Supporting Information (10 pages)

Predicting perfect adaptation motifs in reaction kinetic networks

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Derivation of the Transfer function $H_{B,k_2}(s)$ for motif S1, Eq. 5

Consider motif S1 (main paper):

$$\underset{k_{-1}}{\overset{k_1}{\rightleftarrows}} \mathrm{A} \underset{k_{-2}}{\overset{k_2}{\rightleftarrows}} \mathrm{B} \underset{k_{-3}}{\overset{k_3}{\rightleftarrows}} \tag{S1}$$

with the rate equations:

$$\frac{dA(t)}{dt} = \dot{A}(t) = k_1(t) - k_2(t)A(t) + k_{-2}(t)B(t) - k_{-1}(t)A(t) \tag{A1}$$

$$\frac{dB(t)}{dt} = \dot{B}(t) = k_2(t)A(t) - k_3(t)B(t) - k_{-2}(t)B(t) + k_{-3}(t) \tag{A2}$$

and the rate constants $k_1(t)$, $k_2(t)$, $k_3(t)$, $k_{-1}(t)$, $k_{-2}(t)$ and $k_{-3}(t)$.

The steady state concentrations A_{ss} and B_{ss} are given by Eq. A3 and no perfect adaptation can exist, because both A_{ss} and B_{ss} depend on all rate constants such that for all k_i we have nonzero control coefficients, i.e., $C_{k_i}^{A_{ss}} \neq 0$ and $C_{k_i}^{B_{ss}} \neq 0$.

$$A_{ss} = \frac{k_{-2}(k_{-3}+k_1) + k_1 k_3}{k_3(k_{-1}+k_2) + k_{-1}k_{-2}}; B_{ss} = \frac{k_{-3}(k_{-1}+k_2) + k_1 k_2}{k_3(k_{-1}+k_2) + k_{-1}k_{-2}} \tag{A3}$$

However, by introducing an irreversible *input* to the system (as indicated in the main paper), for example by setting $k_{-1} = 0$, robust perfect adaptation in $B(t)$ with respect to a step-wise change in k_2 (or k_{-2}) can be observed. The reason for this is that $B(t)$ is now independent of k_2 or k_{-2}, because $B_{ss} = \frac{k_1+k_{-3}}{k_3}$. However, $B(t)$ is still connected to k_2 and k_{-2} as can be seen by inspecting the transfer function $H_{B,k_2}(s)$ for the entire scheme S1, Eq. 5 (see below).

A_{ss}, however, still depends on the remaining rate constants and therefore shows no perfect adaptation. Introducing irreversibility in scheme S1 by setting $k_{-2} = 0$ or/and $k_{-3} = 0$, will not lead to perfect adaptation for neither A nor B, because also in these cases A_{ss} and B_{ss} still depend on all the other rate constants.

In order to find the transfer function matrix $H(s)$, the system is linearized around the steady states values A_{ss} and B_{ss}, and the rate constants k_1, k_2, k_3, k_{-1}, k_{-2} and k_{-3}, which gives the following linear model

$$\begin{aligned}\Delta\dot{A}(t) =& -(k_2+k_{-1})\Delta A(t) + k_{-2}\Delta B(t)\\ &+ \Delta k_1(t) - A_{ss}\Delta k_2(t) - A_{ss}\Delta k_{-1}(t) + B_{ss}\Delta k_{-2}(t) \qquad \text{(A4)}\\ \Delta\dot{B}(t) =& k_2\Delta A(t) - (k_3+k_{-2})\Delta B(t)\\ &+ A_{ss}\Delta k_2(t) - B_{ss}\Delta k_3(t) - B_{ss}\Delta k_{-2}(t) + \Delta k_{-3}(t) \qquad \text{(A5)}\end{aligned}$$

or, in matrix form

$$\begin{bmatrix}\Delta\dot{A}(t)\\ \Delta\dot{B}(t)\end{bmatrix} = \begin{bmatrix}-(k_2+k_{-1}) & k_{-2}\\ k_2 & -(k_3+k_{-2})\end{bmatrix}\begin{bmatrix}\Delta A(t)\\ \Delta B(t)\end{bmatrix} + \begin{bmatrix}1 & -A_{ss} & 0 & -A_{ss} & B_{ss} & 0\\ 0 & A_{ss} & -B_{ss} & 0 & -B_{ss} & 1\end{bmatrix}\begin{bmatrix}\Delta k_1(t)\\ \Delta k_2(t)\\ \Delta k_3(t)\\ \Delta k_{-1}(t)\\ \Delta k_{-2}(t)\\ \Delta k_{-3}(t)\end{bmatrix} \qquad \text{(A6)}$$

The transfer function matrix can be found from the relationship (Eq. 3, main paper):

$$H(s) = \frac{\Delta\mathbf{I}(s)}{\Delta\mathbf{k}(s)} = (sI - A)^{-1}B \qquad \text{(A7)}$$

where $\Delta\mathbf{k}(s) = [\Delta k_1(s),\ \ \Delta k_2(s),\ \ \Delta k_3(s),\ \ \Delta k_{-1}(s),\ \ \Delta k_{-2}(s),\ \ \Delta k_{-3}(s)]^T$, and $\Delta\mathbf{I}(s) = [\Delta A(s),\ \ \Delta B(s)]^T$. Applying Eq. A7 to the linear model in Eq. A6 gives the following transfer function matrix

$$H(s) = \begin{bmatrix} s+k_2+k_{-1} & -k_{-2} \\ -k_2 & s+k_3+k_{-2} \end{bmatrix}^{-1} \begin{bmatrix} 1 & -A_{ss} & 0 & -A_{ss} & B_{ss} & 0 \\ 0 & A_{ss} & -B_{ss} & 0 & -B_{ss} & 1 \end{bmatrix}$$

$$= \frac{1}{(s+k_2+k_{-1})(s+k_3+k_{-2}) - k_2k_{-2}} \cdot \begin{bmatrix} s+k_3+k_{-2} & k_{-2} \\ k_2 & s+k_2+k_{-1} \end{bmatrix} \cdot \begin{bmatrix} 1 & -A_{ss} & 0 & -A_{ss} & B_{ss} & 0 \\ 0 & A_{ss} & -B_{ss} & 0 & -B_{ss} & 1 \end{bmatrix}$$

$$= \frac{1}{(s+k_2+k_{-1})(s+k_3+k_{-2}) - k_2k_{-2}} \cdot \left[\begin{matrix} s+k_3+k_{-2} & -A_{ss}(s+k_3) & -B_{ss}k_{-2} & \dots \\ k_2 & A_{ss}(s+k_{-1}) & -B_{ss}(s+k_2+k_{-1}) & \dots \end{matrix} \right.$$
$$\left. \begin{matrix} \dots & -A_{ss}(s+k_3+k_{-2}) & B_{ss}(s+k_3) & k_{-2} \\ \dots & -A_{ss}k_2 & B_{ss}(s+k_{-1}) & s+k_2+k_{-1} \end{matrix} \right]$$

from which the element $H_{B,k_2}(s)$ is found as

$$H_{B,k_2}(s) = \frac{\Delta B(s)}{\Delta k_2(s)} = \frac{A_{ss}(s+k_{-1})}{(s+k_2+k_{-1})(s+k_3+k_{-2}) - k_2k_{-2}} \tag{A8}$$

which is identical to Eq. 5 in the main paper.

Amount of Released/Absorbed B during Adaptation in Motif M1* (Fig. 4)

Setting Eqs. A1 and A2 (see above) to zero, with the additional condition that $k_{-1} = k_{-2} = k_{-3} = 0$, we get the steady state concentrations in A and B as $A_{ss} = k_1/k_2$ and $B_{ss} = k_1/k_3$, respectively. At $t = 0$ we assume that $A(0) = A_{ss}$ and $B(0) = B_{ss}$, and that k_2 undergoes a step-wise change to $f{\cdot}k_2$ with $f > 0$ and $f \neq 1$.

For $t \geq 0$ the response kinetics of A and B are calculated as:

$$A(t) = \frac{A_{ss}}{f}\left(1 + (f-1)e^{-fk_2t}\right) \tag{A9}$$

$$B(t) = B_{ss} + \frac{k_1(f-1)}{k_3 - fk_2}\left(e^{-fk_2t} - e^{-k_3t}\right) \tag{A10}$$

The amount of released or absorbed B during the robust perfect adaptation of B is calculated by using the integral

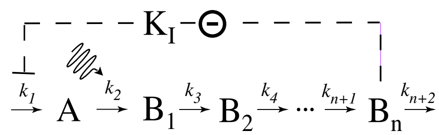
$$I(t) = \int \Big(B(t) - B_{ss}\Big)dt = \frac{k_1(f-1)}{k_3 - fk_2}\Big(\frac{e^{-k_3t}}{k_3} - \frac{e^{-fk_2t}}{fk_2}\Big) + C \tag{A11}$$

Because $\lim_{t\to\infty} I(t) = 0$ and $I(0) = -\frac{f-1}{f}\cdot\frac{k_1}{k_2k_3}$ we get

$$\int_0^\infty \Big(B(t) - B_{ss}\Big)dt = \lim_{t\to\infty} I(t) - I(0) = \frac{f-1}{f}\cdot\frac{k_1}{k_2k_3} \tag{A12}$$

Influence of Negative Feedback and Positive Feedforward on Adaptation

Feedback and feedforward loops are common regulatory elements. We illustrate here with a few examples how negative feedback and positive feedforward can affect adaptation (a description of all possible combinations of negative/positive feedback/feedforward loops is beyond the scope of this paper). First we consider an extension of motif M2 in the main paper by including a negative feedback from intermediate B_n to k_1:



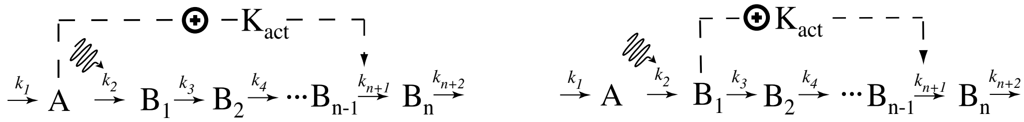
(M6)

with the inhibition term (to be multiplied with k_1) $\frac{1}{K_I+B_n}$. K_I is the inhibition constant. The legends of Figs. S1a and b give the rate equations and numerical values for K_I and rate constants. The transfer function from k_2 to B_n ($n \geq 2$), $H_{B_n,k_2}(s)$, is given by:

$$H_{B_n,k_2}(s) = \frac{\Delta B_n(s)}{\Delta k_2(s)} = \frac{A_{ss}\cdot s\prod_{i=3}^{n+1} k_i}{\frac{1}{(K_I+B_{n,ss})^2}\prod_{i=1}^{n+1} k_i + \prod_{i=2}^{n+2}(s+k_i)} \tag{A13}$$

From Eq. A13 we get $s = 0$ as the only solution to the numerator polynomial $n(s) = 0$, i.e., a zero in origo independent of the rate constants. The transfer functions for intermediates B_1 to B_{n-1} show slightly different structures, but they all have a zero in origo (data not shown). Hence, a step in k_2 results in robust perfect adaption for each B_i-intermediate. $H_{B_n,k_2}(s)$ has both real and complex-conjugated poles, which results in an underdamped response (over- and undershooting) in the perfect adapted B_i's (Fig. S1a). For high K_I values the response becomes overdamped (Fig. S1b), which is due to the fact that the real pole dominates over the complex-conjugated poles as K_I increases. In case the negative feedback from B_n is acting downstream of k_2, at k_i's with $i > 2$, all B_i's show robust perfect adaptation. If the perturbing step is applied to k_{n+2}, i.e., at the end of the network, none of the B_i's show perfect adaptation.

To illustrate the influence of positive feedforward, we consider two cases each shown in scheme M7.



(M7)

In the left scheme of M7 A acts positively on k_{n+1}, while in the right scheme B_1 acts positively on k_{n+1}. In each case the positive feedforward loop is realized by multiplying in the rate equations k_{n+1} with the factors $K_{act}A$ or $K_{act}B_1$, respectively, where K_{act} is an "activation constant". In the case A acts positively on k_{n+1}, concentration B_{n-1} is not perfectly adapted, because A does not show perfect adaptation. All other B_i's show robust perfect adaptation. The transfer function from k_2 to B_n, $H_{B_n,k_2}(s)$ for $n \geq 3$, is given as:

$$H_{B_n,k_2}(s) = \frac{\Delta B_n(s)}{\Delta k_2(s)} = \frac{-K_{act}{\cdot}A_{ss}{\cdot}s{\cdot}k_{n+1}\left(B_{n-1,ss}\prod_{i=3}^{n}(s+k_i) - A_{ss}\prod_{i=3}^{n}k_i\right)}{\left(\prod_{i=2}^{n}(s+k_i)\right)\left(s+K_{act}{\cdot}A_{ss}{\cdot}k_{n+1}\right)\left(s+k_{n+2}\right)} \tag{A14}$$

$H_{B_n,k_2}(s)$ has several zeros. Based on the fact that there is a zero in origo ($s=0$) to the solution $n(s)=0$, independent of the rate constants, B_n shows robust perfect adaptation. However, the total response in B_n will be influenced by the other zeros as indicated in Fig. 2 of the main paper.

In case intermediate B_1 acts positively on k_{n+1} (the right scheme in M7) all B_i's are robust perfectly adapted. The transfer function from k_2 to B_n, $H_{B_n,k_2}(s)$ for $n \geq 4$, is given as:

$$H_{B_n,k_2}(s) = \frac{\Delta B_n(s)}{\Delta k_2(s)} = \frac{K_{act}{\cdot}A_{ss}{\cdot}s{\cdot}k_{n+1}\left(B_{n-1,ss}{\cdot}s\prod_{i=4}^{n}(s+k_i) + B_{ss}\prod_{i=3}^{n}k_i\right)}{\left(\prod_{i=2}^{n}(s+k_i)\right)\left(s+K_{act}{\cdot}B_{ss}{\cdot}k_{n+1}\right)\left(s+k_{n+2}\right)} \tag{A15}$$

Figs. S1c and d show response kinetics of the two cases in M7 for $n=4$.

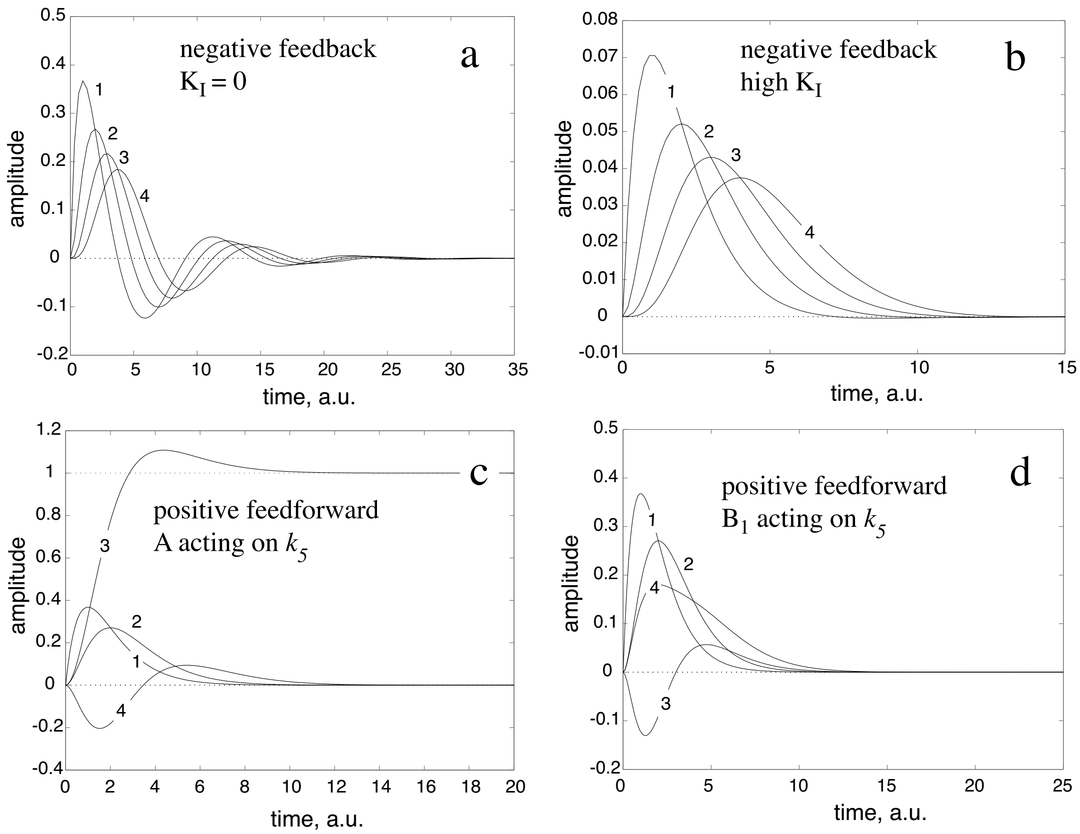


Fig. S1. Perfect adaptation kinetics in B_1 to B_4 for negative feedback scheme M6 ($n = 4$). The step perturbation is applied to k_2 with (a) $K_I = 0$ (strong inhibition) and (b) $K_I = 5.0$ (weak inhibition). The rate equations are: $dA/dt = \frac{k_1}{K_I+B_4} - k_2{\cdot}A, dB_1/dt = k_2{\cdot}A - k_3{\cdot}B_1$, and $dB_i/dt = k_{i+1}{\cdot}B_{i-1} - k_{i+2}{\cdot}B_i, i = 2, \ldots 4$ with all rate constants equal to 1.0. (c) Adaptation kinetics for left scheme in M7 ($n = 4$, and step-wise perturbation of k_2) where A is acting positively on k_5 with rate equations: $dA/dt = k_1 - k_2{\cdot}A, dB_1/dt = k_2{\cdot}A - k_3{\cdot}B_1, dB_2/dt = k_3{\cdot}B_1 - k_4{\cdot}B_2, dB_3/dt = k_4{\cdot}B_2 - K_{act}{\cdot}A{\cdot}k_5{\cdot}B_3, dB_4/dt = K_{act}{\cdot}A{\cdot}k_5{\cdot}B_3 - k_6{\cdot}B_4$. All rate constant values and K_{act} are set to 1.0. (d) Similar system as in (c) but B_1 is acting positively on k_5 (right scheme in M7, $n = 4$). All rate constants and K_{act} are set to 1.0. Note that all B_i show robust perfect adaptation, but B_3 is the only intermediate which shows different adaptation kinetics (undershooting).

Derivation of Eq. 9 for motif M5

From motif M5 the following relationship holds at steady state

$$A_{ss} = \frac{k_1}{k_2} \tag{A16}$$

$$I_{ss} = \frac{k_5}{k_6} \tag{A17}$$

Moreover, the following differential equation can be found for the dynamics of intermediate R_a^*:

$$\frac{dR_a^*(t)}{dt} = -k_4\frac{k_5}{k_6}R_a^*(t) + k_3\frac{k_1}{k_2}R_i(t) \tag{A18}$$

Assuming steady state, i.e. $\frac{dR_a^*(t)}{dt} = 0$ gives

$$k_2k_4k_5R_{a,ss}^* = k_1k_3k_6R_{i,ss} \tag{A19}$$

Using the fact that

$$R_{tot} = R_{a,ss}^* + R_{i,ss} \tag{A20}$$

gives

$$k_2k_4k_5R_{a,ss}^* = k_1k_3k_6(R_{tot} - R_{a,ss}^*) \tag{A21}$$

which again can be organized as

$$R_{a,ss}^* = \frac{k_1k_3k_6}{k_1k_3k_6 + k_2k_4k_5}R_{tot} \tag{A22}$$

Derivation of the Transfer functions for motif M5

According to Table 1 there are six $k_j \rightarrow k_i$ substitutions that give perfect adaptation in both R_a^* and R_i. In the following the individual substitutions and corresponding transfer functions for perfect adaptation sites are presented in more detail.

1. Substitution $k_2 \rightarrow k_3$ where $k_2 = \alpha k_3$ produces the following transfer function from $\Delta k_3(s)$ to $\Delta R_a^*(s)$

   $$H(s) = \frac{\Delta R_a^*(s)}{\Delta k_3(s)} = \frac{A_{ss}R_{i,ss}\cdot s}{(s+\alpha k_3)(s + k_3A_{ss} + k_4I_{ss})} \tag{A23}$$

   As can be seen, this transfer function has a zero in origo.

2. Substitution $k_2 \rightarrow k_6$ where $k_2 = \alpha k_6$ produces the following transfer function from $\Delta k_6(s)$ to $\Delta R_a^*(s)$

$$
\begin{aligned}
H(s) &= \frac{\Delta R_a^*(s)}{\Delta k_6(s)} \\
&= -\bigg(\frac{(k_3 \alpha R_{i,ss} A_{ss} + k_4 I_{ss}(R_{i,ss} - R_{tot}))\cdot s}{(s + \alpha k_6)(s + k_3 A_{ss} + k_4 I_{ss})(s + k_6)} \\
&\qquad + \frac{k_3 \alpha k_6 R_{i,ss} A_{ss} + k_4 \alpha k_6 I_{ss}(R_{i,ss} - R_{tot})}{(s + \alpha k_6)(s + k_3 A_{ss} + k_4 I_{ss})(s + k_6)}\bigg) \qquad \text{(A24)}
\end{aligned}
$$

In order to show that this transfer function actually has a zero in origo, we have to use Eq. A19 and Eq. A20 such that the real part of the denominator of Eq.A24 can be written as (using $k_2 = \alpha k_6$, Eq. A17 and Eq. A16)

$$
\begin{aligned}
k_3 \alpha k_6 R_{i,ss} A_{ss} + k_4 \alpha k_6 I_{ss}(R_{i,ss} - R_{tot}) &= k_3 k_2 R_{i,ss} \frac{k_1}{k_2} + k_4 k_2 \frac{k_5}{k_6}(R_{i,ss} - (R_{a,ss}^* + R_{i,ss})) \\
&= k_1 k_3 k_6 R_{i,ss} - k_2 k_4 k_5 R_{a,ss}^* \qquad \text{(A25)}
\end{aligned}
$$

Hence, by inserting Eq. A19 into Eq. A25, it is shown that the real part of the denominator is zero, and the solution to $n(s) = 0$ in Eq. A24 is $s = 0$. Therefore, the transfer function in Eq. A24 can be written

$$
H(s) = \frac{\Delta R_a^*(s)}{\Delta k_6(s)} = -\frac{(k_3 \alpha R_{i,ss} A_{ss} + k_4 I_{ss}(R_{i,ss} - R_{tot}))\cdot s}{(s + \alpha k_6)(s + k_3 A_{ss} + k_4 I_{ss})(s + k_6)} \qquad \text{(A26)}
$$

which has a zero in origo.

3. Substitution $k_4 \rightarrow k_1$ where $k_4 = \alpha k_1$ produces the following transfer function from $\Delta k_1(s)$ to $\Delta R_a^*(s)$

$$
\begin{aligned}
H(s) &= \frac{\Delta R_a^*(s)}{\Delta k_1(s)} \\
&= \frac{\alpha I_{ss}(R_{i,ss} - R_{tot})\cdot s + k_3 R_{i,ss} + k_2 \alpha I_{ss}(R_{i,ss} - R_{tot})}{(s + k_2)(s + k_3 A_{ss} + \alpha k_1 I_{ss})} \qquad \text{(A27)}
\end{aligned}
$$

In order to show that this transfer function actually has a zero in origo, we have to use Eq. A19 and Eq. A20 such that the real part of the denominator of Eq.A27 can be written as (using $k_5 = \alpha k_1$ and Eq. A17)

$$
\begin{aligned}
k_3 R_{i,ss} + k_2 \alpha I_{ss}(R_{i,ss} - R_{tot}) &= k_3 R_{i,ss} + k_2 \alpha I_{ss}(R_{i,ss} - (R_{a,ss}^* + R_{i,ss})) \\
&= k_3 R_{i,ss} - k_2 \alpha I_{ss} R_{a,ss}^* \\
&= k_1 k_3 R_{i,ss} - \alpha k_1 k_2 I_{ss} R_{a,ss}^* \\
&= k_1 k_3 k_6 R_{i,ss} - k_2 k_4 \frac{k_5}{k_6} k_6 R_{a,ss}^* \\
&= k_1 k_3 k_6 R_{i,ss} - k_2 k_4 k_5 R_{a,ss}^* \qquad \text{(A28)}
\end{aligned}
$$

Hence, by inserting Eq. A19 into Eq. A28, it is shown that the real part of the denominator is zero, and the solution to $n(s) = 0$ in Eq. A27 is $s = 0$. Therefore, the transfer function in Eq. A27 can be written

$$H(s) = \frac{\Delta R_a^*(s)}{\Delta k_1(s)} = \frac{\alpha I_{ss}(R_{i,ss} - R_{tot}) \cdot s}{(s + k_2)(s + k_3 A_{ss} + \alpha k_1 I_{ss})} \tag{A29}$$

which has a zero in origo.

4. Substitution $k_4 \to k_6$ where $k_4 = \alpha k_6$ produces the following transfer function from $\Delta k_6(s)$ to $\Delta R_a^*(s)$

$$H(s) = \frac{\Delta R_a^*(s)}{\Delta k_6(s)} = \frac{\alpha I_{ss}(R_{i,ss} - R_{tot}) s}{(s + k_3 A_{ss} + \alpha k_6 I_{ss})(s + k_6)} \tag{A30}$$

As can be seen, this transfer function has a zero in origo.

5. Substitution $k_5 \to k_1$ where $k_5 = \alpha k_1$ produces the following transfer function from $\Delta k_1(s)$ to $\Delta R_a^*(s)$

$$\begin{aligned} H(s) &= \frac{\Delta R_a^*(s)}{\Delta k_1(s)} \\ &= \frac{(k_3 R_{i,ss} + \alpha k_4 (R_{i,ss} - R_{tot})) \cdot s + k_3 k_6 R_{i,ss} + \alpha k_2 k_4 (R_{i,ss} - R_{tot})}{(s + k_2)(s + k_3 A_{ss} + k_4 I_{ss})(s + k_6)} \end{aligned} \tag{A31}$$

In order to show that this transfer function actually has a zero in origo, we have to use Eq. A19 and Eq. A20 such that the real part of the denominator of Eq.A31 can be written as (using $k_5 = \alpha k_1$)

$$\begin{aligned} k_3 k_6 R_{i,ss} + \alpha k_2 k_4 (R_{i,ss} - R_{tot}) &= k_3 k_6 R_{i,ss} + \alpha k_2 k_4 (R_{i,ss} - (R_{a,ss}^* + R_{i,ss})) \\ &= k_3 k_6 R_{i,ss} - \alpha k_2 k_4 R_{a,ss}^* \\ &= k_1 k_3 k_6 R_{i,ss} - \alpha k_1 k_2 k_4 R_{a,ss}^* \\ &= k_1 k_3 k_6 R_{i,ss} - k_2 k_4 k_5 R_{a,ss}^* \end{aligned} \tag{A32}$$

Hence, by inserting Eq. A19 into Eq. A32, it is shown that the real part of the denominator is zero, and the solution to $n(s) = 0$ in Eq. A31 is $s = 0$. Therefore, the transfer function in Eq. A31 can be written

$$H(s) = \frac{\Delta R_a^*(s)}{\Delta k_1(s)} = \frac{(k_3 R_{i,ss} + \alpha k_4 (R_{i,ss} - R_{tot})) \cdot s}{(s + k_2)(s + k_3 A_{ss} + k_4 I_{ss})(s + k_6)}$$

which has a zero in origo.

6. Substitution $k_5 \rightarrow k_3$ where $k_5 = \alpha k_3$ produces the following transfer function from $\Delta k_3(s)$ to $\Delta R_a^*(s)$

$$H(s) = \frac{\Delta R_a^*(s)}{\Delta k_3(s)} = \frac{A_{ss} R_{i,ss} \cdot s + A_{ss} R_{i,ss} k_6 + k_4 \alpha (R_{i,ss} - R_{tot})}{(s + k_3 A_{ss} + k_4 I_{ss})(s + k_6)} \tag{A33}$$

In order to show that this transfer function actually has a zero in origo, we have to use Eq. A19 and Eq. A20 such that the real part of the denominator of Eq.A33 can be written as (using $k_5 = \alpha k_3$ and Eq.A16)

$$\begin{aligned} A_{ss} R_{i,ss} k_6 + k_4 \alpha (R_{i,ss} - R_{tot}) &= A_{ss} R_{i,ss} k_6 + k_4 \alpha (R_{i,ss} - (R_{a,ss}^* + R_{i,ss})) \\ &= A_{ss} R_{i,ss} k_6 - k_4 \alpha R_{a,ss}^* \\ &= \frac{k_1}{k_2} R_{i,ss} k_6 - k_4 \alpha R_{a,ss}^* \\ &= k_1 k_3 k_6 R_{i,ss} - k_2 k_4 \alpha k_3 R_{a,ss}^* \\ &= k_1 k_3 k_6 R_{i,ss} - k_2 k_4 k_5 R_{a,ss}^* \end{aligned} \tag{A34}$$

Hence, by inserting Eq. A19 into Eq. A34, it is shown that the real part of the denominator is zero, and the solution to $n(s) = 0$ in Eq. A33 is $s = 0$. Therefore, the transfer function in Eq. A33 can be written

$$H(s) = \frac{\Delta R_a^*(s)}{\Delta k_3(s)} = \frac{A_{ss} R_{i,ss} \cdot s}{(s + k_3 A_{ss} + k_4 I_{ss})(s + k_6)} \tag{A35}$$

which has a zero in origo.