SUPPORTING INFORMATION

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Abstract

This note provides supporting information for *"Complex-linear invariants of biochemical networks"*. It should be read in conjunction with the paper, which provides further details. The material is organised in the order in which it appears in the paper but the table of contents provides more information.

Contents

1 Preamble

The symbolic linear algebra calculations that follow may be readily undertaken in any computer algebra system. We used Mathematica, for which we wrote custom functions that compute the relevant matrices automatically from a description of the reaction network.

2 Invariants that are not of type 1

Consider the hypothetical reaction network in Figure 1A. While such chemistry is unlikely, it illustrates the mathematical issues. The network has three species, S_1, S_2, S_3 and nine complexes $C_1, \cdots, C_9$, ordered as in Figure 1C. The ODEs are

$$\begin{aligned} \frac{dx_1}{dt} &= k_1 x_1 \\ \frac{dx_2}{dt} &= k_4 x_1 x_3 - k_2 x_2^2 \\ \frac{dx_3}{dt} &= k_5 x_1 x_2 - k_3 x_3^2 \,. \end{aligned} \tag{1}$$

With the given ordering, the matrix $M = Y.\mathcal{L}(G)$ is

$$\begin{pmatrix} k_1 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\ 0 & 0 & -k_2 & 0 & 0 & 0 & k_4 & 0 & 0 \\ 0 & 0 & 0 & 0 & -k_3 & 0 & 0 & k_5 & 0 \end{pmatrix}.$$

Focussing on the complexes C_1, C_3, C_5, C_7, C_8, Paper Proposition 1 shows that the space of type 1 complex-linear invariants has dimension three. However, it is easy to see from (1) that the only steady state of the network is when $x_1 = x_2 = x_3 = 0$. Hence, for any values of $a, b, c, d, e \in \mathbb{R}$, the polynomial expression

$$ax^{C_1} + bx^{C_3} + cx^{C_5} + dx^{C_7} + ex^{C_8}$$

always vanishes in any steady state. Hence, the space of complex-linear invariants on C_1, C_3, C_5, C_7, C_8 has dimension five.

3 Corollary 1

The nine species and thirteen complexes in the EnvZ/OmpR network in Paper Figure 1 are ordered as follows.

S_1	EnvZ-P-OmpR	C_1	S_8	EnvZ-ADP
S_2	EnvZ-ATP-OmpR-P	C_2	S_4	EnvZ
S_3	EnvZ-ADP-OmpR-P	C_3	S_7	EnvZ-ATP
S_4	EnvZ	C_4	S_9	EnvZ-P
S_5	OmpR	C_5	$S_9 + S_5$	EnvZ-P + OmpR
S_6	OmpR-P	C_6	S_1	EnvZ-P-OmpR
S_7	EnvZ-ATP	C_7	$S_4 + S_6$	EnvZ + OmpR-P
S_8	EnvZ-ADP	C_8	$S_7 + S_6$	EnvZ-ATP + OmpR-P
S_9	EnvZ-P	C_9	S_2	EnvZ-ATP-OmpR-P
		C_{10}	$S_7 + S_5$	EnvZ-ATP + OmpR
		C_{11}	$S_8 + S_6$	EnvZ-ADP + OmpR-P
		C_{12}	S_3	EnvZ-ADP-OmpR-P
		C_{13}	$S_8 + S_5$	EnvZ-ADP + OmpR

With this ordering and with the rate constants as in Paper Figure 1C, the matrix $M = Y.\mathcal{L}(G)$ is

$$\begin{pmatrix} 0 & 0 & 0 & 0 & k_6 & -k_7-k_8 & k_9 & 0 & 0 & 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & 0 & 0 & 0 & k_{10} & -k_{11}-k_{12} & 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & k_{13} & -k_{14}-k_{15} & 0 \\ k_1 & -k_2-k_3 & k_4 & 0 & 0 & k_8 & -k_9 & 0 & 0 & 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & -k_6 & k_7 & 0 & 0 & k_{12} & 0 & 0 & k_{15} & 0 \\ 0 & 0 & 0 & 0 & 0 & k_8 & -k_9 & -k_{10} & k_{11} & 0 & -k_{13} & k_{14} & 0 \\ 0 & k_3 & -k_4-k_5 & 0 & 0 & 0 & 0 & -k_{10} & k_{11}+k_{12} & 0 & 0 & 0 & 0 \\ -k_1 & k_2 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & -k_{13} & k_{14}+k_{15} & 0 \\ 0 & 0 & k_5 & 0 & -k_6 & k_7 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \end{pmatrix}$$

A basis for the kernel of M can then be calculated to make up the columns of a matrix B.

$$\begin{pmatrix} 0 & \frac{k_2(k_4+k_5)k_{15}}{k_1k_3k_5} & 0 & \frac{k_2(k_4+k_5)k_{12}}{k_1k_3k_5} & 0 & 0 \\ 0 & \frac{(k_4+k_5)k_{15}}{k_3k_5} & 0 & \frac{(k_4+k_5)k_{12}}{k_3k_5} & 0 & 0 \\ 0 & \frac{k_{15}}{k_5} & 0 & \frac{k_{12}}{k_5} & 0 & 0 \\ 0 & 0 & 0 & 0 & 0 & 1 \\ 0 & \frac{(k_7+k_8)k_{15}}{k_6k_8} & 0 & \frac{(k_7+k_8)k_{12}}{k_6k_8} & \frac{k_7k_9}{k_6k_8} & 0 \\ 0 & \frac{k_{15}}{k_8} & 0 & \frac{k_{12}}{k_8} & \frac{k_9}{k_8} & 0 \\ 0 & 0 & 0 & 0 & 1 & 0 \\ 0 & 0 & 0 & \frac{k_{11}+k_{12}}{k_{10}} & 0 & 0 \\ 0 & 0 & 0 & 1 & 0 & 0 \\ 0 & 0 & 1 & 0 & 0 & 0 \\ 0 & \frac{k_{14}+k_{15}}{k_{13}} & 0 & 0 & 0 & 0 \\ 0 & 1 & 0 & 0 & 0 & 0 \\ 1 & 0 & 0 & 0 & 0 & 0 \end{pmatrix}$$

Paper Corollary 1 focusses on the complexes C_1, C_3, C_8, C_{11}, so that $k = 4$. These are not the first four complexes in the ordering, as was assumed for convenience in the main paper. We can imagine that the columns of M and the rows of B have been permuted so that these complexes are now the first in the ordering but we will not bother to write out these new matrices. We note that columns 2 and 4 of B have non-zero entries in the relevant four rows, while the remaining columns have zero entries. We can undertake elementary column operations on B, as described in the paper (in fact, only interchange of columns is required), to bring B into lower-triangular block form. The resulting 4×2 sub-matrix, B', in Paper Equation 4, is then given by

$$\begin{pmatrix} \frac{k_2(k_4+k_5)k_{15}}{k_1k_3k_5} & \frac{k_2(k_4+k_5)k_{12}}{k_1k_3k_5} \\ \frac{k_{15}}{k_5} & \frac{k_{12}}{k_5} \\ 0 & \frac{k_{11}+k_{12}}{k_{10}} \\ \frac{k_{14}+k_{15}}{k_{13}} & 0 \end{pmatrix}$$

The columns of this are linearly independent, so that rk $B' = 2$. It follows from Paper Proposition 1 that the dimension of the space of type 1 complex-linear invariants on C_1, C_3, C_8, C_{11} is 2, as claimed. To generate the invariants, we note that rows 2 and 3 of B' are linearly independent, so that we can follow the prescription in the paper and take $U = \{2, 3\}$ and $V = \{1, 4\}$. Then

$$B'_U = \begin{pmatrix} \frac{k_{15}}{k_5} & \frac{k_{12}}{k_5} \\ 0 & \frac{k_{11}+k_{12}}{k_{10}} \end{pmatrix}, \qquad B'_V = \begin{pmatrix} \frac{k_2(k_4+k_5)k_{15}}{k_1k_3k_5} & \frac{k_2(k_4+k_5)k_{12}}{k_1k_3k_5} \\ \frac{k_{14}+k_{15}}{k_{13}} & 0 \end{pmatrix}$$

Since $\Psi(x)_U = (x^{C_3}, x^{C_8})^\dagger$ and $\Psi(x)_V = (x^{C_1}, x^{C_{11}})^\dagger$, the two linearly independent type 1 complex-linear invariants may be read off from Paper Equation 6,

$$\begin{pmatrix} x^{C_1} \\ x^{C_{11}} \end{pmatrix} = \begin{pmatrix} \frac{k_2(k_4+k_5)}{k_1k_3} & 0 \\ \frac{k_5(k_{14}+k_{15})}{k_{13}k_{15}} & -\frac{k_{10}k_{12}(k_{14}+k_{15})}{k_{13}k_{15}(k_{11}+k_{12})} \end{pmatrix} \begin{pmatrix} x^{C_3} \\ x^{C_8} \end{pmatrix}$$

to yield the expressions in Paper Corollary 1, as claimed.

4 Corollary 2

The eight species and fourteen complexes of the PFK-2/FBPase-2 network in Paper Figures 2 and 3 are ordered as follows.

S_1	F2,6BP	C_1	S_5	E
S_2	F6P	C_2	S_7	E-ATP
S_3	E-ATP-F6P	C_3	$S_7 + S_2$	E-ATP + F6P
S_4	E-ATP-F6P-F2,6BP	C_4	S_3	E-ATP-F6P
S_5	E	C_5	$S_5 + S_1$	E + F2,6BP
S_6	E-F2,6BP	C_6	S_6	E-F2,6BP
S_7	E-ATP	C_7	$S_5 + S_2$	E + F6P
S_8	E-ATP-F2,6BP	C_8	S_8	E-ATP-F2,6BP
		C_9	$S_7 + S_1$	E-ATP + F2,6BP
		C_{10}	$S_3 + S_1$	E-ATP-F6P + F2,6BP
		C_{11}	S_4	E-ATP-F6P-F2,6BP
		C_{12}	$S_8 + S_2$	E-ATP-F2,6BP + F6P
		C_{13}	$S_6 + S_1$	E-F2,6BP + F2,6BP
		C_{14}	$S_3 + S_2$	E-ATP-F6P + F6P

With this ordering and with the rate constants in Paper Figure 3A, the matrix $M = Y.\mathcal{L}(G)$ is

$$\begin{pmatrix} 0 & 0 & 0 & k_5 & -k_6 & k_7 & 0 & k_{13} & -k_{12} & -k_{14} & k_{15}+k_{18} & 0 & 0 & 0 \\ 0 & 0 & -k_3 & k_4 & 0 & k_8 & 0 & k_{11} & 0 & 0 & k_{17}+k_{19} & -k_{16} & 0 & 0 \\ 0 & 0 & k_3 & -k_4-k_5 & 0 & 0 & 0 & 0 & 0 & -k_{14} & k_{15}+k_{19} & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & k_{14} & -k_{15}-k_{17}-k_{18}-k_{19} & k_{16} & 0 & 0 \\ -k_1 & k_2 & 0 & k_5 & -k_6 & k_7+k_8 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & k_6 & -k_7-k_8-k_{10} & 0 & k_9 & 0 & 0 & k_{18} & 0 & 0 & 0 \\ k_1 & -k_2 & -k_3 & k_4 & 0 & 0 & 0 & k_{11}+k_{13} & -k_{12} & 0 & 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & 0 & k_{10} & 0 & -k_9-k_{11}-k_{13} & k_{12} & 0 & k_{17} & -k_{16} & 0 & 0 \end{pmatrix}$$

and a matrix B, whose columns form a basis for the kernel of M, can then be calculated as

$$\begin{pmatrix} 0 & 0 & \frac{k_8-k_{10}}{k_1k_{10}} & \frac{k_{19}}{k_1} & \frac{(-k_8+k_{10})k_{12}}{k_1k_{10}} & \frac{k_8k_9+k_{10}k_{11}}{k_1k_{10}} & 0 & \frac{k_2}{k_1} \\ 0 & 0 & 0 & 0 & 0 & 0 & 0 & 1 \\ 0 & 0 & \frac{-k_5k_{10}+(k_4+k_5)k_8}{k_3k_5k_{10}} & \frac{-k_4k_{18}+(k_4+k_5)k_{19}}{k_3k_5} & -\frac{(k_4+k_5)k_8k_{12}}{k_3k_5k_{10}} & \frac{(k_4+k_5)(k_8k_9+k_{10}k_{11})}{k_3k_5k_{10}} & 0 & 0 \\ 0 & 0 & \frac{k_8}{k_5k_{10}} & \frac{-k_{18}+k_{19}}{k_5} & -\frac{k_8k_{12}}{k_5k_{10}} & \frac{k_8k_9+k_{10}k_{11}}{k_5k_{10}} & 0 & 0 \\ 0 & 0 & \frac{k_7+k_8+k_{10}}{k_6k_{10}} & -\frac{k_{18}}{k_6} & -\frac{(k_7+k_8+k_{10})k_{12}}{k_6k_{10}} & \frac{(k_7+k_8)k_9}{k_6k_{10}} & 0 & 0 \\ 0 & 0 & \frac{1}{k_{10}} & 0 & -\frac{k_{12}}{k_{10}} & \frac{k_9}{k_{10}} & 0 & 0 \\ 0 & 0 & 0 & 0 & 0 & 0 & 1 & 0 \\ 0 & 0 & 0 & 0 & 0 & 1 & 0 & 0 \\ 0 & 0 & 0 & 0 & 1 & \frac{k_{11}+k_{13}}{k_{12}} & 0 & 0 \\ 0 & 0 & -\frac{1}{k_{14}} & \frac{k_{15}+k_{18}+k_{19}}{k_{14}} & 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 1 & 0 & 0 & 0 & 0 \\ 0 & 0 & \frac{1}{k_{16}} & \frac{k_{17}}{k_{16}} & 0 & 0 & 0 & 0 \\ 0 & 1 & 0 & 0 & 0 & 0 & 0 & 0 \\ 1 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \end{pmatrix}$$

Paper Corollary 2 focusses on the complexes $C_1, C_2, C_4, C_6, C_8, C_{11}$, so that $k = 6$. As before, we can imagine that the columns of M and the rows of B have been permuted to make these complexes first in the ordering. Only columns $3, 4, 5, 6, 8$ of B have non-zero entries in the relevant rows and, when restricted to these rows, column 5 is a scalar multiple of column 3. As before, we can interchange columns to bring B into lower-triangular block form, with the resulting 6×4 sub-matrix, B', in Paper Equation 4 given by

$$\begin{pmatrix} \frac{k_8-k_{10}}{k_1k_{10}} & \frac{k_{19}}{k_1} & \frac{k_8k_9+k_{10}k_{11}}{k_1k_{10}} & \frac{k_2}{k_1} \\ 0 & 0 & 0 & 1 \\ \frac{k_8}{k_5k_{10}} & \frac{-k_{18}+k_{19}}{k_5} & \frac{k_8k_9+k_{10}k_{11}}{k_5k_{10}} & 0 \\ \frac{1}{k_{10}} & 0 & \frac{k_9}{k_{10}} & 0 \\ 0 & 0 & 1 & 0 \\ 0 & 1 & 0 & 0 \end{pmatrix}$$

with B' evidently of full rank 4. It follows from Paper Proposition 1 that the space of type 1 complex-linear invariants on $C_1, C_2, C_4, C_6, C_8, C_{11}$ has dimension two, as claimed. To generate the invariants, we can take $U = \{2, 4, 5, 6\}$ and $V = \{1, 3\}$ so that

$$B'_U = \begin{pmatrix} 0 & 0 & 0 & 1 \\ \frac{1}{k_{10}} & 0 & \frac{k_9}{k_{10}} & 0 \\ 0 & 0 & 1 & 0 \\ 0 & 1 & 0 & 0 \end{pmatrix} , \quad B'_V = \begin{pmatrix} \frac{k_8-k_{10}}{k_1k_{10}} & \frac{k_{19}}{k_1} & \frac{k_8k_9+k_{10}k_{11}}{k_1k_{10}} & \frac{k_2}{k_1} \\ \frac{k_8}{k_5k_{10}} & \frac{-k_{18}+k_{19}}{k_5} & \frac{k_8k_9+k_{10}k_{11}}{k_5k_{10}} & 0 \end{pmatrix}$$

B'_U is evidently non-singular. The two linear-independent type 1 complex-linear invariants can then be read off from Paper Equation 6,

$$\begin{pmatrix} x^{C_1} \\ x^{C_4} \end{pmatrix} = \begin{pmatrix} \frac{k_2}{k_1} & \frac{(k_8-k_{10})}{k_1} & \frac{(k_9+k_{11})}{k_1} & \frac{k_{19}}{k_1} \\ 0 & \frac{k_8}{k_5} & \frac{k_{11}}{k_5} & \frac{(-k_{18}+k_{19})}{k_5} \end{pmatrix} \begin{pmatrix} x^{C_2} \\ x^{C_6} \\ x^{C_8} \\ x^{C_{11}} \end{pmatrix}$$

to yield the expressions in Paper Corollary 2, as claimed.

5 Example of the systematic procedure

The example in §2.6 of the Paper is a modification of that in Corollary 1. As shown in Paper Figure 5 it has nine species and fifteen complexes which are ordered as follows.

S_1	EnvZ-P-OmpR	C_1	S_8	EnvZ-ADP
S_2	EnvZ-ATP-OmpR-P	C_2	S_4	EnvZ
S_3	EnvZ-ADP-OmpR-P	C_3	S_7	EnvZ-ATP
S_4	EnvZ	C_4	S_9	EnvZ-P
S_5	OmpR	C_5	$S_9 + S_5$	EnvZ-P $+$ OmpR
S_6	OmpR-P	C_6	S_1	EnvZ-P-OmpR
S_7	EnvZ-ATP	C_7	$S_4 + S_6$	EnvZ $+$ OmpR-P
S_8	EnvZ-ADP	C_8	$S_7 + S_6$	EnvZ-ATP $+$ OmpR-P
S_9	EnvZ-P	C_9	S_2	EnvZ-ATP-OmpR-P
		C_{10}	$S_7 + S_5$	EnvZ-ATP $+$ OmpR
		C_{11}	$S_8 + S_6$	EnvZ-ADP $+$ OmpR-P
		C_{12}	S_3	EnvZ-ADP-OmpR-P
		C_{13}	$S_8 + S_5$	EnvZ-ADP $+$ OmpR
		C_{14}	S_6	OmpR-P
		C_{15}	S_5	OmpR

With this ordering and with the rate constants shown in Paper Figure 5A, the matrix M extends that for Corollary 1 with a 9×2 block on the right:

$$\begin{pmatrix}
0 & 0 & 0 & 0 & k_6 & -k_7-k_8 & k_9 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\
0 & 0 & 0 & 0 & 0 & 0 & 0 & k_{10} & -k_{11}-k_{12} & 0 & 0 & 0 & 0 & 0 & 0 \\
0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & k_{13} & -k_{14}-k_{15} & 0 & 0 & 0 \\
k_1 & -k_2-k_3 & k_4 & 0 & 0 & k_8 & -k_9 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\
0 & 0 & 0 & 0 & -k_6 & k_7 & 0 & 0 & k_{12} & 0 & 0 & k_{15} & 0 & k_{16} & 0 \\
0 & 0 & 0 & 0 & 0 & k_8 & -k_9 & -k_{10} & k_{11} & 0 & -k_{13} & k_{14} & 0 & -k_{16} & 0 \\
0 & k_3 & -k_4-k_5 & 0 & 0 & 0 & 0 & -k_{10} & k_{11}+k_{12} & 0 & 0 & 0 & 0 & 0 & 0 \\
-k_1 & k_2 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & -k_{13} & k_{14}+k_{15} & 0 & 0 & 0 \\
0 & 0 & k_5 & 0 & -k_6 & k_7 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0
\end{pmatrix}.$$

A matrix B, whose columns form a basis for $\ker M$, has the following form.

$$B = \begin{pmatrix} 0 & \frac{k_2(k_4+k_5)k_{16}}{k_1k_3k_5} & 0 & \frac{k_2(k_4+k_5)k_{15}}{k_1k_3k_5} & 0 & \frac{k_2(k_4+k_5)k_{12}}{k_1k_3k_5} & 0 & 0 \\ 0 & \frac{(k_4+k_5)k_{16}}{k_3k_5} & 0 & \frac{(k_4+k_5)k_{15}}{k_3k_5} & 0 & \frac{(k_4+k_5)k_{12}}{k_3k_5} & 0 & 0 \\ 0 & \frac{k_{16}}{k_5} & 0 & \frac{k_{15}}{k_5} & 0 & \frac{k_{12}}{k_5} & 0 & 0 \\ 0 & 0 & 0 & 0 & 0 & 0 & 0 & 1 \\ 0 & \frac{(k_7+k_8)k_{16}}{k_6k_8} & 0 & \frac{(k_7+k_8)k_{15}}{k_6k_8} & 0 & \frac{(k_7+k_8)k_{12}}{k_6k_8} & \frac{k_7k_9}{k_6k_8} & 0 \\ 0 & \frac{k_{16}}{k_8} & 0 & \frac{k_{15}}{k_8} & 0 & \frac{k_{12}}{k_8} & \frac{k_9}{k_8} & 0 \\ 0 & 0 & 0 & 0 & 0 & 0 & 1 & 0 \\ 0 & 0 & 0 & 0 & 0 & \frac{k_{11}+k_{12}}{k_{10}} & 0 & 0 \\ 0 & 0 & 0 & 0 & 0 & 1 & 0 & 0 \\ 0 & 0 & 0 & 0 & 1 & 0 & 0 & 0 \\ 0 & 0 & 0 & \frac{k_{14}+k_{15}}{k_{13}} & 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 1 & 0 & 0 & 0 & 0 \\ 0 & 0 & 1 & 0 & 0 & 0 & 0 & 0 \\ 0 & 1 & 0 & 0 & 0 & 0 & 0 & 0 \\ 1 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \end{pmatrix} .$$

Consider the initial set of complexes, $Z = \{C_7, C_8, C_{11}, C_{14}\}$, suggested by the procedure in the Paper. The corresponding rows of B have only a single non-zero entry, each of which occurs in a distinct column, so that the four rows constitute a sub-matrix of full rank. It follows from Proposition 1 in the Paper that the space of type 1 complex-linear invariants is empty. Next consider adding in turn each of the complexes C_1, C_2 and C_3 to Z. Consider first $Z \cup \{C_1\}$. The rows of B may be permuted so that the rows corresponding to complexes $C_1, C_8, C_{11}, C_{14}, C_7$ are placed first and in that order. Permuting the columns of B so that the columns $2, 4, 6, 7$ are placed first yields a matrix in lower-triangular block form, with the top left block, B', given by

$$B' = \begin{pmatrix} \frac{k_2(k_4+k_5)k_{16}}{k_1k_3k_5} & \frac{k_2(k_4+k_5)k_{15}}{k_1k_3k_5} & \frac{k_2(k_4+k_5)k_{12}}{k_1k_3k_5} & 0 \\ 0 & 0 & \frac{k_{11}+k_{12}}{k_{10}} & 0 \\ 0 & \frac{k_{14}+k_{15}}{k_{13}} & 0 & 0 \\ 1 & 0 & 0 & 0 \\ 0 & 0 & 0 & 1 \end{pmatrix} .$$

It is evident that B' is in block diagonal form, with the last row and column, corresponding to complex C_7, forming an identify matrix. It is then easy to see from Paper Equation (6) that C_7 cannot appear in any invariant. In other words, it is sufficient to work with C_8, C_{11}, C_{14}, when adding C_1. Before doing this, note that the C_1, C_2 and C_3 rows of B may be written in the form

$$\alpha_i (0 \quad k_{16} \quad 0 \quad k_{15} \quad 0 \quad k_{12} \quad 0 \quad 0)$$

where

$$\alpha_1 = \frac{k_2(k_4 + k_5)}{k_1 k_3 k_5} , \quad \alpha_2 = \frac{(k_4 + k_5)}{k_3 k_5} , \quad \alpha_3 = \frac{1}{k_5} . \tag{2}$$

Accordingly, we can do all three calculations at once by omitting C_7 and re-writing B' in the form

$$B' = \begin{pmatrix} \alpha_i k_{16} & \alpha_i k_{15} & \alpha_i k_{12} \\ 0 & 0 & \frac{k_{11}+k_{12}}{k_{10}} \\ 0 & \frac{k_{14}+k_{15}}{k_{13}} & 0 \\ 1 & 0 & 0 \end{pmatrix},$$

with α_i given by (2) for $i = 1, 2, 3$. It is evident that rk $B' = 3$ and we can choose $U = \{1, 2, 3\}$ and $V = \{4\}$, so that

$$B'_U = \begin{pmatrix} \alpha_i k_{16} & \alpha_i k_{15} & \alpha_i k_{12} \\ 0 & 0 & \frac{k_{11}+k_{12}}{k_{10}} \\ 0 & \frac{k_{14}+k_{15}}{k_{13}} & 0 \end{pmatrix}, \qquad B'_V = (1 \quad 0 \quad 0)\,.$$

It follows that

$$(B'_U)^{-1} = \begin{pmatrix} \frac{1}{\alpha_i k_{16}} & -\frac{k_{10}k_{12}}{(k_{11}+k_{12})k_{16}} & -\frac{k_{13}k_{15}}{(k_{14}+k_{15})k_{16}} \\ 0 & 0 & \frac{k_{13}}{k_{14}+k_{15}} \\ 0 & \frac{k_{10}}{k_{11}+k_{12}} & 0 \end{pmatrix}.$$

Note that α_i only appears in a single entry. Using Paper Equation (6), with $\Psi(x)_U = (x^{C_i}, x^{C_8}, x^{C_{11}})^\dagger$ for $i = 1, 2, 3$, and $\Psi(x)_V = (x^{C_{14}})$, we recover the invariants in Paper Equation (8).

6 Failure of ACR for the example in Paper §2.6

The steady states of the example just discussed can be algebraically simplified as follows. The differential equations governing the system can be obtained from the matrix M in §5 above by using the fundamental decomposition of CRNT, $dx/dt = M.\Psi(x)$. With the notation in the Table in §5, this gives

$$\frac{dS_1}{dt} = k_6S_5S_9 - (k_7 + k_8)S_1 + k_9S_4S_6 \tag{3}$$

$$\frac{dS_2}{dt} = k_{10}S_6S_7 - (k_{11} + k_{12})S_2 \tag{4}$$

$$\frac{dS_3}{dt} = k_{13}S_6S_8 - (k_{14} + k_{15})S_3 \tag{5}$$

$$\frac{dS_4}{dt} = k_1S_8 - (k_2 + k_3)S_4 + k_4S_7 + k_8S_1 - k_9S_4S_6 \tag{6}$$

$$\frac{dS_5}{dt} = k_7S_1 - k_6S_5S_9 + k_{12}S_2 + k_{15}S_3 + k_{16}S_6 \tag{7}$$

$$\frac{dS_6}{dt} = k_8S_1 - k_9S_4S_6 - k_{10}S_6S_7 + k_{11}S_2 - k_{13}S_6S_8 + k_{14}S_3 - k_{16}S_6 \tag{8}$$

$$\frac{dS_7}{dt} = k_3S_4 - (k_4 + k_5)S_7 - k_{10}S_6S_7 + (k_{11} + k_{12})S_2 \tag{9}$$

$$\frac{dS_8}{dt} = k_2S_4 - k_1S_8 - k_{13}S_6S_8 + (k_{14} + k_{15})S_3 \tag{10}$$

$$\frac{dS_9}{dt} = k_5S_7 - k_6S_5S_9 + k_7S_1\,. \tag{11}$$

It can be checked that these equations (or, equivalently, the matrix M from which they are derived) satisfy two conservation laws, corresponding to the total amounts of sensor and response regulator,

$$\begin{aligned} S_1 + S_2 + S_3 + S_4 + S_7 + S_8 + S_9 &= K_1 \\ S_1 + S_2 + S_3 + S_5 + S_6 &= K_2 \,, \end{aligned} \tag{12}$$

where $K_1, K_2 \in \mathbb{R}_{>0}$ are constants determined by the initial conditions.

The steady states are obtained by setting the right hand sides of equations (3)-(11) to zero. The nine variables may be partitioned into two subsets, $\{S_1, S_2, S_3, S_7, S_8\}$ and $\{S_4, S_5, S_6, S_9\}$, in such a way that the variables in the first subset can be written in terms of the variables in the second subset. Using equation (3),

$$S_1 = \left(\frac{k_6}{k_7 + k_8}\right) S_5 S_9 + \left(\frac{k_9}{k_7 + k_8}\right) S_4 S_6 \,. \tag{13}$$

Combining equations (4) and (9), we get

$$S_7 = \left(\frac{k_3}{k_4 + k_5}\right) S_4 \tag{14}$$

and, similarly, combining equations (5) and (10) we get

$$S_8 = \left(\frac{k_2}{k_1}\right) S_4 \,. \tag{15}$$

Using equation (4) together with (14) we get

$$S_2 = \left(\frac{k_{10}}{k_{11} + k_{12}}\right) \left(\frac{k_3}{k_4 + k_5}\right) S_4 S_6 \tag{16}$$

and, similarly, using equations (5) and (15) we get

$$S_3 = \left(\frac{k_{13}}{k_{14} + k_{15}}\right) \left(\frac{k_2}{k_1}\right) S_4 S_6 \,. \tag{17}$$

Equations (13)-(17) describe $\{S_1, S_2, S_3, S_7, S_8\}$ in terms of $\{S_4, S_5, S_6, S_9\}$.

If we now substitute in equation (7) for S_1, S_2 and S_3 using equations (13), (16) and (17), respectively, and simplify, we get

$$(\alpha S_4 + k_{16}) S_6 = \left(\frac{k_6 k_8}{k_7 + k_8}\right) S_5 S_9 \tag{18}$$

where α depends only on the rate constants and can be conveniently represented as $\alpha = \beta + \gamma$, where

$$\beta = \left(\frac{k_7 k_9}{k_7 + k_8}\right) \,, \quad \gamma = \left(\frac{k_{10} k_{12}}{k_{11} + k_{12}}\right) \left(\frac{k_3}{k_4 + k_5}\right) + \left(\frac{k_{13} k_{15}}{k_{14} + k_{15}}\right) \left(\frac{k_2}{k_1}\right) \,.$$

If we also substitute in equation (11) for S_1 and S_7 using equations (13) and (14), respectively, and simplify, we get

$$\left(\frac{k_3 k_5}{k_4 + k_5}\right) S_4 + \beta S_4 S_6 = \left(\frac{k_6 k_8}{k_7 + k_8}\right) S_5 S_9 \,. \tag{19}$$

Combining equations (18) and (19) we can express S_6 as a rational function of S_4,

$$S_6 = \frac{k_3 k_5 S_4}{(k_4 + k_5)(\gamma S_4 + k_{16})} \,. \tag{20}$$

Finally, substituting this expression into equation (18), we can express S_5 as a rational function of S_4 and S_9,

$$S_5 = \frac{k_3 k_5 (k_7 + k_8)(\alpha S_4 + k_{16}) S_4}{k_6 k_8 (k_4 + k_5)(\gamma S_4 + k_{16}) S_9} \,. \tag{21}$$

If S_4 and S_9 are given arbitrary positive values, the values of all the other variables are determined by equations (21), (20) and (13) to (17). It can be checked that these values form a positive steady-state of the system. The free quantities S_4 and S_9, in terms of which the other variables have been parameterised, implicitly determine the values of the conserved quantities K_1 and K_2 in equation (12). It can be seen from equation (20) that S_6, which is the concentration of the activated response regulator, $S_6 =$ [OmpR-P], varies with the choice of S_4 and, hence, does not exhibit ACR.

It also follows from equation (20) that

$$[\text{OmpR-P}] = S_6 < \frac{k_3 k_5}{(k_4 + k_5)\gamma} \,, \tag{22}$$

which shows, as deduced in the Paper, that OmpR-P has a robust upper bound. The bound in (22) is half the harmonic mean of the robust bounds in Paper Equation 10 and is therefore tighter than either of the latter. This is evident from equation (20), where it is clear that S_6 asymptotically approaches the bound in (22) as S_4 increases. Hence, (22) is the best possible bound on S_6.

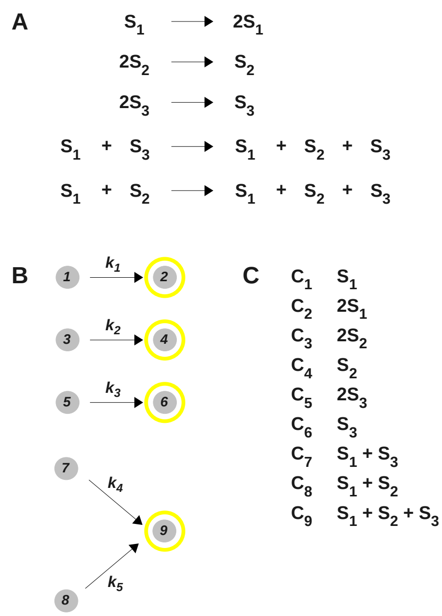

Figure 1: Network with complex-linear invariants that are not of type 1. **A** Hypothetical reaction network. **B** Labelled, directed graph on the complexes, with the terminal strongly-connected components outlined in yellow. **C** Numbering scheme for the complexes.