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Membrane functions genetically programmed in synthetic cells: A barrier to conquer

David Garenne and Vincent Noireaux[1]

Abstract

Integrating molecular components into active synthetic cells is arising as a major goal of bioengineering and, by and large, multidisciplinary research. The encapsulation of cell-free transcription–translation systems into liposomes stands out of the current approaches because it enables programming synthetic cells with genes, as in real living cells, at the relevant biological scale and within the natural physical boundary. Although recapitulating certain biological functions in such minimal settings has been successful, serious barriers are still hard to overcome. In particular, implementing biological functions located at the lipid membrane remains one of the most challenging elements in bottom-up synthetic cell engineering. In this work, we review the current state-of-the-art of the membrane functions that have been achieved by cell-free expression, either on supported lipid bilayers, in the presence of membranes added to reactions or inside liposomes. We extend the considerations to the membrane functions sought in other types of artificial compartments.

Addresses

University of Minnesota, Physics and Nanotechnology, 115 Union Street SE, Minneapolis, MN, 55455, USA

Corresponding author: Noireaux, Vincent (noireaux@umn.edu)
[1] https://orcid.org/0000-0002-5213-273X.

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Introduction

Cell-free DNA-directed synthetic biology is flourishing as a versatile method to construct biochemical systems *in vitro* [1–3]. Integrating biomolecular components from the ground up into active or responsive biological materials programmed by genetic circuits has proven a reliable approach to deepen our basic understanding of living systems [4,5], engineer biology for targeted applications [6] or create alternatives to natural systems for specific purposes [7]. As the cell-free synthetic biology landscape strengthens and broadens, still greater goals are envisioned. Building genetically programmed synthetic cells capable of recapitulating advanced biological functions geared toward the creation of a self-reproducing minimal cell has become a sensible objective of multidisciplinary research [8,9]. The availability of the molecules of life and techniques to assemble them into cell-sized compartments has rendered this endeavor rational, in addition to providing new experimental environments that extend the traditional grounds of the major fields, namely biology, chemistry, and physics. Along this road, the potential for devising truly serviceable biotechnologies are also many.

Cell-free transcription–translation (TXTL) is an ideal technology for such high-risk high-reward undertakings because it enables programming biochemical reactions with DNA in isolation without endogenous genomic background (the genomic DNA is removed during lysate preparation). Furthermore, carrying out TXTL reactions inside liposomes has become the archetype synthetic cell prototype [10] as it shelters gene expression at the relevant biological scale ($\sim$1–30 μm) within a closed phospholipid bilayer, which is the natural boundary of living cells. Although TXTL-based minimal cells are the central approach to synthetic cell research and the scope of this review, outstanding results have also been obtained for compartments that are not programmed genetically. The reconstitution of the Min oscillations in liposomes has provided a wealth of quantitative information about this system [11]. The construction of photosynthetic organelles from pure components is a spectacular achievement [12,13]. The demonstration that these protocellular systems are capable of driving the polymerization of a mechanically active network of actin filaments supports that DNA-less synthetic cells can house rather sophisticated biological functions. The lack of programmability, however, does not empower the full picture of a minimal cell that can, at some stages, reproduce sustainably.

On a broad perspective, encapsulating and executing genetic programs into cell-sized liposomes generally follows one of the following two intentions: (i) either addressing fundamental questions about life at the scale

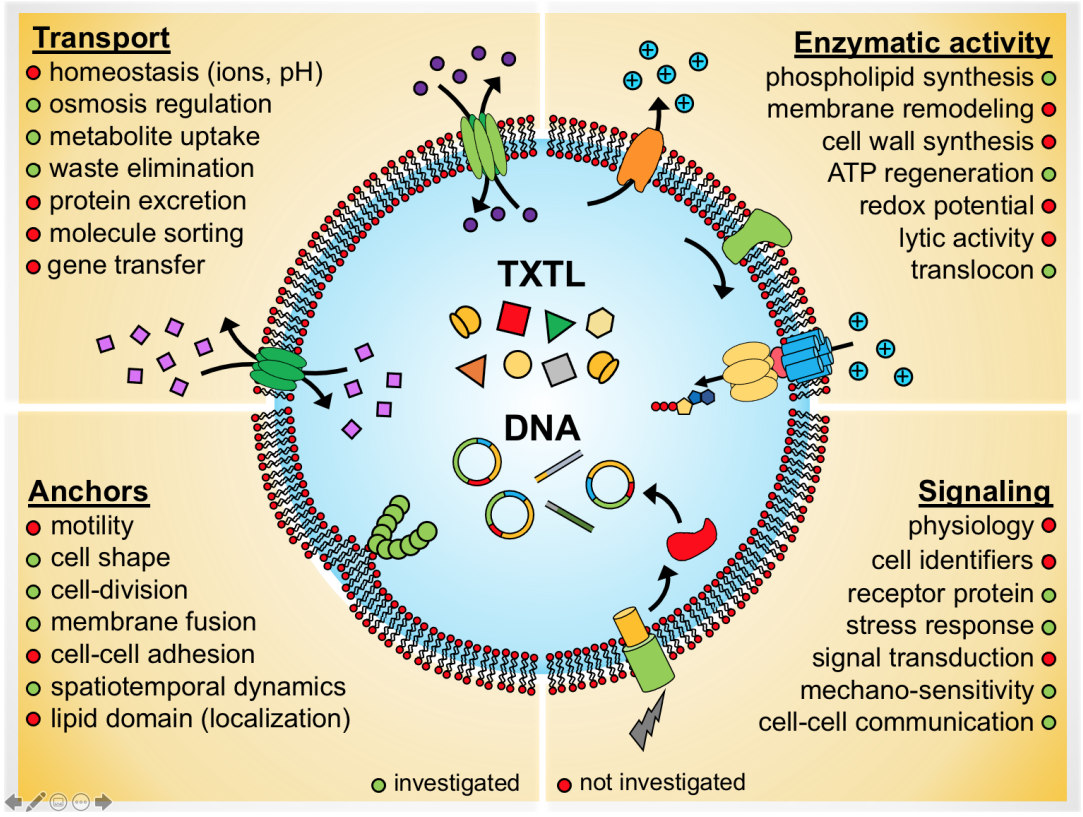
of a cell in a minimal setting, or (ii) working toward a specific application that tackles a societal problem related, for instance, to human health and the environment [3,14]. On the one hand, and as we shall see in this review, working in genetically programmed synthetic cells has already proven useful to gain basic knowledge, either on the biophysics or biology of cell membranes [15,16]. On the other hand, although no application has been demonstrated yet, the field has worked on developing the necessary steps toward truly functional synthetic cells. For instance, engineering gene circuits in TXTL that could serve as synthetic regulatory mechanisms to guide the expression of biological functions in cell-sized compartments has been one of the major foci [17–21]. This particular emphasis is due to several technical advantages: encapsulation or lipid membranes are not necessary, assembling circuits is fast, and in test tubes, one can use high-throughput methods to prototype hundreds of gene circuits simultaneously in one day [18,22]. Constructing biological functions that necessitate a lipid membrane, however, is far more complicated and slower because no high-throughput methods exist to perform fast engineering and screening of rather large parameter spaces. Although expressing membrane proteins (MPs) in batch mode reactions is well established [23], programming and achieving membrane functions in synthetic cells, such as sensing and transport, is still a major bottleneck.

In a bacterium like *Escherichia coli*, the insertion of MPs into the membrane is driven by interactions between ribosomes, membrane-embedded translocons and some chaperonins [24]. Interestingly, insertion of cell-free synthetized MPs in lipid bilayers does not require such complex molecular machineries and interactions [25]. Even in this simplified context, however, developing, by cell-free expression (CFE) only, a lipid membrane interface that hosts proteins reproducing elaborate biological functions is challenging for several other reasons. First, characterizing the activity of MPs is not an easy task. The activity of cell-free expressed MPs is hard to truly assess in many cases because developing rigorous assays that exceed simple reporter protein measurements is not straightforward. Second, it is not clear how the activity of MPs expressed in a cell-free system depends on the TXTL platform used and, more importantly, on the liposome method used. This aspect is still controversial. Third, little is known about the relationship between the biochemical and biophysical properties of lipid membranes and the activity of MPs expressed in TXTL. Recent CFE studies are addressing such lack of knowledge [26]. Yet, the properties of lipid membranes have not been extensively exploited to create sophisticated active membrane functions in synthetic cells. Fourth, the development of an active membrane depends also on the properties of the external environment, an aspect that has been poorly studied as the current minimal cells are developed in ideal laboratory conditions far from natural habitats. Taken altogether, the bottom-up construction of minimal cells programmed genetically for integrating advanced membrane functionalities remains a major challenge (Figure 1). In this article, we review the progress made recently in this area. Our discussion includes membrane functions developed by CFE only, either entailing MPs or extrinsic MPs (EMPs) and for three cases: on supported lipid bilayer, with membranes added to a TXTL reaction or inside liposomes. Our review categorizes membrane functions into four groups: (i) transport, either passive or active; (ii) enzymatic activity; (iii) anchors; and (iv) signaling (Figure 1; Table 1). On a technical aspect, CFE systems, especially the bacterial ones, have proven effective for expressing MPs [27,28], including the protein synthesis using recombinant elements (PURE) system [29]. The advantage of lysate-based CFE systems for producing MPs is the strength of expression (a few mg/ml in batch mode), which is 10–20 times greater than the PURE system (0.1–0.5 mg/ml in batch mode).

Synthesis of MPs in batch mode reactions

The expression of MPs in batch mode TXTL reactions has been extensively studied, although some aspects of this process are still not well understood. The utilization of surfactants was the first method developed to synthesize MPs *in vitro* [30,31]. This method, however, does not reproduce the natural environment of MPs (e.g. lipid composition, inside and outside of a compartment). Remarkably, translocons or chaperons are not necessary to synthesize some MPs in the presence of lipid membranes added to a TXTL reaction as the co-translational association of MPs with bilayers is sufficient [25]. However, the amount of MPs produced varies a lot depending on the type of lipid membrane supplied to the reaction. Surprisingly, the addition of liposomes to CFE reactions does not allow the synthesis of MPs at high yield (not more than a few μM). The reasons why this method is not effective are not well understood, the instability of the liposomes in TXTL reactions being one of the problems [25]. The major improvement to MPs synthesis in test tubes was the invention of nanodiscs, which are soluble lipid rafts of diameter 10–20 nm stabilized by proteins. Nanodiscs are now used as the main tool for the cell-free synthesis of MPs in batch mode [32,33]. Nanodiscs have proven to be useful for the study of membrane transporters [34], MPs involved in enzymatic pathways [32], and signaling MPs such as GPCR (G protein-coupled receptor) [35]. Although nanodiscs enable many applications, they do not offer a true minimal cell setting with an outside environment and a closed compartment, and they remain expensive for routine experiments. Besides, the complexity of the phospholipid mixtures that can be reproduced by nanodiscs is rather

Figure 1



Schematic of the basic membrane functions sorted in four categories, and integrated in a synthetic cell system programmed genetically with a TXTL system. Note that other functions such as cell–cell recognition and intercellular joining are not necessary in the first place for synthetic cells and thus are not shown in this figure.

limited at this point (up to two different lipids can be mixed together) [33].

Molecular transport functions across the membrane

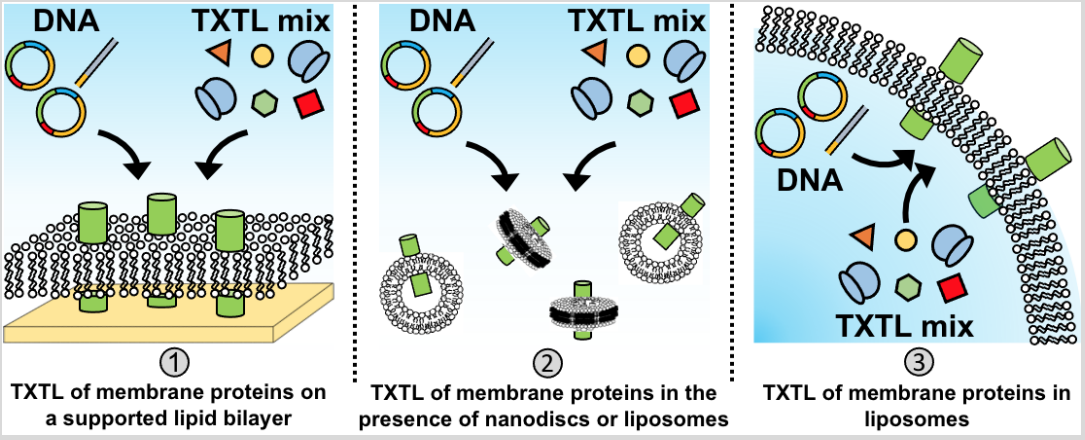
Although expressing membrane channels (passive and nonselective transport) or membrane transporters (active and selective transport) in a cell-free reaction supplied with liposomes is not very efficient, the DNA-directed synthesis of membrane channels or transporters into liposomes is effective and has been achieved for several proteins. The dynamical synthesis of the alpha-hemolysin channel, for instance, creates a positive feedback loop that enables feeding the cytoplasm of minimal cells with building blocks from the outside and eliminating TXTL reaction byproducts from the inside, thus extending CFE inside liposomes to one day or more [36,37]. In a comparable setting, the synthesis of the *E. coli* multidrug channel EmrE into liposomes [38,39] shows that specific molecular transporters can be produced. The CFE of specific transporter genes is still at the very beginning and much of the work has to be done to show that synthetic cells can integrate several of them. Three transport functions have not been addressed or attempted so far in a TXTL-based synthetic cell setting: protein excretion, molecule sorting, and gene transfer (Figure 1). On a broader scale, homeostasis, which certainly involves many different MPs with different functions, has not been truly tackled too. Different from a minimal cell setting, the CFE of MPs on supported lipid bilayers is a practical approach to developing membrane functions. The cell-free synthesis of the potassium channel KcsA on a lipid bilayer has proven useful to characterize the binding of the tetramer to lipid bilayers [40]. Although not critical in the early development of genetically programmed synthetic cells, the control of the orientation of the molecular transporters could be a bottleneck at some stages [41] (Table 2).

Signaling functions through the membrane

Signaling functions are essential to creating minimal cells capable of receiving and responding to external signals and stresses, and thus capable of gradually embodying a 'self.' At the technical level, the cell-free synthesis and incorporation of several active GPCRs into lipid membranes is a milestone [41,42], even if the whole repertoire of GPCRs is far from being explored. The fact that one can synthesize complex receptors

Table 1

Membrane functions in TXTL in the presence of lipid membranes.



DNA program	Mechanism, process, and results	#	Ref.
TRANSPORT			
• Linear DNA: 0.3 kbp 1 gene,1 promoter	Synthesis of EmrE transporter in liposomes (**POPC liposomes, PURE system, T7 promoter, western blot analysis**)	3	[39]
• Plasmids: 0.5 kbp 1 gene, 1 promoter	Cell-free synthesis and activity of KscA **(on-chip, DMPC/DMPG/biotin labelled PE, *E. coli* TXTL, *E. coli* promoter, SPR response**)	1	[40]
SIGNALING			
• Plasmids: 0.5 kbp 1 gene, 1 promoter	Intercommunication between synthetic cell and *P. aeruginosa* (**POPC liposomes, PURE system, T7 promoter, Firefly Luciferase)**	3	[44]
• Plasmids: 3 kbp 4 promoters, 4 genes	Cell-free synthesis of MreB in response of mechanical stress (**DOPC/DOPE/ cholesterol liposomes, *E. coli* TXTL, *E. coli* and T7 promoter, eGFP**)	3	[45]
• Linear DNA: 1–5 kbp 2 genes, 2 promoters	Intercommunication between synthetic cell and *E. coli* (**Test tubes, POPC cholesterol liposomes, *E. coli* TXTL, T7 promoter, alpha-hemolysin**)	3	[43]
ENZYMATIC ACTIVITY			
• Plasmids: 1 kbp 1 gene,1 promoter	Analysis of MraY enzymatic activity in nanodiscs (**Test tube, DMPC/DMPG nanodiscs, *E. coli* TXTL, T7 promoter, western blot analysis**)	2	[32]
• Linear DNA: 1 kbp 1 gene,1 promoter	GFP synthesis with energy from ATP synthase activity (**POPC/Cholesterol/ PE-PEG2000 liposomes, PURE system, T7 promoter, GFP**)	2/3	[47]
• Linear DNA: 1–2 kbp 3–5 genes, 3–5 promoters	Reconstitution of the Sec translocon in liposomes (**POPC liposomes or PC liposomes, PURE system, T7 promoter, FACS/Western blot)**	2/3	[50] [51]
• Plasmids: 8 kbp 9 genes, 3 promoters	Synthesis of PE and PG in liposomes **(DOPC/DOPE/DOPG/CL/DSPE-PEG-biotin liposomes, PURE system, T7/SP6 promoters, GFP**)	3	[16]
ANCHOR			
• Linear DNA: 1 kbp 2 genes, 2 promoters	Synthesis of Min proteins that regulate FtsZ patterns (**on chip, DOPC/ DOPE/DOPG/CL/PE-PEG-biotin, PURE system, T7 promoter, GFP**)	1/3	[53]
• Plasmids: 3 kbp 3 genes, 2 promoters	Deformation of liposomes by MreB from spheres to rods, membrane crowding (**Egg PC/PE-PEG5000 liposomes, *E. coli* TXTL, *E. coli* promoters, deGFP**)	3	[15]
• Linear DNA: 3 kbp 3 genes,3 promoters	Synthesis of cell division protein FtsZ, FtsA and ZipA (**POPC/POPG/Rhod-DOPE liposomes, PURE system, T7 promoter, sfGFP**)	3	[52]
• Plasmids: 3 kbp 1 gene, 1 promoter	Expression of Cldn2 in polymersomes (**on-chip, PBd-PeO polymersomes, wheat germ TXTL, T7 promoter, SPR response**)	1/2	[57]

The **left column** includes information about the type DNA (linear dsDNA or plasmid), the total length of the coding sequences composing the DNA program (promoters, operators, UTRs, genes, terminators), the number of genes and promoters. Ranked by size of the DNA program in each of the three sections. The **column in the middle** provides a short description of the work, including reaction setup, type of TXTL, type of promoters, and reporter used. The **third column** labeled as # shows the type of experiment 1, 2, or 3 with respect to the three schematics above.

from mammalian cells is also noteworthy. On a more conceptual level, the communication between synthetic cells, programmed for the biosynthesis of chemotactic compounds, and living cells is also an important step toward making liposomes as signal emitters [43,44]. Interestingly, such communication between liposomes and bacteria is achieved via the biosynthesis of chemicals that diffuses passively through the membrane without the need for a membrane channel. Conversely, the demonstration that

Table 2

Examples of limitations and possible extensions.

Current limitations and challenges	Potential improvements and extensions
TRANSPORT	
• **Transport of molecules is generally not specific (e.g. alpha-hemolysin, MscL).**	• **Report the basic properties and characteristics (strength, limitations) of TXTL system used.**
• Excretion of macromolecules (exocytosis process) like proteins larger than a pore has not been achieved yet.	• Develop quantitative and high-throughput methods to express membrane proteins in cell-sized liposomes.
• Synthesis of membrane proteins in TXTL reactions supplemented with liposomes is low compared to using nanodiscs.	• Combine membrane protein activity assay (e.g. leak) with physical measurement (e.g. circular dichroism) to couple protein activity with folding.
• Orientation of proteins in the membrane is usually not investigated which could be a problem with active transporters.	• Demonstrate that one can synthesize different transporters specifics to different chemicals to achieve transport multiplexing.
• Minimal cells are far from recapitulating the complexity of protein composition found in living cell membranes.	• Investigate the physical properties of artificial membranes or interfaces (e.g. diblock copolymers, coacervates, liquid phases, hydrogels) to figure out how membrane proteins could be well folded and functional.
• Activity of pores in compartment other than liposomes has not been clearly demonstrated yet.	
SIGNALING	
• **Sensing demonstrated so far is specific to a few types of chemical and mechanical stresses and based on one or two proteins.**	• **Develop minimal cells capable of processing different types of signals with a proper response for each different signal to achieve signal multiplexing.**
• Response is generally simple (expression of a reporter) and not useful for potential applications.	• Develop system of sensing to detect hazardous chemicals in the medium.
ENZYMATIC ACTIVITY	
• **Synthesizing complex cascades involving several different proteins is still problematic. The strength of most of the cell-free systems is not large enough to express complex enzymatic cascades. The purification of proteins is required.**	• **Assemble larger DNA programs that include multiple genes (minigenome) to optimize the expression of complex cascades.**
• The insertion of membrane proteins in lipid bilayers is still not optimal in all synthetic cell experiments.	• Develop assays to quantify the activity of all the proteins involved in enzymatic cascades (e.g. lipid synthesis).
• Expression, folding and integration of quaternary superstructures like ATP synthase in membranes still constitute a huge bottleneck.	• Be more quantitative about enzymatic activities.
	• Report TXTL composition (by mass spectrometry) to continue optimizing the performances of TXTL.
	• Develop a TXTL with chaperones proteins to help the folding and the insertion of proteins in the membrane.
ANCHOR	
• **Comprehensive studies of the physical properties of the compartment (e.g. size) and of membranes for cytoskeletal processes (e.g. division, shape) are still lacking.**	• **Develop a better understanding of the biophysical parameters for cytoskeleton functions (volume to surface ratio, surface tension, curvature, osmotic pressure).**
• The complexity of the membrane composition, which is a crucial parameter for membranes functions, is still far from the ones found in living cells.	• Develop new methods to control precisely the composition and the complexity of lipid membranes.
• Reconstruction of proteins with the right topology.	• Broaden the studies on molecular crowding at the membrane to understand the extent of this effect on other self-assembly processes at the membrane.
• Complex processes like division involve many proteins (>10 proteins) in living cells, but the methodology used so far for synthetic cells is based on 2 or 3 proteins only.	• Develop high-throughput methods (e.g. microfluidic) to test membrane anchor functions.

The **left column** lists some of the current limitations and challenges in membrane functions achieved by cell-free expression, also including some missing capabilities. The **right column** lists some of the possible improvements and extensions to TXTL that would improve developing membrane functions in synthetic cells. In each column and section, one bullet is highlighted as one of the most critical features.

TXTL-based minimal cells can adjust the tension of the lipid membrane in response to osmotic stress via the expression of the mechanosensitive channel MscL is also a preliminary step toward more sophisticated signaling systems [45,46]. In this experiment, the synthetic cell subsequently integrates a chemical signal through the MscL channel to induce the synthesis of the cytoskeleton protein MreB that self-assembles at the inner membrane to increase the mechanical robustness of the liposome. So far, the signaling functions built on MPs have been programmed with simple circuits composed of one or two genes. Enabling minimal cells to process multiple signals simultaneously is still far in the future. The development of efficient gene circuit regulations seems necessary to program and integrate such functions.

Membrane enzymatic activity

Two enzymatic pathways have been the focus of interest in recent years. First, encouraging steps toward light-driven regeneration of ATP have been accomplished by constructing a photosynthetic organelle composed of the ATP synthase F_0F_1 and the proton pump Bacteriorhodopsin, which emulates the proton motive force. When these organelles are added to a TXTL reaction (PURE system), the regenerated ATP fuels the synthesis of the reporter protein GFP inside liposomes [47]. In a similar setup, coupling the respiratory mitochondrial complex I, the F-type ATP synthase and the alternative oxidase into proteoliposomes produces a minimal organelle that emulates the energy-converting catalytic reactions of the mitochondrial respiratory chain: NADH oxidation, ubiquinone cycling, oxygen reduction, proton pumping, and ATP synthesis [48]. This ATP regeneration system fuels the cell-free synthesis of the reporter protein SEpHluorin inside the small liposomes. In these works, the enzymes were either purified or pre-expressed because CFE was not strong enough to carry out the production of all the proteins in the pathways (Table 2). In any case, the demonstration that synthetic cells can be equipped with a photosynthetic energy regeneration system is an important step forward. Such a physical energy regeneration system, based on light, appears to be more autonomous and versatile than the conventional chemical ATP regeneration pathways, which require harvesting nutrients, such as carbon sources, from the environment. The synthesis of phospholipids is the second pathway that has been the center of attention, with significant advances made recently. The *in vitro* production of phospholipids was achieved in a batch mode reaction comprised of seven purified enzymes from *E. coli* and two building blocks (glycerol 3-phosphate and acyl-CoA), providing a lot of information about this pathway and the conditions to accomplish phospholipid synthesis and self-assembly into liposomes [49]. This work was recently adapted into a TXTL-based minimal cell in which the enzymes are expressed into liposomes and the synthesis of different lipids observed and quantified [16]. The physical growth of the liposomes, however, is not achieved. The assembly of five genes into a minigenome is an original and promising approach to optimize the synthesis of complex enzymatic pathways. Although not necessary to cell-free synthesize active MPs at biologically relevant levels, the *E. coli* Sec translocon was fully produced in operative form using the PURE system, which could facilitate developing multiple and complex membrane functions in minimal cells [50,51].

Membrane anchors

Essential biological functions are also carried out by EMPs. EMPs are critical for molecular mechanisms involved in processes like cell shape and cell division, for instance. Several recent studies in this area have delivered concrete technical advances and fundamental understanding of the *E. coli* cytoskeleton, bringing minimal cells closer and closer to division. Membrane deformations are observed when ZipA, FtsA, and FtsZ (proteins involved in the division ring formation) are coexpressed inside liposomes [52]. In a comparable setting, FtsZ self-assembles into rings capable of pinching the membrane of liposomes without full division [53]. The oscillations produced by the Min system, involved in centering the FtsZ fission ring, were recently reconstituted in liposomes [54] using the PURE system too. The importance of the osmotic pressure settings to soften the membrane has yet to be clarified in these experiments though. In both studies, the strength of the CFE system (PURE system) does not permit the synthesis of all the proteins involved in the oscillations or the minimal cytoskeleton assumed to be sufficient to achieve cell division. Hybrid methods including both purified and dynamically produced proteins are necessary to reach these phenotypes making the approach to minimal cells less programmable. Major advances in installing a mechanically active MreB cytoskeleton into minimal cells have also been made recently. When synthesized in lipid vesicles, MreB alone self-assemble spontaneously into a sturdy cytoskeleton filament network at the inner membrane that deforms the spherical liposomes into rods looking like *E. coli* [15]. As importantly, this phenotype is only observed when molecular crowding, emulated with PEG-lipids, is present at the membrane. A consecutive study demonstrates that the stimulation of MreB self-assembly by two-dimensional membrane crowding is independent of the type of macromolecule attached to the lipid bilayer [55]. Taken as a whole, it is the comprehension of the biophysics that seems to be critical for building genetically programmed synthetic cells capable of developing active cytoskeletal functions. Other biochemical and biophysical parameters, such as the membrane fluidity and the lipid composition, have not been thoroughly investigated and could be critical for cytoskeletal functions anchored at the lipid bilayer. Motility and cell−cell adhesion functions have not been investigated yet in minimal cells and seem to be a distant goal (Figure 1).

Membrane functions in artificial cells

By definition, artificial cells incorporate non-natural components in their structure as opposed to minimal cells built from natural biomolecules [9]. Several artificial cell systems can integrate MPs when they are dynamically synthesized in a CFE system. Polymersomes, specifically, are promising cell-sized compartments for applications because they are mechanically more robust than liposomes [56] and thus could be deployed in natural environments in the near future. The CFE of an MP and its direct integration into a polybutadiene-polyethylene oxide (PBD-b-PEO) membrane paved the way toward functionalizing polymersomes [57]. The thickness of PBD-b-PEO bilayers,

however, is typically larger than lipidic membranes (~4–5 nm), which somewhat limits this approach. An alternative to pure polymersomes is a hybrid artificial cell that incorporates both PBD-b-PEO block copolymers and natural lipids. Such a composite membrane can host MPs like MscL, although the activity of MscL is not fully determined in those hybrid compartments [58]. Microfluidic chips are the other prevalent technologies to make artificial cells. Microfluidic technologies offer almost limitless possibilities to fabricate compartments of any size and shape, in addition to making large arrays of cell-sized chambers. The recent demonstration that microfluidic chip chambers hosting TXTL reactions can be closed with phospholipid bilayers is an interesting step toward the high-throughput development and characterization of membrane functions [59]. Although programming polymersomes or microfluidic chips genetically toward sustainable self-replication seems hardly conceivable at this time, these artificial cell systems have true technological potential in biotechnologies and medicine. Lastly, another potential artificial cell system is the one based on protein shells, in which the membrane functions are achieved by the proteins making the shell. Certain classes of peptides like ELPs (elastin-like peptide) have properties prone to make such synthetic cells [60]. Natural bacterial microcompartments could also be exploited to make lipid-free genetically programmable artificial cells with new properties and tunable molecular permeability [61,62].

Concluding remarks

The membrane of bottom-up engineered synthetic cells comes forth as a critical component in the early stages of development. To rapidly enable necessary gain of functions, such as physical robustness, biosynthesis of the membrane, energy regeneration, or metabolite uptake, DNA-directed minimal cells must synthesize MPs and EMPs to acquire basic capabilities at or through the bilayer, whether active, passive, mechanical, or enzymatic. Efforts toward integrating essential functionalities are hampered by the difficulty of characterizing membrane functions, which necessitates quantitative approaches and techniques far beyond the standard fluorescent reporter assays (Table 2). Working with lipid membranes and CFE systems renders this process rather slow, and so the progression. Nonetheless, as covered in this review, quantitative works approaching this goal are emerging [15,47,54]. Novel approaches and ideas are necessary to alleviate the challenges specific to these studies (Table 2). In particular, the strength of CFE is often the limiting factor toward true membrane function acquisition in synthetic cells. In return, the gain is potentially considerable. On the one hand, the minimal cell setting will facilitate delivering new fundamental information on membrane functions, also relevant to living cells. On the other hand, making genetically programmed synthetic cells acquire a truly functional membrane is a prerequisite to using them for practical applications.

Conflict of interest statement

Nothing declared.

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