Supplementary information S4 (box): Motif H

**Purpose:** to prove that motif (H) (below, left), for which both the two-component and three-component feedback loops are positive, can exhibit bistability but cannot generate stable oscillations by a Hopf bifurcation.

\[
\begin{array}{c}
Y \\
\downarrow \\
\downarrow \\
\downarrow \\
X
\end{array}
\]

\[
J = \begin{pmatrix}
-a_x & a_y & \pm a_z \\
b_x & -b_y & 0 \\
0 & \pm c_y & -c_z
\end{pmatrix}
\]

The sign pattern of the Jacobian matrix for motif (H) is given above (right), where the \(a\)'s, \(b\)'s and \(c\)'s are all > 0. The stability of the steady state depends on the eigenvalues, \(\lambda\), of the Jacobian matrix, which are the roots of the characteristic equation:

\[
0 = \det \begin{pmatrix}
-a_x - \lambda & a_y & \mp a_z \\
b_x & -b_y - \lambda & 0 \\
0 & \mp c_y & -c_z - \lambda
\end{pmatrix}
\]

\[
0 = (\lambda + a_x)(\lambda + b_y)(\lambda + c_z) - a_x b_y (\lambda + c_z) - a_x b_z (\lambda + b_y)
\]

\[
0 = \lambda^3 + 3 \lambda^2 (a_x + b_y + c_z) + \lambda \left(2 a_x b_y + b_x c_z + a_x c_z - a_x b_z\right) + a_x b_y c_z - a_x b_z c_y - a_x b_y c_z
\]  
(S4.1)

In order for a Hopf bifurcation to occur, this algebraic equation must have a pair of pure imaginary roots, \(\lambda = \pm i \omega\). The necessary and sufficient condition for pure imaginary roots to equation (S4.1) is

\[
a_x b_y c_z - a_x b_z c_y - a_x b_y c_z = \left(a_x + b_y + c_z\right)\left(a_x b_y + b_x c_y + a_x c_z - a_x b_z\right)
\]

\[
a_x b_x (a_x + b_y) - a_x b_x c_y = 2a_x b_y c_z + a_x^2 (b_y + c_z) + b_x^2 (a_x + c_z) + c_y^2 (a_x + b_y)
\]

(S4.2)

If \(a_x b_y - a_x b_z > 0\), then equation (S4.2) cannot be satisfied for any choice of \(a_x\), etc. Hence, if motif (H) is to generate limit cycle oscillations by a Hopf bifurcation, then \(a_x b_y - a_x b_z\) must be < 0. But, in that case, the characteristic equation (S4.1) must have a real positive root, \(\lambda_1 > 0\), as well as a pair of pure imaginary eigenvalues. The bifurcating limit cycles must be unstable. We conclude that it is impossible for motif (H) to generate stable oscillations by a Hopf bifurcation.

On the other hand, \(\lambda = 0\) is a possible solution of equation (S4.1), if \(a_x b_y c_z = a_x b_y c_z + a_x b_z c_y\). Hence, motif (H) can generate multiple steady states by saddle-node bifurcations.
By a similar argument, we can come to the same conclusion for motif (H’) below:

\[
J = \begin{pmatrix}
-a_x & -a_y & 0 \\
-b_x & -b_y & \mp b_z \\
\mp c_x & 0 & -c_z
\end{pmatrix}
\]