Trends in Chemistry

CellPress

Review

Engineering strategies for sustainable synthetic cells

Xuejing Wang,[1] Shuqi Wu,[1] T.-Y. Dora Tang,[2,3] and Liangfei Tian [1,4,5,*]

An ongoing grand challenge in bottom-up synthetic biology is designing and constructing synthetic cells with life-like properties. Despite the significant advances, even the most highly integrated synthetic cell does not come close to living entities. One of the main differences is that biological cells are dynamic and show self-regulating behavior, while synthetic cellular mimics will eventually reach a static state and exhibit unidirectional responses to perturbation. To this end, the next milestone for bottom-up synthetic biology will be the development of sustainable synthetic cellular systems that can show functional dynamic behavior. Here, we review the engineering strategies to design and construct such functional synthetic cellular systems and the challenges and potential future opportunities in this field are discussed.

Why construct sustainable synthetic cells?

A grand challenge in **bottom-up synthetic biology** (see Glossary) is designing and constructing synthetic cell-like entities (synthetic cells) with life-like properties. A wide range of strategies are now available to generate different forms of synthetic cell-like compartments, including **lipid vesicles** [1–6], polymersomes [7,8], inorganic **colloidosomes** [9], semipermeable protein–polymer microcapsules (**proteinosomes**) [10], and membrane-free **coacervate microdroplets** [11–13]. These are readily integrated with chemical and biochemical reaction pathways to impart ever-more complex functionalities based on kinetic behavior, representing diverse cellular processes, such as growth and division [3,4], communication with the environment via modification of membrane permeability [9], collective information processing [14], **quorum sensing** [15], and cellular differentiation [16].

Despite the significant advances in this field, even the most highly integrated synthetic cell does not come close to living entities. One of the main differences is that biological cells operate in an out-of-equilibrium state compared with these artificial cell-like entities [17]. By continuously consuming energy, biological cells can maintain steady internal, physical, and chemical conditions, show autonomous dynamics in the time domain, and sustain their structural integrity in response to perturbation [18,19]. Synthetic cellular mimics will eventually reach a passive and static state and exhibit unidirectional response to a perturbation(s). To achieve this objective, the next milestone for bottom-up synthetic biology will be the development of substantiable synthetic cellular systems capable of exhibiting self-regulating behavior under non-equilibrium conditions.

Although several reviews have been published in this field [20,21], there is not yet a dedicated review to summarize the recent progress on the development of synthetic cellular systems that can operate far from thermodynamic equilibrium. Here, we systematically review different engineering strategies that can be used to build sustainable synthetic cells. Recent advancements in the development of energy-production modules are examined, along with the engineering methodologies for designing the protometabolic network in synthetic cellular systems. Different functional

Highlights

The incorporation of energy-producing modules within synthetic cellular systems offers an efficient route for energy generation, which represents a major step towards replicating biological compartmentalized out-of-equilibrium systems.

The integration of the complex reaction networks in synthetic cellular systems is crucial to acquire a dynamic homeostatic state to persistent environmental changes.

The establishment of homeostasis, programmable biochemical oscillators, quorum sensing, spontaneous pattern formation, and directional movement in synthetic cellular systems provide clues for better understanding living organisms.

Programming hierarchically assembled and spatiotemporally connected functional modules within synthetic cellular systems that can lead to complex behaviors remains a challenge.

[1]Key Laboratory of Biomedical Engineering of Ministry of Education, Zhejiang Provincial Key Laboratory of Cardio-Cerebral Vascular Detection Technology and Medicinal Effectiveness Appraisal, Department of Biomedical Engineering, Zhejiang University, Hangzhou, 310027, China
[2]Max Planck Institute of Molecular Cell Biology and Genetics, Pfotenhauerstraße 108, 01307 Dresden, Germany
[3]Cluster of Excellence Physics of Life, TU Dresden, Dresden, 01602, Germany
[4]Department of Ultrasound, Women's Hospital, School of Medicine, Zhejiang University, Hangzhou, 310027, China
[5]Innovation Center for Smart Medical Technologies & Devices, Binjiang Institute of Zhejiang University, Hangzhou, 310027, China

*Correspondence:
liangfei.tian@zju.edu.cn (L. Tian).

© 2022 Elsevier Inc. All rights reserved.

dynamic behaviors are presented. The challenges and potential prospects in this field are then discussed.

Energy production in synthetic cellular systems

All out-of-equilibrium systems require a continual source of energy for their sustenance. The production of ATP, as the cell's energy currency, is a biological example of an out-of-equilibrium reaction network. The ATP produced is then used for a myriad of different processes within the cell, such as transcription and translation processes. Techniques that supply continuous energy input throughout time are required to induce out-of-equilibrium behaviors in synthetic cells. For that reason, adding energy-generating modules into synthetic cellular systems is crucial.

Energy-generation modules in synthetic cells

Different energy-generation modules, such as cell-free extracts [22–28], enzymes [29–34], natural organelles [35–37], and artificial energy-generation modules with catalytic activities [38–40], have been integrated into synthetic cellular systems (Figure 1A). These energy-generation modules can be driven either by different chemical precursors or light. For example, different cell-free extracts have been added to synthetic cells, allowing the cell-free synthesis of various proteins [41–44] and lipid compounds [45]. Godino and colleagues demonstrated that the cell division proteins MinD and MinE could be expressed utilizing a recombinant elements (**PURE**) **system** [42]. They observed different types of oscillations, including the pulsing, pole-to-pole, and circling modes at various time points (Figure 1B). Besides the cell-free protein expression systems, different enzymes have also been incorporated to produce energy [29–34,46,47]. For example, Otrin and colleagues constructed a hybrid polymer/lipid vesicle embedded with ATP synthase and cytochrome bo_3 quinol oxidase using dithiothreitol (DTT) from the exterior of the vesicle to produce a proton gradient within the vesicle [46]. Moreover, natural energy generation organelles such as thylakoid [35] and mitochondria [36,37] have been enclosed in synthetic cells. For instance, Li and colleagues showed that the mitochondria could be used to control the polymerization and depolymerization of actin filaments in a giant lipid vesicle colony paving the way for the development of sustainably self-powered artificial tissues [37].

In addition to the aforementioned biologically inspired modules, artificial energy-production systems [38–40] have also been developed and encapsulated in synthetic cells (Figure 1C). For example, colloidosomes supported by silica nanoparticles contained hierarchically ordered metalized peptide/porphyrin/Pt nanofilaments (Figure 1D–F) [40]. This photo-responsive synthetic cell could convert light to chemical energy through nicotinamide adenine dinucleotide (NADH). In addition to this hierarchically arranged photo-responsive nanofilament, TiO_2 nanoparticles [39] and microporous organic semiconducting polymer nanoparticles [48] were also incorporated into synthetic cells to realize the transformation of enzyme cofactors due to photocatalytic activity. It can be envisioned that different artificial energy-production systems, such as nano-enzymes [49], could be combined with synthetic cellsto produce energy.

Engineering membrane permeability for continuous nutrient supply

Several complicated energy-consuming processes [41,50] have been included in artificial systems based on these energy-generation modules. Such systems have an inherent drawback due to the separation of the inner reaction space from the outer volume by an impermeable membrane, limiting the number of chemical resources used to power the reaction. The capability to continuously provide energy to the system over time remains a challenge for the long-term production of critical molecules. In recent years, numerous strategies have been created to combat this drawback. By tailoring the membrane's permeability [9,51,52], for instance, chemical resources from the environment can permeate into the compartment from the exterior.

Glossary

Bottom-up synthetic biology: a field of science that begins with simple molecular bricks and builds complex biological structures and well-defined functional modules, to synthesize an artificial cell with life-like properties.
Coacervate microdroplets: a membrane-free model formed by liquid–liquid phase separation through electrostatic or hydrophobic interactions.
Colloidosomes: microcapsules formed by the self-assembly of colloidal particles at the interface of emulsion droplets.
Homeostasis: a self-regulating process in organisms that regulates internal variables to resist changes caused by the external environment.
Lipid vesicle: a type of synthetic cell model that is assembled by phospholipids in aqueous solution, with the hydrophilic portions of phospholipids facing the aqueous solution.
Positive and negative feedback loops: a system in which one variable increases the quantity of another hence increasing (decreasing) the quantity/occurrence of the first variable.
Proteinosomes: microcompartments with shells that are composed of a closely packed monolayer of conjugated protein-polymer building blocks.
Protoorganelles: artificial organelles, including membranous organelles with a membrane composed of artificial building blocks such as lipids, fatty acids, and proteins; and membrane-free organelles, such as coacervates, formed by polyelectrolytes, proteins, peptides, or nucleotides.
PURE system: a cell-free reconstructed translation system that contains all necessary translation factors, purified with high specific activity and allowing efficient protein production.
Quorum sensing: the regulation of gene expression in response to fluctuations in cell population density.

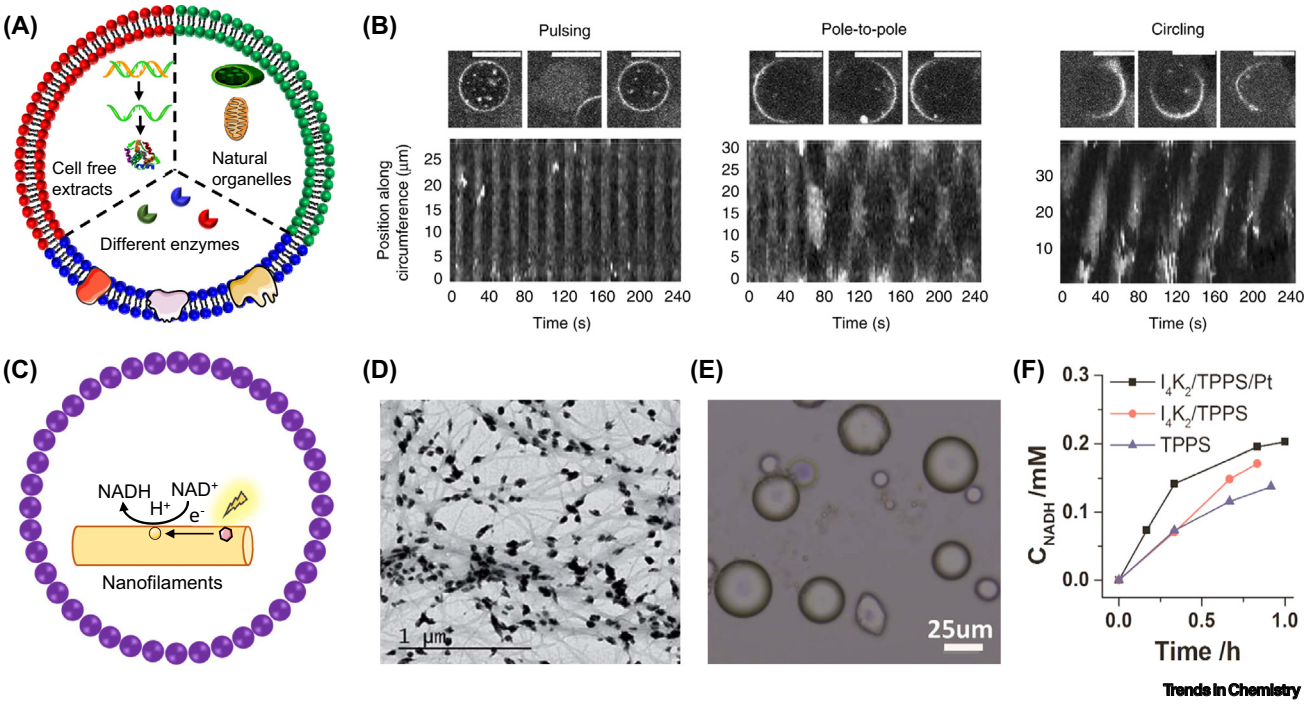

Figure 1. Biologically inspired and artificial energy-generation modules in synthetic cells. (A) Schematic illustration of a liposome that is encapsulated with biologically inspired energy-generation modules. (B) Microscope images showing pulsing, pole-to-pole, and circling modes of oscillations about 1.5 h after triggering gene expression. Scale bars for (B): 10 μm. Adapted, with permission, from [42]. (C) Schematic illustration showing the artificial photo-responsive colloidosomes containing nanofilaments, which could result in photo-mediated reduction of NAD^+ to NADH. (D) Transmission electron microscope image showing nanofilaments of I_4K_2/TPPS/Pt. Scale bar for (D): 1 μm. (E) Optical microscopy image of colloidosomes encapsulating I_4K_2/TPPS/Pt in aqueous phase. Scale bar for (E): 25 μm. (F) Plot showing increases in NADH concentration with time for TPPS, I_4K_2/TPPS, I_4K_2/TPPS/Pt exposed to light. Adapted, with permission, from [40].

Incorporating a membrane protein(s) [22,53–55] into synthetic cellular systems is one way to tune membrane permeability. For instance, α-hemolysin was expressed inside unilamellar vesicles to generate selective nutrient permeability, sustaining GFP expression for up to 5 h [22]. The direct addition of membrane protein offers another alternative method to tune the membrane permeability of the synthetic cell. For example, Li and colleagues inserted melittin into a multicompartment synthetic cell containing two **protoorganelles** (Figure 2A). Through the melittin pore, glucose molecules from an external solution infiltrated the lipid bilayer. They diffused into one protoorganelle (1) to produce hydrogen peroxide, which subsequently diffused to the other protoorganelle (2) to stimulate a cascade enzymic catalytic reaction (Figure 2B) [53].

High membrane permeability can also be imparted by directly altering the intrinsic membrane properties by light or pH [56,57]. For instance, by introducing a light activated azo-benzene moiety to the alkyl chain of the lipid molecule, the transition between the *cis* and *trans* conformation of the azo-benzene moiety in response to the light leads to the transformation from a fluid- to a gel-like membrane [58]. The transition to a more fluid-like membrane from a gel-like membrane will permit increased diffusion of small molecules across the membrane interface. The use of light to infer an irreversible change in lipid membrane properties has also been exploited [59]. Instead of utilizing an azo-benzene moiety on the alkyl chain, a diacetylene functional group was introduced to the lipid molecule. Upon UV radiation, enyne conjugates are generated via 1,4 cycloaddition of the diacetylene functional groups, resulting in lipid clustering and the formation of pores (Figure 2C). Hindley and colleagues demonstrated that light-activated pore formation allowed the real-time outflow of substrates to feed the enzymatic reaction enclosed within the vesicle (Figure 2D,E).

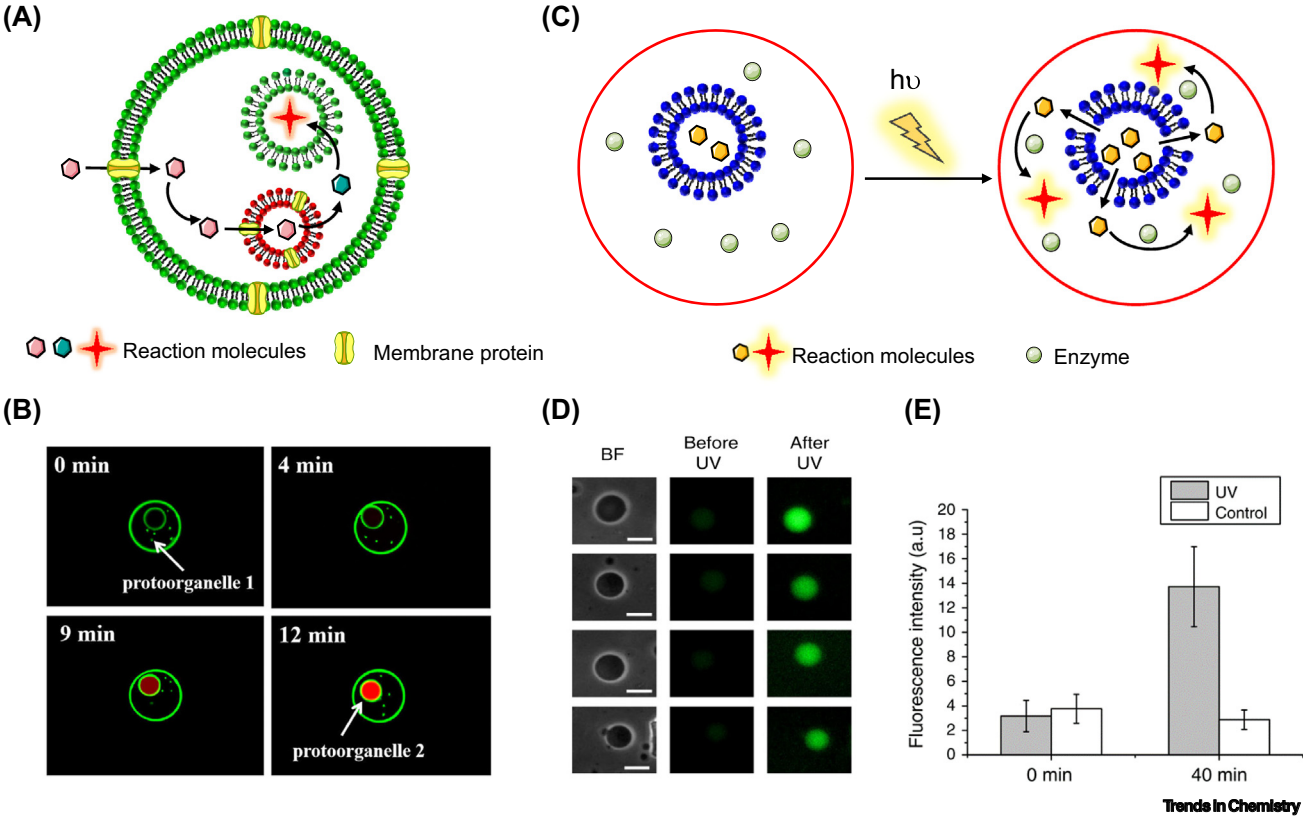

Figure 2. Engineering membrane permeability. (A,B) Schematic illustration showing that the membrane permeability of a synthetic cell can be tuned by direct addition of membrane protein (A), and corresponding fluorescence images revealing that the addition of melittin could facilitate the diffusion of glucose from the environment, which subsequently triggers the enzymatic cascade reaction between two protoorganelles inside of the synthetic cell (B). Adapted, with permission, from [53]. (C–E) Schematic illustration showing that photopolymerization of the inner compartment membranes results in an increase of membrane permeability and subsequent release of the substrate for enzymatic reaction (C). Corresponding bright field (BF) and fluorescence images (D), and plots of the fluorescence intensities (E) of the UV-responsive synthetic cell before and after UV irradiation. Scale bars for (D): 25 μm. Adapted, with permission, from [59].

Building on these light-activated systems, an obvious next step would be to allow transient pore formation by activating and relaxing the functional group to permit the efflux of substrates and nutrients from an external reservoir on cue.

Steps towards continuous energy input in synthetic cellular systems

Although different strategies for the modular incorporation of both biological and artificial energy-producing systems into synthetic cells have been developed, there are limitations in both systems. For instance, one challenge associated with cell-free protein synthesis is the DNA degradation by endogenous nucleases in the cell extract. Also, the ability to produce multiple proteins in cell-free systems should be improved. The artificial energy-producing modules may have better stability, while the low efficiency hinders their application. Therefore, improvements in the efficiency and durability of energy-producing modules will be needed to construct long-lasting synthetic cells. Furthermore, previous investigations have concentrated on the intake of nutrients from the environment that can serve as energy to support their out-of-equilibrium behaviors. However, little attention has been paid to eliminate waste products. For future advancements in this field, highly regulated membrane transporters must be integrated into synthetic cells.

Reconstruction of the protometabolic network in synthetic cells

Integrating energy-generation modules into synthetic cellular systems gives the energy resources required to sustain a variety of biological functions. However, this is insufficient to construct a robust synthetic cell capable of dynamically sensing and adapting to changes in their external conditions. Living cells use sophisticated metabolic networks to manage the functioning of biomolecules in space and time to adapt to physicochemical changes. At the molecular level, these complex metabolic networks are composed of thousands of chemical species that form complex networks of chemical reactions operating far from equilibrium conditions [60]. These networks show complex and cooperative behaviors, which can help living cells persist through irregular environmental changes and potentially display emergent network dynamics to drive many key processes, such as spontaneous pattern formation [61] and periodic oscillation [62–65] in living systems.

Despite the tremendous complexity of biological networks, several fundamental elements of these reaction networks can be identified, providing an opportunity to develop synthetic systems with similar capabilities. For example, these biological reaction networks generally follow nonlinear dynamics, which indicates that the change in output is not proportional to the change in input, resulting in complex dynamical behaviors not seen in linear reaction networks. Adopting an engineer's approach to control systems, these complicated biological reaction networks can be decomposed into simple regulatory motifs, which can be used as dynamic building blocks to program dynamic behaviors in synthetic cellular systems [66]. For instance, positive or negative feedback can introduce promotion or inhibition to the overall output following nonlinear chemical laws (Figure 3A). Diffusion, however, provides the system with concentration gradients stimulating local responses and enhancing reaction variety (Figure 3B). Unlike the linear modules, nonlinear systems adopt the strategy of selectively amplifying or suppressing the input signal. For example, a cascade reaction is a signal amplification process from cell sensing to phenotype transformation, relying on bimolecular nonlinear interactions [67]. However, cell noise inevitably accumulates due to the random fluctuations of genetic reactions [68]. The ordered progression of biological activities illustrates that cell noise can be decreased by dampening the input signal under nonlinear kinetic control. Due to their ability to improve response sensibility and interference resistance in the biological system, integrating similar regulatory motifs is crucial for the endowment of adaptive capacity in the synthetic cell. It lays the foundation for the reproduction of emergent behaviors in synthetic cellular systems.

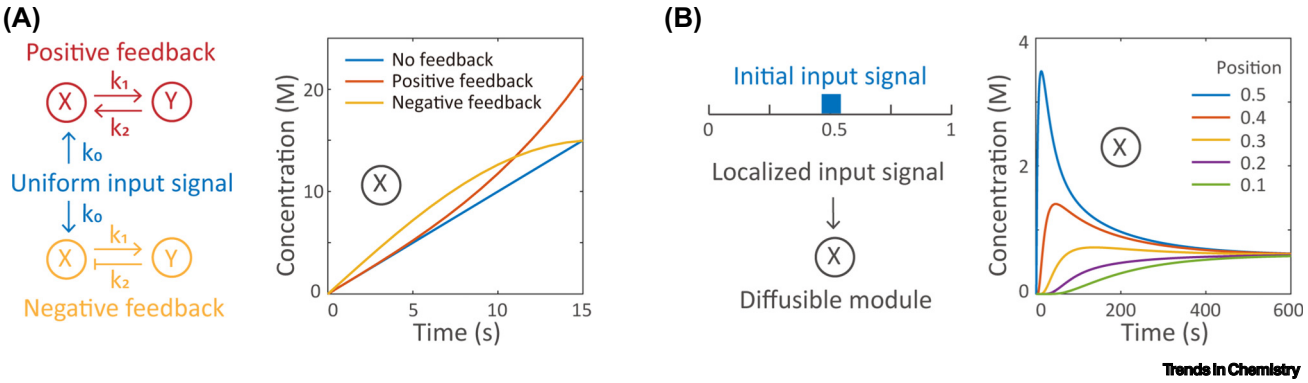

Figure 3. Representative regulatory motifs for the generation of nonlinear dynamics. (A) Systems with or without feedback. In a feedback-free system, the generation of X by input signal was linear following the zero-order reaction. In a positive (or negative) feedback system, the concentration of X was also affected by the promotion (or inhibition) of Y, resulting in nonlinear dynamics. In the simulation, the concentration of reactants was determined by the reaction network in (A), where k_0 was set 1 M s^{-1}; k_1 was set 0.1 × C(Y); k_2 was set 0.1 × C(X); k_3 was set 0.5–0.1 × C(Y) [C(Y) < 5]. (B) Systems with diffusible input signal. Diffusion introduces temporospatial nonlinear modules to the system. Distinct localization of network components generates different nodes gradients, thus increasing the system complexity. In simulation, the concentration of diffusible components was determined by Fick's Law, with the diffusion coefficient setting 10 cm^2 s^{-1}.

The integration of network motifs in synthetic cellular systems

Undoubtedly, imitating biological systems offers a plausible solution to designing such protometabolic reaction networks in synthetic cells. One clear advantage of such systems is to retain the metabolic activity of the naturally existing system; these can be used as a model system to understand the operating principles of biological cells. To this end, the enormous, complicated biological system has identified different network motifs comprising several **positive and negative feedback loops**. Beneyton and colleagues developed out-of-equilibrium artificial microcompartments by combining a minimal metabolism of nicotinamide adenine dinucleotide (NAD)-dependent enzymatic reaction and an NAD-regeneration module [69]. This system is dissipative and can maintain out-of-equilibrium for a finite time until the substrate is completely consumed and can be activated by the addition of fresh substrate molecules.

However, due to the limited number of available network motifs, an alternative approach has been developed combining different currently existing biological reaction network motifs. One of the challenges is that most reaction network motifs may not share common building blocks, with potentially incompatible reactions and conditions, such as different pH or ionic strength. One strategy to overcome this is to couple biological and synthetic modules together (Figure 4A). Miller and colleagues integrated thylakoid membranes into cell-sized droplets [70]. An artificial pathway [crotonyl-CoA/ethylmalonyl-CoA/hydroxybutyryl-CoA (CETCH) cycle] for the fixation of CO_2 was combined with a thylakoid membrane-based energy module (TEM) (Figure 4B). CO_2 was converted to glycolate by CETCH under light with the concomitant production of NADPH and ADP. The fluorescent signal of NADPH in TEM-containing microdroplets increased with the illumination time (Figure 4C,D) [70]. This strategy combines natural and synthetic modules and offers a novel approach to develop new reaction networks rationally. It significantly expands the range of biological reaction network motifs already in use and opens up the possibility of creating out-of-equilibrium systems that might be more effective than biological systems.

Besides naturally existing reaction networks, integrating artificial reaction networks, such as an autocatalytic reaction network or DNA dynamic circuit into synthetic cells offers a different approach to constructing reaction networks in synthetic cellular systems (Figure 4A). For instance, enzyme-free DNA strand-displacement circuits have been widely employed as an artificial network motif due to their programmability [14,71–73]. Based on this principle, Peng and colleagues showed that membrane permeability could be switched between an open and closed state by encapsulating an artificial DNA reaction network (Figure 4E) [73]. To achieve this, they constructed a DNA nano-gatekeeper that could be switched to the open state in the presence of ATP. Sequentially, the zinc and strontium ions, which diffused through the channel, could activate the feedback pathway by releasing a blocker leading the nanogatekeeper back to the closed state (Figure 4F). Joesaar and colleagues designed a highly programmable synthetic messaging system using toehold-mediated strand displacement processes by pre-encapsulating biotinylated DNA gate strand in proteinosome [14]. The DNA logic circuit-based synthetic cells could sense, process, and respond to DNA-based messages and could perform cascaded amplification, bidirectional communication, and distributed computational operations.

Engineering strategies for the functional protometabolic network in synthetic cells

Notwithstanding these recent examples, the construction of protometabolic reaction networks in synthetic cellular systems is still in its infancy. This is because the functional pathway engineering currently in place was developed using static control strategies [74,75]. However, natural metabolic pathways have relied on dynamic control strategies, leading to self-regulation to perturbation. One limitation of this goal is that there are a few available network motifs. For this purpose, the new exploitation of new reliable network motifs [76], or the recombination of existing

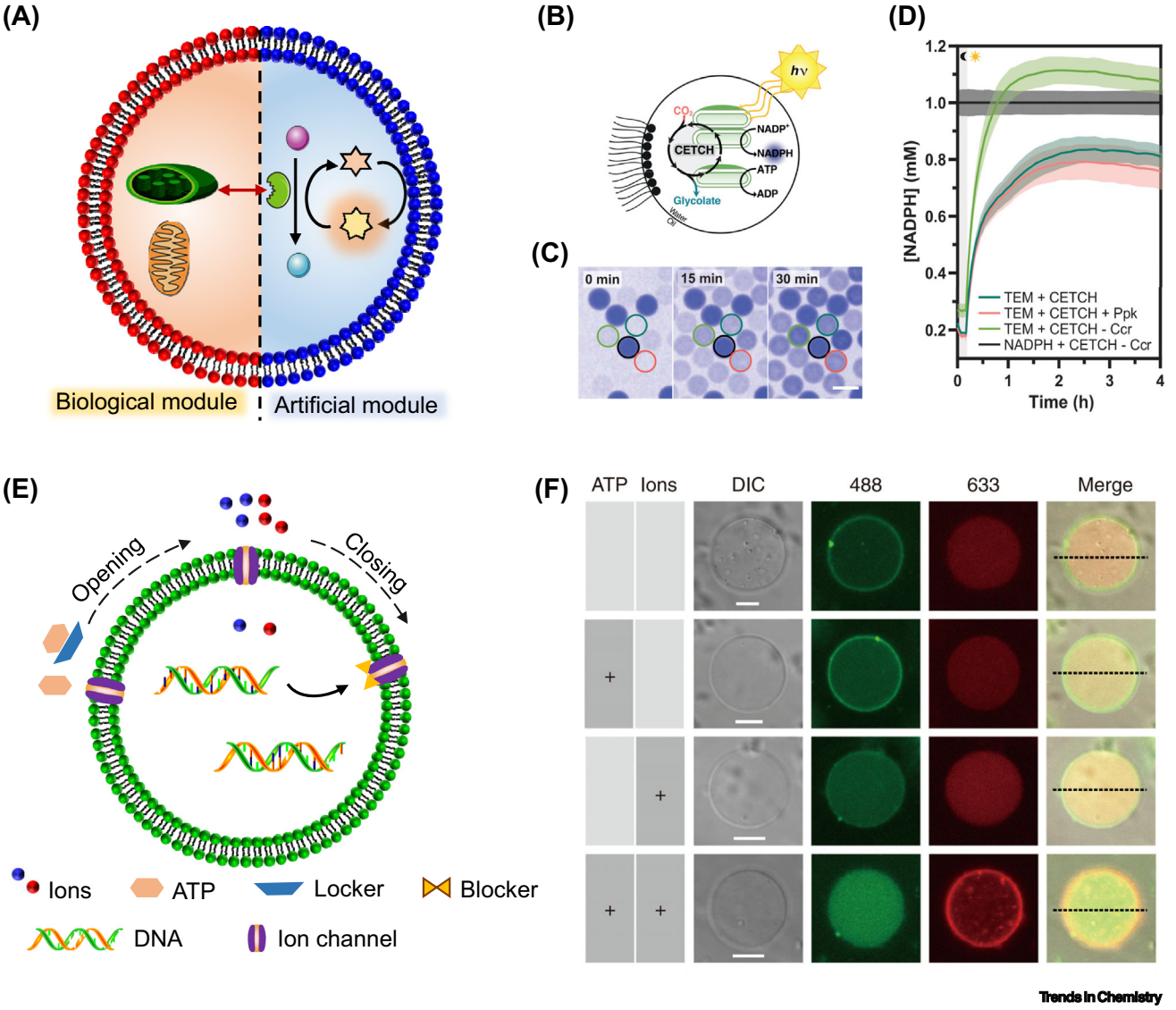

Figure 4. Reconstruction of the protometabolic network. (A) Schematic illustration of protometabolic network containing artificial module and/or biological module. (B–D) Scheme of the NADPH generation module operating inside microdroplets (B), and corresponding microscope images (C) of the microdroplets before illumination and after 15 and 30 min of illumination (from left to right). Scale bar for (C): 100 μm. The plots of the fluorescence intensity of the NADPH show that the efficiency of NADPH generation is the highest when thylakoid membrane-based energy module (TEM) and crotonyl-CoA/ethylmalonyl-CoA/hydroxybutyryl-CoA (CETCH) components, except for Ccr, are enclosed into microdroplet (D). Adapted, with permission, from [70]. (E,F) Schematic representation of the control of membrane permeability using an artificial DNA reaction network (E), and corresponding bright field and fluorescence images showing that the reaction network could only be activated in the presence of both ATP and ion influx. The DNA nanogatekeeper on the membrane was labeled with Fluro 488 (green fluorescence) and the blocker was labeled with Cy5 (red fluorescence). Scale bar for (F): 5 μm. Adapted, with permission, from [73].

reaction network motifs [77], is required. In addition, reaction network motifs only provide temporal dynamics. In contrast, spatial dynamics rely on biomolecular diffusion and increased potential for multiplexing. This is crucial for generating compartmentalized reaction networks for prolonged out-of-equilibrium behavior since it provides a kinetic framework for networks of different complexity. Moreover, creating multicompartmentalized synthetic cells [78,79], in which distinct functional reaction network motifs can be spatially separated into distinct subcompartments, provides alternative strategies to integrate reaction networks into compartments.

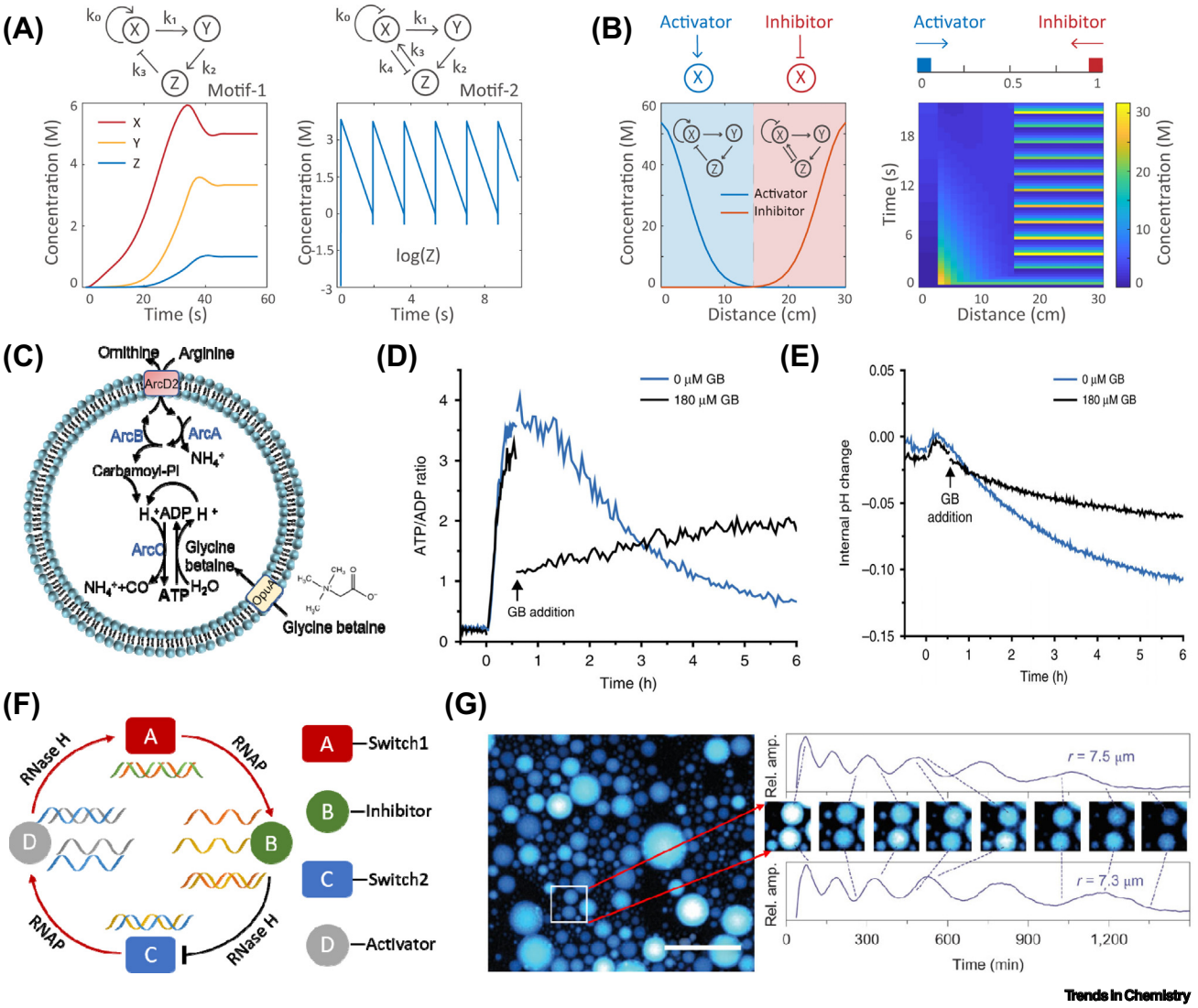

Figure 5. Feedback modules create out-of-equilibrium synthetic systems. (A) Steady state or oscillation was generated and maintained by constructing a three-node reaction network, in which negative feedback was an indispensable module. Distinct states can be also created in the same network motif with different parameters (e.g., reaction rates). To generate steady state, k_0 was set 1 M s^{-1}; k_1 was set 0.1 s^{-1}; k_2 was set 0.15 s^{-1}; k_3 was set 0.1 M^{-1} s^{-1} in motif-1. To generate oscillation, k_0 was set 6×10^5 M^{-1} s^{-1}; k_1 was set 1.5×10^3 s^{-1}; k_2 was set 910 s^{-1}; k_3 was set 0.28 s^{-1}; k_4 was set 2×10^8 M^{-1} s^{-1} in motif-2. (B) Coupled feedback and diffusion modules drive pattern formation. Activator and inhibitor were produced at both ends, forming competitive concentration gradients. In the activator-dominated regions, motif-1 was activated, resulting in steady state, while in the inhibitor-dominated regions, motif-2 was activated, resulting in oscillation. The diffusion coefficients of activator and inhibitor were both set 10 cm^2 s^{-1} and the reaction rate was set 1 M^{-1} s^{-1} in the simulation. (C–E) Schematic representation of a protometabolic network, including an arginine breakdown pathway, and the importation of glycine betaine (GB) pathway via OpuA followed by consuming ATP; the ATP/ADP ratio can keep stable for about 6 h (D), and the internal pH maintains relatively constant (E) after the addition of GB. Adapted, with permission, from [81]. (F,G) Schematic representation of an *in vitro* transcriptional oscillator, including a two-switch negative-feedback oscillator circuit. A and C in the squares represent two transcriptional switches, SW21 and SW12, composed of two double-stranded DNA templates (T21A1 and T12A2). B in the circle represents inhibitor (rI2), which can inhibit transcription from SW12. D in the circle represent activator (rA1), which can activate SW21 by releasing A1 strands. Corresponding fluorescence images and plots of the fluorescence intensities in the microdroplets showed that oscillations were different in amplitude and frequency from droplet to droplet due to stochastic reaction dynamics (G). Scale bar for (G): 100 μm. Adapted, with permission, from [82]. Abbreviation: Rel. amp., relative amplitude.

Program functional synthetic cellular behaviors under non-equilibrium conditions

The functional dynamic behaviors in living systems are maintained through a continuous influx of energy combined with complex reaction networks. Hence, the development of similar energy-

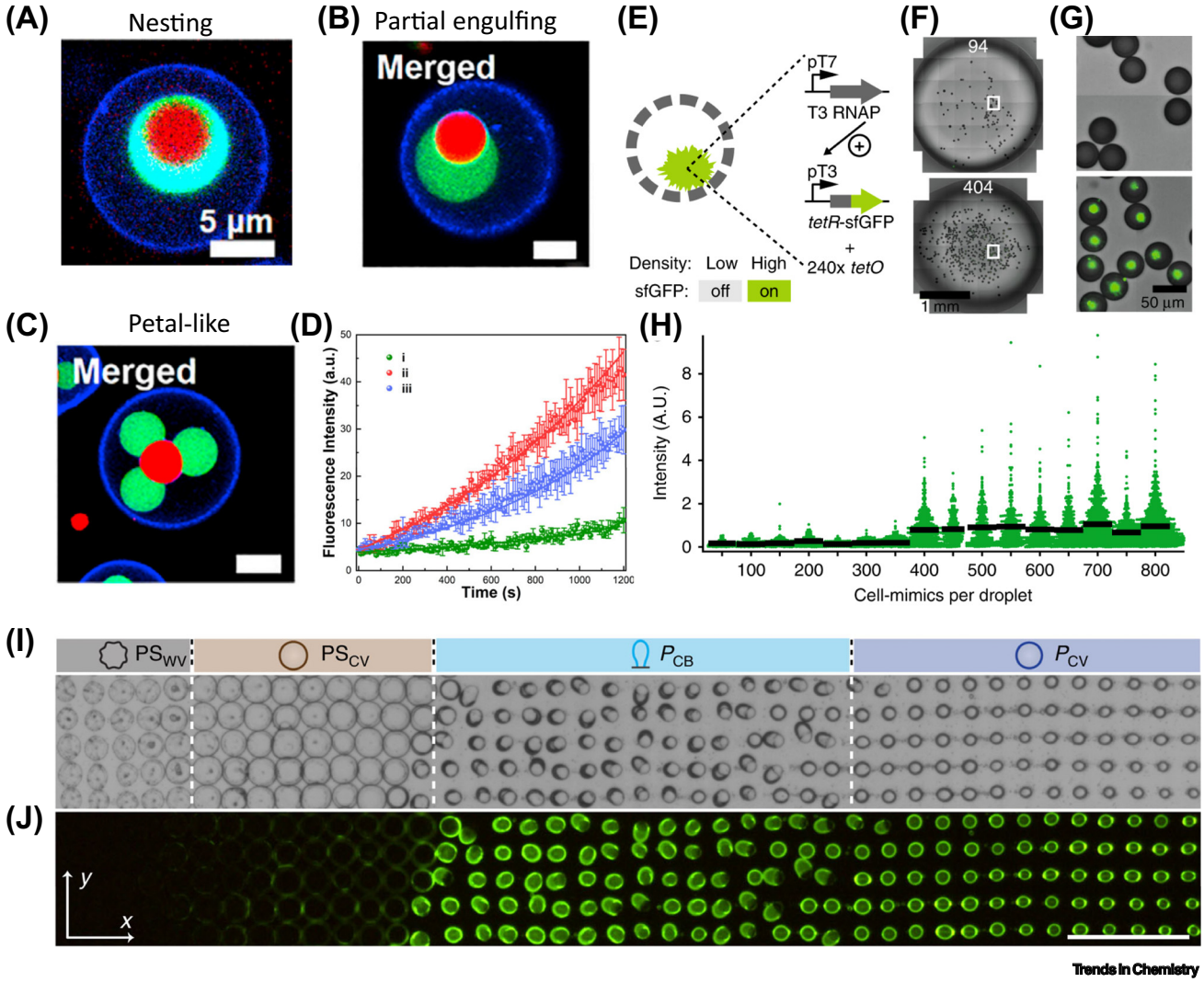

Figure 6. Spatial organization of subcompartments within synthetic cells. (A–D) Confocal fluorescence images of nesting, partial engulfing, and petal-like multi-microcompartments inside proteinosomes after the addition of PEG with molecular weight of 20, 20, 8, 2 kDa, respectively. Scale bar in (A): 5 μm. Scale bars in (B) and (C): 3 μm. (D) Analysis of the fluorescence in different enzyme reaction experiments. Fluorescent labeled HRP located at the dextran phase (i), mixed phase composed of dextran phase and dissociated coacervate (ii), and over the entire lumen of proteinosome after all dissociation (iii). Adapted, with permission, from [89]. Functional dynamic synthetic cellular behaviors under reaction diffusion fields. (E) Schematic of artificial quorum-sensing artificial cell containing T3 activation cascade DNA templates and 240× *tetO* plasmids. (F,G) Microscope images of artificial cells in 4.5 μl of transcription and translation solution. Scale bars in (F) and (G): 1 mm and 50 μm. (H) Scatter dot plot of fluorescence intensities in individual artificial cell at different densities. Adapted, with permission, from [15]. (I,J) Optical (I) and corresponding fluorescence microscopy images (J) showing 2D array of differentiated protocells. Adapted, with permission, from [99].

generation modules and functional network motifs, in principle, will enable us the opportunities to emulate such dynamic behaviors in synthetic cellular systems. Recent progress in the construction of chemical reaction network have showed that simple motifs with a few positive and negative feedback loops and concentration gradient field can produce an out-of-equilibrium synthetic system with sustainable steady state or oscillation behavior (Figure 5A) [80] and spontaneous pattern formation (Figure 5B). To this end, different synthetic cellular systems have been developed to imitate the functional behavior of living systems by using various regulatory motifs.

Regeneration of sustainable, dynamic states in synthetic cellular systems

Living things can be kept in an optimal condition, one of their distinguishing qualities. Numerous parameters, including pH, ionic strength, and solute composition, are maintained within predetermined ranges (homeostatic) and can endure environmental changes. **Homeostasis** in synthetic cells has been rebuilt using those aforementioned synthetic cellular systems with various reaction networks. For instance, Pols and colleagues showed that by integrating an arginine degradation pathway into lipid vesicles, ATP could be used to control the flux of solute (Figure 5C) [81]. The lipid vesicle could maintain a constant ATP/ADP ratio (Figure 5D) and relatively constant pH (Figure 5E) for 6 h after adding glycine betaine. Moreover, programmable functional biochemical oscillators have been incorporated into synthetic cells. Negative feedback loops were created and exhibited dynamic behavior, including sustained oscillating concentrations of active enzyme trypsin [65] or DNA strands [82]. Typically, a programmable two-switch transcriptional oscillator system was compartmentalized into microdroplets. The two gene switches constituted an overall negative feedback loop and presented oscillatory behavior for appropriate parameter settings (Figure 5F). Large populations of microdroplets were measured and showed major variations in the amplitude, frequency, and damping of the oscillations (Figure 5G) [82]. The maintenance of metabolic physicochemical homeostasis in a synthetic cell is an essential advanced functional reconstitution of a chemically defined network that aids in the development of complex synthetic cell models with adaptive behavior in terms of lipid and protein synthesis, cell growth, and intercellular communication.

Spatial organization of subcompartments within synthetic cells

Multicompartmentalization is another characteristic in living systems, in which distinct subcompartments have specialized microenvironments with selectively partitioning of biomolecules. The dynamic spatiotemporal control of the assembly and disassembly of different subcompartments, such as membraneless organelles, plays a key role to regulate the localization of functional biomolecules, which can be used to control biochemical reactions and maintain cellular homeostasis [83–85]. The construction of their synthetic analogs has also been achieved in various synthetic cellular systems by encapsulating membraneless organelles into various synthetic cellular systems [56,86–91]. For instance, Li and colleagues showed that the encapsulated membraneless organelles exhibited three typical spatial configurations, including nesting, partial engulfing, and petal-like configuration in synthetic cells by varying the interfacial tensions between each phase and each phase showed distinct microenvironments with selective hosting of various biomacromolecules (Figure 6A–C) [89]. The programmable spatial organization of different sub-microcompartments was realized upon regulation of external environmental changes, which was used to control the reaction rates of biochemical reactions (Figure 6D) or signaling processes in synthetic cells [89,90]. Advances in this direction will provide new strategies for the hierarchical spatial organization of various biomacromolecules and organelles inside synthetic cells and contribute to the design of novel microreactors with programmable release of various biomacromolecular payloads and spatiotemporal regulation of biochemical reactions.

Collective behavior via population dynamics

In single synthetic cell mimics, sustained biochemical processes are recapitulated as mentioned above. Combining functional network motifs with reaction–diffusion processes has also been possible to produce collective behaviors in a collection of synthetic cells. For instance, quorum sensing, in which microbes communicate cellular information based on population density [92], has also been recreated utilizing populations of synthetic cells and between synthetic cells and biologic cells [93–97]. To this end, Devaraj and colleagues [15] showed that artificial quorum sensing could be reconstructed in synthetic cells using the activation circuit (T3 RNA polymerase, T3 RNAP) and reporter constructs (TetR-sfGFP and *tet*O plasmids). The synthetic cell community showed a collective response where fluorescence accumulated only at high densities while

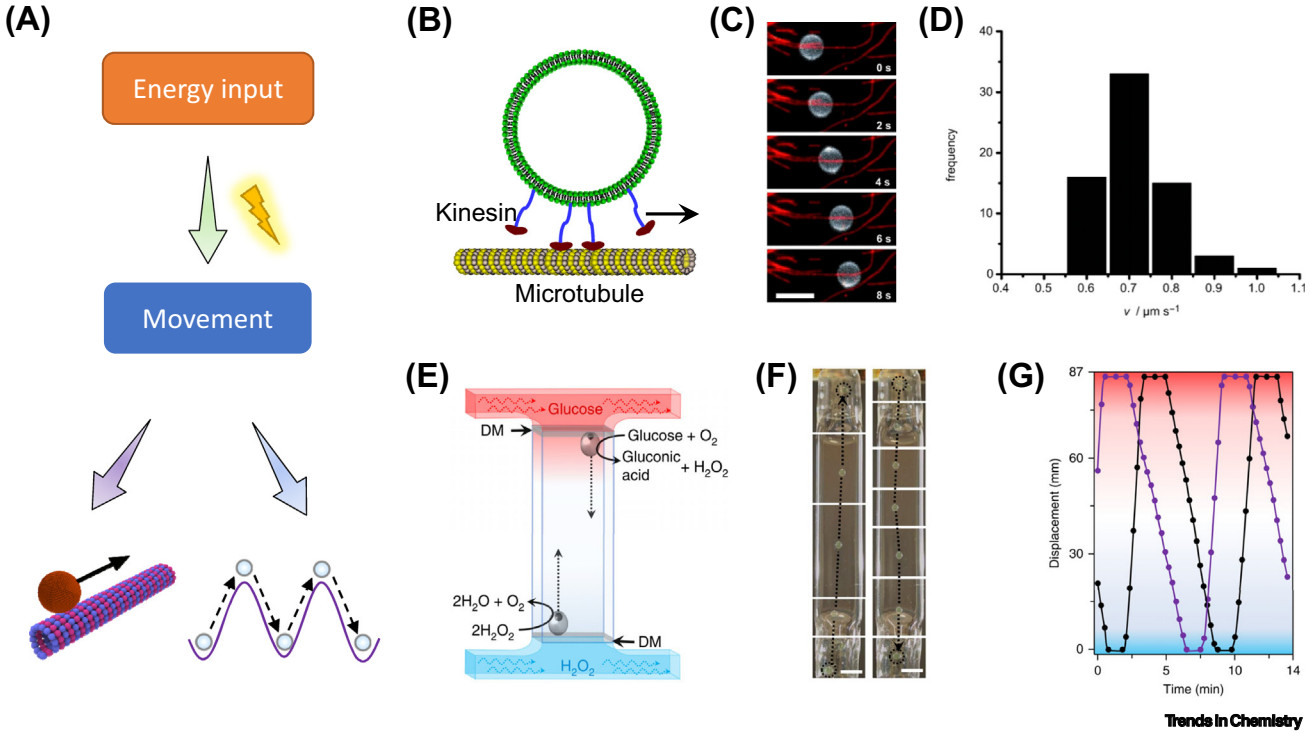

Figure 7. Directional movements in synthetic cellular systems. (A) Schematic representation of different directional movements such as orientation motility and oscillatory movement. (B–D) Schematic representation of vesicle transport along microtubules using kinesin motors and corresponding microscope images of a typical transport event (C), a histogram of the vesicle-transport velocities *v* (D). Scale bar for (C): 5 μm. Adapted, with permission, from [101]. (E–G) Schematic representation of a sustainable oscillatory movement using catalase/GOx-containing organoclay/DNA synthetic cell and corresponding microscope images and plots of the vertical displacement for two synthetic cells of a typical enzyme-mediated oscillatory movement. Scale bar in (E): 5 mm. Adapted, with permission, from [102].

fluorescent signals were not detectable at low artificial cell density (Figure 6E–H). The synthetic cell density threshold approximated bacterial quorum-sensing responses to cell density. The release of T3 RNAP from the synthetic cells caused the collective reaction to the density of synthetic cells. T3 RNAP was diluted in a relatively large sample volume at low density. In contrast, a sufficient quantity of transcriptional activators accumulates at a high density to activate reporter to turn on expression.

Spontaneous pattern formation under concentration gradient fields

Spontaneous pattern formation is another dynamic behavior in various biological systems and plays vital physiological roles in the development of the body plan. The ability to program pattern formation in synthetic cellular systems will offer a better understanding of the emergence of patterns in nature. It could be used to build chemically based communication networks that respond to fluctuating inputs. Tian and colleagues developed an ultrasound-based technology to assemble synthetic cells in 2D spatial arrays [13]. They showed that the organized synthetic cell array could dynamically sense encoded information from the chemical concentration gradients [98]. Moreover, they demonstrated that the spontaneous pattern formation could be generated in these 2D synthetic cell assemblies under uni-directional and counter-directional reaction–diffusion gradients of artificial morphogens sodium dodecylsulfate (SDS) and polyoxometalate (POM) (Figure 6I,J) [99]. Dynamic reconfiguration of the synthetic cell in the morphogen gradients produced a diversity of membrane-bounded vesicles spontaneously segregated into multimodal populations with differentiated enzyme activities. These novel organized synthetic cell arrays provide a promising platform to integrate complex spatial and time-dependent behaviors of synthetic cell consortia by controlling the concentration gradients and their directionality in the reaction field.

Directional movements in synthetic cellular systems

Another important dynamic process is the motility of the biological cell, which is a fundamental process for embryonic development and wound healing. Complex behavior reconstitution, such as directional motility and oscillatory movement, has been documented in a synthetic cellular system (Figure 7A). For example, by coating kinesin motors on their membrane, the unidirectional movement of synthetic cells along microtubules (Figure 7B–D) was observed, which convert the chemical energy to mechanical work by the hydrolysis of ATP [100,101]. Furthermore, repetitive oscillatory movements have been induced in a stable concentration gradient. Kumar and colleagues achieved continuous oscillatory movement within the synthetic cell by encapsulating catalase and glucose oxidase (GOx) in stable glucose and hydrogen peroxide concentration gradients (Figure 7E) [102]. The oxygen-microbubble-generating microcapsules can ascend, harnessing the buoyant force to move against gravity. Moreover, when the microcapsules move to the top of the column, the GOx-mediated conversion of glucose to gluconic acid consumes the oxygen-microbubble, decreasing the buoyant force such that the capsule descends under gravity (Figure 7F,G). The oscillatory movement in the vertical displacement of microcapsules could be sustained for 4–5 h.

Combinational approach towards substantiable synthetic cellular systems

Even though several synthetic cellular systems have been developed to imitate some of the most fundamental functions of living systems, using these artificial systems to create an autonomous, sustained synthetic cellular system is still difficult, because the current, too simplified models fall short in many ways. One way to solve this problem is to increase the complexity of the functional modules. While living cells have developed intricate metabolic pathways with numerous interconnected pieces, the simple amalgamation of various core parts would not be sufficient to mimic sophisticated cell behaviors. Therefore, it would be crucial to look into the durability of such artificial systems and how to construct and spatiotemporally connect various core modules hierarchically.

Concluding remarks

The emergence of cellularity from different functional parts is the ultimate goal for bottom-up synthetic biology. These synthetic systems with life-like properties (synthetic cells) provide a conceptual pathway to explore the transition from nonliving matter to living cells, strongly impacting different scientific disciplines. This grand challenge to construct a minimal living system has been ongoing for many years and we are still far from the ultimate goal. In this review, we focus on the recent progress in the design and construction of out-of-equilibrium synthetic cellular systems and discuss the various engineering strategies that can provide a continuous energy source and reconstitute complex metabolic networks in synthetic cellular systems. Various functional dynamic behaviors arising from the integration of energy input modules and reaction network motifs within the synthetic cellular systems have been summarized.

There are challenges to accomplishing these objectives (see Outstanding questions). For instance, ongoing efforts are made to increase the complexity of the functional modules that can be reconstituted in the synthetic cellular system, such as the energy supply modules and various reaction networks, as the current simplified systems fall short in capturing many crucial biological processes in living systems, such as autonomous cell division. Moreover, more attention must be paid to how to remove the waste products during the reaction networks in synthetic cells. Whereas, many functional systems are intertwined, combining different fundamental parts might not be enough to imitate complicated cell behaviors. Therefore, it will be crucial to investigate how to spatially and temporally connect several core modules inside the synthetic cellular systems. Due to our incomplete understanding of the molecular functioning of living cells, this undertaking is incredibly challenging. Although specific biological nonlinear kinetic processes

Outstanding questions

How can different fundamental modules within synthetic cellular systems be hierarchically assembled and spatiotemporally connected?

How can systematic formulations of energy transduction in metabolic networks be developed?

How can waste products formed during reaction networks in synthetic cells be removed?

Can more complex spatiotemporal chemical signals networks, evaluated using Turing theory, be integrated into synthetic cellular systems?

How can we develop new technologies to reproducibly spatially organize different synthetic cells and further construct higher-order synthetic cell assemblies?

have been identified thus far, connecting these nonlinear reactions to synthetic cells is still challenging. Research in this area will significantly contribute to the study of basic biology and may disclose previously unknown cellular pathways. Additionally, it may result in developing novel artificial techniques that do not necessarily exist in natural systems, thereby significantly broadening the application of current biological systems and opening the door to establishing synthetic systems that might be more effective than biological ones. It could be applied to enhance future microscale technologies that exhibit essential characteristics of living systems, which have enormous potential in biotechnology and biomedical engineering, including drug delivery, cell therapies, and biosensing.

Acknowledgments

L.T. acknowledges the financial support provided by National Natural Science Foundation of China (32171302), Hundred Talents Program of Zhejiang University, Open Research Projects of Zhijiang Lab (2022RD0AB01), and ICGEB Research Grant (CRP/CHN21-04_EC). T.Y.D.T. acknowledges financial support from the MPG and MaxSynBio Consortium, jointly funded by the Federal Ministry of Education and Research (Germany) and the Max Planck Society and the MPI-CBG, Deutsche Forschungsgemeinschaft (DFG, German Research Foundation) under Germany´s Excellence Strategy – EXC-2068 – 390729961– Cluster of Excellence Physics of Life of TU Dresden and EXC-1056- Center for Advancing Electronics Dresden, Volkswagen Foundation, and Horizon 2020 Marie Sklodowska-Curie Actions Innovative Training Network (ITN)-Protomet ITN (Grant number 813873). X.W. acknowledges financial support provided by China Postdoctoral Science Foundation (No. 2020TQ0564).

Declaration of interests

The authors declare no conflict of interest.

References

1. Walde, P. *et al.* (2010) Giant vesicles: preparations and applications. *ChemBioChem* 11, 848–865
2. Weiss, M. *et al.* (2018) Sequential bottom-up assembly of mechanically stabilized synthetic cells by microfluidics. *Nat. Mater.* 17, 89–96
3. Zhu, T.F. and Szostak, J.W. (2009) Coupled growth and division of model protocell membranes. *J. Am. Chem. Soc.* 131, 5705–5713
4. Zong, W. *et al.* (2017) A fissionable artificial eukaryote-like cell model. *J. Am. Chem. Soc.* 139, 9955–9960
5. Wang, X. *et al.* (2020) Chemical information exchange in organized protocells and natural cell assemblies with controllable spatial positions. *Small* 16, 190394
6. Wang, X. *et al.* (2019) Chemical communication in spatially organized protocell colonies and protocell/living cell microarrays. *Chem. Sci.* 10, 9446–9453
7. Kamat, N.P. *et al.* (2011) Engineering polymersome protocells. *J. Phys. Chem. Lett.* 2, 1612–1623
8. Jiang, W. *et al.* (2015) Hyperbranched polymer vesicles: from self-assembly, characterization, mechanisms, and properties to applications. *Chem. Soc. Rev.* 44, 3874–3889
9. Li, M. *et al.* (2013) Electrostatically gated membrane permeability in inorganic protocells. *Nat. Chem.* 5, 529–536
10. Huang, X. *et al.* (2013) Interfacial assembly of protein–polymer nano-conjugates into stimulus-responsive biomimetic protocells. *Nat. Commun.* 4, 2239
11. Koga, S. *et al.* (2011) Peptide-nucleotide microdroplets as a step towards a membrane-free protocell model. *Nat. Chem.* 3, 720–724
12. Aumiller, W.M. and Keating, C.D. (2016) Phosphorylation-mediated RNA/peptide complex coacervation as a model for intracellular liquid organelles. *Nat. Chem.* 8, 129–137
13. Tian, L. *et al.* (2016) Spontaneous assembly of chemically encoded two-dimensional coacervate droplet arrays by acoustic wave patterning. *Nat. Commun.* 7, 13068
14. Joesaar, A. *et al.* (2019) DNA-based communication in populations of synthetic protocells. *Nat. Nanotechnol.* 14, 369–378
15. Niederholtmeyer, H. *et al.* (2018) Communication and quorum sensing in non-living mimics of eukaryotic cells. *Nat. Commun.* 9, 5027
16. Dupin, A. and Simmel, F.C. (2019) Signalling and differentiation in emulsion-based multi-compartmentalized in vitro gene circuits. *Nat. Chem.* 11, 32–39
17. Merindol, R. and Walther, A. (2017) Materials learning from life: concepts for active, adaptive and autonomous molecular systems. *Chem. Soc. Rev.* 46, 5588–5619
18. Schwille, P. *et al.* (2018) MaxSynBio: avenues towards creating cells from the bottom up. *Angew. Chem. Int. Ed.* 57, 13382–13392
19. Yewdall, N.A. *et al.* (2018) The hallmarks of living systems: towards creating artificial cells. *Interface Focus* 8, 20180023
20. Guindani, C. *et al.* (2022) Synthetic cells: from simple bio-inspired modules to sophisticated integrated systems. *Angew. Chem. Int. Ed.* 61, e202110855
21. Wang, C. *et al.* (2021) Modularize and unite: toward creating a functional artificial cell. *Front. Mol. Biosci.* 8, 781986
22. Noireaux, V. and Libchaber (2004) A vesicle bioreactor as a step toward an artificial cell assembly. *Proc. Natl. Acad. Sci. U. S. A.* 101, 17669–17674
23. Ishikawa, K. *et al.* (2004) Expression of a cascading genetic network within liposomes. *FEBS Lett.* 576, 387–390
24. Tawfik, D.S. and Griffiths, A.D. (1998) Man-made cell-like compartments for molecular evolution. *Nat. Biotechnol.* 16, 652–656
25. Shum, H.C. *et al.* (2008) Microfluidic fabrication of monodisperse biocompatible and biodegradable polymersomes with controlled permeability. *J. Am. Chem. Soc.* 130, 9543–9549
26. Torre, P. *et al.* (2014) Multiphase water-in-oil emulsion droplets for cell-free transcription-translation. *Langmuir* 30, 5695–5699
27. Dora Tang, T.-Y. *et al.* (2015) In vitro gene expression within membrane-free coacervate protocells. *Chem. Commun.* 51, 11429–11432
28. Li, M. *et al.* (2011) In vitro gene expression and enzyme catalysis in bio-inorganic protocells. *Chem. Sci.* 2, 1739–1745
29. Calhoun, K.A. and Swartz, J.R. (2007) Energy systems for ATP regeneration in cell-free protein synthesis reactions. In *In Vitro Transcription and Translation Protocols* (Grandi, G., ed.), pp. 3–17, Humana Press
30. Otrin, L.C. *et al.* (2019) Artificial organelles for energy regeneration. *Adv. Biosyst.* 3, e1800323

31. Feng, X. *et al.* (2016) Coassembly of photosystem II and ATPase as artificial chloroplast for light-driven ATP synthesis. *ACS Nano* 10, 556–561
32. Kleineberg, C. *et al.* (2020) Light-driven ATP regeneration in diblock/grafted hybrid vesicles. *ChemBioChem* 21, 2149–2160
33. Altamura, E. *et al.* (2017) Highly oriented photosynthetic reaction centers generate a proton gradient in synthetic protocells. *Proc. Natl. Acad. Sci. U. S. A.* 114, 3837–3842
34. Chen, Z. *et al.* (2019) Light-gated synthetic protocells for plasmon-enhanced chemiosmotic gradient generation and ATP synthesis. *Angew. Chem. Int. Ed.* 58, 4896–4900
35. Wei, Z. *et al.* (2018) Thylakoid containing artificial cells for the inhibition investigation of light-driven electron transfer during photosynthesis. *ACS Synth. Biol.* 7, 945–951
36. Westensee, I. *et al.* (2021) Mitochondria encapsulation in hydrogel-based artificial cells as ATP producing subunits. *Small* 17, 2007959
37. Li, C. *et al.* (2022) Reversible deformation of artificial cell colonies triggered by actin polymerization for muscle behavior mimicry. *Adv. Mater.* 34, 2204039
38. Lin, S. *et al.* (2018) Bioinspired design of alcohol dehydrogenase@ nano TiO microreactors for sustainable cycling of NAD^+/NADH coenzyme. *Nanomaterials* 8, 127
39. Summers, D.P. and Rodoni, D. (2015) Vesicle encapsulation of a nonbiological photochemical system capable of reducing NAD(+) to NADH. *Langmuir* 31, 10633–10637
40. Wang, S. *et al.* (2017) Design and construction of artificial photoresponsive protocells capable of converting day light to chemical energy. *J. Mater. Chem. A* 5, 24612–24616
41. Berhanu, S. *et al.* (2019) Artificial photosynthetic cell producing energy for protein synthesis. *Nat. Commun.* 10, 1325
42. Godino, E. *et al.* (2019) De novo synthesized Min proteins drive oscillatory liposome deformation and regulate FtsA-FtsZ cytoskeletal patterns. *Nat. Commun.* 10, 4969
43. Nies, P. *et al.* (2018) Self-replication of DNA by its encoded proteins in liposome-based synthetic cells. *Nat. Commun.* 9, 1583
44. Kai, L. and Schwille, P. (2019) Cell-free protein synthesis and its perspectives for assembling cells from the bottom-up. *Adv. Biosyst.* 3, 1800322
45. Exterkate, M. *et al.* (2018) Growing membranes in vitro by continuous phospholipid biosynthesis from free fatty acids. *ACS Synth. Biol.* 7, 153–165
46. Otrin, L. *et al.* (2017) Toward artificial mitochondrion: mimicking oxidative phosphorylation in polymer and hybrid membranes. *Nano Lett.* 17, 6816–6821
47. Li, Y. *et al.* (2019) Supramolecularly assembled nanocomposites as biomimetic chloroplasts for enhancement of photophosphorylation. *Angew. Chem. Int. Ed.* 58, 796–800
48. Ma, B.C. *et al.* (2019) Polymer-based module for NAD^+ regeneration with visible light. *ChemBioChem* 20, 2593–2596
49. Wu, J. *et al.* (2019) Nanomaterials with enzyme-like characteristics (nanozymes): next-generation artificial enzymes (II). *Chem. Soc. Rev.* 48, 1004–1076
50. Lee, K.Y. *et al.* (2018) Photosynthetic artificial organelles sustain and control ATP-dependent reactions in a protocellular system. *Nat. Biotechnol.* 36, 530–535
51. Zhou, P. *et al.* (2016) Programmable modulation of membrane permeability of proteinosome upon multiple stimuli responses. *ACS Macro Lett.* 5, 961–966
52. Zheng, Y. *et al.* (2021) Ordered conformation-regulated vesicular membrane permeability. *Angew. Chem. Int. Ed.* 60, 22529–22536
53. Li, S. *et al.* (2019) Chemical signal communication between two protoorganelles in a lipid-based artficial cell. *Anal. Chem.* 91, 6859–6864
54. Elani, Y. *et al.* (2014) Vesicle-based artificial cells as chemical microreactors with spatially segregated reaction pathways. *Nat. Commun.* 5, 5305
55. Tang, T.-Y.D. *et al.* (2018) Gene-mediated chemical communication in synthetic protocell communities. *ACS Synth. Biol.* 7, 339–346
56. Love, C. *et al.* (2020) Reversible pH-responsive coacervate formation in lipid vesicles activates dormant enzymatic reactions. *Angew. Chem. Int. Ed.* 59, 5950–5957
57. Last, C. Mart *et al.* (2020) pH-controlled coacervate-membrane interactions within liposomes. *ACS Nano* 14, 4487–4498
58. Urban, P. *et al.* (2020) A lipid photoswitch controls fluidity in supported bilayer membranes. *Langmuir* 36, 2629–2634
59. Hindley, J.W. *et al.* (2018) (2018) Light-triggered enzymatic reactions in nested vesicle reactors. *Nat. Commun.* 9, 1093
60. Van Roekel, H.W.H. *et al.* (2015) Programmable chemical reaction networks: emulating regulatory functions in living cells using a bottom-up approach. *Chem. Soc. Rev.* 44, 7465–7483
61. Gines, G. *et al.* (2017) Microscopic agents programmed by DNA circuits. *Nat. Nanotechnol.* 12, 351–359
62. Semenov, S.N. *et al.* (2016) Autocatalytic, bistable, oscillatory networks of biologically relevant organic reactions. *Nature* 537, 656–660
63. Roszak, R. *et al.* (2019) Propagation of oscillating chemical signals through reaction networks. *Angew. Chem. Int. Ed.* 58, 4520–4525
64. Srinivas, N. *et al.* (2017) Enzyme-free nucleic acid dynamical systems. *Science* 358, eaal2052
65. Semenov, S.N. *et al.* (2015) Rational design of functional and tunable oscillating enzymatic networks. *Nat. Chem.* 7, 160–165
66. Lyu, Y. *et al.* (2020) Protocells programmed through artificial reaction networks. *Chem. Sci.* 11, 631–642
67. Wu, H. (2013) Higher-order assemblies in a new paradigm of signal transduction. *Cell* 153, 287–292
68. Thattai, M. and van Oudenaarden, A. (2002) Attenuation of noise in ultrasensitive signaling cascades. *Biophys. J.* 82, 2943–2950
69. Beneyton, T. *et al.* (2018) Out-of-equilibrium microcompartments for the bottom-up integration of metabolic functions. *Nat. Commun.* 9, 2391
70. Miller, T.E. *et al.* (2020) Light-powered CO_2 fixation in a chloroplast mimic with natural and synthetic parts. *Science* 368, 649–654
71. Lyu, Y. *et al.* (2018) Constructing smart protocells with built-in DNA computational core to eliminate exogenous challenge. *J. Am. Chem. Soc.* 140, 6912–6920
72. Liu, H. *et al.* (2019) Artificial signal feedback network mimicking cellular adaptivity. *J. Am. Chem. Soc.* 141, 6458–6461
73. Peng, R. *et al.* (2020) DNA-based artificial molecular signaling system that mimics basic elements of reception and response. *Nat. Commun.* 11, 978
74. Cress, B.F. *et al.* (2015) Sensitive cells: enabling tools for static and dynamic control of microbial metabolic pathways. *Curr. Opin. Biotechnol.* 36, 205–214
75. Holtz, W.J. and Keasling, J.D. (2010) Engineering static and dynamic control of synthetic pathways. *Cell* 140, 19–23
76. Erb, T.J. (2019) Structural organization of biocatalytic systems: the next dimension of synthetic metabolism. *Emerg. Top. Life Sci.* 3, 579–586
77. Maguire, O.R. and Huck, W.T.S. (2019) On the importance of reaction networks for synthetic living systems. *Emerg. Top. Life Sci.* 3, 517–527
78. Peters, R.J.R.W. *et al.* (2014) Cascade reactions in multicompartmentalized polymersomes. *Angew. Chem. Int. Ed.* 53, 146–150
79. Liu, X. *et al.* (2016) Hierarchical proteinosomes for programmed release of multiple components. *Angew. Chem. Int. Ed.* 55, 7095–7100
80. Epstein, I.R. and Showalter, K. (1996) Nonlinear chemical dynamics: oscillations, patterns, and chaos. *J. Phys. Chem.* 100, 13132–13147
81. Pols, T. *et al.* (2019) A synthetic metabolic network for physicochemical homeostasis. *Nat. Commun.* 10, 4239
82. Weitz, M. *et al.* (2014) Diversity in the dynamical behaviour of a compartmentalized programmable biochemical oscillator. *Nat. Chem.* 6, 295–302
83. Wang, X. *et al.* (2021) Spatiotemporal organization of coacervate microdroplets. *Curr. Opin. Colloid. In.* 52, 101420
84. Banani, S. *et al.* (2017) Biomolecular condensates: organizers of cellular biochemistry. *Nat. Rev. Mol. Cell Biol.* 18, 285–298
85. Shin, Y. and Brangwynne, C. (2017) Liquid phase condensation in cell physiology and disease. *Science* 357, eaaf4382
86. Booth, R. *et al.* (2019) Spatial positioning and chemical coupling in coacervate-in-proteinosome protocells. *Angew. Chem. Int. Ed.* 58, 9120–9124
87. Deshpande, S. *et al.* (2019) Spatiotemporal control of coacervate formation within liposomes. *Nat. Comm.* 10, 1800

88. Zhao, H. *et al.* (2020) Dynamic spatial formation and distribution of intrinsically disordered protein droplets in macromolecularly crowded protocells. *Angew. Chem. Int. Ed.* 59, 11028–11036
89. Li, J. *et al.* (2022) Programmable spatial organization of liquid-phase condensations. *Chem* 8, 784–800
90. Mu, W. *et al.* (2021) Membrane-confined liquid-liquid phase separation toward artificial organelles. *Sci. Adv.* 7, eabf9000
91. Yin, Z. *et al.* (2022) Spontaneous membranization in a silk-based coacervate protocell model. *Angew. Chem. Int. Ed.* 61, e202202302
92. Ng, W.-L. and Bassler, B.L. (2009) Bacterial quorum-sensing network architectures. *Annu. Rev. Genet.* 43, 197–222
93. Lentini, R. *et al.* (2014) Integrating artificial with natural cells to translate chemical messages that direct *E. coli* behaviour. *Nat. Commun.* 5, 4012
94. Gardner, P.M. *et al.* (2009) Sugar synthesis in a protocellular model leads to a cell signalling response in bacteria. *Nat. Chem.* 1, 377–383
95. Rampioni, G. *et al.* (2018) Synthetic cells produce a quorum sensing chemical signal perceived by *Pseudomonas aeruginosa*. *Chem. Commun.* 54, 2090–2093
96. Lentini, R. *et al.* (2017) Two-way chemical communication between artificial and natural cells. *ACS Cent. Sci.* 3, 117–123
97. Chen, C. *et al.* (2021) Construction of protocell-based artificial signal transduction pathways. *Chem. Commun.* 57, 12754–12763
98. Tian, L. *et al.* (2018) Nonequilibrium spatiotemporal sensing within acoustically patterned two-dimensional protocell arrays. *ACS Cent. Sci.* 4, 1551–1558
99. Tian, L. *et al.* (2019) Artificial morphogen-mediated differentiation in synthetic protocells. *Nat. Commun.* 10, 3321
100. Song, W. *et al.* (2009) Assembled capsules transportation driven by motor proteins. *Biochem. Biophys. Res. Commun.* 379, 175–178
101. Herold, C. *et al.* (2012) Long-range transport of giant vesicles along microtubule networks. *ChemPhysChem* 13, 1001–1006
102. Kumar, B.V.V.S.P. *et al.* (2018) Enzyme-powered motility in buoyant organoclay/DNA protocells. *Nat. Chem.* 10, 1154–1163