Supplementary material to

“Steady states and stability in metabolic networks without regulation”
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Appendix A: Detailed information on the examples

Example 2

We assume constant inflows $v_1 = v_2$. Reaction rates $v_3(x)$ and $v_4(x)$ are assumed to obey convenience kinetics (Libemeister and Klipp, 2006), and the reaction $v_5(x)$ is assumed to obey Michaelis-Menten kinetics. The reaction rate equations for the metabolic network (14) are:

$$v_3(x) = \frac{v_{f1}\frac{x_1}{K_1}\frac{x_2}{K_2} - v_{r1}\frac{x_3}{K_3}\frac{x_4}{K_3}}{(1+\frac{x_1}{K_1})(1+\frac{x_2}{K_2}) + (1+\frac{x_3}{K_3})(1+\frac{x_4}{K_4}) - 1}$$

$$v_4(x) = \frac{v_{f2}\frac{x_3}{K_5}\frac{x_4}{K_6} - v_{r2}\frac{x_5}{K_7}}{(1+\frac{x_3}{K_5})(1+\frac{x_4}{K_6}) + \frac{x_5}{K_7}},$$

$$v_5(x) = \frac{v_{f3}x_5}{x_5 + K_8}.$$

The parameters for the set of equations above are: $v_1 = 1, v_{f1} = v_{f2} = v_{f3} = v_{r1} = 2,\ v_{r2} = 1,$ $K_1 = ... = K_8 = 1.$ The steady states for the metabolic network (14) are

$$x_1^* = \phi_1(x_2^*, x_4^*) = \frac{2 - x_1 + 10x_3 + x_1^* x_3^* + 4(x_3^*)^2}{(x_1^* - 1)(x_3^* - 1)}$$

$$x_4^* = \phi_2(x_3^*) = \frac{x_3^* + 3}{x_3^* - 1}$$

$$x_5^* = 1$$

Example 4

The parameters for the reaction rates (26) are: $v_{f1} = 1,\ K_1 = \ldots K_4 = 1,\ v_1 = v_{f2} = \ldots = v_{f4} = 1.$

Example 5

The rate equations for the metabolic network (27) are

$$v_i = \frac{v_{\max,i} x_i}{x_i + K_i}, i = 2, 3, 4, 5$$

The parameters are $K_1 = K_3 = 1$, $K_2 = 0.5$, $K_4 = 0.01$, $v_1 = 0.01$, $v_{f1} = v_{f2} = v_{f3} = 1$, $v_{f4} = 0.1$. There are two steady states in this metabolic network: $\{0.0162,\ 0.0015,\ 0.006\}$ and $\{0.1728,\ 0.0369,\ 0.1592\}$. The corresponding eigenvalues of the Jacobian matrix are: $\{-9.5, -1.67,\ -0.335\}$ and $\{0.16, -1.9 + 0.85i, -1.9 - 0.85i\}$.

Example 7

The equations for reaction rates for the metabolic network (45) are:

$$v_2 = \frac{v_{f1} x_1}{K_1 + x_1},\ v_3 = \frac{v_{f2} \frac{x_2}{K_2} \frac{x_3}{K_3}}{(1 + \frac{x_2}{K_2})(1 + \frac{x_3}{K_3}) - 1},\ v_4 = \frac{v_{f3} \frac{x_2}{K_4} \frac{x_3}{K_5}}{(1 + \frac{x_2}{K_4})(1 + \frac{x_3}{K_5}) - 1}$$

$$v_5 = \frac{v_{f4} x_4}{x_4 + K_6},\ v_6 = \frac{v_{f5} x_5}{x_5 + K_7}$$

The parameters for the equations above are: $v_1 = 1$, $K_1 = \ldots K_7 = 1$, $v_{f1} = \ldots = v_{f5} = 3$.
The eigenvalues of the corresponding Jacobian matrix (45) are:
$\{0.0,\ -746.7,\ -4.17,\ -2.52,\ -2.52\}$.

Example 8

The equations for reaction rates for the metabolic network (47) are:

$$v_2 = \frac{v_{f2} \left(\frac{x_1}{K_1}\right)^2 \frac{x_3}{K_3}}{(1 + \frac{x_2}{K_2} + \left(\frac{x_2}{K_2}\right)^2)(1 + \frac{x_3}{K_3}) - 1},\ v_3 = \frac{v_{f2} \frac{x_2}{K_4} \left(\frac{x_3}{K_5}\right)^2}{(1 + \frac{x_2}{K_4})(1 + \frac{x_3}{K_5} + \left(\frac{x_3}{K_5}\right)^2) - 1},$$

$$v_4 = \frac{v_{f1} x_1}{x_1 + K_1}, v_5 = \frac{v_{f4} x_4}{x_4 + K_6}, v_6 = \frac{v_{f5} x_5}{x_5 + K_7}$$

The parameters for the equations above are: $v_1 = 0.5, v_{f1} = v_{f5} = 1$, $K_2 = K_5 = 0.1$, $K_1 = K_3 = K_4 = K_6 = K_7 = 1$. The only positive steady state is: $x_1^* = 1$, $x_2^* = x_3^* = 3$, $x_4^* = x_5^* = 0.2$. Eigenvalues of the system are: $\{0.266, -0.25, -0.7, -0.7,\ -1.77\}$.

Appendix B: Stoichiometric matrix of the citric acid cycle

The set of reactions of citric acid cycle is

$$\begin{aligned}
&\text{Oxaloacetate} + \text{Acetyl CoA} + H_2O \rightarrow \text{Citrate} + \text{CoA-SH}\\
&\text{Citrate} \rightarrow \textit{cis}\text{-Aconitate} + H_2O\\
&\textit{cis}\text{-Aconitate} + H_2O \rightarrow \text{Isocitrate}\\
&\text{Isocitrate} + \text{NAD}^{+} \rightarrow \text{Oxalosuccinate} + \text{NAD} + H^{+}\\
&\text{Oxalosuccinate} \rightarrow \alpha\text{-Ketuglutarate} + CO_2\\
&\alpha\text{-Ketuglutarate} + \text{NAD}^{+} + \text{CoA-SH} \rightarrow \text{Succinyl-CoA} + \text{NADH} + H^{+} + CO_2\\
&\text{Succinyl-CoA} + \text{GDP} + P_i \rightarrow \text{Succinate} + \text{CoA-SH} + \text{GTP}\\
&\text{Succinate} + \text{Ubiquinone (Q)} \rightarrow \text{Fumarate} + \text{Ubiquinol}(QH_2)\\
&\text{Fumarate} + H_2O \rightarrow \textit{L}\text{-Malate}\\
&\textit{L}\text{-Malate} + \text{NAD}^{+} \rightarrow \text{Oxaloacetate} + \text{NADH} + H^{+}
\end{aligned}$$

The corresponding stoichiometric matrix is given by:

$$S = \begin{array}{cc}
\begin{pmatrix}
-1 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 1 & 0 & 0 & 0 & 0 & 0\\
-1 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0\\
1 & -1 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0\\
1 & 0 & 0 & 0 & 0 & -1 & 1 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0\\
0 & 1 & -1 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0\\
0 & 0 & 1 & -1 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0\\
0 & 0 & 0 & -1 & 0 & -1 & 0 & 0 & 0 & -1 & 0 & 0 & 0 & 0 & 0\\
0 & 0 & 0 & 1 & -1 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0\\
0 & 0 & 0 & 1 & 0 & 0 & 0 & 0 & 0 & 0 & -1 & 0 & 0 & 0 & 0\\
0 & 0 & 0 & 1 & 0 & 1 & 0 & 0 & 0 & 1 & 0 & -1 & 0 & 0 & 0\\
0 & 0 & 0 & 0 & 1 & -1 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0\\
0 & 0 & 0 & 0 & 0 & 1 & -1 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0\\
0 & 0 & 0 & 0 & 0 & 1 & 0 & 0 & 0 & 1 & 0 & 0 & -1 & 0 & 0\\
0 & 0 & 0 & 0 & 0 & 0 & -1 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0\\
0 & 0 & 0 & 0 & 0 & 0 & 1 & -1 & 0 & 0 & 0 & 0 & 0 & 0 & 0\\
0 & 0 & 0 & 0 & 0 & 0 & 1 & 0 & 0 & 0 & 0 & 0 & 0 & -1 & 0\\
0 & 0 & 0 & 0 & 0 & 0 & 0 & -1 & 0 & 0 & 0 & 0 & 0 & 0 & 0\\
0 & 0 & 0 & 0 & 0 & 0 & 0 & 1 & -1 & 0 & 0 & 0 & 0 & 0 & 0\\
0 & 0 & 0 & 0 & 0 & 0 & 0 & 1 & 0 & 0 & 0 & 0 & 0 & 0 & -1\\
0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 1 & -1 & 0 & 0 & 0 & 0 & 0
\end{pmatrix}
&
\begin{array}{c}
Oxaloacetate\\
Acetyl-CoA\\
Citrate\\
CoA-SH\\
cis-Aconitate\\
Isocitrate\\
NAD^{+}\\
Oxalosuccinate\\
NAD\\
H^{+}\\
\alpha-Ketoglutarate\\
Succinyl-CoA\\
NADH\\
GDP\\
Succinate\\
GTP\\
Ubiquinone\,(\mathrm{Q})\\
Fumarate\\
Ubiquinol\,(\mathrm{QH_2})\\
L-Malate
\end{array}
\end{array}$$

We constructed the stoichiometric matrix for this network, not taking into account H_2O and CO_2 as variables, without inflow reactions and with outflows of H^+, NADH, GTP and ubiquinol. The stoichiometric matrix consists of 20 rows, representing different metabolites and 15 columns, representing different reactions (including four outflow reactions).

The rank deficiency of this matrix is five. Therefore, it is possible that this system has a five-dimensional manifold of steady states.

Appendix C: Stability in branched SS-SP networks

The Jacobian matrix for branched single-substrate-single-product metabolic networks with general stoichiometry is

$$J(x)=\begin{pmatrix} \boxed{P} & s_{p,p+1}g_{p+1,p+1} & s_{p,y+1}g_{y+1,y+1} \\ s_{p+1,p+1}g_{p+1,p} & \boxed{Y} & 0 \\ s_{p,y+1}g_{y+1,y+1} & 0 & \boxed{M} \end{pmatrix}. \qquad \text{(C. 1)}$$

Here the square $p\times p$ diagonal submatrix P is

$$P=\begin{pmatrix} -s_{1,2}g_{2,1} & s_{1,2}g_{2,2} & & \\ s_{2,2}g_{2,1} & \ddots & \ddots & \\ & \ddots & \ddots & s_{p-1,p}g_{p,p} \\ & & s_{p,p}g_{p,p-1} & -s_{p,p}g_{p,p}-s_{p,p+1}g_{p+1,p}-s_{p,y+1}g_{y+1,p} \end{pmatrix}, \qquad \text{(C. 2)}$$

with row and column indices in range from 1 to p.
Square diagonal submatrix Y is

$$Y=\begin{pmatrix} -s_{p+1,p+1}g_{p+1,p+1}-s_{p+1,p+2}g_{p+2,p+1} & s_{p+1,p+2}g_{p+2,p+2} & & \\ s_{p+2,p+2}g_{p+2,p+1} & \ddots & \ddots & \\ & \ddots & \ddots & s_{y-1,y}g_{y,y} \\ & & s_{y,y}g_{y,y-1} & -s_{y,y}g_{y,y}-s_{y,m+1}g_{m+1,y} \end{pmatrix}, \qquad \text{(C. 3)}$$

with row and column indices in range from $p+1$ to y. The square diagonal submatrix M is

$$M=\begin{pmatrix} -s_{y+1,y+1}g_{y+1,y+1}-s_{y+1,y+2}g_{y+2,y+1} & s_{y+1,y+2}g_{y+2,y+2} & & \\ s_{y+2,y+2}g_{y+2,y+1} & \ddots & \ddots & \\ & \ddots & \ddots & s_{m-1,m}g_{m,m} \\ & & s_{m,m}g_{m,m-1} & -s_{m,m}g_{mm}-s_{m,m+2}g_{m+2,m} \end{pmatrix}. \qquad \text{(C. 4)}$$

with row and column indices in range from $y+1$ to m.

The entries $J_{p,p+1}(x)=s_{p,p+1}g_{p+1,p+1}$ and $J_{p+1,p}(x)=s_{p+1,p+1}g_{p+1,p}$ that 'connect' submatrices P and Y are located on the upper and lower diagonals respectively.

The Jacobian matrix (C. 1) has negative diagonal entries and nonnegative off-diagonal entries as in the case of the Jacobian matrix for linear networks (18). The matrix (C. 1) is not tridiagonal. In particular, there are two off-tridiagonal terms $J_{y+1,p}(x)=s_{y+1,y+1}g_{y+1,p}$ and $J_{p,y+1}(x)=s_{p,y+1}g_{y+1,y+1}$.

To prove that the Jacobian matrix (C. 1) is a negative M-matrix we need to prove that all its leading principal minors $f_1,..., f_m$ alternate in sign starting from negative $f_1 < 0$. Since the Jacobian matrix (C. 1) is tridiagonal, except for two entries, there are several steps in which we prove that all leading principal minors f_i alternate in sign: 1) $i < p$, 2) $i = p$, 3) $i = p+1$, 4) $p+1 < i < y$, 5) $i = y$, 6) $i = y+1$, 7) $y+1 < i < m$, 8) $i = m$.

1) $\underline{i < p}$

Leading principal minors until f_{p-1} are the same as for linear networks (19):

$$f_{p-1} = (-1)^p \prod_{i=1}^{p-1} s_{i,i+1} g_{i+1,i}. \tag{C. 5}$$

Accordingly, leading principal minors until f_{p-1} alternate in sign, starting from negative.

2) $\underline{i = p}$

We calculate the leading principal minor f_p using recursion equation for a determinant of a tridiagonal matrix (El-Mikkawy, 2004):

$$\begin{aligned} f_p &= (-s_{p,p} g_{p,p} - s_{p,p+1} g_{p+1,p} - s_{p,y+1} g_{y+1,p}) f_{p-1} - s_{p-1,p} g_{p,p} s_{p,p} g_{p,p-1} f_{p-2} \\ &= (-s_{p,p} g_{p,p} - s_{p,p+1} g_{p+1,p} - s_{p,y+1} g_{y+1,p})(-1)^p \prod_{i=1}^{p-1} s_{i,i+1} g_{i+1,i} \\ &\quad - s_{p-1,p} g_{p,p} s_{p,p} g_{p,p-1} (-1)^{p-1} \prod_{i=1}^{p-2} s_{i,i+1} g_{i+1,i} \\ &= (-s_{p,p+1} g_{p+1,p} - s_{p,y+1} g_{y+1,p}) f_{p-1}. \end{aligned} \tag{C. 6}$$

Clearly, minor f_p has the opposite sign of the minor f_{p-1}.

3) $\underline{i = p+1}$

The leading principal minor f_{p+1} is

$$\begin{aligned} f_{p+1} &= (-s_{p+1,p+1} g_{p+1,p+1} - s_{p+1,p+2} g_{p+2,p+1}) f_p - s_{p,p+1} g_{p+1,p+1} s_{p+1,p+1} g_{p+1,p} f_{p-1} \\ &= (-s_{p+1,p+1} g_{p+1,p+1} - s_{p+1,p+2} g_{p+2,p+1})(-s_{p,p+1} g_{p+1,p} - s_{p,y+1} g_{y+1,p}) f_{p-1} \\ &\quad - s_{p,p+1} g_{p+1,p+1} s_{p+1,p+1} g_{p+1,p} f_{p-1} \\ &= -s_{p+1,p+2} g_{p+2,p+1} f_p + s_{p,y+1} g_{y+1,p} s_{p+1,p+1} g_{p+1,p+1} f_{p-1} \end{aligned} \tag{C. 7}$$

Since, f_p has the opposite sign of f_{p-1}, and f_{p+1} has the same sign as f_{p-1}, then f_{p+1} has the opposite sign of f_p.

4) $\underline{p+1<i<y}$

Leading principal minors after f_{p+1} until f_{y-1} are of the form

$$f_{p+r}=-s_{p+r,p+r+1}g_{p+r+1,p+r}f_{p+r-1}+(-1)^{r+1}s_{p,y+1}g_{y+1,p}f_{p-1}\prod_{j=1}^{r}s_{p+j,p+j}g_{p+j,p+j}. \tag{C. 8}$$

We prove the equation (C. 8) by induction. It holds for $r=1$ (equation C. 7). Assuming that the equation (C. 8) is true, we calculate the next leading principal minor using the recursion equation for a determinant of a tridiagonal matrix:

$$\begin{aligned}
f_{p+r+1}&=(-s_{p+r+1,p+r+1}g_{p+r+1,p+r+1}-s_{p+r+1,p+r+2}g_{p+r+2,p+r+1})f_{p+r}\\
&\quad-s_{p+r,p+r+1}g_{p+r+1,p+r+1}s_{p+r+1,p+r+1}g_{p+r+1,p+r}f_{p+r-1}\\
&=(-s_{p+r+1,p+r+1}g_{p+r+1,p+r+1}-s_{p+r+1,p+r+2}g_{p+r+2,p+r+1})[-s_{p+r,p+r+1}g_{p+r+1,p+r}f_{p+r-1}\\
&\quad+(-1)^{r+1}s_{p,y+1}g_{y+1,p}f_{p-1}\prod_{j=1}^{r}s_{p+j,p+j}g_{p+j,p+j}]\\
&\quad-s_{p+r,p+r+1}g_{p+r+1,p+r+1}s_{p+r+1,p+r+1}g_{p+r+1,p+r}f_{p+r-1}\\
&=-s_{p+r+1,p+r+2}g_{p+r+2,p+r+1}f_{p+r}+(-1)^{r+2}s_{p,y+1}g_{y+1,p}f_{p-1}\prod_{j=1}^{r+1}s_{p+j,p+j}g_{p+j,p+j}
\end{aligned} \tag{C. 9}$$

The equation (C. 9) is according to the equation (C. 8), which concludes the proof. Therefore, the leading principal minors in range of $p+1<i<y$ alternate in sign.

From the equation (C. 8) by setting $p+r=y-1$ we can express the leading principal minor f_{y-1}:

$$f_{y-1}=-s_{y-1,y}g_{y,y-1}f_{y-2}+(-1)^{y-p}s_{p,y+1}g_{y+1,p}f_{p-1}\prod_{j=1}^{y-p-1}s_{p+j,p+j}g_{p+j,p+j}. \tag{C. 10}$$

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5) $\underline{i=y}$

The leading principal minor f_y is

$$\begin{aligned}
f_y&=(-s_{y,y}g_{y,y}-s_{y,m+1}g_{m+1,y})f_{y-1}-s_{y-1,y}g_{y,y}s_{y,y}g_{y,y-1}f_{y-2}\\
&=(-s_{y,y}g_{y,y}-s_{y,m+1}g_{m+1,y})[-s_{y-1,y}g_{y,y-1}f_{y-2}+(-1)^{y-p}s_{p,y+1}g_{y+1,p}f_{p-1}\prod_{j=1}^{y-p-1}s_{p+j,p+j}g_{p+j,p+j}]\\
&\quad-s_{y-1,y}g_{y,y}s_{y,y}g_{y,y-1}f_{y-2}\\
&=-s_{y,m+1}g_{m+1,y}f_{y-1}+(-1)^{y-p+1}s_{p,y+1}g_{y+1,p}f_{p-1}\prod_{j=1}^{y-p}s_{p+j,p+j}g_{p+j,p+j}
\end{aligned} \tag{C.11}$$

The first term has the opposite sign of f_{y-1} and the second term has the sign $(-1)^y$. Overall, the leading principal minor f_y has the opposite sign of f_{y-1}.

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6) $\underline{i=y+1}$

The leading principal minor f_{y+1} is

$$\begin{aligned} f_{y+1} &= (-s_{y+1,y+1}g_{y+1,y+1} - s_{y+1,y+2}g_{y+2,y+1})f_y \\ &\quad + (-1)^{y+p+1}s_{p,y+1}g_{y+1,y+1}(-1)^{y+p}s_{y+1,y+1}g_{y+1,p}f_{p-1}\tilde{f}_y \\ &= (-s_{y+1,y+1}g_{y+1,y+1} - s_{y+1,y+2}g_{y+2,y+1})f_y - s_{p,y+1}g_{y+1,y+1}s_{y+1,y+1}g_{y+1,p}f_{p-1}\tilde{f}_y. \end{aligned} \tag{C. 12}$$

Here $\tilde{f}_y$ is the determinant of the submatrix Y (C. 3). The determinant $\tilde{f}_{y-1}$ is

$$\tilde{f}_{y-1} = -s_{y-1,y}g_{y,y-1}\tilde{f}_{y-2} + (-1)^{y-p}\prod_{j=1}^{y-p-1}s_{p+j,p+j}g_{p+j,p+j}. \tag{C. 13}$$

The equation (C. 13) can be proved by induction in the same manner as the equation (C. 10). Here we omit the proof of equation (C. 13).

The determinant $\tilde{f}_y$ therefore is

$$\begin{aligned} \tilde{f}_y &= (-s_{y,y}g_{y,y} - s_{y,m+1}g_{m+1,y})\tilde{f}_{y-1} - s_{y-1,y}g_{y,y}s_{y,y}g_{y,y-1}\tilde{f}_{y-2} \\ &= (-s_{y,y}g_{y,y} - s_{y,m+1}g_{m+1,y})[-s_{y-1,y}g_{y,y-1}\tilde{f}_{y-2} + (-1)^{y-p}\prod_{j=1}^{y-p-1}s_{p+j,p+j}g_{p+j,p+j}] \\ &\quad - s_{y-1,y}g_{y,y}s_{y,y}g_{y,y-1}\tilde{f}_{y-2} \\ &= -s_{y,m+1}g_{m+1,y}\tilde{f}_{y-1} + (-1)^{y-p+1}\prod_{j=1}^{y-p}s_{p+j,p+j}g_{p+j,p+j} \end{aligned} \tag{C. 14}$$

Using the equations (C. 12) and (C. 14) we can calculate the leading principal minor f_{y+1}:

$$\begin{aligned} f_{y+1} &= (\underline{-s_{y+1,y+1}g_{y+1,y+1}} - s_{y+1,y+2}g_{y+2,y+1})\times \\ &\quad \times[-s_{y,m+1}g_{m+1,y}f_{y-1} + \underline{(-1)^{y-p+1}s_{p,y+1}g_{y+1,p}f_{p-1}\prod_{j=1}^{y-p}s_{p+j,p+j}g_{p+j,p+j}}] \\ &\quad - \underline{s_{p,y+1}g_{y+1,y+1}s_{y+1,y+1}g_{y+1,p}f_{p-1}}\times \\ &\quad \times[-s_{y,m+1}g_{m+1,y}\tilde{f}_{y-1} + \underline{(-1)^{y-p+1}\prod_{j=1}^{y-p}s_{p+j,p+j}g_{p+j,p+j}}] \\ &= -s_{y+1,y+2}g_{y+2,y+1}f_y + s_{y+1,y+1}g_{y+1,y+1}s_{y,m+1}g_{m+1,y}f_{y-1} \\ &\quad + s_{p,y+1}g_{y+1,y+1}s_{y+1,y+1}g_{y+1,p}s_{y,m+1}g_{m+1,y}f_{p-1}\tilde{f}_{y-1}. \end{aligned} \tag{C. 15}$$

Then we expand f_{y-1} from the equation (C. 10) and $\tilde{f}_{y-1}$ from the equation (C. 13) and cancel the similar terms (underscored) in (C. 13). Then the terms including f_{y-2} and $\tilde{f}_{y-2}$ are left and we expand this terms further. We continue so until we get to the terms f_{p+1} and $\tilde{f}_{p+1}$. Denoting

$$f_{x-2} = (-1)^{y-p-2}\prod_{j=3}^{y-p}s_{p+j-1,p+j}g_{p+j,p+j-1},$$

we get

$$f_{y+1} = -s_{y+1,y+2} g_{y+2,y+1} f_y + s_{y+1,y+1} g_{y+1,y+1} s_{y,m+1} g_{m+1,y} f_{x-2} f_{p+1}$$
$$+ s_{p,y+1} g_{y+1,y+1} s_{y+1,y+1} g_{y+1,p} s_{y,m+1} g_{m+1,y} f_{x-2} \tilde{f}_{p+1}. \quad \text{(C. 16)}$$

Here $\tilde{f}_{p+1}$ is the first diagonal term of the submatrix (C. 3), $\tilde{f}_{p+1} = -s_{p+1,p+1} g_{p+1,p+1} - s_{p+1,p+2} g_{p+2,p+1}$. Inserting the expressions for $\tilde{f}_{p+1}$ and f_{p+1} (equation C. 7) we get

$$\begin{aligned} f_{y+1} &= -s_{y+1,y+2} g_{y+2,y+1} f_y + s_{y+1,y+1} g_{y+1,y+1} s_{y,m+1} g_{m+1,y} f_{x-2} \times \\ &\quad [-s_{p+1,p+2} g_{p+2,p+1} f_p + s_{p,y+1} g_{y+1,p} s_{p+1,p+1} g_{p+1,p+1} f_{p-1}] \\ &\quad + s_{p,y+1} g_{y+1,y+1} s_{y+1,y+1} g_{y+1,p} s_{y,m+1} g_{m+1,y} f_{x-2} f_{p-1} [-s_{p+1,p+1} g_{p+1,p+1} - s_{p+1,p+2} g_{p+2,p+1}] \quad \text{(C. 17)} \\ &= -s_{y+1,y+2} g_{y+2,y+1} f_y + s_{y+1,y+1} g_{y+1,y+1} s_{y,m+1} g_{m+1,y} f_{x-2} [-s_{p+1,p+2} g_{p+2,p+1} f_p] \\ &\quad + s_{p,y+1} g_{y+1,y+1} s_{y+1,y+1} g_{y+1,p} s_{y,m+1} g_{m+1,y} f_{x-2} f_{p-1} [-s_{p+1,p+2} g_{p+2,p+1}] \end{aligned}$$

Inserting the equation (C. 6) for f_p we get

$$\begin{aligned} f_{y+1} &= -s_{y+1,y+2} g_{y+2,y+1} f_y + s_{y+1,y+1} g_{y+1,y+1} s_{y,m+1} g_{m+1,y} f_{x-2} f_{p-1} \times \\ &\quad \times [-s_{p+1,p+2} g_{p+2,p+1} (-s_{p,p+1} g_{p+1,p} - s_{p,y+1} g_{y+1,p})] \\ &\quad + s_{p,y+1} g_{y+1,y+1} s_{y+1,y+1} g_{y+1,p} s_{y,m+1} g_{m+1,y} f_{x-2} f_{p-1} [-s_{p+1,p+2} g_{p+2,p+1}] \\ &= s_{y+1,y+2} g_{y+2,y+1} f_y + s_{y+1,y+1} g_{y+1,y+1} s_{y,m+1} g_{m+1,y} s_{p+1,p+2} g_{p+2,p+1} s_{p,p+1} g_{p+1,p} f_{x-2} f_{p-1}. \end{aligned}$$

Denoting

$$f_x = (-1)^{y-p} \prod_{j=1}^{y-p} s_{p+j-1,p+j} g_{p+j,p+j-1}, \quad \text{(C. 18)}$$

we get the expression for the leading principal minor f_{y+1}

$$f_{y+1} = -s_{y+1,y+2} g_{y+2,y+1} f_y + s_{y+1,y+1} g_{y+1,y+1} s_{y,m+1} g_{m+1,y} f_x f_{p-1}. \quad \text{(C. 19)}$$

The first term has the opposite sign to the sign of f_y and the second term has the sign $(-1)^{y-p}(-1)^2(-1)^{p-1} = (-1)^{y-1}$, which is the opposite to the sign of f_y. Overall, the leading principal minor f_{y+1} has the opposite sign of f_y.

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7) $\underline{y+1 < i < m}$

The leading principal minor f_{y+2} is

$$\begin{aligned} f_{y+2} &= (-s_{y+2,y+2} g_{y+2,y+2} - s_{y+2,y+3} g_{y+3,y+2}) f_{y+1} - s_{y+1,y+2} g_{y+2,y+2} s_{y+2,y+2} g_{y+2,y+1} f_y \\ &= (-s_{y+2,y+2} g_{y+2,y+2} - s_{y+2,y+3} g_{y+3,y+2}) \times \\ &\quad \times [-s_{y+1,y+2} g_{y+2,y+1} f_y + s_{y+1,y+1} g_{y+1,y+1} s_{y,m+1} g_{m+1,y} f_x f_{p-1}] \quad \text{(C. 20)} \\ &\quad - s_{y+1,y+2} g_{y+2,y+2} s_{y+2,y+2} g_{y+2,y+1} f_y \\ &= -s_{y+2,y+3} g_{y+3,y+2} f_{y+1} - s_{y+2,y+2} g_{y+2,y+2} s_{y+1,y+1} g_{y+1,y+1} s_{y,m+1} g_{m+1,y} f_x f_{p-1}. \end{aligned}$$

The sign of f_{y+2} is the opposite of f_{y+1}.

Leading principal minors after f_{y+1} until f_{m-1} are of the form

$$f_{y+d} = -s_{y+d,y+d+1}g_{y+d+1,y+d}\,f_{y+d-1} - (-1)^d s_{y,m+1}g_{m+1,y}\,f_x\,f_{p-1}\prod_{i=1}^{d} s_{y+i,y+i}g_{y+i,y+i}. \qquad \text{(C. 21)}$$

The proof of the equation (C. 21) is by induction as for the previous similar case of the equation (C. 8). The sign of the first term in (C. 21) is the opposite of f_{y+d-1}. The sign of the second term is $(-1)^{d+1}(-1)^{y-p}(-1)^{p-1} = (-1)^{y+d}$, which is also the opposite of f_{y+d-1}.

From the equation (C. 21) by setting $y+d = m-1$ we get $d = m-y-1$ and the leading principal minor f_{m-1}:

$$f_{m-1} = -s_{m-1,m}g_{m,m-1}\,f_{m-2} - (-1)^{m-y-1}s_{y,m+1}g_{m+1,y}\,f_x\,f_{p-1}\prod_{i=1}^{m-y-1} s_{y+i,y+i}g_{y+i,y+i}. \qquad \text{(C. 22)}$$

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8) $\underline{i = m}$

The last leading principal minor is

$$\begin{aligned}
f_m &= (s_{m,m}y_{m,m} - s_{m,m+2}g_{m+2,m})\,f_{m-1} - s_{m-1,m}g_{m,m}s_{m,m}g_{m,m-1}\,f_{m-2} \\
&= (s_{m,m}y_{m,m} - s_{m,m+2}g_{m+2,m})\times \\
&\quad \times[-s_{m-1,m}g_{m,m-1}\,f_{m-2} - (-1)^{m-y-1}s_{y,m+1}g_{m+1,y}\,f_x\,f_{p-1}\prod_{i=1}^{m-y-1} s_{y+i,y+i}g_{y+i,y+i}] \qquad \text{(C. 23)} \\
&\quad - s_{m-1,m}g_{m,m}s_{m,m}g_{m,m-1}\,f_{m-2} \\
&= -s_{m,m+2}g_{m+2,m}\,f_{m-1} - (-1)^{m-y}s_{y,m+1}g_{m+1,y}\,f_x\,f_{p-1}\prod_{i=1}^{m-y} s_{y+i,y+i}g_{y+i,y+i}.
\end{aligned}$$

The sign of the first term is the opposite of f_{m-1}. The sign of the second term is $(-1)^{m-y+1}(-1)^{y-1} = (-1)^m$, which is also different from the sign of f_{m-1}. Overall, all leading principal minors of the Jacobian matrix (C. 1) alternate in sign starting with negative. Combining this with the fact that off-diagonal entries of (C. 1) are non-positive results in that the Jacobian matrix (C. 1) is a negative M-matrix.

Appendix D: Stability in networks with tree topology

We present a part with two branches of a metabolic network with tree. Two branching points are at metabolites X_p and X_w (D. 1). The first branch ends up on the metabolite X_y, the second branch ends up on the metabolite X_z, and the network potentially branches more.

$$\xrightarrow{v_1} X_1 \xleftrightarrow{v_2} \ldots \xleftrightarrow{v_p} X_p \xleftrightarrow{v_{p+1}} \ldots \xleftrightarrow{v_y} X_y \xrightarrow{v_{m+1}}$$

$$X_p \xleftrightarrow{v_{y+1}} \ldots \xleftrightarrow{v_w} X_w \xleftrightarrow{v_{w+1}} \ldots \xleftrightarrow{v_z} X_z \xrightarrow{v_{m+2}}$$

$$X_w \xleftrightarrow{v_{z+1}} \ldots$$

(D. 1)

Below we present a general structure of the Jacobian matrix for metabolic network with tree topology (D. 2). This part of the Jacobian matrix corresponds to two branches part of metabolic network with tree topology (D. 1), and continues with the similar structure if network branches more.

$$J(x)=\begin{pmatrix}
\boxed{P} & s_{p,p+1}g_{p+1,p+1} & s_{p,y+1}g_{y+1,y+1} & & \\
s_{p,y+1}g_{y+1,y+1} & \boxed{Y} & 0 & & \\
s_{p,y+1}g_{y+1,y+1} & 0 & \boxed{W} & s_{w+1,w}g_{w+1,w+1} & \\
 & & s_{w+1,w+1}g_{w+1,w} & \ddots \quad \ddots & \\
 & & & \ddots \quad \ddots & \\
 & & & & \boxed{M}
\end{pmatrix}$$

(D. 2)

Here the square diagonal submatrix W is

$$W=\begin{pmatrix}
-s_{y+1,y+1}g_{y+1,y+1}-s_{y+1,y+2}g_{y+2,y+1} & s_{y+1,y+2}g_{y+2,y+2} & & \\
s_{y+1,y+2}g_{y+2,y+2} & \ddots & \ddots & \\
 & \ddots & \ddots & s_{w-1,w}g_{w,w} \\
 & & s_{w,w}g_{w,w-1} & -s_{w,w}g_{w,w}-s_{w,w+1}g_{w+1,w}-s_{w,z+1}g_{z+1,w}
\end{pmatrix}. \quad \text{(D. 3)}$$

The diagonal submatrix *W* has the same structure as the submatrix *Y*, except for the presence of additional negative term $-s_{w,z+1}g_{z+1,w}$ in the last diagonal entry, which corresponds to the second branching, similar to the last diagonal entry in the submatrix *P* (C. 2). Depending on the number of next branches the structure of the matrix repeats further with *Y* and *W* submatrices, and with additional tridiagonal entries. Therefore, the matrix (D. 1) simply repeats the structure of the matrix (C. 1) with additional repetitions of it for every branch. Since the matrix (D. 1) repeats the structure of the matrix (C. 1) the proof for the alternation of signs of leading principal minors is the same. The presence of additional negative term in the diagonal entry of *W* submatrix does not influence the alternation of sign of leading principal minors. Combining this with the fact that off-diagonal entries of (D. 1) are nonnegative we conclude that the Jacobian matrix for SSSP metabolic networks with general stoichiometry and tree topology is negative M-matrix.

Appendix E: Stability in cyclic SS-SP networks

We prove Theorem 4 by showing condition for the Jacobian matrix (22) to be an M-matrix, and therefore the uniqueness and local asymptotic stability of steady state if it exists. The diagonal entries of $J(x)$ (22) are negative while the off-diagonal entries are positive. By showing condition for which all leading principal minors of $-J(x)$ are positive we get the condition for $-J(x)$ to be an M-matrix. Since leading principal minors of $-J(x)$ (22) are lower triangular with positive diagonal entries, they are always positive until the last one, which is the determinant of the Jacobian matrix. It remains to determine the condition for the determinant of $-J(x)$ to be positive.

The determinant of negative of the Jacobian matrix (22) matrix is

$$\begin{aligned}\det(-J(x)) &= (-1)^{1+m}(-s_{1,m+1}g_{m+1,m})(-1)^{m-1}\prod_{i=2}^{m} s_{i,i}g_{i,i-1} \\ &\quad + (-1)^{m+m} s_{m,m+1}g_{m+1,m} f_{m-1}.\end{aligned} \tag{E. 1}$$

Here the leading principal minor f_{m-1} is lower triangular and is equal to

$$f_{m-1} = (s_{k,k+1}g_{k+1,k} + s_{k,m+2}g_{m+2,k})\prod_{i=2,i\neq k}^{m} s_{i-1,i}g_{i,i-1}. \tag{E. 2}$$

Combining the equations (E. 1) and (E. 2) we get

$$\begin{aligned}\det(-J(x)) &= -s_{1,m+1}g_{m+1,m}\prod_{i=2}^{m} s_{i,i}g_{i,i-1} \\ &\quad + s_{m,m+1}g_{m+1,m}(s_{k,k+1}g_{k+1,k} + s_{m,m+2}g_{m+2,m})\prod_{i=2,i\neq k}^{m} s_{i-1,i}g_{i,i-1}.\end{aligned} \tag{E. 3}$$

The determinant is positive if

$$(s_{k,k+1}g_{k+1,k} + s_{m,m+2}g_{m+2,m})\prod_{i=2,i\neq k}^{m+1} s_{i-1,i} > s_{1,m+1}g_{k+1,k}\prod_{i=2}^{m} s_{i,i}. \tag{E. 4}$$

The determinant is positive if the inequality (23) in the Theorem 4 holds.