Biol Theory
DOI 10.1007/s13752-013-0132-0

THEMATIC ISSUE ARTICLE: SYNTHESIS (σύνθεσις)

# Simulating a Model of Metabolic Closure

**Athel Cornish-Bowden · Gabriel Piedrafita · Federico Morán · María Luz Cárdenas · Francisco Montero**

Received: 14 March 2012 / Accepted: 12 February 2013


**Abstract** The goal of synthetic biology is to create artificial organisms. To achieve this it is essential to understand what life is. Metabolism-replacement systems, or ($M$, $R$)-systems, constitute a theory of life developed by Robert Rosen, characterized in the statement that *organisms are closed to efficient causation*, which means that they must themselves produce all the catalysts they need. This theory overlaps in part with other current theories, including autopoiesis, the chemoton, and autocatalytic sets, all of them invoking some idea of *closure*. A simple model of an ($M$, $R$)-system has been implemented in the computer, and behaves in ways that may shed light on the requirements for a prebiotic self-organizing system. In addition to a trivial steady state in which nothing happens, it can establish a non-trivial steady state in which all intermediates have finite concentrations, with their rates of degradation balanced by their rates of synthesis. The system can be regenerated from the set of food components plus a single intermediate, and maintain itself in that state indefinitely, despite continuous degradation. At the very low compartment volumes that may have existed in prebiotic conditions, for example in cavities in minerals, or in micelles formed by simple amphiphiles, statistical fluctuations in the numbers of molecules need to be taken into account. With the stochastic approach there is no non-trivial steady state in strict mathematical terms, because the system will always collapse to the trivial state after sufficient time. However, the average time before collapse is so long for volumes greater than $10^{-19}$ L (much smaller than the volume of the order of $10^{-15}$ L for a typical bacterial cell) that for practical purposes the self-maintaining state of non-null concentrations becomes significant, recalling the situation of bistability that is observed in deterministic analysis. In turn, there exists a minimum size below which the self-organizing system cannot maintain itself on chemically relevant time scales. The value of the critical volume depends on the particular concentrations and rate constants assumed, but the principle could apply generally.

**Keywords** Autopoiesis · Chemoton · Hypercycles · Metabolic closure · ($M$, $R$)-systems · Self-organization · Simulation

> Le corps humain est une Machine qui monte elle-même ses ressorts; vivante image du mouvement perpétuel. Les aliments entretiennent ce que la fièvre excite. Sans eux l'Âme languit, entre en fureur, et meurt abattue.
>
> –Julien Jean Offray de la Mettrie (1748)

> To whatever degree we might imagine our knowledge of the properties of the several ingredients of a living body to be extended and perfected, it is certain that no mere summing up of the separate actions of those elements will ever amount to the action of the living body itself.
>
> –John Stuart Mill (1846)

A. Cornish-Bowden (✉) · M. L. Cárdenas
Unité de Bioénergétique et Ingénierie des Protéines, Centre National de la Recherche Scientifique, Aix-Marseille Université, Marseille, France
e-mail: acornish@imm.cnrs.fr

G. Piedrafita · F. Morán · F. Montero
Facultad de Ciencias Químicas, Universidad Complutense de Madrid, Madrid, Spain

Published online: 09 August 2013

## Introduction

Synthetic biology is commonly regarded as a new field that has emerged in the past few years, but the term itself is more than 100 years old, as Leduc (1912) used it as the title of his book *La Biologie Synthétique*, in which the main focus was on osmotic phenomena that produce structures resembling the forms of living organisms. For many years his work was believed to have little relevance to life, but it is now being rehabilitated in efforts to recreate the conditions that led to the emergence of life at the end of the Hadean aeon (Barge et al. 2012). However, despite this and other current attempts to create artificial life, most theories of life itself can be traced to the pioneering work of Schrödinger (1944). These include *metabolism-replacement systems*, or (*M*, *R*) systems (Rosen 1991), *autopoiesis* (Maturana and Varela 1980), the *chemoton* (Gánti 2003), the *hypercycle* (Eigen and Schuster 1977), and *autocatalytic sets* (Kauffman 1986).

As recently reviewed (Letelier et al. 2011), these theories overlap in important respects, but differ in others, and each of them lacks at least one feature that could be regarded as essential in an ideal theory. Unfortunately their principal architects have systematically failed to recognize the degree of overlap, or even to refer to one another's publications, and as a result the similarities are often obscured by inconsistent terminology. In particular, they all incorporate some idea of closure, an idea foreshadowed as early as the 18th century by Mettrie (1748). It is most tidily expressed by Rosen's statement that "an organism is closed to efficient causation." What this means is that all of the catalysts that allow an organism to remain alive must be produced by the organism itself; there is no external efficient cause. The same idea is embodied in a strange-looking equation (Letelier et al. 2005):

$$f(f) = f$$

in which $f$ successively fulfils the roles of function, argument, and result: metabolism acts on metabolism to produce metabolism.

Closure to efficient causation does not conflict with thermodynamic principles, which require that the energy needed by an organism must come from irreversible conversion of food into excretion products: there is no suggestion that an organism is closed to material causation. For this reason the Ouroboros, the dragon that nourishes itself by eating its own tail, is a misleading metaphor of metabolic circularity, and thus of life, however appealing it may seem at first sight.

A conclusion Rosen drew from closure to efficient causation is that it is impossible to construct a model of an organism. This has proved to be hotly contested, and the argument continues (Cárdenas et al. 2010). Chemero and Turvey (2006) have even maintained that the whole idea of closure to efficient cause is mistaken, but this is a misunderstanding due to a failure to understand the definition of catalysis. Although they attributed their definition to Kauffman (1986) they actually inverted it: where he had argued (reasonably) that every catalyst in metabolism must be a product of metabolism, they took it to mean that every product of metabolism must be a catalyst. That is clearly absurd, and is certainly not what Kauffman (1986) meant, but it illustrates the dangers that can arise when drawing conclusions about biological organization without using the basic terminology with sufficient care. In this article we shall first discuss the distinction that Rosen made between models and simulations, and then show how the behavior of an (*M*, *R*) system can be simulated.

## Models and Simulations

Rosen made an essential distinction between a *model* of an organism, which he said was impossible, and a *simulation*, which is possible. During the course of his career he was more interested in models than in simulations, but in two of his less well-known papers (Rosen 1971, 1973) described how a simulation might be made. In his terminology, a model of a machine incorporates understanding of how the machine works; it does more than simply mimic its behavior. A simulation, on the other hand, allows prediction of how the machine will respond to changes in its environment without any knowledge of how the real machine achieves its behavior. As an illustration of the difference, consider the following equation:

$$F(0.95, 1, n) = 3.836 + \frac{1}{\frac{0.08889502}{n} - 0.018868 + 0.0100613n}$$

which allows the 5 % point of an $F$ distribution at 1 and $n$ degrees of freedom to be calculated with an accuracy of ± 0.15 %.[1] But what does it tell about the theoretical basis of the $F$ test? Obviously nothing: it is simply an arithmetical trick that gives the right result. This makes it a simulation in Rosen's sense, and not a model of the $F$ test. In this case the appropriate theory is known, but tedious to apply. Such simulation is also possible, however, when the theory is unknown or incomplete, but the actual behavior is known with great precision. For example, Briggs (1962) determined experimentally the relationship between measurements of starch-iodine color in a spectrophotometer

[1] This sort of approximation was useful when computer time and storage were orders of magnitude more expensive than they are now. Today one would simply store the entire table, or calculate the appropriate value to any desired precision with the proper statistical theory.

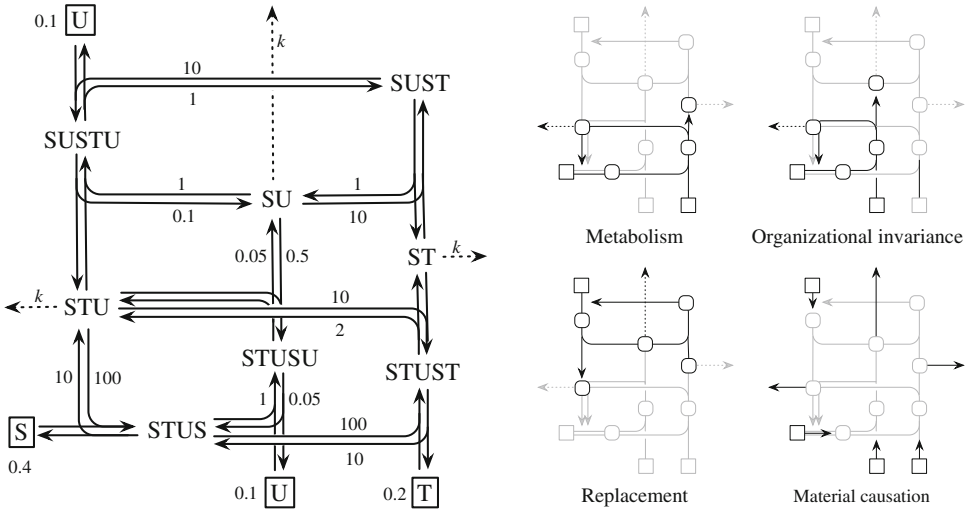


**Fig. 1** Model for simulating an (*M*, *R*) system. The three catalytic cycles, metabolism, replacement, and organizational invariance, allow the three intermediates ST, STU, and SU to be synthesized and maintained, in spite of irreversible loss through the three degradation reactions represented by *dashed arrows*. The system is thus closed to efficient causation, as no external catalysts are needed. However, it is open to material causation, as the three precursors S, T, and U (contained in *rectangles*) are converted irreversibly into degradation products. For the simulations the rate constants were assigned the numerical values shown (in $s^{-1}$ for first-order rate constants and in $mM^{-1}\ s^{-1}$ for second-order rate constants), and the concentrations of the precursor molecules were fixed at the values shown (in mM). Various values in the range $0 - 0.6\ s^{-1}$ were considered for *k*, the degradation rate constant, which was taken to be the same in all three reactions

and the glucose equivalents giving rise to the color. Although one could convert the intensity of color to the concentration of glucose equivalents by means of a standard curve, it proved more convenient to use an arbitrary equation that provided the correct result with a programmable calculator (Smith et al. 1979). Again, the equation just simulates the true relationship, and does not model it.

In terms of this distinction between models and simulations, the results that we shall discuss in the remainder of this paper are clearly not computer models of organisms, but they can still be models of (*M*, *R*) systems, which themselves incorporate some (but not all) of our understanding of the way an organism is organized.

## Model of an (*M,R*) System

We used the model illustrated in Fig. 1 to study the range of behavior possible in a simple (*M*, *R*)-system (Piedrafita et al. 2010, 2012a, b). It is based on one proposed earlier (Letelier et al. 2006; Cornish-Bowden et al. 2007) that was itself derived from that of Morán et al. (1996). However, it differs in that not only the "catalytic" intermediates STU and SU are degraded irreversibly, but also the "metabolic product" ST is subject to degradation as well, with the same kinetics. This change was made for logical consistency: as ST has a similar structure to the other two there is no reason to postulate that it is indefinitely stable.

Notice that the identities of the degradation products are not specified in Fig. 1. Although it may seem tempting to assume that they are simply the external molecules S, T, and U, so that ST is degraded to S + T, for example, this cannot be correct,[2] because if it were there would be no thermodynamic driving force for the whole process. It is better to think of them as non-activated forms s, t, and u that cannot be transformed back into S, T, and U unless the system is coupled to an external source of energy (such as electromagnetic radiation, chemical energy, etc.).

The initial simulations (Piedrafita et al. 2010) were deterministic, done in terms of concentrations, so the numbers of molecules were essentially infinite and no statistical fluctuations were considered. These simulations were done with the commercial software MatLab and checked with COPASI (Hoops et al. 2006), or vice versa. In deterministic conditions the system could exist in two different steady states, i.e., it showed bistability. There was a trivial steady state in which all rates and all concentrations of intermediates were zero, and the system could obviously remain in this state for infinite time. More interesting, however, was a non-trivial steady state in which all of the concentrations and all of the rates were non-zero. The system could remain in this state indefinitely

[2] It is an everyday observation that the chemical output of any organism is different from the food taken in, and it should be obvious from thermodynamic considerations that that must be the case.

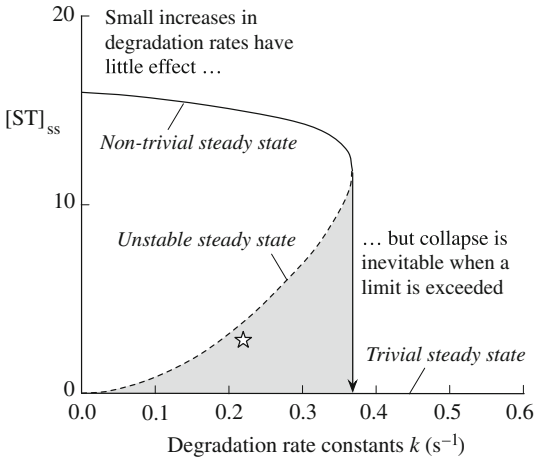


**Fig. 2** Deterministic steady states. The steady state concentrations of the "metabolic" intermediate ST are shown as functions of the degradation rate constants. The trivial steady state is coincident with the abscissa axis. The behavior in stochastic simulations is qualitatively similar, but with points scattered about the *lines*. If the initial state of the system is in the shaded region below the line for the unstable steady state, collapse to the trivial steady state is certain in deterministic simulations. Recovery remains possible, however, in the stochastic case, albeit with decreasing probability as the state is further from the unstable steady state. The significance of the point shown by a star is discussed in the text

as long as the degradation rates were small enough to be matched by resynthesis of the intermediates from the fixed reservoirs of the source molecules S, T, and U. If the rate constants for degradation were gradually increased (as might happen, for example, in a real system if the ambient temperature increased) the steady-state concentrations of the metabolic intermediates decreased, very slowly at first, but then more steeply, and once a threshold was passed the system collapsed irreversibly to the trivial steady state (Fig. 2).[3]

From the point of view of self-organization, the most interesting result from these simulations is that the system proved to be capable of creating itself from (almost) nothing, and then maintaining itself in a non-trivial state: provided reservoirs of the food molecules S, T, and U were present at fixed concentrations in the range $0.1 - 0.4$ mM then if just one of the intermediates was present, at a concentration above some threshold value,[4] the whole system would reach the non-trivial steady state. Any intermediate apart from ST and SU could act in this way as seeds. The two exceptions are readily explainable by the fact that neither of these have any partner to react with if no other intermediates are present, so they can do nothing. However, a mixture of ST and SU together proved able to act as seed.

## Stochastic Simulations

In small volumes, such as those that might have existed during prebiotic evolution, molecular components are bound to have small population sizes, even at high concentrations. In these situations, statistical fluctuations will predominate and must be expected to have important consequences for the dynamics of the system. It follows, therefore, that study of the behavior of the model of Fig. 1 at small volumes requires stochastic simulation, as we shall now describe.

Simulations in terms of molecules rather than concentrations were done with the Monte Carlo method of Gillespie (1976, 1977) implemented in MatLab. For system volumes greater than about $10^{-19}$ L the behavior of the model was very similar to that in the deterministic simulations,[5] apart from the expected presence of statistical fluctuations around the lines representing the time courses. In this case, however, the apparent non-trivial steady state is not a real steady state, because after sufficient time there must be a fluctuation large enough to bring about collapse to the trivial steady state. This is more of a theoretical than a practical point, because at these or higher volumes the waiting time before collapse is so extremely long that this state can be regarded as quasi-stationary.

Decreasing the volume had various effects. The fluctuations were, of course, much bigger, with the consequence that collapse after the quasi-steady state was reached could occur with higher probability at any time. In addition, the existence of such large fluctuations could imply that the stochastic system could recover from near collapse at concentrations where the deterministic system would proceed inexorably to extinction. This is illustrated by the point shown as a star in Fig. 2. A deterministic system at this point would move with 100 % certainty towards the trivial steady state, but (for the particular numerical values assumed when constructing the figure) the stochastic system would have a 5 % probability of moving out of the shaded area and to high concentrations characteristic of the non-trivial quasi-stationary state. As the origin of life almost certainly required a highly improbable event, this means that even an initial state far from the self-maintained state could still have been sufficient for it to be reached.

The larger fluctuations at small volumes also meant that the quasi-final state (whether trivial or non-trivial) was

[3] The collapse would not be totally irreversible if we allow uncatalyzed production of minimal amounts of intermediates: in this case the system could regain the non-trivial steady state if all of the degradation rate constants were sufficiently low.

[4] For example, for $k = 0.1$ s$^{-1}$, a starting concentration of STU of at least 0.135 mM would allow the whole system to construct itself.

[5] This is much smaller than the volume of more than $10^{-15}$ L (1 μm$^3$) for a bacterium such as *Escherichia coli*.

reached much more quickly than at large volumes. A system of infinite volume could in principle remain in the unstable steady state for infinite time, but the smaller the volume the more rapidly it must move away from this state.

For the particular numerical values assumed for the concentrations of the food sources and the values of the rate constants, there was a *critical volume* of about $4 \times 10^{-20}$ L. Below this volume the system could not maintain itself for any significant time. Above this volume the quasi-steady state became essentially stable, with an extremely steep dependence of the time to extinction on the volume. For example, at $10^{-19}$ L the average time to extinction could be estimated to be about $10^{10}$s, or about 300 years.

In general the volumes assumed in scenarios for prebiotic evolution (Hanczyc et al. 2003; Martin and Russell 2003; Walde 2006) are larger than $10^{-19}$ L, so the problems due to stochastic fluctuations would not arise for a system as simple as the one in Fig. 1 for the values of the concentrations and rate constants that we have assumed.[6] However, prebiotic self-organized systems can hardly have been as simple as the one in Fig. 1 and the concentrations of some necessary components may well have been much less than 1 mM. As it is the number of molecules that is important, not the concentration as such, it is evident that, say, a component present at a concentration of just 1 μM would need a volume orders of magnitude higher for statistical fluctuations to be insignificant.

## Decay and Self-Maintenance in Prebiotic Conditions

Up to this point we have left the chemical nature of the diverse molecules involved in the model unspecified. The building blocks S, T, and U could in principle be different amino acids, different nucleotides to be condensed, or even be oligomer modules such as those that may have interacted in a RNA world scenario (Briones et al. 2009). Although this would not affect the general conclusions of the model, a deeper chemical characterization would allow a realistic range of values for the degradation rate constants to be determined, and would indeed help to identify if they are consistent with those conditions leading to maintenance of a non-trivial steady state or not. This analysis would in turn shed light on the sort of compounds that could achieve this type of organization at the origins of life.

This issue is, however, complex and deserves more attention, as the degradation rate constants and the system volume are not the only critical parameters determining the behavior of the system. Actually, the other rate constants, which have so far gone more unnoticed (as their values were assumed to be constant), would be important as well. In general terms, we can argue that the emergence and self-maintenance, and thus the prebiotic relevance of this model, would depend on a trade-off between sufficiently high efficiencies of catalysis (i.e., the urge to construct itself) and relatively low degradation rates, such as might have occurred for other autocatalytic systems (Szathmáry 2007). In this sense, even though a catalytically closed metabolism consisting of structurally simple intermediates would probably exhibit modest catalytic efficiencies, another one involving complex oligomer modules could potentially show higher efficiencies (and specificities) but would probably be at the same time subject to higher degradation rates that could threaten its maintenance. Hordijk and Fontanari (2003) have demonstrated how the decay can affect the maintenance of metabolic networks involving long polymers while preserving others formed by smaller oligomers.

Another important factor to consider in prebiotic conditions is the ambient temperature, which would obviously affect the kinetic rate constants. More specifically, if the interactions between the diverse intermediates in the model are non-hydrophobic, an increase in temperature would not only increase the characteristic degradation rate constants, but also all those of dissociation processes, thus decreasing the equilibrium constants of condensation reactions. In any case, the net effect will be a drift toward the destabilization (collapse) of the system, with an opposite trend if the temperature decreases. As a consequence, this correlation between temperature and ability for self-maintenance would allow a new bifurcation diagram to be envisaged (Fig. 3) that would presumably resemble (in qualitative terms) that for the degradation rate constants.

In Fig. 2 the point marked with a star represents a state in which an event of probability 5 % could produce a non-trivial steady state. However, if we are concerned with events that need to have happened only once in many millions of years then we can consider vastly lower probabilities to be reasonable for the appearance of the first self-maintaining system.[7] In a small-enough volume the chance appearance of a few molecules of a seed intermediate such as STU (resulting perhaps from collisions at very unlikely velocities between S, T, and U molecules) could be

[6] In their recent paper on osmotic structures resembling Leduc's osmotic gardens, Barge et al. (2012) do not give estimates of the internal volumes of their structures. They have informed us (Barge, personal communication, 8 March 2012) that although these volumes are very difficult to estimate with any accuracy they are certainly orders of magnitude larger than the volumes we are considering here.

[7] It is easy to forget that supposedly improbable events occur very frequently. In a human population of $10^9$ individuals the probability that one of them will experience an event of probability $10^{-9}$ at any particular moment is close to certainty.

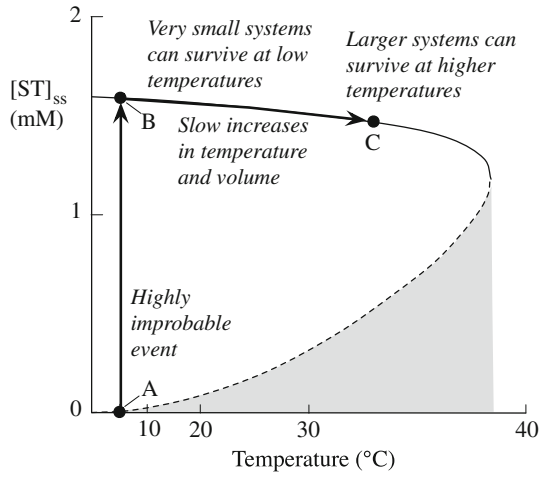


**Fig. 3** Scenario for the appearance of a self-maintaining system. A highly improbable event when a very small system is in the trivial steady state at point A, resulting in chance appearance of a few molecules of STU or one of the other intermediates that can act as seeds, could propel the system to point B, in the non-trivial steady state. Subsequent increases in temperature and volume could then allow progression towards point C, with a stable self-maintaining system at a higher temperature and a volume large enough to allow a long life at that temperature. The temperature values are arbitrary, but the implied doubling of rate constants for each 10 °C increase is in accordance with typical experimental observations

sufficient to bring a system to the non-trivial state. However, in a very small volume it would also have a high probability of collapsing, for the reasons set out in the preceding section, unless the temperature was low enough for the degradation rates to be very small. We can deduce, however, that once the state of relatively high concentrations was reached, an adaptation to a bigger volume of the system would allow the survival at a wider range of temperatures; the system could thus arrive at a state that would be very difficult to produce in a single step. The general scenario is illustrated in Fig. 3.

## Structural Closure

In addition to closure to efficient causation, and openness to material causation, already mentioned, there is a third kind of closure that is different from these, and is also important. We refer to a physical boundary, which would not only allow a self-organizing system to avoid being diluted out of existence into the bulk liquid or keep it separated from diverse toxic molecules, as a passive container would do, but if actively produced by the system itself it would be crucial for completing the individualization and gaining a proper control over the internal set of metabolic processes. This aspect is explicitly part of autopoiesis (Maturana and Varela 1980) and the chemoton (Gánti 2003), but is at best only implicit in (*M*, *R*)-systems (Rosen 1991) and autocatalytic sets (Kauffman 1986).[8] There is no explicit structural closure in Fig. 1, and this is clearly therefore a point that will need to be addressed in future work, whether we consider an enclosing membrane produced by the system itself, as required by autopoiesis and the chemoton, or a spontaneously formed compartment such as a lipid vesicle or a mineral cavity. In both of these we need to consider, among other aspects, the capacity of food molecules to enter the compartment at rates sufficient to maintain the system. In the case of lipid vesicles we have measured the rates at which small molecules can cross membranes constituted by lauric acid on the one hand, or by a mixture of oleic acid and glycerol monooleate on the other (Piedrafita et al. 2012b), and found that the model we propose is at least in principle viable.

Any such primitive compartment would have initially been causally independent of the internal self-organizing protometabolic system. That is why we have treated these structures only implicitly, as mere external constraints, focusing only on the way they can affect the functioning of an internal chemical network (for example, by limiting the reaction volume or the reaction rates). On the other hand, sooner or later, the compartment must have become coupled to the internal network (for example, by means of an internal reaction producing the membrane components, thus modifying the membrane from within to regulate the metabolism, etc.), becoming strictly part of the system organization: we can then talk about a higher-order organization with structural closure, distinct from the initial event producing structural closure. This distinction is made in various publications (Szostak et al. 2001; Chen and Walde 2010) and allows a "transition from self-assembling compartments to proper protocells" to be considered (Ruiz-Mirazo et al. 2011).

## Relationship to Synthetic Biology

We have strayed rather far from the original theme of synthetic biology, so we should finish by reconnecting it. Much of it as presently practiced consists of modifying existing organisms rather than creating new ones. Engineering new microbial consortia (Brenner et al. 2008) or using TAL proteins to edit genomes (Miller et al. 2011) can be very useful technologically, but they do not involve fundamental new concepts. Even the synthesis of a complete genome followed by its insertion in a new host

[8] This is just one illustration of the point that all current theories of life lack some features that an ideal theory ought to have (Letelier et al. 2011). On the other hand the chemical nature of the catalysts—absolutely necessary for the specificity that any self-organizing set of reactions must have—is too vague in autopoiesis and the chemoton to be satisfactory.

depends on the biochemical capacity of the host (Gibson et al. 2010). However, synthetic biology cannot really be considered to have been achieved until an entire organism has been synthesized from raw materials. Just as organic chemists insist on total synthesis as a definitive proof of structure (Sánchez-Izquierdo et al. 2007) claims of synthetic biology should also be based on total synthesis, and this will certainly involve a complete theory of life. Once wholly new organisms that can survive outside the laboratory become a reality various ethical questions will need to be answered, but there will be ample time to resolve these before they become of practical importance.

Genuine synthetic biology must, at the beginning at least, deal with construction of self-organizing systems far simpler than any living organisms that still exist in the biosphere, such as those studied by Barge and co-workers (2012). However, once artificial self-organizing systems have been made they will help to clarify the nature of ($M$, $R$) systems, and, conversely, understanding ($M$, $R$) systems will likewise be helpful for the creation of artificial self-organizing systems. As we have noted, these will inevitably be simple systems, and a recent paper (Chiarabelli et al. 2012) begins with the following words: "To date, the construction of a synthetic cell containing about two hundred genes is out of reach. Also, it is not useful to try to construct a very complex system without understanding how a simpler one works."

We agree with this, and references in the same paper point to efforts by various groups to construct such simpler systems. The model of Fig. 1 is in principle constructible, if suitable chemical components for S, T, and U can be identified. As it stands, however, it makes no allowance for structural closure, but the studies of the permeability of lipid membranes (Piedrafita et al. 2012b) should allow this gap to be filled. Conversely, we believe that the principles developed in the various theories we have mentioned, and in particular that of ($M$, $R$)-systems, will be needed to make synthetic biology a practical proposition: an artificial living cell that does not incorporate metabolic circularity will fail: just assembling a set of molecules in a micelle will not be enough. Perhaps more important, synthetic biology, like exobiology, needs a definition of life. At present no accepted definition exists, but without one it will be impossible to judge the success of efforts to create life or to detect it elsewhere in the universe. As Maturana and Varela (1980), and more recently Luisi (2006), have discussed, when we are limited to the forms of life that we know on earth it is usually easy to decide whether a particular entity is alive or not—trees and coral are alive, but clouds and crystals are not—but it is less easy to convert that understanding into a definition that does not, for example, exclude mules or post-reproductive humans, or include computer programs such as genetic algorithms. However, if it is difficult for the forms of life that we know, how much more so is it for truly exotic forms of life? So although a definition based on Rosen's ($M$, $R$) systems may not satisfy everyone, it is certain that *some* definition is needed.

**Acknowledgements** This work was supported by grant BFU2009-12895-C02-02 (Ministerio de Ciencia e Innovación, Spain). GP acknowledges support from PhD scholarship FPU (Formación del Profesorado Universitario, Ministry of Education, Spain). AC-B and MLC were supported by the Centre National de la Recherche Scientifique.

## References

Barge LM, Doloboff IJ, White LM, Stucky GD, Russell MJ, Kanik I (2012) Characterization of iron–phosphate–silicate chemical garden structures. Langmuir 28(8):3714–3721 doi:10.1021/la203727g

Brenner K, You L, Arnold FH (2008) Engineering microbial consortia: a new frontier in synthetic biology. Trends Biotechnol. 26(9):483–489 doi:10.1016/j.tibtech.2008.05.004

Briggs DE (1962) Simplification of assay of enzymes using non-linear reaction-progress graphs. Enzymologia 24(2):97–104

Briones C, Stich M, Manrubia SC (2009) The dawn of the RNA World: toward functional complexity through ligation of random RNA oligomers. RNA Publ RNA Soc 15(5):743–749 doi:10.1261/rna.1488609

Cárdenas ML, Letelier JC, Gutierrez C, Cornish-Bowden A, Soto-Andrade J (2010) Closure to efficient causation, computability and artificial life. J Theor Biol 263(1):79–92 doi:10.1016/j.jtbi.2009.11.010

Chemero A, Turvey MT (2006) Complexity and closure to efficient cause. In: Ruiz-Mirazo K, Barandiaran R (eds) ALIFE X: workshop on artificial autonomy. MIT Press, Cambridge, MA, pp 13–18

Chen IA, Walde P (2010) From self-assembled vesicles to protocells. Cold Spring Harbor Perspect. Biol. 2(7) doi:10.1101/cshperspect.a002170

Chiarabelli C, Stano P, Anella F, Carrara P, Luisi P (2012) Approaches to chemical synthetic biology. FEBS Lett 586(15):2138–2145 doi:10.1016/j.febslet.2012.01.014

Cornish-Bowden A, Cárdenas ML, Letelier JC, Soto-Andrade J (2007) Beyond reductionism: metabolic circularity as a guiding vision for a real biology of systems. Proteomics 7(6):839–845 doi:10.1002/pmic.200600431

de la Mettrie JJO (1748) L'Homme machine. Elie Luzac, Leyden

Eigen M, Schuster P (1977) The hypercycle—a principle of natural self-organization. A. Emergence of the hypercycle. Naturwissenschaften 64(11):541–565 doi:10.1007/BF00450633

Gánti T (2003) The principles of life. Oxford University Press, Oxford

Gibson DG, Glass JI, Lartigue C, Noskov VN, Chuang RY, Algire MA, Benders GA, Montague MG, Ma L, Moodie MM, Merryman C, Vashee S, Krishnakumar R, Assad-Garcia N, Andrews-Pfannkoch C, Denisova EA, Young L, Qi ZQ, Segall-Shapiro TH, Calvey CH, Parmar PP, Hutchison III, CA, Smith HO, Venter JC (2010) Creation of a bacterial cell controlled by a chemically synthesized genome. Science 329(5987): 52–56

Gillespie DT (1976) A general method for numerically simulating stochastic time evolution of coupled chemical-reactions. J Comput Phys 22(4):403–434 doi:10.1016/0021-9991(76)90041-3

Gillespie DT (1977) Exact stochastic simulation of coupled chemical-reactions. J Phys Chem 81(25):2340–2361 doi:10.1021/j100540a008

Hanczyc MM, Fujikawa SM, Szostak JW (2003) Experimental models of primitive cellular compartments: encapsulation, growth, and division. Science 302(5645):618–622 doi:10.1126/science.1089904

Hoops S, Sahle S, Gauges R, Lee C, Pahle J, Simus N, Singhal M, Xu L, Mendes P, Kummer U (2006) COPASI—a Complex pathway simulator. Bioinformatics 22(24):3067–3074 doi:10.1093/bioinformatics/btl485

Hordijk W, Fontanari JF (2003) Catalytic reaction sets, decay, and the preservation of information. In: Hexmoor H (ed) International conference on integration of knowledge intensive multi-agent systems: KIMAS'03: Modeling, exploration, and engineering. IEEE, New York, pp 133–138 doi:10.1109/KIMAS.2003.1245035

Kauffman SA (1986) Autocatalytic sets of proteins. J Theor Biol 119(1):1–24 doi:10.1016/S0022-5193(86)80047-9

Leduc S (1912) La Biologie Synthétique. Poinat, Paris

Letelier JC, Cárdenas ML, Cornish-Bowden A (2011) From *L'Homme machine* to metabolic closure: steps towards understanding life. J Theor Biol 286:100–113 doi:10.1016/j.jtbi.2011.06.033

Letelier JC, Kuboyama T, Yasuda TH, Cárdenas ML, Cornish-Bowden A (2005) A self-referential equation, $f(f) = f$, obtained using the theory of ($M$, $R$) systems: overview and applications. In: Anai H, Horimoto K (eds) Algebraic biology. Universal Academy Press, Tokyo, pp 115–126

Letelier JC, Soto-Andrade J, Guíñez Abarzua F., Cornish-Bowden A., Cárdenas ML (2006) Organizational invariance and metabolic closure: analysis in terms of ($M$, $R$) systems. J Theor Biol 238(4):949–961 doi:10.1016/j.jtbi.2005.07.007

Luisi PL (2006) The emergence of life. Cambridge University Press, Cambridge

Martin W, Russell MJ (2003) On the origins of cells: a hypothesis for the evolutionary transitions from abiotic geochemistry to chemoautotrophic prokaryotes, and from prokaryotes to nucleated cells. Phil Trans R Soc B 358(1429):59–83 doi:10.1098/rstb.2002.1183

Maturana H, Varela F (1980) Autopoiesis and cognition: the realisation of the living. D. Reidel Publishing Company, Dordrecht

Mill JS (1846) A system of logic. Harper & Brothers, New York, pp 211

Miller JC, Tan S, Qiao G, Barlow KA, Wang J, Xia DF, Meng X, Paschon DE, Leung E, Hinkley SJ, Dulay GP, Hua KL, Ankoudinova I, Cost GJ, Urnov FD, Zhang HS, Holmes MC, Zhang L, Gregory PD, Rebar EJ (2011) A TALE nuclease architecture for efficient genome editing. Nat Biotechnol 29(2):143–148 doi:10.1038/nbt.1755

Morán F, Moreno A, Minch E, Montero F (1996) Further steps towards a realistic description of the essence of life. In: Langton CG, Shimohara K (eds) Artificial life V. MIT Press, Cambridge, MA, pp 255–263

Piedrafita G, Montero F, Morán F, Cárdenas ML, Cornish-Bowden A (2010) A simple self-maintaining metabolic system: robustness, autocatalysis, bistability. PLoS Comput Biol 6(8):e1000,872 doi:10.1371/journal.pcbi.1000872

Piedrafita G, Cornish-Bowden A, Morán F, Montero F (2012a) Size matters: influence of stochasticity on the self-maintenance of a simple model of metabolic closure. J Theor Biol 300:143–151

Piedrafita G, Ruiz-Mirazo K, Monnard PA, Cornish-Bowden A, Montero F (2012b) Viability conditions for a compartmentalized protometabolic system: a semi-empirical approach. PLoS ONE 7:e39,480

Rosen R (1971) Some realizations of ($M$, $R$)-systems and their interpretation. Bull Math Biophys 33(3):303–319 doi:10.1007/BF02476776

Rosen R (1973) On the dynamical realization of ($M$, $R$)-systems. Bull Math Biol 35(1-2):1–9

Rosen R (1991) Life itself. Columbia University Press, New York

Ruiz-Mirazo K, Piedrafita G, Ciriaco F, Mavelli F (2011) Stochastic simulations of mixed-lipid compartments: from self-assembling vesicles to self-producing protocells. Adv Exp Med Biol 696:689–696

Sánchez-Izquierdo F, Blanco P, Busqué F, Alibés R, de March P, Figueredo Galimany M, Font J, Parella T (2007) Total synthesis of the putative structure of stemonidine: the definitive proof of misassignment. Org Lett 9(9):1769–1772 doi:10.1021/ol070486p

Schrödinger E (1944) What is life? Cambridge University Press, Cambridge, UK

Smith MT, Cornish-Bowden A, Briggs DE (1979) Determination of barley α-amylase activity. J Inst Brew 85(4):157–159

Szathmáry E (2007) Coevolution of metabolic networks and membranes: the scenario of progressive sequestration. Philos Trans R Soc 362(1486):1781–1787 doi:10.1098/rstb.2007.2070

Szostak JW, Bartel DP, Luisi PL (2001) Synthesizing life. Nature 409(6818):387–390

Walde P (2006) Surfactant assemblies and their various possible roles for the origin(s) of life. Orig Life Evol Biosphere 36(2):109–150 doi:10.1007/s11084-005-9004-3