

Additional file 1

Spontaneous Formation and Evolution of Autocatalytic Sets within Compartments

Chrisantha Fernando, Vera Vasas, Mauro Santos, Stuart Kauffman, and
Eörs Szathmáry

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A – Autocatalytic sets

A1 Definitions of autocatalytic sets

The essential feature of so-called autocatalytic sets is that they can collectively self-replicate (grow exponentially), even if none of their component molecular species can individually self-replicate [1]. It is necessary to distinguish the various –sometimes contradictory– definitions of what an autocatalytic set is. Kauffman [2] defines an autocatalytic set as a set of molecules in which “every member of the autocatalytic set has at least one of the possible last steps in its formation catalyzed by some member of the set, and that connected sequences of catalyzed reactions lead from the maintained ‘food set’ to all members of the autocatalytic set”.¹ This is more formally defined by Hordijk and Steel [4], who state that an R (sub)set of reactions is called (i) reflexively autocatalytic (RA) if every reaction in R is catalyzed by at least one molecule involved in any of the reactions in R , (ii) food-generated (F) if every reactant in R can be constructed from a small “food set” by successive applications of reactions from R , and (iii) reflexively autocatalytic and food-generated (RAF) if both RA and F . Note that Kauffman’s definition [2] excluded autocatalytic sets that could not be constructed from the food set and thus corresponds to a RAF . Other work has defined autocatalytic sets more abstractly in terms of the catalytic graph: “an autocatalytic set is a graph, each of whose nodes has at least one incoming link from a node belonging to the same graph” [5], analogous to an RA set. In a catalytic graph, nodes depict molecules and edges are catalytic actions.

A2 Structure of autocatalytic sets in Kauffman’s polymer chemistry

Kauffman’s [2] original mathematical model assumes the following: (i) there exists a large “food set” of abundant polymers naturally formed in the environment, up to some low level of complexity; i.e. up to length M consisting of B types of monomers; (ii) each molecule has a certain probability P of catalyzing each ligation-cleavage reaction. The model assumes infinite specificity, in other words, a molecule either does or does not catalyze a particular reaction without quantitative variation in efficiency. It was

¹ In an associated paper [3] it is stated that an autocatalytic set is a “set of polymers such that each polymer is produced by at least one catalytic reaction involving only other members of the autocatalytic set”. However, in their Figure 2. a species that catalyses its own formation is considered to be a type of autocatalytic set. Therefore, it appears that they use the same definition as Kauffman [2].

demonstrated that above a certain catalytic probability threshold a chain reaction is triggered and due to catalytic closure autocatalytic sets appear.²

Hordijk and Steel [4] verified this claim by generating random networks of reversible ligation/cleavage reactions between strings up to length $n = 20$, where each molecule had the probability P of catalyzing each reaction. At low values of P they found unconnected *RAFTs* utilizing separate food sets, but at higher values a percolation phenomenon produced a fully connected *RAFT* sets³.

Our results after implementing Farmer et al. [7] model (see section B – Methods) are in agreement with those of Hordijk and Steel [4]. With small P the number of molecular species is small compared to the size of the food set and they form jets with acyclic catalytic graphs. Catalytic closure is reached when all food species are catalyzed (non-food species can only appear if catalyzed) and the catalytic graph consists of one strongly connected component (core) and its periphery (Figure S1). However, autocatalytic cores consist mainly of suicidal autocatalysts rather than viable autocatalytic loops (Figure S2).

² The proof itself contains a mistake. Kauffman argues that because any specific longest polymer of length M can be constructed in $M-1$ possible end condensation reactions (e.g.: aabb can be constructed in three condensation reactions: aab+a, aa+ba, a+aba), and all reactions can be catalyzed by any of the $N = 2^{M+1}$ molecules, the probability that none of the reactions is catalyzed by any of the molecules equals $\bar{P}_{all} = \bar{P}^{(M-1) \times N}$. This implies that as M increases, \bar{P}_{all} decreases, meaning that the longer the polymer we consider, it becomes less and less likely that its production is not catalyzed. However, this is only true if all end condensation reactions ($M-1$) are possible as all precursors are also catalyzed and if all possible catalysts (N) are present, i.e. the probability calculation for one specific longest polymer assumes that all other species are present. This is neither the case nor, as a matter of fact, the goal.

³ A note on Lifson’s [6] criticism: in Kauffman’s model, P remains constant and so the average number of reactions catalyzed by one molecular species increases rapidly with network size. In Lifson’s [6] interpretation, P is inversely proportional to the total number of possible reactions and the average number of reactions that a molecule catalyses is constant. Hordijk and Steel [4] have shown that a linear growth of P with system size is sufficient for *RAFT* sets to appear with high probability.

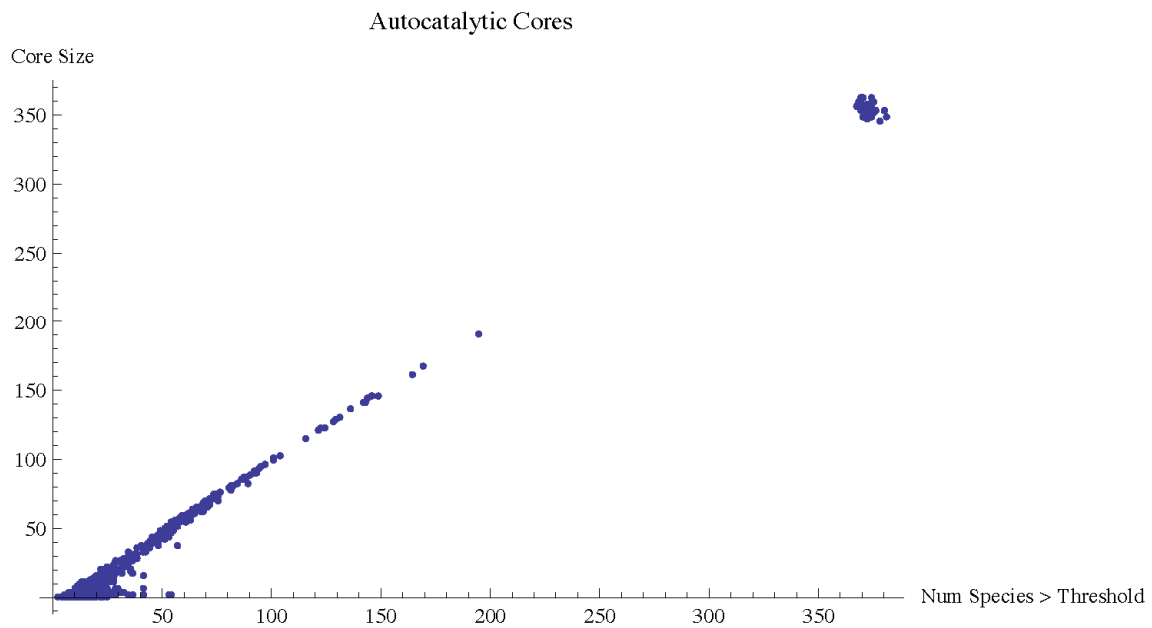


Figure S1. Distribution of sizes of autocatalytic cores in Farmer et al. [7] model. For large networks most species maintained above threshold are in one autocatalytic core (strongly connected component in the catalytic graph). Each dot corresponds to a different chemical network generated with $P = 0.00022$.

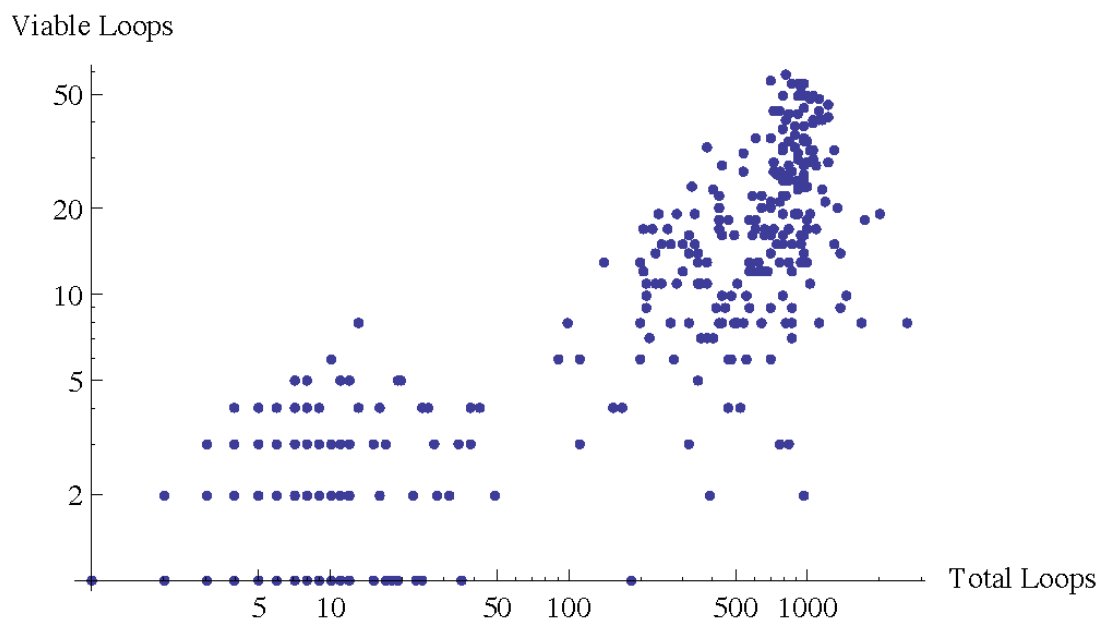


Figure S2. Frequency of viable loops in Farmer et al. [7] model. Only a minority of catalytic loops are viable. Each dot corresponds to a different chemical network generated with $P = 0.00022$.

B – Methods

B1 Reimplementation of Farmer et al.'s model

In Farmer et al. [7] a simplified model of catalyzed linear polymer reactions was devised consisting of polymers of alphabet size $B = 2$. Reversible, ligation (condensation) and cleavage reactions were permitted. Only reactions catalyzed by another polymer were modeled. Spontaneous stochastic production of uncatalyzed species whilst ignored in the model would be expected to occur in a realistic reactor (see below). Reactions were of the form:



where R_1 and R_2 are the concentrations of reactants, P_1 is the concentration of the product of a condensation reaction, and c is the catalyst. Cleavage involves P_1 breaking down into R_1 and R_2 , the bi-directional arrow indicating reversibility. There is no attempt to relate the structure (i.e. sequence) of a polymer to its catalytic or reactive function. The reactions in which a polymer will participate are determined randomly. The catalyst for the reaction is also chosen randomly. From the initial food set (each member of which is present at initial concentration F_c and added continuously at rate F_{input}) the maximum number of possible distinct condensation and cleavage reactions that could theoretically occur is determined. Because at the outset, all species below and including length M are present, cleavage reactions within the food set are not modeled. The food set species are placed into the inner ring of species, S_I .

The number of new reactions to be generated is determined by multiplying the number of possible reactions by probability P . The number of possible reactions is a function of the numbers of species in the inner (S_I) and outer (S_O) rings that are present above the concentration threshold, T . The method for generating these rings is described below. Note: it would have been more realistic to determine the number of reactions using a Binomial distribution with probability of success per possible reaction equal to P , and where the sample size n equals the population size (number of possible reasons) N . If a novel species is formed in a new reaction then it is placed into the outer ring of species, S_O . The reaction kinetics are then simulated for long enough for them to reach steady-state for that particular reaction network.

The main loop of the simulation is then entered. This involves again calculating all new reactions between the newly produced species in S_O and the old species in S_I , and placing new species in a temporary ring, S_T . The outer ring species are then moved to the inner ring, and the temporary species are moved to the outer ring. Then the dynamics are run for one hundred time-steps. If this results in a species in the inner ring obtaining a concentration greater than threshold T (for the first time) then that species is moved to the outer loop where it can take part in the next round of novel reaction calculations (and its candidate status is updated to active). This simulates a minimum concentration below which no molecule of the species exists in the reactor. A concentration of 10^{-9}M corresponds to a single atom in a compartment the size of a bacterium. Higher thresholds have to be used in simulations, due to the difficulty of simulating large reaction networks, e.g. we typically use 10^{-5}M as a concentration threshold. This loop repeats until the simulation is terminated if there are too many species or too many condensation reactions to simulate, or if the maximum simulation time is reached.

The chemical kinetics approximates the behavior of catalyzed reactions, assuming identical binding velocity for all intermediates. Only the catalytic rates, v_o , vary in magnitude from 10 to 1000. The concentration of each species is separated into a bound, $[S]_b$, and a free part, $[S]_f$. The bound concentration represents the sum of all concentrations of intermediates in which S participates. The forward rate of a condensation reaction (e.g. Eq. 1) is given by

$$v_f = v_o k_f [C]_f [R_1]_f [R_2]_f \quad [2]$$

and the backward rate by

$$v_b = v_o k_b [C]_f [P_1]_f \quad [3]$$

where k_f and k_b are the forward and backward equilibrium constants, respectively. The change in free concentration of the species due to reactions are given by the reaction terms

$$\frac{[R_1]_f}{dt} = -v_f \quad [4.1]$$

$$\frac{[R_2]_f}{dt} = -v_f \quad [4.2]$$

$$\frac{[P_1]_f}{dt} = -v_b \quad [4.3]$$

$$\frac{[C]_f}{dt} = -v_b - v_f \quad [4.4]$$

When addition of the food set, formation of bound intermediates, and decay of species is considered, the total change of concentration of a species is given by

$$\frac{[S]_f}{dt} = \sum [\text{reactions}] + k_u [S]_b - k_d [S]_f + F_{input} H(M - L) \quad [5]$$

where k_u is the unbinding velocity or dissociation constant and k_d is the decay rate. The last term is the Heaviside function $H(x) = 1$ for $x > 0$, $H(x) = 0$ for $x < 0$; L is the length of species S ; and F_{input} is the rate of food input. The change in bound concentrations due to a reaction are given by

$$\frac{[R_1]_b}{dt} = v_b \quad [6.1]$$

$$\frac{[R_2]_b}{dt} = v_b \quad [6.2]$$

$$\frac{[P_1]_b}{dt} = v_f \quad [6.3]$$

$$\frac{[C]_b}{dt} = v_b + v_f \quad [6.4]$$

and the corresponding total change of concentration for bound species is

$$\frac{[S]_b}{dt} = \sum [\text{reactions}] - k_u [S]_b - k_d [S]_b \quad [7]$$

The full details of the algorithm and parameters used are described in Table 1 and Algorithm 1.

Table 1. Parameters, initial values and variables used in Farmer et al. [7].

Parameter	Symbol	Value/Range
Alphabet Size	B	2
Firing Disk Radius	M	4
Probability of Catalysis	P	0.0032 - 0.00028
Concentration Threshold	T	0.00001
Time step	τ	0.0001
Iterations until steady-state	Φ	100
Catalytic velocity per catalyst	v_o	$10 < v_o < 1000$
Condensation rate constant	k_f	1
Cleavage rate constant	k_b	10
Decay rate constant	k_d	1.0
Dissociation rate constant	k_u	1000
Initial Value		
Initial firing disk species concentration	F_c	2.0
Food set input rate	F_{input}	2.0
Variables/States		
Species	S	
Species in inner disk	S_I	
Species in outer disk	S_O	
Species in temporary disk	S_T	
Species Length	S_L	$1 < S_L < \infty$
Species concentration	$[S]$	$0 < [S] < \infty$
Species concentration (free)	$[S]_f$	$0 < [S] < \infty$
Species concentration (bound)	$[S]_b$	$0 < [S] < \infty$
Species catalyses a reaction	S_{cat}	1 / 0
Species candidate status	S_{cand}	1 / 0
Reactant x / Product x	R_x / P_x	

Algorithm 1: An interpretation of the algorithm described in Farmer et al. [7].

1. Initialize firing disk

Create all firing disk polymers $\leq M$ in length, at initial concentration F_c .

2. Create new condensation reactions

Number of condensation reactions in firing disk (R_{cond}) = $P.c.S^2$

c = num of possible catalysts in inner disk, i.e. the subset of S_i satisfying conditions: $[S_i] > T$, $S_{cat} = 0$.

For R_{cond}

Choose two random species satisfying conditions: $[S_i] > T$.

Create random condensate at concentration 0, $S_{cand} = 1^4$, catalysed by a random species satisfying conditions: $[S_i] > T$, $S_{cat} = 0$. Set $S_{cat} = 1$

Assign new condensate species to outer disk S_o

End For

3. Run dynamics for Φ steps with time-step τ

Reset all temporary concentrations

For each condensation reaction $R_1 + R_2 \rightarrow P_1$ where $[C] > T$ & ($[R_1] \& [R_2] > T \parallel [P_1] > T$)

For each catalyst j that catalyses this reaction

$$[R_1]_{f_temp} = [R_1]_{f_temp} - \tau k_f v_o [C_j]_f [R_1]_f [R_2]_f$$

$$[R_2]_{f_temp} = [R_2]_{f_temp} - \tau k_f v_o [C_j]_f [R_1]_f [R_2]_f$$

$$[P_1]_{f_temp} = [P_1]_{f_temp} + \tau k_b v_o [C_j]_f [P_1]_f$$

$$[C_j]_{f_temp} = [C_j]_{f_temp} + \tau v_o [C_j]_f (-k_b [P_1]_f - k_f [R_1]_f [R_2]_f)$$

$$[R_1]_{b_temp} = [R_1]_{b_temp} + \tau k_b v_o [P_1]_f$$

$$[R_2]_{b_temp} = [R_2]_{b_temp} + \tau k_b v_o [P_1]_f$$

$$[P_1]_{b_temp} = [P_1]_{b_temp} + \tau k_f v_o [C_j]_f [R_1]_f [R_2]_f$$

$$[C_j]_{b_temp} = [C_j]_{b_temp} + \tau v_o [C_j]_f (k_b [P_1]_f + k_f [R_1]_f [R_2]_f)$$

End For

End For

For each cleavage reaction $R_1 \rightarrow P_1 + P_2$, where $[C] > T$ & ($[P_1] \& [P_2] > T \parallel [R_1] > T$)

For each catalyst j that catalyses this reaction

$$[P_1]_{f_temp} = [P_1]_{f_temp} - \tau k_f v_o [C_j]_f [R_1]_f [R_2]_f$$

$$[P_2]_{f_temp} = [P_2]_{f_temp} - \tau k_f v_o [C_j]_f [P_1]_f [P_2]_f$$

$$[R_1]_{f_temp} = [R_1]_{f_temp} - \tau k_b v_o [C_j]_f [R_1]_f$$

$$[C_j]_{f_temp} = [C_j]_{f_temp} + \tau v_o [C_j]_f (-k_b [R_1]_f - k_f [P_1]_f [P_2]_f)$$

⁴ A species is a candidate until it has achieved a concentration above the threshold. Therefore when all species are created in new reactions (except for spontaneous new reactions) they are candidates.

$$\begin{aligned}
[P_1]_{b_temp} &= [P_1]_{b_temp} + \tau k_b v_o [R_1]_f \\
[P_2]_{b_temp} &= [P_2]_{b_temp} + \tau k_b v_o [R_1]_f \\
[R_1]_{b_temp} &= [R_1]_{b_temp} + \tau k_f v_o [C_j]_f [P_1]_f [P_2]_f \\
[C_j]_{b_temp} &= [C_j]_{b_temp} + \tau v_o [C_j]_f (k_b [R_1]_f + k_f [P_1]_f [P_2]_f)
\end{aligned}$$

End For

End For.

Adjust distribution between bound and unbound species

For all Species

$$\begin{aligned}
[S]_{f_temp} &= [S]_{f_temp} + \tau (k_u [S]_b - k_d [S]_f) \\
[S]_{b_temp} &= [S]_{b_temp} + \tau (-k_u [S]_b - k_d [S]_b) \\
\text{If } (S_L \leq M) \text{ then } [S]_{f_temp} &= [S]_{f_temp} + \tau \cdot F_{input}
\end{aligned}$$

End For

$$[S]_{b/f} += [S]_{b_temp/f_temp}$$

4. MAIN LOOP

For N iterations or until Stop = 1

4.1. Assign condensations between one old and one new species, and between two new species, catalysed by old or new species.

$$\text{Num new condensations} = S_o^2 + 2S_i S_o \text{ for all } [S] > T$$

$$\text{Num possible catalysts} = S_i + S_o, \text{ where } S_{cand} = 0$$

$$\text{Num reactions} = P \times \text{Num possible catalysts} \times \text{Num new condensations}$$

Create reactions: $S_{cat} = 1$ for the catalysts used. Put New species in S_{temp} .

4.2. Assign cleavages of new species, catalysed by old or new species.

$$\text{Num new cleavages} = \text{Total number of bonds in } S_o$$

$$\text{Num possible catalysts} = S_i + S_o, \text{ where } S_{cand} = 0$$

$$\text{Num reactions} = P \times \text{Num possible catalysts} \times \text{Num new condensations}$$

Create reactions: $S_{cat} = 1$ for the catalysts used. Put New species in S_{temp} .

4.3. Assign condensations of old species, catalysed by new species.

$$\text{Number reactions} = P \times S_o \times S_i^2$$

Create reactions: $S_{cat} = 1$ for the catalysts used. Put New species in temp store.

4.4. Assign cleavages of old species, catalysed by new species.

$$\text{Num Reactions} = P \times S_o \times \text{Number of bonds in } S_i$$

Create reactions: $S_{cat} = 1$ for the catalysts used. Put New species in S_{temp} .

4.5. Move S_o to S_i and move S_{temp} to S_o .

4.6. Run step (as in 3).

4.7. If $[S_i] > T$ for the first time: $S_{cand} = 0$, move to S_o

End For

B2 Chemical network analysis algorithms

The first part of the algorithm for characterizing the structure of the catalytic reaction network obtained is to determine all the strongly connected components of the catalytic reaction graph. In a catalytic graph, nodes depict molecules and edges are catalytic actions. The strongly connected components (SCCs) correspond to the viable cores. SCCs were detected using a standard function from the *Boost* graph library [8].

The next step was to detect all the directed cycles in the catalytic graph. Cycles can only exist within SCCs. Detection of cycles is achieved using a distributed salesman algorithm on a catalytic graph. The catalytic graph is first modified by giving a number to each chemical type (node). Then the following algorithm is undertaken to detect all directed cycles.

Algorithm 2: Detecting cycles in a catalytic graph .

```
0  For each strongly connected component
2.      Choose a random node in the SCC and place a new salesman on it
3.      Number this salesman according to the number of the node
4.      Place a pointer to the node into its visitedList
5.      Place the salesmen in the oldSalesman list
6.      While (oldSalesman list is not empty)
7.          Make empty newSalesman list
8.          For each salesman S in oldSalesman list
9.              For each reachable node X from the node of salesman S
10.                 If (If reachable node number X >= S number)
11.                     Make new salesman S' with same number
12.                     and visitedList as parent S
13.                     Add pointer to node X to S' visitedList
14.                     If (cycle detected in visitedList)
15.                         If cycle is new store in cycleList
16.                         Kill Salesman S'
17.                 delete oldSalesman List
18.                 copy newSalesman List to oldSalesman list
```

Once all the possible cycles have been detected in the catalytic graph it is necessary to determine the viability of each cycle. For a cycle to be viable, it must be possible to obtain from the food set a minimal subset of the substrates from which the members of the cycle can be catalyzed by other members of the cycle. Only if this is the case is there

the potential for autocatalysis, i.e. for exponential growth of the cycle. The algorithm for detecting viable cycles is shown below.

Algorithm 3: Identifying viable cycles.

- 1 For each cycle
- 2 Initialize extendedFoodSet with the food set
- 3 Grow extendedFoodSet with species created from reactions of species in extendedFoodSet
 whose product is not a loop member
- 4 For each member of the cycle
- 5 Determine all reactions in which this member is produced as a product
- 6 If at least one of the necessary set of reactants for production of this
 member is in the extendedFoodSet, mark this member as validly producible.
- 7 If all members of the cycle are validly producible, then mark cycle as valid.

C – Examples of networks with novel viable loops

Example 1 (used in main text)

Reaction network prior to spontaneous reactions

abaa + aaab - aab -> abaaaaab
abbb + abbb - abaa -> abbbabbb
abbb + babb - baba -> abbbbabb
aabb + bba - aa -> aabbbba
baaa + aaba - a -> baaaaaba
abbbabbb + b - aaaa -> abbbabbbb
aabbbba + bbab - baa -> aabbbbabbab
baa + aaba - abbbbabb -> baaaaaba
abbbabbbb + abbbbabb - aaba -> abbbabbbbabbbbabb
abbbabbbb + bab - bb -> abbbabbbbabb
bbba + baba - abbbabbbb -> bbbababa
bbbababa + baba - aaab -> bbbababababa
bbbababa + abbbabbb - ab -> bbbababaabbbabbb
bbbababababa + aa - bbbababa -> bbbababababaaa
bbbababaabbbabbb + ab - bba -> bbbababaabbbabbbab
bbbababababaaa + bbb - bab -> bbbababababaaaabbb
bbbababaabbbabbbab + abaaaaab - babb -> bbbababaabbbabbbabababaaaaab
bbbababababaaaabbb + aba - bab -> bbbababababaaaabbbaba
abaa + bbbababaabbbabbb - bbbababababaaaabbb -> abaabbbababababababbb

New reactions incorporated after a spontaneous species appears

abaa + aaab - aab -> abaaaaab
abbb + abbb - abaa -> abbbabbb
abbb + babb - baba -> abbbbabb
aabb + bba - aa -> aabbbba
baaa + aaba - a -> baaaaaba
abbbabbb + b - aaaa -> abbbabbbb
aabbbba + bbab - baa -> aabbbbabbab
baa + aaba - abbbbabb -> baaaaaba
abbbabbbb + abbbbabb - aaba -> abbbabbbbabbbbabb
abbbabbbb + bab - bb -> abbbabbbbabb
bbba + baba - abbbabbbb -> bbbababa
bbbababa + baba - aaab -> bbbababababa
bbbababa + abbbabbb - ab -> bbbababaabbbabbb
bbbababababa + aa - bbbababa -> bbbababababaaa
bbbababaabbbabbb + ab - bba -> bbbababaabbbabbbab
bbbababababaaa + bbb - bab -> bbbababababaaaabbb
bbbababaabbbabbbab + abaaaaab - babb -> bbbababaabbbabbbabababaaaaab
bbbababababaaaabbb + aba - bab -> bbbababababaaaabbbaba
abaa + bbbababaabbbabbb - bbbababababaaaabbb -> abaabbbababababababbb
bbba + bbbabababababa - baaaa -> bbaabbbabababababa
bbaabbbabababababa + baaaaaba - abbbabbbb -> bbaabbbababababababaaaaaba
b + aaaa - bbaabbbabababababa -> baaaa
aabb + aaa - abbbbba -> aabbbaa
aabbbaa + bab - baaa -> aabbbaabab
b + abbb - aabbbaa -> babb
aaa + abaa - babb -> aaaabaa
abaaaaab + abaa - aaaabaa -> abaaaaababaa
abab + ba - aabbbaabab -> ababba
abb + bbaa - abaaaaababaa -> abbbbbaa

New cores

bbaabbbababababa -> baaaa -> bbaabbbababababa
 abbbbbaa -> aabbaaa -> babbb -> aaaabaa -> abaaaaababaa -> abbbbbaa

Example 2

Reaction network prior to spontaneous reactions

abaa + bab - abbb -> abaabab
 aba + aaba - aab -> abaaaba
 aa + aaaa - aba -> aaaaaa
 aabb + baa - bbba -> aabbbbaa
 aabb + aab - abbb -> aabbaab
 aabbbbaa + aabbbbaa - aaaaaa -> aabbbbaaaabbbbaa
 abaabab + b - abab -> abaababb
 aaaa + ab - aaaaaa -> aaaaab
 aabbbbaaaabbbbaa + abb - bbbb -> aabbbbaaaabbbbaaabb
 abaababb + aaaaaa - b -> abaababbbaaaaaa
 aaa + bab - abaababb -> aaabab
 aaabab + abba - aaa -> aaabababba
 abaababbbaaaaaa + a - aaabab -> abaababbbaaaaaa
 aaabababba + bab - bbab -> aaabababbabab
 aaabababbabab + abb - bbba -> aaabababbabababb
 aaabababbabababb + bbbb - aaabababbabab -> aaabababbabababbbbbbb

New reactions incorporated after a spontaneous species appears

babb + baab - aaaaa -> babbbbaab
 babbbbaab + babbbbaab - aaab -> babbbbaabbabbbbaab
 aaaaa + b - babbbbaab -> aaaaab
 babbbbaabbabbbbaab + aaaaa - a -> babbbbaabbabbbbaabaaaaa
 babbbbaabbabbbbaabaaaaa + abaababbbaaaaaa - aba ->
 babbbbaabbabbbbaabaaaaaabaababbbaaaaaa

New core

aaaaa -> babbbbaab -> aaaaa

Example 3

Reaction network prior to spontaneous reactions

b + aa - aaaa -> baa
 aaa + aaab - bab -> aaaaaab
 abaa + bbab - aaab -> abaabbab
 aba + abaa - bba -> abaabaa
 abaabbab + aaba - bbba -> abaabbabaaba
 ab + baaa - abaabaa -> abbbaa
 abbbaa + abb - bbb -> abbbaaabb

New reactions incorporated after a spontaneous species appears

aaaaa + ab - aaaaa -> aaaaaab

New core

aaaaa -> aaaaa

Example 4

Reaction network prior to spontaneous reactions

a + aaaa - baa -> aaaaa
baa + baaa - bbba -> baabaaa
bbb + aba - bbb -> bbbaba
aaaa + baaa - bbbb -> aaaabaaa
bbbaba + bbba - ab -> bbbababbaa
aaaaa + bb - b -> aaaaabb
aba + a - aaaabaaa -> abaa
aaaaabb + aaaaa - aabb -> aaaaabbaaaaa
bba + aaaa - bbbababbaa -> bbaaaaaa
bbaaaaa + aaab - bba -> bbaaaaaaaab
bbba + bb - aaaaabbaaaaa -> bbbabb
bbaaaaaaaab + aaaa - bbbaba -> bbaaaaaaaabaaaa
abb + abaa - bbaaaaaaaab -> abbabaa
abbabaa + abaa - bbba -> abbabaaabaa
abbabaaabaa + aaaaabbaaaaa - bbbababbaa -> abbabaaabaaaaaabbaaaaa
bbaaaaaaaabaaaa + aaaaabb - ba -> bbaaaaaaaabaaaaaaabb

New reactions incorporated after a spontaneous species appears

aaa + aaba - bababbbaaaaaab -> aaaaaba
aaaaaba + babb - bbaaaaaaaab -> aaaaabababb
abaa + bba - aaaaabababb -> abaabba
abaabba + bab - abb -> abaabbabab
baba + bbba - abaabba -> bababbbba
bababbbba + aaaa - bb -> bababbbbaaaaa
bbba + aab - bababbbba -> bbbbaaab
bbbbaaab + bbb - ab -> bbbbaabbbb
bababbbbaaaaa + ab - bbbbaabbbb -> bababbbbaaaaaab
aaaaabbbaaaaa + aaaa - bbbbaabbbb -> aaaaabbbaaaaaaa
bbbaba + aab - abaabbabab -> bbbabaaab
bbbabaaab + ab - aaa -> bbbabaaabab
bbaaaaaaaab + a - bbbabaaab -> bbaaaaaaaaba
bbbabaaabab + bbbbaabbbb - abaabbabab -> bbbabaaababbbbaabbbb
bbaaaaaaaaba + baba - abaabbabab -> bbaaaaaaaabababa
bbaaaaaaaabababa + bbbaba - abab -> bbaaaaaaaabababbbba
aabb + aaaa - bbaaaaaaaabababa -> aabbbaaa
aabbbaaa + aaba - aaba -> aabbbaaaaaaba
aaaaa + aaab - aabbbaaa -> aaaaaaaab
aaaaaaaab + abaabba - aaba -> aaaaaaaabababaabba
aaaa + bbbabb - aaaaaaaab -> aaaabbbabb
aaaaa + bbbababbaa - aabbbaaaaaaba -> aaaaabbbababbaa
aaaabbbabb + aabb - aaaaabbbaaaa -> aaaabbbabbaabb
aaaabbbabb + aaaaabb - bbbabb -> aaaabbbabbaaaaabb
abaa + bbaaaaaaaaba - aaaabbbabb -> abaabbbaaaaaaaaba

New core

bababbbbaaaaaab -> aaaaaba
aaaaaba -> aaaaabababb
aaaaabababb -> abaabba->
abaabba-> bababbbba
bababbbba -> bababbbbaaaaa, bbbbaaab

bbbaaab -> bbbaaabbbb
bababbbaaaaa, bbbaaabbbb -> bababbbaaaaaab

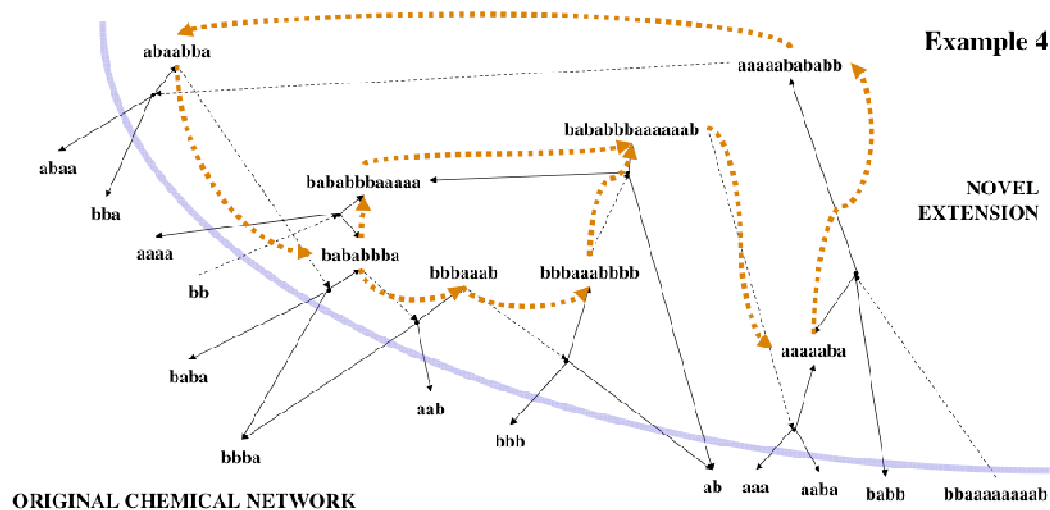
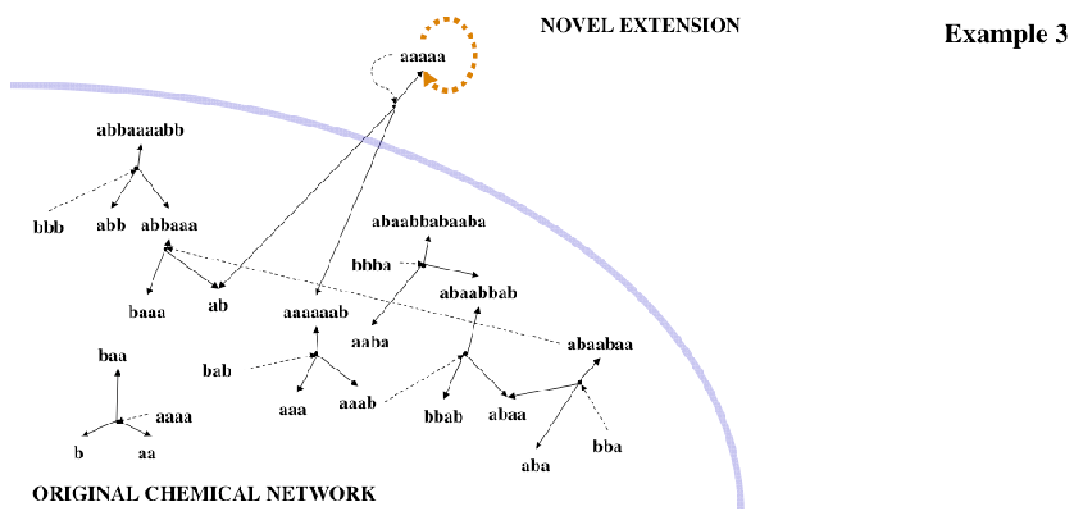
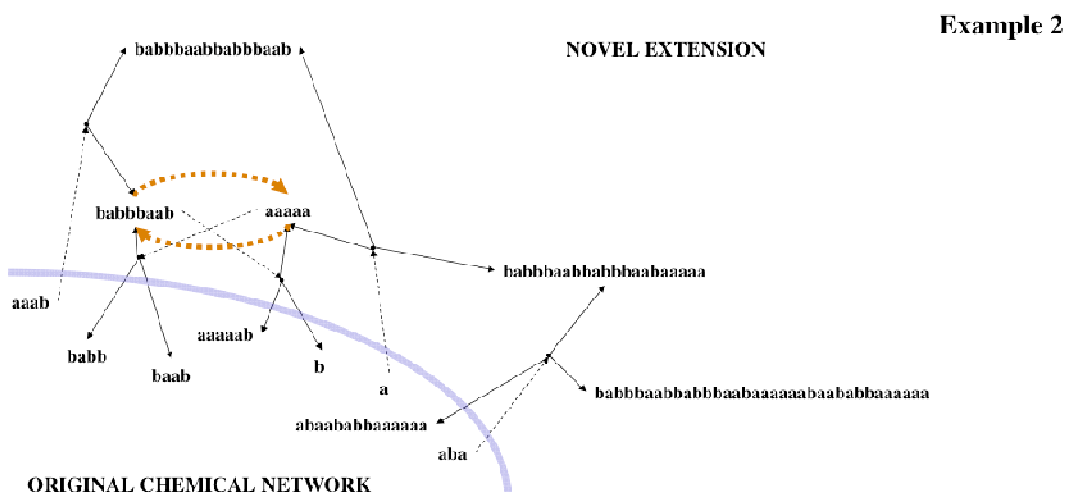


Figure S3. Examples of viable loop organization. Solid lines: reactions; dotted lines: catalytic activities. Orange dotted lines show the superimposed selfreproducing loop. The original network is not shown in detail.

D – Further results on evolvability

D1 Alternative attractors

The original Farmer-type networks of catalyzed cleavage and ligation reactions (generated with $P = 0.00022$) have only one attractor; i.e. are not capable of variation and cannot be a basis for pre-template based heredity. Networks with inhibition ($P = 0.00024$, $K = 0.005$) exhibit multiple attractors. In the five cases of networks in which the addition of spontaneous new species resulted in the ignition of a novel viable loop, there were multiple attractors in each case found after random shuffling of concentrations (Figure S4).

Networks with inhibition are only very rarely able to spontaneously transit between attractors, and even then they transit periodically or chaotically. On the other hand, spontaneous transitions occur between attractors of the networks that have non-food generated viable loops that were ignited by spontaneously produced shadow species. In both types of network the separate attractors are of different stabilities. Most of the time is spent in one attractor, with only occasional generations spent in the less stable attractor (Figure S5).

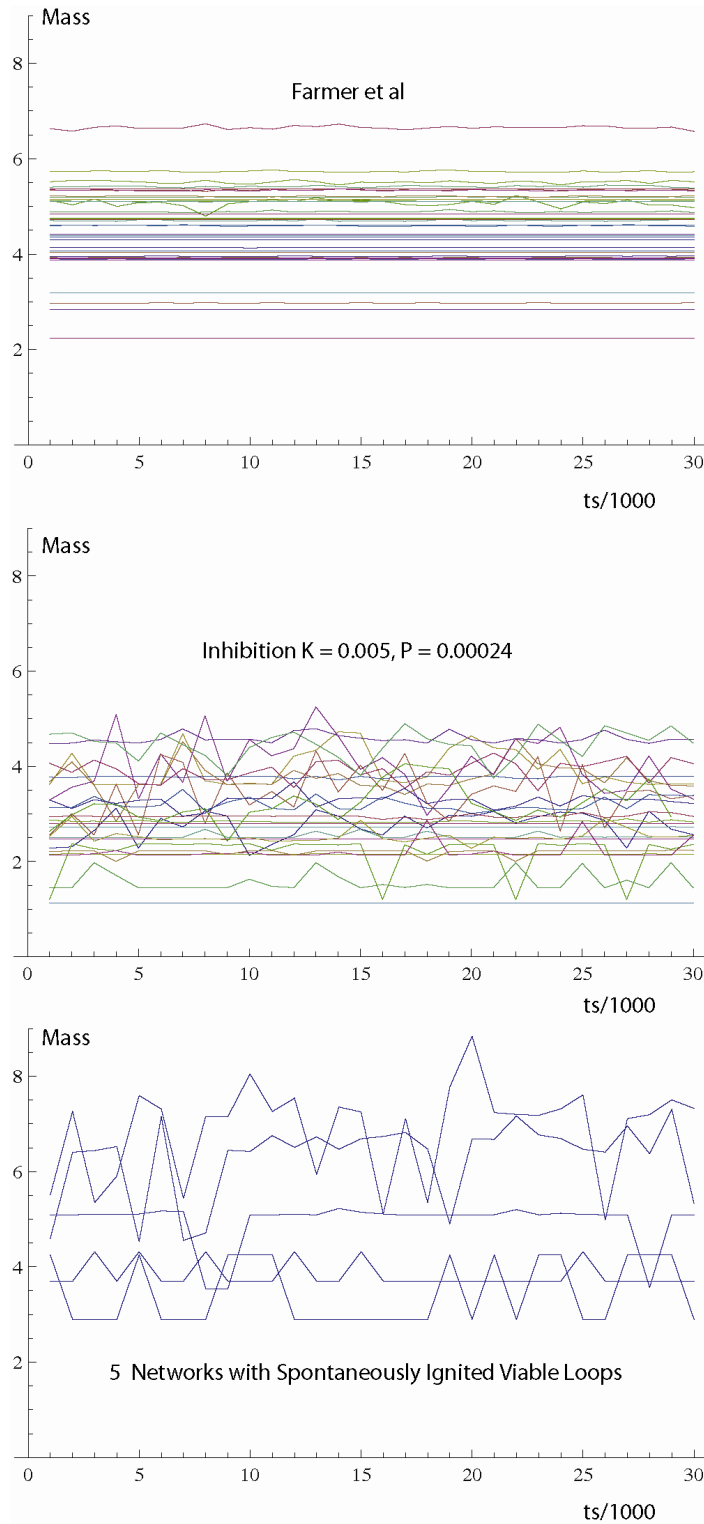


Figure S4. Searching for different attractors. Each line shows a different run where the concentrations of molecular species were shuffled every 1000 time steps for the same chemical network. (Top) In Farmer et al.'s [7] original model, there are no major changes in mass after shuffled concentrations. (Middle) However, where there is inhibition, shuffling results in a different mass 1000ts after shuffling, indicating the presence of a distinct attractor. (Bottom) In the five cases where a network was produced that had increased mass due to incorporation of a novel viable loops, there are always clearly distinct (typically two) attractors that the system settles into.

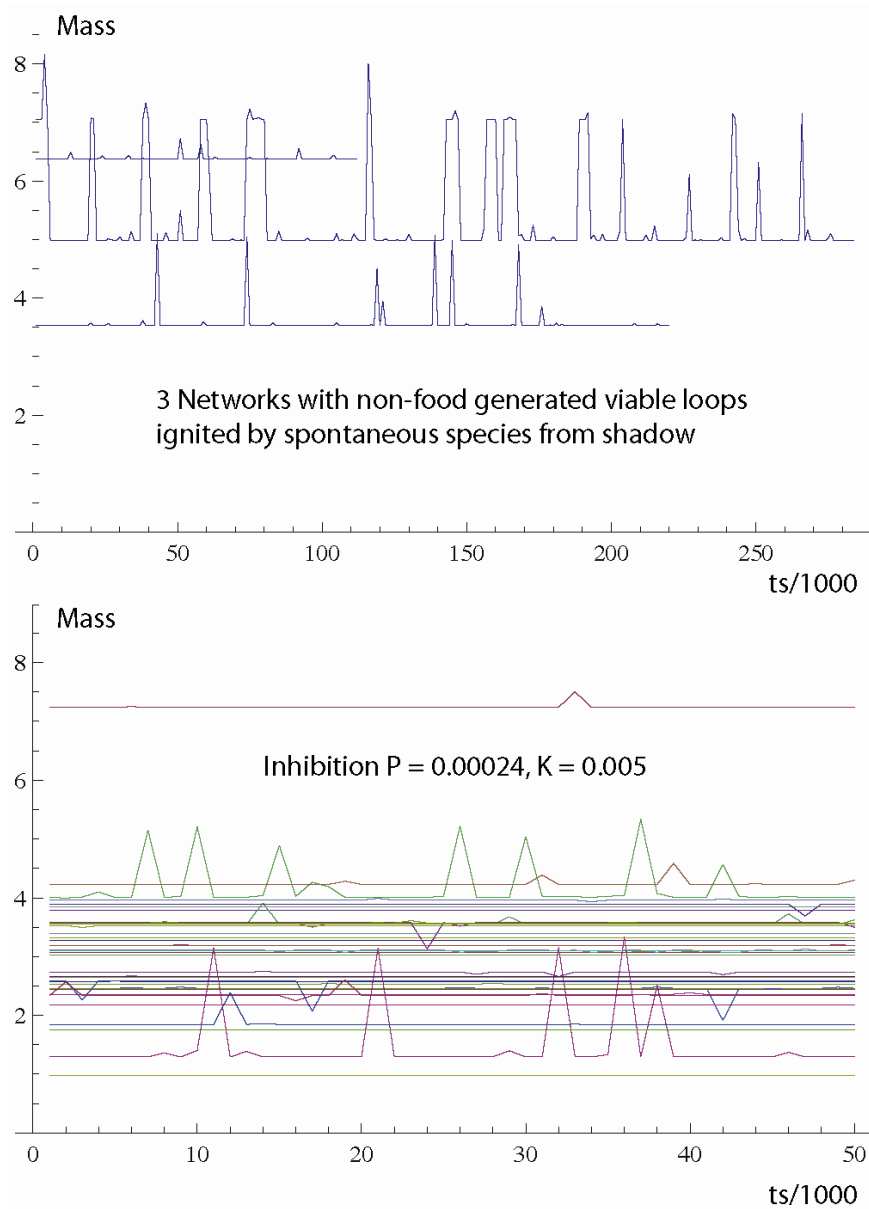


Figure S5. Spontaneous transitions between attractors. Growth and division of compartments result in transitions between existing attractors due to sampling noise during division and spontaneous reappearance of lost molecular species. (Top) Networks with non-food generated viable loops are typically able to transit between attractors. (Bottom) Networks with inhibition are only rarely able to spontaneously transit between attractors.

D2 Artificial selection

We applied artificial selection to a population of replicating individuals to test the selectability of alternative attractors. Networks with inhibition could not be stable selected (Figure S6). On the other hand, reaction networks that had obtained a novel viable loop ignited by a spontaneous appearance of a shadow species responded to selection. In the absence of selection the population fluctuated more or less randomly between the two attractors (Figure S7 bottom). However, when artificial selection was imposed the population moved in the expected direction; that is, towards the high mass attractor when total mass of non-food species was defined as the fitness criterion (Figure S7 top), or toward the low mass attractor when fitness was defined as the reciprocal of total mass of non-food species (Figure S7 middle).

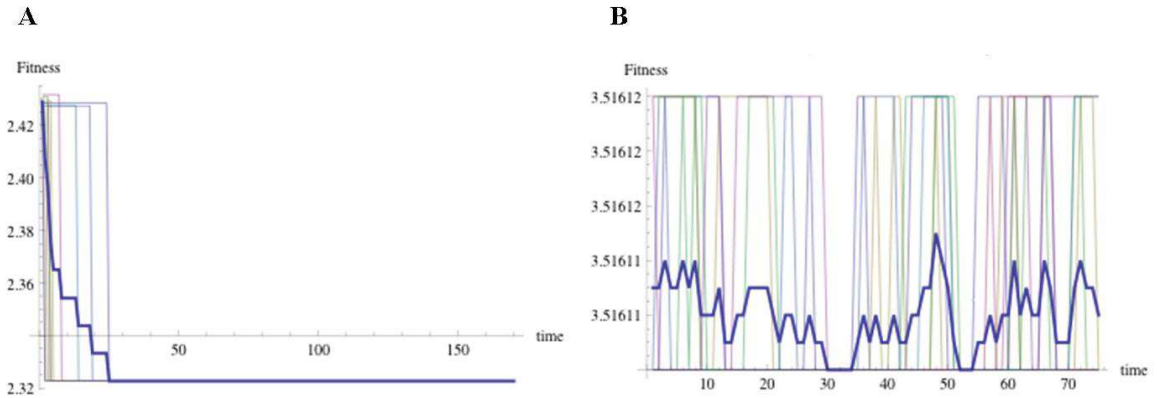


Figure S6. Artificial selection for increased non-food mass in networks with inhibition. Narrow lines: fitness (total non-food set mass) of each compartment; thick line: average fitness. The population typically settled down into one equilibrium (A) or fluctuated stochastically or periodically between attractors (B). In both cases selection had little effect. $K = 0.008$, $P = 0.1$, $P'' = 0.0032$, $M = 4$, $B = 2$.

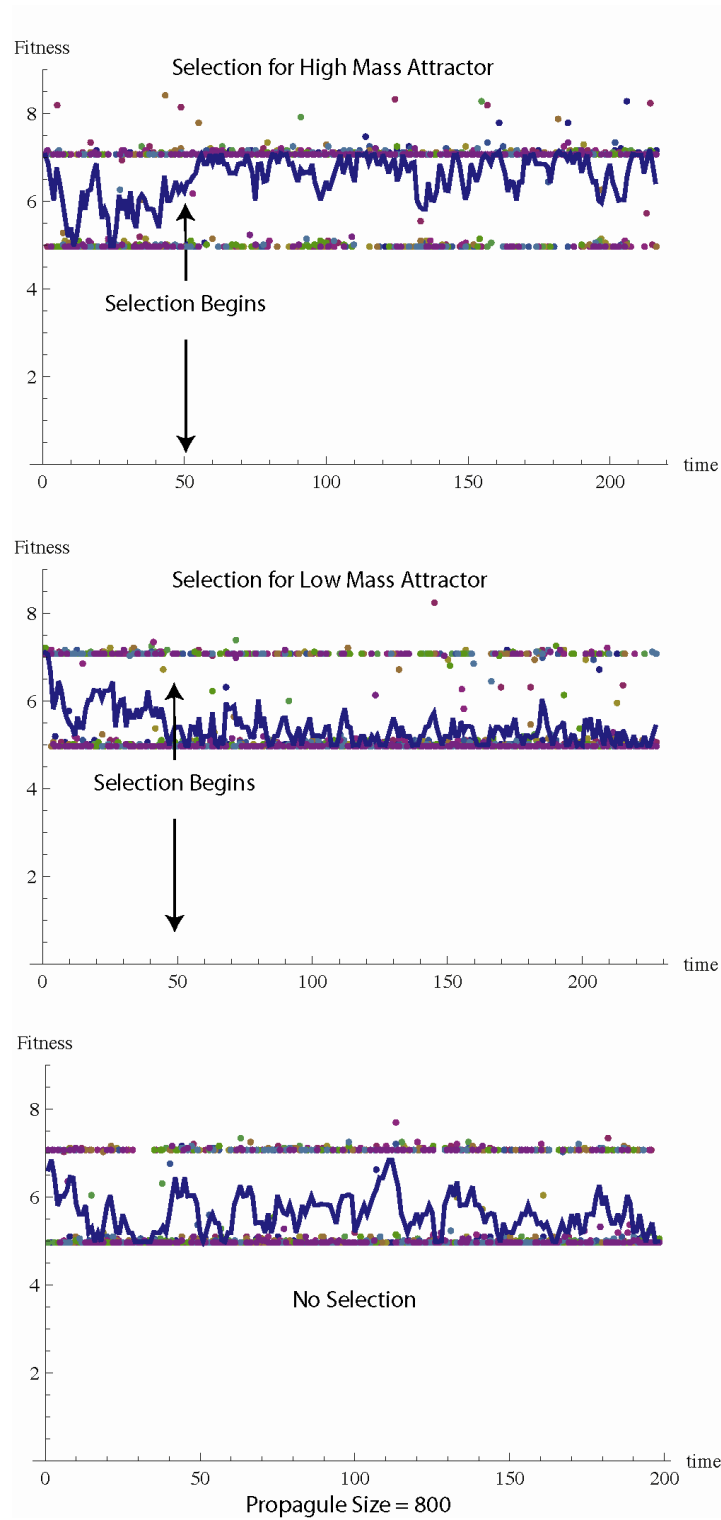


Figure S7. Artificial selection for increased and decreased non-food mass in networks containing a non-food generated viable loop. In both cases there was a response to selection and the population moved towards the high mass or the low mass attractor. Selection started after 50 generations. Dots: fitness (total non-food set mass) of each compartment; thick line: average fitness. (Top) Selection for high mass attractor. (Middle) Selection for low mass attractor. (Bottom) No selection throughout the entire run.

References

1. Krishna S (2003) Formation and destruction of autocatalytic sets in an evolving network model. Center for Theoretical Studies, Bangalore, Indian Institute of Science. PhD.
2. Kauffman SA (1986) Autocatalytic sets of proteins. *J Theor Biol* 119: 1–24.
3. Bagley RJ, Farmer JD, Fontana W (1992) Evolution of a metabolism. . In: Langton CG, Farmer JD, Rasmussen S, Taylor C, editors. *Artificial Life II: A Proceedings Volume in the Santa Fe Institute Studies in the Science of Complexity Vol 10*. Reading, Massachusetts: Addison-Wesley. pp. 141-158.
4. Hordijk W, Steel M (2004) Detecting autocatalytic, self-sustaining sets in chemical reaction systems. *J Theor Biol* 227: 451–461.
5. Jain S, Krishna S (1998) Autocatalytic Sets and the growth of complexity in an evolutionary model. *Phys Rev Lett* 81: 5684-5687.
6. Lifson S (1997) On the crucial stages in the origin of animate matter. *J Mol Evol* 44: 1-8.
7. Farmer JD, Kauffman SA, Packard NH (1986) Autocatalytic replication of polymers. *Physica D* 22: 50-67.
8. Siek JG, Lee L-Q, Lumsdaine A (2002) *The Boost Graph Library: User Guide and Reference Manual*. New York: Addison-Wesley Professional. 321 p.
9. Bäck T (1996) *Evolutionary Algorithms in Theory and Practice* Oxford: Oxford Univ Press. 314 p.