Supplementary Material

Derivation of Eq. 1a. Assume J_j is a function of the rate constants for the N component reactions:

$$J_j = J_j\,(k_1, k_2, \ldots, k_i, \ldots, k_N) \tag{4}$$

The derivative with respect to temperature T is calculated by using the chain rule:

$$\frac{dJ_j}{dT} = \sum_i \left(\frac{\partial J_j}{\partial k_i}\right)\left(\frac{\partial k_i}{\partial T}\right) \tag{5}$$

The last term in eq. 5 can be explicitly calculated from the Arrhenius equation, i.e.,

$$k_i = A_i e^{-\frac{E_a^i}{RT}} \qquad \Rightarrow \qquad \frac{\partial k_i}{\partial T} = \frac{E_a^i}{RT^2}\left(A_i e^{-\frac{E_a^i}{RT}}\right) = \frac{E_a^i}{RT^2} k_i \tag{6}$$

which gives

$$\frac{dJ_j}{dT} = \sum_i \left(\frac{\partial J_j}{\partial k_i}\right)\frac{E_a^i}{RT^2} k_i = \sum_i \left(\frac{\partial J_j}{\partial \ln k_i}\right)\frac{E_a^i}{RT^2} \tag{7}$$

by using $\partial k_i / k_i = \partial \ln k_i$. Multiplying eq. 7 by T/J_j and observing that $dJ_j/J_j = d\ln J_j$, and $T/dT = 1/d\ln T$, Eq. 7 can be written as:

$$\frac{T}{J_j}\frac{\partial J_j}{\partial T} = \frac{d\ln J_j}{d\ln T} = \frac{1}{RT}\sum_i \left(\frac{\partial \ln J_j}{\partial \ln k_i}\right) E_a^i = \frac{1}{RT}\sum_i {}^{*} C_i^{J_j} E_a^i \tag{8}$$

This shows that the activation enthalpy of a pathway flux is equal to the weighted average of the activation enthalpies of the steps, where the enthalpies are normalized by RT and the weighting factors are the flux control coefficients.

Derivation of Eq. 1b. Each reaction step is catalyzed by an enzyme and for the sake of simplicity we assume that each step can be described by Michaelis-Menten kinetics with non-saturating enzymes following first-order kinetics with respect to substrates, i.e.

$$J_j = J_j(\frac{k_1 e_1}{K_1}, \frac{k_2 e_2}{K_2}, \ldots.., \frac{k_i e_i}{K_i}, \ldots..., \frac{k_N e_N}{K_N}) \tag{9}$$

where k_i is the turnover number, e_i the concentration of enzyme *i*, and K_i is a dissociation constant of a rapid equilibrium (or a dynamic equilibrium constant) between enzyme and substrates. Using the chain rule one obtains

$$\frac{d\ln J_j}{dT} = \frac{1}{J_j}\sum_i \left(\frac{\partial J_j}{\partial k_i}\right)\left(\frac{\partial k_i}{\partial T}\right) + \frac{1}{J_j}\sum_m \left(\frac{\partial J_j}{\partial e_m}\right)\left(\frac{\partial e_m}{\partial T}\right) + \frac{1}{J_j}\sum_l \left(\frac{\partial J_j}{\partial K_l}\right)\left(\frac{\partial K_l}{\partial T}\right) \tag{10}$$

Because k_i and K_l have an Arrhenius-type temperature dependence [1, 21], the first and the last terms in Eq. 10 can be written as (see Eqs. (6)-(8))

$$\frac{d\ln J_j}{dT} = \sum_i \frac{E_a^{k_i}}{RT^2}\left(\frac{\partial \ln J_i}{\partial \ln k_i}\right) + \frac{1}{J_j}\sum_m \frac{e_m}{e_m}\left(\frac{\partial J_j}{\partial e_m}\right)\left(\frac{\partial e_m}{\partial T}\right) + \sum_l \frac{E_a^{K_i}}{RT^2}\left(\frac{\partial \ln J_j}{\partial \ln K_l}\right) \tag{11}$$

Because $\frac{e_m}{J_j}\frac{\partial J_j}{\partial e_m} = \frac{k_m}{J_j}\frac{\partial J_j}{\partial k_m} = \frac{\partial \ln J_j}{\partial \ln k_m} = C_{k_m}^{J_j}$ we can finally write

$$\frac{d\ln J_j}{dT} = \sum_i \frac{E_a^{k_i}}{RT^2} C_{k_i}^{J_j} + \frac{1}{J_j}\sum_m C_{k_m}^{J_j}\left(\frac{d\ln e_m}{dT}\right) + \sum_l \frac{E_a^{K_i}}{RT^2}\left(\frac{\partial \ln J_j}{\partial \ln K_l}\right) \tag{12}$$

Multiplication with *T* and using the definition of the response coefficients

$R_T^{e_m} = \frac{d\ln e_m}{d\ln T}$, $R_{K_l}^{J_j} = \frac{d\ln J_j}{d\ln K_l}$ yields Eq. 1b.

Reaction schemes where *J'* cannot be temperature compensated (Fig. 2b)

Scheme (1). We consider the irreversible case, but the analysis can easily be applied to the reversible situation. The rate equations at steady state conditions are:

$$\begin{aligned} \frac{d[A]}{dt} &= k_1 - k_2[A] = 0 \\ \frac{d[B]}{dt} &= k_2[A] - k_3[B] = 0 \\ \frac{d[C]}{dt} &= k_3[B] - k_4[C] = 0 \\ \frac{d[D]}{dt} &= k_4[C] - J' = 0 \end{aligned} \tag{13}$$

Summing up the four equations leads to $J'=k_1$ showing that $C_1=1$, while the other control coefficients are zero.

Scheme (2). We are looking at the irreversible clockwise loop. The rate equations at steady state conditions are:

$$\begin{aligned} \frac{d[A]}{dt} &= k_1 + k_5[D] - k_2[A] = 0 \\ \frac{d[B]}{dt} &= k_2[A] - k_3[B] = 0 \\ \frac{d[C]}{dt} &= k_3[B] - k_4[C] = 0 \\ \frac{d[D]}{dt} &= k_4[C] - J' - k_5[D] = 0 \end{aligned} \tag{14}$$

Summing up the four equations leads to $J'=k_1$ showing that $C_1=1$, while the other control coefficients are zero.

Scheme (3). Although this scheme contains a branch point, flux $J' = k_1$ showing that $C_1=1$, while the other control coefficients are zero.

Scheme (4). Reaction species D inhibits process *2* by replacing k_2 by $\frac{k_2}{K_I + [D]^m}$.

$$\begin{aligned}
\frac{d[A]}{dt} &= k_1 - \frac{k_2[A]}{K_I + [D]^m} = 0 \\
\frac{d[B]}{dt} &= \frac{k_2[A]}{K_I + [D]^m} - k_3[B] = 0 \\
\frac{d[C]}{dt} &= k_3[B] - k_4[C] = 0 \\
\frac{d[D]}{dt} &= k_4[C] - J' = 0
\end{aligned} \tag{15}$$

The sum of the four equations leads to $J'=k_1$ showing that $C_1=1$, while the other control coefficients are zero.

Scheme (5). Like in scheme (4), the contributions of the (positive) feedback loop cancel and flux $J'=k_1$ leading to $C_1=1$ and the other C_i's = 0.

Scheme (6). We consider a negative feedforward to the output flux *J'*. The rate equations read:

$$\begin{aligned}
\frac{d[A]}{dt} &= k_1 - k_2[A] = 0 \\
\frac{d[B]}{dt} &= k_2[A] - k_3[B] = 0 \\
\frac{d[C]}{dt} &= k_3[B] - k_4[C] = 0 \\
\frac{d[D]}{dt} &= k_4[C] - \frac{k'}{K_I + [B]^m}[D] = 0
\end{aligned} \tag{16}$$

The steady state output flux *J'* is given by:

$$J' = \frac{k'}{K_I + [B]_{SS}^m}[D]_{SS} = k_1 \tag{17}$$

showing that $C_1=1$, while the other control coefficients are zero.

Scheme (7). Reaction species *A* activates reaction *4* (positive feedforward).

$$\begin{aligned}\frac{d[A]}{dt} &= k_1 - k_2[A] = 0\\ \frac{d[B]}{dt} &= k_2[A] - k_3[B] = 0\\ \frac{d[C]}{dt} &= k_3[B] - k_4 k[A]^n[C] = 0\\ \frac{d[D]}{dt} &= k_4 k[A]^n[C] - J' = 0\end{aligned} \tag{18}$$

The sum of the four equations leads to $J'=k_1$ showing that $C_1=1$, while the other control coefficients are zero. The same result we get for the activation of reaction *4* by *B* or the activation of reaction *3* by *A*.

The rate equations of a positive feedforward from *B* to an output flux read:

$$\begin{aligned}\frac{d[A]}{dt} &= k_1 - k_2[A] = 0\\ \frac{d[B]}{dt} &= k_2[A] - k_3[B] = 0\\ \frac{d[C]}{dt} &= k_3[B] - k_4[C] = 0\\ \frac{d[D]}{dt} &= k_4[C] - k' k[B]^n[D] = 0\end{aligned} \tag{19}$$

The steady state output flux *J'* is given by:

$$J' = k' k[B]_{SS}^n [D]_{SS} = k_1 \tag{20}$$

leading to $C_1 = 1$, while the other control coefficients are zero.

Reaction schemes where *J'* can be temperature compensated (Fig. 2c)

Scheme (8). Calculating the steady state values:

$$\begin{aligned}
\frac{d[A]}{dt} &= k_1 - k_2[A] = 0 && \Rightarrow [A]_{SS} = \frac{k_1}{k_2} \\
\frac{d[B]}{dt} &= k_2[A] - (k_3 + k_4)[B] = 0 && \Rightarrow [B]_{SS} = \frac{k_1}{k_3 + k_4} \\
\frac{d[C]}{dt} &= k_3[C] - k_5[C] = 0 && \Rightarrow [C]_{SS} = \frac{k_3}{k_5}[B]_{SS} \\
\frac{d[D]}{dt} &= k_4[B] - J' = 0 && \Rightarrow [D]_{SS} = \frac{k_4}{k'}[B]_{SS} = \frac{k_1 k_4}{k'(k_3 + k_4)}
\end{aligned} \tag{21}$$

Because $J'=k'[D]_{SS}$, we get $J' = \frac{k_1 k_4}{k_3 + k_4}$. We can write $C_3 = \frac{\partial \ln J'}{\partial \ln k_3} = \frac{k_3}{J'} \cdot \frac{\partial J'}{\partial k_3} = -\frac{k_3}{k_3 + k_4}$ and $C_4 = \frac{\partial \ln J'}{\partial \ln k_4} = \frac{k4}{J'} \cdot \frac{\partial J'}{\partial k_4} = +\frac{k_3}{k_3 + k_4}$ showing that $C_3=-C_4$. Likevise it can be shown that $C_1=1$.

Scheme (9).

$$\begin{aligned}
\frac{d[A]}{dt} &= k_1 - (k_2 + k_6)[A] = 0 \\
\frac{d[B]}{dt} &= k_2[A] - (k_3 + k_7)[B] = 0 \\
\frac{d[C]}{dt} &= k_3[B] - (k_4 + k_8)[C] = 0 \\
\frac{d[D]}{dt} &= k_4[C] - (k' + k_9)[D] = 0
\end{aligned} \tag{22}$$

By successive elimination of the steady state values for A, B, C, and D in the equations, we get:

$$J' = k'[D]_{SS} = k' \frac{k_1 k_2 k_3 k_4}{(k_2 + k_6)(k_3 + k_7)(k_4 + k_8)(k' + k_9)} \tag{23}$$

Calculating the C_i's, one get that $C_1=1$ and

$C_2 = -C_6 = \frac{k_6}{k_2 + k_6}$; $C_3 = -C_7 = \frac{k_7}{k_3 + k_7}$; $C_4 = -C_8 = \frac{k_8}{k_4 + k_8}$; $C' = -C_9 = \frac{k_9}{k' + k_9}$. In order to temperature compensate fluxes J_6, J_7, J_8 and J' simultaneously, vi observe that when compensating first J_6, this flux depends only on k_1, k_2 and k_6 with $C_1^{J_6} = 1$ and

$C_2^{J_6} = -C_6^{J_6} = -\frac{k_2}{k_2 + k_6}$ while all the other control coefficients are zero. This leads to the balance equation

$$E_1 + C_2^{J_6}(E_2 - E_6) = 0 \tag{24}$$

which determines the values of E_1, E_2 and E_6. The condition to temperature compensate J_7 is:

$$E_1 + C_2^{J_7}(E_2 - E_6) + C_3^{J_7}(E_3 - E_7) = 0 \tag{25}$$

Because $C_2^{J_6}$ is generally different from $C_2^{J_7}$, the sum $E_1 + C_2^{J_7}(E_2 - E_6)$ in Eq. 20 need not to be zero, but E_3 and E_7 can be chosen such that Eq. 20 is satisfied. In this way J_6, J_7 and J_8 and J' can be simultaneously temperature compensated when solving the balance equations 19-22.

$$E_1 + C_2^{J_8}(E_2 - E_6) + C_3^{J_8}(E_3 - E_7) + C_4^{J_8}(E_4 - E_8) = 0 \tag{26}$$

$$E_1 + C_2^{J'}(E_2 - E_6) + C_3^{J'}(E_3 - E_7) + C_4^{J'}(E_4 - E_8) + C^{J'}(E' - E_9) = 0 \tag{27}$$

Scheme 10. The rate equations of this cyclic scheme with steady state conditions read:

$$\begin{aligned}
\frac{d[A]}{dt} &= k_1 - k_2[A] + k_5[D] = 0\\
\frac{d[B]}{dt} &= k_2[A] - (k_3 + k_6)[B] = 0\\
\frac{d[C]}{dt} &= k_3[B] - (k_4 + k_7)[C] = 0\\
\frac{d[D]}{dt} &= k_4[C] - (k' + k_5)[D] = 0
\end{aligned} \tag{28}$$

By successively eliminating the steady state values $[A]_{SS}$, $[B]_{SS}$ and $[C]_{SS}$, an expression for $[D]_{SS}$ and J' can be obtained:

$$J' = k' \frac{k_1 k_3 k_4}{N} \tag{29}$$

where $N = k'k_6k_7 + k'k_4k_6 + k'k_3k_7 + k'k_3k_4 + k_5k_6k_7 + k_4k_5k_6 + k_3k_5k_7$. Calculating the control coefficients as $C_i = \frac{k_i}{J'}\left(\frac{\partial J'}{\partial k_i}\right)$, we get:

$C_1 = 1$, $C_2 = 0$, $C_3 = \frac{k_6(k'k_7 + k'k_4 + k_5k_7 + k_5k_4)}{N}$, $C_4 = \frac{k_7(k'k_6 + k'k_3 + k_5k_6 + k_5k_3)}{N}$,

$C_5 = -\frac{k_5(k_6k_7 + k_6k_4 + k_3k_7)}{N}$, $C_6 = -C_3$, $C_7 = -C_4$, $C' = -C_5$ with $\sum_{i=1}^{7} C_i + C' = 1$.

Scheme (11). Inhibition of J_1 by any intermediate. Vi take as an example the inhibition of J_1 by C with Hill coefficient $m = 1$. The rate equations with steady state conditions are:

$$\begin{aligned}
\frac{d[A]}{dt} &= \frac{k_1}{K_I + [C]^1} - k_2[A] = 0 \\
\frac{d[B]}{dt} &= k_2[A] - k_3[B] = 0 \\
\frac{d[C]}{dt} &= k_3[B] - k_4[C] = 0 \\
\frac{d[D]}{dt} &= k_4[C] - k'[D] = 0
\end{aligned} \tag{30}$$

leading to $J' = k'[D]_{SS} = \dfrac{k_1}{K_I + [C]_{SS}}$. Eliminating the variables A and B and determining the positive solution of the steady state value of C:

$$[C]_{SS} = \frac{-k_4K_I + \sqrt{(k_4K_I)^2 + 4k_1k_4}}{2k_4} \tag{31}$$

J' can be expressed as:

$$J' = \frac{2k_1k_4}{k_4K_I + \sqrt{k_4M}}; \qquad M = (k_4K_I{}^2 + 4k_1) \tag{32}$$

Calculating the control coefficients as $C_i = \dfrac{k_i}{J'}\left(\dfrac{\partial J'}{\partial k_i}\right)$, we get:

$$C_1 = \frac{k_4(K_I\sqrt{k_4M} + k_4K_I{}^2 + 2k_1)}{(k_4K_I + \sqrt{k_4M})\cdot\sqrt{k_4M}},\ C_4 = \frac{2k_1k_4}{(k_4K_I + \sqrt{k_4M})\cdot\sqrt{k_4M}},\ C_{K_I} = -\frac{k_4K_I}{(k_4K_I + \sqrt{k_4M})};$$

$C_2 = C_3 = C' = 0$. Note, temperature compensation is only possible because the inhibitor constant K_I (which can be interpreted as a dissociation constant in rapid equilibrium between the enzyme that catalyzes step *1* and the inhibitor C) is assumed to be temperature dependent. The temperature dependence of K_I can be described analogously to the Arrhenius equation by substituting the activation enthalpy with the enthalpy of formation ΔH_I^0. In this case the pre-exponential factor A_I can still be treated as temperature independent with $A_I = \exp(-\Delta S_I^0/R)$ [1]. It should also be noted that when treating K_I as a temperature dependent parameter, then the sum of the control coefficients is generally not one. In this case we get:

$$\sum_{i=1}^{4} C_i + C_{K_I} + C' = \frac{4k_1k_4}{(k_4K_I + \sqrt{k_4M})\sqrt{k_4M}} \tag{33}$$

Scheme (12). Activation of J_1 by any intermediate. In order to avoid an exponential increase of concentrations, *n* needs to be lower than 1. As an example, we look at the activation of reaction *1* by intermediate *C*. The rate equations with steady state conditions are:

$$\begin{aligned}
\frac{d[A]}{dt} &= k_1 k[C]^n - k_2[A] = 0, \qquad 0 < n < 1 \\
\frac{d[B]}{dt} &= k_2[A] - k_3[B] = 0 \\
\frac{d[C]}{dt} &= k_3[B] - k_4[C] = 0 \\
\frac{d[D]}{dt} &= k_4[C] - k'[D] = 0
\end{aligned} \qquad (34)$$

Summing up all steady state equations leads to

$$J' = k'[D]_{SS} = k_1 k[C]_{SS}^n \qquad (35)$$

When adding only the first three equations, we get $k_1 k[C]_{SS}^n - k_4[C]_{SS} = 0$. Solving for $[C]_{SS}$ from the last equation and multiplying $[C]_{SS}$ with k_4, we get:

$$J' = k_3 \left(\frac{k_1 k}{k_3} \right)^{-\frac{1}{n-1}} \qquad (36)$$

Calculating the control coefficients as $C_i = \frac{k_i}{J'} \left(\frac{\partial J'}{\partial k_i} \right)$, we get: $C_1 = C_k = -\frac{1}{n-1}$, $C_3 = \frac{n}{n-1}$, while all other C_i are zero. The sum $\sum_{i=1}^{4} C_i + C' + C_k = \frac{n-2}{n-1}$ shows that in case of a feedback activation of an input flux using n < 1 the summation theorem is not obeyed.

A Simple Representation of the Calvin Benson Cycle

Fig. 5c shows a simple representation of the Calvin Benson cycle with its 3 stages: (i) the reduction phase (flux J_2) due to influx of ATP and NADPH produced by the light reaction together with the output flux of P_i, (ii) the regeneration phase (flux J_3) with the output flux J_6 forming carbohydrates, and (iii) the ATP/NADPH-driven carboxylation phase (flux $J_4^{CO_2}$) assimilating CO_2 [32]. The rate constant $k_4^{CO_2}$ is assumed to be dependent on the partial but constant CO_2 pressure.

The rate equations and steady state conditions are given by the following equations:

$$\begin{aligned}\frac{d[A]}{dt} &= k_3[C] - (k_4^{CO_2} + k_6)[A] = 0\\ \frac{d[B]}{dt} &= k_4^{CO_2}[A] + k_1 - k_2[B] = 0\\ \frac{d[C]}{dt} &= k_2[B] - (k_3 + k_5)[C] = 0\end{aligned} \tag{37}$$

Eliminating variables C and B and calculating $J_4^{CO_2} = k_4^{CO_2}[A]_{SS}$ leads to

$$J_4^{CO_2} = \frac{k_4^{CO_2} k_1 k_3}{L}, \quad L = k_5 k_4^{CO_2} + k_5 k_6 + k_3 k_6 \tag{38}$$

The control coefficients are $C_1 = 1$, $C_2 = 0$, $C_3 = -C_5 = \frac{k_5(k_4 + k_6)}{L}$, and

$C_4 = -C_6 = \frac{k_6(k_5 + k_3)}{L}$. The assimilation of CO_2 can be temperature compensated because of the ”balance” between the input and output fluxes.