Abstract

A living organism must not only organize itself from within; it must also maintain its organization in the face of changes in its environment and degradation of its components. We show here that a simple (M,R)-system consisting of three interlocking catalytic cycles, with every catalyst produced by the system itself, can both establish a non-trivial steady state and maintain this despite continuous loss of the catalysts by irreversible degradation. As long as at least one catalyst is present at a sufficient concentration in the initial state, the others can be produced and maintained. The system shows bistability, because if the amount of catalyst in the initial state is insufficient to reach the non-trivial steady state the system collapses to a trivial steady state in which all fluxes are zero. It is also robust, because if one catalyst is catastrophically lost when the system is in steady state it can recreate the same state. There are three elementary flux modes, but none of them is an enzyme-maintaining mode, the entire network being necessary to maintain the two catalysts.

Introduction

Several theories of life [1–5] coincide in the importance that they give to metabolic closure, the necessity for all of the catalysts essential for survival of an organism to be produced internally, as an organism cannot rely on any external agent for maintaining it. The same considerations must apply to the self-maintaining systems at the origin of life [6,7]. Rosen [1] expressed this idea that catalysts must be produced by the system itself by saying that it must be closed to efficient causation. These differences occur in their details, and each includes important points missing from the others. Among them the theory of (M,R)-system s, or metabolism–replacement systems, perhaps comes closest to a complete explanation of life, but it is usually presented in abstract terms [1] that make it difficult to relate it to any ordinary ideas of chemistry, metabolism and catalysis.

To give concrete expression to the idea of an (M,R)-system, and to evaluate its possible relevance to the origin of life, we proposed [8–10] a simple system of three interlocking cycles: a metabolic process $S + T \rightarrow ST$ produces a metabolite $ST$ from external precursors $S$ and $T$ in a reaction catalyzed by a component $STU$ that is itself the product of a replacement process $ST + U \rightarrow STU$, in which $U$ is another external precursor. The replacement process is necessary because $STU$, as a biological molecule, cannot be assumed to have an infinite lifetime, and even if it did it would be diluted by growth of the system and by other processes. Moreover, replacement also needs a catalyst, which also needs to be replaced. To escape immediately from the implied infinite regress we proposed that replacement is catalyzed by a similar type of molecule, $SU$, that results from a secondary reaction catalyzed by $STU$, $S + U \rightarrow SU$. This system, illustrated in Fig. 1, is closed to efficient causation, because each of the three reactions is catalyzed by a product of the system itself. In our original proposal we assumed that only $STU$ and $SU$ were subject to unavoidable degradation [see Fig. 1b of [10]], but there is no logical reason to suppose that the other product of the system, $ST$, is indefinitely stable, especially as it is assumed to be a molecule similar to $SU$. In Fig. 1, therefore, there is a third degradation reaction, reaction 11.

A controversial aspect of Rosen’s analysis is his contention that a system closed to efficient causation cannot have computable models [11–14]. Many aspects of biological systems can, of course, be simulated in the computer, and many examples of metabolic simulation can be found in the literature, but typically these examples do not simulate systems that are closed to efficient causation. In the recent simulation of aspartate metabolism in Arabidopsis thaliana [15], for example, the enzymes were taken as given; their production was not simulated. We discuss this controversy elsewhere [16] and will not do so here, apart from noting that there is no obvious reason why the system illustrated in Fig. 1 should not be simulated. On the contrary, it can certainly be simulated, as we shall show, with results that shed light of the conditions that need to be fulfilled by a self-maintaining system.

We shall show that a simple (M,R)-system can be robust, capable of a recovering from the loss of most of its catalysts, and in addition has the interesting property of bistability. As Delbrück [17] emphasized many years ago, multistability is also an important property for all but the simplest living organisms because it is essential for differentiation, an idea that has subsequently been developed by other authors [18].
can arise in systems considerably smaller and simpler [19] than the one we discuss in this paper, but we are concerned here with (M,R)-systems, which must be closed to efficient causation.

Model

For the system to be simulated it needs to be defined in precise numerical terms, and for doing this it is convenient to expand the catalytic processes shown in Fig. 1a into the cycles of chemical reactions shown in Fig. 1b [9]. There is no fundamental difference in this model between catalysts (“enzymes”) and metabolites, and elsewhere [10] we have argued that no fundamental difference exists: all enzymes are products of the system in which they participate, and are thus metabolites, and many conventional metabolites (for example, ornithine in the urea cycle) participate in cycles of reactions, and thus satisfy the definition of a catalyst.

As we shall be supposing that the system in Fig. 1 can continue in operation indefinitely, despite containing irreversible degradation steps, we need to consider the thermodynamic feasibility of what we propose. In effect, we assume that the overall chemical reactions $S + T \rightarrow$ degradation products, $S + U \rightarrow$ degradation products and $S + T + U \rightarrow$ degradation products are irreversible, that synthesis of $ST$ in the reaction $S + T \rightarrow ST$ is thermodynamically favored, and that the concentrations of the external molecules $S$, $T$ and $U$ are constant, either because the quantities consumed by the system are too small to have any effect on their concentrations, or because they are buffered by external chemistry. External constraints on a system of chemical reactions can be applied in two main ways, either with constant external concentrations or with constant input fluxes. In this model we have chosen the former approach, primarily to facilitate comparison with earlier work [8–10].

In this context it is important to note that organizational closure does not imply thermodynamic closure, or vice versa. In the Aristotelean terminology favored by Rosen [1], closure to efficient causation is not the same as closure to material causation [10]. An

![Figure 1. A model of an (M,R)-system.](image)

(a) The metabolites shown inside squares (input) are considered to be “external” and to have fixed concentrations. The reactions shown in red constitute the metabolic process, those in blue the replacement process, and in gray the replacement of the replacement catalyst. (b) Expanded version of the model in which each catalyzed reaction is expanded into a cycle of three chemical reactions with explicit rate constants. Each forward rate constant refers to the reaction in the direction of the arrow, and the three degradation reactions, steps 4, 8 and 11, are assumed to be uncatalyzed and irreversible. All rate constants are treated as constant with the values shown, apart from $k_4$, $k_9$ and $k_{11}$, which are varied (but kept equal to one another) in the range 0.0–0.6. The three external reactants $S$, $T$ and $U$ are assumed to have the constant concentrations shown. All other concentrations are variable. All units are arbitrary, but they are consistent (i.e. the same units of time and quantity of matter apply throughout) and the model can be written in dimensionless form, if desired. In addition, the numerical values assigned to the rate constants and external concentrations are also arbitrary. doi:10.1371/journal.pcbi.1000872.g001
organism must clearly be open to material causation — it “feeds on
negative entropy”, in Schrödinger’s words [22] — but it can still
synthesize all of its catalysts, and thus be closed to efficient causation.
In a third type of closure, independent from both of these, an
individual organism must be structurally closed, separated from other
individuals by a skin or other barrier. This aspect was given almost no
attention by Rosen [1], and we shall not discuss it further here, but it
is clearly necessary, and it forms an important element of other
theories of life such as autopoiesis [3].

Results
Stationary solutions and self-maintenance of the (M,R)
system
The concentration evolution of the different metabolites in Fig. 1b
can be described by a series of ordinary differential equations:

\[
\frac{d(STU)}{dt} = -k_1[STU][S] + k_{-1}[STU] + k_3[STUST]
\]

\[
- k_{-3}[ST][ST] - k_4[STU] + k_7[SUSTU]
\]

\[
- k_{-7}[STU][SU] + k_{10}[STUSU] - k_{-10}[STU][SU]
\]

\[
\frac{d(STUS)}{dt} = k_1[STU][S] - k_{-1}[STUS] - k_2[STUS][T]
\]

\[
+ k_{-2}[STUST] - k_9[STUS][U] + k_{-9}[STUSU]
\]

\[
\frac{d(STUST)}{dt} = k_3[STUST] - k_{-3}[STU][ST]
\]

\[
- k_5[ST][SU] + k_{-5}[SUST] - k_{11}[ST]
\]

\[
\frac{d(STUSU)}{dt} = k_5[STU][S] - k_{-5}[STUS] - k_6[STUS][T]
\]

\[
- k_6[SUST][U] + k_{-6}[SUSTU]
\]

\[
\frac{d(STUSTU)}{dt} = k_6[STUS][U] - k_{-6}[STUSTU]
\]

\[
- k_{17}[STUSTU] + k_{-7}[STU][SU]
\]

\[
\frac{d(SU)}{dt} = k_7[SUSTU] - k_{-7}[STU][SU] - k_8[ST][SU]
\]

\[
+ k_{-5}[SUST] - k_9[SU] + k_{10}[STUSU]
\]

\[
- k_{-10}[STU][SU]
\]

\[
\frac{d(STUSU)}{dt} = k_9[STUS][U] - k_{-9}[STUSU]
\]

\[
- k_{10}[STUSU] + k_{-10}[STU][SU]
\]

The simple non-linear terms in these equations arise from applying
simple mass action kinetics to the bimolecular steps.

Stationary solutions of the system of Fig. 1b were obtained by
two different methods, numerical integration of the previous set of
differential equations, and analytical solution of the nonlinear
algebraic equations. Both revealed the existence of a region with
three distinct steady states, one trivial and two non-trivial. It is
obvious that the system shown in Fig. 1 cannot undergo any
reactions if no form of any catalyst is present. Less obvious is
whether it can construct itself and maintain itself indefinitely if it is
seeded with a sufficient quantity of one catalyst. This has been
tested in the first instance with various values in the range 0–0.6 of
the degradation rate constants k_4, k_8 and k_{11}, other rate constants
as defined in Fig. 1, and various initial concentrations of one
intermediate, STU, all other intermediate concentrations being set
initially to zero.

For k_4 = k_8 = k_{11} \geq 0.367 the system cannot construct itself or
maintain itself despite seeding with large or small initial
concentrations of STU, and it always ends in a trivial steady state
with all concentrations and all rates zero. However, with smaller
degradation rate constants it can reach either the trivial steady
state or a non-trivial steady state with all concentrations and rates
non-zero, i.e. a self-maintaining regime. The results are summar-
ized in Fig. 2 for k_4 = k_8 = k_{11} = 0.3 and different initial
concentrations of STU. For 0<[STU]_{0} < 11.5 the system
reached a trivial steady state with all concentrations zero, but at
any [STU]_{0} > 11.5 it reached a non-trivial steady state with
[STU]_{\infty} = 14.3 and all other concentrations and all rates non-zero.
Hence there is a none-to-all transition at this critical point, as
indicated by the broken line in Fig. 2a.

STU is not of course the only catalytic intermediate that could
be used for seeding the system, and results with each of the others,
and for some pairs of intermediates, are shown in Table 1, for two
values of k_4 = k_8 = k_{11}. Two important points are evident in this
table: first, any metabolite apart from ST or SU can be separately
used to seed the system, and although the concentration of seed
metabolite necessary to drive it to a non-trivial steady state varies
with the seed, the steady state reached depends only on the
degradation rate constants, and is independent of the identity of
the seed. We have also made simulations with various mixtures of
metabolites used as seeds and these generalizations remain true.

The reason why ST and SU cannot act as seed can be seen by
inspection of Fig. 1b: neither of these metabolites reacts directly
with any of the external reactants S, T and U, and so no reaction
can take place if none of the other metabolites are present.
However, ST and SU can react with one another to give a product
SUST capable of participating in additional reactions and closing
all the loops. Not surprisingly therefore, the system can be seeded
with a mixture of ST and SU even though neither of them is
effective alone.

Bistability and hysteretic behavior
To verify the stability of the steady states, the Jacobian matrices
were evaluated at the steady states obtained, and the eigenvectors
and eigenvalues calculated. For those conditions in which three
steady states were obtained, k_4 = k_8 = k_{11} < 0.367, the trivial and
one of the non-trivial solutions always have all eigenvalues with
negative real parts, and thus are asymptotically stable. Obviously,
they correspond to those reached by numerical integration
experiments. The additional non-trivial steady state calculated by
the analytical solution of the non-linear algebraic equations has,
however, one of the eigenvalues with positive real part, and is
therefore an unstable steady state (a saddle point), so in this region
the system exhibits bistability. Beyond the critical value,
only the trivial steady solution exists and is asymptotically stable, i.e. each of its eigenvalues has a negative real part. These results are summarized in the bifurcation diagram illustrated in Fig. 3.

The diagram of Fig. 3 predicts a sort of hysteretic behavior: if the system is in the stable non-trivial steady state with small values of the decay rate constants, it remains in the same state when these constants are increased, until it abruptly collapses to the trivial steady state when the critical point is reached. Once in the trivial steady state, it remains there even when the decay rate constants are decreased below the critical point. The hysteretic cycle cannot be completed unless we allow the possible appearance of trace quantities of any intermediate (such as might result from external chemistry) that could allow the system to recover the non-trivial steady state when close enough to the equilibrium condition.

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The unstable steady state that appears in those conditions of bistability, , belongs to a separating barrier that constitutes a hypersurface limiting the attractor regions of both trivial and non-trivial stable steady states. A planar region of the phase diagram is illustrated in Fig. 4 for . Different initial conditions close enough to the separating barrier could drive the system either to one stable steady state or the other, as shown in Fig. 5.

Robustness of the stable non-trivial steady state

It is clear that the system as described is capable of reaching a stable non-trivial steady state with finite fluxes and finite concentra-
equilibrium with separated by an unstable steady state. When the system is at
bistability in which both trivial and non-trivial stable steady states are
direction of movement determines the specific behavior: the jump is
appearance of small fluctuations in the concentrations. In brief, the
approaching this condition can it experience a large jump after the
increases in metabolite concentrations, for example, when ST was
the same non-trivial steady state. It can equally resist very large
generalizable to combinations of metabolites), it always returns to
perturbations. Unless it is perturbed to such a large extent that the
as tested by analysis of the Jacobian matrix, but also for large
functions return to the previous steady-state values.

Figure 3. Bifurcation plot. For \( k_4 = k_9 = k_{11} < 0.367 \) there is a region
of bistability in which both trivial and non-trivial stable steady states are
separated by an unstable steady state. When the system is at
equilibrium with \( k_4 = k_9 = k_{11} = 0 \), the only possible stable steady state
is the non-trivial steady state with \( \langle S T \rangle = 16 \), as indicated by the arrows.
If the decay constants are increased (proceeding to the right in the plot), the system remains in a non-trivial steady state until it falls
abruptly to zero — the trivial steady state — exactly when leaving the
bistability region. However, when starting from these final conditions,
with every concentration zero, the initial trajectory cannot be reversed,
because the system cannot “climb” to the non-trivial steady state until
it is close to the equilibrium condition \( (k_4 = k_9 = k_{11} = 0) \). Only when
approaching this condition can it experience a large jump after the
appearance of small fluctuations in the concentrations. In brief, the
direction of movement determines the specific behavior: the jump is
detected at \( k_4 = k_9 = k_{11} = 0 \) when going to the left and at
\( k_4 = k_9 = k_{11} = 0.367 \) when going to the right.

Stoichiometric network analysis

With the use of MetaTool [21] we have analyzed the structure
of the model by means of an approximation to a stoichiometric
analysis in the steady state. In this analysis, S, T and U are
considered as external metabolites, the others being considered
internal. With the rates \( v_i \) numbered as in Fig. 1b the reaction
subsets \( R \) are as follows:

\[
R = \begin{pmatrix}
  v_1 & v_2 & v_3 & v_4 & v_5 & v_6 & v_7 & v_8 & v_9 & v_{10} & v_{11}
\end{pmatrix}
\]

\[
= \begin{pmatrix}
  1 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0
  0 & 1 & 1 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0
  0 & 0 & 1 & 1 & 1 & 0 & 0 & 0 & 0 & 0 & 0
  0 & 0 & 0 & 0 & 0 & 0 & 1 & 1 & 0 & 0 & 0
  0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 1
\end{pmatrix}
\]

As seen in this equation, subsets of reactions operate at the same rate
in the steady state, i.e. \( v_2 = v_3, v_4 = v_5 = v_6 = v_7 \) and
\( v_8 = v_9 = v_{10} \), as illustrated in Fig. 7a. Notice that the degradation
rates \( v_4 \) and \( v_5 \) for the two catalysts STU and SU are in the same
subsets as the corresponding replacement reactions: \( v_4 \) with \( v_5 \), \( v_6 \)
and \( v_7 \); but \( v_8 \) with \( v_9 \) and \( v_{10} \). This explains how the replacement
can efficiently balance the decay of each catalyst in the steady
state.

The resulting convex basis can be expressed in the following way:

\[
= \begin{pmatrix}
  1 & 1 & 1
  0 & 1 & 1
  0 & 1 & 0
  0 & 1 & 0
  0 & 0 & 1
  0 & 0 & 0
  1 & 0 & 0
\end{pmatrix}
\]

\[
\begin{pmatrix}
  b_1 & b_2 & b_3
\end{pmatrix}
\]

\[
= \begin{pmatrix}
  1 & 0 & 0
  1 & 0 & 0
  0 & 1 & 0
  0 & 1 & 0
  0 & 0 & 1
  0 & 0 & 1
  1 & 0 & 0
\end{pmatrix}
\]

\[
\begin{pmatrix}
  \lambda_1 & \lambda_2 & \lambda_3
\end{pmatrix}
\]

\[
= \begin{pmatrix}
  \lambda_1 + \lambda_2 + \lambda_3 & \lambda_1 & \lambda_2 & \lambda_3 & \geq 0
  0 & 1 & 0
  0 & 0 & 1
  0 & 0 & 1
  1 & 0 & 0
\end{pmatrix}
\]

All three basis elements are shown schematically in Fig. 7b. The
first, \( b_1 \), includes the reactions involved in the metabolism
Figure 4. Planar section of the multidimensional phase diagram. The calculation refers to $k_4 = k_8 = k_{11} = 0.3$. The point shown in green corresponds to the unstable steady state, which is contained in a barrier separating the attraction areas of the trivial steady state (point shown in red) and the non-trivial steady state (point shown in blue). The brown arrows represent a schematic illustration of the orbits followed in approaching the steady states. The inset illustrates schematically that the main plot is a two-dimensional slice of a multidimensional reality.

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Figure 5. Time evolution from starting points close to the unstable steady state. Simulations were done with $k_4 = k_8 = k_{11} = 0.3$. The initial concentration of ST was 6.6 (red curve) or 7.2 (blue curve), and other concentrations were set to those in the unstable steady state. The inset shows the time dependences at very low times, which are in the opposite directions from the long-term trends.

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process, $b_2$ corresponds to both the metabolic and replacement cycles, and the third, $b_3$, is the pathway that replaces the replacement catalyst SU. As previously shown in the subsets of reactions, the rate $v_{11}$ of decay of ST does not share the same rate with any other reaction. However, it also is compensated as a consequence of the performance of the metabolic reactions $v_1$, $v_2$ and $v_3$, as deduced from the inspection of the first element of the convex basis.

To study the relative contributions of the basis elements to the steady-state flux distribution, $\lambda_1$, $\lambda_2$ and $\lambda_3$ were evaluated from the numerical integration results for different values of the degradation rate constants (Fig. 8). The optimum operating rate value is obtained when $k_4 = k_8 = k_{11}$ is in the range 0.2–0.3, rather closer to the conditions for bifurcation than those for equilibrium (Fig. 8a). The contribution of $b_1$ turns out to be around double that of $b_2$ over most of the range. Nevertheless, as shown in Fig. 8b,
Thus, the enzyme-maintaining mode [23] because none of the elements of the convex basis. Nevertheless, none of them is an enzyme-maintaining mode [23] because none of them could indefinitely function alone, i.e. STU acting in $b_1$ and $b_3$ could not possibly function alone, i.e. STU acting in $b_1$ and $b_3$ could indefinitely function alone, i.e. STU acting in $b_1$ and $b_3$ needs $b_2$ to be replaced but at the same time ST and SU in $b_2$ need the replacement function in $b_1$ and $b_3$, respectively (Fig. 7b).

The relative contributions of the three elements are illustrated over the same range of values of $k_4 = k_8 = k_{11}$.

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Discussion

A simple model of an (M,R)-system consisting of three catalytic cycles organized so that all catalysts are products of reactions within the system is able to establish and maintain a non-trivial steady state capable of resisting degradation of all the catalysts, provided that this degradation is not so fast that the catalysts are eliminated faster than they are regenerated. This model was originally proposed as a way of giving concrete expression to the abstract view of life embodied in Rosen’s (M,R)-system s [1]. It does of course oversimplify some aspects of his analysis, but we consider that it is helpful for understanding the nature of his concept of closure to efficient causation. It shows that a small system in which all catalysts are produced internally can not only organize itself into a non-trivial steady state, but it can also recover from large perturbations, such as complete loss of a catalyst. In favorable conditions and with a large amount of time available, the system in stable steady state can create itself from essentially nothing — a few suitable reactants present in vanishingly small amounts. As mentioned above, no elementary flux mode in this model is independently capable of maintaining itself. We are conscious that this does not constitute a proof of the simplicity of this model. In fact the model in the form originally proposed [8] did not allow for degradation of ST (reaction 11 in Fig. 1), and in a sense, therefore, represented a simpler system. However, the inclusion of this decay process is more realistic when considering the capacity of ST to be driven to new processes of increasing complexity and thus the evolutionary potential of the model.

As our original model [8] was designed to be self-maintaining the demonstration here that it is indeed capable of self-maintenance confirms our prediction. The bistability that it also shows was not consciously designed. This leads to more complex dynamics, and the advantages of multistability for a living organism have been discussed previously [17,18]. The model is composed of various interconnected reactions, and it can be decomposed into individual circuits according to either logical or stoichiometric criteria; it was, in fact, constructed logically, with interplay of three basic building blocks, as described in the Introduction. These three cycles have both structural and dynamic roles in the self-maintenance of the entire system, and they exert constraints on the conditions for a “living”, non-trivial steady state, as discussed already and illustrated by Fig. 8. We have checked that none of them exhibits bistability by itself, and the occurrence of multistationarity is a consequence of the combined action of all of them: no “living” steady state is achieved in the system if any reaction (other than a degradation step) of the model is eliminated.

As mentioned in the Introduction, a smaller system [19] than the one in Fig. 1 can show bistability: this was presented as the smallest chemical reaction system with bistability, but it is not a model of an organism because it includes no mechanism for regenerating the catalyst, and if this is lost, for whatever reason, no recovery is possible. We do not claim to have demonstrated that the model studied here is the simplest system capable of self-maintenance.

The simplicity of this robust self-maintaining system and its capacity to be easily seeded may allow us to regard it as a plausible prebiotic system. Specifically, the establishment of a reflexive autocatalysis, i.e. autocatalysis that results from the structure of the whole network rather than from specifically autocatalytic components, is a typical common feature of models that illustrate recent theories of the origin of life; for example, the “lipid-world” scenario [24] and the theory of autocatalytic sets of proteins [25] share this property. Although the chemical nature of the components in the system analyzed in this paper is not specified, its autocatalytic organization is sufficient to satisfy the definition of an autocatalytic set: STU catalyzes synthesis of SU and vice versa. Of course, the difficulty of spontaneously developing a realistic {STU, SU} dual set of molecules performing such a special task of autocatalysis is arguable, but no other simple model of organizational closure escapes this criticism either. In any case,
the essential postulate is that acquisition of some kind of recursive autocatalytic network should have been a necessary step at the very beginning of prebiotic evolution, before the development of more complex infrabiological systems [26].

In this analysis we have effectively assumed that a primitive self-maintaining system has metabolism but does not have information processing, in other words a metabolism-first scenario for the origin of life. All of the principal current theories of life [1–5] incorporate metabolism, but only a minority [2,5] explicitly incorporates storage of information; even the autocatalytic sets [4] treat RNA molecules only as catalysts, not as information stores. Particularly interesting is that this simple (M,R)-system shows that depending on the arrangement of elements in its intermediates: multiple components have the same composition but different functions, depending on the arrangement of their elements, e.g. SUSTU and STUSU are isomers with different activities, and the same is true of STUS and SUST. As the model is drawn, the structural differences are differences in sequence, suggesting sequence-dependent information storage even in a metabolism-first model of the origin of life: thus the borderline between replication-first and metabolism-first approaches to the origin of life may not be absolute. Indeed, this typical dichotomy may be blurred when considering simple organizational recursive systems in which the different chemical intermediates necessarily have parts of their structures in common.

Author Contributions
Conceived and designed the experiments: G. Piedrafita, F. Montero, F. Morán, M.L. Cárdenas, A. Cornish-Bowden. Performed the experiments: G. Piedrafita. Analyzed the data: G. Piedrafita, F. Montero, F. Morán, M.L. Cárdenas, A. Cornish-Bowden. Wrote the paper: M.L. Cárdenas, A. Cornish-Bowden.

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