THE JOURNAL OF PHYSICAL CHEMISTRY B

ARTICLE

pubs.acs.org/JPCB

On the Relationship between Sensitivity Coeffcients and Transfer Functions of Reaction Kinetic Networks

Tormod Drengstig,[†] Thomas Kjosmoen,[†] and Peter Ruoff[*,‡]

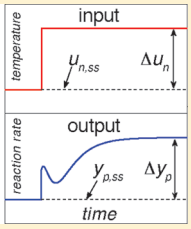
[†]Department of Electrical Engineering and Computer Science and [‡]Centre for Organelle Research, Faculty of Science and Technology, University of Stavanger, Stavanger, Norway

S *Supporting Information*

ABSTRACT: Metabolic control analysis (MCA) and biochemical systems theory (BST) have become established methods when analyzing the behavior/kinetics of biochemical reaction systems. While the usage of MCA and BST involves the determination of sensitivities, e.g., steady state control coefficients (CCs), typically between reaction rates and concentrations/fluxes, transfer functions (TFs) from control engineering allow to analyze the *connectivity* between arbitrary input signals (e.g., rate constants or temperature) and arbitrary output signals (e.g., concentrations or fluxes) in the complex-valued *s*- or frequency domain. As CCs generally do not provide information about the connectivity between input and output signals, we became interested in the question of how CCs and TFs, or more generally, how arbitrary sensitivity coefficients (SCs) and TFs are related to each other. In this work, we describe a general relationship between SCs and their corresponding TFs from a general kinetic (state space) approach and show that the state space approach can describe the SC-TF relationship by a *single* equation. During our work, we became aware of an alternative method which relates CCs and TFs by using a stoichiometric network approach. In this work, we describe a procedure to identify conditions to determine whether a receptor-mediated input to a reaction kinetic network can show robust (perturbation independent) or nonrobust (balanced or perturbation dependent) adaptive or homeostatic behavior in an output. Compared to the stoichiometric network approach, the here described method allows for dealing with arbitrary (including empirically identified) kinetic expressions.

$$C_{u_n}^{y_p} = H_{y_p,u_n}(0)\cdot\frac{u_{n,ss}}{y_{p,ss}}$$

$$\begin{pmatrix}\text{sensitivity}\\ \text{coefficient}\end{pmatrix} = \begin{pmatrix}\text{transfer}\\ \text{function}\end{pmatrix}\cdot\left(\frac{\text{input}}{\text{output}}\right)$$



INTRODUCTION

Methods such as general and biological systems theory,[1−3] biochemical systems theory,[4,5] and metabolic control analysis and its extensions[6−16] provide important tools in the analysis of complex oscillatory or nonoscillatory reaction systems.[17−20]

We became interested in how adaptation processes in reaction kinetic networks can be characterized and identified by using both metabolic control analysis and methods from control engineering.[21−26] For example, a condition for robust (rate parameter independent) perfect adaptation for a certain network site requires that the respective sensitivity coefficient between an input (for example, a changed rate constant) and an output (for example, a concentration) becomes zero. Using methods from control engineering, a sufficient condition for robust perfect adaptation can be formulated, which requires that the transfer function between input and output has a zero in origo independently of rate constant values.[26] Moreover, if the transfer function has a zero in origo only for a given combination of rate constants or activation energies, the output still shows perfect adaptation, but since the adaptation behavior depends on certain parameter values, this situation is "nonrobust". A question which repeatedly occurred to us was how control coefficients in particular, or sensitivity coefficients in general, may be related to transfer functions. In this work, we describe the relationship between sensitivity coefficients and their corresponding transfer functions from a general kinetic (state space) perspective, i.e., considering an open system model with independent rate constant parameters or temperature as input and species concentrations or fluxes as output. Ingalls[15] analyzed the relationship between response/control coefficients and transfer functions on the basis of stoichiometric network presentation.[9] However, as we show in the Supporting Information, Ingalls' results may differ from the input/output state space formulation, because in the stoichiometric network formulation perturbations are not applied to individual rate constants but to reaction velocities and their linear combinations. The formulation of control coefficients by using stoichiometric networks[15] is dependent on the choice of model output, e.g., whether the outputs are concentrations or fluxes, and no formulation has been provided for how other inputs than reactions rates, for example temperature, can be included in the analysis.

Received: January 19, 2011
Revised: March 25, 2011

 © XXXX American Chemical Society

COMPUTATIONAL METHODS

The symbolic equations were analyzed by using MATLAB (www.mathworks.com) and MAPLE (www.maplesoft.com) as described in ref 27.

RESULTS

Transfer Functions of Reaction Kinetic Networks. *State Space Modeling Approach.* Consider a reaction kinetic network with M chemical components $I_1,...,I_M$ with concentrations $x_1,...,x_M(t)$ and N reactions steps associated with a rate constant, i.e. $k_1(t),...,k_N(t)$. The kinetics will generally be of first or second order, but empirical kinetics can also be considered. The network can be perturbed by changing one of the rate constants $k_n(t)$, $n\in\{1,...,N\}$ by means of, for example, a step function or any other time-dependent signal. Such a perturbation may occur due to an signal coming from a receptor acting specifically on $k_n(t)$. The network can also be stimulated by temperature changes by assuming that each rate constant depends on temperature using the Arrhenius equation, i.e., $k_n(t) = A_n \cdot e^{(-E_n)/(RT(t))}$.

In this work, we use $u_n(t)$ as a general notation for a time varying input signal and for a vector of inputs we use $\mathbf{u}(t) = [u_1(t),...,u_N(t)]$. Further, we use the symbol $\boldsymbol{\alpha}$ for the L-dimensional vector of parameters and $\mathbf{x}(t) = [x_1(t),...,x_M(t)]$ for a vector of state variables, i.e., concentrations. The kinetics of the network are described by the rate equations for each chemical component as follows:

$$\frac{d\mathbf{x}(t)}{dt} = \mathbf{f}(\mathbf{x}(t), \mathbf{u}(t), \boldsymbol{\alpha}) \tag{1}$$

where $\mathbf{f}$ is a vector of nonlinear functions $\mathbf{f} = [f_1, ..., f_M]^T$. From this model we define a P-dimensional vector of model outputs $\mathbf{y}(t) = [y_1(t),...,y_P(t)]^T$ as follows:

$$\mathbf{y}(t) = \mathbf{g}(\mathbf{x}(t), \mathbf{u}(t), \boldsymbol{\alpha}) \tag{2}$$

where $\mathbf{g}$ is a vector of nonlinear functions $\mathbf{g} = [g_1, ..., g_P]^T$. These model outputs are properties of the network we want to investigate, for example concentrations, fluxes, or other network properties which depend on concentrations and/or rate constants.

In order to find the *transfer function matrix* $\mathbf{H}(s)$ from the vector of inputs to the vector of outputs, eqs 1 and 2 are first linearized around a working point, i.e., the unperturbed steady state values of $\mathbf{u_{ss}} = [u_1, ..., u_N]$, $\mathbf{x_{ss}} = [x_1, ..., x_M]$ and $\mathbf{y_{ss}} = [y_1, ..., y_P]$ (note the independence of time t of the vector elements to indicate steady state values). This gives the following linear state space model:

$$\Delta\dot{\underline{\mathbf{x}}}(t) = A\cdot\Delta\mathbf{x}(t) + B\cdot\Delta\mathbf{u}(t) \tag{3}$$

$$\Delta\mathbf{y}(t) = C\cdot\Delta\mathbf{x}(t) + D\cdot\Delta\mathbf{u}(t) \tag{4}$$

where $\Delta\mathbf{u}(t) = [\Delta u_1(t), ..., \Delta u_N(t)]^T$, $\Delta\mathbf{x}(t) = [\Delta x_1(t), ..., \Delta x_M(t)]^T$ and $\Delta\mathbf{y}(t) = [\Delta y_1(t), ..., \Delta y_P(t)]^T$ are vectors of small deviations around $\mathbf{u_{ss}}$, $\mathbf{x_{ss}}$ and $\mathbf{y_{ss}}$, respectively. The $(M \times M)$ state matrix A is defined by $A_{ij} = (\partial f_i)/(\partial x_j)|_{ss}$, the $(M \times N)$ input matrix B is defined by $B_{ij} = (\partial f_i)/(\partial u_j)|_{ss}$, the $(P \times M)$ output matrix C is defined by $C_{ij} = (\partial g_i)/(\partial x_j)|_{ss}$, and the $(P \times N)$ direct transmission matrix D is defined by $D_{ij} = (\partial g_i)/(\partial u_j)|_{ss}$. The names of the matrices are terminology from control engineering.[28]

Laplace transforming the linearized model in eqs 3 and 4, gives the $(P \times N)$ *transfer function* matrix $\mathbf{H}(s)$ as follows:[21]

$$\mathbf{H}(s) = C(sI - A)^{-1}B + D \tag{5}$$

where I is the $(M \times M)$ identity matrix. $\mathbf{H}(s)$ describes the relationship between *a small change* in all possible inputs, i.e. the array of Laplace transformed inputs $\Delta\mathbf{u}(s) = [\Delta u_1(s),..., \Delta u_N(s)]^T$, and the resulting *changes* in all possible outputs, i.e. $\Delta\mathbf{y}(s) = [\Delta y_1(s),...,\Delta y_P(s)]^T$. Note that we use the same symbol for both time- and Laplace domain signals. The element of the transfer function matrix in eq 5 corresponding to the relationship between an arbitrary input $u_n(t)$, $n\in\{1,...,N\}$ and an arbitrary output $y_p(t)$, $p\in\{1,...,P\}$ is in general given as follows:

$$H_{y_p,u_n}(s) = \frac{\Delta y_p(s)}{\Delta u_n(s)} = \frac{K\cdot\prod_{r=1}^{m}\left(-\frac{1}{z_r}s+1\right)}{\prod_{q=1}^{k}\left(-\frac{1}{p_q}s+1\right)} \tag{6}$$

where z_r are defined as the transfer function's zeros, p_q are the poles, K is the DC-gain, m is the number of zeros and k is the number of poles.[28]

System Identification Approach. In control engineering, the field of system identification uses statistical methods to build empirical mathematical models of dynamical systems from measured data. System identification also includes the optimal design of experiments for efficiently generating informative data for fitting such models.[29] One reason for doing system identification is that modeling from first principles may prove difficult in that the regulatory processes may be poorly understood. As such, most identified models are "black-box models" which only describe the observed input-output relationship without any knowledge about the state variables. These black-box models may be represented as state space models, transfer functions (with and without time-delays), nonlinear models, spectral models, correlations models, and more.[29]

We will not go into detail about system identification methods, but only point out that a (more or less accurate) transfer function $H_{y_p,u_n}(s)$ or transfer function matrix $\mathbf{H}(s)$ can be estimated from a set of measured input/output data. These transfer functions are therefore similar to eqs 5 and 6, respectively, applicable to the relationship which is described in the next section.

The Relationship between Transfer Functions and Relative Sensitivity Coefficients. An *absolute* or *unscaled* sensitivity coefficient is the partial derivative of a variable (for example flux J_i) with respect to variations in a parameter value (for example rate constant k_j) as $(\partial J_i)/(\partial k_j)$, whereas the *relative* or *scaled* sensitivity coefficient is defined as follows:[30]

$$\frac{\partial J_i}{\partial k_j}\cdot\frac{k_j}{J_i} = \frac{\partial \ln J_i}{\partial \ln k_j} = C_{k_j}^{J_i} \tag{7}$$

Two of the most frequently employed relative sensitivity coefficients from MCA are the concentration and flux control coefficients, describing the (steady state) relative sensitivity between the reaction rate v and concentration or flux, respectively.[4,6,8,10,11,31]

When considering a kinetic network as described by eqs 1 and 2, the relative sensitivity coefficient between an arbitrary input $u_n(t)$, $n\in\{1,...,N\}$ and an arbitrary output $y_p(t)$, $p\in\{1,...,P\}$ becomes:

$$C_{u_n}^{y_p} = \frac{\partial \ln y_p}{\partial \ln u_n} = \frac{\partial y_p}{\partial u_n}\cdot\frac{u_n}{y_p} \tag{8}$$

The corresponding transfer function for the u_n-y_p input-output system is given by eq 6. In general, a transfer function is defined for a sinusoidal input of arbitrary frequency, whereas

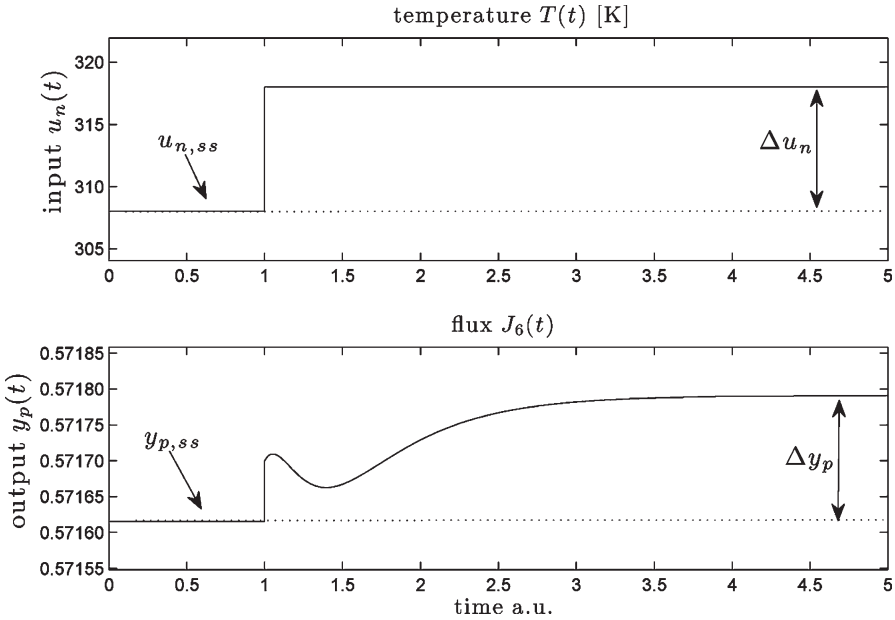


Figure 1. Example of how simulation results (or experimental results) can be used to empirically estimate the transfer function DC-gain $H(0) = K = (\Delta y_p)/(\Delta u_n)$ and thereby the steady state relative sensitivity coefficient $C_{u_n}^{y_p}$.

absolute/relative sensitivity/control coefficients are defined for (frequency independent) stepwise input perturbations.

In order to identify the relationship between a *frequency independent* (steady state) relative sensitivity coefficient $C_{u_n}^{y_p}$ (eq 8) and a corresponding *frequency dependent* transfer function $H_{y_p,u_n}(s)$ (eq 6), we first note from eq 6 that as the frequency of the sinusoidal input goes to zero, i.e., $s = jw = 0$, we get an expression for the steady state gain as $H_{y_p,u_n}(0) = K$. In general, the same steady state gain K is also obtained as the amplification of a small stepwise input signal Δu_n around the steady state value $u_{n,ss}$, resulting in an output change Δy_p around $y_{p,ss}$, i.e., $K = (\Delta y_p)/(\Delta u_n)$. This is shown in Figure 1 for the single-input single-output (SISO) system of motif M1 where the input is temperature $u_n(t) = T(t)$, and the output is flux $y_p(t) = J_6(t)$. See section Illustrating the Principles below and Supporting Information for details about the simulation.

Since Δu_n can be an infinite small step around the working point $u_{n,ss}$, the relationship $(\Delta y_p)/(\Delta u_n)$ can be interpreted as the absolute sensitivity coefficient $(\partial y_p)/(\partial u_n)$ and thereby:

$$C_{u_n}^{y_p} = H_{y_p,u_n}(0)\cdot\frac{u_{n,ss}}{y_{p,ss}} \tag{9}$$

Note that this expression is general and applies to either flux or concentration sensitivities depending on the definition of input $u_n(t)$ and output $y_p(t)$. From the input/output relationship in Figure 1, the steady state relative sensitivity coefficient, i.e., $C_{u_n}^{y_p} = C_T^{J_6}$, is straightforward to estimate as follows:

$$C_{u_n}^{y_p} = H_{y_p,u_n}(0)\cdot\frac{u_{n,ss}}{y_{p,ss}} = \frac{\Delta y_p}{\Delta u_n}\cdot\frac{u_{n,ss}}{y_{p,ss}}$$
$$\approx \frac{(0.57179 - 0.571615)}{(318 - 308)}\cdot\frac{308}{0.571615} = 0.0943 \tag{10}$$

The determination of $H_{y_p,u_n}(0)$ in eq 10 is actually a very simple form of system identification.

Generalizing the results in eq 9 for a multiple input multiple output (MIMO) system, gives the following relative sensitivity matrix as (using element-wise multiplication, or so-called Hadamard matrix multiplication $\circ$[32]):

$$\mathbf{C}_{\mathbf{u}}^{\mathbf{y}} = \frac{\partial \ln \mathbf{y}_{ss}}{\partial \ln \mathbf{u}_{ss}} = \begin{bmatrix} \frac{\partial \ln y_1}{\partial \ln u_1} & \frac{\partial \ln y_1}{\partial \ln u_2} & \cdots & \frac{\partial \ln y_1}{\partial \ln u_N} \\ \frac{\partial \ln y_2}{\partial \ln u_1} & \frac{\partial \ln y_2}{\partial \ln u_2} & \cdots & \frac{\partial \ln y_2}{\partial \ln u_N} \\ \vdots & \vdots & \vdots & \vdots \\ \frac{\partial \ln y_P}{\partial \ln u_1} & \frac{\partial \ln y_P}{\partial \ln u_2} & \cdots & \frac{\partial \ln y_P}{\partial \ln u_N} \end{bmatrix}$$
$$= \begin{bmatrix} \frac{\partial y_1}{\partial u_1} & \frac{\partial y_1}{\partial u_2} & \cdots & \frac{\partial y_1}{\partial u_N} \\ \frac{\partial y_2}{\partial u_1} & \frac{\partial y_2}{\partial u_2} & \cdots & \frac{\partial y_2}{\partial u_N} \\ \vdots & \vdots & \vdots & \vdots \\ \frac{\partial y_P}{\partial u_1} & \frac{\partial y_P}{\partial u_2} & \cdots & \frac{\partial y_P}{\partial u_N} \end{bmatrix} \circ \begin{bmatrix} \frac{u_{1,ss}}{y_{1,ss}} & \frac{u_{2,ss}}{y_{1,ss}} & \cdots & \frac{u_{N,ss}}{y_{1,ss}} \\ \frac{u_{1,ss}}{y_{2,ss}} & \frac{u_{2,ss}}{y_{2,ss}} & \cdots & \frac{u_N}{y_{2,ss}} \\ \vdots & \vdots & \vdots & \vdots \\ \frac{u_{1,ss}}{y_{P,ss}} & \frac{u_{2,ss}}{y_{P,ss}} & \cdots & \frac{u_{N,ss}}{y_{P,ss}} \end{bmatrix} = \frac{\partial \mathbf{y}_{ss}}{\partial \mathbf{u}_{ss}} \circ \frac{\mathbf{u}_{ss}}{\mathbf{y}_{ss}} \tag{11}$$

Similarly, as for the SISO case in eq 9, we identify the $(\partial \mathbf{y}_{ss})/(\partial \mathbf{u}_{ss})$ matrix in eq 11 as the steady state gain matrix $\mathbf{H}(0)$, giving the following relationship between the (steady state) relative sensitivity coefficient matrix and the transfer function matrix:

$$\mathbf{C}_{\mathbf{u}}^{\mathbf{y}} = \mathbf{H}(0)\circ\frac{\mathbf{u}_{ss}}{\mathbf{y}_{ss}} \tag{12}$$

This relationship also holds for the frequency domain, with $s = j\omega$ and where ω is the frequency

$$\mathbf{C}_{\mathbf{u}}^{\mathbf{y}}(s) = \mathbf{H}(s)\circ\frac{\mathbf{u}_{ss}}{\mathbf{y}_{ss}} \tag{13}$$

and where the steady state sensitivity coefficient matrix in eq 12 becomes a special case of eq 13. Hence, the *connectivity* that we stated to exist only in the transfer functions, actually exists in the *frequency dependent* relative sensitivity coefficients as well. It should also be emphasized that the dimensions of the two matrices $\mathbf{C}_{\mathbf{u}}^{\mathbf{y}}(s)$ and $\mathbf{H}(s)$ are the same, making it easy to compare

them element-wise. Comparing eq 7 and eq 9 indicates that the transfer function is actually the absolute or unscaled sensitivity coefficient.

Stoichiometric Network Approach. A frequency domain approach to sensitivity analysis was formulated by Ingalls[15] based on stoichiometric network theory.[9] This approach is described in more detail in the Supporting Information together with a comparison with the state space approach described above.

Conditions for Different Adaptation Types. It was previously stated[26] that a zero control coefficient (in a steady state relative control coefficient matrix) is only a necessary but not sufficient condition for robust (rate parameter independent) perfect adaption. This result is generally true for any steady state sensitivity coefficient since a zero valued element does not carry information about the connectivity or adaptive behavior between input and output. However, the connectivity information is provided by the transfer function $\mathbf{H}(s)$, or the frequency dependent scaled sensitivity coefficient $\mathbf{C}_{\mathbf{u}}^{\mathbf{y}}(s)$. Before we analyze the connectivity information, note that a transfer function element as in eq 6 can be rewritten as the relationship between two polynomials:

$$H_{y_p,u_n}(s) = \frac{b_m s^m + b_{m-1}s^{m-1} + \ldots + b_0}{s^k + a_{k-1}s^{k-1} + \ldots + a_0} \tag{14}$$

where the parameters $b_0, \ldots, b_m$ will generally be functions of the input vector $\mathbf{u_{ss}}$ and parameter vector $\boldsymbol{\alpha}$ as $b_j = h_j(\mathbf{u_{ss}},\boldsymbol{\alpha})$ $\forall\, j\in\{0,\ldots,m\}$. From eq 13, we calculate $C_{u_n}^{y_p}(s)$, which can be formulated as the relationship between a numerator polynomial $n_{u_n}^{y_p}(s)$ and a denominator polynomial $d_{u_n}^{y_p}(s)$ as follows:

$$C_{u_n}^{y_p}(s) = H_{y_p,u_n}(s)\cdot\frac{u_{n,ss}}{y_{p,ss}} = \frac{n_{u_n}^{y_p}(s)}{d_{u_n}^{y_p}(s)} \tag{15}$$

The numerator polynomial $n_{u_n}^{y_p}(s)$ of eq 15 can generally be written as follows:

$$n_{u_n}^{y_p}(s) = q(\mathbf{u_{ss}},\boldsymbol{\alpha})\cdot\sum_{j=0}^{m} h_j(\mathbf{u_{ss}},\boldsymbol{\alpha})s^j \tag{16}$$

where $q(\mathbf{u_{ss}},\boldsymbol{\alpha})$ comes from the term $(u_{n,ss})/(y_{p,ss})$.

On the basis of $C_{u_n}^{y_p}(s)$ and $n_{u_n}^{y_p}(s)$, the following scenarios describe the *conditions* for an output y_p (e.g., flux or concentration) to be either (1) robustly disconnected, (2) nonrobustly disconnected, (3) robust perfectly adapted, (4) nonrobust perfectly adapted, or (5) near nonrobust perfectly adapted, to changes in an input u_n (e.g., rate constant or temperature):

1 The output y_p is *robustly disconnected* from the input u_n if the following condition is fulfilled:
   (a) $C_{u_n}^{y_p}(s)\equiv 0$
   The corresponding element of the steady state relative sensitivity coefficient matrix $C_{u_n}^{y_p}$ will always be zero.

2 The output y_p is *nonrobustly disconnected* to changes in input u_n if all the following conditions are fulfilled ($\exists$ means *there exists*,: means *such that*, $\forall$ means *for all*):
   (a) $C_{u_n}^{y_p}(s) \not\equiv 0$
   (b) $\exists\,(\mathbf{u_{ss}},\boldsymbol{\alpha}):q(\mathbf{u_{ss}},\boldsymbol{\alpha}) = 0$
   (c) $\exists\, j\in\{0,\ldots,m\}: h_j(\mathbf{u_{ss}},\boldsymbol{\alpha}) \neq 0\ \forall\,(\mathbf{u_{ss}},\boldsymbol{\alpha})$
   This means that there is actually a connection between the input and output (since one or more of the functions $h_j(\mathbf{u_{ss}},\boldsymbol{\alpha}) \neq 0$), but this connectivity information is hidden when $q(\mathbf{u_{ss}},\boldsymbol{\alpha}) = 0$ for a certain combination of $\mathbf{u_{ss}}$ and $\boldsymbol{\alpha}$. The corresponding element of the steady state relative sensitivity coefficient matrix $C_{u_n}^{y_p}$ can in general be either zero or nonzero, depending the numerical values of $\mathbf{u_{ss}}$ and $\boldsymbol{\alpha}$.

3 The output y_p is *robust perfectly adapted* to changes in input u_n if all of the following conditions are fulfilled:
   (a) $C_{u_n}^{y_p}(s) \not\equiv 0$
   (b) $\exists\, j\in\{1,\ldots,m\}:q(\mathbf{u_{ss}},\boldsymbol{\alpha})\cdot h_j(\mathbf{u_{ss}},\boldsymbol{\alpha}) \neq 0\ \forall\,(\mathbf{u_{ss}},\boldsymbol{\alpha})$
   (c) $h_0(\mathbf{u_{ss}},\boldsymbol{\alpha}) = 0\ \forall\,(\mathbf{u_{ss}},\boldsymbol{\alpha})$
   This means that there will be a zero in origo since $h_0(\mathbf{u_{ss}},\boldsymbol{\alpha}) = 0$ regardless of the numerical values of $\mathbf{u_{ss}}$ and $\boldsymbol{\alpha}$. The corresponding element of the steady state relative sensitivity coefficient matrix $C_{u_n}^{y_p}$ will therefore always be zero.

4 The output y_p is *nonrobust perfectly adapted* to changes in input u_n if conditions (3a), (3b) and the following condition are fulfilled:
   (c) $\exists\,(\mathbf{u_{ss}},\boldsymbol{\alpha}):h_0(\mathbf{u_{ss}},\boldsymbol{\alpha}) = 0\ \forall\, u_n$
   This means that there exists a solution to $h_0(\mathbf{u_{ss}},\boldsymbol{\alpha}) = 0$ only for a certain combination of $\mathbf{u_{ss}}$ and $\boldsymbol{\alpha}$ and that the solution is *independent of* the actual input u_n. The corresponding element of the steady state relative sensitivity coefficient matrix $C_{u_n}^{y_p}$ can in general be either zero or nonzero, depending the numerical values of $\mathbf{u_{ss}}$ and $\boldsymbol{\alpha}$. This is in the literature sometimes referred to as balancing.[33]

5 The output y_p is *near nonrobust, perfectly adapted* to changes in input u_n (referred to as static compensation in ref 33) if conditions (3a), (3b) and the following condition are fulfilled:
   (c) $\exists\,(\mathbf{u_{ss}},\boldsymbol{\alpha}):h_0(\mathbf{u_{ss}},\boldsymbol{\alpha}) = 0$
   This implies that there exists a solution to $h_0(\mathbf{u_{ss}},\boldsymbol{\alpha}) = 0$ only for a certain combination of $\mathbf{u_{ss}}$ and $\boldsymbol{\alpha}$ and that the solution is *dependent on* the actual input u_n. The corresponding element of the steady state relative sensitivity coefficient matrix $C_{u_n}^{y_p}$ can in general be either zero or nonzero, depending the numerical values of $\mathbf{u_{ss}}$ and $\boldsymbol{\alpha}$.

These scenarios are applicable to both the state space and the stoichiometric network approaches. As mentioned in context with motifs M2 and M3 in the Supporting Information, if reactions rates are lumped in the modeling, as is often done in the stoichiometric network approach,[15] the dimensions of the control coefficient matrices will differ from the relative sensitivity coefficients from the state space approach, and hence, the results from the two methods with respect to various kinds of adaptation sites are not comparable.

■ ILLUSTRATING THE PRINCIPLES

We will use three different motifs (one here and two in the Supporting Information) to illustrate the conditions described in the previous chapter for the different kinds of adaptation. We will use superscript 1−5 to indicate which matrix element is robustly disconnected (scenario 1), nonrobustly disconnected (scenario 2), robust perfectly adapted (scenario 3), nonrobust perfectly adapted (scenario 4) or near nonrobust perfectly adapted (scenario 5), respectively.

Motif M1. Motif M1 shown below is used to find the relative sensitivity coefficients between temperature as input and fluxes or concentrations as outputs, and we show how to identify near nonrobust perfect adaptation (i.e., temperature compensation)

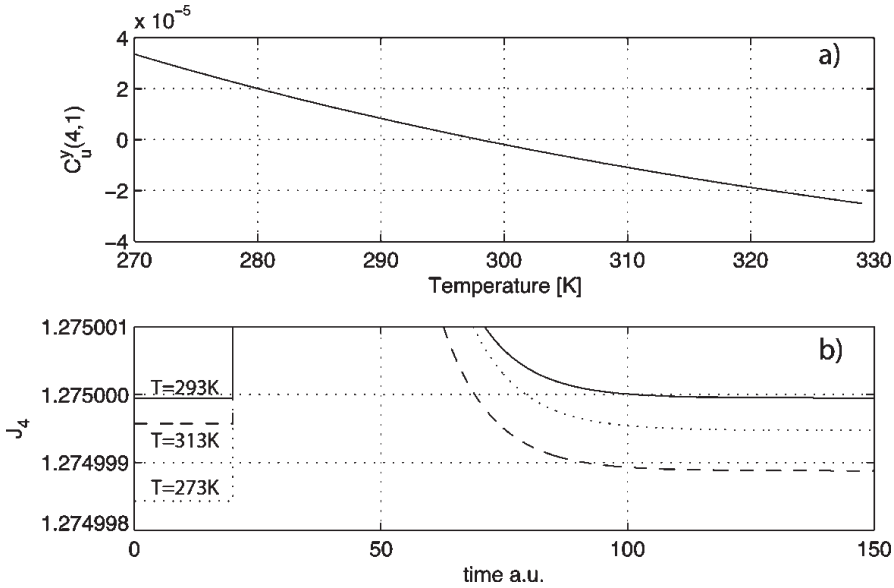
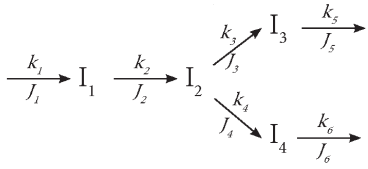


Figure 2. Near nonrobust perfect adaptation behavior. The parameters values are $\boldsymbol{\alpha} = [A_1, ..., A_6, E_1, ..., E_6, R]$, where the vector of activation constants are $[A_1, ..., A_6] = [1.72, 0.10, 0.52, 1.51, 1.40, 0.70]$, activation energies $[E_1, ..., E_6] = [24.5, 34, 120, 22, 98, 12]$ and $R = 8.314$. Note that these values are not identical to the ones in ref 33 as there are many possible combinations. The temperature is $\mathbf{u}_{ss} = T = 298$ K. (a) Temperature dependence of the relative sensitivity coefficient matrix element $\mathbf{C}_\mathbf{u}^\mathbf{y}(4,1)$ from temperature to flux J_4. (b) Partial, nonrobust, and overadaptation in flux J_4 as a function of a stepwise perturbation of 10 K at $t = 20$ au in the input, when the preperturbation value of the temperature is 273, 293, or 313 K, respectively. The peak value of the fluxes J_4 after the step is approximately 1.25755 (data not shown). The step response for $T = 293$ K shows nonrobust perfect adaptation since the step goes from 5 K below the balancing temperature of $T = 298$ K, to 5 K above this temperature.

sites.[14,33−35] We do not identify the corresponding control coefficients used in MCA.



(M1)

The input to the model is the scalar temperature $\mathbf{u}(t) = T(t)$ and the output from the model is the fluxes $\mathbf{y}(t) = [k_1(t),\ k_2(t)x_1(t),\ k_3(t)x_2(t),\ k_4(t)x_2(t),\ k_5(t)x_3(t),\ k_6(t)x_4(t)]^T$. The dynamic model of this motif is given in the Supporting Information.

In ref 33, it was shown that this network actually exhibits near nonrobust perfect adaptation (termed static temperature compensation in ref 33) in flux J_6 for a certain combination of activation energies. In this work, we find the same result from analyzing the numerator polynomials of $\mathbf{C}_\mathbf{u}^\mathbf{y}(s)$.

As an example, consider $n_{u_1}^{y_3}(s)$ where $\mathbf{u}_{ss} = T$ and $\boldsymbol{\alpha} = [A_1, ..., A_6, E_1, ..., E_6, R]$, the following functions are found:

$$q(\mathbf{u}_{ss}, \alpha) = 1 \tag{17}$$

$$h_2(\mathbf{u}_{ss}, \alpha) = E_3 \tag{18}$$

$$h_1(\mathbf{u}_{ss}, \alpha) = A_2 e^{-E_2/RT} E_3 + A_3 e^{-E_3/RT} E_2 + A_4 e^{-E_4/RT}(E_2 + E_3 - E_4) \tag{19}$$

$$h_0(\mathbf{u}_{ss}, \alpha) = A_2 e^{-E_2/RT}(A_3 e^{-E_3/RT} E_1 + A_4 e^{-E_4/RT}(E_1 + E_3 - E_4)) \tag{20}$$

As can be seen, conditions (3a) and (3b) are fulfilled, whereas (3c) is not. Proceeding to scenario 4, we also see that condition (4c) cannot be fulfilled. However, condition (5c) can be fulfilled since the function $h_0(\mathbf{u}_{ss},\boldsymbol{\alpha})$ is dependent on the actual input $u_1 = T$ and a solution to $h_0(\mathbf{u}_{ss},\boldsymbol{\alpha}) = 0$ exists for a certain temperature T and certain parameters $\boldsymbol{\alpha}$.

The complete steady state relative sensitivity coefficient matrix $\mathbf{C}_\mathbf{u}^\mathbf{y}$ is shown in eq 21.

$$\mathbf{C}_\mathbf{u}^\mathbf{y} = \begin{pmatrix} \frac{E_1}{RT} \\ \frac{E_1}{RT} \\ \frac{E_1}{RT} + \frac{A_4 e^{-E_4/RT}(E_3 - E_4)}{RT(A_3 e^{-E_3/RT} + A_4 e^{-E_4/RT})} \\ \frac{E_1}{RT} - \frac{A_3 e^{-E_3/RT}(E_3 - E_4)}{RT(A_3 e^{-E_3/RT} + A_4 e^{-E_4/RT})} \\ \frac{E_1}{RT} + \frac{A_4 e^{-E_4/RT}(E_3 - E_4)}{RT(A_3 e^{-E_3/RT} + A_4 e^{-E_4/RT})} \\ \frac{E_1}{RT} - \frac{A_3 e^{-E_3/RT}(E_3 - E_4)}{RT(A_3 e^{-E_3/RT} + A_4 e^{-E_4/RT})} \end{pmatrix}^S \tag{21}$$

Since both elements $\mathbf{C}_\mathbf{u}^\mathbf{y}(3,1)$ and $\mathbf{C}_\mathbf{u}^\mathbf{y}(5,1)$ are equal, the condition for near nonrobust perfect adaptation in J_3 and J_5 is found from condition (5c) where a solution to $h_0(\mathbf{u}_{ss},\boldsymbol{\alpha}) = 0$ can be found:

$$A_3 e^{-E_3/RT} E_1 + A_4 e^{-E_4/RT}(E_1 + E_3 - E_4) = 0 \tag{22}$$

Similarly, since both elements $\mathbf{C}_\mathbf{u}^\mathbf{y}(4,1)$ and $\mathbf{C}_\mathbf{u}^\mathbf{y}(6,1)$ are also equal, the condition for near nonrobust perfect adaptation in J_4 and J_6 is:

$$A_4 e^{-E_4/RT} E_1 + A_3 e^{-E_3/RT}(E_1 + E_3 - E_4) = 0 \tag{23}$$

What we in this work term near nonrobust perfect adaptation is actually either partial, nonrobust, or overadaptation as described in ref 26, where the actual response depends on the preperturbation steady state value of the input. This is shown in

Figure 2a) where the relative sensitivity coefficient matrix element $\mathbf{C}_{\mathbf{u}}^{\mathbf{y}}(4,1)$ is plotted as a function of temperature T. The responses in Figure 2b) are examples of partial, nonrobust and overadaptation in flux J_4 as a function of a stepwise perturbation of 10 K at $t = 20$ au in the input, when the preperturbation value of the temperature is 273, 293, or 313 K, respectively.

As already mentioned, the SISO-system response in Figure 1 is based on a simulation (shown in Supporting Information) of the nonlinear model of motif M1 for a stepwise increase in temperature of 10 K from 308 to 318 K. The corresponding analytical values of $\mathbf{C}_{\mathbf{u}}^{\mathbf{y}}$ (eq 21) are calculated to be as follows:

$$\mathbf{C}_{\mathbf{u}}^{\mathbf{y}} = \begin{bmatrix} 0.02616 \\ 0.02616 \\ 0.04806 \\ 0.00975 \\ 0.04806 \\ 0.00975 \end{bmatrix} \tag{24}$$

Recall from eq 10 that the empirical steady state relative sensitivity coefficient from temperature $u_n(t) = T(t)$ to flux $y_p(t) = J_6(t)$ was estimated to $C_{u_n}^{y_p} \approx 0.00943$. The difference between this empirical value and the analytical value of element (6,1) in eq 24 is due to the relative large step of 10 K temperature increase in Figure 1. A smaller stepwise increase in temperature will reduce the difference, and this actually demonstrates the nonlinearity of the system.

DISCUSSION

We have shown that the transfer function matrix $\mathbf{H}(s)$ and the *frequency dependent* relative sensitivity coefficient matrix $\mathbf{C}_{\mathbf{u}}^{\mathbf{y}}(s)$ both provide connectivity information between inputs and outputs in reaction kinetic networks. This information is lacking in *steady state* relative sensitivity coefficient matrix $\mathbf{C}_{\mathbf{u}}^{\mathbf{y}}$ in general and in control coefficients in particular. Moreover, we have shown that the transfer function is the same as the absolute or unscaled sensitivity coefficient.

From the numerator polynomial of the relative sensitivity matrix $\mathbf{C}_{\mathbf{u}}^{\mathbf{y}}(s)$ we have identified five scenarios describing conditions to determine whether a receptor-mediated input to a reaction kinetic network can show robust (perturbation independent) or nonrobust (balanced or perturbation dependent) adaptive or homeostatic behavior in an output.

We have also shown (Supporting Information) that care should be taken when lumping reversible reactions together (as in the Stoichiometric Network Approach), as the independence of two rate constants is reduced to one, and may therefore not reflect the actual independence of such rate constants.

The state space approach presented here has the advantage that it describes the relationship between transfer functions and the corresponding relative sensitivity coefficients by a single equation independent of the choice of inputs and outputs. This makes an automated flux and concentration analysis of network perturbations straightforward.

ASSOCIATED CONTENT

Supporting Information. The Supporting Information contains derivations of the transfer function matrices and relative sensitivity coefficients for motif M1, M2, and M3. This material is available free of charge via the Internet at http://pubs.acs.org.

AUTHOR INFORMATION

Corresponding Author

*Tel.: (47) 5183-1887; Fax: (47) 5183-1750; E-mail: peter.ruoff@uis.no.

REFERENCES

(1) Milsum, J. H. *Biological Control Systems Analysis*; McGraw-Hill: New York, 1966.
(2) Rosen, R. *Dynamical System Theory in Biology. Vol. I: Stability Theory and its Applications*; Wiley: New York, 1970.
(3) von Bertalanffy, L. *Perspectives on General System Theory*; George Braziller: New York, 1975.
(4) Savageau, M. A. *Nature* **1971**, *229* (5286), 542–4.
(5) Savageau, M. A. *Biochemical Systems Analysis. A Study of Function and Design in Molecular Biology*; Addison-Wesley: Reading, 1976.
(6) Kacser, H.; Burns, J. A. *Symp. Soc. Exp. Biol.* **1973**, *27*, 65–104.
(7) Kacser, H.; Burns, J. A. *Biochem. Soc. Trans.* **1979**, *7* (5), 1149–60.
(8) Burns, J. A.; Cornish-Bowden, A.; Groen, A. K.; Heinrich, R.; Kacser, H.; Porteous, J. W.; Rapoport, S. M.; Rapoport, T. A.; Stucki, J. W.; Tager, J. M.; Wanders, R. J. A.; Westerhoff, H. V. *Trends Biochem. Sci.* **1985**, *19*, 16.
(9) Reder, C. *J. Theor. Biol.* **1988**, *135*, 175–210.
(10) Heinrich, R.; Schuster, S. *The Regulation of Cellular Systems*; Chapman and Hall: New York, 1996.
(11) Fell, D. *Understanding the Control of Metabolism*; Portland Press: London and Miami, 1997.
(12) Hofmeyr, J. H. S. In Yi, T. M., Hucka, M., Morohashi, M., Kitano, H., Eds., *Proceedings of the 2nd International Conference on Systems Biology*; Omnipress: Madison,WI, 2001; pp 291−300.
(13) Ingalls, B. P.; Sauro, H. M. *J. Theor. Biol.* **2003**, *222* (1), 23–36.
(14) Ruoff, P.; Christensen, M. K.; Wolf, J.; Heinrich, R. *Biophys. Chem.* **2003**, *106* (2), 179–92.
(15) Ingalls, B. P. *J. Phys. Chem. B* **2004**, *108*, 1143–1152.
(16) Ingalls, B. P. In *Control Theory and Systems Biology*; Iglesias, P. A., Ingalls, B. P., Eds.; The MIT Press: Cambridge, MA, 2010; Chapter 8, pp 145−168.
(17) Deutsch, A., Howard, J., Falke, M., Zimmermann, W, E., Eds. *Function and Regulation of Cellular Systems*; Birkhauser: Basel, 2004.
(18) Ross, J.; Schreiber, I.; Vlad, M. O. *Determination of Complex Reaction Mechanisms. Analysis of Chemical, Biological and Genetic Networks*; Oxford University Press: Oxford, 2006.
(19) Szallasi, Z., Stelling, J., Periwal, V., E., Eds. *System Modeling in Cellular Biology*; MIT Press: Cambridge, MA, 2006.
(20) Ross, J. *J. Phys. Chem. A* **2008**, *112*, 2134–2143.
(21) Wilkie, J.; Johnson, M.; Reza, K. *Control Engineering: An Introductory Course*; Palgrave: New York, 2002.
(22) Wolkenhauer, O.; Sreenath, S. N.; Wellstead, P.; Ullah, M.; Cho, K. H. *Biochem. Soc. Trans.* **2005**, *33* (Pt 3), 507–15.
(23) Wolkenhauer, O.; Ullah, M.; Wellstead, P.; Cho, K. *FEBS Lett.* **2005**, *579* (8, Sp. Iss. SI), 1846–1853.
(24) Ingalls, B. P.; Yi, T.-M.; Iglesias, P. A. In *System Modeling in Cellular Biology*; Szallasi, Z., Stelling, J., Periwal, V., Eds.; MIT Press: Cambridge, MA, 2006; pages 243−267.
(25) Ingalls, B. P. In *Proceedings of the 45th IEEE Conference on Decision and Control*, pp 2116−2121, 2006.
(26) Drengstig, T.; Ueda, H. R.; Ruoff, P. *J. Phys. Chem. B* **2008**, *112*, 16752–16758.
(27) Drengstig, T.; Kjosmoen, T.; Ruoff, P. In *Yeast Genetic Networks*; Becskei, A., Ed.; Springer: New York, 2010; Chapter 8; Methods in Molecular Biology.
(28) Ogata, K. *Modern Control Engineering*, 4th ed.; Prentice Hall: New York, 2002.

(29) Ljung, L. *System Identification Theory For the User*, 2nd ed.; PTR Prentice Hall: New York, 1999.
(30) Kohn, M. C. *Risk Anal.* **2002**, *22* (3), 623–631.
(31) Heinrich, R.; Rapoport, T. A. *Eur. J. Biochem.* **1974**, *42*, 89–95.
(32) Lutkepohl, H. *Handbook of Matrices*; John Wiley & Sons: New York, 1996.
(33) Ruoff, P.; Zakhartsev, M.; Westerhoff, H. V. *FEBS J.* **2007**, *274* (4), 940–50.
(34) Ruoff, P. *J. Interdisciplinary Cycle Res.* **1992**, *23*, 92–99.
(35) Ruoff, P.; Loros, J. J.; Dunlap, J. C. *Proc. Natl. Acad. Sci. U. S. A.* **2005**, *102* (49), 17681–6.