Supporting Information

Metabolic dynamics restricted by conserved carriers:
jamming and feedback

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1 Original full model

Ordinary differential equations (ODEs) of the simplest model of the carrier cycling cascade (CCC) are given as equations of mass action kinetics, as follows:

\[
\begin{align*}
\frac{dm_0}{dt} &= k_{\text{in}} - k_{b0}[m_0][c] + k_{d0}[cm_0] - k_{\text{leak}}[m_0], \quad (1a) \\
\frac{dcm_0}{dt} &= k_{b0}[m_0][c] - k_{d0}[cm_0] - k_c[cm_0], \quad (1b) \\
\frac{dm_1}{dt} &= k_c[cm_0] - k_{b1}[m_1][c^*] + k_{d1}[c^*m_0], \quad (1c) \\
\frac{d[c^*m_1]}{dt} &= k_{b1}[m_1][c^*] - k_{d1}[c^*m_0] - k_p[c^*m_0], \quad (1d) \\
\frac{dm_2}{dt} &= k_p[c^*m_0] - k_{\text{out}}[m_2], \quad (1e) \\
\frac{dc}{dt} &= -k_{b0}[m_0][c] + k_{d0}[cm_0] + k_p[c^*m_0], \quad (1f) \\
\frac{d[c^*]}{dt} &= -k_{b1}[m_1][c^*] + k_{d1}[c^*m_0] + k_c[cm_0], \quad (1g)
\end{align*}
\]

where \( m_i \) represents the \( i \)-th metabolite, \( c \) and \( c^* \) are the active and inactive carriers, respectively, and \([x]\) denotes the concentration of \( x \). \( m_0 \) and \( m_1 \) can form complexes with the active and inactive carriers as \( cm_0 \) and \( c^*m_1 \) with the association rates \( k_{b0} \) and \( k_{b1} \), respectively, and these complexes can decompose with the dissociation rates \( k_{d0} \) and \( k_{d1} \), respectively. Active carriers are consumed with the rate \( k_c \) when \( m_1 \) is transformed from \( m_0 \) and are produced with rate \( k_p \) when \( m_2 \) is transformed from \( m_1 \). \( m_0 \) is supplied and diluted with rates \( k_{\text{in}} \) and \( k_{\text{leak}} \), respectively, and \( m_2 \) is diluted with rate \( k_{\text{out}} \). If we assume that \( k_c, k_p \ll k_{b1}, k_{d1} \), these equations can be reduced to five ODEs, and we calculated the five-variable model.

Here, we use the parameters presented in S1 Table unless otherwise noted.
Table S. 1: Parameters used in the simple CCC model.

| Parameter | Value |
|-----------|-------|
| $c_{\text{pool}}$ | 2.0 |
| $c_{\text{sum}}$ | 2.0 |
| $k_{\text{in}}$ | 1.0 |
| $k_c$ | 1.0 |
| $k_p$ | 1.0 |
| $k_{\text{leak}}$ | 0.001 |
| $K_0$ | $10^{-3}$ |
| $K_1$ | $10^{-3}$ |

2 Reversible model

ODEs of the reversible model are given as follows:

\[
\frac{d[m_0]}{dt} = k_{\text{in}} - k_c \frac{[m_0][c]}{K_0 + [c]} + k_{r1} \frac{[m_1][c^*]}{K_{r1} + [c^*]} - k_{\text{leak}} [m_0],
\]

(2a)

\[
\frac{d[m_1]}{dt} = k_c \frac{[m_0][c]}{K_0 + [c]} - k_{r1} \frac{[m_1][c^*]}{K_{r1} + [c^*]} - k_p \frac{[m_1][c^*]}{K_1 + [c^*]} + k_{r2} \frac{[m_2][c]}{K_{r2} + [c]},
\]

(2b)

\[
\frac{d[m_2]}{dt} = k_p \frac{[m_1][c^*]}{K_1 + [c^*]} - k_{r2} \frac{[m_2][c]}{K_{r2} + [c]} - k_{\text{out}} [m_2],
\]

(2c)

\[
\frac{d[c_t]}{dt} = -k_c \frac{[m_0][c]}{K_0 + [c]} + k_{r1} \frac{[m_1][c^*]}{K_{r1} + [c^*]} + k_p \frac{[m_1][c^*]}{K_1 + [c^*]} - k_{r2} \frac{[m_2][c]}{K_{r2} + [c]},
\]

(2d)

\[
\frac{d[c^*_t]}{dt} = k_c \frac{[m_0][c]}{K_0 + [c]} - k_{r1} \frac{[m_1][c^*]}{K_{r1} + [c^*]} - k_p \frac{[m_1][c^*]}{K_1 + [c^*]} + k_{r2} \frac{[m_2][c]}{K_{r2} + [c]},
\]

(2e)

\[
[c_t] = [c] + \frac{[m_0][c]}{K_0 + [c]} + \frac{[m_2][c]}{K_{r2} + [c]},
\]

(2f)

\[
[c^*_t] = [c^*] + \frac{[m_1][c^*]}{K_1 + [c^*]} + \frac{[m_1][c^*]}{K_{r1} + [c^*]},
\]

(2g)

where $k_{r1}$ and $k_{r2}$ are the speed of reactions from $m_1$ to $m_0$ and from $m_2$ to $m_1$, respectively. $K_{r1}$ and $K_{r2}$ are the dissociation constants between $m_1$ and $c^*$ and between $m_2$ and $c$, respectively. Here, we use the parameters presented in S2 Table.
Table S. 2: Parameters used in the reversible model.

| Parameter | Value |
|-----------|-------|
| $c_{\text{pool}}$ | 2.0 |
| $c_{\text{sum}}$ | 2.0 |
| $k_c$ | 1.0 |
| $k_p$ | 1.0 |
| $k_r1$ | 0.1 |
| $k_r2$ | 0.1 |
| $k_{\text{leak}}$ | 0.001 |
| $K_0$ | $10^{-3}$ |
| $K_1$ | $10^{-3}$ |
| $K_{r1}$ | $10^{-3}$ |
| $K_{r2}$ | $10^{-3}$ |

3 Analytical calculation of $k_{\text{in}}^{\text{th}}$

The reduced CCC model is given as:

\[
\frac{d[m_0]}{dt} = k_{\text{in}} - k_c \frac{[m_0][c]}{K_0 + [c]} - k_{\text{leak}}[m_0], \quad (3a)
\]

\[
\frac{d[m_1]}{dt} = k_c \frac{[m_0][c]}{K_0 + [c]} - k_p \frac{[m_1][c^*]}{K_1 + [c^*]}, \quad (3b)
\]

\[
[c] = \frac{-([m_0] + K_0 - c_{\text{sum}} + [m_1])}{2} + \sqrt{([m_0] + K_0 - c_{\text{sum}} + [m_1])^2 + 4K_0(c_{\text{sum}} - [m_1])}, \quad (3c)
\]

\[
[c^*] = \frac{-(K_1 + c_{\text{sum}} - c_{\text{pool}})}{2} + \sqrt{(K_1 + c_{\text{sum}} - c_{\text{pool}})^2 + 4K_1(c_{\text{pool}} - c_{\text{sum}} + [m_1])}, \quad (3d)
\]

Here, we obtained $k_{\text{in}}^{\text{th}}$ analytically. When $k_{\text{leak}}$ is zero, a nullcline for $[m_0]$ is analytically calculated as:

\[
[m_1] = c_{\text{sum}} - \frac{k_{\text{in}}}{k_c} + \frac{K_0k_{\text{in}}}{k_{\text{in}} - k_c[m_0]}.
\]
This nullcline converges into \( [m_1] = c_{\text{sum}} - \frac{k_{\text{in}}}{k_c} \) for \( [m_0] \to \infty \). Although a nullcline for \( [m_1] \) becomes too complicated, analysis of the limit of \( [m_0] \to \infty \) is helpful. In these limits, the first term of the right side in Eq.(3b) becomes \( k_c(c_{\text{sum}} - [m_1]) = k_c[c_1] \), because the maximum speed can be obtained as a multiplication of the turnover rate of the enzyme and the active carrier concentration. Thus, the nullcline in the limit of \( [m_0] \to \infty \) is obtained as:

\[
[m_1] = \frac{1}{2(k_c + k_p)} \left\{ c_{\text{sum}}(2k_c + k_p) - c_{\text{pool}}k_p - k_p\alpha \right. \\
+ \left. k_p\sqrt{(c_{\text{pool}} - c_{\text{sum}} + \alpha)^2 + 4c_{\text{sum}}\alpha} \right\},
\]

where \( \alpha = k_cK_1/(k_c + k_p) \). Therefore, from Eqs.(4) and (5), \( k_{\text{in}}^{\text{th}} \) is obtained as:

\[
k_{\text{in}}^{\text{th}} = \frac{k_ck_p}{2(k_c + k_p)} \left\{ c_{\text{pool}} + c_{\text{sum}} + \alpha \right. \\
+ \left. \sqrt{(c_{\text{pool}} - c_{\text{sum}} + \alpha)^2 + 4c_{\text{sum}}\alpha} \right\}.
\]

(6)

For the limit of \( K_1 \to 0 \), i.e., in the case where \( m_1 \) can perfectly bind to \( c^* \) and never unbind, Eq.(6) becomes:

\[
k_{\text{in}}^{\text{th}} = \begin{cases} 
\frac{k_c k_p c_{\text{sum}}}{(k_c + k_p)} & \text{if } c_{\text{pool}} > c_{\text{sum}}, \\
\frac{k_c k_p c_{\text{pool}}}{(k_c + k_p)} & \text{if } c_{\text{pool}} < c_{\text{sum}}.
\end{cases}
\]

(7a)

### 4 Analytical calculation of the frequency response of the CCC model

To obtain the frequency response analytically, we calculated the nullclines using the large \( [m_0] \) limit. Using the limit of \( [m_0] \to \infty \), the speed of the active coenzyme-consuming reaction becomes \( k_c(c_{\text{sum}} - [m_1]) = k_c[c_1] \). Therefore, the ODE and the nullcline for \( [m_0] \) can be approximated for large \( [m_0] \) as

\[
\frac{d[m_0]}{dt} \simeq k_{\text{in}} - k_c(c_{\text{sum}} - [m_1]) - k_{\text{leak}}[m_0],
\]

(8)

\[
[m_1] \simeq \frac{k_{\text{in}}}{k_c} [m_0] - \frac{k_c c_{\text{sum}} - k_{\text{in}}}{k_c}.
\]

(9)
respectively. A nullcline for \([m_1]\) in the large \([m_0]\) and small \(K_1\) limit is calculated from Eq.(5) as

\[ [m_1] \simeq \frac{k_c c_{\text{sum}}}{k_c + k_p} = \frac{k_{\text{in}}^{\text{th}}}{k_p}. \]  

Therefore, from Eqs.(9) and (10), the fixed point value of \([m_0]\) is given as:

\[ [m_0]^* = \frac{k_{\text{in}}}{k_{\text{leak}}} - \frac{k_c k_{\text{p}} c_{\text{sum}}}{k_{\text{leak}} (k_c + k_p)} \]

\[ = \frac{k_{\text{in}} - k_{\text{in}}^{\text{th}}}{k_{\text{leak}}}. \]  

(11)

When \(k_{\text{leak}}\) is small, two nullclines are close to the fixed point, and then the speed of change in \([m_0]\) on the nullcline for \([m_1]\) is much slower than that approaching the nullcline and is proportional to the distance between two nullclines. Following this, the two-dimensional dynamics (Eqs.(3a) and (3b)) can be reduced into one-dimensional dynamics of \([m_0]\) around the fixed point as

\[ \frac{d[m_0]}{dt} = -\frac{k_{\text{leak}}}{k_c} [m_0] - \frac{k_c c_{\text{sum}} - k_{\text{in}}(t)}{k_c} + \frac{c_{\text{sum}}}{k_c + k_p}. \]  

(12)

When \(k_{\text{in}}(t)\) is given as a sinusoidal function \(k_{\text{in}}(t) = A_{\text{in}} \cos(2\pi ft) + k_{\text{in}}^0\), \([m_0](t)\) at the steady state is calculated as

\[ [m_0](t) = A_{\text{in}} \frac{[(k_{\text{leak}}/k_c) \cos(2\pi ft) + 2\pi f \sin(2\pi ft)]}{(k_{\text{leak}}/k_c)^2 + (2\pi f)^2} + \frac{k_{\text{in}}^0 - k_{\text{in}}^{\text{th}}}{k_{\text{leak}}}. \]  

(13)

Here, the above approximations are feasible when \([m_0]\) is higher than \([m_0]^{\text{th}} = k_{\text{in}}^{\text{th}}/k_c\) due to saturation, which is the maximal \(m_0\) concentration processed in the first reaction per unit of time. When \([m_0]\) becomes lower than \([m_0]^{\text{th}}\) due to changes in the influx rate, the fixed point is drastically changed and the concentrations of \([m_1], [c]^1, \) and \([c]^3\) are altered. Therefore, the cut-off frequency is given as the frequency at which the minimal value of Eq.(13) is the same as \([m_0]^{\text{th}}\), as follows:

\[ 2\pi f = \frac{k_{\text{leak}} k_c A_{\text{in}}}{k_c k_{\text{in}}^0 - (k_c + k_{\text{leak}}) k_{\text{in}}^{\text{th}}} - k_{\text{leak}} k_c. \]  

(14)

The estimated cut-off frequency fits well with the result of our simulation (Fig. 4B in the main text), suggesting that the complex dynamics can be reduced into one-dimensional dynamics due to saturation, and the need for robustness against external fluctuation is determined by the conditions underlying this saturation.
5 Internal fluctuation of the metabolite concentration

We considered the stochastic dynamics of the number of $m_1$ molecules, $n$. First, we calculated the steady-state distribution of $n$ in a non-saturated condition, i.e., the $m_0$ concentration is lower than the maximal coenzyme concentration $c_{\text{max}}$. Here, we consider the following conditions: $c_{\text{sum}} < c_{\text{pool}}$ and $c_{\text{max}}$ is the same as $c_{\text{sum}}$. Using the limits of $K_0 \to 0$ and $K_1 \to 0$, i.e., the metabolites bind to coenzymes perfectly, the production rate of $m_1$ becomes $k_c[m_0]$, and the consumption rate of $m_1$ becomes $k_p n$ when the number of $m_1$ molecule is $n$. Here, only the consumption rate is proportional to $n$, while the production rate is not, so that the master equation can be given as follows:

$$\frac{dp_0}{dt} = k_p p_1 - k_c[m_0] p_0,$$
$$\frac{dp_n}{dt} = k_p (n+1)p_{n+1} + k_c[m_0]p_{n-1} - (k_p n + k_c[m_0]) p_n,$$
\hspace{1cm} (15)

where $p_n$ is the probability that the number of $m_1$ molecule is $n$. The steady-state distribution is the Poisson distribution:

$$p^*_n = \frac{1}{n!} \left( \frac{k_c[m_0]}{k_p} \right)^n e^{- \frac{k_c[m_0]}{k_p}}. \hspace{1cm} (16)$$

Therefore, both $<n>$ and $\sigma^2$ are given as $k_c[m_0]/k_p$ and $\sigma^2/<n>$ becomes 1, which is similar to a previously reported condition [1].

Under the saturated condition, i.e., the $m_0$ concentration is higher than $c_{\text{max}}$, because of coenzyme conservation, the production rate becomes $k_c(c_{\text{max}} - n)$ while the consumption rate remains the same as the non-saturated condition. Here, both the production and consumption rates are proportional to $n$. The master equation is thus given as follows:

$$\frac{dp_0}{dt} = k_p p_1 - k_c c_{\text{max}} p_0,$$
$$\frac{dp_n}{dt} = k_p (n+1)p_{n+1} + k_c(c - n + 1) p_{n-1} - \{k_p n + k_c(c - n)\} p_n,$$
$$\frac{dp_{c_{\text{max}}}}{dt} = k_c p_{c_{\text{max}}-1} - k_p c_{\text{max}} p_{c_{\text{max}}},$$
\hspace{1cm} (17)

The steady-state distribution is the binomial distribution:

$$p^*_n = \binom{c_{\text{max}}}{n} K^n (1 + K)^{-c_{\text{max}}}, \hspace{1cm} (18)$$
where $K$ is $k_c/k_p$. The moment-generating function is given as:

$$G(k) = \left( \frac{1 + Ke^k}{1 + K} \right)^{c_{\text{max}}},$$

(19)

and then,

$$< n > = G'(0) = \frac{c_{\text{max}} k_c}{k_c + k_p},$$

(20)

$$< n^2 > = G''(0) = \frac{c_{\text{max}} k_c}{k_c + k_p} \left\{ 1 + \left( \frac{c_{\text{max}} - 1}{k_c + k_p} \right) \right\},$$

(21)

$$\sigma^2 = \frac{c_{\text{max}} k_c}{k_c + k_p} \left( 1 - \frac{k_c}{k_c + k_p} \right),$$

(22)

$$< n > = 1 - \frac{k_c}{k_c + k_p}.$$  

(23)

Therefore, the fluctuation can be reduced depending on the $k_c$ and $k_p$ values (see Fig. 5B in the main text). The carrier cycling can improve the signal-to-noise ratio by feedback regulation through the conserved concentration of a carrier, and this effect does not depend on the concentration of a coenzyme as long as the metabolite is saturated before the coenzyme-consuming step. This suggests that the feedback in the CCC model can reduce the fluctuations of the active and inactive carrier concentrations because of the conserved quantities between $[c]_t$ and $[m_1]$.

To investigate the differences in the CCC from ordinal Michaelis-Menten type reactions, we considered a double Michaelis-Menten model; i.e., reactions from $m_0$ to $m_1$ and $m_1$ to $m_2$ are catalyzed by different catalysts. Under the non-saturated condition, the behavior of the double Michaelis-Menten model is similar to that of the CCC model, and the Fano factor is approximately 1. However, under the saturated condition, the concentration of $m_1$ shows a random walk (Fig. S6) when a similar parameter set as used for the CCC model was used, as demonstrated in Fig. 5A in the main text. As shown for the saturated condition, the Fano factor of the $m_1$ concentration never decreases in the double Michaelis-Menten model.

Furthermore, in the double Michaelis-Menten model, under the non-saturated condition, the master equation is the same as that of the CCC model, and a steady-state distribution is given as the Poisson distribution.
However, under the saturated condition, the master equation is given as

\[
\frac{dp_0}{dt} = k_p c_2 p_1 - k_c c_1 p_0, \\
\frac{dp_n}{dt} = k_p c_2 p_{n+1} + k_c c_1 p_{n-1} - \{k_p c_2 + k_c c_1\} p_n,
\]

(24)

where \(c_1\) and \(c_2\) represent the catalysis for the first and second step reactions, respectively. The steady-state distribution is given as

\[
p_n^s = \left( \frac{k_c c_1}{k_p c_2} \right)^n p_0^s.
\]

(25)

Here, if \(k_c c_1/k_p c_2\) is higher than 1, the time evolution of the number of \(m_1\) is governed by the asymmetric random walk and the number of \(m_1\) will diverge. If \(k_c c_1/k_p c_2\) is 1, the time evolution of the number of \(m_1\) is governed by the symmetric random walk from 0 to \(\infty\), as shown in S6 Fig. If \(k_c c_1/k_p c_2\) is lower than 1, the steady-state distribution can be given as a geometric distribution:

\[
p_n^s = \left( 1 - \frac{k_c c_1}{k_p c_2} \right) \left( \frac{k_c c_1}{k_p c_2} \right)^n.
\]

(26)

The average and variance are:

\[
< n > = \frac{k_c c_1}{k_p c_2 - k_c c_1},
\]

(27)

\[
\sigma^2 = \frac{k_c c_1 k_p c_2}{(k_p c_2 - k_c c_1)^2},
\]

(28)

\[
\frac{\sigma^2}{< n >} = \frac{k_p c_2}{k_p c_2 - k_c c_1} = \frac{1}{1 - k_c c_1/k_p c_2} > 1.
\]

(29)

Therefore, in the double Michaelis-Menten model, \(\sigma^2/ < n >\) should be higher than 1 in all cases.

### 6 Coupled carrier cycling cascade (CCCC) model

We considered the condition in which two carrier cycling cascades are coupled through a common coenzyme pool. Association and dissociation reactions
between the coenzyme and substrates are eliminated adiabatically, as in the single cascade model, and the ODEs can be given as:

\[
\begin{align*}
\frac{d[m_0]}{dt} &= k_{i_{\text{in}}} - k_{i_{\text{c}}} \frac{[m_0][c]}{K_0 + [c]} - k_{i_{\text{leak}}}[m_0], \\
\frac{d[m_1]}{dt} &= k_{i} \frac{[m_0][c]}{K_0 + [c]} - k_{i_{\text{p}}} \frac{[m_1][c^*]}{K_1 + [c^*]}, \\
\frac{d[m_2]}{dt} &= k_{i_{\text{p}}} \frac{[m_1][c^*]}{K_1 + [c^*]} - k_{i_{\text{out}}}[m_2], \\
\frac{d[c]}{dt} &= \sum_{i=1}^{N} \left\{ -k_{i_{\text{c}}} \frac{[m_0][c]}{K_0 + [c]} + k_{i_{\text{p}}} \frac{[m_1][c^*]}{K_1 + [c^*]} \right\}, \\
\frac{d[c^*]}{dt} &= \sum_{i=1}^{N} \left\{ k_{i_{\text{c}}} \frac{[m_0][c]}{K_0 + [c]} - k_{i_{\text{p}}} \frac{[m_1][c^*]}{K_1 + [c^*]} \right\},
\end{align*}
\]

\[
\begin{align*}
[c]_t &= [c] + \sum_{i=1}^{N} \frac{[m_0][c]}{K_0 + [c]}, \\
[c^*]_t &= [c] + \sum_{i=1}^{N} \frac{[m_1][c^*]}{K_1 + [c^*]},
\end{align*}
\]

where \( i \) represents an index of cascade numbers. Although we set \( N \) to 2, i.e., two CCCs are coupled (S7 Fig), the obtained results do not change for higher \( N \) when the following conditions are satisfied.

The CCCC shows a qualitatively similar response to the environmental changes as the CCC when one cascade is a major pathway. Here, we considered two examples:

1. \( c \) and \( m_2^0 \) with a larger dissociation constant than \( c \) and \( m_1^0 \).
2. The influx rate (leak rate) of \( m_2^0 \) is smaller (larger) than that of \( m_1^0 \).

In both cases, cascade 1 is the major cascade, and the CCCC displays responses to environmental changes in a similar way as observed for the CCC (S8 Fig). Therefore, our findings should be applicable to more complicated metabolic networks as well.

For these, we used the parameter set presented in S3 Table, unless otherwise specified.
Table S.3: Parameter set used in a CCCC model

| Parameter | Value |
|-----------|-------|
| $c_{pool}$ | 2.0   |
| $c_{sum}$  | 2.0   |
| $k_{in}^{2}$ | 1.0   |
| $k_{c}^{1}$ | 1.0   |
| $k_{c}^{2}$ | 1.0   |
| $k_{p}^{1}$ | 1.0   |
| $k_{p}^{2}$ | 1.0   |
| $k_{leak}^{1}$ | 0.001 |
| $k_{leak}^{2}$ | 0.001 |
| $K_{0}^{1}$ | $10^{-3}$ |
| $K_{0}^{2}$ | $10^{-3}$ |
| $K_{1}^{1}$ | $10^{-3}$ |
| $K_{1}^{2}$ | $10^{-3}$ |

References

[1] Levine E, Hwa T. Stochastic fluctuations in metabolic pathways. Proc Natl Acad Sci USA. 2007; 104: 9224-9229.

7 Supporting Figures
Fig S. 1

Fig S. 2
Fig S. 3

Fig S. 4
Fig S. 5

Fig S. 6

Fig S. 7
FigS. 8