SUPPLEMENTARY MATERIAL

Autocatalytic networks in biology: structural theory and algorithms
Mike Steel, Wim Hordijk and Joana C. Xavier

1. Proof of Theorem 1

Proof. A key observation throughout is that in an elementary CRS $\mathcal{Q}$, any nonempty subset $\mathcal{R}'$ of reactions automatically satisfies the F–generated property, so $\mathcal{R}'$ forms a RAF for $\mathcal{Q}$ if and only if $\mathcal{R}'$ satisfies the reflexively autocatalytic (RA) property. By the manner in which $\mathcal{D}_{\mathcal{Q}}$ is constructed, the RA property means that the induced subgraph $\mathcal{D}_{\mathcal{Q}}|\mathcal{R}'$ has the property that each vertex has in-degree at least 1.

In particular, $\mathcal{R}$ has a RAF if and only if $\mathcal{D}_{\mathcal{Q}}$ has a directed cycle. The 'if' direction of this claim is clear. For the 'only if' direction, suppose that $\mathcal{R}'$ is a RAF and $r \in \mathcal{R}'$. By the assumption that each vertex in $\mathcal{D}_{\mathcal{Q}}$ has in-degree at least 1, there is a directed walk of length k (for any $k \geq 1$) involving vertices in $\mathcal{R}'$ and ending in r. Since $\mathcal{R}'$ is finite if we take $k > |\mathcal{R}'|$ then two vertices on this directed walk must coincide and the resulting sub-walk between this vertex to itself gives a directed cycle in $\mathcal{D}_{\mathcal{Q}}$. Moreover, $\mathcal{D}_{\mathcal{Q}}|\mathcal{R}'$ contains a directed cycle if and only if this sub-digraph contains a chordless cycle (again, the 'if' direction is clear and the 'only if' direction follows by the finiteness of $\mathcal{R}$, so shortening each directed cycle by following a chord leads to a sequence of cycles of decreasing length that eventually terminates on a chordless cycle). This establishes Part (i).

For Part (ii), a subset $\mathcal{R}'$ of $\mathcal{R}$ has the property that $\mathcal{D}_{\mathcal{Q}}|\mathcal{R}'$ is a chordless cycle, which implies (by Part (i)) that $\mathcal{R}'$ is a RAF. It is also an irrRAF; otherwise, the cycle would have a chord. Conversely, if $\mathcal{R}'$ has the property that $\mathcal{D}_{\mathcal{Q}}|\mathcal{R}'$ is not a chordless cycle, then either $\mathcal{D}_{\mathcal{Q}}$ does not contain a cycle (in which case it is not a RAF) or it contains a cycle which either has a chord or has other vertices reachable from it, in which case $\mathcal{R}'$ is not an irrRAF. This establishes the first sentence of Part (ii). The arguments for the second and third sentences follow similar lines.

For Part (iii), it is clear that the union of one or more cores is a RAF; however, the resulting set of reactions is closed if and only if all reactions that are reachable from that set are also included.

For Part (iv), suppose that a core c' is reachable from another core c (by definition, c is not reachable from c'). Any closed RAF $\mathcal{R}'$ that contains both c and c' is thus not minimal, since we could delete c' and all the reactions that are reachable from c' but not from c and obtain a strict subset of $\mathcal{R}'$ that is also a closed RAF. On the other hand, if $\mathcal{R}'$ has the property described in Part (iv), then it is a closed RAF by Part (iii) and it is also minimal, since any closed RAF must contain at least one core, alongside all the reactions that are reachable from it.

Part (v) follows from Part (iv), since each minimal closed RAF is associated with exactly one core, and since cores are strongly connected components of $\mathcal{D}_{\mathcal{Q}}$ these cores are vertex-disjoint (i.e. two cores share no reaction). Consequently, the number of cores is bounded above by the number of reactions in the maxRAF of $\mathcal{D}_{\mathcal{Q}}$. Moreover, finding the strongly connected components of any digraph can be done in polynomial time in the size of the digraph [3]. Each of these strongly connected components can then be tested in polynomial time to determine if it is a core; if so, one can then determine in polynomial time which other vertices are reachable from it. Thus the minimal closed RAFs can be listed in polynomial time in the size of $\mathcal{Q}$.

Part (vi) follows from Part (v) since $\mathcal{Q}$ contains a closed subRAF if and only if it contains a minimal closed subRAF.

□

2. Proof of Proposition 1

Proof. Consider the probability p_r that a single reaction r has an arc to itself (such an event is sufficient but not necessary for $\mathcal{D}_{\mathcal{Q}}$ to contain a directed cycle). If r produces $m \geq 1$ products, we have $p_r = 1-(1-p)^m \geq pm \geq p = \lambda/|\mathcal{R}|$. The probability that no reaction has an arc to itself is therefore $\left(1-\frac{\lambda}{|\mathcal{R}|}\right)^{|\mathcal{R}|}$. Since $(1-x/n)^n \sim e^{-x}$, we obtain the result claimed.

□

3. Proof of Proposition 2

Proof. Part (i) follows from Part (i) of Theorem 1, combined with the fact that the adjacency matrix A of an acyclic directed graph is nilpotent (i.e. specifically, A^{l+1} is the all-zero matrix when l is the length of a longest path in the directed graph) and thus all the eigenvalues of A are equal to zero [1]. For Part (ii), if $\mathcal{Q}$ contains a RAF, then $\mathcal{D}_{\mathcal{Q}}$ has a minimal (chordless) directed cycle (which could just be a loop on a vertex). Let w be the vector that has value 1 for each vertex in this minimal directed cycle and is zero otherwise. Then w is both a left and right eigenvector for A_Q with eigenvalue 1. For Part (iii), let $\mathcal{R}' = \{r \in \mathcal{R} : w_r > 0\}$. The condition $wA_Q = \lambda w$ translates as $\sum_{r\in\mathcal{R}} w_r A_{rr'} = \lambda w_{r'}$. Since the right-hand side is non-zero for each reaction $r' \in \mathcal{R}'$, it follows that $w_r A_{rr'} \neq 0$ for at least one reaction $r \in \mathcal{R}'$; In other words, each reaction is $\mathcal{R}'$ is catalysed by the product of at least one reaction in $\mathcal{R}'$. Since $\mathcal{Q}$ is elementary, this implies that $\mathcal{R}'$ is a RAF.

Notice that Part (iii) of Proposition 2 does not hold if left eigenvectors are substituted for right ones. A counterexample is given by the elementary CRS for which $A_{\mathcal{Q}}$ is the 2×2 matrix with both rows equal to $[0,1]$; in this case, $A_{\mathcal{Q}}$ has a principal eigenvalue of $+1$ but the associated right eigenvector is a column vector with strictly positive entries, but this does not correspond to a RAF for $\mathcal{Q}$.

□

4. Proof of Lemma 1

Proof. Part (i) follows by definition, since each set in the generating sequence is the closure of a subRAF of $\mathcal{Q}$ and is therefore a closed RAF for $\mathcal{Q}$, and a genRAF is the final set in its generating sequence.

Part (ii): For each reaction $r \in \mathcal{R}$, let $\rho(r)$ denote the set of reactants of r. We prove Part(ii) by induction on k. For $k = 2$, suppose that $r \in \mathcal{R}_1$. Then $\rho(r) \subseteq F$ and there exists some molecule type $x \in F \cup \pi(\mathcal{R}_1)$ that catalyses r. Since $\mathcal{R}_2$ is a closed RAF, and since the reactants and at least one catalyst (namely x) are available in the enlarged food set for $\mathcal{R}_2$, namely $Y_2 = F \cup \pi(\mathcal{R}_1)$, then $r \in \mathcal{R}_2$. Thus Part (ii) holds for $k = 2$. Suppose now that Part (ii) holds for $k = m$ and that $\mathcal{R}_1, \mathcal{R}_2, \ldots, \mathcal{R}_{m+1}$ is a generating sequence for $\mathcal{Q}$. We need to show that $\mathcal{R}_m \subseteq \mathcal{R}_{m+1}$. To this end, suppose that $r \in \mathcal{R}_m$. Then $\rho(r) \subseteq \pi(\mathcal{R}_{m-1})$ and there exists a molecule type $x \in F \cup \pi(R_m)$ that catalyses r. Now $\mathcal{R}_{m-1} \subseteq \mathcal{R}_m$ by induction and so $\rho(r) \subseteq \pi(\mathcal{R}_m)$. Thus the reactants and at least one catalyst of r are in $Y_{m+1} = F \cup \pi(R_m)$, and so, by the closure property, $r \in \mathcal{R}_{m+1}$. This establishes the induction step, and thereby Part (ii).

□

5. Proof of Theorem 2

Proof. For the first claim in Part (i), if $\mathcal{R}_1 = \emptyset$ then $\mathcal{Q}|F$ contains no RAF and so $\mathcal{Q}$ has no genRAF. Suppose that $\mathcal{R}_1 \neq \emptyset$. Then $\overline{\mathcal{R}}(\mathcal{Q})$ is a genRAF for $\mathcal{Q}$ since it has the generating sequence $\overline{\mathcal{R}}_i$ $(i \geq 1)$ (noting that $\overline{\mathcal{R}}_i$ is the closure in $\mathcal{Q}$ of $\mathcal{R}_i$ which is the maxRAF (and so a RAF) for $\mathcal{Q}|F$ when $i = 1$, and for $\mathcal{Q}|(F \cup \pi(\overline{\mathcal{R}}_{i-1})$, when $i > 1$). For the second claim in Part (i), suppose that $\mathcal{R}'$ is a genRAF for $\mathcal{Q}$; will show that $\mathcal{R}' \subseteq \overline{\mathcal{R}}(\mathcal{Q})$. Let $(\mathcal{R}'_i, i \geq 1)$ be a generating sequence for $\mathcal{R}'$. We show by induction on i that $\mathcal{R}'_i \subseteq \overline{\mathcal{R}}_i$ for all $i > 1$. The base case $i = 1$ holds since $\mathcal{R}_1$ is the maxRAF of $\mathcal{Q}|F$ which contains any other RAF of $\mathcal{Q}|F$, and so the closure of $\mathcal{R}_1$ in $\mathcal{Q}$, namely $\overline{\mathcal{R}}_1$ contains the closure in $\mathcal{Q}$ of any other RAF of $\mathcal{Q}|F$. Suppose the induction hypothesis holds for all values of i up to $j \geq 1$. Then $\mathcal{R}_{j+1}$ is the maxRAF of $\mathcal{Q}|(F \cup \pi(\overline{\mathcal{R}}_j))$ and so it contains any RAF of $\mathcal{Q}|(F \cup \pi(\mathcal{R}'_j))$ since $\mathcal{R}'_j \subseteq \overline{\mathcal{R}}_j$ (by the induction hypothesis) and so $F \cup \pi(\mathcal{R}'_j) \subseteq F \cup \pi(\overline{\mathcal{R}}_j)$. Consequently, the closure of $\mathcal{R}_{j+1}$ in $\mathcal{Q}$, namely, $\overline{\mathcal{R}}_{j+1}$, contains the closure in $\mathcal{Q}$ of any RAF of $\mathcal{Q}|(F \cup \pi(\mathcal{R}'_j))$. Thus the induction hypothesis holds, which establishes that $\mathcal{R}' \subseteq \overline{\mathcal{R}}(\mathcal{Q})$.

For Part (ii), observe that $\overline{\mathcal{R}}(\mathcal{Q}')$ is a genRAF for $\mathcal{Q}'$ (by Part (i)) and so if $\mathcal{R}' = \overline{\mathcal{R}}(\mathcal{Q}')$ then $\mathcal{R}'$ is a genRAF for $\mathcal{Q}'$. Since $\mathcal{R}'$ is a closed RAF for $\mathcal{Q}$, $\mathcal{R}'$ is also a genRAF for $\mathcal{Q}$ (since the closure in $\mathcal{Q}$ of any subset of reactions from $\mathcal{R}'$ is a subset of $\mathcal{R}'$). Conversely, suppose that $\mathcal{R}'$ is a genRAF for $\mathcal{Q}$. Then since $\mathcal{R}'$ is a closed RAF for $\mathcal{Q}$, $\mathcal{R}'$ is also a genRAF for $\mathcal{Q}'$. Now, $\overline{\mathcal{R}}(\mathcal{Q}') \subseteq \mathcal{R}'$, and since $\overline{\mathcal{R}}(\mathcal{Q}')$ contains any other genRAF for $\mathcal{Q}'$ (in particular, $\mathcal{R}'$) by Part (i), we have $\overline{\mathcal{R}}(\mathcal{Q}') = \mathcal{R}'$, as required.

For Part (iii), the proof of the claim (regarding the construction of $\overline{\mathcal{R}}(\mathcal{Q})$) follows from the fact that the maxRAF (of $\mathcal{Q}_i$), and its closure (in $\mathcal{Q}$) can be computed in polynomial time in the size of the CRS [2]. The the second claim then follows from Part (ii).

For Part (iv), consider the following algorithm. Given a closed RAF $\mathcal{R}'$ for $\mathcal{Q}$, let $\overline{\mathcal{R}}'_1, \overline{\mathcal{R}}'_2, \ldots$ be the generating sequence for $\overline{\mathcal{R}}(\mathcal{Q}')$ (described above, but with $\mathcal{R}$ replaced by $\mathcal{R}'$ and $\mathcal{Q}$ by $\mathcal{Q}' = (X, \mathcal{R}', C, F)$). From Part (ii) we have $\overline{\mathcal{R}}(\mathcal{Q}') = \mathcal{R}'$, and so $\overline{\mathcal{R}}'_1, \overline{\mathcal{R}}'_2, \ldots$ is a generating sequence for $\mathcal{R}'$.

Now, let $\mathcal{Q}'_1 = \mathcal{Q}'|F$ and for $i > 1$, let $\mathcal{Q}'_i = \mathcal{Q}|(F \cup \pi(\overline{\mathcal{R}}'_{i-1}))$. Notice that $\mathcal{Q}'_1$ is an elementary CRS, and for $i > 1$ we can regard $\mathcal{Q}'_i$ as an elementary RAF with enlarged food set $F \cup \pi(\overline{\mathcal{R}}'_{i-1})$. Thus we can apply Part (v) of Theorem 1, and in polynomial time in $|\mathcal{Q}|$ search all the minimal closed RAFs for $\mathcal{Q}_j$ and determine whether the closure in $\mathcal{Q}$ of any of these results in a strict subset (say $\mathcal{R}''$) of $\mathcal{R}'$. When such a set $\mathcal{R}''$ exists, its closure is clearly a closed RAF for $\mathcal{Q}$ that is a strict subset of $\mathcal{R}'$. However, if no such set $\mathcal{R}''$ is located then we claim that $\mathcal{R}'$ contains no closed RAF for $\mathcal{Q}$ as a strict subset. To see why, suppose that there is a closed RAF for $\mathcal{Q}$ that is strictly contained within $\mathcal{R}'$. In that case there exists a minimal closed RAF for $\mathcal{Q}$ that is strictly contained in $\mathcal{R}'$, and we denote such a minimal closed RAF as $\mathcal{R}_*$. Let j be the smallest value of i for which $\mathcal{R}_*$ is contained in $\overline{\mathcal{R}}'_i$ as a strict subset (this is well defined, since $\mathcal{R}_*$ is strictly contained in $\mathcal{R}'$). Then $\mathcal{R}_*$ is a closed RAF for $\mathcal{Q}_j$ also, and its closure in $\mathcal{Q}$ is a strict subset of $\mathcal{R}'$, so the closure in $\mathcal{Q}$ of any minimal closed RAF for $\mathcal{Q}_j$ that lies strictly within $\mathcal{R}_*$ would also be a strict subset of $\mathcal{R}'$.

□

6. Proof of Theorem 3

Proof. Let $\mathcal{L} = \{f(x,r) : (x,r) \in C\}$, and let $M = \max \mathcal{L}$. Consider the CRS $\mathcal{Q}' = (X', \mathcal{R}^*, C^*, F)$ obtained from $\mathcal{Q}$ by first deleting any uncatalysed reaction and then replacing each reaction r that is catalysed by (say) $k \geq 1$ molecule types with k distinct copies of this reaction $r_1, \ldots, r_k$, each of which is catalysed by a different one of the k molecule types. Thus each reaction r in $\mathcal{R}^*$ is catalysed by exactly one molecule type, which we will denote as $x(r)$. For the associated catalysis set $C^* = \{(x(r), r) : r \in \mathcal{R}^*\}$, let f' be the rate function induced by f (i.e. if $r \in \mathcal{R}$ is replaced by $r_1, \ldots, r_k \in \mathcal{R}^*$ then $f'(x(r_i), r_i) := f(x(r), r)$). For each $\ell \in \mathcal{L}$ let:

$$\mathcal{R}^*_\ell = \{r \in \mathcal{R}^* : f'(x(r), r) \geq \ell\}.$$

In other words, $\mathcal{R}^*_\ell$ is the set of catalyst-reaction pairs $(x(r), r)$ where the rate of reaction r when catalysed by the molecule type $x(r)$ is at least ℓ (as specified by the rate function f).

Now, let $\widetilde{\mathcal{R}}$ be the maxRAF of $\mathcal{R}^*_\ell$ for the largest value of $\ell \in \mathcal{L}$ for which maxRAF$(\mathcal{R}^*_\ell)$ is nonempty. This set is well-defined, since $\mathcal{R}^* = \mathcal{R}^*_\ell$ when $\ell = 0$, and because $\mathcal{R}$ (and thereby $\mathcal{R}^*$) is assumed to have a RAF. Notice that $\widetilde{\mathcal{R}}$ can be efficiently determined, by starting at $\ell = M$ and decreasing ℓ through the (at most $|\mathcal{L}| \leq |C|$) possible values it can take until a nonempty maxRAF first appears (alternatively, one could start at $\ell = 0$ and increase ℓ until the last value for which a nonempty maxRAF is present).

Claim: $\widetilde{\mathcal{R}}$ is a RAF that has the largest possible φ–value of any RAF for $\mathcal{Q}'$, and $\widetilde{\mathcal{R}}$ contains any other RAF for $\mathcal{Q}'$ with this maximal φ–value.

To establish this claim, suppose that $\widetilde{\mathcal{R}} = $ maxRAF$(\mathcal{R}_\ell)$ for $\ell = t$ and that maxRAF$(\mathcal{R}_\ell) = \emptyset$ for $\ell > t$ (i.e. t is the largest value of ℓ in $\mathcal{L}$ for which $\mathcal{R}_\ell$ has a (nonempty) maxRAF). For each reaction r in $\widetilde{\mathcal{R}}$, we then have $f'(x(r), r) \geq t$, and for at least one reaction r in $\widetilde{\mathcal{R}}$, $f'(x(r), r) = t$ (otherwise, a larger value of ℓ would support a maxRAF). It follows that $\varphi(\widetilde{\mathcal{R}}) = t$. Now if $\mathcal{R}'$ is any other RAF for $\mathcal{Q}'$, let t' be the minimal value of $f'(x(r), r)$ over all choices of $r \in \mathcal{R}'$. Then $t' \leq t$ otherwise, $\mathcal{R}_\ell$ would have a nonempty maxRAF for a value $\ell = t'$ that is greater than t, contradicting the maximality of t. Thus $\mathcal{R}' \subseteq \mathcal{R}_t^*$ and so

$$\mathcal{R}' = \text{maxRAF}(\mathcal{R}') \subseteq \text{maxRAF}(\mathcal{R}_t^*) = \widetilde{\mathcal{R}},$$

which shows that $\widetilde{\mathcal{R}}$ contains any other RAF with this maximal value.

This establishes the above Claim, and thereby Theorem 3 for $\mathcal{Q}'$. However, the subset of reactions of $\mathcal{R}$ whose copies are present in $\widetilde{\mathcal{R}}$ provides a RAF for $\mathcal{Q}$ that has the largest possible φ–value (namely t) and which contains any other RAF for $\mathcal{Q}$ with this φ–value.

□

7. An example to show that the number of minimal closed RAFs in a (general, nonelementary) CRS is not bounded polynomially in the size of $\mathcal{Q}$.

Consider the CRS $\mathcal{Q}_k := (X, \mathcal{R}, C, F)$ where

$$X = \{f, x, \theta\} \cup \{x_1, x_1', \ldots, x_k, x_k'\} \cup \{\theta_1, \ldots, \theta_k\}, F = \{f\},$$

and for $[k] = \{1, 2, \ldots, k\}$, the reaction set is:

$$\mathcal{R} = \{r_x, r_\theta\} \cup \{r_i : i \in [k]\} \cup \{r_i', i \in [k]\} \cup \{\overline{r_i} : i \in [k]\} \cup \{\overline{r_i'} : i \in [k]\},$$

where these reactions are described as follows (with catalysts indicated above the arrows):

$$r_x : f \xrightarrow{\theta} x,$$

$$r_\theta : \theta_1 + \theta_2 + \cdots + \theta_k \xrightarrow{\theta} \theta,$$

and for all $i \in [k]$:

$$r_i : x \xrightarrow{x_i} x_i, \ r_i' : x \xrightarrow{x_i'} x_i',$$

$$\overline{r_i} : x_i \xrightarrow{\theta_i} \theta_i, \ \overline{r_i'} : x_i' \xrightarrow{\theta_i} \theta_i.$$

Thus, $\mathcal{Q}_k$ has a food set of size 1, a reaction set of size $4k+2$, and $3k+3$ molecule types. Fig. 1 provides a graphical representation of $\mathcal{Q}_3$.

Proposition 1. *The minimal closed RAFs of $\mathcal{Q}_k$ coincide with the irrRAFs for $\mathcal{Q}_k$, and there are 2^k of them. More precisely, $\mathcal{R}'$ is a minimal closed RAF of $\mathcal{Q}_k$ if and only if $\mathcal{R}'$ contains r_x and r_θ and for each $i \in [k]$, $\mathcal{R}'$ contains either (i) r_i and $\overline{r_i}$ but neither r_i' nor $\overline{r_i'}$, or (ii) r_i' and $\overline{r_i'}$ but neither r_i nor $\overline{r_i}$.*

Proof. The 'if' direction in the second sentence is clear, since any such $\mathcal{R}'$ is easily seen to be a closed subRAF, as well as being an irrRAF, and thus is a minimal closed RAF. For the 'only if' direction, a subset $\mathcal{R}'$ of $\mathcal{R}$ is a RAF of $\mathcal{Q}_k$ precisely if the following two properties hold: (a) $\mathcal{R}'$ contains r_x and r_θ, and (b) for each i, $\mathcal{R}'$ contains either r_i and $\overline{r_i}$ or r_i' and $\overline{r_i'}$ (in order to

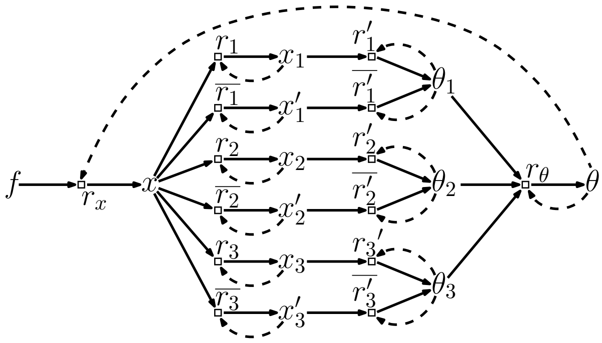

Figure 1. The CRS $\mathcal{Q}_3$.

generate θ_i, which is required by r_θ). Unless $\mathcal{R}'$ satisfies the stronger condition (i) or (ii) (for each $i \in [k]$) listed in the statement of Proposition 1, $\mathcal{R}'$ is not minimal.

$\square$

References

[1] D. M. Cvetković, M. Doob, and H. Sachs, *Spectra of Graphs, third ed.*, Barth, Heidelberg, 1995.

[2] W. Hordijk and M. Steel, *Detecting autocatalyctic, self-sustaining sets in chemical reaction systems*, J. Theor. Biol., 227 (2004), pp. 451–461.

[3] R. E. Tarjan, *Depth-first search and linear graph algorithms*, SIAM J. Comput., 1 (1972), pp. 146–160.