Origins of Life

DOI: 10.1002/anie.201204968

The Origins of Life: Old Problems, New Chemistries**

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origins of life · protocells · soft matter · synthetic life · systems chemistry

Introduction

It is ironic that modern biology—considered by many to be the pre-eminent science of the 21st century—tells us everything we know about life as it exists today, but nothing substantial about its origin on the early Earth some 3.5–3.8 billion years ago.[1] Biology is underscored by two great principles: that all life is interconnected through a Darwinian landscape of random variation and selective retention, and that cellularity is the fundamental and universal organizational unit of life. We know much about the details of these principles and their molecular basis; in particular, how they depend on out-of-equilibrium energization, informational capacity, and matter/energy throughput,[2,3] and how material embodiment is maintained throughout eons by processes of self-replication, metabolism and compartmentalization.[4] But a study of biology offers no illumination on the origin of life—on how life first emerged in a physical universe. If anything, it compounds this problem by showcasing an overarching commonality in which the phylogenetic histories of known organisms can be traced through the molecular archives of ribosomal RNA to a putative last universal common ancestor (LUCA) with most of the central biochemical machinery of extant cells still in place. Although the level of detail emanating from this molecular historicity is remarkable—for example, the recent unraveling of the origin and evolution of the ribosome[5]—there remains an intractable discontinuity at the base of the reconstructed tree of life, where all current knowledge of biology becomes effectively bottlenecked such that the origin of life appears impenetrable and mysterious. Metaphorically speaking, the tree of life appears rootless.

We are therefore left with two momentous challenges: how did the transition from inanimate matter to the first forms of living matter occur on the early earth? And can a similar transition be realized ex novo in the laboratory? These are profound etiological questions that most biologists justifiably walk away from; should chemists do so too? Understandably, most chemists are resistant to undertaking research in this area for several reasons. To a cynic it may be that there is simply no critical level of funding. But there are more fundamental problems, principally to do with epistemology and methodology. Chemistry is viewed on the whole as an ahistorical science, unlike biology and earth sciences for example, and from this perspective there are strong objections to the study of the origin of life on the early Earth. This is compounded by the absence of statistically significant, reproducible and empirical data. Indeed, it is reasonable to question whether a systematic and meaningful investigation can ever be undertaken if no trace of life before the LUCA can be acquired. Thus, the irrevocable erasing of prebiotic signatures by Archean geochemistry, the fragmentary and rudimentary nature of models of the early Earth atmosphere and oceans, the sheer impossibility of reconstructing local chemical conditions, and the perceived weakness of the underlying theories are sufficient reasons to halt a concerted chemical approach to solving the origin of life.

In a perfect world, many of these concerns might simply evaporate if we had a robust mathematical theory to describe the transition from inanimate to living matter. (In fact, there are many computational models[6,7] and theories,[8–10] but none which provide an overarching description). Then the study of the origin of life would sit comfortably alongside mathematical theories of the origin of the universe. The latter are so sufficiently advanced that they drive high-cost, large-scale, multi-national research activities, such as the Large Hadron Collider at CERN, that seek to recreate the conditions just after the Big Bang. In contrast, investigations into the origin of life are poorly funded, and the study of prebiotic mechanisms and an attendant protobiology has remained essentially low-key and confined to a limited number of laboratories around the world. It is time to re-evaluate this predicament.

Re-evaluation

In the first place, questions concerning the origin of life on the Earth are becoming increasingly acute in a range of intersecting fields often grouped under the label of astrobiology (exobiology).[11,12] This field is burgeoning and chemists need to get involved. Significantly, explorations of adverse environments on the earth have continued to reenforce the perception that life as we know it is ubiquitous, robust, and therefore possibly easier to initiate than we once thought. Moreover, it is generally accepted that organic

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[**] I am indebted to my research group for many interesting discussions, and to the Radcliffe Institute for Advanced Study, Harvard University, USA, for the award of an International Fellowship during 2011–2012.

matter is present throughout much of the cosmos, and that extrasolar planets (exoplanets), as identified with increasing frequency by the Kepler space telescope, are widespread in our galaxy.[13] As a consequence, the possible existence of extra-terrestrial life has to be seriously considered in the context of a universal biology. This leads to a deep question: is it possible for life to emerge through fundamentally different organizational, operational and evolutionary mechanisms, or are the core criteria of terrestrial biology—membrane-based cellularity, semi-conservative DNA/RNA-mediated self-replication, protein-regulated metabolism, Darwinian evolution, non-equilibrium energization—invariant and axiomatic? This wider perspective necessitates an intellectual shift away from the historical impasse associated with the study of the origin of life specifically on Earth to a broader perspective concerned with the generic transformation of inanimate matter to a life-like state. And by focusing attention towards the possibility of generating alternative models of life in the laboratory that are essentially devoid of historical content—that is, without needing to anticipate too many unknown boundary conditions—it should be possible for chemists to contribute significantly to understanding the *origins* of life as a general physical phenomenon, even if the actual *origin* of life as it occurred on the early Earth remains unresolved. Put another way, reframing an etiological problem with an ontological one (the nature of living matter as a special form of material existence) reveals a deep challenge that chemists in particular should be engaged in. After all, chemists are specialists in arranging matter into new representations—in effect, new aspects of existence—and are therefore centrally placed, along with colleagues in synthetic biology, complexity science and systems engineering, to begin to tackle the possibility of constructing representations of life in the laboratory.

Is it possible therefore to find pathways that might ultimately lead to synthetic constructs of life?[14–17] As a start, one can seek new perspectives by abstracting problems implicit to the deep past and addressing them in terms of future technologies based on notions of synthetic cellularity. Several key scenarios of "life before biology"—that is, an alternative living world that existed prior to the LUCA of organisms as we know them—are available (Figure 1), and serve as an imaginative source of new ideas that can be advanced experimentally. Crucially, there has been a shift away from "Stanley Miller-type" experiments in which highly speculative scenarios of early Earth reaction conditions are probed, to more judicious and systematic investigations that are breaking new ground by attempting to solve old problems with new chemistries. For example, rather than following the conventional retrosynthesis route of deconstructing a ribonucleotide into nucleobase, sugar and phosphate units, followed by disassembly into primary molecules (CO, HCN, HCHO, etc.), Sutherland and colleagues[18,19] based their disconnection on reactive fragments of the nucleobase and sugar, which could be successfully re-assembled in the presence of phosphate to hybrid intermediates that underwent additional reactions and processing to produce an enantiomerically pure pyrimidine-activated nucleotide. In a similar vein, alternative biochemistries based on expanding the genetic code through

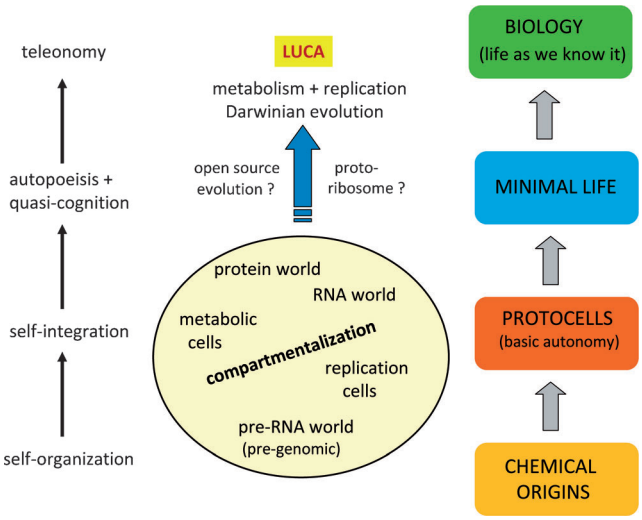

Figure 1. Life before biology? Scheme showing possible scenarios of protobiological events prior to the emergence of the LUCA. Arrows indicate progression in complexity over time. General grades of life, types of protobiological worlds and mechanisms, and overarching concepts are shown in the right, middle and left panels, respectively. For details of chemical origins from abiotic geochemistry see reference [97]. See references [88–90] for details concerning the concepts of autopoiesis, quasi-cognition and teleonomy, respectively.

new types of replicable base pairs,[20–22] inter-strand interactions[23–25] and backbone linkages[26–28] have developed with increasing momentum in recent years, partly in response to the centrality of the informational/catalytic RNA world theory, but also because of breathtaking changes in the scope of in vitro molecular evolution techniques such as SELEX.[29] Significantly, by adopting these approaches, it is possible to couple key enigmas facing the origin of life research community—for example, why is the informational basis of life based on ribofuranosyl nucleic acids?—to new technological breakthroughs focused on extending the genetic alphabet in areas as diverse as DNA-based logic gates,[30,31] bioinspired engineering[32] and materials nanofabrication.[33]

Similar kinds of etiological problems are inspiring chemical innovations with regard to the complex mechanisms responsible for oligoribonucleotide synthesis and replication. The idea of self-instructed replication is based on the demonstration that ribozymes with RNA-catalyzed, RNA-mediated copying ability can be generated in the laboratory using in vitro evolution methods.[34,35] However, the low efficiency and fidelity of these ribozymes, along with the high temperatures required to separate the synthesized strand from the template, are currently viewed by many as insurmountable factors that mitigate against a model of self-replication based on a RNA replicase. In response, a chemical scenario of non-enzymatic-mediated RNA replication is being reconsidered in a positive light.[36] After many years of concerted effort that showed only partial success, for example by using activated monomers with an oligo-GC template,[37,38] or by periodic refreshing of the reaction solutions,[39] attempts to induce the non-enzymatic replication of arbitrary, mixed ribonucleotide sequences have generally failed.[40,41] Given these limitations, alternative chemistries

have been developed for high-yielding non-enzymatic copying by using activated monoribonucleotides comprising unnatural bases with enhanced base-pairing propensities (e.g. 5-propyluridine or 2,6-diaminopurine) or by using primers with strongly nucleophilic terminal groups.[42] Significantly, in an attempt to provide a blueprint for future work, Szostak has recently outlined the key problems—low levels of regiospecificity for the 3′,5′ linkage, unfavorable strand separation and annealing, high error rates, slow reaction kinetics, and requirements for chemically activated monoribonucleotides, high divalent ion concentrations and primers—and offered insights into their resolution.[36]

Towards Integration

In the end, addressing questions concerning the transition of inanimate matter to the living state necessitates a new paradigm in chemistry that moves away from a radical form of reductionism to a more integrative, reconstructive approach. In this respect, the advent of systems chemistry represents a bold and timely move to address many of the challenges related to the understanding of the structural and dynamical basis for chemical self-replication and molecular evolution,[43] chiral symmetry breaking[44] and autocatalytic networks.[45,46] For example, systems-based studies using directed evolution methods have shown that nucleic acid molecules can be exploited to address issues of molecular cooperation and competition,[47,48] and that cross-catalysis—in which different RNA enzymes interact such that the product of one promotes the activity of another and vice versa—appears to be a core criterion for sustainable rates of in vitro replication.[49–51] Indeed, one can justifiably argue that the emergence of the field of evolutionary chemistry as a counterpart to evolutionary biology has sharpened our focus on the possible pathways that could be responsible for transitions from nonliving to living matter. But it is clear that molecular engineering through in vitro evolution has to take place in a wider, more integrative context if the process of self-catalyzed self-replication is to be endowed with constitutional meaning.[52] After all, informational capacity must be conveyed and utilized if it is to be the basis of selective advantage; otherwise, the codes remain dormant and unmaterialized. Information therefore has to be nested within the organizational logic of an ensemble of higher-order processes that can be embodied in forms of synthetic cellularity and its attendant processes of compartmentalization, energization, and evolutionary capacity.

As a consequence, it is important to address models of chemical compartmentalization and construct simplified systems of interacting molecular components derived from biological or non-biological sources, or both.[53–55] Significantly, establishment of artificial cells will require cooperative behavior between the components in order to generate ensembles that are dynamically persistent. This is a major challenge because synthetic cells will inevitably fail if there is excessive chemical competition between the entrapped components, or if the molecular trajectories are driven inextricably towards thermodynamic equilibrium ("dead-end" products). As a consequence, a minimal level of integration that can be maintained under energized, non-equilibrium conditions has not yet been realized. However, progress has been made by using alternative strategies in which semi-synthetic cells based on the mimicking of specific cellular functions have been constructed by incorporating within the self-organized aqueous micro-compartments of phospholipid or fatty acid vesicles, known biological reactions and processes including gene circuitry,[56–58] enzyme-mediated chemical transformations,[59] polymerase chain reaction (PCR) amplification,[60] and enzymatic[61,62] or non-enzymatic[56] template-directed synthesis of oligonucleic acids.

A significant breakthrough in this exciting approach hinges on the coupling of the replicative and biosynthetic capacity of the encapsulated reactions and processes to membrane properties such as molecular uptake, bilayer stability, growth and fusion.[63] This level of coupling demands a high degree of chemical design because the aim is to integrate the (bio)chemical networks in a way that increases the dynamical stability of the entire physicochemical system, and in so doing, open up the possibility of increasing the viability of the compartmentalized construct as a self-referential chemical entity. As a starting point, one could consider incorporating a gene cascade within the intravesicular environment that generates amongst several components certain proteins, such as α-hemolysin[64] or lipid acyltransferases,[65] which modulate the permeability or growth of the enclosing membrane, and thereby provide a regulatory mechanism for influencing the behavior of the entire compartmentalized system. Alternatively, one can couple, albeit indirectly, self-reproduction and self-replication within a protocell model by the self-production of new molecules within the vesicle that influence the materials properties of the lipid membrane.[66] As a consequence, the design of viable reaction networks in synthetic cells requires not only a consideration of the associated kinetics and thermodynamics, but also how such factors are integrated into the physical properties of the system as a whole. Thus, materials and soft matter chemistry play a central role in advancing research in the construction of synthetic protocells.

Materiality

It is self-evident that life at its most fundamental level is a materialistic phenomenon, and as such, mechanisms that attempt to bridge the divide between inanimate and animate matter should not underestimate the importance of physical states in determining the operation of cells and their synthetic counterparts. For instance, the functioning of living cells is very much dependent on the material properties of membranes, cytoplasmic fluids, cytoskeletal scaffolds, extracellular matrices and tissues, and strategies focused on biomimetic modeling of protocells will need to incorporate aspects of soft matter and materials chemistry into their design principles.[67,68] Thus, the dielectric constant, density and viscoelasticity of compartmentalized media are important criteria that need to be considered, particularly in regard to their influence on encapsulated reaction networks. A key challenge is to

produce and regulate these properties through reactions or phase transitions taking place within the protocell interior. In this respect, supramolecular organic chemistry can play an important role as it provides a wide range of possibilities for preparing synthetic compartments comprising hydrogel structures generated by non-covalent, reversible self-assembly. For example, intra-vesicular enzyme-mediated reactions can be used to generate a localized concentration of functionalized amino acid molecules that spontaneously self-assemble into nanofilaments to produce a supramolecular hydrogel specifically within the vesicle interior (Figure 2a–d).[69] The hydrogel, which contains embedded proteins and molecular substrates, is constrained by the surrounding lipid bilayer so that gel-to-sol transitions in the supramolecular matrix can be coupled to temperature-dependent changes in the vesicle morphology.

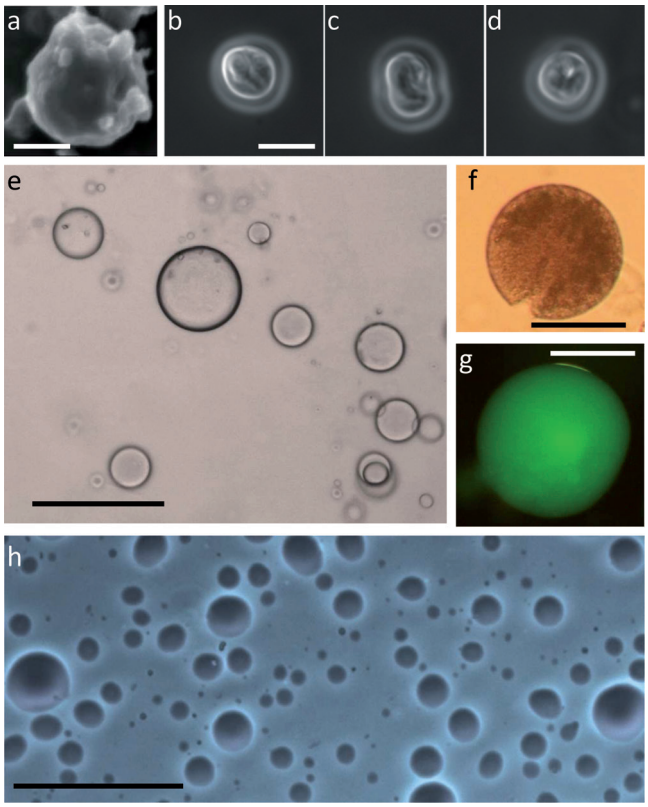

Figure 2. Alternative protocell models based on materials assembly. a) SEM image showing a single phospholipid vesicle with a supramolecular hydrogel interior produced by enzyme-mediated self-assembly of the functionalized amino acid, FMOC-tyrosine; scale bar = 5 μm. b–d) Time sequence of phase contrast microscopy images showing reversible fluctuations in morphology for an individual vesicle prepared as in (a) and maintained at the gel–sol transition (40 °C) for 4 (b), 7 (c) and 8.5 min (d); scale bar = 10 μm.[69] e–g) Optical/fluorescence microscopy images of silica nanoparticle-stabilized water droplets (colloidosomes) in dodecane (e), after transfer to a bulk water phase with encapsulated protein (ferritin, red coloration) (f), and after cell-free gene expression of green fluorescent protein (GFP) within the colloidosome interior (g) (fluorescence image excited in blue light and recorded after 24 h incubation at 37 °C); scale bars = 50, 100 and 100 μm in (e), (f) and (g), respectively.[77] h) Optical image of membrane-free droplets prepared at pH 8 in water from mixtures of polylysine and ATP.[78] The cationic dye, methylene blue, is preferentially sequestered into the droplets; scale bar = 50 μm.

Similarly, it is also possible to induce higher-level properties within a model protocell by using phase separation processes located specifically within a membrane-delineated micro-environment. For example, temperature- or concentration-induced aqueous phase separation of two neutral macromolecules (dextran and poly(ethylene glycol) (PEG)) from a single-phase mixture can be exploited to generate sub-compartments within the internal volume of a phospholipid vesicle.[70] By using the non-ideal aqueous solution behavior of macromolecular mixtures, it is possible to create chemically distinct microdomains—one enriched in dextran, the other in PEG—within a protocellular construct, and as a consequence, a range of interesting cytomimetic behaviors, such as molecular crowding,[71] protein localization and phase transfer,[72] and vesicle budding and division into compositionally different daughter vesicles[73,74] have been demonstrated.

The fact that such apparently complex phenomena can be induced in compartmentalized mixtures of macromolecules, and that this behavior can be coupled at a higher level with vesicle polarity and asymmetric division in the absence of genetic or metabolic machinery, hold much promise for advancing our understanding of the interface between non-living and living forms of matter. Thus, steps towards synthetic cellularity are very likely to involve innovative breakthroughs in chemically driven self-organization, as demonstrated by recent studies in which novel types of self-assembled compartments have been introduced as alternative paradigms for the design and construction of artificial cell-like entities based on polymer self-assembly,[75] layer-by-layer deposition,[76] inorganic nanoparticle self-organization[77] or spontaneous micro-droplet formation.[78] The scope for these new approaches seems very extensive indeed. For example, block copolymer vesicles (polymersomes) can be used as robust compartmentalized platforms for the co-localization and site-specific positioning of biomolecular components for use in light-driven ATP generation,[79] and enzymatic cascade reactions.[80–82] These studies illustrate how proto-metabolic networks might be constructed in synthetic polymeric compartments, although the low membrane permeability and nanoscale dimension of many polymersomes could restrict their development as gene-containing synthetic cells. However, devices for generating high yields of uniform micrometer-sized double emulsions have been recently developed using amphiphilic block copolymers,[83] suggesting that it may be possible to undertake self-replication reactions inside polymersomes by exploiting microfluidic technologies. Alternatively, semi-permeable, membrane-bounded microscale compartments can be prepared by the layer-by-layer deposition of oppositely charged polyelectrolytes on sacrificial solid microspheres, and this reproducible but laborious procedure has considerable potential for the design of synthetic cells.[76]

Whilst the use of organic polymers and macromolecules as membrane components of synthetic cell-like entities appears judicious and highly sensible, it may also be perfectly tenable that synthetic cells could be delineated by semi-permeable, nanometer-thin shells of inorganic components. For example, the self-assembly of amphiphilic inorganic nanoparticles at

water droplet/oil interfaces has been recently demonstrated as the basis for semi-permeable compartmentalization systems capable of functioning as confined reaction environments for cell-free gene expression[77] and enzyme-mediated catalysis[77,84] (Figure 2e–g). Semi-permeable inorganic-based membranes can also be prepared by careful injection of aqueous droplets containing large polyoxometalate anions into aqueous solutions containing large organic/transition metal complex cations.[85] Immediate precipitation at the liquid–liquid interface produces millimeter-sized closed compartments that partition the two components, and which can be inflated or deflated, or arranged into nested structures by further processing. The membranes self-heal when ruptured, exhibit selective permeabilities to alkylammonium cations, and have potential redox-activity, suggesting that it should be possible to exploit a wide range of inorganic materials in the design of artificial cell-like microstructures.

Although membrane-based synthetic approaches clearly mimic the cellularity of living systems, one can take a more radical approach and ask whether a membrane is needed at all for effective compartmentalization. Such a notion has been recently developed both as an alternative protocell model of pre-biotic organization,[78] and as an innovative route towards synthetic cell-like entities.[86] In both cases, aqueous suspensions of micro-droplets comprising high concentrations of cationic peptides (oligo/poly-lysine) or polyelectrolytes and mononucleotides (ATP, CTP, etc.) are prepared by microphase separation (coacervation) under conditions close to charge neutralization (Figure 2h). The droplets are stable up to 95 °C, and across a wide range of ionic strength and pH values, even though there is no surrounding membrane. The studies indicate that even in the absence of a membrane, processes such as protein and small-molecule partitioning, peptide secondary structure formation, induced supramolecular stacking, and nanoparticle- or enzyme-mediated catalysis can be undertaken specifically in the micro-droplets. Together, these studies suggest that the core criteria required for the onset of synthetic cellularity might not necessitate the formation of membrane-delineated reaction volumes.

Outlook

We began this Essay with the epistemological and methodological difficulties inherent in a scientific study of the origin of life on the early Earth, and by re-framing these problems within a more general question concerning the transition from non-living to living matter *independent of its context* were able to progress towards deep challenges that are addressable by the development of new chemistries positioned at the interface with a range of cognate disciplines. In essence, such an endeavor—we might call it "protolife science" (Figure 3)—represents the search for the minimal organizational logic that is sufficient for the emergence of matter with a basic level of systems autonomy, ultimately capable of undergoing evolutionary change. A key feature is that the integrated coupling of chemical networks preserves the structural and dynamical integrity of the whole as well as the parts of a synthetic cell, and that the recursive action of

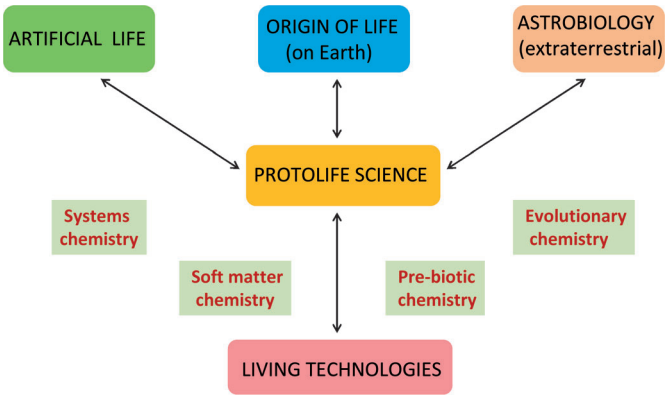

Figure 3. Scheme showing relationship of protolife science with cognate disciplines, including various sub-disciplines of chemistry.

such self-defining units endows the system with a persistent self-identity.[4] Similarly, systems autonomy[87] is fundamentally dependent on self-referential processes such as the internalized production and maintenance of components (autopoesis),[88] indigenous generation and regulation of informational capacity, and delineation of an outer boundary as an interactive quasi-cognitive interface with the environment.[89] The latter process places the system in contradistinction to the surrounding milieu in terms of the flow of energy and matter, non-equilibrium status of its persistence and viability, and evolutionary capacity via natural selection pressures. As a consequence, a key defining criteria of a protolife construct—and arguably the most striking feature of life as we know it—is that it would appear to an observer to follow an agenda; that is the system is endowed with an apparent purposefulness (teleonomic behavior),[90] even though there is no cognisant intentionality.

By outlining how exciting opportunities arise from new perspectives on old but profound problems, I hope that this Essay provides some encouragement for chemists (and funding agencies) to get involved in the development of protolife research. In this respect, a roadmap for addressing the transformation of inanimate matter into living systems can be drawn up by merging prebiotic chemistry to evolutionary chemistry, and incorporating this intellectual amalgam into systems chemistry through the study of unbounded reaction networks and then by integration of these processes within compartmentalized media to generate chemical cells and protocellular constructs in the laboratory. In more general terms, this approach represents a fundamental shift away from present day chemistry with its focus on linear reactivity and supramolecular self-organization to a paradigm based on the functional self-integration and material embodiment of highly orchestrated, non-linear chemical networks. As a consequence, an overarching objective is to progress towards synthetic cellularity; that is, the design and construction of non-equilibrium chemical microsystems capable of basic levels of autonomy (self-identity) as expressed through rudimentary forms of dynamical persistence and self-processing, quasi-cognitive and teleonomic behavior, and Darwinian evolution.

Undertaking such a challenge not only provides a major stimulus in fundamental research in chemistry at the interface with biology, but also opens up novel possibilities for new disruptive technologies based on soft, wet, chemical microsystems with adaptive and self-referential properties. Indeed, the ability to address new technological futures by abstracting problems implicit to the deep past is of special and unprecedented importance. For example, one can envisage artificial cells that are designed for specific applications in which the properties of biological systems, such as self-organization, nanoscale efficiency and adaptability, are compartmentalized at a relatively low cost to produce new miniaturized agents for applications in DNA sequencing and molecular screening,[91] soft matter biotechnology,[92] energy conversion in microscale batteries[93–95] and pharmacology and medical diagnostics.[96] Such microstructures might also recognize and sequester specific molecules from the external solution, and in so doing trigger pre-defined sets of ameliorating responses in the presence of certain signature molecules. Or they might possibly regulate the exchange of materials with the local environment, generate a supporting metabolic network, transduce external energy into chemical energy, and synthesize desired biosynthetic products in response to challenges placed on their basic autonomy.

Significantly, in contradistinction to more radical forms of synthetic biology, the chemical construction of artificial cells provides an approach to life-like constructs with minimal evolutionary capacity, and as such would be more ethically acceptable in diverse biotechnological, environmental and medical applications. Thus, from a technological perspective the design of synthetic cellularity should be primarily focused on basic autonomy in terms of maintaining functional/structural viability under diverse conditions, rather than attempting to build-in highly responsive modes of evolution. Indeed, if basic autonomy can be achieved in single synthetic cells, it would then be possible to study the organizational properties of communities of such entities—how they might communicate chemically to undergo collective tasks in response to changes in the environment, for example—and the mechanism by which they respond as a population to changes in applied selection pressures. Although we are a long way from advances of this kind, the etiological perspective accompanying the notion of life before biology as we know it spans many imaginative scenarios, which although epistemologically problematic, provide a fertile source of new ideas that can be systematically formulated and experimentally advanced. Steps towards minimal forms of synthetic life are being put in place, and chemistry should be at the heart of this endeavour.

Received: June 25, 2012
Published online: December 3, 2012

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