

Submitted to BioSystems Special Issue, December 2006

The Origin of Molecular Autonomous Agents by Natural Selection

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Revised March 2007

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Abstract

Kauffman defines an autonomous agent to be “a system capable of self-reproduction and at least capable of performing one thermodynamic work cycle”. The first such agents were likely to have been chemical automata in the fluid phase. We present a hypothesis to explain the origin of molecular autonomous agents. On the primitive earth there arose at least one recycling flow-reactor containing spontaneously formed ‘oil droplets’ or lipid aggregates. These were capable of a basal growth rate by simple incorporation of lipid phase material, and division by external agitation. This system was the first implementation of a natural selection algorithm, albeit with limited heredity. Macroevolution was possible by the selection for rarely occurring chemical reactions that produced holistic autocatalytic molecular replicators capable of doubling at least as fast as the lipid aggregate, and which were also capable of benefiting the growth of the lipid aggregate container. No nucleotides or monomers capable of modular heredity were required. To explicitly state this hypothesis, a computer model was developed that employed an artificial chemistry exhibiting conservation of mass and energy, incorporated in a population of lipid aggregates. This model evolved increasingly complex metabolic networks whose structure is different from that predicted by previous models of the origin of metabolism.

Keywords: Origin of Metabolism; Artificial Chemistry; Autonomous Agents; Natural Selection; Chemical Evolution

Introduction

There has been no experimental demonstration of a process capable of producing molecular autonomous agents. For example, the prebiotic synthesis experiment of Miller (1953), although initially yielding formic acid, glycine, glycolic acid, alanine, etc, p85 Miller and Orgel (1974), Folsome (1978) ultimately produced a combinatorial explosion of random polymers that degraded into a kind of tar or resin, p 44-45 Cairns-Smith (1993), Schuster (2000).

What modifications must be made to Miller type experiments to allow at least one of the following outcomes: ‘open-ended evolution’, Bedau et al (2000); the origin of basic autonomy, i.e. a dissipative system capable of the recursive generation of functional constraints, Ruiz-Mirazo, Pereto, & Moreno (2004); a process ultimately capable of the production of nucleic acids or other modular replicators with unlimited heredity potential, Maynard-Smith & Szathmary (1995), Szathmary, (2000); identification of “the course of evolution by which the determinate order of biological metabolism developed out of the chaos of inter-crossing reactions”, Oparin (1964); the coupled cycling of bioelements, Morowitz (1968); the maximization of entropy production by a biosphere, Kleidon (2004); the minimal unit of life, Ganti (2003a,b); an autopoietic unit, Maturana & Varela (1992); or the production of molecular autonomous agents, Kauffman (2003)?

The two factions of ‘prebiotic chemistry’, Ganti (2003), Hogeweg and Takeuchi (2003), give very different answers to this question. The metabolism first group, Oparin (1924), Fox and Dose (1977), Dyson (1985), Morowitz (1992), claim that self-organizing principles must have acted prior to hereditary variation by micro-mutation, i.e. prior to template replication. The replicators first school, Haldane (1954), Miller and Orgel (1974),

Eigen and Schuster (1979) believe template replicators of some sort arose spontaneously. However, this position is increasingly untenable. Even replicator first proponents now admit that “relatively pure, complex organic molecules might have been made available in large amounts via a self-organizing, autocatalytic cycle” and that this “might, in principle, help to explain the origin of the component monomers” necessary for the existence of modular replicators, Orgel (2000).

What explanations do metabolism first theorists give for the origin of molecular autonomous agents? Wächtershäuser detailed a metabolism-first model in which an autocatalytic cycle self-organizes on a mineral surface, Wächtershäuser (1992). Based on Eigen’s proposal in 1971 of a reflexively autocatalytic protein set, Eigen (1971), Stuart Kauffman extended Freeman Dyson’s idea of a phase transition resulting in the spontaneous formation of such sets, Kauffman (1986), Dyson (1985). Walter Fontana and Leo Buss in their λ -calculus model, claimed that “organizations arise in a system lacking any formulation of Darwinian selection”, p1 Fontana and Buss (1994) when a generative chemistry was placed in a well-mixed flow reactor. Harold Morowitz argued that systems ‘driven by radiant energy’ would attain complexity in the form of coupled cycling of material, p121 Morowitz (1992). Tibor Ganti proposed that a formose cycle metabolism could exist in the absence of encoded catalytic channelling, within a membrane bound proliferating microsphere, Ganti (2003a). Later we discuss some problems with these theories.

We propose an individual first hypothesis. To describe this hypothesis we must first define metabolism, and how individuals can (and perhaps must) exist prior to metabolism. Some

definitions of metabolism presuppose the existence of a physically distinct individual, Boden (1999) whilst others define it in terms of organizational closure, Moreno & Ruiz-Mirazo, (1999). We urge the use of a definition that pre-supposes a physically distinct individual. Why? If one is given a set of chemical reactions with no notion of a physically discrete entity, how can one identify the metabolic subset, in contrast to the non-metabolic subset? Is a definition of metabolism possible purely at the chemical network level? Chemical organization theory has attempted such a definition (Dittrich and Speroni d. F., 2006), as has Kauffman using his notion of catalytic closure, Kauffman, (1995) in which “every molecule in the system either is supplied from the outside as “food” or is itself synthesized by reactions catalyzed by molecular species within the autocatalytic system.” The former is insufficient to capture fully the unique properties of the metabolic networks of extant organisms. For example, there is no instance of a metabolic network in an organism that is a closed and self-maintaining set, i.e. waste products are produced, and recycled outside but not inside the organism. Metabolism is also simultaneously more than a closed and self-maintaining set since there is exponential growth, and eventually the material is transformed into a physically distinct offspring. Kauffman’s notion of catalytic closure also does not apply to extant organisms because recently it has been shown using metabolic scope analysis, Heinrich ([date?]), that there exists in all known cases, a subset of molecules (e.g. ATP) that is neither produced *de novo* by reflexive autocatalysis, nor available in the food set, but which must be present in at least one copy to produce more copies of itself, i.e. a holistic autocatalytic core (Eors Szathmary, personal communication). Therefore, such notions of metabolism introduce a conceptual bias that may (and probably does) exclude important potential metabolic organizations.

We propose that the notion of a physically separate individual is logically anterior to the notion of metabolism as the term is used naturally. We define metabolism as the network of chemical reactions, that is channelled by autocatalytic chemical species contained within an individual, and that *contributes* to the production of this physically distinct individual. We say *contributes* because we see that it is possible for an individual, e.g. a lipid aggregate, to form without chemical reactions and without autocatalytic molecular replicators; i.e. it may form by phase separation alone. Thus, discrete individuals can exist as units prior to metabolism. We then propose that metabolism arises (as Wächtershäuser suggests) through stochastic chemical avalanches, which we describe later. However, in contrast to Wächtershäuser's model, individual level natural selection at the level of the lipid aggregate preserves those metabolic networks that benefit the growth of the lipid aggregate. The organizational features of the evolved metabolism will be described, and we will see their relation to previous formal notions of what constitutes metabolism. Our model demonstrates that for a particular set of chemical reactions (metabolism) to be maintained indefinitely across lipid aggregate generations, there *must* exist autocatalytic chemical species that can at least double in concentration per lipid aggregate generation. This chemical species may *either* catalyse a chemical reaction, *or* may be directly involved in a productive chemical reaction. Thus, the metabolism of each lipid aggregate may consist of different chemical reactions, if there is a variety of autocatalytic chemical species that may either catalyse or be consumed in chemical reactions. Note that our notion of an individual is less than the bounded autopoietic individual, proposed by autopoietic theory, Maturana & Varela (1992). It

is sufficient to be merely an oil droplet to be an individual, in our case. In summary, the first individuals capable of undergoing natural selection were non-metabolic, i.e. formed due to phase separation alone, in the absence of metabolism.

Theories of Chemical Self-Organization

The reader already familiar with the problems of previous metabolism-first theories that suggest self-organizing principles independent of natural selection, may safely skip this section and proceed to the description of the assumption of the model.

Reflexive Autocatalytic Sets

Based on Eigen's proposal in 1971 of a reflexively autocatalytic protein set, Eigen (1971), Stuart Kauffman extended Freeman Dyson's idea of a phase transition resulting in the spontaneous formation of such sets, Kauffman (1986), Dyson (1985). Kauffman's mechanism assumes: i. a large set of abundant food molecules from which members of the set are produced; ii. molecules have a certain probability of catalysing the production of *other* molecules; iii. each species in the set is reflexively autocatalytic, Farmer, Kauffman & Packard (1986), Bagley & Farmer (1991). In reality, this is far from the situation found at the end of the Miller experiment, which although containing polymers of amino acids, does not contain polymer replicators. Kauffman's model assumes a significant extent of self-organization to produce the high numbers of successful autocatalysts capable of co-existence in the same reactor, when this is in fact what we must explain. However, the killer blow to Kauffman's theory comes not from the minutia of a debate about the probability of catalysis or autocatalysis, Jain and Krishna (1998), but from the fact that it

ignores the problem of side-reactions, Szathmary (2000). It does not consider that as well as a probability of catalysed *addition* of a species to the set, there should be an even greater probability of the catalysed *removal* of a species from the set, if such catalysts are indeed randomly generated. Furthermore, because there are no mass constraints, species cannot be lost from current models of reflexive autocatalytic sets, so depletion of species due to side-reactions is neglected. Neither has this objection been addressed in more recent derivative work, Mossel and Steel (2005). In practice, even by using encoded molecules, Lee et al (1998), it has not been possible to produce a reflexive autocatalytic set, Szathmary (2005). Thus, Kauffman has proposed a self-organizing principle distinct from natural selection that cannot work if we take the idea of side-reactions seriously.

λ -calculus

Another model of chemical evolution is Walter Fontana and Leo Buss' λ -calculus, in which they claim "organizations arise in a system lacking any formulation of Darwinian selection", p1 Fontana and Buss (1994). They admit that their model is competent *only* to explore the logical possibilities that follow from the construction of new molecules upon reaction and the equivalence of reactions with respect to products, p 11 Fontana and Buss (1994), however, the absence of conservation of mass or energy brings into doubt whether their findings could be replicated in a physical well-mixed chemical flow reactor¹. In the λ -calculus, λ -objects collide and react in a flow reactor. Random removal of λ -objects is a selective pressure at the level of λ -objects, so if they become capable of multiplication and variation they will be subject to natural selection. Indeed this is observed in the formation

¹ Our model introduces mass and energy conservation, and despite lacking an arbitrary notion of equivalence of reactions with respect to products, it assumes that catalysis arises in one step only if there is reformation of reactants as products in a random bimolecular rearrangement reaction.

of L0 organizations consisting of self-copiers of form $\lambda x.x$, which form unstable hypercycles. When the authors introduce the assumption that single-step copy operations are disallowed, L1 organizations form consisting of sets of self-reproducing λ -objects, which are stable against the introduction of L0 self-copiers at a later stage. They claim that no natural selection could have taken place *between* L1 organizations, so arguing for a self-organizing principle that could establish increasingly complex 'self-reproducing' L1 organizations in the absence of natural selection. It is not *necessarily* the case that only one L1 organization can exist in the flow-reactor, and therefore that natural selection could in fact take place, Szathmary (1995). Even single constituents, if lost, may compromise an entire L1 organization. In any case, it is not clear whether even natural selection between multiple L1 organizations would be sufficient in a well-mixed flow reactor to allow continuing chemical organisation. For example, Decker maintained a formose cycle in a flow reactor, but he found no tendency for continued organization, as Fontana and Buss' model predicts, Decker (1982). One reason is the production of tapping (polymeric) side-products that are not themselves autocatalytic, but which increase the food threshold of the autocatalytic constituents (i.e. the food set concentration required to prevent depletion of the autocatalytic constituents), Decker (1982), King (1982). This is the paradox of metabolic specificity; no neat (high specificity) metabolic reactions without encoded catalysis, but no encoded catalysis without neat metabolic networks. The tapping side reaction is a problem inherent to systems with conservation of matter. In any case, in the world of λ -calculus, limiting the maximum length of expressions has artfully prevented the equivalent of such tapping by elongation reactions. Thus, yet another claim to have demonstrated a self-organizing principle independent of natural selection must be rejected.

Energy Flux as an 'Organizing Principle'.

Another self-organizing principle has been put forward by Harold Morowitz who argues that life is 'driven by radiant energy' to attain complexity in the form of coupled cycling of material, p121 Morowitz (1992). Although Morowitz is careful to point out that "complexity alone is an insufficient measure for characterizing the transition from nonliving to living", p123 Morowitz (1992) he does go on to claim that the Miller type experiments "indicate the great potential of a directed energy input to organize a system". Organization being defined as compressible complexity, p127 Morowitz (1992), McGregor & Fernando (2005). The logical mistake is quite clear in the following quote

"Continuing non-equilibrium systems must thus be maintained by a flow of energy through the systems which counters the disordering due to dissipative processes. This is a statement of a necessary condition. There appears to be associated with this a statement of sufficiency that the flow of energy from a source to a sink through an intermediate system always leads to an ordering of the intermediate system.", Morowitz (1971).

The last statement does not follow from the first. The continued steady state flux through a Benard cell or a cloud does not arise *because* the physical properties of the Benard cell or the cloud were ordered by the heat flux. Rather, it is the response of a material that makes up these systems to convection, inertia, and gravity that serendipitously permit these *particular* systems to maintain a steady-state heat flux. Morowitz writes correctly "If energy flows in from the source at a fixed rate, a steady state is ultimately reached where the influx of high frequency energy is just balanced by the flow of heat to the reservoir." However, depending on the system concerned, this steady-state flow may not be

maintained, and may decrease (e.g. as non-recyclable polymers are produced). If one randomly chooses a physical system and applies some energy to it, it may reach a higher steady-state energy flux, but does this allow one to claim that the energy itself has acted as a ‘driving force’? The confusion rests in the ambiguity of the term ‘driving-force’, for example, is the driving force of a car the engine or the driver? It seems that Morowitz’s energy flux is partly analogous to the engine in a car, but not to the organizing principle that directs the motion of the car, i.e. the driver who selects which route the car will take. We propose that steady state energy flux is a necessary but not sufficient condition to explain the origin of autonomous agents.

Morowitz’ idea that energy flow through a system “acts to organize that system”, p 73 Folsome (1979), has not been without influence. For example, since Folsome asks the question “How did aqueous coupled bioelemental cycles driven by radiant energy to become more complex come to exist?” she believes that “the complexity of energy-driven systems must increase”, p77 Folsome (1979). The fact that “steady-state organic reaction networks maintained away from equilibrium by the input of electronic excitation will be characterised by flows of matter around closed loops in the network.”, p117 Morowitz (1992) does not imply that all systems that are driven by the supply of external energy become increasingly organized; most will lose the capacity for recycling, by the formation of enlarging and non-recyclable sinks, and Morowitz does not explain the mechanism by which a small subset of systems which are driven by external energy actually increase their rate of recycling. We propose that *natural selection* is the only known means of producing the increasingly complex cyclic systems capable of increased steady state energy flux, and that vice versa, an initial steady state energy flux is necessary to sustain the first

spontaneously formed natural selection machine. Thus, we do not agree with Morowitz' hypothesis that energy flux is an organizing principle, other than the requirement of steady state energy flux for the maintenance of a 'natural selection machine'.

Maximization of Entropy Production Principle

Recently, Dewar, who seeks to give a thermodynamic explanation for MaxEP, Dewar (2005), has made the claim that systems with sufficient degrees of freedom, driven by an external energy source, maximize entropy production as an inevitable statistical consequence. It is implied that this is an organizing 'selection principle'. So far Dewar's model has been shown to apply to a very limited set of cases, e.g. convection systems, and his theory does not seem to predict increasing organization in chemical systems in which side-reactions can increase the size of the sink. In the later kind of system, our model predicts that reactors that sustain 'biospheres' undergoing natural selection will tend to exhibit an increase in the rate of entropy production.

The Oparin School

Natural selection is an algorithm that operates in populations of entities capable of multiplication, variation and heredity, Maynard-Smith (1986). What is the simplest 'machine' capable of implementing the natural selection algorithm, and how likely was this implementation to arise spontaneously? Oparin proposed natural selection between spontaneously formed dissipative coacervates, structures composed of initially randomly aggregated polypeptides, p190 Oparin (1938). In an early presentation, Oparin distinguished the coacervates made in the lab (artificial coacervates) from coacervates that may have formed naturally, the later being capable of internal reactions and rare chemical

transformations, with selection between coacervates favouring those capable of absorbing material from the outside medium more strongly, Oparin (1938). Later, Oparin suggested that polypeptide composition would be the substrate for variation, p70 Oparin (1964). However, no replicating coacervates were demonstrated. Sidney Fox experimented with microspheres composed of polypeptides, working along essentially the same principle whereby division depends on multiplication of the constituents, or at least the non-random polycondensation of amino acids. Budding occurred, but with undetermined heredity, p248, Fox and Dose (1977). In another variant of this theme, Folsome observed that the “thin oily scum” on the water surface of Miller type experiments formed microstructures that she claimed grew exponentially and then sank to the bottom of the flask, Folsome (1979). In all the above cases, the proposed but not successfully realized mechanism of natural selection was the aggregation of partly hydrophobic constituents in aqueous systems, their multiplication with heredity and variation, and selection based on some dilution effect. Folsome proposed that the first microstructures grow by light driven heterotrophic anhydrous polycondensation of amino acids driven by pyrophosphates’ conversion to phosphate; pyrophosphate being produced in ‘photosynthesis by the primary metabolism of protocells’, p110 Folsome (1979). Such an ordered system could not form spontaneously, and exists so far only in theory. Krishna Bahadur in India, working at the same time as Fox, showed that formaldehyde, ammonium phosphate, mineral salts and ammonium molybdate, when exposed to sunlight, formed spherical microstructures called “Jeewanu”, p329 Ganti (2003), Bahader (1954), Bahadur and Ranganayaki (1983), with catalytic activates. Arthur Weber recently described a similar synthesis of proliferating microspherules from sugars and ammonia, Weber (2005). Almost a century of research shows there are many ways to produce phase-separated individuals. In contrast to the

model presented here, recent models of the spatial dynamics of phase-separation have not considered the prebiotic co-evolution of phase separated compartments with holistic metabolic replicators, but rather the co-evolution of synthetic phase separated structures and modular template replicators, McCaskill et al, (2006).

The Lipid World

It is important to distinguish the mechanism of lipid aggregate growth and variation proposed here, from the idea of ‘composome’ based heredity in the lipid world model, Segre et al (2000, 2001), which is akin to a population of reflexively autocatalytic sets isolated in compartments, and that is not capable of stable heredity. Our model differs in that chemical avalanches produce autocatalysts that do not *necessarily* contribute to the growth of the lipid aggregate by catalysing the production of other autocatalysts in a reflexive manner (as in the GARD model), but by contributing to a common intermediary metabolism. The network diagrams in the Results section reveal the evolution of a completely different chemical organization to that proposed in the ‘composome’ model.

Assumptions of the Model

Inspired principally by the experimental work of Oparin, Fox and Dose, Folsome, etc..., we favour the hypothesis that the natural selection machine capable of forming the first cells was a geophysical process capable of spontaneously forming phase separated ‘individuals’, e.g. lipid aggregates, undergoing *division by externally imposed agitation*, i.e. arguably replication rather than self-replication. In addition to the reliable sustenance of a reactor in which lipid aggregates can grow and multiply, a source of internal lipid aggregate variation would have been required. Guenter Wächtershäuser has considered in

some detail the capacity for pre-template variation in a more complex surface bound three phase 'semi-cell', Wächtershäuser (1992). Wächtershäuser writes "Abandoning determinism (Popper, 1972, 1974, 1983c, 1990a) we come to see that chemical reality covers more than high-propensity ("deterministic") reactions. In fact, low-propensity ignitions of autocatalytic cycles (with high-propensity propagations once they have been ignited) seem to be the very chemical stuff of biochemical evolution.", p89 Wächtershäuser (1992). We model precisely such a process, referring to it as "variation by chemical avalanches". Szathmary classifies this process as holistic or attractor based heredity, Szathmary (2000).

The natural selection machine instantiated in a laboratory is shown in figure 1.

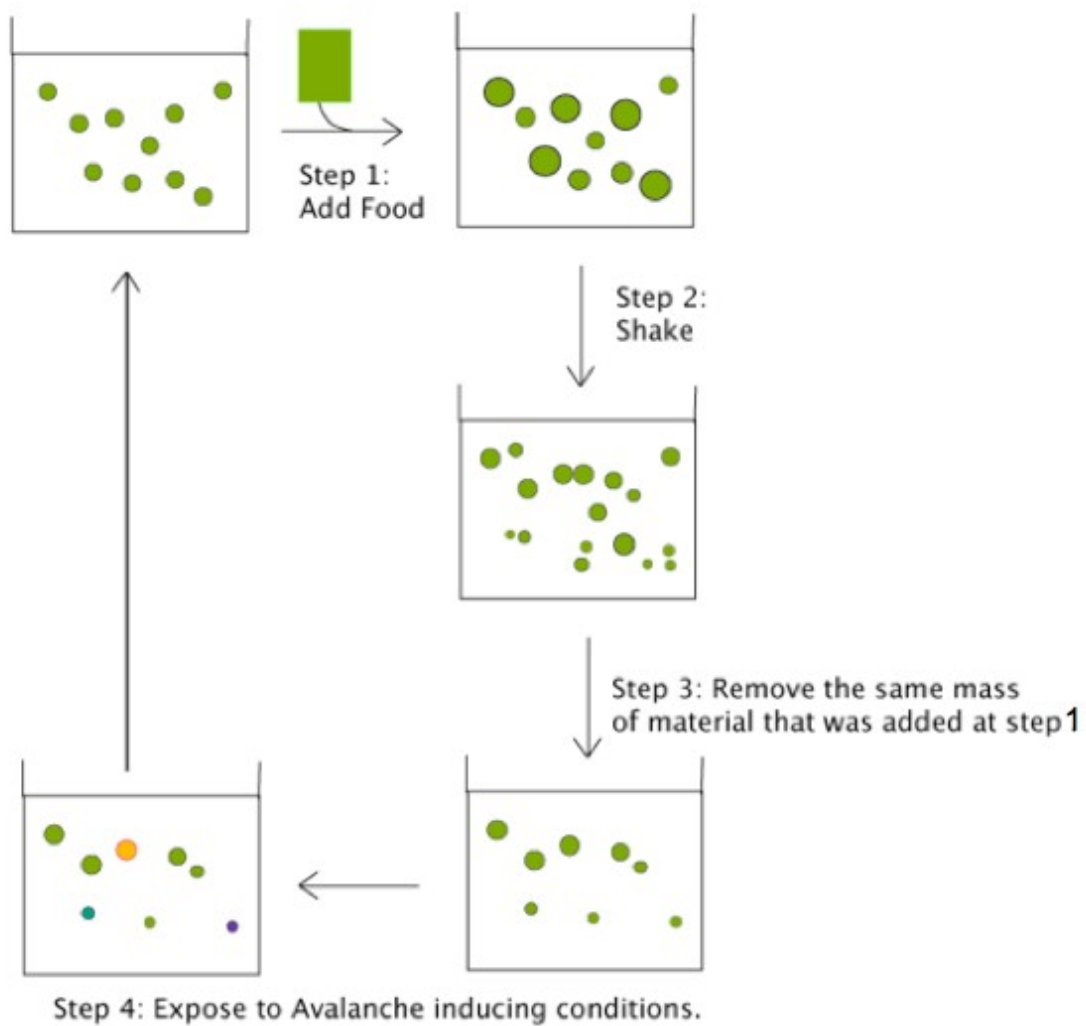


Figure 1: Start with a solution of lipid aggregates. Initially all grow at some base rate when lipid is added, and divide stochastically when the reactor is vigorously shaken in step 2. After this, a reaper removes 50% of the hydrophobic material from the reactor. Conditions are changed to favour rare novel chemical reactions, e.g. high pressure. During this phase a chemical avalanche may occur, which may produce an autocatalyst that may increase the production of food set molecules and so increases the fitness of the lipid aggregate that contains it.

The assumptions made are offered below. It is important to distinguish conceptually three systems, i. the real geophysical system, ii. the laboratory model of the geophysical system, shown in figure 1, iii. a simplified computer model of the laboratory model in which is modelled only *one* lipid aggregate and its surrounding water phase food molecules.

Assumption 1: *A reactor ('natural selection machine') capable of sustaining replicating phase-separated compartments arises spontaneously.* Heterotrophically replicating lipid aggregates are assumed to exist, possibly on a surface as in Wachtershauser's hypothesis of the Iron-Sulphur world. However, the dynamics considered here assume simply the replication of *some* phase-separated individual, e.g. a lipid aggregate, due to the incorporation of external food matter, resulting in proliferation. The crucial difference between this model and that of Wachtershauser's is that we assume no specific intra-lipid aggregate chemical reactions are necessary for a base rate of lipid aggregate replication. Such a robust geophysical process of generating "virgin" lipid aggregates and proliferating existing lipid aggregates by incorporation of 'food molecules' is central to this hypothesis. We assume that some geochemical cycle is capable of spontaneously arising that continues the supply of 'food material' (the food set) to a reactor containing such lipid aggregates. Further, we assume that these lipid aggregates divide due to external agitation. This should be called replication not self-replication, nevertheless it satisfies the requirement of the natural selection algorithm for multiplication, Maynard-Smith (1986), see figure 1 , step 1 and 2. We assume there is some method of removal of lipophilic matter that imposes selective pressure, see figure 1 step 3.

Assumption 2: *'Random' rare novel species are produced by rare reactions within the lipid aggregate.* Novel rare reactions arise between species in the hydrophobic phase that may result in the production of a novel species at extremely low copy number, step 4.

Assumption 3: *There is an avalanche of effects due to a novel species.* Some novel species

may undergo reactions that occur at sufficiently high rates that they influence the dynamics of a lipid aggregate within the average basal doubling period of the lipid aggregate. Very rarely produced novel species present at very low copy number can only have a significant effect if it is autocatalytic since otherwise its copy number will not increase beyond one, and it will be lost by segregation instability. Most novel rare species will not be autocatalytic. By autocatalytic we mean that the species grows exponentially due to the existence of an autocatalytic cycle which may be many chemical reactions long, for example it may be the case that the two ‘copies’ of the autocatalytic species are produced at two distant points in the reaction network. Furthermore, even if the novel species is autocatalytic, other reactions in which it participates may not be of benefit to the lipid aggregate. A novel species has a certain probability of being in the lipid phase (so remaining within the lipid aggregate), and a certain probability of being in the water phase, so being extruded from the lipid aggregate. Side-reactions occur as avalanches that randomly explore the adjacent possible chemical space. These avalanches consist of additions to the stoichiometric matrix, and are not restricted to catalysis, although catalysis may emerge due to the generation of cycles of production. Alternatively, avalanches may ‘poison’ catalytic cycles, again this is an emergent process in our model. Typically, an avalanche, even if it produces an autocatalytic topology will not be capable of sustaining that autocatalyst since the decay rate of the novel autocatalyst may be too high due to side-reactions also produced in the avalanche.

Assumption 4: Lipid aggregate fitness is proportional to the rate of production of a subset of lipophilic molecules (the growth set). In reality, the fitness of the lipid aggregate is a complicated function of its composition, but for purposes of modelling we assume that

lipid aggregate growth is proportional to the rate of production of some subset of lipid phase species by the reactions occurring between the lipid aggregate constituents and the externally supplied food set. We assume an artificial selection type experiment in which a batch reactor (envisioned to be a microfluidic reactor) is initialized with a supply of food molecules and a lipid aggregate. For computational simplicity we model *only* one lipid aggregate rather than a population, and we only model lipid phase reactions, assuming that the environment consists only of a bolus of food set molecules, replenished at each generation. The fitness in the computer model is the extent of growth by the single lipid aggregate within the batch reactor initialized with a food bolus at the start.

Assumption 5: Inheritance of lipid aggregate constituents occurs at division. During division imposed at fixed time periods due to external agitation, approximately half of the lipophilic constituents of the lipid aggregate are inherited by each daughter cell. The water phase constituents, not being so inherited, are assumed to have been washed away in the water phase in which lipid aggregates exist (A. Moreno, personal communication).

Assumption 6: A potential autotrophic reaction exists that is capable of utilizing light energy to drive an otherwise non-spontaneous reaction. There is conservation of mass and energy in chemistry. This imposes important constraints that define the properties of reaction networks. All chemical species have a defined free energy of formation, and a defined material composition. The food set has a defined energy, as does the growth set. The autotrophic reaction may be ‘discovered’, i.e. if the reactants undergoing the autotrophic reaction happen to become synthesised due to a beneficial avalanche. As with any other avalanche, the avalanche may be ‘fixed’ if it contributes to the growth of the lipid

aggregate utilizing that reaction.

Methods

A simplified version of figure 1 was modelled using a computer program. Only one lipid aggregate was modelled at a time, along with its enclosing water phase compartment containing food molecules. Simulated lipid aggregates were selected on the basis of their supra-basal growth rates resulting from the production of a predefined set of growth molecules. A hill-climbing algorithm, Rich & Knight (1991) was used to assess the efficiency with which natural selection could generate ‘adaptations’ under the above variation and selection constraints. This hill-climbing algorithm worked by testing the fitness of a parent, and generating offspring from that parent until an offspring was produced whose fitness was greater than the fitness of the parent, in which case the offspring replaced the parent, and the algorithm iterated.

Overview of the algorithm

Algorithm 1 An Overview

Require: $generation = 1$. $t = 0$. Initialize reactor with food set and initial reactions. Assume initial fitness of ‘virgin’ lipid aggregate = 0. $Generation_time = 10,000 \times 0.0001$ seconds. Time-step = 0.0001 seconds.

1: **for** $generation = 1$ to Max_Gen **do**

2: *Create offspring* reactor by taking 50% of liposome phase material present at end of parental generation, and replenishing the food set to its original concentration.

3: *Generate Avalanche:* Randomly (but according to thermodynamic and mass conservation constraints

described later) create novel rare reactions and the subsequent high flux reaction avalanche. Initialize each novel species at very low concentration (e.g. 10^{-7} mM).

4: *Simulate Offspring*: Using the novel reaction network and initial concentrations, use Euler Integration to simulate *generation_time* seconds of reaction dynamics, measuring fitness at 1000 time-step intervals.

4.1: Simulate 3 offspring consecutively produced from the original offspring (with no chemical avalanches between divisions), in order to exclude non-inheritable avalanches. Calculate fitness only of the 3rd offspring.

5: **if** Offspring fitness > Parent fitness x 1.1 (i.e. offspring fitness must be at least 10% greater than parent)

6: *Replace* Parental reactor with Offspring reactor.

7: **end if**

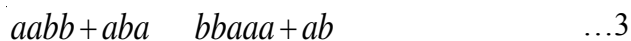
8: **end for**

The artificial chemistry and initialization

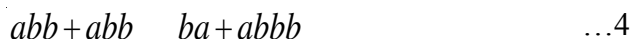
The artificial chemistry consists of ‘molecules’ which are 1D strings composed of the following ‘atomic’ units ‘*a, b, c* and ‘*d*’. Reactions are reversible and irreversible bimolecular rearrangements of the form



in which C + D are random rearrangements of the letters in A and B, for example



The irreversible reactions are of two types; a light absorbing reaction, which is the only reaction defined at the outset...



and novel irreversible reactions, the need for which is described later. Each species has a defined free energy of formation G^f which is either assigned at initialization or generated when a new reaction is produced. All novel reversible reactions are constrained to be spontaneous, that is, $\Delta G^f = G^f_{products} - G^f_{reactants} < 0$. The equilibrium K of a reversible reaction is defined as $K = e^{-\Delta G^f/RT}$, where R is the gas constant ($8.3144 \text{ J mol}^{-1}\text{K}^{-1}$) and T is the temperature in Kelvin. Since thermodynamics underdetermines kinetics, we assume for simplicity that $k_b = 0.01$ and $k_f = 0.01K$. In addition, each species is assumed to be either lipophilic, in which case it is capable of taking part in simulated reactions (type 1 or lipophilic) or it is lipophobic in which case it is extruded from the lipid aggregate and we assume it has a concentration of zero (type 0 or lipophobic). In most experiments there is a probability on 0.8 of a species being lipophilic. A third type, species extruded from the lipid aggregate but capable of taking part in surface reactions, is completely ignored for now. Type 0 molecules are not a part of the set of molecules inherited by offspring. Thus, the production of a lipophobic product can act to make a reaction effectively irreversible, even though chemically the equilibrium may not have been biased towards production of products.

The initial chemical reactor consists in most experiments of the following food set molecules, each provided at concentration 100 mM: $\{aab, aaab, aabb, bbbb, aaaab,$

$aaabb$, $aabbb$ }. All food set molecules are given a $G^f = 1 \text{ kJ}$. The growth set is defined as the following set of molecules: $\{abb, abbb, abbbb, abbbbbb, abbbbbbb, abbbbbbbb, abbbbbbbbbb, abbbbbbbbbb\}$. No molecules of the growth set are present at initialization. The energy of growth set molecules differs. Those comprising the light absorbing reaction have energies defined as follows: $G^f_{abb}=0.1 \text{ kJ mol}^{-1}$, $G^f_{abbb}=0.01 \text{ kJ mol}^{-1}$, $G^f_{ba}=5 \text{ kJ mol}^{-1}$. Thus, light is assumed to contribute *approx* 5 kJ of energy per mole of photons, and is present at 2 moles of photon per litre, and is treated effectively as a non-depleting species that undergoes a tri-molecular reaction with two abb molecules. The other members of the set have increasing G^f with length, i.e. $G^f_{abbbb} = 1 \text{ kJ mol}^{-1}$, $G^f_{abbbbbb} = 2 \text{ kJ mol}^{-1}$, $G^f_{abbbbbbb} = 3 \text{ kJ mol}^{-1}$, etc.

Generation of avalanches

In line 3 of **algorithm 1**, a chemical avalanche is generated as follows. Figure 2 shows how an avalanche is produced.

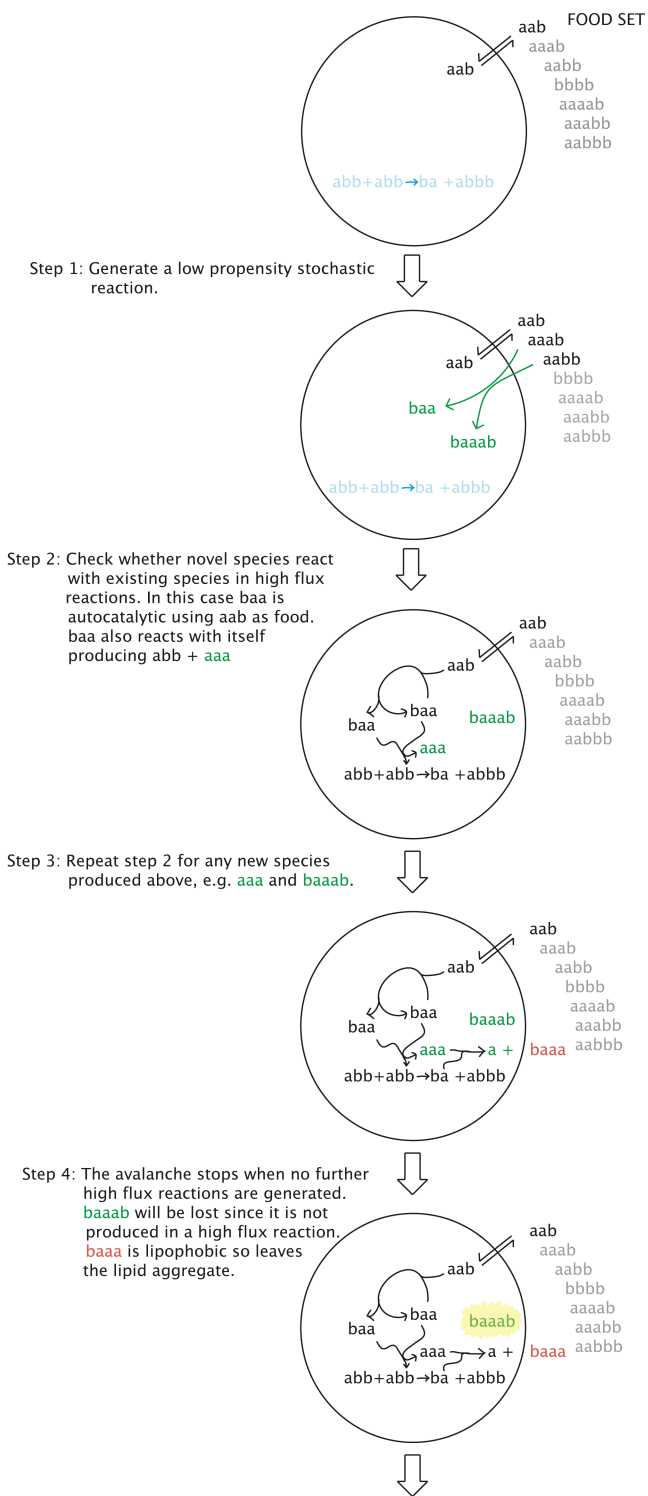


Figure 2: The lipid aggregate initially harbours no chemical reactions. It is formed by phase separation of food set molecules from the surrounding aqueous medium. Note the existence of the ‘platonic’ light absorbing chemical reaction $abb + abb \rightarrow ba + abbb$, shown in pale blue. It is platonic (at this stage) because, so far, no instances of molecule *abb* actually exist. Step 1: After division, the offspring undergoes a chemical avalanche. Species are ‘randomly’ chosen to undergo a low propensity reaction (green arrows) that produces (as a one off) a very low concentration of products (green species *baa* and *baaab*). In Step 2

we must determine which new reactions will be allowed to occur at high propensity due to the presence of these novel species. Novel species are assumed to react with a low probability in high propensity reactions with existing species. These reactions are generated stochastically, according to rules shown in **algorithm 3**. For example, *baa* undertakes two reactions $\{aab + baa \rightarrow baa + baa, baa + baa \rightarrow aaa + abb\}$. Since *baa* may now potentially be preserved over lipid aggregate generations since it is autocatalytic, we colour it black. Application of the stochastic reaction rule resulted in *baaab* not taking part in any further reactions. The high propensity rule produced novel species *aaa*, and an existing species *abb*. The reactions and energy of *abb* has been specified already. Step 3: Repetition of step 3 is required to determine the reactions of *aaa*, which turns out to be $\{aaa + ba \rightarrow a + baaa\}$, and again a repetition of step 2 is required for the new species 'a' which undertakes no new reactions. *baaaa* is stochastically determined to be lipophobic and so leaves the lipid aggregate. Novel species and reactions that may have non-zero flux over an indefinite number of aggregate generations (given appropriate kinetics) are coloured black. *baaab* is inevitably lost. This concludes one possible chemical avalanche. Next the novel reaction network is simulated by numerical integration of its ordinary differential equations using the Euler method.

Algorithm 2: Generate Avalanche

```

0: for N = 1 to num_low_propensity_reactions.
1: Choose two existing species (r1, r2) to react in a low propensity reaction ( $k_f \sim 0, k_b \sim 0$ ) to produce two
potential products p1 and p2.
2: Generate the free energies of p1 and p2 (if they don't already exist) so that the following relation holds,
 $G_{p1} + G_{p2} + \text{heat} = G_{r1} + G_{r2}$ , by portioning energies according to a uniform random distribution, heat being
positive.
3: if it is not possible to satisfy this relation, e.g. because  $G_{p1} > G_{r1} + G_{r2}$ , then the reaction is not permitted,
and no new products are created.
4: else if the condition that the reaction is spontaneous can be satisfied, create novel products at low
concentration (e.g.  $10^{-7}$  M) and increment newSpec ++ //Store the number of novel species produced.
5:  $k_f = k_b = 0$ .
6: end if
7: end for
8: while newSpec != 1 do
9:   for i = 1 to newSpec
10:     for j = 1 to number of species currently existing
11:       if rand() < PROB_HIGH_FLUX x 1/(species[i].length)2
12:         *See tempNewSpecies = output of Algorithm 3
//Make high flux reaction with new species[i] and a random species
13:         //Store tempNewSpecies produced in that high flux reaction.

```

```
14:                 end if
15:             end for
16:     end for
17: newSpec = tempNewSpecies, tempNewSpecies = 0.
18: end while
```

Each novel species is produced at an initial very low concentration. One must then check whether it reacts with other species, and so on, so generating the chemical avalanche. Line 11 describes our assumption that the longer the novel species, the less likely it is to react with other species. If the condition on line 11 is passed, algorithm 3 is executed to generate a potential high flux reaction involving the novel species. Note that it is an emergent feature of the above algorithm that longer molecules are less likely to be autocatalytic because random rearrangements of longer reactants are less likely to reproduce themselves as products in one rearrangement.

Algorithm 3: Calculate a high flux reaction

```
1: Choose species to react with rare species i as follows.
2: for j = 1 to num species
3: score += 1/(species[j].length - species [i].length)2 x (species[j].length)2
4: end for
5: Use roulette wheel selection to find species j biased by the above scores.
6: Once r1 = i and r2 have been chosen, generate random p1 and p2 products based on a bimolecular rearrangement of r1 and r2. This reaction can be biased in various ways, e.g. let the probability of a catalytic reaction, i.e. where r1 = p1, or r2 = p2, be related linearly to the proportion of 'b' atoms in r1 or r2. etc... Many such structure specific probabilistic rules may be applied.
7: Check that Gp1 + Gp2 + heat = Gr1 + Gr2 can be satisfied, and only if it can, store this new high flux reaction, and set kf = rand(C) x 0.01K, and
```

9: **if** p1 and p2 already exist, $k_b = 0$ //C = 100 or 1000 and is a uniformly randomly assigned rate. A log normal distribution has also been used Logrand(C).

10: **else** $k_b = \text{rand}(C) \times 0.01$. //This is to ensure that the exploration of the adjacent possible is correct

11: Return the number of new species produced legitimately, i.e. 0, 1 or 2.

The definition of fitness

Fitness is defined as the integral over the trial of the product of $[\text{species}[i]] \times \text{length of Species } i$, where i is a molecule in the growth set. The second term introduces a biomass effect. This is approximated by sampling concentrations at intervals of 1000 time steps. All trials are of fixed duration. The bolus of food molecules is allowed to deplete if the chemical avalanche has produced species that react with the bolus, or if material has been inherited that reacts with the bolus. This replicates the effect of a potential microfluidic experiment in which a single lipid aggregate is isolated in a small compartment containing a bolus of food molecules, and its size measured after a fixed duration. In many of the trials, fitness is assessed not on the first offspring produced after a chemical avalanche, but on the 3rd post-avalanche offspring. This is to increase the probability that any fitness benefit due to an avalanche is heritable, rather than beneficial only to the offspring in which the avalanche occurs.

Results

The reader may refer to a recent paper describing the same model that presents results from an independent evolutionary run (Fernando and Rowe, 2007).

[Obtain new results]

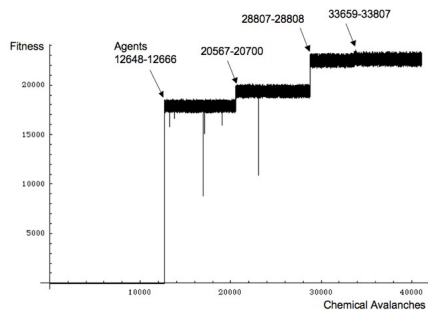


Figure 2 : 4 distinct punctuations are observed. Initial fitness is zero since no *abb* or *abb* exists. Therefore there is effectively random search for an avalanche that is capable of producing one or more of the food set. The first adaptive avalanche is the 12648th. Following this, the frequency of adaptive avalanches is increased for the next three punctuations. Each punctuation consists of a relatively rapid succession of offspring that have higher fitness than the parent that started the punctuation. This may be because the original avalanche takes many generations to fully manifest itself. After a punctuation, the offspring subjected to further chemical avalanches never return to the original fitness of zero. Note that there is a distinct population of harmful avalanches that reduce the fitness below the mean fitness of offspring produced after a punctuation. These avalanches are those that produce an autocatalyst with a harmful effect on the liposome. Most avalanches do not produce an autocatalyst, and the fitness of such offspring contribute most to the mean fitness of post-punctuation offspring. Note that there are no outlying low fitness offspring after generation 28807, which *may* be due to the absence of autocatalyst production.

Fitness increase is punctuated, each punctuation followed by a rapid succession of offspring fitter than its parent. This is due to either the adaptive chemical avalanche requiring several generations to fully establish itself, or due to noise in the initial concentration of food set molecules provided.

The behaviour of the lipid aggregates, one from each of the four fitness levels, is shown in figure 3.

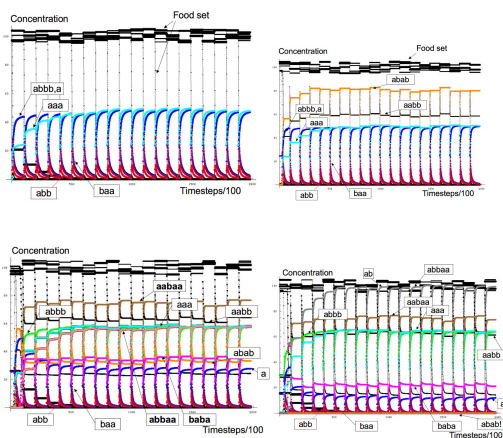


Figure 3. Each graph shows the behaviour of a lipid aggregate (Agent) during 20 replication events in the absence of avalanches. Each generation lasts 10,000 time-steps. At each replication event, the lipophilic molecule concentration is halved and passed to the offspring, and the food bolus re-introduced at 100mM \pm 10mM per food molecule. **Top Left (part a):** Agent 12648. **Top Right (part b):** Agent 20567. **Bottom Left (part c):** Agent 28807. **Bottom Right (part d):** Agent 33659. Note that with successively evolved lipid aggregate (agent) there is an increasingly complex concentration profile, i.e. more (non-food-set) species are maintained at high concentrations, that would be *impossible* without some form of ‘memory’ capable of withstanding dilution at each generation. For example between agent 12648 and 20567 *abab* has obtained the capacity to survive generational dilution. Between agent 20567 and 28807, three more species obtain high concentrations, *abaa*, *abbaa* and *baba*. However, between agent 28807 and 33659 (admittedly a small fitness increase), we see a loss of species, not a gain, i.e. the concentration of *abab* has become negligible. Since there is elitism in the hill-climbing algorithm, each agent is fitter than the previous agent. All agents gain fitness from the concentration of *abb* (red line) and *abbb* (green line). See how the height of *abbb* increases with each successive agent. Also note that *abbb* is initially at the same concentration as *a*, but that in the last two agents, *abbb* and *a*, have different concentration profiles, *a* decreasing in concentration, and *abbb* increasing in concentration. No more complex growth set molecule is produced, e.g. *abbbb*, *abbbbb* are completely absent.

Successive lipid aggregates evolved in the evolutionary run were increasingly complex, i.e. consisted of more species and more reactions, due to the accumulation of chemical avalanches. Table 1 shows the number of species and reactions that constitute the space of the chemical network utilized by accumulated chemical avalanches.

Agent I.D.	Num. Species	Num. Reactions
12648	61	47
20567	99	63
28807	122	76
33659	147	89

Table 1: Total number of species and number of reactions evolved in the four evolved lipid aggregates (agents) studied.

Note that most of these species will be present at very low concentration due to the fact that most will not have been autocatalytically produced, and would have been present initially at very low concentration following an avalanche. Correspondingly, most reactions will experience very low flux.

It is possible, by hand, to identify the core components of the networks that are responsible for the behaviours shown in figure 3. Figure 4 shows this analysis for agent 12648.

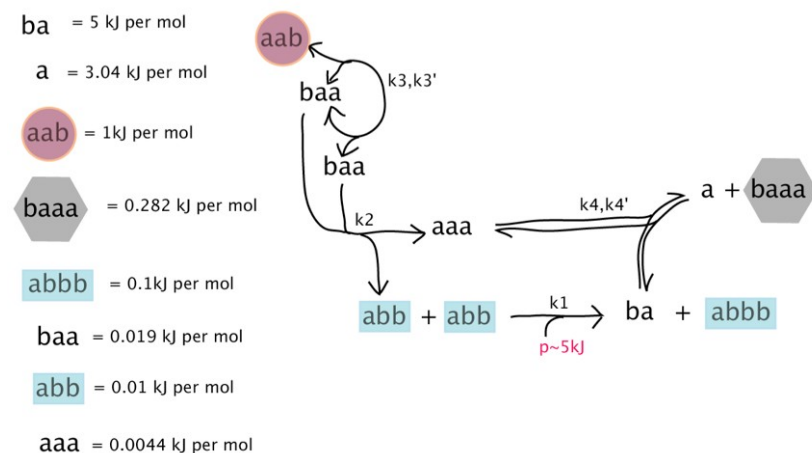


Figure 4. The core autocatalytic network of agent 12648. Red circle = food molecule, blue rectangle = growth molecule, grey hexagon = lipophobic. All other reactions are not shown. The full network is available online at www.chrisantha.com. *aab* is the only food molecule utilized. *baa* is the autocatalyst in one step. Two *baa* molecules are used to produce *abb* and *aaa*. *abb* then utilizes the existing light absorbing reaction to produce *ba* and *abbb*. *ba* reacts with *aaa* to produce *a* + *baaa*. Since *baaa* is lipophobic, it leaves the liposome and would have driven this reaction in the forward direction, so encouraging the production of *abbb* from *abb*. However, since the light absorbing reaction is irreversible, this extrusion reaction has no effect here. The *k* values represent the catalytic factors, i.e. rand© in **algorithm 3**. These must be applied to the expression in **algorithm 3** to obtain the actual rates. $k_1 = 1.0$, $k_2 = 54.2136$, $k_3 = 66.3937$, $k'_3 = 66.3937$. $k_4 = k'_4 = 99.16$.

The core reaction network responsible for supra-basal fitness is due to the autocatalyst *baa*

that is capable of using food molecule *aab*. *baa* at the same time reacts with itself to produce *abb* + *aaa*. *abb* is then transformed by the external energy absorbing reaction that was defined at the outset, to produce *ba* + *abbb*. *aaa* reacts with *ba* producing *a* + *baaa*, *baaa* being extruded. Figure 5 shows how agent 20567 is an improvement upon agent 12648.

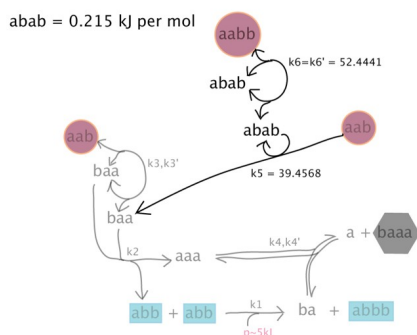


Figure 5. The adaptation of agent 20567 is a new autocatalytic particle with cross-catalytic effects to convert a food molecule into an existing reaction intermediate. The new reactions are shown in bold, and the old ones of agent 12648 more faintly. There are two important new reactions evolved in agent 20567. The first is a one step autocatalysis of *abab* and the next is a catalysis by *abab* of the conversion of food molecule *aab* into *baa*, which then enters the same pathway as used by agent 12648 to produce *abb* and *abbb*.

A new single step autocatalytic constituent, *abab*, has been discovered by avalanche, along with a cross-catalysis of the direct production of an existing reaction intermediate (and autocatalyst!) *baa*, from a food molecule, *aab*. We see in figure 3 the high concentration of *abab* that is maintained by autocatalysis, and the lower concentration of *aabb* that is a consequence of this. Figure 6 shows how agent 28807 has further improved upon agent 20567.

In all evolutionary runs, multiple autocatalytic cycles arose that were organized in various ways to produce the growth set. Several points are evident from the model.

Firstly, the theoretical argument of Wachtershauser is confirmed in this model (Wachtershauser, 1992). All the evolutionary runs (see supplementary material) demonstrate that selection at the level of the lipid aggregate results in survival of lipid aggregates in which underlying molecular replicators came into existence. *Natural selection acting at the level of the higher-order unit (the lipid aggregate) has stabilized novel lower-order units of selection (molecular autocatalysts)*. Why? Because only by autocatalysis is it possible to sustain a novel high flux reaction between generations, i.e. autocatalysts are the sole basis of chemical memory. This type of autocatalysis should be called *engram autocatalysis*, to distinguish it from *growth autocatalysis*. Growth autocatalysis refers to the autocatalytic production of *abbb*, which is defined as increasing the growth of the lipid aggregate, and so is a property at the level of the higher order unit. Natural selection at the level of the lipid aggregate is required to select the autocatalysts that benefit the lipid aggregate. In the absence of natural selection, parasitic autocatalysts could arise that destroyed higher order chemical organizations.

Secondly, a shorter molecule of fewer atom types is more likely to produce itself in a random rearrangement reaction than is a longer molecule with more atom types, therefore the former have a greater probability of being autocatalytic constituents in the current model. This is critically dependent on the structural assumptions of the model. In real chemistry, an equivalence class of longer molecules may be capable of higher probability autocatalysis than some other equivalence class of shorter molecules. Further elaborations

to our model of artificial chemistry are necessary to embody these more realistic structural influences, which in reality we conclude must be essential in continued adaptations. Importantly, only one, two, or three step autocatalytic cycles were evolved. This supports G.A.M. King's notion of a symbiotic process in which independently evolved small autocatalytic cycles come together displaying by-product mutualism (King, 1976). We have not experimented with symbiosis of lipid aggregates, but such variation would occur naturally, and is a subject for further study.

Thirdly, independent autocatalysts can be sustained within the same lipid aggregate, especially if they utilize different food molecules, e.g. *baba* uses *aabb*, and *baa* uses *aab* as food and confer fitness to the lipid aggregate. There will be material constraints on the number of autocatalysts that can be maintained if these autocatalysts must compete for the same food molecule, as demonstrated in the final agent 33659. In this case the autocatalyst with the lowest 'food threshold' will survive. Thus, in early systems we expect the original engram autocatalysts to be fed by different food molecules.

Fourthly, autocatalysts can produce lipid aggregate level fitness by either catalytic reactions (e.g. *abab*), or by being consumed in reactions, that produce the growth set (e.g. *baba*). Both mechanisms are observed in the above experiment.

Fifthly, the build-up of lipophilic side-reactants such as *aabaa* produced reversibly in an OR-reaction tapping an autocatalytic cycle can actually be beneficial to the lipid aggregate since this reduces the net efflux of *baba* from the cycle. Conversely, the removal of a hydrophilic product can shift the equilibrium towards the products, e.g. potentially *baaa*. In

the absence of large differences in chemical potential, random production of lipophilic and hydrophilic species can be a ‘poor man’s irreversibility’ by physical rather than chemical potentials.

Sixthly, the autocatalytic cycles are coupled not by a cross-catalytic requirement for each other’s production, but by the fact that they contribute to the same intermediary metabolism that produces the growth set. We did observe that *baa* became secondarily the product of a new autocatalytic cycle, but its production never became entirely dependent on that novel cycle, in fact the novel cycle was lost. Parasitic autocatalysts, i.e. autocatalysts that use another autocatalyst as their food molecule, can be more easily sustained when the food molecules are of higher free energy.

Seventhly, the probability of producing an autocatalytic cycle in an avalanche increases as the diversity of molecules in the food set increases. This is because there are more chances that a multiple reaction autocatalytic cycle will be successfully driven at each step. This is because each step can utilize an external molecule rather than depending on internal recycling of *both* reactants in the bimolecular rearrangement reaction, see figure 7.

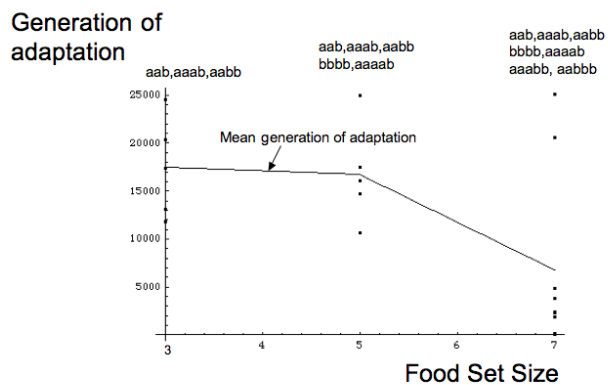


Figure 7. Generation of adaptation (Y-axis) as a function of the number of species provided in the food set

(X-axis). The points show the time of generation of an adaptation during one run conducted for each food set size.

Eighthly, the frequency of autocatalyst production decreases with each chemical avalanche, see figure 8 (middle).

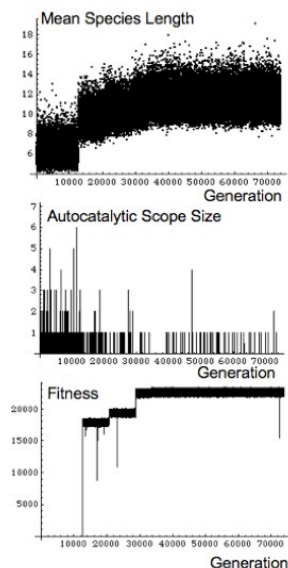


Figure 8. **Top:** Mean species length produced during an avalanche, over the course of apx 70,000 generations. There is an increase in the mean length of species produced. **Middle:** The extent of influence of autocatalytic reactions upon the chemical network, and the frequency of autocatalytic reactions, can be visualized by plotting the number of novel species whose concentration reaches at least 0.0001mM within one generation. Note that all novel species are initialized at a concentration of 10^{-7} mM. **Bottom:** Fitness.

Energy dissipation tends to increase with increasing fitness.

Since this model has conservation of energy, we can ask; is there a relationship between fitness and energy dissipation within a lifetime, or between fitness and the “energetic inheritance” transferred to offspring during the division event? Figure 9 (middle) shows that the lower bound of energy dissipation increases with increasing fitness, but that high energy dissipation does not inevitably result in high fitness, for example, offspring with fitness zero show high rates of energy dissipation, but this is due to reactions that do not

produce the growth set and so do not increase fitness.

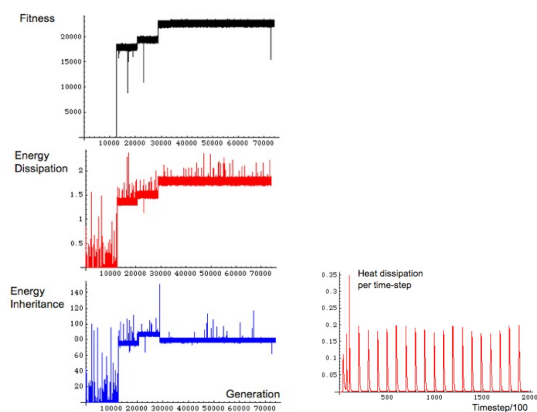


Figure 9. (Part a) Top: Fitness of agents during the evolutionary run of the hill climbing algorithm. **Middle:** Total energy dissipation per generation, measured as the sum over all reactions, of flux through reaction i \times free energy change in reaction i . **Bottom:** Energy inherited, measured as the sum of concentration \times free energy of all lipophilic species at the beginning of a generation, not including the food set. **(Part b) Right:** Energy dissipation per time step over 20 divisions of agent 33659. No distinction between heat and entropy components of energy has been made in this model.

Figure 9, (right) shows the energy dissipation over the course of 20 division events in agent 33659. Obviously, most of the energy dissipation occurs at the beginning of a lifetime since this is when the food bolus is supplied.

Why does energy dissipation increase with increasing fitness? There are two quite distinct causes of energy dissipation, the first is the production of growth set molecules from food molecules, and the second is the maintenance of engram autocatalysts, required to sustain the network capable of producing growth set molecules. The total energy dissipation per generation must inevitably increase as more growth set molecules are produced. Since fitness is here defined as proportional to the mass of the growth set molecules produced after a fixed time period, this *component* of energy dissipation must inevitably increase

with increasing fitness. In reality, fitness is not directly related to the mass of material that constitutes the organization of the individual, because units of selection can evolve to divide at a lower mass. Also, organisms may evolve to make the free energy of the growth set greater than that of the food set, in which case external energy sources other than food will have to be utilized, and there will be a net gain of energy, rather than a net loss. However, in the current model, the high free energy growth set molecules were not discovered. This is partly because their production in a spontaneous reaction occurring in a chemical avalanche is less likely than the production of a low free energy molecule (see figure 10, bottom right), but also because they are longer.

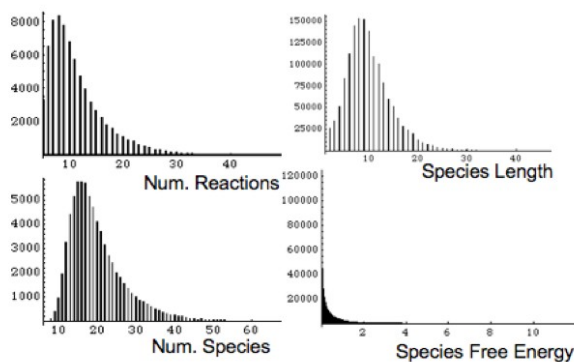


Figure 10. Histograms of avalanche properties. **Top Left:** Histogram of frequency vs. number of reactions produced in avalanches, over the entire course of evolution in figure 1. **Top Right:** Histogram of species lengths produced in each avalanche. **Bottom Left:** Histogram of number of species produced in each avalanche. **Bottom Right:** Histogram of species free energies produced in each avalanche.

The second *component* of energy dissipation is due to engram autocatalysis. Unlike the component of energy dissipation due to production of the growth set, this component does not necessarily have to increase with increasing fitness, because an engram autocatalyst of higher free energy may maintain a reaction by cross-catalysis, better than another engram

autocatalyst with a lower free energy of formation. Alternatively, a lipid aggregate may have higher fitness with fewer well-placed autocatalysts than another lipid aggregate that uses a greater number of energetically costly but poorly placed autocatalysts.

Despite these reasons why an increase of energy dissipation is not inevitable, it is nevertheless observed. The main reason being that more low energy molecules are produced in chemical avalanches than high energy molecules, since reactions in chemical avalanches must occur spontaneously. In addition, in the current hill-climbing algorithm, there is no selection pressure for more efficient lipid aggregates, i.e. for lipid aggregates that produce the growth set with the minimal quantity of food molecules. A fitness function that explicitly rewarded efficiency of growth set production would be expected to produce lipid aggregates with lower energy dissipation per generation. Such a fitness function would simulate conditions of competition for limited food molecules. However, we expect the total energy dissipation of the biosphere to increase, even though individual organisms would be selected that could utilize the novel energy source more efficiently, since there is no selective pressure for efficiency due to competition between biospheres.

Finally, the total free energy of chemicals transmitted to offspring does not necessarily increase with fitness (see figure 9, bottom).

Properties of avalanches relate to the frequency of adaptations.

Figure 8 reveals why no adaptation arises after generation 34000. The number of autocatalytically growing molecules produced in an avalanche decreases with each

adaptation, as does the frequency of producing at least one autocatalyst per avalanche. This is because the mean length of species produced in an avalanche increases with each adaptation. Avalanches become less effective at producing adaptations as they begin to produce more divergent reactions with fewer cycles.

Figure 10 shows 4 histograms of avalanche properties over the course of the evolutionary run, specifically: number of reactions added in the avalanche, number of species added in the avalanche, mean species length of species produced in the avalanche, and species free energy of species produced in avalanches. Note the log-normal type distribution of reaction sizes and species numbers per avalanche. Most species have low free energies of formation, with only a few species having high free energies. Note that most of the data points will inevitably come from avalanches that were non-adaptive, i.e. that resulted in offspring rejected in the hill-climbing algorithm.

Conclusion

We hypothesised a geophysical natural selection machine capable of sustaining chemical evolution by natural selection between lipid aggregates in which heterotrophically formed individuals replicate by external agitation at a base rate and experience chemical avalanches which affect their growth rate. Initially the individuals are not molecular autonomous agents according to Kauffman's definition that requires the agent to undertake a chemical work cycle, Kauffman (2003). We modelled an artificial selection experiment using a hill-climbing algorithm in which lipid aggregates are selected for maximization of the production of a subset of chemicals that defines their growth set, from a food set supplied as a bolus in each generation. We found that autocatalytic cycles evolved in the

lipid aggregates because they were necessary for the maintenance of chemical reaction networks that were beneficial at the lipid aggregate level. At this stage the entities satisfy Kauffman's definition of autonomous agent since firstly they act in their own best interests as a consequence of having been subjected to natural selection, and secondly they contain self-maintaining chemical cycles that utilize external chemical energy sources to produce their constituents.

The segregation of the population into lipid aggregates allows harmful avalanches to be isolated to a compartment. This is especially important in primitive evolutionary systems in which most chemical avalanches produce harmful side-reactions. Fontana's L1 organizations and Kauffman's reflexive autocatalytic set models do not demand a population of individuals because they ignore material-energetic constraints that would lead to side-reactions destroying the entire system unless they could be isolated.

The idealization of two distinct kinetic time scales (fast and slow) is a convenience for the model. In fact, chemical avalanches would be expected to occur according to a more complex distribution of periods. The rate of chemical avalanches would have to be sufficiently low to allow a liposome experiencing a successful avalanche to reach fixation before its progeny were wiped out by the much more frequent harmful avalanches. The capacity for variation by chemical avalanches depends on there being requisite variety, but this is an assumption that probably cannot be avoided in any successful constructive dynamical system, Ashby (1956).

Chemists should be encouraged to pursue experiments involving heterotrophically

produced phase separated chemical units as units of selection. However, if the effect of autocatalysts is small, then very large population sizes are required. Furthermore, if the frequency of chemical avalanches is low, then some means of accelerating this process must be devised, e.g. the exposure of lipid aggregates to high pressure between generations, or the capacity to reduce generation times by automation.

Acknowledgements

Thanks to Prof. Eors Szathmary and Dr. Kepa-Ruiz Mirazo for useful criticism and discussion. Thanks to the European 6th Framework ESIGNET grant for funding this research.

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