

# Compositional complementarity and prebiotic ecology in the origin of life

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## Summary

We hypothesize that life began not with the first self-reproducing molecule or metabolic network, but as a prebiotic ecology of co-evolving populations of macromolecular aggregates (composomes). Each composome species had a particular molecular composition resulting from molecular complementarity among environmentally available prebiotic compounds. Natural selection acted on composomal species that varied in properties and functions such as stability, catalysis, fission, fusion and selective accumulation of molecules from solution. Fission permitted molecular replication based on composition rather than linear structure, while fusion created composomal variability. Catalytic functions provided additional chemical novelty resulting eventually in autocatalytic and mutually catalytic networks within composomal species. Composomal autocatalysis and interdependence allowed the Darwinian co-evolution of content and control (metabolism). The existence of chemical interfaces within complex composomes created linear templates upon which self-reproducing molecules (such as RNA) could be synthesized, permitting the evolution of informational replication by molecular templating. Mathematical and experimental tests are proposed. *BioEssays* 28:399–412, 2006.

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## Introduction

This paper discusses the emergence of life from simple constituents, emphasising a general process based on

physical principles and physicochemical constraints rather than particular chemistries or chemical components. The key feature of our viewpoint is that life had its inception neither in individual, complex molecules, such as self-replicating RNA, nor as a metabolic network devoid of the capacity to replicate: rather we posit that metabolic and replicative functions emerged hand-in-hand in networks of interactions best described as a *pre-biotic ecology*. Thus we consider life to have evolved as a diverse interacting *community of molecules* from the start, and not as a single replicating entity, or as a unique primordial species, with divergent offspring. Within this ecological context, the all-important problem is how to explain the emergence of a collection of entities with simple constituents that allow Darwinian evolution of content, function and control.

In previous publications, we have individually posited a set of partially overlapping alternative scenarios under such disparate titles as “a cells-first” equilibrium approach involving lipid domains in a fission–fusion scenario,<sup>(1)</sup> a “lipid world” non-equilibrium approach involving “composomes”,<sup>(2)</sup> “autocatalytic networks”,<sup>(1,2)</sup> “cross-catalyzed networks”,<sup>(3)</sup> and “molecular complementarity systems”.<sup>(4)</sup> None of us realized how our individual pieces fit into a common puzzle until we met in person for a week during December 2004. The result of that meeting is this paper, in which we propose a common, integrated approach to the origin of life problem. We propose an evolutionary process by which complexity can be bootstrapped from relatively simple organic molecules that self-assemble and interact with each other through non-covalent forces governed by the principles of molecular complementarity. These first compositionally diverse, non-homogeneous, non-covalent assemblies we call *composomes*. They exist as dynamic entities that can undergo fusion and fission. In this context, we address a set of issues that we consider to be at the heart of debates over the origin of life (Table 1):

1. the ways in which the first set of composomes formed;
2. how composomes acquired capacities such as growth, fission, and fusion;
3. how this set of composomes acquired catalytic and other functional properties;
4. how control of metabolism and autocatalysis evolved;

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**Table 1.** Problems addressed by proposed theory

1. composome formation via molecular complementarity
2. composome growth, variation and replication
3. acquisition of catalytic and other functions
4. evolution of metabolism and control
5. from compositional replication to linear templates
6. metabolite selection, retention and semi-permeability
7. surviving at the edge of equilibrium
8. adapting energy bandwidth to metabolic function
9. networks, hierarchical order and emergent properties

5. how compositional replication by fission gave rise to informational replication based on molecularly complementary, linear templates;
6. how the concentration of components was focused on biologically useful molecules and semi-permeability evolved;
7. how composomal evolution permitted the emergence of systems that could be maintained away from equilibrium and that could undergo transition between equilibrium and non-equilibrium states;
8. how and why the “bandwidth” of usable energy was narrowed and coupled to metabolic function;
9. how newly emergent properties and more complex regulatory networks evolved from simple composomes through hierarchical ordering of modular subunits.

We choose to ignore other crucial problems, such as the origin of homochirality and the origin of the genetic code, while recognizing that the solution to such problems is as important as those that we address.

### Self-assembly and the origins of composomes

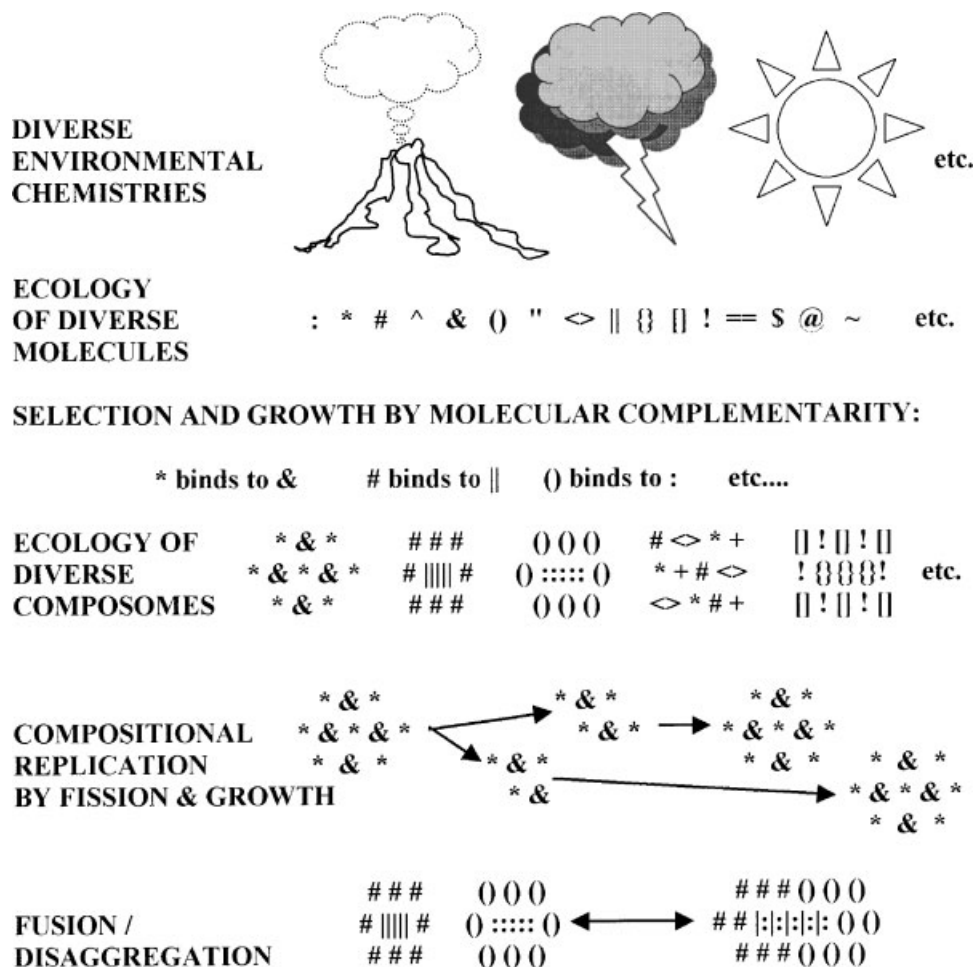
The first problem is how to generate sets of interactive molecules that form the compositionally diverse, functional aggregates that we call composomes. There is no shortage of abiotic mechanisms to generate a huge diversity of molecular species given a flux in which molecules are created and destroyed. These mechanisms include volcanic activity (both terrestrial and suboceanic), lightning, solar heat and ultraviolet light. Molecules may be formed abiotically together in the same locations (hot springs, underwater volcanic vents, tidal pools), or separately in different locations (terrestrial or extraterrestrial) and then brought together by diffusion or transport (streams and rain feeding ponds, precipitation on mineral or clay substrates in the ocean near volcanic vents, cometary infall, etc) (Fig. 1). We propose that from amongst this huge mixture of compounds, various subsets (composomes) would be selected for their ability to interact, stabilize each other against degradation, and buffer their local environment against rapid change. Such basic chemical interactions are clearly essential to metabolism-first models since the catalytic closure required to generate an auto-

catalytic web requires that key constituents must interact before they are degraded. In this respect, composome formation shares with RNA-world scenarios the need for non-random aggregation of the components to be replicated.

The components of composomal assemblies are often thought of as lipid-like or amphiphilic molecules<sup>(1,2,5)</sup> that interact in an energetically favorable manner due to hydrophobic interactions in an aqueous environment. However, such aggregates might also have been based on proteinoids and other amphiphiles, or autophiles (see below). For example, the key chemical constituents may have been diverse entities closely related to the formose reaction (C3-sugars, amino acids, small peptides) that later incorporated lipids and nucleotides into their compositional diversity.<sup>(4)</sup> What is essential is the ability to create micelle-like<sup>(1,2)</sup> or gel-like<sup>(6)</sup> aggregates.

Specific interactions between small molecules in aqueous solution lead to self-assembly.<sup>(7–9)</sup> Lipids, phospholipids, proteolipids and glycolipids will all spontaneously form micelles.<sup>(1,2,6,10–12)</sup> Nucleotides and polynucleotides will bind to cationic lipids<sup>(13,14)</sup> and polynucleotides, polyamines and lipids will all associate to form regular structures with some similarity to bacteriophage.<sup>(15,16)</sup> Micelle-to-vesicle transitions are promoted by the presence of peptides and proteins<sup>(17)</sup> and the interactions are component-selective.<sup>(18,19)</sup> Contrary to intuition, partial incompatibility of molecules enhances self-assembly<sup>(20)</sup> as does increasing temperature near ambient temperature.<sup>(21)</sup> Peptides can self-assemble into non-covalently bound polymers<sup>(22,23)</sup> and can self-aggregate or form heterocomplexes through complementarity.<sup>(24–27)</sup> Peptides can selectively bind to other small molecules such as catecholamines, indoleamines and sugars with binding constants in the micromolar range.<sup>(28–33)</sup> Even molecules as small as ascorbic acid can bind to epinephrine with mid-micromolar affinity.<sup>(34,35)</sup>

The result of such binding is to form complexes that are stabilized against oxidation, pH, heat denaturation and other degradative processes<sup>(4,13,29,34,35)</sup> thereby conferring upon the constituents of such complexes an evolutionary survival advantage so that they may accumulate.<sup>(4)</sup> Hydrogen bonding, ionic interactions, charge transfer complexation and van der Waals bonding promote self-assembly processes and specificity of interaction.<sup>(4)</sup> Homo- (or self-) and heterocomplementarity are therefore important selection criteria in our model of the origins of life. We explicitly acknowledge that such aggregates might also be formed on mineral and clay substrates<sup>(36–38)</sup> and stress that the formation of composomes is compatible with diverse chemical processes in different ecological niches—a point that increases the probability of such a mechanism operating during the origins of life. In general, diversity is essential to the prebiotic ecology that we posit during the origins of life.



**Figure 1.** The emergence of an ecological system of reproducible composomes. Composomes are molecular aggregates of molecules available through the diversity of chemistries present in the prebiotic world. We have used keyboard symbols, rather than chemical symbols or models, to portray composomal components in order to emphasize our point that our theory is not limited to any particular chemistry. Composomal components may have included a very wide range of compounds, including but not limited to, lipids, peptides, saccharides, nucleic acids, and polymers or copolymers of these. Molecular complementarity produces a wide variety of “species” of composomes that represent a prebiotic molecular ecosystem. Complexation buffers composomes against degradation processes so that they persist in the environment for longer periods of time (“survive”) than do their component molecules or molecules not engaged in composome formation. Composomes grow by the continuous aggregation of molecules from the environment. Studies of liposomes show that as such aggregates reach a certain size, they spontaneously fission, producing “offspring”. As long as the component molecules within a composome are present in statistically large numbers, then the “offspring” will have the same range of components as the “parent”. Fusion of composomes will lead to novel compositions that may have emergent properties not found in the original composomes.

### Composomal diversity and replication

The second and third problems to be addressed are that composomes must have diverse properties and be able to replicate with reasonable fidelity in order for natural selection to act upon them. Two sources of composomal variability exist. One is the chemical diversity available in the environment, which leads to diverse sets of compositional aggregates each with differing properties. The other is that some of the aggregates are able to fuse with each other to form more complex aggregates, exhibiting properties not found in the

original individual compositions (i.e. emergent properties). Darwinian evolution will act upon both the simple chemical diversity of the composomes and upon their more complex aggregate properties.

Composomes must also be able to replicate in order to evolve. Early replicators need not have been covalent molecular assemblies. Information can be “encoded” compositionally.<sup>(1,2,5)</sup> In contrast to the elaborate covalent polymerization reactions necessary to transfer information to progeny by nucleic acid base pairing, non-covalent assemblies are

## Hypotheses

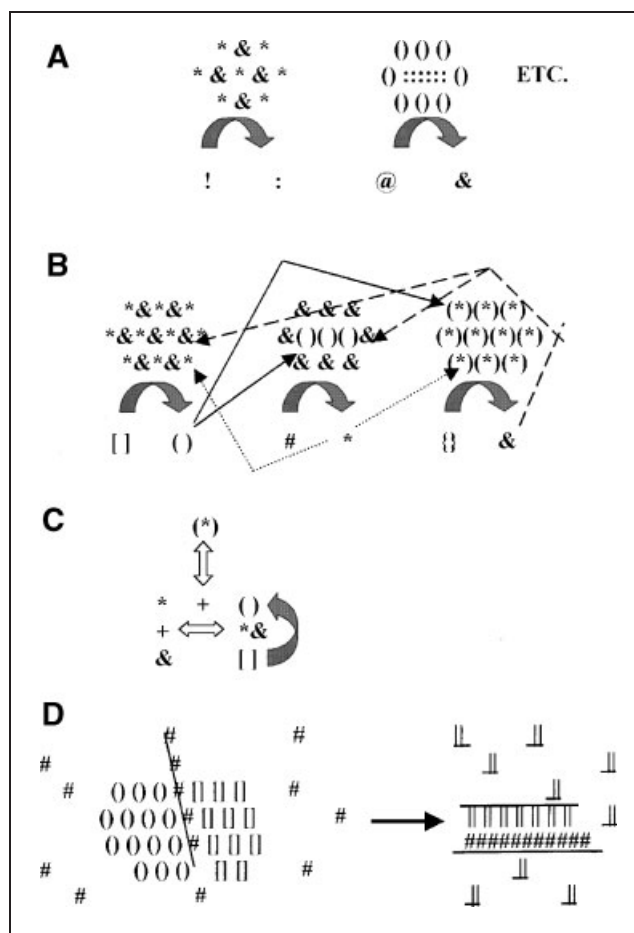
proposed to transmit compositional, structural, network recognition and catalytic information by straightforward fission of the composome.<sup>(1,2)</sup> Fission occurs spontaneously in liposomes in the presence of a ready supply of amphiphilic constituents<sup>(36)</sup> and would therefore occur in growing composomes as well (Fig. 1). For such a mechanism to produce reliable replication, it is sufficient that there is an adequate number of every component of the composome to ensure the probabilistic inclusion of every component in both fission products.<sup>(2)</sup> The stochastic distribution of cellular components such as Golgi apparatus, mitochondria, etc, observed in cells today can be thought of as recapitulating the reproduction properties of the proposed earlier life forms that lacked discretely encoded replication information and were without specific genetic segregation mechanisms. Notably, division need not yield identical daughters to preserve the range of phenotypes in a population:<sup>(40)</sup> all that is needed is for each daughter to generate progeny that generate the full range of phenotypes. Even this requirement can be relaxed in the context of a population of composomes that recombine with one another by fusion.

We propose that the encoding of what we presently call genetic information (i.e. replication based on linear biopolymers) arose only as a secondary step following compartmentalization of structure/function in composomes, which in turn allowed specific polymerization reactions to be carried out (see below). Thus, the split between genotype and phenotype emerged late in pre-biotic evolution.

### The evolution of composomal function

The next problem that must be addressed is the evolution of function within networks of composomal aggregates. If composomes were merely stable associations (even self-reproducing ones) then they would have the non-evolutionary effect of locking up prebiotic material and removing it from the environment. In order to constitute a step towards living systems, composomes had to manifest secondary properties, such as catalytic activity, buffering capacity and metabolic control, which had to be encoded in their composition and functionally available to interact with the environment. In other words, composomes had to *co-evolve* with prebiotic ecologies.

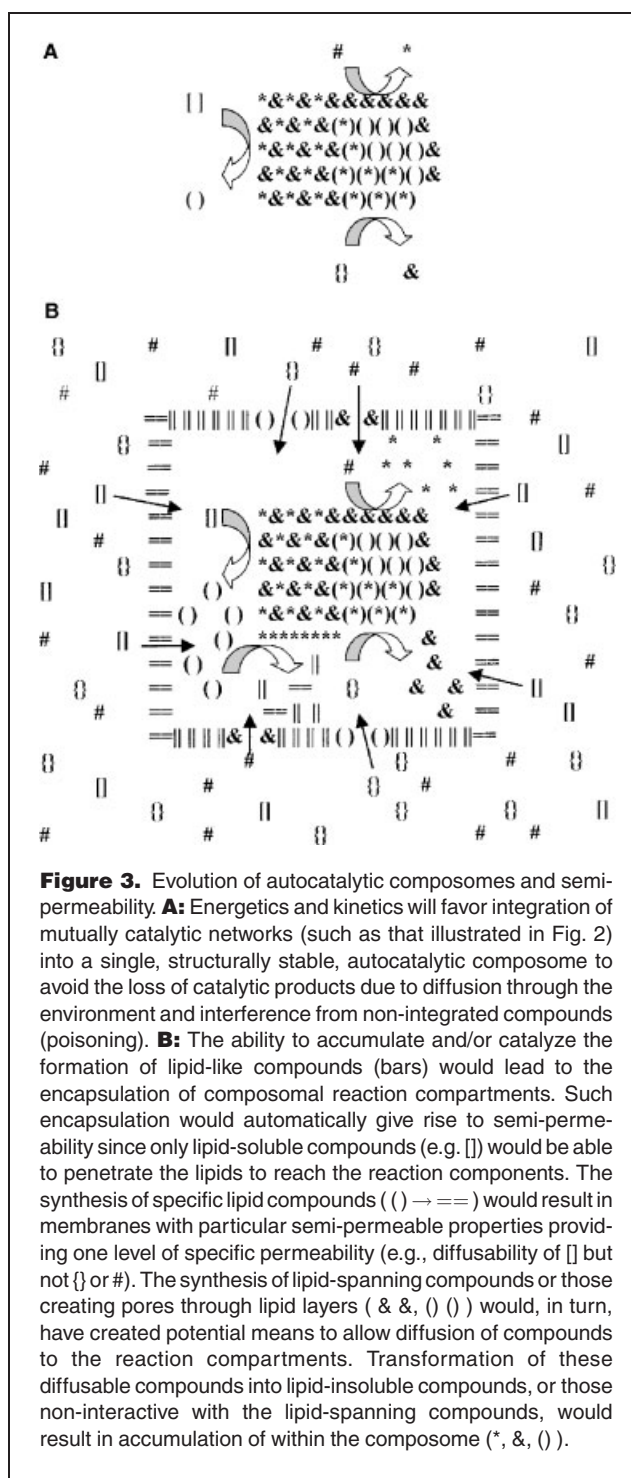
**Figure 2.** Selection for composome function and evolution of mutually catalytic networks of composomes and negative feedback. **A:** Novel composome functions can emerge from novel aggregates of component molecules (see Figure 1B). Some composomes will have surfaces capable of catalyzing reactions (gray block arrows). **B:** Among these, networks of catalytic reactions will emerge. Some of these networks will become mutually catalytic, as illustrated by the three composomes here. Each composome catalyzes one reaction that is required to create the components of another composome. **C:** As a result of molecular complementarity, negative feedback systems will evolve within catalytic networks. \* binds reversibly (white arrows) to & to catalyze the conversion of [ ] into ( ). \* also binds reversibly to the ( ), resulting in stable complexes (\*). The more ( ) catalyzed by the \*& complex, the more (\*) will be formed, and the less \* will be available to form \*& catalytic complex. Thus the reaction is self-regulating through a feedback inhibition mechanism. **D:** Other composomes will have chemical interfaces due to self-aggregation of constituents, or due to modularity, and these may act as linear arrangements at which subunits may align prior to polymerization (bottom). Once polymers become abundant as constituents of composomes, a new round of composomal evolution will begin in which simple molecular complementarity will be replaced increasingly with more complex forms of complementarity between polymers and simple molecules (the origins of enzymes and receptors) and between polymers and other polymers. The result of polymer-polymer complementarity will be selection for systems of complementarity that can accurately encode polymer constituent information. The encoding of complementary polymer constituent information, in turn, permits the emergence of linear replication *in addition to* composomal replication.



We assume that some of the myriad composomes will have catalytic activity (Fig. 2). This is a reasonable assumption since the micellar catalysis of many reactions has been described in the chemical literature.<sup>(41–45)</sup> Moreover, small peptides (e.g. glutathione) and even molecules as simple as proline and histidine have been demonstrated to have catalytic activity.<sup>(46–54)</sup> Our fourth problem is not therefore simply to explain the emergence of catalysis and its control, but to understand the selection criteria by which it evolves.

Simple metabolic control could have been performed in prebiotic conditions by simple chemical kinetics or by means of chemical complementarity, in which pairs of products or substrates could regulate each other's availability by mutual binding.<sup>(3,4,26)</sup> Thus, the product of a reaction could bind to its own substrate, providing negative feedback control, or the products of two different reactions could precipitate each other, driving both reactions toward completion—a form of positive feedback control. Eventually selection for a web of cross-interactions would result in a mutually catalytic network that yielded homeostatic growth.<sup>(3)</sup> Even in webs with predominantly negative (inhibitory) interactions, indirect loops would have enhancing effects. An inhibitor to an inhibitor is effectively an activator in such webs. Self-sustained cross-enhancing webs could result even without the presence of direct autocatalytic entities.<sup>(3)</sup> We further imagine networks of composomes having differing catalytic properties evolving to supply each other with their varied constituents and simultaneously regulating this ecology of chemical species by the kinds of feedback and feed-forward systems just outlined (Fig. 2).

The fourth problem (the evolution of autocatalytic, controlled metabolism) is therefore one of population ecology. In general, we expect catalytic activity to lead to a growing, diverse and increasingly integrated *population* of composomes. In this context, the evolution of spatially diffuse or disseminated autocatalytic networks or ecologies of composomes might have evolved prior to the localization of a fully autocatalytic network within a single composome, or “protocell” (Fig. 3).<sup>(3–6)</sup> The evolution of structural compartmentalization, cell-like organization, membranes with semi-permeable properties, etc would all be late developments in our schema. Spatial co-localization of autocatalytic and mutually catalytic networks would have provided obvious benefits in terms of metabolic efficiency, the concentration of metabolites, and the control over disseminated networks or ecologies (kinetic benefits). The evolution of semi-permeable membranes would have increased concentrations of useful molecules, while decreasing concentrations of molecules that could interfere with efficient network function. In contrast, mathematical analyses of molecular assemblies demonstrate that they can evolve mutually catalytic properties, thereby integrally linking metabolic and reproductive functions from the outset.<sup>(2,3,55–57)</sup> Such mutual catalysis would also wean



composomes off any substrates (such as clays or crystals) upon which they may originally have formed and move such systems further from equilibrium with their environment.

Incorporation of diverse components into composomes, either due to diversity within the environmentally available pool

of molecules or as a result of the emergence of new catalytic functions, would result in the formation of domains or compartments within composomes. For example, micelles composed of several lipids spontaneously form organized domains dominated by the different lipid species.<sup>(2,3,39)</sup> Such domains or compartments would concentrate some components in preference to others and form discrete boundaries of composition within the aggregate. Boundary formation in turn would permit catalysis at the interfaces between such compartments generating more novel materials and functions. Molecular evolution and structural evolution are thereby coupled.

### The evolution of linearly encoded replication

Our fifth problem is that two, quite different, forms of replication must be accounted for in any complete theory of the origin of life: genetic replication and cellular component replication. In modern cells, mitochondrial and chromosomal DNA is the only component (along with the centriole) that is replicated and segregated, with dedicated mechanisms, as a single covalent entity. All other components are stochastically distributed into daughter cells. Either these components are numerous and spatially distributed (e.g. ribosomes) so that stochastic distribution is trivial, or they are low-copy (e.g. unique animal Golgi apparatus or yeast vacuoles), in which case they are broken down into numerous modules prior to division, stochastically distributed, and reassembled in each daughter cell. Our theory provides a general model for how stochastic replication originated and why it is used so universally and broadly within all living systems.

Our theory also accounts for the roles that specific protein and lipid composition play in modern cell division. Although composome division was at first presumably simple vesicle splitting, for example from forced percolation through FeS crystal layers or through simple growth and splitting, controlled division was a major leap forward in the evolution of life. Such primitive directed mechanisms for division still have relics in present-day cell division. Membrane domains of specific lipid and protein composition are implicated in division in both prokaryotes and eukaryotes<sup>(58)</sup> whilst the prokaryotic cell division protein MinD and the nitrogen-splitting enzyme nitrogenase are structural homologs,<sup>(59)</sup> consistent with the idea that primitive biomimetic precursors of these proteins may have been bound to FeS or membranes at a very early stage in composome evolution. This may have been a prelude to modern prokaryotic and eukaryotic cell division mechanisms.<sup>(60)</sup>

In addition, our theory can also explain how encoded, linear replication evolved secondarily as part of the general evolution of catalyzed polymerization reactions. Some polymerization reactions can occur spontaneously in solution. Examples include the transformation of amines such as epinephrine into polymers such as melanins,<sup>(61)</sup> polysaccharide formation or

the polymerization of amino acids in the presence of hydrogen sulfide.<sup>(62)</sup> Such reactions could have provided subunits for composome formation. Many polymerization reactions of the aforementioned types result in complexly branched products that are inappropriate for storing linear information. The formation of linear polymers often requires a surface or interface upon which to order the monomers.<sup>(63–67)</sup> In our model, compositional information would eventually have resulted in discrete localization of functions within composomes, providing the structural basis for linearization of information storage through polymerization reactions on or at modular or compartmental interfaces (Fig. 2).

We may note that linear information transfer need not have resided initially, or solely, in polynucleotides when it first emerged.<sup>(68,69)</sup> It is possible to envision means by which peptides or proteins could have helped each other replicate under prebiotic conditions,<sup>(70–72)</sup> and the transfer of conformational information by prions today<sup>(73)</sup> may be a remnant of such a replication strategy.

### Semipermeability and compartmentalisation

The sixth problem to be addressed is that in modern cells, biological membranes and their associated pumps and permeases, are extremely selective in what they allow to enter and leave. One essential role of highly selective permeability is to concentrate substrates for more efficient chemical reactions and to stabilize some structures. Another is to allow for controlled flux of matter and energy. While the molecular complementarity underlying the formation of composomes may have provided some degree of selectivity, highly specific permeability could not have existed in early life forms. Therefore, to reach the present state, membranes had to gradually increase specific permeability and decrease general permeability to produce the dynamic equilibria and homeostasis mechanisms that characterize the cellular *milieu intérieur*.<sup>(74)</sup> This problem goes beyond the issue of membranes: microcompartmentation within the aqueous phase and preferential partitioning of solutes into gels<sup>(75,76)</sup> are other ways to create local concentration effects essential to metabolic regulation.

To take a simple example, glyceraldehyde is only moderately soluble in water, since it prefers to make hydrogen bonds to itself rather than to water: it is an autophil<sup>(77,78)</sup> that forms 'liquid' domains. Such autophilic liquid regions present a defined surface (or set of dynamic surfaces) to the aqueous medium. Most of what we have said about accumulation of compounds into information-containing regions in a presumptive membrane may have analogs in autophilic surfaces.

A primitive composome would exchange compounds with the surroundings much more easily than a bilayers-enclosed entity, but it would contain a strong pressure toward selective uptake, which is where a selection pressure toward

amphiphilic compounds would enter. Thus a long evolutionary phase may have preceded the emergence of bilayer membranes as we now know them. This may also address the awkward fact that the widely separated groups of prokaryotes and archaea have two different types of lipids (glycerol esters and ethers, respectively) with two widely (in evolutionary space) separated sets of proteins catalyzing their respective synthesis.<sup>(79)</sup> These differences suggest multiple paths toward membrane formation during pre-biotic evolution that are more consistent with a diverse pre-biotic ecology than with a single common ancestor.

In our view, compartmentalisation leading towards membrane formation can arise by three, mutually reinforcing processes (Fig. 3). The first process is that as micelles grow, those composed of appropriate subunits will spontaneously encapsulate aqueous materials, thereby creating a protected aqueous compartment. The second is that compartmentalisation will have arisen within the lipid membrane itself due to self-assortment among different components including lipids, peptides, saccharides, etc. For example, quite simple molecules, such as polyhydroxybutyrate and polyphosphate, can form ion transfer pores in such membranes.<sup>(80)</sup> Such inhomogeneities in the composomal membrane would have provided means for selective uptake, excretion, and concentration of compounds. The third is that molecular complementarity among some molecular species would have provided mechanisms for storage via precipitation, decreased or increased solubility in either aqueous or lipid phases, or decreased or increased permeability into or through composomal domains. A critical result of such mechanisms would be to maintain the composomal composition stably and robustly away from that of the environment. Selection over a long period of time would have resulted in the protein-studded lipid bilayers that currently characterize cells.

### **Capturing free energy to move away from equilibrium**

The seventh problem that our composomal-ecosystems theory helps to elucidate is how systems such as bacteria and viruses evolved that can be maintained away from thermodynamic equilibrium and yet make transitions between equilibrium and non-equilibrium states. We believe that such transitions must have been common during the origins of life. Control over energy usage and over equilibrium–non-equilibrium transitions arose as a natural consequence of the molecular interactions that hold composomal structures together. Just as water absorbs energy prior to a phase transition, so will the components of a composome absorb energy prior to disaggregation, gel–sol transitions, etc. Thus, the forces determining composomal structure themselves buffer the system against changes in concentration of components, disaggregation due to energy fluxes, etc. This buffering will maintain the system away from equilibrium within

reasonable limits set by the binding energies of the components.

We propose that the first polymers also endowed composomes with non-equilibrium properties in addition to providing improved means of specific information storage. First, polymerisation stores constituents and energy, too (if these constituents can be broken down to yield energy); second, the ordered, equilibrium structures formed by polymers give reproducible, catalytic surfaces for the production of the constituents of polymers. Indeed, the regularity of the higher-order structure of certain polymers might be the key factor in such catalysis by lowering the thermodynamic degrees of freedom of the reactants as they interact with the ordered surface. Third, the contraction that cytoplasm-containing polymers undergo in sol–gel transitions has force-generating properties that confer mechanical stability to composomes and could ensure structural integrity in the absence of a flow of energy. Conversion from equilibrium to non-equilibrium structures involving these polymers is facilitated by their regular ordering in low-energy states. Examples include polyphosphates binding to poly- $\beta$ -hydroxybutyrate and polyamines to DNA. Such ordered interactions may lead to new properties. An interesting example is the assembly of polynucleotides into liquid crystalline phases whose binding characteristics to other polymers (e.g. polypeptides) significantly differ from those revealed by dispersed DNA molecules.<sup>(81,82)</sup>

Finally, maintenance of composomal systems away from equilibrium would have been fostered by the evolution of energy-trapping systems in addition to polymerisation. Light energy, for example, could be captured by composomes by means of complexes of simple molecules. For example, flavins and riboflavins will react with indoles and indoleamines in the presence of ultraviolet light.<sup>(83)</sup> Ion–aromatic molecule complexes can also absorb light at different frequencies than the aromatic molecules alone<sup>(84)</sup> and, if complexed to appropriate receptor molecules, become means of energy transfer of the sort that is now seen in chlorophylls. Thus, many simple reactions (including perhaps the more-common heme reactions) would have been available from the outset of prebiotic chemistry for capturing usable energy.

In sum, self-organization, reproduction and inheritable variations emerge from composomal systems when they are kept away from equilibrium by coupling their assembly, chemistry and fission–fusion processes to an external free energy source in such a way as to evolve compartmentalisation and autocatalysis<sup>(2–4)</sup> (Figs. 1–3).

### **Limiting the bandwidth of energy used**

The eighth problem that needs to be addressed is the restricted “bandwidth” of energy used by modern cellular systems. Prebiotic synthesis of compounds is universally assumed to have occurred by high-energy mechanisms, such as the extreme heat of undersea volcanic vents or hot springs,

ultraviolet radiation, etc which are generally not tolerated by modern forms of life. In modern cells, catabolic reactions display standard free energy increments that are in the order of the energy liberated upon breaking a phosphate bond. This narrow energetic bandwidth explains the high yield of today's metabolism, as one of several classical energy tokens (ATP, GTP, NADPH) is phosphorylated for each catabolic step. It is likely that the energy bandwidth used by living systems has therefore narrowed significantly over evolutionary times.

One possible way in which the energy bandwidth could have been narrowed is related to the constraints imposed by the development of a large mutually or autocatalytic web of reactions. Catalysis, by definition, lowers the activation energy necessary to produce a given reaction. The evolution of catalytic composites would therefore have expanded the environmental range in which composites could survive away from high-energy environments to lower energy environments. The migration of composites into lower energy environments would, simultaneously, have increased compositomal survival by removing them spatially from high-energy sources that would decrease their stability. Thus, energy fluxes that would initially have been too small to produce the chemical substrates for composites would have become available with selection for ever-more-efficient catalytic function so as to exploit the energy supplied by light or by a spatial concentration gradients of ions or temperature or by a temporal changes in a parameter such as humidity (hydration–dehydration). Conversely, as composites became ever-more efficient at using lower-energy sources to run their metabolic processes, selection against the use of high-energy sources would have emerged for the simple reason that the higher the energy source, the greater the number of adverse chemical reactions that source will permit. The greater the control over energy bandwidth, the greater the control composites had over reaction products, and thus the greater the fidelity of their function, composition and replication.

The emergence of modularity may also have acted as a selection factor to restrict energy bandwidth. Non-covalently bound molecular structures, such as composites (or ribosomes, Golgi apparatus, etc in modern cells) can exist stably only within a limited range of free energies. Modularity therefore acts as a *structural buffer* against changes in free energy.

A related aspect of the energy bandwidth problem is adaptation to local variations in energy availability by means of structural and chemical equilibrium/non-equilibrium conversions. Prebiotic systems had to contend with changes in their environment, not just extreme conditions. One extreme is the relative lack of usable free energy, as might have occurred if composites experienced low temperatures in tidal pools during winter or as they diffused away from hot suboceanic vents. The ability to make the transition between inactive, structurally stable, highly ordered (equilibrium-like) states and

active, structurally dynamic (non-equilibrium) states, as do modern viruses and bacteria, is therefore a critical feature of the origins of life that must be explained (Fig. 4).

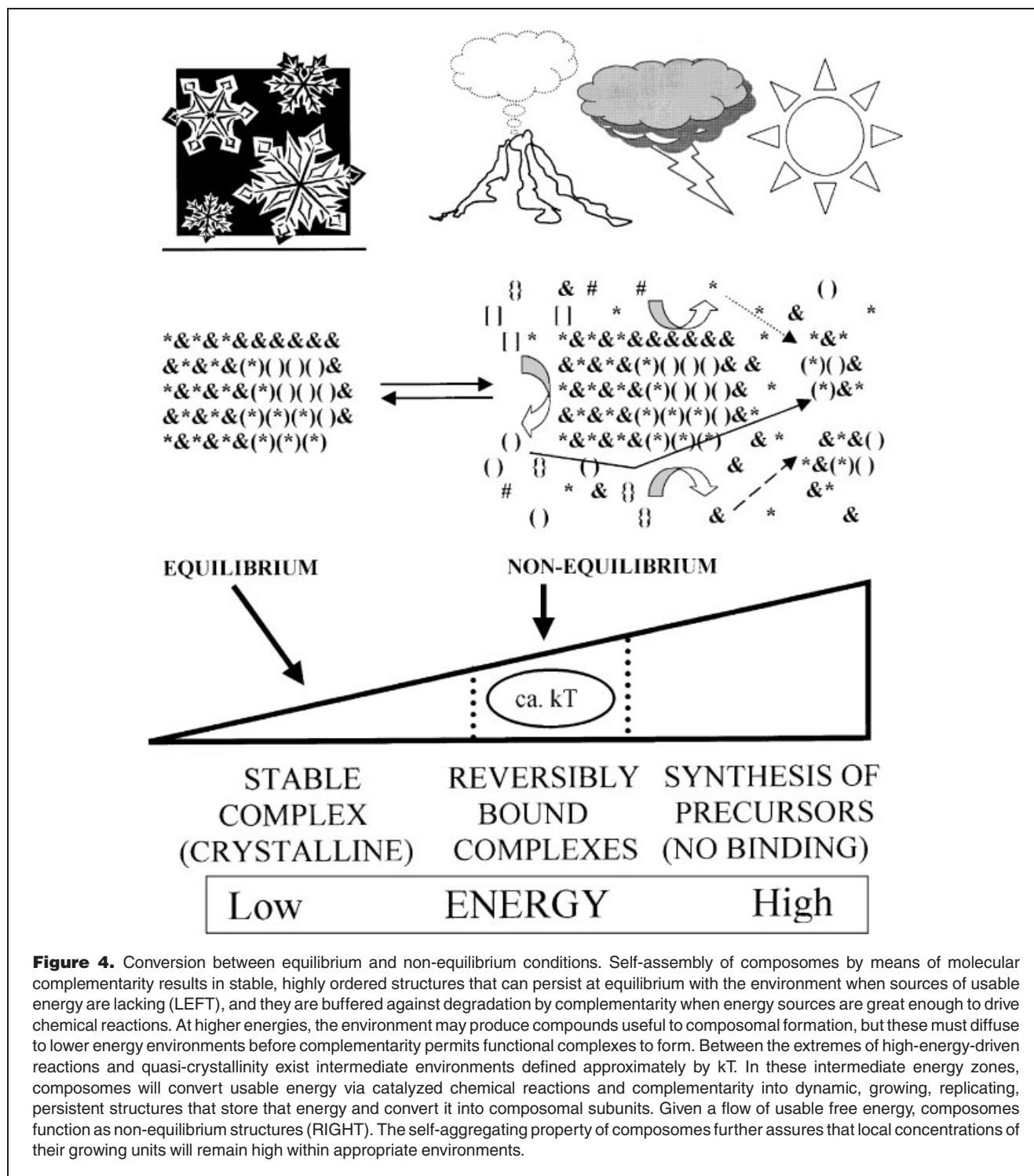
One relevant example of the conversion from equilibrium to non-equilibrium structures is displayed by biopolymers such as actin when cytoplasm undergoes in sol–gel transitions. Such transitions are facilitated by the regular ordering of the polymers. Such ordering produces force-generating properties that could have conferred mechanical stability to composites and could ensure structural integrity in the absence of a flow of energy. For example, divalent ions might stabilise a charged polymer in a regular matrix such that a lowering in the concentration of the ions would allow a progressive phase transition in the form of a cooperative unzipping of the polymer from the matrix. Given that association and complementarity are at the heart of our model, it is significant that modern polymers associate with one another: polyphosphates associate with poly- $\beta$ -hydroxybutyrate whilst polyamines associate with DNA.

Control over gel–sol and polymer-phase transitions would result in sigmoidal (non-linear) system control. Such sigmoid control is present already on simple crystal surfaces such as platinum crystal catalysts,<sup>(85)</sup> and similar processes are likely to emerge on compositomal surfaces, external as well as internal.

### Modularity and emergent properties

Finally, our ninth problem is to explain how increasing complexity and newly emergent properties constantly evolve. A problem with both metabolism-first and RNA-world theories of life is that once a functional set of chemical reactions, or a functional set of autocatalytic RNAs emerge, it is not evident why evolution should continue. What drives a metabolic system to eventually develop a genetic system? What drives an RNA autocatalytic network to require the evolution of the complex metabolic support that exists in all cells? These problems do not exist within our theory because networks of both metabolism and replication exist from the outset, as do specific means for bootstrapping ever-increasing complexity.

Bootstrapping of complexity and emergent properties result from modularity, which in turn is driven by molecular and higher order forms of complementarity.<sup>(4)</sup> Simon has demonstrated that the probability of any complex system being assembled, or reassembled from its disassembled parts, increases dramatically with increased stability of the modular subunits and with increased hierarchical organisation of these subunits.<sup>(86)</sup> Molecular complementarity naturally produces sets of stable modularity within any diverse array of compounds (Figs. 1–4) thereby providing a means of buffering compositomal subunits both structurally and functionally against environmental stresses. As noted above, some complexes will have functions not found in their individual



constituents (e.g. catalytic functions, selective uptake capacity, etc). Equally importantly, each module becomes a new element that can itself be the subject of variation and selection. In this way, modularity permits the emergence of novel properties and functions among composites. Composite

fission–fusion is a recombination-like process that creates novel permutations of properties from the reproducible modules represented by the fission products. Thus, even incomplete fission that fails to produce functional copies of the parent composite may provide modular components that

can be of evolutionary value when these fuse with other modules, or even whole composomes, to produce novel structures and functions. The diversity of chemicals that gives rise to the diversity of initial composomal units thereby provides a source of ever-more-complex functional modules, each of which has new properties that can be combined with additional modules to produce yet more novel properties.

Note, however, that modularity also acts as a conservative force. Adaptive modular structures and functions will tend to be retained through evolution. Novelty will be built in general from mixing and matching previously adaptive modules into ever-more elaborate hierarchies of complexity.<sup>(87–90)</sup>

### Experimental tests of composomal ecology

Many possible tests, some retrodictive (that is regarding patterns that should exist within data already available) and some predictive, exist to differentiate our theory from other theories of the origins of life. We propose only a handful here.

One retrodiction made by our theory is that the functionality of small molecules should be a reflection of molecular complementarity. Within standard Darwinian evolution, it is possible to imagine that any molecule was randomly selected for the function that it currently plays in living systems. But if prebiotic metabolism was regulated by molecular complementarity from the outset, then every molecule incorporated into a functional composome would have been constrained by the selection pressure of complementarity. Thus, we predict (1) that every functional molecule will have at least one molecular complement within living systems, (2) that such molecular complements will be identifiable by the fact that the molecules bind to each other specifically, and (3) that these binding molecules will behave functionally as physiological complements, either antagonizing or synergizing, each other's activity. Conversely, where molecules are found to interact functionally, they will also be found to be molecular complements.<sup>(4)</sup> Preliminary evidence for such pairings of structure–function have already been reported in several systems, most notably the advanced glucose regulatory system, within which glucose binds to insulin, insulin binds to glucagon, and the respective receptor and transporter systems utilize these small molecule interactions as modules around which the proteins have been built.<sup>(27,89–93)</sup> We predict that analyses such as that outlined here will reveal much more extensive use of molecular complementarity as a determinant of function across all cellular systems once the connection between complementarity and function is recognized.<sup>(87,88)</sup>

Predictions made by our theory are also amenable to mathematical and experimental tests. The chemical mechanisms that we have proposed for the evolution of compomes are clearly compatible with the multi-level selection theories of Maynard-Smith and Szathmary,<sup>(94)</sup> Buss<sup>(95)</sup> and others, as well as the mathematical models of the evolution of such self-maintaining organizations previously published by King,<sup>(96)</sup>

Kauffman,<sup>(97)</sup> and Fontana and Buss,<sup>(98)</sup> among others. One mathematical test would be to address a major problem posed by the relative instability of these previous models of metabolic (hyper)cycles when challenged by side reactions, poisoning reactions, environmental instability and increasing complexity.<sup>(38)</sup> We believe that using molecular complementarity as the basis for composomal structure–function will obviate instability. We predict that kinetic modeling will show that the result of molecular complementarity is to drive useful reactions towards completion by effectively and selectively “precipitating” the end products out of metabolic cycles. Such “precipitation” should minimize side reactions and poisoning of metabolic cycles by maximizing the kinetic stability of pathways while fostering the accumulation of metabolites as bound complexes that can buffer the system both chemically and structurally. The functional result should be systems that are far more kinetically stable across a much wider range of parameters than the systems previously modeled by Fontana, Kauffman and King. The type of error minimization system that we are proposing is very similar to the stochastic corrector model previously proposed by Szathmary and, like his, does not require hypercycles to achieve environmental stability.<sup>(99,100)</sup> At the same time, our mechanism for error minimization is compatible with hypercycle models.

Experimental tests of the ecological aspects of the theory are also possible. Most previous experiments on micelles and vesicles have focused almost exclusively on either proteinaceous or lipid components. Researchers performing RNA-world experiments utilize only RNA and its precursors, excluding amino acids, peptides, sugars, polysaccharides, lipids, polyphosphates, polyamines and polyhydroxybutyrate, all of which are known to play key roles in cellular processes. In consequence, previous experiments have largely been unable to explore the results of possible interactions between RNAs and other types of compounds. We believe that RNA-world experiments need to be combined with micelle and vesicle experiments. We would predict that such interactions will have profound consequences, including catalysis of specific reactions, selection for particular sequences, preference for one chiral form over another,<sup>(101)</sup> emergence of new properties, etc. We predict (1) that complex mixtures of peptides, lipids, polysaccharides, polyphosphates, polyamines, polyhydroxybutyrate and nucleic acids in various combinations will self-assemble into stable complexes of limited diversity (excluding some materials available in the “environment”) as a result of molecular complementarity, (2) that some of these stable complexes will be able to catalyze reactions that aggregates formed from their pure components cannot, and (3) that chemical interactions between mixtures of chemical species will result in reactions and products that the pure species cannot. In particular, we suggest that it would be instructive to observe what would happen if the components of the formose reaction<sup>(36,102)</sup> were combined with a variety of

peptides (perhaps derived from relevant enzyme active sites) in the presence of a variety of polysaccharides and lipids. Might a formose-catalyzing complex emerge? Would RNA-world (hyper)cycles be kinetically stabilized over wider ranges of environmental conditions in the presence of lipids, peptides or more complex mixtures than the cycles are when only RNA and its precursors are present? What is the relative kinetic stability of RNA or DNA in the presence and absence of peptides, polysaccharides, polyamines, polyphosphates, polyhydroxybuterate and/or lipids in oxidizing or high-energy environments?

Such experiments may also permit tests of Maturana and Varela's<sup>(103)</sup> concept of cellular "cognition", which is to say the ability of (in this case) composomes to selectively discriminate between environmentally available compounds and components that the emerging life system can effectively utilize. Can vesicles and/or micelles that incorporate specific polyhydroxybuterate, polyamine, polyphosphate, peptide, polysaccharide and/or nucleic acid components evolve the ability to selectively take up and exclude components from the environment? Does such selection maintain composomes away from equilibrium?

Our theory also addressed the so-called "concentration problem".<sup>(68,94,104)</sup> How do sufficient concentrations of prebiotic molecules become localized in order to give rise to non-equilibrium gradients that can drive chemical reactions? The most-common hypothesis is that reactions occur on catalytic inorganic surfaces.<sup>(105–109)</sup> The problem with this proposed solution to the concentration problem is that reaction products will rapidly diffuse away from the surface and be "lost" to further reaction.<sup>(38)</sup> Complementarity once again provides a possible solution to this problem. If reaction products are selected for their ability to bind to other products (or reactants), and if these bound molecules either form aggregates or have a higher affinity for existing aggregates such as micelles or vesicles, then the reaction products will be concentrated rather than lost. Moreover, the fact that some of the complexes so formed will have properties (e.g. catalytic) that do not exist in the individual components will ensure that the reaction pathway never reaches equilibrium by utilizing the products to create novel processes and compounds at higher levels of metabolic organization.

In particular, molecular complementarity makes possible the concentration of prebiotic molecules through aggregate formation in the joint temperature–concentration gradient that will exist in prebiotic environments such as volcanic vents. (1) High temperatures near vents are accompanied by the highest concentrations of compounds, but these are too energetic to bind to one another, (2) further away from the vent, concentrations begin to drop and binding between molecules becomes kinetically possible, (3) complementary molecules aggregate forming composomes, and (4) these aggregates become ever more stable as available energy decreases with

distance or time from the energy source, but rather than being lost by diffusion to the environment, composomal aggregates will remain intact and available for prebiotic functions if (or when) the available ambient energy returns to the approximate kT of the binding energies holding the aggregates together (Fig. 4). Such aggregates may be concentrated by binding to inorganic surfaces as well.

Specific tests of our solution to the concentration problem can be performed by modifying the prebiotic peptide synthesis schemes of Wachtershauser<sup>(108,109)</sup> or Orgel<sup>(62)</sup> or the more general chemical scheme proposed by Martin and Russell.<sup>(38)</sup> We suggest synthesizing peptides in the presence of potential catalysts/templates such as ordered lipid micelles, complementary peptides or nucleic acids (codons or anticodon sequences, in particular). Can appropriate lipids or phospholipids or lipopolysaccharides, or mixtures of these with polyphosphates, polyhydroxybuterate,<sup>(110,111)</sup> or polyamines, replace the mineral surfaces in Wachtershauser's reactions? Can a peptide or nucleic acid-based complement act as a template or catalyst to enhance Orgel's reaction and push it toward completion by partially "precipitating" the product as a complex?

Similarly, RNA-world experiments could be carried out in the presence of lipids, peptides, polyamines, polyphosphates, polyhydroxybuterate and/or polysaccharides to determine whether any of these help to accumulate, stabilize or catalyze particular species of RNAs and to search for RNA catalysts of lipid, peptide and polysaccharide formation (primitive ribozymes). We predict that a sufficient search through these various possibilities should yield a mixture of composomal systems in which RNAs help to catalyze peptides and peptides help to catalyze RNAs, giving rise to primitive virus-like particles very much along the lines proposed by Koonin and Martin.<sup>(112)</sup> In addition, evidence of mutual peptide–RNA (or DNA) catalysis may provide significant clues as to the basis of the origin of the genetic code.<sup>(71)</sup>

### Conclusion: an ecosystems-first theory of the origins of life

The theme of this paper is that the co-evolution of populations of molecularly diverse aggregates (composomes) selected on the basis of molecular complementarity and the emergence of modular functionality forms a basis for addressing some of the issues raised by the origin of life and for recognizing some of the outstanding problems left unaddressed by other theories of the origins of life (Table 1). In this picture, life emerged as a functioning ecological system through a process of integration from diverse components, not as a single entity that subsequently evolved by an as-yet-unknown process into an ecologically diverse system. In our model, there is no identifiable point at which life emerged. Rather, we have described a continuous *process* by which increasingly complex, integrated, self-replicating, autocatalytic, modular sys-

tems evolve new properties in tandem with their environments. We can summarize our theory by saying that it posits an “ecosystems-first” origin of life, rather than a metabolism or gene-first origin.

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