ACCOUNTS
of chemical research

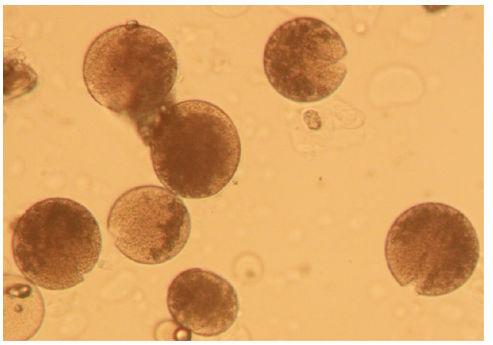
Systems of Creation: The Emergence of Life from Nonliving Matter

STEPHEN MANN*

Centre for Organized Matter Chemistry, School of Chemistry, University of Bristol, Bristol BS8 1TS, United Kingdom

RECEIVED ON NOVEMBER 4, 2011

CONSPECTUS



The advent of life from prebiotic origins remains a deep and possibly inexplicable scientific mystery. Nevertheless, the logic of living cells offers potential insights into an unknown world of autonomous minimal life forms (protocells). This Account reviews the key life criteria required for the development of protobiological systems. By adopting a systems-based perspective to delineate the notion of cellularity, we focus specific attention on core criteria, systems design, nanoscale phenomena and organizational logic.

Complex processes of compartmentalization, replication, metabolism, energization, and evolution provide the framework for a universal biology that penetrates deep into the history of life on the Earth. However, the advent of protolife systems was most likely coextensive with reduced grades of cellularity in the form of simpler compartmentalization modules with basic autonomy and abridged systems functionalities (cells focused on specific functions such as metabolism or replication). In this regard, we discuss recent advances in the design, chemical construction, and operation of protocell models based on self-assembled phospholipid or fatty acid vesicles, self-organized inorganic nanoparticles, or spontaneous microphase separation of peptide/nucleotide membrane-free droplets. These studies represent a first step towards addressing how the transition from nonliving to living matter might be achieved in the laboratory. They also evaluate plausible scenarios of the origin of cellular life on the early Earth. Such an approach should also contribute significantly to the chemical construction of primitive artificial cells, small-scale bioreactors, and soft adaptive micromachines.

1. Introduction

At the most fundamental level, life as we know it is a materialistic phenomenon, which generates and maintains its existence as a distinct system by internalized processes that are self-regulated and coupled to the external environment. Significantly, the origin of life and the advent of cellularity on the early Earth appear to be coupled at the deepest level. Cellular structures reminiscent of photosynthetic bacteria have been discovered in Archean rocks[1] as far back as around 3.5×10^9 years (Ga) ago. Given that the age of the earth is estimated to be 4.5 Ga and that the initial stages of planetary geochemistry are considered inhospitable to the emergence of life, the transition from nonliving to living matter is delineated by a time window of around 500 million years. Assuming that operational cellular forms were not seeded on the early Earth during this period from extraterrestrial sources such as meteorites and comets, it follows that this stage in the Earth's history was marked not only by the emergence of prebiotic chemistries with replicative and evolutionary potential but also by the advent of protocellular constructs comprising primitive life-like functions.

10.1021/ar200281t

While the nature and diversity of this hypothetical "life before biology" may never be known, the universality of cellular life on the Earth strongly suggests that the onset of protolife was contingent on the emergence of viable archetypes of cell-like construction and operation. But how did the first cells emerge in a world devoid of biological evolution? Solving this long-standing mystery is of deep significance because understanding the origin of cellularity bridges the conspicuous disconnection between nonliving and living manifestations of matter and provides a unifying theory for the emergence of biology within a physical universe. Moreover, can the abiogenic transition of nonliving to living matter be realized in the laboratory using synthetic protocols?

The foundation of modern research on the origin of life is based on the concept of molecular evolution as a chemical progenitor of biological evolution.[2,3] While much attention is being focused on the molecular origins of chemical evolution (see articles in this Special Issue) and alternative chemical worlds based on RNA[4] or proteins and peptides (metabolism-first scenario),[5] less emphasis has been placed from a chemical perspective on the criticality of higher-order processes for the emergence of life. In this Account, we adopt a more systems-based perspective to first delineate the notion of cellularity, with specific attention focusing on core criteria, systems design, and organizational logic and emphasis being placed on the central importance of basic autonomy and nanoscale phenomena in the origin of cellular systems. We then review and discuss recent advances in the emerging field of what could be called *protobiology*,[6] with an emphasis on the design, construction, and operation of protocell models. We highlight three key strategies: use of synthetic vesicles prepared by the self-assembly of phospholipids or long-chain fatty acids, self-organization of amphiphilic inorganic nanoparticles to produce enclosed semipermeable inorganic vesicles, and spontaneous membrane-free compartmentalization based on charge matching between simple peptides and mononucleotides. The main conclusions are presented in the final section.

2. Cellularity

2.1. Core Criteria. Living cells can be considered as soft, wet machines encoded in the language of chemistry, and as such, they exist in the form of highly dynamic and complex biochemical networks. It is useful therefore to distill this complexity into a set of universal principles that capture the essential operational properties of life as we know it. In brief, the key features of modern cells include the following:[7]

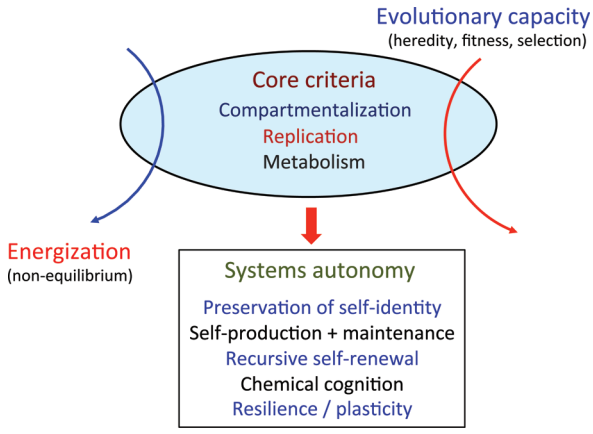


FIGURE 1. Cellularity: core criteria and systems autonomy in living organisms (see sections 2.2 and 2.3 for further details).

- *Compartmentalization*: A semipermeable membrane physically encloses the internalized constituents of the cell and acts as a selective barrier between the external environment and cell interior; as a consequence, the influx and efflux of materials and energy is highly regulated. Compartmentalization is also of key importance for the spatial coupling of genotype to phenotype and provides protection against parasitic attack.
- *Replication*: Genetic information is carried in the form of double-stranded molecules of DNA that are inherited by daughter cells during cell division. Template-directed polymerization is used as the universal mechanism to copy hereditary information. This takes place by protein-mediated transcription of the genetic information stored in DNA into RNA and translation of RNA into proteins (the so-called "central dogma" of biology).
- *Metabolism*: Protein-based catalysts (enzymes) are used in myriad chemical transformations for self-maintenance and self-renewal, as well as in informational processing (transcription, translation, and DNA replication). The feedback loop between DNA and protein biochemistry is the basis of the self-reproducing capacity of living cells.
- *Energization*: The cell is maintained in a dynamic steady state (*homeostasis*) arising from nonequilibrium conditions that require a continuous influx and transduction of energy from the surroundings to sustain life and generate growth and division.
- *Evolutionary capacity*: Considered in terms of population genetics, cells exhibit the ability to adapt to changes in

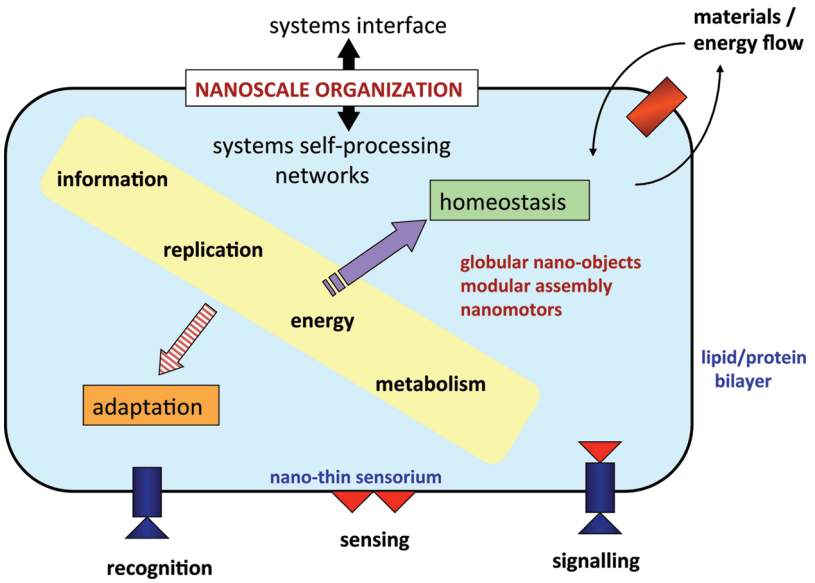


FIGURE 2. Systems of cellular life.

their environment through evolutionary processes involving the interplay of heredity, variation, fitness, and selection pressures.

Given the wide diversity of these criteria, it follows that their integration and collective operation, rather than their individual pre-eminence, mark out the essence of cellularity and hence the phenomenon of life. Thus life can be considered as a *systems property* that is internally maintained and regenerated under nonequilibrium conditions by flows of energy, matter, and information (Figure 1). Moreover, the system persists locally in time and space in the form of self-defining autonomous units and across millennia as species sculpted by evolution. As a consequence, even the simplest cellular systems are endowed with a basic autonomy exhibiting a range of emergent properties (see box in Figure 1), which together give rise to highly organized and complex behaviors in the absence of any underlying intentionality.

2.2. Systems Design. According to the above criteria, a living cell represents a spatially enclosed autonomous chemical system that is continually undergoing internally directed self-generation and self-maintenance through the action and interaction of myriad metabolic processes, which are orchestrated by the flow of genetic information and energy gradients that operate under nonequilibrium conditions. From a systems perspective, it follows that the cell comprises two key operational features: (i) an internalized systems network for dynamical self-construction and self-processing and (ii) a systems interface for coupling the internal networks via active exchange with the external environment (Figure 2). The former is involved with the storage and generation of energy and information, metabolic activity, gene replication, and various ancillary activities associated with cellular logistics (protein sorting, trafficking, servicing, etc). In contrast, the latter, which is in the form of a nanometer-thin phospholipid-based bilayer with embedded or peripherally attached proteins, not only serves as a semipermeable barrier for the containment, transfer, and exchange of materials and energy but, significantly, is a highly advanced sensorium for cell/molecule and cell/cell recognition and signaling. Together, these processes constitute the basis of cellular autonomy,[8] which is maintained as a nonequilibrium system that is intrinsically self-referential and dependent on nanoscale organization (see section 3 for further details). In this way, cellularity is different from more conventional nonequilibrium dissipative structures, because the flow of energy and matter through the latter is not regulated by the internal organization of the system but is dependent on physical boundary conditions in the external environment. In contrast, living organisms are internally geared at the deepest level to the preservation of systems integrity.

At the level of the individual cell, the coupling between internal and external systems operations manifests in a pseudo-steady-state of materials and energy fluxes, which is maintained through hierarchical loops and networks that are capable of passively or actively assimilating novel environmental inputs into the pre-existing pathways without undermining viability. This implicit "adaptive robustness" is achieved by a high degree of systems vigilance and tolerance associated with maintaining a metabolically off-line

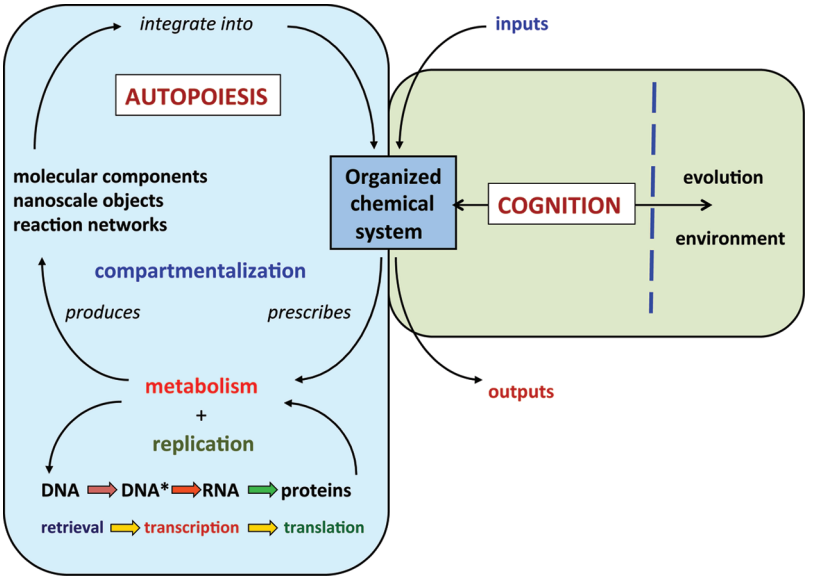


FIGURE 3. The organizational logic of modern cellular systems (adapted in part from ref 3). The boxes refer to different domains of organization based on autopoietic or cognition processes.

genetic code, high-fidelity mechanisms of error correction and repair, and efficient levels of molecular degradation and removal. The key feature of this resilience when viewed from a systems perspective is that it is based on an underlying plasticity in the operations taking place both internally and at the cell/environment interface. As such, the cell has the capacity to adopt a multitude of potential internal states in response to the immediate environment. This plasticity, which is based on the coupling of molecular recognition and systems-determined agency, suggests that even the simplest organisms exhibit *basic cognition*.[3,9] This does not imply that cells are merely information processing systems; on the contrary, they comprise embodied knowledge that has been retained by natural selection and that is accessible to the organism in the form of predetermined responses given the appropriate chemically based triggers.

2.3. Organizational Logic. The mechanistic complexity and wide ranging landscape of cellular biochemistry can be reduced to a common systems-based form of organizational logic that serves not only as a universal feature of life but also as an archetype for the realization of minimal as well as synthetic forms of living matter. Viewed as a fundamental unit of life, the cell comprises a system that is distinguished by the continuous constitution of an autonomous identity through an inherent capacity for adaptive self-maintenance and self-construction. As discussed above, this occurs via recursive processes that are internally placed but coupled at a deep level with the environment via chemical cognition at the cell surface. Significantly, the function and constitution of such a system are indissolubly intertwined, such that the activity of the system is always self-referential in the sense that the operational unit is constructed from the continuous production and regeneration of its constituent processes and components. Such a system has been termed *autopoietic*[10,11] and is considered to represent the basis for the organizational logic of living matter. As shown in Figure 3, such a system is recursive and operationally closed from the perspective of the individual cell because the overriding function is to maintain the persistence of the very processes that produce the organizational constraints; that is, the system represents a form of self-realization in that it is its own cause and effect. Moreover, this logic is coupled at the deepest level to the environment through the sensorium of the systems interface, with the result that unlike inanimate matter, living organisms are endowed with teleonomic properties arising from an apparent purposefulness in the absence of a central organizational agent.[12] As a consequence, a key aspect of the organizational logic of the cell is that autonomy at the level of the individual is inseparable from the evolutionary capacity of life and the dynamics of population genetics.

2.4. The Origin of Cellularity. The above considerations have a profound consequence for the mechanistic understanding of the origin of cellularity and in achieving plausible representations of the transition from nonliving to living forms of matter in the laboratory. It seems reasonable to propose that given a prebiotic world replete with organic macromolecules with potential informational and catalytic

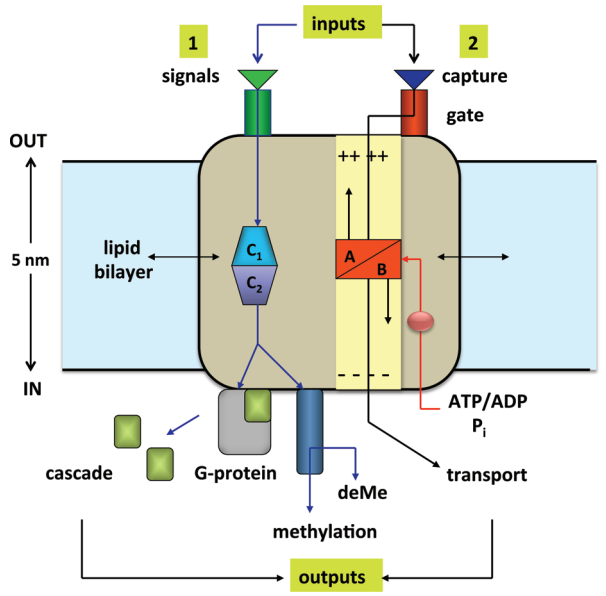


FIGURE 4. Cellular cognition via nanoscale operations. The schematic illustrates the key operations of integral membrane proteins housed within the 5 nm thick phospholipid bilayer of the cell membrane. Information flows across the membrane via receptor-mediated (1) or transport-mediated (2) pathways. In pathway 1, extracellular binding of small molecule chemosensors induces conformational rearrangements (C_1, C_2) in the membrane-bound receptor that influence intracellular signal transmission by glutamate methylation/demethylation (deMe) or binding/release of G-proteins/αGTP-bound subunits on the cytoplasmic side of the receptor. In pathway 2, extracellular binding of selected ions and molecules results in transmission of information via gated responses determined by proton gradients, electrochemical potentials, auxiliary ligand binding, or photoinduced conformational changes. Typically, antiport transport of species (A, B) is switched on or off by phosphorylation (P_i) reactions involving chemical activation via ATP binding. See ref 16 for more details.

properties, the advent of self-assembled compartmentalized microstructures (vesicles, aerosol droplets, foam-like inorganic minerals, etc.) that were capable of accumulating and integrating diverse combinations of these reaction components would be a logical step toward chemically based systems exhibiting basic autonomy. In this scenario, molecules that are essentially noninteracting prior to encapsulation become functionally important when corralled into the confined reaction media. As a consequence, the operational viability of the compartmentalized system as a sustainable reaction environment critically depends not only on the chemical composition but also on the spatial organization and time-dependent exchange of matter and energy with the surrounding environment. Thus, while the notion of a RNA world is certainly attractive,[13] compartmentalization of protoribozymes and RNA replicases would be a necessary condition to provide the energization required for even the most primitive replication processes, as well as to increase the efficiency of information transfer within a diffusion-limited environment. Moreover, confinement provides a mechanism for forging the intimate connection between genotype and phenotype and helps to curtail the combinatorial explosion of possible chemical reactions and reduce parasitic scrambling of the system.[14]

While the transition from the advent of autonomy to the origin of cellularity requires the unfolding of the evolutionary capacity of informationally rich compartmentalized systems, the possibility that the progenitors to life were essentially protometabolic and devoid of significant genetic content cannot be ruled out.[15] Indeed, one could argue that only in the presence of a metabolic context does the replication of information have any functional meaning.

3. Life as a Nanoscale Phenomenon

A characteristic feature of living systems is that they are contingent on the emergence of nanoscale components and operations.[16] This stems from the inevitable consequence of the up-scaling required for gathering, storage, processing, and transmission of chemical information based primarily on the input and reactivity of small molecules (Figure 4). Thus nanoscale organization is a natural prerequisite for self-renewal and adaptive mechanisms to emerge in chemically cognitive systems and, as such, places significant constraints on the structural evolution of the cell membrane and modes of operation of early metabolic and information processing networks.

Based on our current knowledge of the stability of biomacromolecules, we can make the general proposition that the evolution of an integrated and functional cell membrane, as well as the emergence of metabolic processing networks based on globular polypeptides, appears to be dependent on the up-scaling of molecular interactions to length scales beyond 3 and 2.5 nm, respectively.[16] These boundary conditions are imposed by structural and energy instabilities associated, respectively, with phospholipids or polypeptide chains of insufficient length and amphiphilicity, which in turn necessitate the optimization of scale-dependent parameters under distinct physicochemical constraints. For example, factors such as membrane fluidity, bending/rigidity, and conformational matching/mismatching between lipid chains and integral membrane proteins appear to be optimized for a membrane thickness of around 5–6 nm.[17,18] This corresponds to a mean acyl chain length of 16–18 carbon atoms. Much smaller chains, for example, comprising less than 9–10 carbon atoms, destabilize the

bilayer toward micelle formation,[19] while longer hydrophobic tails generate thicker membranes with reduced fluidity, making them less sensitive to transmembrane activities involving signaling and transport. As a consequence, there appears to be an optimum length scale in the thickness of the lipid bilayer that is coextensive with the evolution, integration, and operation of integral membrane proteins.

Similarly, the optimum domain size for globular proteins is approximately 4.5 nm (150 amino acids) due to nanoscale constraints on the folding of polypeptide secondary structures.[20] Reducing the sequence length below this threshold value to 20−25 residues results in insufficient domains of amphiphilicity in the amino acid side chains necessary for formation of a persistent globular architecture with delineated reaction spaces and integrated conformational dynamics. Because these properties are required for the controlled processing of small organic molecules, this boundary condition, which occurs at around 2.5 nm, sets a critical constraint on the up-scaling associated with the emergence of activities involved with enzyme-mediated self-processing and signal transduction-based cellular cognition.

4. In Search of Protobiology

It seems reasonable to propose that the first cells to appear on the early Earth (assuming they were not derived from advanced extraterrestrial sources!) were much simpler systems than contemporary cells. In the absence of advanced nanoscale machinery, basic cellular functions would be more strongly dependent on physicochemical and geochemical interactions and constraints operating between molecules housed within the primitive cells and those in the surrounding environment. It therefore seems reasonable to propose that one of the key steps in the formation of a hypothetical protocell involved the spontaneous self-ordering of a mixture of abiogenic molecules under appropriate conditions into compartmentalization modules capable of primitive forms of replication or metabolism, or both. This notion constitutes the basis of bottom-up approaches to the laboratory construction of protocell models exhibiting minimal representations of the core criteria of life (see section 4.1). In contrast, top-down approaches, in which contemporary cells are progressively simplified by removing genes not considered necessary to sustain the essential properties of cellular life, are also being actively pursued. This is a synthetic biology approach that aims to engineer a minimal cell comprising the lowest number of genes necessary to maintain basic cellularity (see ref 21 for further details). Taken together, the bottom-up and top-down strategies are complementary approaches to the modeling of protocells and span a wide spectrum of organization and complexity ranging from retro-engineered modern cells to ensembles of simple nonliving molecules.

4.1. Organic Membrane-Based Models of Minimal Cellularity. A key element of the bottom-up approach to protocell construction is the development of appropriate models of minimal membrane formation and function. As discussed above, compartmentalization is a necessary criterion for the implementation of processing and cognition networks within modern cellular systems, and this view holds for the emergence of primitive cells with viable replication and metabolic strategies. In particular, an enclosed, semipermeable barrier is required to restrict the transport and accumulation of nutrients from the environment into the protocell interior, and as a consequence, only certain molecules of small size and appropriate polarity have sufficient membrane permeability to become enriched within the interior. This then opens up the potential for novel chemistries within the protocell that are separated from but connected to the ambient conditions. In principle, concentration and electrochemical gradients across the protocell membrane can be induced by partitioning the molecules between the inside and outside of the protocell membrane and used to drive internalized reactions against free energy. If these reactions produce energy-rich molecules capable of promoting polymerized products with low membrane permeability, then self-assembly and entrapment of structures with supramolecular and nanometer length scales could extend the chemistry within the protocell interior beyond that possible with a library of small molecules.

With these objectives in mind, the facile entrapment of aqueous microvolumes associated with the spontaneous self-assembly of amphiphilic molecules, such as phospholipids or long chain fatty acids, into spherical bilayer vesicles has had a profound impact on the modeling of protocellular systems. Indeed, many researchers consider this mechanism of compartmentalization, which is based on simple physiochemical forces, to represent a key step in the formation of early cells.[22] As a consequence, several pioneering studies have been undertaken using synthetic vesicles as the basis of orchestrating aspects of cellular function in artificial systems (Figure 5). In each case, the protocell models aim to mimic just a few of the core criteria of living cells, typically demonstrating aspects of minimal replication, metabolism, membrane uptake, growth, or division. However, the complexity of phospholipid synthesis and the low permeability of phospholipid

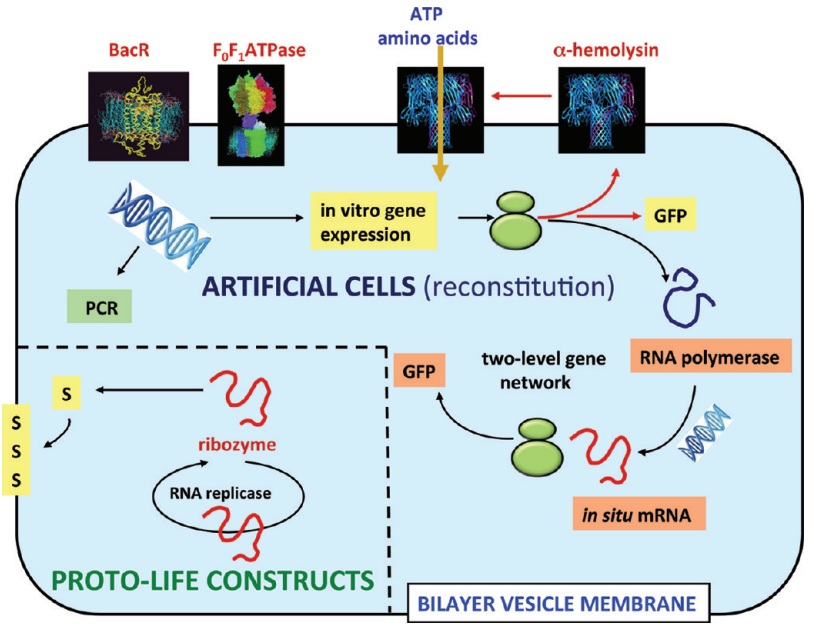


FIGURE 5. Protocell models based on phospholipid or fatty acid vesicles (see text for details and references). The models involve the reconstitution and operation of functional biocomponents (artificial cells) or prebiotically relevant components (proto-life constructs). Artificial cells comprising single genes or simple gene networks are used to express proteins and enzymes that have functional relevance as fluorescent markers (GFP), membrane porins (α-hemolysin), or catalysts for mRNA synthesis (RNA polymerase). The translation of mRNA into proteins occurs via entrapped ribosomes (green structures). In addition, encapsulated DNA strands can be amplified by temperature cycling using polymerase chain reaction (PCR) procedures. Proton transport proteins such as bacteriorhodopsin (BacR) and F_0F_1ATPase can be incorporated into the vesicle membrane by direct addition of the biomolecules. Protolife constructs comprise prebiotically plausible components such as catalytically active strands of RNA (ribozymes) or polynucleic acid templates that participate in nonenzymatic extension to produce double-stranded informational polymers. Entrapment of a single RNA molecule that could self-replicate (RNA replicase) and act as a ribozyme for the synthesis of new membrane components (S) could in principle represent the simplest model of cellularity.

membranes suggest that such molecules are not very plausible as constituents of protocellular compartments. In contrast, fatty acid bilayer membranes permit the passive diffusion of ions and small molecules and undergo fast exchange between the vesicle membrane and monomers/micelles in solution.[23] As a consequence, the vesicles interact readily with solutes in the external environment and can incorporate new amphiphiles into the bilayer membrane leading to growth and division of the self-assembled compartments.[24] Together, these properties suggest that it may be possible in the future to design and construct protocell models with an active systems interface capable perhaps of minimal chemical cognition by exploiting the responsive and adaptive nature of vesicles prepared from mixtures of single chain amphiphiles.

The range of reported studies involving protocell models based on vesicles is steadily increasing.[25] For example, phospholipid vesicles capable of supporting the gene expression of single components[26] or cascading networks,[27] polymerase chain reaction (PCR)-induced DNA amplification,[28] RNA replication,[29] or biochemical transformations[30] have been described. For example, 100 or more components comprising a green fluorescent protein (GFP) gene-expression system were encapsulated simultaneously within the vesicle internal microenvironment to produce a compartmentalized model of informationally directed protein synthesis.[27] However, the protocell model was not self-sustaining and terminated once the entrapped amino acids and activated mononucleotides were depleted. To circumvent this, a second gene that expressed the membrane protein α-hemolysin was encapsulated along with the GFP gene and cell-free expression components to generate a simple protocol that was consistent in principle with the organizational logic necessary for synthetic cellularity. The expressed α-hemolysin was incorporated into the vesicle membrane to produce molecular pores that facilitated the influx of amino acids and nucleotides from the external environment. As a consequence, GFP expression in the vesicles was maintained for up to 4 days. Significantly, these studies illustrate how a form of basic autonomy might be incorporated within a protocell model.

Other studies have attempted to simulate notions of autopoiesis into protocell models. One possibility is to encapsulate genes or enzymes within the vesicles that are responsible for phospholipid/fatty acid synthesis such that

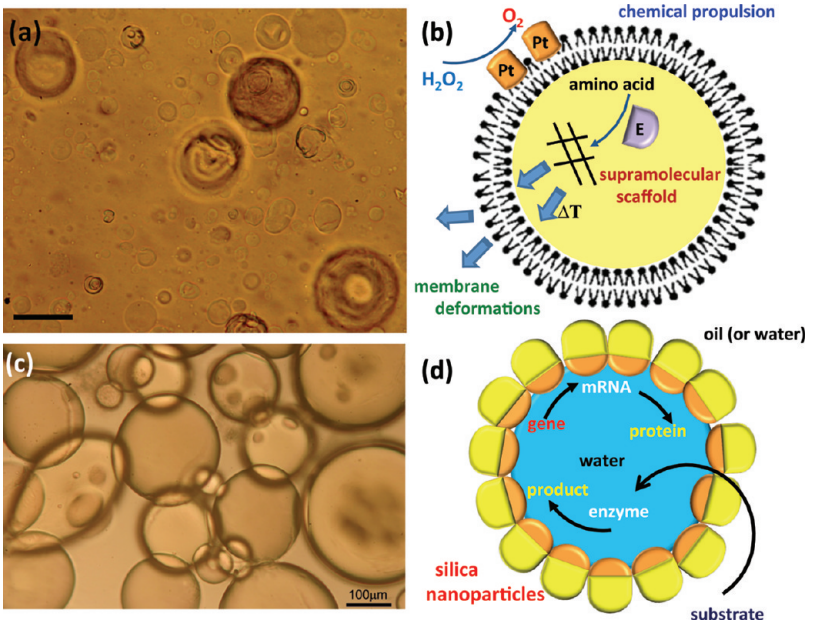


FIGURE 6. Membrane-based protocell models. (a, b) Phospholipid vesicles with cytoskeletal-like interiors. (a) Optical microscopy image of prepared vesicles. Scale bar = 20 μm. (b) Scheme showing associated design principles (see ref 36 for details). (c, d) Bioinorganic protocells comprising nanoparticle-based membranes. (c) Optical microscopy image of silica nanoparticle-stabilized water droplets suspended in oil. The mineral-coated droplets can be subsequently transferred into water as intact aqueous compartments by chemical modifications of the silica shell. Scale bar = 100 μm. (d) Scheme showing use of bioinorganic protocells for *in vitro* gene expression or enzyme-mediated transformations. See ref 37 for details.

new amphiphilic building blocks can be generated internally and then integrated into the existing bilayer membrane to promote vesicle self-reproduction. While very challenging, an initial step in this direction has been reported.[31] Alternatively, as demonstrated very recently, PCR amplification of DNA within phospholipid vesicles can promote self-reproduction of the vesicles.[32] Growth of the vesicles was associated with integration of new membrane molecules from the external solution via scission of soluble precursors by catalytic components in the phospholipid bilayer. Significantly, increasing the concentration of DNA within the vesicles by increasing the number of PCR cycles promoted vesicle division in the growing compartments by membrane-based interactions between the oppositely charged polynucleotide and new membrane molecules. Overall, the effect was to couple, albeit indirectly, self-reproduction and self-replication processes within the protocell model. Significantly, these studies suggest that by extending the complexity of coupling, it should be possible to design protocells with interesting emergent properties.

Because vesicles prepared from fatty acids are considered more prebiotically relevant than their phospholipid counterparts,[33] there have been several reports of protocell models based on single-chain amphiphile self-assembly. For example, enzymatic[34] and nonenzymatic[35] extension of homopolynucleotide templates has been demonstrated in vesicles prepared from mixtures of oleic acid/oleate or decanoic acid/decanol/decanoic acid glycerol monoester, respectively. In the former case, polynucleotide phosphorylase was encapsulated in the vesicles, and ADP was then added to the external medium. Slow diffusion of ADP across the vesicle membrane resulted in an increase in the intravesicular concentration, and as a consequence polyadenosine was produced specifically within the enclosed compartment. Interestingly, because polyadenosine remained inside the vesicles, the enzyme-driven polymerization reaction was terminated by the increase in osmotic pressure inside the compartment. In the case of vesicles prepared from mixtures of decanoic acid/decanol/decanoic acid glycerol monoester, a single-stranded polycytosine template with an attached DNA primer was entrapped, and membrane-permeable imidazole-activated mononucleotides were added to the external solution. Replication was terminated when the entire population of template molecules had been converted into double-stranded DNA, indicating that a mechanism of separating the duplex would be required if successive cycles of replication were to be achieved.

Aspects of primitive nanoscale organization have been integrated into vesicle-based protocell models. For example, the intravesicular self-assembly of an internalized cytoskeletal-like network based on small-molecule building units has been recently reported (Figure 6a,b).[36] The nanostructured interior was produced by *in situ* self-assembly of

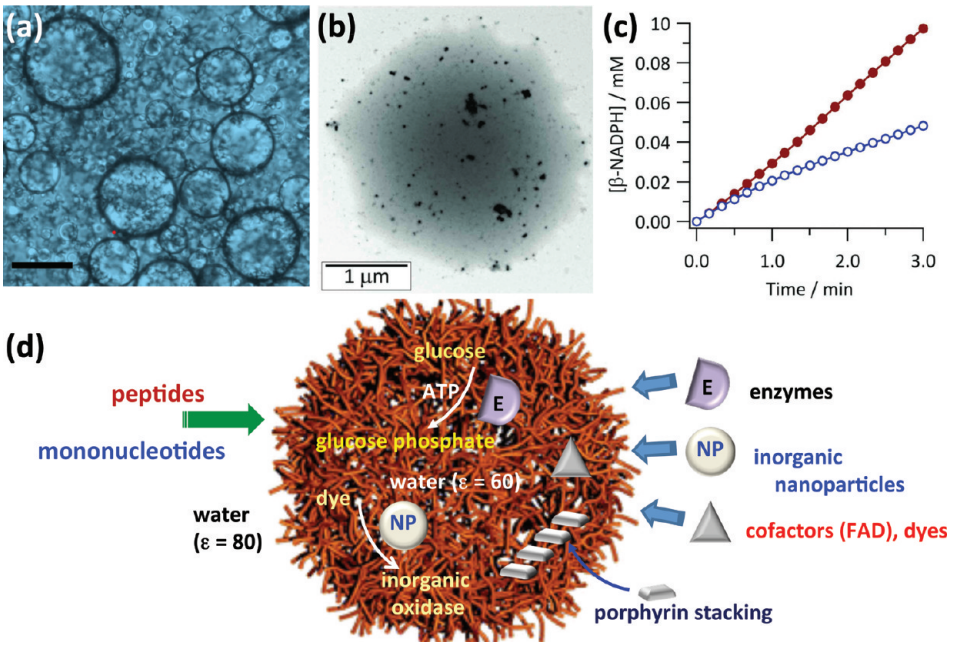


FIGURE 7. Protocell models based on membrane-free compartmentalization. (a) Optical image showing water-containing peptide/ATP microdroplets; scale bar = 50 μm. (b) TEM image of a single oligolysine/nucleotide microdroplet showing encapsulated gold nanoparticles (small dark spots); scale bar = 1 μm. (c) Time profile of increase in product (β-NADPH) in the presence (filled red circles) and absence (open blue circles) of enzyme-containing polylysine/ATP microdroplets. The corresponding reaction rates for glucose phosphorylation were 33 and 16 μM min^{-1}, respectively, indicating a 2-fold kinetic enhancement for the encapsulated enzymes. (d) Scheme summarizing the properties of peptide/mononucleotide microdroplets associated with their use as membrane-free protocell models (ε = dielectric constant). See text and ref 38 for details.

an amino acid-based supramolecular hydrogel using components trapped within the membrane-bounded aqueous compartment. Moreover, changes in temperature were used to influence the viscosity of the entrapped hydrogel such that the vesicles underwent distinct changes in shape at 45 °C. Interestingly, autonomous movement of the protocells in the form of bubble-generated chemical propulsion was achieved by addition of aqueous hydrogen peroxide to vesicles prepared with an exterior coating of platinum nanoparticles.[36]

4.2. Alternative Paradigms for Protocell Construction. Although most attention has been focused on modeling minimal cellularity through the use of self-assembled organic membranes, alternative modes of compartmentalization involving nanoparticle self-organization have been recently described that might have prebiotic relevance. For example, simple inorganic minerals in the form of silica nanoparticles with an appropriate balance of surface hydrophilicity and hydrophobicity have been used to stabilize aqueous microdroplets capable of functioning as confined reaction environments for cell-free gene expression or enzyme-mediated catalysis (Figure 6c,d).[37] Partitioning of the nanoparticles at the surface of the water droplets produced an ultrathin inorganic membrane that was assembled from a closely packed array of the nanoparticles to produce a contiguous but semipermeable shell. Interestingly, the size of the bioinorganic protocells was controlled by the number of nanoparticles used per unit volume of added water, with the consequence that the net flux of small-molecule substrates into droplets containing entrapped enzymes increased as the surface area/volume ratio increased. As a result, decreasing the size of the bioinorganic protocells increased the effective rate of enzymatic turnover.

Recently, the concept of membrane-free compartmentalization has been reintroduced as an alternative model for prebiotic organization based on cell-like entities that may have occurred prior to the emergence of lipid-based compartmentalization on the early Earth. These studies,[38] which were related to the pioneering work of the Russian scientist, Oparin,[2] but which utilized small molecule interactions rather than macromolecular complexation, indicated that spontaneous compartmentalization can occur when low molecular weight positively charged oligolysine peptides are mixed with anionic mononucleotides such as ATP. Charge-matching interactions between the peptide and mononucleotide molecules resulted in spontaneous microphase separation (coacervation) to produce droplets that were highly enriched in the biomolecules and stable across a wide range of ionic strength and pH values, as well as up to temperatures of 95 °C (Figure 7). Interestingly, longer chain peptides such as polylysine spontaneously

adopted an α-helical secondary structure when partitioned within the peptide/ATP compartments, suggesting that higher-order structuration can be induced spontaneously by coacervation. Significantly, the peptide/nucleotide droplets had a lower dielectric constant than water and, as a consequence, could be used to sequester a wide range of water-soluble solutes, many of which, such as anionic porphyrins, organic heterocyclic dyes, and inorganic nanoparticles, have possible prebiotic relevance. Moreover, the uptake of water-soluble porphyrin molecules resulted in supramolecular stacking and formation of photoactive functional nanostructures specifically within the peptide/nucleotide droplets. In addition, enzymes such as hexokinase and glucose-6-phosphate dehydrogenase were preferentially sequestered into the peptide/nucleotide droplets, where they were used for glucose phosphorylation and dehydrogenation at rates approximately twice that measured in bulk aqueous solution under identical conditions. Together, these studies suggest that the core criteria required for the onset of synthetic cellularity might be accessible in membrane-free systems of compartmentalization. In particular, the peptide/nucleotide droplets exhibit highly simplified systems properties, such as internal (component enrichment, nanoparticle/enzyme-mediated catalysis, supramolecular, and nanoscale structuration) and interfacial (molecular sequestration, solute uptake, pH/temperature sensitivity) processing, although their capacity to support information storage and transfer remains to be determined.

5. Conclusions

The transition from nonliving to living matter represents a transformation from molecular/supramolecular self-organization to highly orchestrated chemical systems of functional self-integration. In this Account, we have addressed the core criteria, basic autonomy, and organizational logic of cellularity, paying particular attention to the importance of systems design and nanoscale phenomena in determining the nonlinear interconnectivity essential for the emergence of chemically based processes of autopoiesis and cognition. Cellularity appears as a fundamental aspect of living systems and notions of compartmentalization, replication, metabolism, energization, and evolution provide the framework for a universal biology that penetrates deep into the history of life on the Earth. Thus, it seems reasonable to speculate that the advent of protolife systems was coextensive with reduced grades of cellularity in the form of simpler compartmentalization modules with basic autonomy and abridged systems functionalities (metabolic cells, replication cells, etc.), which with time became integrated possibly through processes of rapid, open-source evolution. Modeling such aspects of cellularity is a deep challenge, and only small steps toward integrating systems-based ideas concerning autopoiesis, cognition, autonomy, and organizational logic have been made. Nevertheless, experimental protocols aimed toward the construction and design of various types of microscale compartments and their application as protocell models capable of supporting replication and enzyme-mediated catalysis are increasing in number and complexity.

Clearly, there are many profound challenges. For example, how do we generate chemically based networks that exhibit autopoietic properties or construct physical interfaces that transform molecular recognition into chemical cognition? What is the minimal degree of molecular complexity required to realize an autonomous organization? How are evolutionary pressures to be integrated into models of basic autonomy to transform self-determining chemical systems into laboratory representations of artificial life? As described in this Account, the design and construction of laboratory based protocell models that mimic different aspects of minimal cellularity represent a first step toward addressing how the transition from nonliving to living matter might be achieved in the laboratory, as well as evaluating plausible scenarios of the origin of life on the early Earth. Such an approach will also contribute significantly to the development of novel chemical strategies geared toward the construction of primitive artificial cells, small-scale bioreactors, adaptive micromachines, and autonomous agents capable of remote sensing and energy conversion.

I am indebted to the Radcliffe Institute for Advanced Study, Harvard University, USA, for the award of an International Fellowship during 2011–2012.

BIOGRAPHICAL INFORMATION

Stephen Mann is Professor of Chemistry, Director of the Centre for Organized Matter Chemistry, and Principal of the Bristol Centre for Functional Nanomaterials at the University of Bristol, U.K.. His research interests are focused on the chemical synthesis, characterization, and emergence of complex forms of organized matter, including models of protocell assembly. He has published over 400 scientific papers and has served on the editorial advisory boards of numerous journals including *Advanced Materials*, *Angewandte Chemie*, *Chemical Science*, and *Small*. Prof. Mann was elected as a Fellow of the Royal Society, U.K., in 2003. In 2011, he received the Royal Society of Chemistry de Gennes Medal and was a recipient of the Chemical Society of France French-British Prize.

He is currently on sabbatical as an International Fellow at the Radcliffe Institute for Advanced Study at Harvard University, USA.

FOOTNOTES

*E-mail: s.mann@bristol.ac.uk.
The authors declare no competing financial interest.

REFERENCES

1 Schopf, J. W.; Kudryavtsev, A. B.; Czaja, A. D.; Tripathi, A. B. Evidence of Archean life: Stromatolites and microfossils. *Precambrian Res.* **2007**, *158*, 141–155.

2 Oparin, A. I. *The Origin of Life*; Macmillan: New York, 1938.

3 Luisi, P. L. *The Emergence of Life*; Cambridge University Press: Cambridge, U.K., 2006.

4 Gilbert, W. Origin of life: The RNA world. *Nature* **1986**, *319*, 618.

5 Lacey, J. C.; Cook, G. W.; Mullins, D. W. Concepts related to the origin of coded protein synthesis. *Chemtracts: Biochem. Mol. Biol.* **1999**, *12*, 398–418.

6 Dzieciol, A. J.; Mann, S. Designs for life: Protocell models in the laboratory. *Chem. Soc.* **2012**, *41*, 79–85.

7 Alberts, B. et al. *Molecular Biology of the Cell*, 5th ed.; Garland Sciences: New York, 2008.

8 Ruiz-Mirazo, K.; Moreno, A. Searching for the roots of autonomy: The natural and artificial paradigms revisited. *Commun. Cognit.—Artif. Intell.* **2000**, *17*, 209–228.

9 Kováč, L. Life, chemistry and cognition. *EMBO Rep.* **2006**, *7*, 562–566.

10 Maturana, H.; Varela, F. J. *Autopoiesis and cognition. The realization of the living*; D. Riedel: Dordrecht, The Netherlands, 1980.

11 Luisi, P. L. Autopoiesis: A review and a reappraisal. *Naturwissenschaften* **2003**, *90*, 49–59.

12 Weber, A.; Varela, F. J. Life after Kant: Natural purposes and the autopoietic foundations of biological individuality. *Phenomenol. Cognit. Sci.* **2002**, *1*, 97–125.

13 Gesteland, R.; Cech, T.; Atkins, J. *The RNA World*, 3rd ed.; Cold Spring Harbor Laboratory Press; Cold Spring Harbor, NY, 2006.

14 Szostak, J. W.; Bartel, D. P.; Luisi, P. L. Synthesizing life. *Nature* **2001**, *409*, 387–390.

15 Pohorhille, A. Early ancestors of existing cells. In *Protocells: Bridging Nonliving and Living Matter* Rasmussen, S., Bedau, M. A., Chen, L., Deamer, D., Krakauer, D. C., Packard, N. H., Stadler, P. F., Eds.; MIT Press: Cambridge, MA, 2009.

16 Mann, S. Life as a nanoscale phenomenon. *Angew. Chem., Int. Ed.* **2008**, *47*, 5307–5320.

17 Rawicz, W.; Olbrich, K. C.; McIntosh, T.; Needham, D.; Evans, E. Effect of chain length and unsaturation on elasticity of lipid bilayers. *Biophys. J.* **2000**, *79*, 328–329.

18 Veld, G. I.; Driessen, A. J. M.; Op den Kamp, J. A. F.; Konings, W. N. Hydrophobic membrane thickness and lipid-protein interactions of the leucine transport system of Lactococcus lactis. *Biochim. Biophys. Acta* **1991**, *1065*, 203–212.

19 Apel, C. L.; Deamer, D. W.; Mautner, M. N. Self-assembled vesicles of monocarboxylic acids and alcohols: conditions for stability and for the encapsulation of biopolymers. *Biochim. Biophys. Acta* **2002**, *1559*, 1–9.

20 Shen, M.; Davis, F. P.; Sali, A. The optimal size of a globular protein domain: a simple sphere packing model. *Chem. Phys. Lett.* **2005**, *405*, 224–228.

21 Luisi, P. L.; Ferri, F.; Stano, P. Approaches to semi-synthetic minimal cells: A review. *Naturwissenschaften* **2006**, *93*, 1–13.

22 Chen, I. A.; Walde, P. From self-assembled vesicles to protocells. *Cold Spring Harbor Perspect. Biol.* **2010**, *2*, 1–13.

23 Monnard, P.-A.; Deamer, D. W. Loading of DMPC based liposomes with nucleotide triphosphates by passive diffusion: a plausible model for nutrient up-take by the protocell. *Orig. Life Evol. Biospheres* **2001**, *31*, 147–155.

24 Stano, P.; Luisi, P. L. Achievements and open questions in the self-reproduction of vesicles and synthetic minimal cells. *Chem. Commun.* **2010**, *46*, 3639–3653.

25 Stano, P.; Carrara, P.; Kuruma, Y.; Souza, T.; Luisi, P. L. Compartmentalized reactions as a case of soft-matter biotechnology: Synthesis of proteins and nucleic acids inside lipid vesicles. *J. Mater. Chem.* **2011**, *21*, 18887–18902.

26 Nomura, S. M.; Tsumoto, K.; Hamada, T.; Akiyoshi, K.; Nakatani, Y.; Yoshikawa, K. Gene expression within cell-sized lipid vesicles. *ChemBioChem* **2003**, *4*, 1172–1175.

27 Noireaux, V.; Libchaber, A. A vesicle bioreactor as a step toward an artificial cell assembly. *Proc. Natl. Acad. Sci. USA.* **2004**, *101*, 17669–17674.

28 Oberholzer, T.; Albrizio, M.; Luisi, P. L. Polymerase chain reaction in liposomes. *Chem. Biol.* **1995**, *2*, 677–682.

29 Oberholzer, T.; Wick, R.; Luisi, P. L.; Biebricher, C. K. Enzymatic RNA replication in self-reproducing vesicles: an approach to a minimal cell. *Biochem. Biophys. Res. Commun.* **1995**, *207*, 250–257.

30 Walde, P.; Ichikawa, S. Enzymes inside lipid vesicles: Preparation, reactivity and applications. *Biomol. Eng.* **2001**, *18*, 143–177.

31 Kuruma, Y.; Stano, P.; Ueda, T.; Luisi, P. L. A synthetic biology approach to the construction of membrane proteins in semi-synthetic minimal cells. *Biochim. Biophys. Acta* **2009**, *1788*, 567–574.

32 Kurihara, K.; Tamura, M.; Shohda, K.; Toyota, T.; Suzuki, K.; Sugawara, T. Self-reproduction of supramolecular giant vesicles combined with the amplification of encapsulated DNA. *Nat. Chem.* **2011**, *3*, 775–781.

33 Dworkin, L. P.; Deamer, D. W.; Sandford, S. A.; Allamandola, L. J. Self-assembling amphiphilic molecules: Synthesis in simulated interstellar/precometary ices. *Proc. Natl. Acad. Sci. U.S.A.* **2001**, *98*, 815–819.

34 Walde, P.; Goto, A.; Monnard, P. A.; Wessicken, M.; Luisi, P. L. Oparin's reactions revisited: Enzymic synthesis of poly(adenylic acid) in micelles and self-reproducing vesicles. *J. Am. Chem. Soc.* **1994**, *116*, 7541–7547.

35 Mansy, S. S.; Schrum, J. P.; Krishnamurthy, M.; Tobe, S.; Treco, D. A.; Szostak, J. W. Template-directed synthesis of a genetic polymer in a model protocell. *Nature* **2008**, *454*, 122–125.

36 Krishna Kumar, R.; Yu, X.; Patil, A. J.; Li, M.; Mann, S. Cytoskeletal-like supramolecular assembly and nanoparticle-based motors in a model protocell. *Angew. Chem., Int. Ed.* **2011**, *50*, 9343–9347.

37 Li, M.; Green, D. C.; Anderson, J. L. R.; Binks, B. P.; Mann, S. In vitro gene expression and enzyme catalysis in bio-inorganic protocells. *Chem. Sci.* **2011**, *2*, 1739–1745.

38 Koga, S.; Williams, D. S.; Perriman, A. W.; Mann, S. Peptide/nucleotide micro-droplets as a step towards a membrane-free protocell model. *Nat. Chem.* **2011**, *3*, 720–724.