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Review

Compartmentalization as a ubiquitous feature of life: from origins of life to biomimetics

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Abstract Life is hypothesized to have emerged in a heterogenous prebiotic soup that potentially comprised a variety of chemical moieties. One relevant consideration in this scenario is that of dilution of pertinent molecules, which would impinge on the emergence and functioning of a self-sustaining chemical machinery. Given this, encapsulation of molecules within a compartment is considered a prerequisite for the origin, sustenance and evolution of living systems. This review discusses two well-studied prebiotically plausible minimal compartment models—membrane-bound liposomes and membraneless liquid–liquid phase separated (LLPS) compartments. Such minimally complex compartments can be used to mimic biomimetic properties like molecular crowding, diffusion of molecules, tunable physical architecture, etc. using a bottom-up approach. The relative ease of tunability of these systems, and their semblance to extant cells, can be used to study a vast array of fundamental processes like metabolism, growth and division using them. In this backdrop, we connect the fundamental role of compartments in origin of life processes with cellular biomimetics, using a synthetic systems biology perspective. More recently, concocting multi-layered hierarchical architecture in protocells has been possible that better mimic cellular spatiotemporal segregation. This overarching review thus bridges fundamental research involving soft matter boundary systems, with translational synthetic biology and biomimetic research.

1 Introduction

Extant cells harbour immensely complicated machinery, carrying within themselves a vast network of chemical reactions necessary to execute essential life processes including metabolism, growth and division. At any given point in time, only a chosen set of chemical reactions proceed within a cell, and this mainly depends on factors including the energy needs of the cell, or the stage of the cell cycle that it is in etc. Such meticulous coordination of reaction pathways would necessitate control over the spatiotemporal organization of the reactants, products, and catalysts (enzymes) within the cell. This detailed orchestration is an elegant evolutionary design imparted by the formation of distinct compartments in a cell. This compartmentalization is a ubiquitous feature of all life and confers significant advantages to efficiently manage a vast array of mutually exclusive and often divergent biochemical networks of interacting molecules over an open bulk system. Pertinently, all forms of life show some kind of compartmentalization highlighting its indispensability to life [1]. Starting from creating and maintaining concentration gradients for metabolism, to protecting and replicating informational molecules, compartments provide the fundamental prerequisite for a Darwinian evolutionary process, which is that of forming an 'entity' separated from the bulk phase. Given these aspects, it is understandable why many research groups are employing various bottom-up approaches to fabricate artificial cells in the lab that allow for mimicking cellular phenomena in a minimal living system to understand them at great depth.

Two most prominent strategies of compartmentalization in a cell are the use of a physical boundary system (as provided by lipid membranes), and membrane-less liquid–liquid phase separated compartments. The former type or biomembranes, are integral to most functions of a cell. Organelles like ER, Golgi, plasma membrane etc. are actively and meticulously controlled by metabolic bias. This helps them to retain distinct membrane

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properties, which are strictly regulated to maintain the organellar identities that, in turn, are governed by the specificity in their lipid composition [2]. This raises the question as to why a modern cell invests so much in stringently retaining its lipid bilayer composition, down to the specific asymmetric distribution of lipids even across the leaflet of a bilayer. Lipidomic studies show that these properties are so strictly modulated that even small tweaks to the lipid bilayer can result in significant functional consequences and sometimes even cell death [3]. This highlights the importance of the nature of the lipids present in the bilayer and what this means for their fundamental physicochemical properties, which is in addition to the intricate functions dictated by the presence of select membrane proteins. Biomimetics that simulate different aspects of cellular functions including endo and exocytosis, membrane signalling and homeostasis of molecular and energy gradients, have all recently been part of significant breakthrough studies [4, 5]. Majority of these studies used a vast array of model systems including giant unilamellar vesicles (GUVs) or liposomes, proteinosomes, single chain amphiphilic (SCA) vesicles (or ufasomes) and polymer-based synthetic membranes, to name some [6]. However, the main underlying idea has been to delineate the role of bilayer compartments and boundary systems as essential functioning entities, effectively performing life-like activities.

In addition to membrane-bound compartments, there exist in cells, a variety of membrane-less compartments too. The most familiar examples of such systems are nucleolus, Cajal bodies, stress granules, P-granules, to name a few [7]. These result mainly due to the phenomenon of liquid–liquid phase separation (LLPS) in cells that occurs as a result of multivalent interactions mainly between proteins and nucleic acids, thereby concentrating these molecules within a confined space. Relevantly, they are liquid-like as they allow ready diffusion of molecules within this LLPS compartment as well as into the surrounding bulk solution, while also allowing biochemical reactions to take place within their interior. It is well known that the cell interior is composed of well-segregated membrane-bound and membrane-less compartments, which communicate extensively amongst each other, facilitating life-sustaining reactions and maintaining homeostasis. But how did such a complex cellular machinery even come into being? Researchers studying the origins of life (OoL) have proposed that life would have emerged in a heterogenous prebiotic soup containing a complex mixture of chemicals. The prebiotic soup is hypothesized to have been present in geochemically pertinent niches on the early Earth, wherein relevant chemistry would have been predominantly driven by the surrounding environmental conditions. In this context, prebiotic chemists have demonstrated pathways by which the building blocks of life (e.g. nucleotides/nucleic acids, lipids, amino acids/peptides and carbohydrates) could have been synthesized abiotically under early Earth conditions. Nonetheless, the formation of a functional biomolecule like the genetic material (nucleic acid), from its constituent nucleotides, would have entailed one or more mechanisms to concentrate these nucleotides to effectively result in functional length molecules. In this larger backdrop, OoL researchers proposed the idea of a protocell, an assembly consisting of a genetic material encapsulated within a lipid bilayer, that is capable of undergoing growth and division. Although this seems similar to what we see in today's cell, an entirely different view on the assembly of a protocell was envisioned by Oparin in 1930s [8]. He had proposed that molecules similar to proteins and carbohydrates could have spontaneously assembled into what he termed as "coacervates". Such molecules could have provided a mechanism to concentrate organic compounds in the prebiotic soup, thereby assisting in the formation of a protocell. It is important to note that the central feature in Oparin's proposal, has indeed been found to be an important mechanism to concentrate biomolecules in extant cells as well.

Given these aforementioned aspects, a focal point of artificial cell/biomimetic cell research has been to glean the fundamental role of compartments using a 'minimal cell' scenario. Bringing together our fundamental understanding of LLPS and liposome systems, which have been researched in isolation in the past, has been crucial to fabricating more complex functional protocell designs and increasing their tunability (Fig. 1). A major part of these synthetic biology research efforts using interdisciplinary approaches, have led to a rapid progress in the last few decades resulting in the assembly of life-like biomimetic systems capable of mimicking many biological functions. In this review, we will be discussing the current understanding of design principles gained from basic research, as applied to biomimetic LLPS and liposome-based compartments. Specifically, recent lessons gleaned from the fundamental understanding of biophysical and biochemical processes of biomimetic compartments, is finally allowing researchers to put together hierarchical synthetic cell models. Using both LLPS and membrane systems, one can systematically fabricate a complex hierarchical compartment that can mimic cellular compartmentalization and complexity in a comprehensive manner (Fig. 1). In terms of understanding a spatio-temporally intricate bioreactor, such conjugate systems have been demonstrated to recapitulate the partitioning of molecules with reasonably high efficiency mimicking a cell-like architecture. Biomimetic functions in a relatively simplistic 'minimal' system can greatly enhance our understanding of interactions underlying various biological phenomena, by essentially decluttering the system. All these endeavours would eventually pave the way towards making a totally self-sustaining minimal cellular system, which has been an ultimate goal and the holy-grail of OoL research. In this review, we aim to tie together a wide range of aspects pertaining to compartments, all the way from the foundational aspects to the application elements. We sincerely hope that with this review, we have been able to bridge between the research happening in the origins of life, synthetic biology and artificial cell realms (Fig. 2).

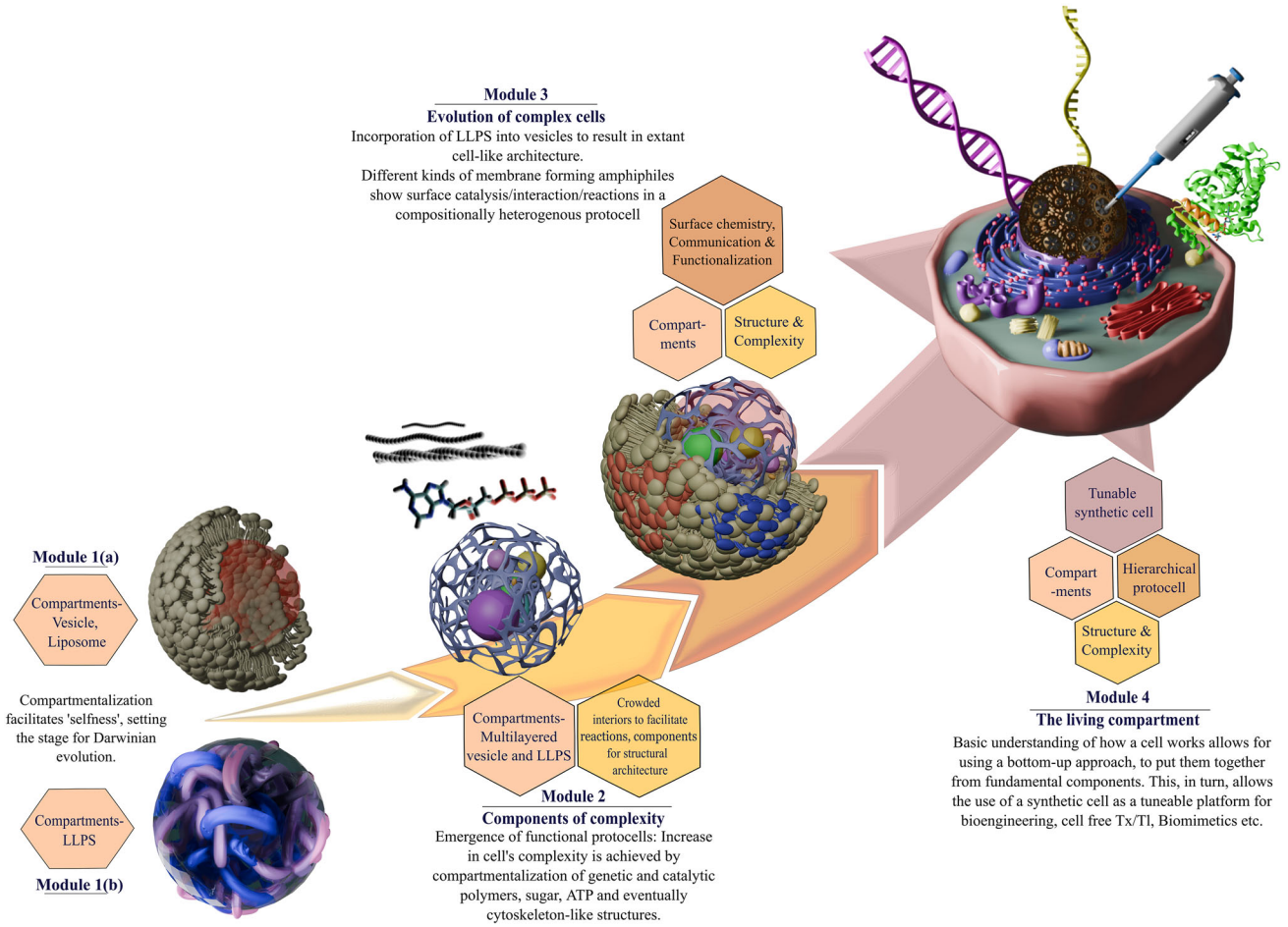

Fig.1 The overarching structure of this review article shows a stepwise increase in complexity among distinct membrane-bound and membrane-less compartments achieved using a bottom-up approach. The final step is a consortium of different spatiotemporally separated compartments working coherently in a synthetic biomimetic system. Module1- Represents the minimally complex liposome (**a**) and liquid–liquid phase separated (LLPS) compartment (**b**) systems, Module 2- Introduction of crowding and structural components (e.g. actin, ATP etc.[39, 169]) to achieve a more defined tuneable architecture. Module 3- LLPS in membrane-bound structures resulting in a multilayered hierarchical compartment module (HCM). Module 4- The crux of increasing complexity and organization to result in a controllable synthetic living system consisting of cell-like architecture that could be used as a tool for biomimetic and synthetic biology applications

1.1 Molecular crowding and reactions in LLPS compartments

One of the central purposes of forming any kind of compartment, be it in the context of OoL processes or in modern cells, is to concentrate molecules of interest in a spatiotemporally regulatable manner. This is pertinent as dilution of molecules that need to frequently interact would give rise to inefficient reactions and stalling of biochemical processes. As a (ubiquitous) solution, compartmentalization is thought to have emerged as a universal mechanism to encapsulate pertinent molecules within a confined space. During life's origin, dilution would have been an especially significant issue as efficient biomachines like modern cells would have been absent, further underscoring the essentiality of invoking such mechanisms that would have allowed for the concentration of relevant molecules. Towards this, a synthetic bottom-up approach that has been effectively studied over the last many decades involves the use of a compartmentalized systems famously referred to as protocells. Protocells are essentially confining systems that allow for the encapsulation and sequestration of molecules from bulk environment within a relatively crowded interior. Different kinds of protocells have been developed and studied according to the requirement of the experimental paradigm. However, two of the major classes are lipid-based vesicles and liquid–liquid phase-separated (LLPS) coacervates or droplets; both of which are found in extant cells as well (Fig. 1).

Regarding LLPS systems, there are two types that result from the interactions of polymers in aqueous solution; associative and non-associative [9]. This is fundamentally based on the type of interactions that occur between the

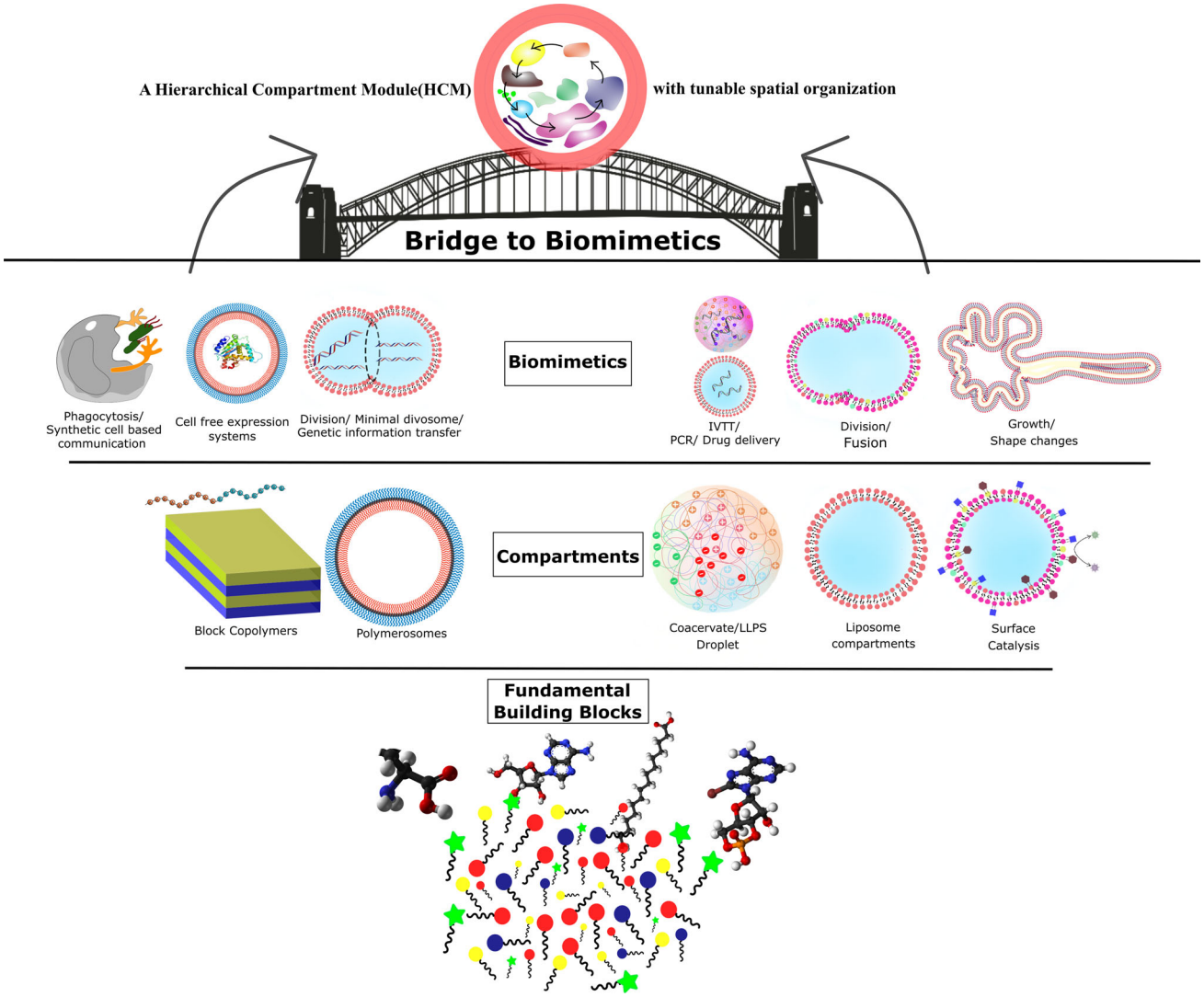

Fig. 2 The bridge that connects fundamental research on compartment systems pertinent to life's origin and evolution, with bottom-up synthetic systems and biomimetics. The outcome of this at the very top depicts an illustration of a self-sustaining chemical system that results from the coming together of multiple compartments resulting in an HCM. The below layers depict the various stages of increasing complexity that result in HCMs using a bottom-up approach, starting with the fundamental biomolecules. The left-hand side shows the approaches involving synthetic biology and biomimetics research, while the right-hand side depicts research from the origins of life field. Each stratum shows the overlapping similarities between these disciplines. *IVTT* In vitro transcription–translation, *LLPS* Liquid–liquid phase separated droplet

polymers involved in these systems. Associative phase separation, also called as coacervation, results from electrostatic attraction between two oppositely charged polymers, e.g., between polylysine and RNA [9, 10]. On the other hand, non-associative phase separation results from repulsive interactions between two non-ionic polymers, for instance between polyethylene glycol (PEG) and dextran. Under a microscope, these phase-separated compartments appear as spherical drops and are often referred to as "droplets". A recent study showed another mechanism of phase separation, demonstrating polyester-based microdroplet formation—a novel system that also shows LLPS behavior [11]. When it comes to studying any LLPS system as a protocell model, they are mainly tested for their ability to concentrate biomolecules, especially RNA, because of its quintessential role in the putative RNA World. In this context, a coacervate system composed of polyamines and nucleotides showed a remarkable enhancement in the concentration of encapsulated RNA (˜ 10,000 fold), magnesium and nucleotides (˜ 300 to 1500-fold, respectively) within the droplets. The partitioning of RNA has been shown to depend on the size of the RNA; longer RNA molecules partition more effectively than the shorter ones [12]. Similar dependence of partitioning on size was observed with peptides too [13].

Aside RNA, a coacervate system composed of polylysine and ATP was also demonstrated to be capable of concentrating other molecules such as porphyrin, a prebiotically plausible metabolite that is a frequently used enzyme scaffold [14]. Also, the same system was shown to prefer uptake of anionic solutes over cationic ones, indicating that the nature and interaction of components that compose the coacervates do play an important role in the compartmentalization of the solutes [14]. Furthermore, partitioning of solutes also depends on other types of interactions including electrostatic, hydrophobic, pi–pi stacking, and van der Waal's interactions [15]. In an aqueous two-phase system (ATPS) composed of PEG and dextran, partitioning of a native protein was shown to happen in the dextran-rich phase while that of the denatured protein happened in the PEG rich phase [16]. Relevantly, polyU RNA present in a coacervate system showed sequestration of polyA RNA (instead of polyU), highlighting the importance of intermolecular interactions in the sequestration of molecules [9]. Moreover, polyesters composed of different alpha-hydroxy acids, e.g. lactic acid, glycolic acid, 3-phenyllactic acid, 2-hydroxy-4-(methylsulfanyl) butanoic acid and leucic acid were recently shown to form droplets [11]. They differed significantly in their capacity to encapsulate RNA; only the droplets formed from 3-phenyllactic acid showed RNA sequestration. Thus, the dependence of partitioning of a molecule into the droplets depends on its interactions with the components of the droplet. Pertinently, this also provides a mechanism for readily establishing various interacting protocell populations composed of chemically distinct components, and thereby, containing within them chemically diverse encapsulants.

Now that the molecules can be concentrated within a compartment, they should also be able to diffuse within and across droplets to facilitate chemical reactions. To understand the diffusion of molecules within droplet systems, techniques such as FRAP (fluorescence recovery after photobleaching) and FCS (fluorescence correlation spectroscopy) are often employed [10, 17–19]. Typically, a fluorescently tagged RNA or protein is encapsulated within the droplet and is subjected to bleaching by shining a high intensity laser. The time taken for recovery of fluorescence after photobleaching is a measure of how fast the fluorescent molecules diffuse within the compartment. Thus, a compartment that can facilitate both concentration and diffusion of molecules within itself would in principle be readily able to enhance the rates of chemical reactions. Indeed, the rates of ribozyme catalysis increased by ˜ 70-fold in a PEG/dextran ATPS and this was attributed to the concentration of the ribozyme strands in the dextran phase, as well as the crowing that aided the folding of the ribozyme [20, 21]. Additionally, nonenzymatic template-directed reactions and ribozyme activities have been studied in coacervates, which resulted in varying product yields, reinforcing the effect of the droplet chemical composition even on prebiotic reactions [22]. As for reactions relevant to metabolism that have been carried out within coacervates, these include the compartmentalization and activity evaluation of hexokinase and glucose-6-phosphatase in polylysine/nucleotide coacervates [23], nonenzymatic oxidation of NAD, and conversion of malonate to 3-Carboxymalate in polyarginine/anionic metabolite coacervates. Also, an increase in reaction rates was observed in aforesaid scenarios due to a high local concentration of solutes such as NADH and ferricyanide [24].

1.2 Membrane compartments and fabricating structurally 'complex' minimal cells

Lipid-based compartments or vesicles made of both phospholipids and single chain amphiphiles (SCAs) have been studied extensively as protocell candidates and for concentrating or crowding molecules [25–27]. Encapsulation of RNA molecules in model protocellular vesicles has been shown to catalyse reaction rates when compared to rates observed in bulk solution (e.g., template-directed replication [28–30]). Similarly, reactions involving ribozymes have been studied inside SCA compartments as well [31]. Protocell compartments have also been shown to act as chaperones, selecting for functional RNAs solely due to the benefit they confer on compartmentalization [32]. One of the major challenges that prebiotic reactions in bulk solutions encounter is the exclusion of 'parasites'; e.g., shorter length RNA sequences that compete for resources and substrates in the vicinity. In this regard, the first pioneering evidence of spontaneous emergence of parasitic sequences was provided by Spiegelman's group in 1967 using Qβ replicase [33]. In this evolution experiment, a Qβ coliphage RNA was subjected to undergo replication using Qβ replicase (an RNA-dependent RNA polymerase). Over time, it was observed that RNA molecules shorter than the original template were formed and replicating much faster than the original RNA. However they did not possess the ability to synthesize the virus particles [33]. Therefore, in such scenarios, elimination of the parasites would become a prerequisite to allow the replication of the functional RNA. In this context, compartmentalization of the functional RNA has been explored as an effective strategy to overcome the competition coming from parasitic sequences [34–36]. Specifically, there is a high chance of generating RNA/DNA sequences during an information transfer reaction that are non-functional but which still are complementary to the template sequence and these can hinder the information transfer process. Significantly, protocell vesicles have been shown to prevent such 'parasitic sequences' from hindering/stalling the replication process [1, 25]. In a study where an encapsulated 'translation-coupled RNA replication' was being evaluated, it was demonstrated systematically that compartmentalization could exclude parasitic sequences and favour functional coding sequences over multiple generations of division cycles [37]. This is fundamentally what a cell does, highlighting the underlying goal of Darwinian evolution of 'self-improvement' by selecting favourable sequences over 'parasitic' ones. Although the advantages of excluding

parasitic sequences are obvious, the 'double origin of life' theory discusses the importance of such parasitic sequences in life's origin. It takes into account the possibility that an organism having a functional metabolism (proteins) but no genetic material (nucleic acid) could have originated first, and the one having only the genetic material (albeit no metabolism) could have been an obligatory parasite on the former [38]. This is relevant to extant biology where certain organisms such as bacteriophages and viruses, possessing only a genetic material, rely on the host protein machinery for synthesizing proteins encoded in their genomes. Thus, for such parasites to exist, the hosts must have been existing from before. To put this theory in context, Freeman Dyson argued that origin of proteins and nucleic acids in a heterogenous prebiotic soup would have had independent origins rather than a simultaneous one. Further, he proposed that 'parasitic' RNA, often harmful for the host itself, could have been synthesized from nucleotides accumulated within primitive cells. These cells that would have lacked genetic material would have contained proteins including enzymes that could oligomerize the accumulated nucleotides. Ultimately over millions of years, the host cells would have evolved to accommodate these parasitic RNA, thereby establishing a symbiotic relationship with the protein-based life, possibly resulting in a functional replication machinery [38]. In this context, it is exciting to consider how lipid vesicles or coacervates could have enabled the concentration of peptides and nucleotides simultaneously, potentially acting as the hosts for the emergence or sequestration/replication of the parasitic RNA molecules. Given this possibility, experimental demonstration of such symbiotic association would allow discerning the evolution of such an assembly into a more complex one. The preceding discussions involving either LLPS systems or membrane-bound compartments, clearly highlight why encapsulating any biologically pertinent system is important while delineating processes and networks that allow us to understand how they actually work. To quote a famous statement credited to Richard Feynman, "What I cannot create, I do not understand". This next section specifically touches upon this creation, as some of the reconstitution efforts by colleagues in the field are allowing us to understand the fundamental design principles that life is based on.

In a living compartment, i.e. a cell, cytoskeleton is an integral part of the cell interior that helps in maintaining shape and spatial organization of molecules. The crucial changes in cell morphology during events like protrusion formation, division or cellular movement, are mainly controlled by cytoskeletal proteins like actin, myosin etc. Although shape changes in protocellular systems have predominantly been studied by inducing them using physicochemical tweaks, recent studies show that using cytoskeletal components makes the process tunable and readily programmable [39]. However, the in vitro reconstitution of the complete cytoskeletal network within protocellular systems is a non-trivial problem. Nonetheless, certain aspects of cytokinesis, cellular deformation and shape changes have been studied using protocells as a model system, e.g., encapsulation of cytoskeleton proteins like actin/myosin, and their subsequent polymerization, have been shown to trigger shape changes in GUVs [40]. Further, these shape changes can be manipulated using patterned surfaces and adherent GUVs to create predictable cytoskeletal polarization [39].

These kinds of bottom-up reconstitution studies have demonstrated the possibility of discerning the fundamental framework of a minimalistic cytoskeletal structure that can support aforementioned functions in a minimal cellular system. Particularly, significant effort has gone into mimicking cellular cortex and actin network due to its crucial role in cell adhesion, cytokinesis and maintenance of the 3D structure of a cell. Majority of the work in this regard has been done using organism-derived or modified membrane anchoring cytoskeletal proteins [41]. Göpfrich and co-workers demonstrated that a similar cytoskeletal organisation could also result in controlled cargo transport through cytoskeletal freight tracks, which they mimicked using DNA nanotube based intravesicular structures [42]. Interestingly, cytoskeletal components themselves (e.g. actin), have also been shown to result in a novel synthetic cell compartment termed 'actinosome', when combined with polylysine [43]. These actinosomes act as a bioreactor to carry out cell-free protein expression. All these studies highlight the array of tunable functions that can be incorporated in minimal systems and the implications these have for using cytoskeletal components in concocting synthetic cells using a bottom-up approach.

2 Role of membrane surfaces in generating functional diversity in minimal cells

In addition to the cell interior, the boundary layer also plays a central role in many cellular functions and their regulation. In addition to lipids, biomembranes consist of different kinds of membrane proteins, lipids and polysaccharides as the main components. To date, thousands of lipid molecules have been discovered and are still being identified across different forms of life [44]. Also, different classes of lipids play crucial roles in various aspects of a living organism, starting from forming membrane boundaries to driving various metabolic processes. Further, diverse lipid molecules have been used to build protocell and synthetic cell membranes, based on the basic research question or for relevant biomimetic applications. Incidentally, the pool of molecules available during life's origins is considered to have been vast and the extent of molecular complexity that has been explored to date in OoL experiments is still really small [45–47].

Given this backdrop, it is not surprising that generating amphiphilic molecules of varied properties that are of biomedical interest, is a happening research area. Also, using membrane-forming molecules that have an interacting

moiety on the head group has been gaining increased traction in OoL research (Fig. 2), e.g., molecular recognition possibility stemming from having complementary nucleobases as the head groups, is being pursued for delineating prebiotically relevant phenomena. Such nucleolipid amphiphiles are essentially hybrid organic molecules consisting of a lipid tail and a nucleobase/nucleoside/nucleotide/oligonucleotide as the head group [48]. They are especially interesting because they inherit the properties of two of the fundamental biomolecules of life. Their amphiphilic nature is required for self-assembly, while the nucleoside component of the head group also confers them with the ability to possess complimentary molecular recognition [49, 50]. Since their discovery in living systems [48], various kinds of nucleolipid molecules containing complex lipid components have been synthesised and studied. Studies have shown that two vesicle populations of nucleolipids that contain Watson–Crick complementary nucleotides can readily interact with each other [51–55]. The mechanism of interaction, however, depends on the structure of the nucleolipids. Berti et al. showed that when complementary nucleolipid vesicles were mixed, they resulted in the formation of hybrid vesicles via lipid flip-flop mechanism [51]. On the other hand, Barthelemy et al. showed that when complementary nucleolipid vesicles were mixed, they can spontaneously fused with each other [53]. Conventionally, membrane-nucleic acid interaction does not happen readily due to electrostatic repulsion between the negatively charged polar head groups of the lipids and the phosphate groups of nucleic acids. It typically requires the presence of divalent cations, like Mg^{+2}, to facilitate the interaction via salt bridges. Nucleolipid bilayer membranes can spontaneously interact with nucleic acids and form non-covalent complexes (lipoplexes), even in the absence of metal ions [55, 56]. Base stacking and hydrogen bonding seem to dominate over electrostatic repulsion while driving these interactions. Interestingly, these aforesaid properties of self-assembly and molecular recognition has also found them being used for concocting systems with interesting material properties useful for drug delivery and as biomaterials [50, 57, 58]. Further, synthetic chemistry studies using nucleolipids have shown the potential of these molecules to self-assemble into vesicles, micelles, hydrogels and organogels [51, 52, 59, 60].

Synthetic functionalization of membranes to result in other kinds of hybrid amphiphiles has also been successfully achieved under abiotic conditions, e.g., synthesis of aminoacylated molecules like *N*-oleoyl glycine under pertinent early Earth conditions, has been recently demonstrated [61]. Similarly, non-biological membrane components like peptide surfactants are also of considerable interest in this context, because integral membrane proteins are critical membrane components in cells [62, 63]. Thus, such hybrid molecules/peptide-conjugated lipids that come in the category of 'peptide amphiphiles' could have also been used in compartments, in prebiotic scenarios. Peptide amphiphiles (PA) are a class of self-assembling peptide-based molecules that have also been shown to assemble into vesicles, micelles, nanotubes, ribbons, nanofibers, bilayers, etc., under specific conditions of temperature, pH and ionic strength [64]. Assembling supramolecular structures of these PAs has been shown to require the participation of functional domains such as a hydrophobic tail, a peptide sequence containing charged amino acids and a functional peptide epitope [65]. Formation of these supramolecular aggregates has been shown to occur above critical aggregation concentration (CAC) similar to what is seen in pure liposomal systems [64].

In aqueous media, such PAs comprising of amino acid residues as polar head groups and aliphatic chains as hydrophobic tails, tend to form single chain compartments [66]. These, vesicles have been shown be very stable and bear close resemblance to that of phospholipid vesicles [67]. Their stability is attributed to the intermolecular H-bonding between the biofunctional peptide epitopes, which also enables the molecules to fold into secondary structures such as α-helices and β-sheets [64–68]. The self-assembly process of PAs to form different nanostructures (to encapsulate molecules) has been modulated using approaches such as using immobilized enzymes, varying surface potentials or formation of hydrogel films [69]. In addition to these unique aspects of PAs, they also demonstrate great capacity for interfacial adsorption and selective affinity to various membrane surfaces. This has made the use of PAs really promising in the field of nanofabrication, biomineralization, membrane protein stabilization, controlled drug/gene release, etc. [63, 70, 71]. The presence of de-novo or biomimetic peptide sequences has been demonstrated for specific encapsulation of molecules of interest, including the encapsulation of hydrophobic fluorophores such as pyrene and drugs like camptothecin [72, 73]. These can be confined within PAs via H-bonding interactions or can be taken up during the self-assembly process. In the OoL context, in prebiotic reactions comprising of both amino acids and lipids, membranes have been shown to catalyse the formation of peptides. This is achieved by concentrating monomers while inhibiting the hydrolysis of the resultant peptides, or by even assisting surface-mediated catalysis through non-covalent bond formation [61, 66].

3 Growth and division in minimal cells: from simple to complex!

Growth, fusion and fission/division are some of the most prominent functions of living membranes. In this context, coacervates have been shown to undergo coalescence, which is a process wherein multiple droplets fuse together to form a single phase that continues to be distinct from the dilute bulk phase [74]. Coalescence is a spontaneous process and is considered as a means of 'passive' growth of model coacervate-based protocellular compartments. Incidentally, this phenomenon also provides an effective mechanism for mixing of contents that have been sequestered in different coacervate droplets present in the same environment. This type of growth, however,

occurs at the expense of a reduction in the overall number of droplets. Therefore, researchers have also studied 'active' growth in coacervates that occurs at the expense of energy [75, 76]. A detailed account of both active and passive growth in coacervates has recently been reviewed elsewhere [74], thus, we mainly focus on the growth and division of fatty acid-based vesicles in this section.

In modern eukaryotic cells or even primitive bacterial and archaeal cells, cell proliferation typically involves growth by means of an increase in cellular volume, followed by division. The division process is strictly regulated, facilitated by a very evolved biological division as against being dependent on any stochastic external processes. However, from a perspective of vesicle-based protocells, the growth is thought to be driven by differential physicochemical membrane parameters, which also leads to an increase in the overall surface area-to-volume ratio, resulting in division/fission (Fig. 2). This process encompasses the splitting of the boundary system to result in two or multiple smaller vesicles or progeny from the mother 'entity'. As for fusion, two or more compartments need to fuse together resulting in the mixing of their contents in the process. Modern cell membranes undergo these complex processes, which are completely dependent on evolved protein machineries [2, 77]. Given the centrality of the aforesaid events to life, mimicking such functions in an artificial minimal protocell has been a very active area of research in the past decade. In this context, different modes of growth and division have been demonstrated from exploiting the lipid membranes' biophysical and biochemical properties, to using external molecular 'implants'.

The simplest mechanism that does not involve any protein machinery was demonstrated by Szostak's group. They showed that a vesicles can grow into a thread like structure and eventually divide by sheer agitation in a process called 'pearling and snapping' [78–80]. This division process was shown to happen in a coupled manner involving the growth of multilamellar protocell vesicles and its subsequent fission could occur [78, 81, 82]. Previous OoL studies indicate that the basic phenomenon, which governs division or replication of a protocell is an increase in entropy. This has been shown to be triggered due to volume/surface ratio imbalance and [83–86] Rayleigh instability generated using physical sheer forces [80, 81, 87, 88]. A phenomenon involving lipid vesicles feeding off of other lipid sources like micelles, to result in growth, has also been shown to involve a physicochemically driven 'minimal' mechanism that involved vesicle growth [89–91]. Interestingly, a bacterial cell (*Bacillus subtilis*) that was stripped off its division machinery showed a very similar mechanism of growth and division, wherein these L-form bacteria were shown to synthesize excess membrane independent of the classical FtsZ machinery [92, 93]. The proliferation in these L-form bacteria is fundamentally dependent on the membrane composition and fluidity and is independent of the cytoskeletal structures; and this is known to be the primary player in cellular shape changes and division [94]. In all, these studies highlight the importance of the underlying biophysical properties of membranes and how these are capable of modulating growth and division aspects of a membrane system that are important to life processes.

Typically, protocell division involving SCA membranes have been shown to possess unique challenges due to their inherent instability and dynamicity. Several mechanisms of vesicle division have been put forth, including the generation of membrane asymmetry by inducing protein crowding [85], or embedding a short DNA catalyst in the membrane that induces membrane curvature [95]. In one study, an elegant mechanism showed some internal control that can be achieved even under these conditions of vesicle division [96]. A model protocell membrane was shown to undergo intrinsically controlled division facilitated by pH change inside the lumen due to an encapsulated internal reaction. The pH change triggered the deprotonation of the SCA head group, resulting in an asymmetry in the membrane that prompted spontaneous division [96]. Achieving membrane fission in phospholipid-based vesicles depends majorly on two important aspects; the shape deformation of the membrane resulting in the formation of a neck, and the subsequent fission of the entity [97]. However, the formation of a neck and the actual separation of the two daughter vesicles is a difficult task in itself due to the intrinsic energy barrier posed by membrane curvature. Previous work involving phospholipid and sphingolipid vesicles has shown that an increase in temperature can cause membrane deformities, leading to neck formation or resulting in dumbbell-shaped vesicles [98]. Also, the varying phase transition temperatures of different lipids in a mixture, wherein some lipids are in liquid-ordered phase while others are in gel phase, has also been demonstrated to result in membrane deformities causing shape changes [83, 99, 100].

Even though growth and division have been demonstrated in reasonably minimal lipidic systems, using a bottom-up approach as detailed above, the involvement of cellular division machinery would allow for more control over the scission process. In such cases, the fabricated artificial cell uses a fine-tuned mechanism that triggers the fission process, resulting in the production of 'consistent' phenotypes in the progeny [101–103]. Cells are known to meticulously control the composition of their membranes and inherits them with high fidelity even after division of the whole cell or of the organelle. The lipid composition bias across two leaflets is also strictly regulated in a cell. In addition to conserving the overall membrane functionality, maintaining such rigorous control is crucial for retaining the microscale physical properties and the functional activity of the embedded proteins present in the membrane [2, 104, 105]. However, achieving such heritable physicochemical properties in a protocell membrane context has been a considerable challenge. In a seminal idea, Doron Lancet and coworkers' theoretical work on catalytic lipid network, discussed the property of autocatalysis or autopoiesis based around the catalytic property of a membrane, terming it a 'lipozyme' [106]. The lipozyme is argued to have functioned in a mutual network within a specific patch of lipid composition present in a supramolecular aggregate. The lipidic chemical information

in these patches are hypothesized to be transferable to another vesicle, as the lipid molecules inherently possess the power of catalysis. These structures were termed 'composomes' or compositional inheritance units. Computational simulation of GARD (Graded Autocatalytic Replication Domain) or amphiphile GARD showed composomes to be catalytically active and capable of transferring information from one generation of lipid structures (e.g. vesicles/micelles) to another. Such systems could have also undergone Darwinian evolution in a prebiotic scenario, which gave rise to the "Lipid world" hypothesis of life's origin [107–110]. However, a crucial empirical hurdle has been the experimental demonstration of compositional inheritance in a non-templated soft-matter system like lipidic vesicles or micelles. Pioneering work by Luisi Luigi's group showed evidence of emergent phenomena like autopoiesis and 'matrix effect'. Subsequently, Stephen Fletcher and co-workers demonstrated the emergence of autocatalytic supramolecular systems from simple building blocks. In a recent article, they also showed multiple amphiphilic species competing for the same source reagent using an out-of-equilibrium reaction setup called 'Continuously Stirring Tank Reactor' (CSTR). Under these conditions, certain species got selected over others, depending on their tendency to phase separate and whether they were able to form a kinetically favoured metastable replicator [111]. Another article from this group also showed how under a relevant selection pressure of pH, vesicles or micellar replicators were preferentially chosen under a hydrolysing condition, while competing for a common resource reactant, by forming dissipative self-assemblies [112]. In another recent computational study, ideas about competition for shared resources were explored. It showed that during growth-division cycles, vesicles could indeed transmit a certain 'compositional state' from one generation to another [113].

4 Communication/signalling circuits between minimal cells

Communication is another key aspect of survival in all organisms living in an ecological niche, ranging from simple bacteria to higher eukaryotes. It regulates a whole range of physiological phenomena [114, 115] including budding, shape transformation, reproduction to even modulating population density [92, 93, 116, 117]. This is chiefly achieved via the transmission of diffusible chemical molecules [118]. Further, the molecular basis of multicellularity and division of labour depends on the ability of individual cells to communicate with the other cells present in the population, which potentially sets the stage for the evolution of cell signaling [119]. Bottom-up synthetic approaches to mimic cell-like communication via small messenger molecules have been of considerable interest to OoL researchers and the cell biology community at large. A new emerging field of cellular bionics deals specifically with synergistically working hierarchical modules of living and non-living artificial systems [120]. An important part of all these bottom-up bioengineering is the crosstalk between different types of compartmentalized systems. Developing synthetic protocells with chemical messenger molecules capable of communicating with each other is considered a big step towards developing a synergistic/antagonistically functional protocellular community [118]. In this regard, the general modus operandi that drives the communication is the release of a transmission signalling molecule from the 'sender' protocell, which is then accepted by a 'receiver' protocell. This is coupled with a downstream signalling pathway and the end product of such a cascade is used as a read out to characterise these processes.

The reaction network generally involves enzymatic cascade(s), gene circuits etc. This mechanism has been broadly mentioned as artificial paracrine or juxtacrine-like signalling in some recent empirical demonstration [121]. The crosstalk using a gene-directed chemical communication between a 'sender' and a 'receiver' protocell is also another approach being used in this bioengineering. Such approaches have been used to understand cell-free gene expression, porin-directed efflux, substrate signalling and enzyme cascade-mediated processing [122]. The importance and implication of such systems comes through in a study where compartmentalized gene circuits and transcription/translation (Tx/Tl) could be carried out in a population of synthetic liposomal protocells. Such synthetic platforms also allow to 'run' mutually non-compatible reactions in different population of compartments, while they can still communicate via the 'sender'- 'receiver' model using a small plasmid as a signalling molecule [123]. Similar approach of gene-mediated communication has been used in a recent study, to mimic host–pathogen interaction, inoculation and immunization in a liposomal vesicle-based protocell [124] using a minimal synthetic cell [125]. This spatial separation of reactions along with controlled yet tuneable communication between them, lays the fundamental groundwork for division of labour by synergy in an interacting population of cells.

Signalling and communication between extant biological cells includes two major types of membrane receptors that play a crucial role, are found embedded inside the plasma membrane: a) G-Protein coupled receptor (GPCR), b) Receptor Tyrosine Kinase (RTK). They transduce the signal by forming dimerised structures or by undergoing structural modification in response to environmental stimuli [126, 127]. Towards this, generation of artificial membrane receptors that mimic such structures, and their incorporation into synthetic cells has been demonstrated [128]. A recent study described one such artificial signal transduction module that is induced by low pH-mediated i-motif formation and dimerization of DNA-based receptors. The dimerization ultimately results in a G-quadruplex/hemin-based fluorescence inside a GUV-based model protocell model used as a proxy for successful downstream signaling [128]. Wu and co-workers have demonstrated how a membrane-anchored synthetic receptor

can undergo structural changes in response to the pH change. This was shown to enable the recruitment of a membrane-proximal protein that initiated a cascade of downstream signaling [124]. Such artificial systems are essential tools to understand the underlying molecular mechanism of biomolecular interactions and could also be used as a potential tool for drug delivery, intelligent biosensing and logic gate sensing etc. In this context, other kinds of DNA structures have also been transformed into an artificial receptor system. Self-assembled DNA-tiles have been integrated inside the lipid bilayer as an artificial receptor [129], while a single membrane-spanning DNA-duplex has been also been shown to act as an ion channel [130]. Certain other types of protocell communication structures have been described in literature with Proto-cellular Nanotube Network (PNN) being the most recent one. A PNN consists of several surface-adhered lipid compartments physically coupled by lipid nanotubes [131]. These are formed by accumulating multilamellar vesicles on to silicon oxide surfaces in an aqueous environment. PNN is used to demonstrated how molecular transport could have been a potential means of communication in protocell communities during life's early stages [131].

Living cells process the chemical signals emerging from the surrounding population, to evoke a collective response similar to what is seen in quorum sensing, morphogenesis, and regeneration. Few emerging applications involving tunable and robust synthetic communication platforms are also being used to study the interactions of synthetic protocells with living biological tissues; a valuable tool for targeted delivery and regenerative medicine. In a recent review, Mukwaya et al., have categorised living cell and synthetic cell communication in three ways: (1) distributed populations and through-space signal processing, (2) nested populations and embedded signalling, (3) interfacially connected populations or contact-dependent signalling pathways [114]. Such interfacing systems of living and non-living systems have been shown to interact by Lentini et al. in their work, between *E. coli* cells and liposomal vesicles that were made of phospholipid and DNA [132]. This involved the use of Tx/Tl machinery and small molecule signalling. On similar lines, the predatory behaviour mimicked by proteinosome-based protocells have also been exploited effectively for controlling or targeted killing of live bacterial cells [133]. Such an approach capable of engineering controllable signalling network might eventually pave way for protocell-based cytomimetic functions and architecture, or tissue-like bio-inspired network formation [118], some prospects of which are mentioned hereafter.

5 Cytomimetic compartments as an emerging frontier in translational applications

As is evident from discussions thus far, compartmentalization has been an essential tool for developing minimal cell-based applications. However, whether these compartments can support all the processes entailing the central dogma of life is yet to be seen. Towards making artificial 'living' cells, and a cell-free Tx/Tl machinery, LLPS systems are being actively pursued as the compartment system of choice given their preference for encapsulating specific kind of molecules and their tunability with respect to releasing the inner contents. Also, applied research involving targeted and more effective cargo delivery systems that could also be used for synthetic bioengineering applications is a big thing in the field of modern biomedicine. Given their potential to facilitate crowding, LLPS systems were recently explored in the context of in vitro transcription and translation (IVTT) reactions. Earlier demonstration of IVTT mainly used water-in-oil emulsion or aqueous two-phase system (ATPS)[134]. Sokolova and co-authors showed that coacervates containing *E. coli* cell lysate could be used to carry out transcription and translation processes to result in the expression of a fluorescent protein (GFP) and at enhanced rates [135].

Further, to mimic closely the cellular environment where multiple phases coexist, cell-free protein expression was carried out in ATPS and A3PS (aqueous three phase system) droplets encapsulated within mineral oil [136]. A complex coacervate system composed of carboxymethyl-dextran/polylysine (CM-dextran/PLys) was also found to facilitate cell-free expression of a fluorescent protein [137]. However, the expression was adversely affected by the presence of PLys alone, which suggests that the interactions between the components of the IVTT machinery and cationic peptide could be unfavourable [137]. This implied that while considering complex coacervates as an artificial cell system, the ratio of charges in a given complex would need to be finetuned depending on the biological function in question. In this context, a recent study used a comprehensive approach to find out the stability of various coacervate systems towards IVTT components, and determined the expression of a fluorescent protein inside the droplets using confocal microscopy [138]. LLPS systems could also be used as drug molecule production modules, given their ability to carry out IVTT reactions. Moreover, the ability to concentrate molecules in a selective fashion has seen coacervation being extensively be used in drug delivery and biopurification too [139]. While considering replication and Tx/Tl in an artificial cell, there is always the threat of invasion by parasitic nucleic acid molecules [37]. This is especially pertinent in LLPS systems because of absence of a boundary system that allows for the ready diffusion of molecules. To address this, translation-coupled RNA replication was carried out, where fluorescently labelled genomic RNA and parasitic RNA were sequestered in different populations of ATPS droplets prior to initiating the reaction. This multiple population approach achieved inhibition of the parasitic RNA replication. Moreover, when present together, the replication of the genomic RNA was more efficient

than the parasitic RNA in the presence of ATPS, suggesting that multiple LLPS compartments could indeed facilitate parasite exclusion [36].

Coacervates have also been explored as drug delivery vehicles because of their following properties: (1) they assemble in aqueous solvents as against organic solvents (the latter affects the bioactivity of the drug or the protein); (2) they carry high cargo loading capacity; (3) coacervates, as mentioned earlier, possess the capacity to selectively partition specific solutes; (4) because of the nano- to micron-level size they are easy to administer[140–142]. Towards targeted drug delivery, elastin-like peptides, chitosan aggregates, and heparin-based coacervates have been used to study solutes ranging from anti-cancer drugs to those that control growth factor activity, and also in oral drug delivery. Since the drug is bound to a polyelectrolyte, its potential side reactions with the host molecules are prevented to a large extent [140]. Furthermore, molecules that are otherwise poorly soluble in water can be sequestered and transported via LLPS systems [141, 143, 144]. LLPS-based delivery systems have also been shown to effectively sequester and release drug molecules to a specific target [145]. Moreover, several LLPS delivery systems are comprised of components such as polylactate, polyglycolate and other aliphatic copolymers, which are non-toxic and shows less untoward side effects to the host and have been shown to degrade within days or a few weeks [141].

Another rapidly emerging field in the applied aspect of such compartments is 'UFAsomes'. Gebicki and Hicks in their seminal work demonstrated the capability of Unsaturated Fatty Acids (UFA) to form bilayer compartment structures, which were termed as ufasomes [146, 147]. Interestingly, they are similar to prebiotically plausible compartment building blocks that might have been present during the genesis of life. Ufasomes are capable of carrying hydrophilic drugs in their lumen and hydrophobic drugs in their lipidic bilayer, making them an excellent delivery system choice for a wide range of drugs. Such membranogenic SCA molecules like oleic acid and linoleic acid have been predominantly shown to be one of the best choices for ufasome-based drug delivery systems. These SCAs are naturally found, not toxic and show high drug retention in vivo [148]. Also, having such compartments delays rapid elimination of the drugs that are otherwise metabolized quickly, thereby decreasing the need for frequent doses. Ufasome-based systems have been used extensively against fungal infection and drug delivery through skin barrier [148, 149]. Stratum corneum of skin is one of the most resilient barriers; use of glycerol oleate-based ufasomes for antifungal treatment against the well-known *Candida albicans* has demonstrated improved efficacy in this regard [149]. Another study highlighted increased uptake of a small proxy molecule carboxyfluoroscein in the rat intestine (independent of endocytosis) when delivered via ufasomes [150].

SCA-based ufasomes can be of various kinds depending on their building blocks and their functions. Given this, understanding the properties of each molecule involved to narrow down the choice of SCA while fabricating ufasomes is a critical step [148]. Since, ufasomes or SCA vesicles are capable of encapsulating genetic materials as well, they are important candidates for targeted gene delivery, genetic medicine and tunable horizontal gene transfer. Also, ufasomes excel as carrier systems as they protect the mRNA/DNA/proteins from degradation of nucleases and proteases in [151]. Oberholzer and colleagues in their study showed the average lumen volume of such ufasomes is enough to host up to 10 dsDNA strands [26, 152]. All such studies clearly underline how ufasomes are an emerging delivery system that can help translate basic research of amphiphiles into biotechnology and drug delivery applications effectively.

6 Where do we go from here?

A substantial body of work has demonstrated the different modes of compartmentalization, and the many pros and cons of such compartments. On the one hand, much of the basic phenomena discussed in the aforementioned sections have been implemented in several applications. These include fabrication of nanoreactor systems, creating biomimetic supramolecular structures, fabricating various kinds of biosensors and drug delivery systems. However, compartmentalization is only one of the hallmark features of life. A more realistic and pragmatic approach to practising the science of synthetic life biology involves the concoction of a hierarchical multi-compartment systems. Such an architecture can readily reorganize spatiotemporally in response to different stimuli like in a true 'living' system. Cells that are encountered in extant biology are very architecturally complex entities with strict spatial organization that is subjected to extensive temporal regulation. A hybrid multi-compartmented architecture consisting of LLPS- and membrane-based modules is what we are referring to here as a hierarchical compartment module (HCM) (Fig. 3). Fundamentally, HCMs are distinct only in terms of the molecules that they harbour and the type of compartment that makes them; the hierarchy is strictly in terms of modularity of the superstructure comprising of both droplets and membrane boundary entities. Examples of bottom-up synthetic systems that can result in the formation of HCMs are nested vesicles (Fig. 3), membranized coacervates and LLPS in polymerosomes, etc. [5, 16, 136, 153]. With the advancement of analytical techniques like FACS and use of high precision tools like microfluidics, the formation of HCM has made good progress. One pertinent area in the context of biomimetic compartments that has not gotten the attention it deserves and is just catching up relates to HCMs that essentially have internalized LLPS/coacervates and are encapsulated in a "biologically relevant" lipid vesicle.

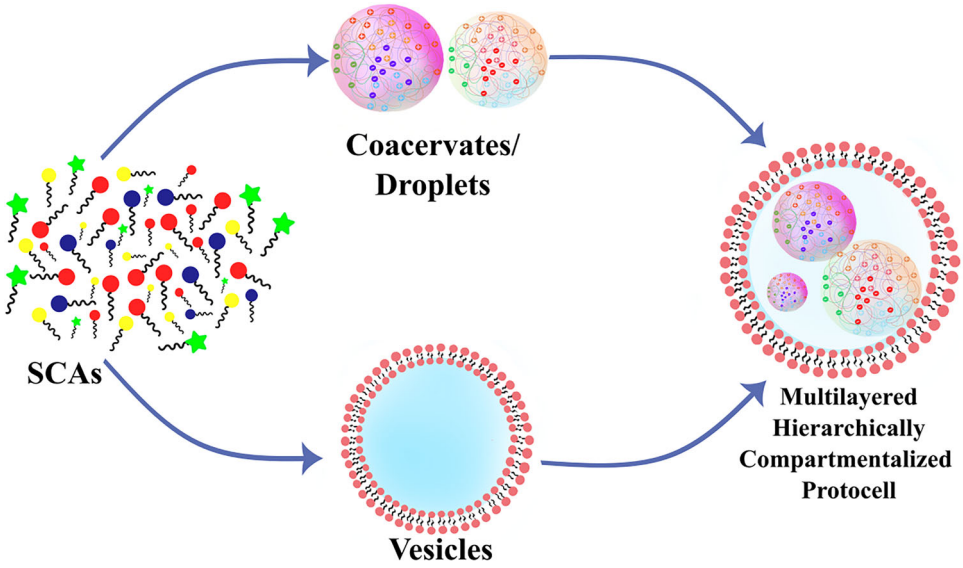

Fig. 3 The schematic representation of how a multi-compartmented hierarchical compartment module (HCM) can be formed, using fundamental components that are also pertinent to origins of life. Single-chain amphiphiles (SCAs) are very relevant candidates in this context that are prebiotically plausible and have been demonstrated to result in both LLPS/droplet and vesicle formation. Taken together, different kinds of SCAs can be used to create robust chemically distinct HCMs, since SCAs can form both vesicle and LLPS/droplets, using a bottom-up approach that involves the fabrication of increasingly complex cellular entities

A good sum of work from Stephen Mann's group has led to the demonstration of a wide array of membranized coacervate systems that have been made with block copolymers, proteinosomes or different types of lipids [5]. These supramolecular aggregates, predominantly made of synthetic molecules [154–156], have been demonstrated to be capable of mimicking relevant functions including membrane-mediated chemical signalling, DNA-based signaling, etc. Demonstration of proto-tissue-like structures using caged coacervates and multi-switch chemical signalling among protocells has also been achieved using varying kinds of coacervates depending on the reaction/pathway studied [5, 118, 157]. For example, in one of the more common scenarios of a coacervate system, which is often charge-based LLPS droplets, is the exclusion of molecules having the same charge due to repulsion. In a recent study involving stimuli-responsive amphiphiles that could form membranes, it was shown that they could result in tunable systems that formed artificial chemical signal releasing organelle-like structures [158]. Specifically, this study showed that thermo- and photo-responsive lipid vesicles can trigger chemical signalling in bacteria.

Single-chain amphiphile (SCA) systems or ufasomes are also known to be environment-sensitive as their intrinsic dynamicity makes them hyper-responsive to environmental fluctuations. Moving from a classical protocell, which is for the most part a bag of interacting biochemical molecules, a spatially separated system of coacervates inside a large vesicular system seems readily amenable to being devised using bottom-up synthetic biology and, more recently, also involving systems chemistry approaches. Invoking straightforward chemical and physical processes, a study showed how a PDDA/Polylysine/ATP coacervate that was coated with oleate amphiphiles resulted in biomimetic compartments capable of molecular uptake and fusion, while also being prebiotically plausible. Fatty acid SCAs have recently been demonstrated to form coacervate droplets as well. These fatty acid coacervates using guanidium myristate form via a unique mechanism by making worm like micelles that fold into droplets [159]. Such fatty acid-based coacervates have been shown to participate in forming hierarchical synthetic protocells and can carry out biomimetic chemical coupling [154]. These studies indicate how fatty acid systems have a lot to offer and are yet to be exploited fully for their varied potential. Fatty acids can form vesicles, micelles and coacervate droplets depending on the experimental conditions (Fig. 3). They have even been shown to be undergo transition between 'open' diffusible coacervates and 'closed' vesicular systems [159]. In future, understanding how different kinds of SCAs can impart specific characteristics on the compartments they make, can facilitate the fabrication of HCM systems using SCAs for forming coacervate cores that can be surrounded by a fatty-acyl membrane. Recently, work from Nicolas Martin's group demonstrated that a sodium oleate, 1-decanol mixed fatty acid membrane system could support such nested protocell formation with an aqueous PEG-dextran droplet inside [160]. Also, taking advantage of the inherent differences in the chemical properties of different building blocks, coacervates have been made that can remain separate without coalescing and can show selective partitioning while coexisting in bulk phase [161].

The aforementioned combinatorial approaches would allow the incorporation of complex functional aspects such as molecular recognition, organizational segregation and regulated functioning, in an enclosed space like what is observed in a complex modern cell. Nonetheless, many aspects still need to be systematically addressed before this is successfully achieved, e.g. selectively permeable boundary system, active membrane transport etc. continue to be challenging problems. The degree of tunability of a membrane system, and the uptake of molecules, both depend greatly on the constituents of the membrane system in question. It is highly unlikely that a single kind of model compartment system would be able to perform all kinds of biomimetic functions, starting from life's origins right up to the formation of a complex living cellular system. In this regard, there remains a sizable knowledge gap that is required to seamlessly bridge the bottom-up approaches of using protocells, with the top-down approaches that use primitive living cell models. The first living system or LUCA (Last Universal Common Ancestor) still seems like a long shot, especially if using only bottom-up approaches, as LUCA still needs hundreds of genes to survive [162, 163]. On the other hand, most of the top-down approaches inspired by the central dogma have been centred around and involve the use of genome sequence. Some of the simplest organisms belong to genus mycoplasma that seem to have the smallest genome. Using a top-down approach, researchers produced an organism with 'minimal' genome comprised of 473 genes (JCVI-syn3.0) using genes from an already modified *Mycoplasma mycoides* [164]. However, natural division was not possible using this minimal strain, as 19 more genes were required. This was incorporated in a strain named JCVI-syn3.0a which can divide phenotypically similar to a wild-type *M. mycoides* cell [165]. However, in all such cases the role of membrane is unclear. In a study that we discussed earlier, researchers achieved a primitive form of bacterial division using L-form bacteria and this was without a classical FtsZ machinery [92, 94]. This mode of top-down division is strikingly similar to a bottom-up division mechanism using SCA vesicles that happens by pearling, blebbing and snapping into randomly sized daughter compartments [78, 87]. An intermediate semi-synthetic approach of using parts of a living cell(e.g. organelles, metabolic pathway components), with non-living vesicle-like compartments, would be a good starting point to merge bottom-up and top-down approaches [166, 167]. Such approaches, which are being referred to as 'middle-out' approaches [166], provide a unique insight of basic life-like processes [102, 168]. Such findings in a larger context indicate that a reconciliation between top-down and bottom-up approaches can indeed provide unique insights into the workings of living phenomena. Therefore, it is really important to understand the design principles and underlying mechanisms that govern the existence and functioning of compartment systems.

In conclusion, we have tried to explore multiple types of compartmentalization and crosstalk between them that would have eventually resulted in the emergence of a complex HCM system architecturally akin to the modern cell. We have comprehensibly discussed various studies that use relatively simple molecules such as fatty acids, polypeptides and nucleotides. These are relevant both biologically and prebiotically, which allows us to bridge the understanding of life's origin with discerning how a synthetic bottom-up approach could enable a deep understanding of the fundamental design principles of compartmentalized living systems. In principle, doing this could open-up an 'exciting' Pandora's box of artificial cell-based technologies, which could shed light on how systems chemistry would have transitioned to complex biology while also enabling a better understanding of the evolution of early cellular complexity.

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Author contributions

SD, GP and SR conceived the overall idea and structure of this review article. SD created the illustrations. SD, GP and SR wrote the article with VS, SM, RR, UB and NNK contributing towards some sections of the manuscript.

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Declarations

Conflict of interest The authors declare no conflict of interest.

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