Supplementary Data for

Analysis of network motifs in cellular regulation: structural similarities, input-output relations and signal integration

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Cross-talk in two-component systems

The phosphorylation of two response regulators (RRs) by a single sensor kinase (HK), as depicted in Fig. 15C, is described by the reaction mechanism

$$\begin{aligned} HK^* + RR_i &\underset{k^-_{t,i}}{\overset{k^+_{t,i}}{\rightleftarrows}} HK^*\text{-}RR_i \overset{k_{t,i}}{\to} HK + RR_i^*, \quad i = 1,2 \\ HK + RR_i^* &\underset{k^-_{p,i}}{\overset{k^+_{p,i}}{\rightleftarrows}} HK\text{-}RR_i^* \overset{k_{p,i}}{\to} HK + RR_i, \quad i = 1,2 \\ HK &\underset{k^-_K}{\overset{k^+_K}{\rightleftarrows}} HK^* \end{aligned}$$

where HK^* and RR_i^* denote the phosphorylated forms of the sensor kinase and the response regulator, respectively. The corresponding ODE system reads

$$\frac{d\,[RR_i^*]}{dt} = k_{t,i}\,[HK^*\text{-}RR_i] - k^+_{p,i}\,[RR_i^*]\,[HK] + k^-_{p,i}\,[HK\text{-}RR_i^*] \tag{S1}$$

$$\begin{aligned} \frac{d\,[HK]}{dt} &= -k^+_K\,[HK] + k^-_K\,[HK^*] + \sum_{i=1}^{2} k_{t,i}\,[HK^*\text{-}RR_i] \\ &\quad - \sum_{i=1}^{2} \left(k^+_{p,i}\,[RR_i^*]\,[HK] - \left(k^-_{p,i} + k_{p,i}\right)[HK\text{-}RR_i^*]\right) \end{aligned} \tag{S2}$$

$$\frac{d\,[HK^*\text{-}RR_i]}{dt} = k^+_{t,i}\,[RR_i]\,[HK^*] - \left(k^-_{t,1} + k_{t,i}\right)[HK^*\text{-}RR_i] \tag{S3}$$

$$\frac{d\,[HK\text{-}RR_i^*]}{dt} = k^+_{p,i}\,[RR_i^*]\,[HK] - \left(k^-_{p,i} + k_{p,i}\right)[HK\text{-}RR_i^*] \tag{S4}$$

where $[RR_1]$, $[RR_2]$ and $\left[HK^P\right]$ have to be replaced according to the conservation relations

$$[RR_1] + [RR_1^*] + [HK^*\text{-}RR_1] + [HK\text{-}RR_1^*] = RR_{1T} \tag{S5}$$

$$[RR_2] + [RR_2^*] + [HK^*\text{-}RR_2] + [HK\text{-}RR_2^*] = RR_{2T} \tag{S6}$$

$$[HK] + [HK^*] + \sum_{i=1}^{2} [HK^*\text{-}RR_i] + \sum_{i=1}^{2} [HK\text{-}RR_i^*] = HK_T. \tag{S7}$$

We assume that both RRs are in excess so that

$$\begin{aligned} [RR_1] &\approx RR_{1T} - [RR_1^*] \\ [RR_2] &\approx RR_{2T} - [RR_2^*]\,. \end{aligned} \tag{S8}$$

Under steady state conditions the enzyme-substrate complexes are given by

$$\begin{aligned}[HK^*\text{-}RR_i] &= \frac{[RR_i]\,[HK^*]}{K_{t,i}} \approx \frac{(RR_{iT} - [RR_i^*])\,[HK^*]}{K_{t,i}} \\ [HK\text{-}RR_i^*] &= \frac{[RR_i^*]\,[HK]}{K_{p,i}}\end{aligned} \tag{S9}$$

where the Michaelis-Menten constants are defined by

$$K_{t,i} = \frac{k_{t,i} + k_{t,i}^-}{k_{t,i}^+} \quad \text{and} \quad K_{p,i} = \frac{k_{p,i} + k_{p,i}^-}{k_{p,i}^+}, \quad i = 1, 2.$$

Addition of Eqs. (S2) and (S4) yields at steady state

$$\begin{aligned}[HK] &= \frac{k_K^-}{k_K^+}\,[HK^*] + \frac{k_{t,1}}{k_K^+}\,[HK^*\text{-}RR_1] + \frac{k_{t,2}}{k_K^+}\,[HK^*\text{-}RR_2] \\ &= \frac{k_K^-}{k_K^+}\left(1 + \frac{k_{t,1}}{k_K^-}\frac{[RR_1]}{K_{t,1}} + \frac{k_{t,2}}{k_K^-}\frac{[RR_2]}{K_{t,2}}\right)[HK^*] \\ &\approx \frac{k_K^-}{k_K^+}\left(1 + \frac{k_{t,1}}{k_K^-}\frac{RR_{1T} - [RR_1^*]}{K_{t,1}} + \frac{k_{t,2}}{k_K^-}\frac{RR_{2T} - [RR_2^*]}{K_{t,2}}\right)[HK^*]\end{aligned} \tag{S10}$$

where we have used the conservation relations Eqs. (S8) in the last line. Similarly, addition of Eqs. (S1) and (S4) yields the steady state relations

$$\begin{aligned}k_{t,1}\,[HK^*\text{-}RR_1] &= k_{p,1}\,[HK\text{-}RR_1^*] \\ k_{t,2}\,[HK^*\text{-}RR_2] &= k_{p,2}\,[HK\text{-}RR_2^*]\,.\end{aligned}$$

Replacing the enzyme-substrate complexes by the relations in Eqs. (S9) and the conservation relations Eqs. (S8) yields

$$\begin{aligned}k_{t,1}\frac{(RR_{1T} - [RR_1^*])\,[HK^*]}{K_{t,1}} &\approx k_{p,1}\frac{[RR_1^*]\,[HK]}{K_{p,1}} \\ k_{t,2}\frac{(RR_{2T} - [RR_2^*])\,[HK^*]}{K_{t,2}} &\approx k_{p,2}\frac{[RR_2^*]\,[HK]}{K_{p,2}}.\end{aligned}$$

Finally, replacing $[HK]$ on the right-hand sides by the expression in Eq. (S10) the factor $[HK^*]$ cancels on both sides of the equation resulting in the steady state equations

$$\begin{aligned}k_{t,1}\frac{(RR_{1T} - [RR_1^*])}{K_{t,1}} &\approx k_{p,1}\frac{[RR_1^*]}{K_{p,1}}\frac{k_K^-}{k_K^+}\left(1 + \frac{k_{t,1}}{k_K^-}\frac{RR_{1T} - [RR_1^*]}{K_{t,1}} + \frac{k_{t,2}}{k_K^-}\frac{RR_{2T} - [RR_2^*]}{K_{t,2}}\right) \\ k_{t,2}\frac{(RR_{2T} - [RR_2^*])}{K_{t,2}} &\approx k_{p,2}\frac{[RR_2^*]}{K_{p,2}}\frac{k_K^-}{k_K^+}\left(1 + \frac{k_{t,1}}{k_K^-}\frac{RR_{1T} - [RR_1^*]}{K_{t,1}} + \frac{k_{t,2}}{k_K^-}\frac{RR_{2T} - [RR_2^*]}{K_{t,2}}\right).\end{aligned} \tag{S11}$$

By defining the rescaled Michaelis-Menten constants $C_{p,i}$ and $C_{t,i}$ through

$$C_{p,i} = \frac{k_K^+}{k_{p,i}} K_{p,i} \quad \text{and} C_{t,i} = \frac{k_K^-}{k_{t,i}} K_{t,i}, \quad i = 1, 2$$

Eqs. (S11) can be written in the form

$$\frac{(RR_{1T} - [RR_1^*])}{C_{t,1}} \approx \frac{[RR_1^*]}{C_{p,1}} \left(1 + \frac{RR_{1T} - [RR_1^*]}{C_{t,1}} + \frac{RR_{2T} - [RR_2^*]}{C_{t,2}}\right) \tag{S12}$$

$$\frac{(RR_{2T} - [RR_2^*])}{C_{t,2}} \approx \frac{[RR_2^*]}{C_{p,2}} \left(1 + \frac{RR_{1T} - [RR_1^*]}{C_{t,1}} + \frac{RR_{2T} - [RR_2^*]}{C_{t,2}}\right). \tag{S13}$$

Taking the ratio of both equations yields

$$\frac{C_{p,1}}{C_{t,1}} \frac{(RR_{1T} - [RR_1^*])}{[RR_1^*]} = \frac{C_{p,2}}{C_{t,2}} \frac{(RR_{2T} - [RR_2^*])}{[RR_2^*]}$$

or

$$[RR_2^*] = \frac{R_{2T}\,[RR_1^*]}{\frac{\varepsilon_p}{\varepsilon_t}\,(R_{1T} - [RR_1^*]) + [RR_1^*]} \tag{S14}$$

where

$$\varepsilon_p \equiv \frac{C_{p,1}}{C_{p,2}} = \frac{k_{p,2}/K_{p,2}}{k_{p,1}/K_{p,1}} \quad \text{and} \quad \varepsilon_t \equiv \frac{C_{t,1}}{C_{t,2}} = \frac{k_{t,2}/K_{t,2}}{k_{t,1}/K_{t,1}}$$

denote the ratios of the kinetic preferences of the HK's phosphatase activity (ε_p) and the HK's phosphotransferase activity (ε_t) with respect to the two RRs.

Substituting the relation

$$R_{2T} - [RR_2^*] = \frac{R_{2T}\frac{\varepsilon_p}{\varepsilon_t}\,(R_{1T} - [RR_1^*])}{\frac{\varepsilon_p}{\varepsilon_t}\,(R_{1T} - [RR_1^*]) + [RR_1^*]}$$

into Eq. (S12) yields a cubic equation for $[RR_1^*]$ which can be written as

$$\left(1 - \frac{\varepsilon_p}{\varepsilon_t}\right)[RR_1^*]^3 - \left(RR_{1T} + C_{p,1} - \varepsilon_p RR_{2T} + C_{t,1} - \frac{\varepsilon_p}{\varepsilon_t}\,(2 \cdot RR_{1T} + C_{p,1} + C_{t,1})\right)[RR_1^*]^2 \tag{S15}$$

$$+RR_{1T}\left(C_{p,1} - \varepsilon_p RR_{2T} - \frac{\varepsilon_p}{\varepsilon_t}\,(RR_{1T} + 2C_{p,1} + C_{t,1})\right)[RR_1^*] + \frac{\varepsilon_p}{\varepsilon_t} C_{p,1} RR_{1T}^2 = 0.$$

By symmetry the steady state equation for $[RR_2^*]$ is given by

$$\left(1 - \frac{\varepsilon_t}{\varepsilon_p}\right)[RR_2^*]^3 - \left(RR_{2T} + C_{p,2} - \frac{1}{\varepsilon_p} RR_{1T} + C_{t,2} - \frac{\varepsilon_t}{\varepsilon_p}\,(2 \cdot RR_{2T} + C_{p,2} + C_{t,2})\right)[RR_2^*]^2 \tag{S16}$$

$$+RR_{2T}\left(C_{p,2} - \frac{1}{\varepsilon_p} RR_{1T} - \frac{\varepsilon_t}{\varepsilon_p}\,(RR_{2T} + 2C_{p,2} + C_{t,2})\right)[RR_2^*] + \frac{\varepsilon_t}{\varepsilon_p} C_{p,2} RR_{2T}^2 = 0.$$

Note that these equations are structurally identical with that for the receptor-ligand complex arising in the competition of two ligands for a receptor binding site in Eq. (35) if one makes the substitutions (cf. Table 1)

$$[RR_1^*] \leftrightarrow [L1.R], \quad RR_{1T} \leftrightarrow L1_T, \quad \frac{\varepsilon_p}{\varepsilon_t} \leftrightarrow \varepsilon, \tag{S17}$$
$$C_{p,1} \leftrightarrow R_T, \quad \varepsilon_p RR_{2T} \leftrightarrow L2_T, \quad C_{t,1} \leftrightarrow K_{d1}$$

in the case of Eq. (S15) and

$$[RR_2^*] \leftrightarrow [L1.R], \quad RR_{2T} \leftrightarrow L1_T, \quad \frac{\varepsilon_t}{\varepsilon_p} \leftrightarrow \varepsilon$$
$$C_{p,2} \leftrightarrow R_T, \quad \frac{1}{\varepsilon_p} RR_{1T} \leftrightarrow L2_T, \quad C_{t,2} \leftrightarrow K_{d1}$$

in the case of Eq. (S16).

In the limit $\varepsilon_p \to \infty$ (with ε_t constant) the dominant terms in Eqs. (S15) are

$$-\frac{\varepsilon_p}{\varepsilon_t}\left[RR_1^*\right]^3 + \left(\varepsilon_p RR_{2T} + \frac{\varepsilon_p}{\varepsilon_t}\left(2 \cdot RR_{1T} + C_{p,1} + C_{t,1}\right)\right)\left[RR_1^*\right]^2$$
$$+RR_{1T}\left(\varepsilon_p RR_{2T} + \frac{\varepsilon_p}{\varepsilon_t}\left(2C_{p,1} + RR_{1T} + C_{t,1}\right)\right)\left[RR_1^*\right] + \frac{\varepsilon_p}{\varepsilon_t} C_{p,1} RR_{1T}^2 \quad \approx \quad 0$$

which can be factorized as

$$\left(\left[RR_1^*\right] - RR_{1T}\right)\left(\left[RR_1^*\right]^2 - \left(RR_{1T} + C_{p,1} + C_{t,1} + \varepsilon_t RR_{2T}\right)\left[RR_1^*\right] + C_{p,1} RR_{1T}\right) \approx 0.$$

Hence, $[RR_1^*] \approx R_{1T}$ or $[RR_1^*]$ is a solution of the **LR**-type equation

$$\left[RR_1^*\right]^2 - \left(RR_{1T} + C_{p,1} + C_{t,1} + \varepsilon_t RR_{2T}\right)\left[RR_1^*\right] + C_{p,1} RR_{1T} \approx 0.$$

In contrast, in the limit $\varepsilon_t \to \infty$ the dominant terms of Eq. (S15) are given by

$$\left(\left[RR_1^*\right]^2 - \left(C_{t,1} + RR_{1T} + C_{p,1} - \varepsilon_p RR_{2T}\right)\left[RR_1^*\right] + RR_{1T}\left(C_{p,1} - \varepsilon_p RR_{2T}\right)\right)\left[RR_1^*\right] \approx 0.$$

Hence, $[RR_1^*] \approx 0$ or $[RR_1^*]$ is a solution of the **LR**-type equation

$$\left[RR_1^*\right]^2 - \left(RR_{1T} + C_{p,1} - \varepsilon_p RR_{2T} + C_{t,1}\right)\left[RR_1^*\right] + \left(C_{p,1} - \varepsilon_p RR_{2T}\right) RR_{1T} \approx 0. \tag{S18}$$

Note that similar as for Eq. (38) the solution of Eq. (S18) is only defined for $C_{p,1} > \varepsilon_p RR_{2T}$ or $k_K^+ > \left(k_{p,2}/K_{p,2}\right) RR_{2T}$. In the opposite case ($k_K^+ < \left(k_{p,2}/K_{p,2}\right) RR_{2T}$) the approximation can be obtained by substituting the corresponding quantities (S17) into Eq. (37) which yields

$$\left[RR_1^*\right] \approx \frac{1}{\varepsilon_t} \frac{RR_{1T} C_{p,1}}{RR_{2T} - C_{p,2}}, \quad k_K^+ < \frac{k_{p,2}}{K_{p,2}} RR_{2T}.$$

For $[RR_2^*]$ as described by Eq. (S16) the dominant terms are can be factorized as

$$[RR_2^*]\left([RR_2^*]^2 - (RR_{2T} + C_{p,2} + C_{t,2})\,[RR_2^*] + RR_{2T}C_{p,2}\right) \approx 0$$

if $\varepsilon_t \to \infty$ and

$$(RR_{2T} - [RR_2^*])\left([RR_2^*]^2 - [RR_2^*]\,(RR_{2T} + C_{p,2} + C_{t,2}) + RR_{2T}C_{p,2}\right) \approx 0.$$

if $\varepsilon_p \to \infty$. Hence, in either limit the steady state of $[RR_2^*]$ is determined by the same quadratic equation which is identical with that of the Batchelor-Goulian model for a single RR (cf. Eq. 61).