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Perspective

Membrane Mimetic Chemistry in Artificial Cells

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ABSTRACT: Lipid membranes in cells are fluid structures that undergo constant synthesis, remodeling, fission, and fusion. The dynamic nature of lipid membranes enables their use as adaptive compartments, making them indispensable for all life on Earth. Efforts to create life-like artificial cells will likely involve mimicking the structure and function of lipid membranes to recapitulate fundamental cellular processes such as growth and division. As such, there is considerable interest in chemistry that mimics the functional properties of membranes, with the express intent of recapitulating biological phenomena. We suggest expanding the definition of membrane mimetic chemistry to capture these efforts. In this Perspective, we discuss how membrane mimetic chemistry serves the development of artificial cells. By leveraging recent advances in chemical biology and systems chemistry, we have an opportunity to use simplified chemical and biochemical systems to mimic the remarkable properties of living membranes.

INTRODUCTION

The development of artificial cells is a principal task of "bottom-up" synthetic biology, which aims to build biomimetic systems using non-living precursors.[1] The effort to reproduce life-like characteristics synthetically will lead to an improved understanding of biological processes and development of next-generation tools in biotechnology and pharmaceuticals.[2] As living cells are extraordinarily complex, the term *artificial cell* has generally been applied to systems that mimic a small subset of the characteristics of living cells—consequently, systems that use this term can vary quite significantly in their properties and may not obviously resemble living organisms. While advances in recombinant transcription/translation systems have led to a surge of interest in cell-free synthetic biology,[3−5] biomimetic systems that lack defined compartments have significant disadvantages. Compartmentalization, or physical separation between and within cells, is an attribute of all known organisms. Compartmentalization appears to serve many purposes in living cells, and its ubiquity suggests that it is of critical importance to life. Crucially, compartmentalization is essential for self-replicating systems to maintain or increase their complexity at the expense of free energy.[6] We observe that, among other things, cellular compartments separate incompatible chemical reactions, house and protect genetic information, increase reaction rates by concentrating substrates and enzymes, and maintain electrochemical gradients for metabolic processes.[7] These functions are key to cellular energy homeostasis, growth, and proliferation. Therefore, systems that attempt to replicate these fundamental biological processes in artificial cells will require the development of synthetic methods for compartmentalization.[8]

Researchers attempting to develop artificial cells will undoubtedly look to biology for inspiration in the effort to reproduce the characteristics of life. While membraneless and protein-exclusive microcompartments have been observed in many organisms, all life forms are defined by compartments consisting of phospholipid bilayer membranes.[7,9,10] Because living cells rely disproportionately on phospholipid membranes for compartmentalization, synthetic biologists have frequently used lipid-based vesicles in synthetic cells.[11] Typically, the lipid vesicles are used as stable compartments that ensure encapsulated material does not disperse, promoting colocalization of reactants. Cell membranes, conversely, are different than simple reconstituted lipid vesicles in significant ways—for instance, they are dynamic, constantly changing in chemical composition. The development of lipid membranes that more closely resemble living membranes is therefore critical to research in bottom-up synthetic biology and the development of artificial cells. Because the task of constructing life-like membranes requires the formation of self-assembling molecules from chemical building blocks, we believe there exists an opportunity to utilize biomimetic chemistry to create artificial membranes that more faithfully mimic the structure and function of living membranes.

Chemistry involving biomimetic membranes is not a new endeavor. The term *membrane mimetic chemistry* was popularized by Janos Fendler in the 1980s.[12] Membrane mimetic chemistry, as described by Fendler, is the effort to utilize lipid nanostructures (e.g., membranes, micelles, vesicles, etc.) to facilitate chemical reactions. Membranes were used to compartmentalize chemistry and template reactions through self-assembly and reagent colocalization.[13] While parallels were drawn to membrane biology (for instance, the role of membranes in photosynthesis),[14] mimicking biological phenomena (division, growth, transport) was not the explicit

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objective. Conversely, chemists interested in building an artificial cell are attempting to *construct* lipid-based compartments that faithfully emulate the properties of living membranes.[15] Given the modern understanding of biological membranes and synthetic chemistry, we suggest a revival of membrane mimetic chemistry with an emphasis on the development of functional artificial cell membranes with life-like properties. Approaches using minimal chemical and/or biochemical elements to mimic cell membranes can be considered. Pursuing membrane mimetic chemistry will greatly advance our understanding of fundamental lipid chemistry, improve our ability to generate biomimetic membranes, and accelerate applications using lipid-based tools.[2]

We believe that membrane mimetic chemistry is an important cornerstone of artificial cell research. While the development of an artificial cell may require reconstituting existing biological macromolecules in liposomes, we hope to emphasize that an artificial cell membrane in principle does not need to contain any material derived from living organisms. Drawing inspiration from origin-of-life research, we suggest that it may be possible to mimic many existing cellular processes using minimal chemical systems that do not obviously resemble their living counterparts.[15] Here we discuss recent advances in membrane mimetic chemistry with respect to properties of phospholipid membranes that we view as fundamental to biomimetic compartments. We discuss methods for the generation and growth of lipid vesicles. As a key property of cells is their ability to reproduce, we then turn our attention to controlled division of artificial membranes. While phospholipid membranes act as barriers, an artificial cell will need to be able to control the exchange of material with the surrounding environment. We comment on methods for controlling membrane permeability to small molecules and ions. Finally, we examine lipid mesophases as a potential alternative to lamellar vesicles for compartmentalization and mimicry of membrane-bound organelle function. Simultaneously, we also look to the near future in identifying unmet needs and potential opportunities, with an aim to inspire research efforts in this emerging area.

MEMBRANE GENERATION AND GROWTH

In the effort to generate life-like membranes in the laboratory, it is helpful to consider the origin of living cell membranes. The current dogma in biology is that all living membranes originate from pre-existing membranes.[16] This is supported by the fact that diacyl phospholipids are generally synthesized in living organisms by membrane-bound acyltransferases—hence, the reactions that produce membrane-forming monomers can only occur within membranes themselves. If this principle of membrane continuity holds true, it suggests that all living phospholipid membranes are descendants of an ancient lipid membrane. The circumstances that led to the genesis of this primordial membrane are obscured by billions of years of evolution, but several possibilities for its origin have been suggested. One hypothesis is that modern cell membranes arose gradually from more primitive cells that possessed membranes composed of simpler amphiphiles.[17] This possibility has prompted researchers to consider alternative membrane-forming amphiphiles and question whether phospholipids are in fact necessary for the assembly of life-like lipid-based membranes.[15]

Several researchers have theorized that the first biological membranes were composed primarily of fatty acids.[18−21] The abiotic generation of fatty acids has been deemed more prebiotically plausible than the formation of diacyl phospholipids. Indeed, fatty acids readily form vesicles, can be formed in Fischer−Tropsch-like reactions, and have even been found on chondraceous meteorites.[22,23] Additionally, fatty acid vesicles have a key advantage over phospholipid vesicles: growth can be induced in fatty acid vesicles by simple addition of monomers (Figure 1A,E).[24] In a seminal study, the Szostak lab observed that addition of fatty acid micelles to multi-lamellar vesicles triggered vesicle growth by the formation of tubular structures.[24] The Szostak group has also demonstrated that fatty acid vesicles can grow due to the osmotic pressure changes that take place upon encapsulation of high concentrations of negatively charged

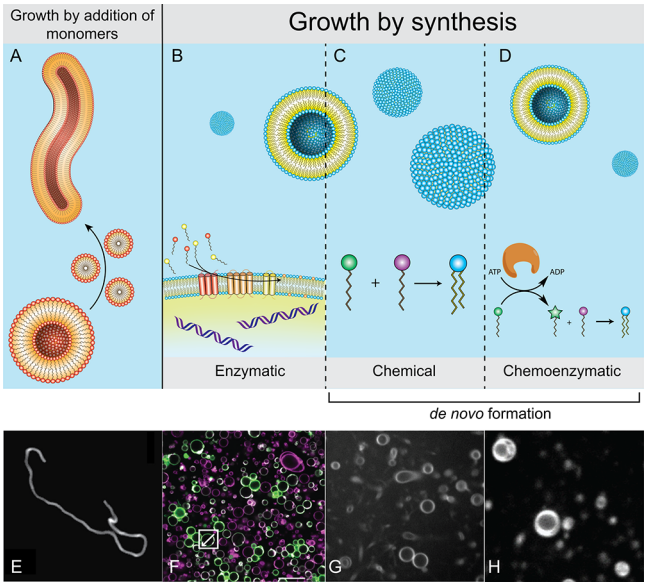

Figure 1. Growth in artificial cell membranes. (A−D) Schematic representations of methods for growth in artificial cell membranes: (A) Growth can be induced in fatty acid vesicles by introducing fatty acid micelles to the extravesicular environment. (B) Synthesis of new phospholipids through reconstitution of membrane-bound enzymes. (C) Formation and growth of membranes by *in situ* chemical synthesis of phospholipids. (D) Formation and growth of membranes through enzymatic activation of lipid precursors followed by chemical coupling. (C, D) Representation of methods for forming phospholipid membranes without the need for pre-existing membranes (*de novo* formation). (E−H) Images of membrane expansion by the methods represented in panels A−D: (E) Growth and tubulation of a fatty acid vesicle containing RNA upon addition of 5 equiv of oleate micelles. [Adapted from ref 24. Copyright 2009 American Chemical Society.] (F) Enzymatic production of membrane-forming phospholipids. Vesicles encapsulate a cell-free expression system and a plasmid encoding phospholipid synthesis enzymes. DOPS is then produced enzymatically when vesicles are supplied with oleoyl-CoA. The phosphatidylserine-binding protein LactC2-eGFP binds to vesicles that successfully produced DOPS, staining such membranes in green. [Adapted with permission from ref 31. Copyright 2020, licensed under CC BY 4.0.] (G) Formation of phospholipid membranes by non-enzymatic synthesis. Esterification of lysophospholipids by synthetic acyl thioesters is carried out in water, leading to *in situ* self-assembly of natural phospholipid vesicles as described in ref 38. (H) Formation of vesicles by chemoenzymatic synthesis. The soluble enzyme FadD10 is used to form fatty acyl adenylates, which react with amine-functionalized lysolipids to yield membrane-forming phospholipids that spontaneously self-assemble in water to form lamellar vesicles. [Adapted with permission from ref 39. Copyright 2020, licensed under CC BY 4.0.]

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biopolymers like RNA.[25] Such simple physical phenomena may explain the emergence of Darwinian evolution among protocells encapsulating ribozymes. Vesicles formed from fatty acids have also been shown to support some simple biochemical reactions, including ribozyme-catalyzed reactions.[26] Facile methods for inducing growth have made fatty acid vesicles important for origin-of-life research and may make them attractive platforms for some synthetic biology applications.

Fatty acid vesicles, however, are not generally capable of harboring many of the reactions that are typically associated with existing life. They suffer from an intolerance to divalent cations, are unable to support proton gradients, and can inhibit the action of polymerases.[15] Furthermore, fatty acids tend to have very high critical vesicle concentrations (e.g., vesicles form from decanoic acid only above 40 mM fatty acid) and show limited pH stability. Some of these issues can be alleviated by using vesicles composed of mixtures of single-chain amphiphiles. For example, it has been shown that low concentrations of long-chain fatty acids can significantly lower the concentration at which vesicles form from short-chain fatty acids.[27] The inclusion of fatty alcohols can also stabilize fatty acid vesicles to pH changes.[20] It remains the case, however, that because many enzymes are incompatible with high concentrations of simple amphiphiles, fatty acid vesicles are unlikely to be adequate platforms for artificial cells that host complex enzyme-dependent reactions.

Because fatty acid vesicles are incompatible with many biochemical reactions, it has been theorized that mixed fatty acid and phospholipid membranes may have represented an intermediate stage in the evolution of modern membranes.[28] Budin et al. have demonstrated that fatty acid vesicles containing a percentage of phospholipid will grow when vesicles containing a higher ratio of fatty acid are added to the surrounding medium.[17] That is, hybrid phospholipid/fatty acid vesicles can accumulate additional fatty acid amphiphiles at the expense of pure fatty acid vesicles, constituting a form of lipid competition between vesicle species. The result suggests that the ability to generate dialkyl lipids would have conferred a selective advantage on protocellular membranes.[28] By coupling recent advances in the generation of phospholipids either chemically or enzymatically, it may be possible to create vesicles that can generate phospholipids catalytically and grow at the expense of vesicles that are unable to generate phospholipids.

While origin-of-life researchers have focused their attention on membranes consisting of single-chain amphiphiles due to prebiotic plausibility, synthetic biologists tend to employ vesicles composed mainly of phospholipids. Methods for generating phospholipid-based liposomes with static membranes have been extensively discussed in a recent review by Wang et al.[29] Growth of liposomes requires either *in situ* formation of monomers from non-membrane-forming precursors or membrane fusion.[30] Here, we focus on recent membrane mimetic chemistry used for producing dynamic lipid membranes capable of growth by lipid synthesis (Figure 1).

A potentially straightforward approach for creating membranes capable of growth and lipid remodeling is to reconstitute the natural cellular lipid synthesis machinery (Figure 1B). Demonstrating this concept, Exterkate et al. recently reconstituted bacterial enzymes involved in membrane synthesis in liposomes, leading to the formation of phospholipids when vesicles were supplied with free fatty acids and glycerol-3-phosphate. In exciting work, Blanken et al. reconstituted enzymatic synthesis using vesicles encapsulating a cell-free expression system (Figure 1F).[31] These researchers reconstituted seven enzymes from the *E. coli* Kennedy lipid synthesis pathway in phospholipid vesicles. Functionality was demonstrated by the formation of natural phospholipids such as phosphatidylethanolamine and phosphatidylglycerol in the liposomal membrane. This work validated that an enzymatic strategy can result in membranes capable of transcriptionally regulated phospholipid synthesis. The method described by these researchers requires that enzymes be expressed inside existing liposomes and that vesicles be supplied with acyl-CoA and glycerol-3-phosphate, which are normally produced enzymatically inside living cells. However, liposomes showed large heterogeneity in lipid production, and no visible vesicle growth could be observed due to the limited amount of phospholipid produced. The reconstitution of an entire lipid synthesis pathway inside liposomes is clearly challenging and may require cofactors that have not yet been characterized. Recent work has demonstrated that lipid synthesis can be highly regulated by accessory proteins, which if absent in cell-free systems might lower the efficiency of reaction.[32]

One way to avoid the challenges of reconstituting lipid synthesis enzymes is to mimic phospholipid synthesis reactions non-enzymatically (Figure 1C). In past work, our lab has generated vesicles composed of unnatural phospholipids using click chemistry and native chemical ligation and showed that these coupling reactions can drive membrane expansion.[33−35] Related research has demonstrated that combining lipid chemical conjugation methods with a phase-transfer catalyst can result in dissipative self-assembly of liposomes.[36] To form natural phospholipids, we attempted to emulate biological coupling reactions. While mimicking all the steps of Kennedy lipid synthesis pathway is daunting, our lab drew inspiration from another lipid synthesis pathway known as the Lands cycle or remodeling pathway.[37] Specifically, a key step of the Lands cycle involves combining a single-chain lysophospholipid with a single-chain thioester derivative of a fatty acid to form a new phospholipid. Neither of the single-chain precursors form membranes, but the coupling product does. We sought to mimic the acylation coupling step non-enzymatically using chemoselective chemistry. By choosing reactions that work in water, the solvent in which lipid self-assembly takes place, we observed spontaneous formation of membrane-bound vesicles during the synthesis reaction. Furthermore, our approach generated lipid membranes *de novo* (i.e., in the absence of preexisting membranes), which, as discussed, is thought to not occur in biology. Ultimately, this method allowed the synthesis of natural, membrane-forming phospholipids under aqueous conditions in the absence of enzymes (Figure 1G).[38] This result suggests that it may have been possible for phospholipid membranes to form without the need for a pre-existing membrane composed of simpler amphiphiles and could be applied to the generation of artificial cell membranes.

However, unlike enzymatic synthesis of diacyl phospholipids, purely chemical methods cannot be easily controlled by transcriptional regulation. Combining enzymatic and non-enzymatic methods can allow high-yielding *de novo* formation of membranes from simple precursors while retaining transcriptional control (Figure 1D). For example, a chemoenzymatic approach demonstrated by Bhattacharya et al. resulted in the *de novo* formation of phospholipid membranes that could be linked to transcription, and vesicle growth could be observed (Figure 1H).[39] Chemoenzymatic synthesis may therefore be an effective method for creating membranes capable of sustained and

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tunable growth.[40] As discussed in a recent review by Liu et al., membranes composed entirely of peptides have also been proposed as platforms for artificial cells.[41] Peptide vesicles could potentially be generated enzymatically, allowing for transcriptionally controlled membrane formation.

MEMBRANE FISSION

The fission of membranes into distinct compartments is essential for many biological processes. In living organisms, membrane fission allows for cellular growth and division and facilitates vesicular transport between and within compartments. It is highly desirable to create artificial cells capable of controlled fission because such systems could be used as models for cellular division or platforms for the *in vitro* reconstitution of biological phenomena such as Darwinian evolution (Figure 2). However, the division of membrane-bound structures requires overcoming a substantial energetic barrier to deform the membrane and eventually achieve separation.[42,43] Fission in living cells is exceptionally complex and involves sophisticated cellular machinery such as FtsZ, actin-myosin rings, ESCRT, or dynamin.[44] Cellular division is a highly controlled fission process that results in two compartments that are approximately equivalent in size and content. Reconstituting division machinery in its entirety in minimal synthetic cells would likely be cumbersome and impractical. Some groups have therefore tried to reconstitute only what are expected to be the most important cellular components for triggering division (Figure 2A). One such approach involves the *in vitro* reconstitution of the prokaryotic divisome. The principal components of this complex molecular machine are the contractile ring structure formed by the bacterial tubulin homolog FtsZ and several proteins, termed Min proteins, which facilitate its polymerization at the equator of the cell.[45,46] Schwille and co-workers first reported the *in vitro* reconstitution of Min proteins on planar lipid bilayers, on which they observed surface wave patterns that are reminiscent of oscillations that have been observed in live bacteria.[47] More recently, Litschel et al. and Godino et al. successfully reconstituted Min proteins in lipid vesicles (Figure 2E). They observed similar oscillatory patterns as well as membrane deformation, suggesting that reconstitution of minimal division machinery can lead to significant membrane perturbations.[48,49] Likely because these proteins work in concert with other proteins, many of which remain uncharacterized, there has thus far been limited success in achieving controllable fission in artificial cell membranes through these methods. Other research, however, has been spent pursuing potential methods for inducing fission in artificial cells by reconstituting processes unrelated to the cell cycle. For example, it was recently demonstrated that proteins involved in vesicular transport could induce fission in liposomes.[50] These simpler systems may provide alternative sources of inspiration for inducing fission in artificial cellular membranes.

To achieve fission of artificial cells, researchers may need to look beyond reconstituting existing machinery from living organisms. Although all known living membranes achieve fission using complex protein-based machines, division in primitive cells likely occurred through simpler mechanisms. Understanding these mechanisms is key to understanding the evolution of life and may also be crucial to inducing fission in minimal cells. Some have suggested that division of early cells could have been driven by mechanical perturbations. Deshpande et al. demonstrated that fission could be induced through microfluidic methods that flow liposomes against a sharp edge.[51] Recent work has also shown that osmotic pressure, generated by manipulating the external solution bathing lipid

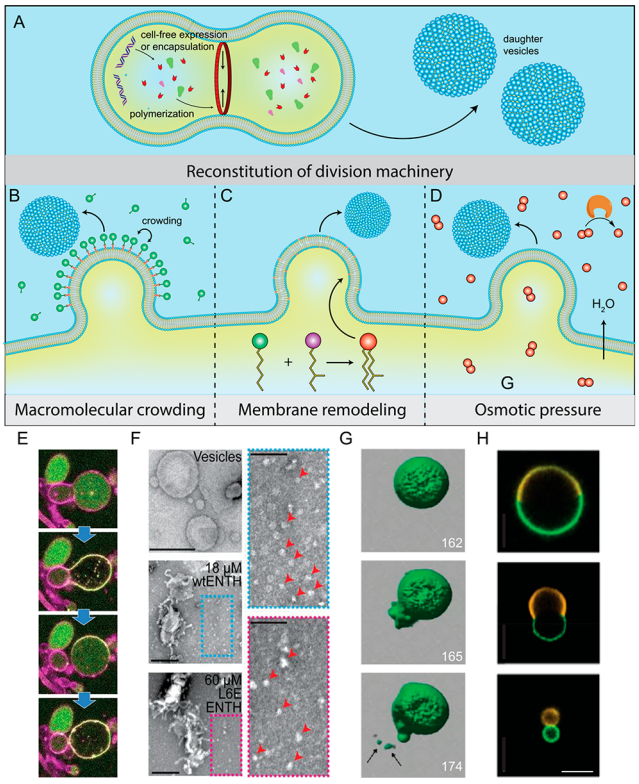

Figure 2. Fission of membrane-bound vesicles. (A–D) Schematic representation of methods that could be harnessed for inducing fission in liposomes: (A) Division caused by reconstitution of cellular division machinery. (B) Membrane perturbations and budding caused by recruitment of high concentrations of macromolecules to the vesicular membrane. (C) Excess membrane synthesis and membrane remodeling can lead to instabilities and membrane scission. (D) Increase in osmolarity in the extravesicular space leading to a reduction of volume encapsulated by vesicles and membrane fission. (E–I) Representative images of membrane deformation and fission demonstrated in artificial cell models: (E) Bacterial Min proteins lead to significant membrane deformation when they are expressed inside vesicles. [Adapted with permission from ref 48. Copyright 2020. licensed under CC BY 4.0.] (F) Membrane tubulation and budding as a result of binding of the clathrin adaptor epsin1 N-terminal homology (ENTH) domain. Binding of ENTH to membranes is thought to increase membrane spontaneous curvature through two possible mechanisms: insertion of an amphipathic helix that causes a reduction in leaflet surface area, or steric crowding at the membrane surface that forces curvature. Binding of ENTH causes membrane deformation even when the amphipathic helix responsible for membrane insertion is mutated (L6E) to impact hydrophobicity, suggesting that deformation can result from crowding alone. [Adapted with permission from ref 60.] (G) Fission by excess membrane synthesis. Phospholipid synthesis is driven by alkyne–azide click chemistry and a self-reproducing membrane-associated copper catalyst. Membrane growth is monitored over time, and daughter vesicles are indicated by black arrows. Numbers indicate time, in minutes, since the start of the experiment. [Adapted with permission from ref 35.] (H) Division of phase-separated vesicles by external modulation of osmotic pressure. The liquid disordered phase is labeled with LissRhod PE (orange, λ_{ex} = 561 nm) and the liquid ordered phase with 6-FAM-labeled cholesterol-tagged DNA (green, λ_{ex} = 488 nm). Vesicles undergo fission when the osmolarity of the extravesicular environment is increased through a simple enzymatic reaction. [Adapted with permission from ref 52. Copyright 2020, licensed under CC BY 4.0.]

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vesicles, can be used to trigger fission in phase-separated giant unilamellar vesicles (Figure 2D,H).[52] While these techniques allow for considerable control over the volume and shape of resulting daughter vesicles, primitive cell membranes capable of autonomous division without external intervention must have arisen early in the evolution of modern cells.[53]

One clue as to how primitive cells may have divided comes from the observations of Errington and co-workers who have shown that a primitive kind of membrane division, which does not result in two equivalent daughter cells, can occur in bacteria that lack a cell wall (L-form bacteria).[54] Such bacteria do not divide using the conventional FtsZ-reliant bacterial division machinery, so they must acquire genetic mutations that allow division while in this state.[55] They showed that proliferation in L-form *B. subtilis* can occur after the bacterium acquires one or more mutations that cause an increase in the abundance of phospholipids, leading to excess membrane synthesis. Previous work showed that membrane scission during proliferation is dependent on the production of branched-chain fatty acids, which increase the fluidity of the resulting membranes.[56] These results suggest a major role for lipid expansion and composition in promoting membrane morphological changes (Figure 2C). This is also supported by observations that phase separated vesicles tend to undergo stimuli dependent division more readily.[52] Logically, changes in the physical properties of membranes produced by changing phospholipid composition would likely help cells overcome the enormous energy barrier required to divide. Even complex multi-cellular organisms include remodeling of the lipid membrane as a crucial part of the process of cytokinesis.[57] Work from our lab and others has shown that membrane expansion can drive vesicle budding at low rates, suggesting that instabilities caused by expanding the membrane surface area while maintaining vesicle volume could potentially be exploited for inducing membrane division (Figure 2G).[35] Finely tuned remodeling of vesicle membranes is expected to be necessary for division of artificial cells and could result in programmed division events. More work will be needed in this area to fully understand the role that membrane composition plays in division and to explore methods for adjusting lipid content of synthetic membranes *in situ*. One possible approach would be to harness chemical mechanisms to remodel artificial cell membranes, either reversibly or driven through the use of chemical fuels and dissipative self-assembly.[36,58,59]

While careful attention to the lipid membrane composition of artificial cells is likely to be necessary for controlled fission, simple interactions between membranes and macromolecules may also drive membrane deformation (Figure 2B).[42] It has been shown that protein crowding at the membrane can impact cell membrane curvature and cause membrane budding. For example, work by Stachowiak and co-workers demonstrated that high concentrations of recombinant clathrin adaptor epsin1 N-terminal homology (ENTH) domain caused division of liposomes.[60] This protein domain was previously understood to aid in fission through shallow insertion of a hydrophobic helix into the membrane, impacting leaflet surface area. However, they showed that binding of ENTH causes membrane deformation even when the amphipathic helix responsible for membrane insertion is mutated (L6E) to impact hydrophobicity, suggesting that deformation can result from high densities of ENTH at the membrane (Figure 2F). They then demonstrated that fission can be driven by recruitment of high concentrations of GFP to the membrane, meaning that macromolecular crowding, regardless of protein identity, may provide a generalized mechanism for inducing fission in artificial cell membranes.[42] More work is needed to explore how these effects could be finely tuned to produce division that is controllable and that leads to daughter cells that are similar in size and content.

Finally, there is an opportunity to circumvent existing paradigms in biology and induce vesicle fission through chemical methods that are apparently unrelated to the mechanisms of membrane fission in living organisms. In one fascinating example, Zhu et al. showed that simple, light-driven chemical reactions could have been involved in early cellular division and might be harnessed for synthetic biology applications.[61] They demonstrated that ultraviolet illumination of filamentous fatty acid vesicles in the presence of thiols resulted in membrane pearling and division. They hypothesized that oxidized (but not reduced) thiols associated with the membrane, causing instabilities that lead to division. More recently, Miele et al. demonstrated fission of hybrid vesicles (i.e., vesicles containing both phospholipids and fatty acids) by increasing the alkalinity of the vesicle lumen through a simple enzymatic reaction.[62] These researchers hypothesized that deprotonation of internal oleic acid molecules caused them to dissociate from the membrane, thus lowering inner leaflet surface area. At the same time, the introduction of urea to the extravesicular environment initially caused osmotic shrinking of vesicles. Together, these effects were sufficient to cause division of giant unilamellar vesicles containing oleic acid. Further developing simple chemical methods for inducing division in lipid vesicles would be would be an interesting future direction of membrane mimetic chemistry.

TRANSPORT ACROSS MEMBRANES

Phospholipid membranes possess several advantages, including their biocompatibility and ability to maintain ion gradients. However, the limited permeability of phospholipid bilayer membranes also presents disadvantages for their use in artificial cells. Steady-state metabolism and growth of living cells requires that they continuously obtain material from the environment such as amino acids, nucleotides, and sugars, much of which does not permeate readily through phospholipid bilayers. Consequently, the comparatively high permeability of fatty acid membranes is attractive to synthetic biologists. However, reduced permeability is also necessary for living cells to maintain ion and metabolite gradients that are required for metabolism. For this reason, researchers have theorized that selective permeability of membranes arose gradually as protocells transitioned from fatty acid membranes to diacyl phospholipid membranes.[28] Vesicle membranes that contain both fatty acid and phospholipid may therefore be appropriate platforms for some applications that require increased permeability. Demonstrating this concept, Zhou et al. recently examined ion permeability in hybrid phospholipid/fatty acid vesicles.[63] Interestingly, they found that hybrid vesicles selectively retained potassium and excluded sodium, establishing transmembrane gradients for these ions in the absence of any protein-based pumps. This suggests that membrane permeability to ions in artificial cells can be modulated by simply adjusting membrane lipid content.

Modern cells evolved selectively permeable membranes by incorporating integral membrane proteins that are permeable to target molecules or ions. Ion gradients are maintained and metabolites are concentrated using ATP-dependent membrane-

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associated pumps. Thus, one of the most readily apparent strategies for affecting the permeability of artificial membranes would involve the reconstitution of these membrane proteins in synthetic cells (Figure 3A). In general, reconstitution of membrane proteins in liposomes is challenging. This is traditionally done by solubilizing membrane proteins in detergent, adding phospholipid, and then removing excess detergent to generate proteoliposomes. This process can be time-consuming, and quantitative removal of detergent is challenging.[64] Trace detergent molecules can inhibit the activity of some transmembrane proteins or can create defects in the membranes. Work in our group has explored reconstitution of membrane proteins in liposomes through alternative methods. For example, we have previously shown that several different integral membrane proteins, including G protein-coupled receptors (GPCRs), can be reconstituted in a functional form during *de novo* membrane synthesis.[65] In this process, the detergents used to solubilize and purify the proteins are themselves reactive. Reaction with lysolipid analogs leads to artificial phospholipid membrane formation and spontaneous membrane protein reconstitution. The method does not require a large excess of reactive detergent, and the high yield of reaction leads to near-quantitative detergent depletion. More work is needed to determine whether proteins can be simultaneously expressed and reconstituted in membranes during *de novo* chemoenzymatic lipid synthesis using cell-free expression systems. In principle, *in situ* reconstitution of membrane proteins can be used to generate semi-permeable artificial cells. However, directionality of membrane proteins is difficult to control, which can impede the formation of proton gradients or membrane potentials in artificial cells using recombinant protein. This is a challenge that will need to be addressed if recombinant membrane proteins are to be used for this purpose. One potential solution was demonstrated by Majumder et al., who showed that linker of nucleoskeleton and cytoskeleton (LINC) complexes could be reconstituted with a preferred functionality through cell-free expression of LINC proteins in the presence of a supported lipid bilayer.[66]

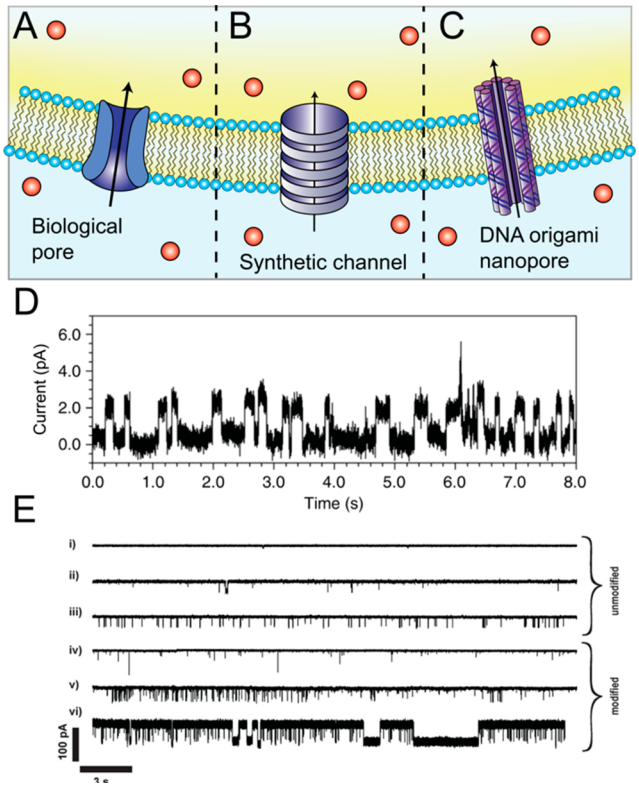

Figure 3. Methods for selectively permeabilizing artificial cell membranes. (A–C) Schematic representations of membrane-permeabilizing reagents for artificial cells: (A) Reconstitution of biological pores can facilitate transport of solutes across phospholipid bilayers. (B) Synthetic mimics of biological pores can insert in membranes and permeabilize them to solutes. (C) DNA origami pores that can be rationally designed to insert into membranes creating pores of a specific diameter. (D,E) Representative current traces from a synthetic ion channel (D) and a DNA origami nanopore (E): (D) Current trace from a multi-block amphiphile inserted into a black lipid membrane (BLM) composed of DOPC using an applied voltage of 100 mV. (E) Representative current traces from electrical characterization of DNA nanopores performed on painted DPhPC (1,2-diphytanoyl-*sn*-glycero-3-phosphocholine) bilayers. DNA nanopores with a heptanucleotide protruding through the middle of the channel (iv–vi) demonstrate "gating" behavior, similar to biological channels, which is not observed in unmodified nanopores (i–iii). [Panel D adapted with permission from ref 68. Copyright 2020, licensed under CC BY 4.0. Panel E adapted with permission from ref 70. Copyright 2012 The American Association for the Advancement of Science.]

Alternatively, phospholipid membranes can be permeabilized using non-protein reagents (Figure 3B,C). Ion channels and pores can permit diffusion of inorganic and organic solutes across hydrophobic membrane regions. Ion carriers, which sequester ions and facilitate their transport across the membrane, also serve this purpose.[67] Significant efforts in the field of supramolecular chemistry have been dedicated to the development of synthetic channels, pores, and carriers for biological research.[67] While molecules capable of increasing membrane permeability have long been identified, recent work has focused on creating molecules that allow transport with a preferred direction (e.g., outside to inside of vesicles) and that are responsive to external cues. Muraoka et al., for example, devised synthetic multi-pass ion channels that could be incorporated in an anisotropic manner into vesicular membranes (Figure 3D).[68] They then demonstrated that these channels could be turned off and on through reversible ligand binding. Others have drawn inspiration from biological channels to design synthetic channels that are sensitive to light, pH, temperature, and mechanical force.[69] An emerging area is the application of DNA origami to rationally create membrane channels. Simmel and co-workers have shown that DNA nanochannels in lipid membranes can show conductances and gating similar to natural ion channels and can discriminate between single DNA molecules (Figure 3E).[70] These advancements will likely be crucial to the development of artificial cells and suggest that there are alternatives to the reconstitution of transmembrane proteins for creating life-like membranes that are selectively permeable, responsive to external cues, and capable of establishing and maintaining gradients.

LIPID MESOPHASES AS SYNTHETIC ORGANELLES

While researchers have tended to employ lipid vesicles in bottom-up synthetic biology, few biological compartments resemble these spherical, lamellar structures. Living cells instead adopt a wide variety of shapes and sizes that are important for their unique functions. Additionally, many organelles in living cells are highly interconnected rather than vesicular structures. These structures facilitate organellar functions that require membrane-dependent reactions and benefit from high mem-

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brane surface area, such as in the Golgi apparatus, the endoplasmic reticulum, and the mitochondria.[71] Controlling the topology of artificial cells as well as their sub-compartments is a significant challenge in synthetic biology.[8]

The shape and size of lipid-based cellular compartments are in part determined by cytoskeletal elements. It would be quite difficult to reconstitute these networks in their entirety in artificial cells to precisely modify the shape and size of artificial organelles. However, properties of lipids themselves can impact physical properties of lipid compartments, including their morphology. The self-assembly of various natural and synthetic lipids into non-lamellar mesophases comprised of highly interconnected bilayer networks, such as cubic and sponge phases, is well-studied *in vitro*.[72] Additionally, the extensive sponge-like morphology of the smooth endoplasmic reticulum (ER) has been documented in early electron microscopy studies, and cubic ER phases have been observed in cells, particularly those that are stressed, diseased, or virally infected (Figure 4D).[73,74] Some lipid mesophases may therefore be appropriate models for organellar functions and could serve as platforms for bottom-up synthetic biology (Figure 4). Saw et al. recently showed that lipid substructures could be carefully tuned by simply adjusting relative amounts of lipids with different physical properties.[75] These studies may be important in the development of artificial membranes and organelles for an artificial cell. Additionally, despite being observed in cells, non-lamellar lipidic structures *in cellulo* are underexplored and their functional roles are poorly understood. Modeling these compartments *in vitro* could help elucidate their biological functions. It should be pointed out that it may not be necessary to identically mimic the observed structures, such as bicontinuous cubic phases, as alternative non-lamellar mesophases may have similar properties. Our group recently developed a novel biocompatible lipid sponge phase droplet capable of confining biological molecules and pathways with great efficiency (Figure 4A–C).[76] Compartmentalization of protein into these bicontinuous sponge droplets is programmable, and remarkably high encapsulation efficiencies can be achieved. Past work has shown that various lipids such as gemini surfactants and natural glycolipids can form droplets consisting of non-lamellar lipid mesophases, and these systems are likely worth revisiting from the context of creating synthetic organelles.[77,78] More work is needed to determine how mesophase lipid droplets could be coupled to synthetic cells to act as hubs for organization of biochemical reactions such as proteolysis and lipid synthesis.

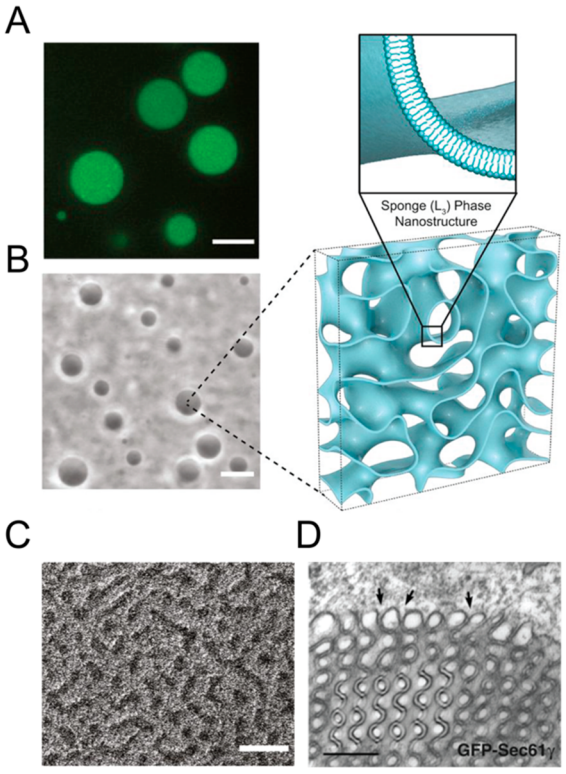

Figure 4. Cubic-phase lipid nanostructures as microcompartments for bottom-up synthetic biology. (A) Lipid sponge droplet encapsulating Alexa Fluor 488-labeled cytochrome *c* oxidase. (B) Phase-contrast image of a typical droplet dispersion and an illustration of the droplets' porous, bilayer-rich, bicontinuous nanostructure. (C) Freeze-fracture cryogenic scanning electron microscopy (cryo-SEM) image of a dispersion showing the sponge phase morphology in lipid droplets from (B). [Panels A–C adapted with permission from ref 76.] (D) Distinct endoplasmic reticulum morphology induced by high levels of protein that are associated with formation of organized smooth endoplasmic reticulum. This morphology is consistent with a bicontinuous cubic lipid mesophase model described in ref 73. [Adapted with permission from ref 74. Copyright Rockefeller University Press 2003.]

CONCLUSION

All forms of life on Earth require lipid membranes. Living cell membranes are highly dynamic structures undergoing constant growth, degradation, division, and remodeling. By embracing membrane mimetic chemistry, there exists an opportunity to design and study synthetic membrane systems that possess dynamic properties rivaling those of living cellular membranes. Membrane mimetic chemistry was initiated to develop new chemistry by drawing inspiration from the compartmentalization of biomembranes. Faithful mimicry of biological phenomena was not the explicit goal—instead, membrane-like environments such as micelles were used to organize reactants and catalyze chemical reactions. Over the past 40 years since these pioneering efforts, there have been tremendous advances in fields such as chemical biology and synthetic biology. Coupled with the growth of sophisticated analytical techniques, there now exists an opportunity to expand the scope of membrane mimetic chemistry with the specific goal of using minimal chemical and biochemical systems to mimic the diverse functional phenomena encountered in biomembranes. Doing so may reveal fundamental phenomena related to cell membranes, may create new opportunities for applying membranes in biotechnology, and will accelerate progress in the rapidly developing research area of artificial cells.

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Notes

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