dynamic transitions remain underexplored. It is vital to develop more conceptual and experimental methods for quantitative characterization of the biophysical properties of protein condensates<sup>85</sup>. Such extensive characterizations are urgently needed to correlate the phase behaviors with dynamic functions of the synthetic protein condensates in living cells and to reveal physiochemical cues and cellular processes that regulate the correlations<sup>86</sup>. Second, the design principles underlying protein phase transitions have aided the rapid development of new membraneless organelles. However, how individual molecules contribute to the emergent material properties remains underexplored, and strategies for direct manipulation of phase dynamics are still limited<sup>85,87,88</sup>. Third, although the availability of chemo- and opto-stimuli responsive blocks and switchable condensate systems has improved greatly in the past decade, controllable manipulation of these responsive systems remains a challenge, possibly due to the nonspecific interactions between the diverse scaffolding, client and responsive blocks. In particular, monitoring and engineering synthetic condensates in bacteria are

recognized to be more challenging when compared to their eukaryotic counterparts, as the bacterial cells are relatively small in size (submicrons to a few microns) and highly crowded in intracellular environments<sup>89,90</sup>. The third challenge may be alleviated by synthetic biology approaches, such as modular and orthogonal optimization of the protein component architecture, and temporal control over formation of these components with engineered gene circuits. Through these efforts, we anticipate that designer protein condensates will evolve to be a powerful platform for reprogramming cellular and metabolic processes with diverse applications.

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## Author contributions

X.-X.X. and Z.-G.Q. conceived of the manuscript. Z.-G.Q. and X.-X.X. wrote the paper. S.-C.H. reviewed the literature and edited the paper.

## Competing interests

The authors declare no competing interests.

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