Modular Interaction Strengths in Regulatory Networks; An Example

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Abstract. Cellular networks composed of metabolic, signalling, and genetic subnetworks, comprise many distinct intermediates. However, only a subset of the latter, referred to as 'communicating intermediates', mediate cross-talk between individual modules. Here, this characteristic feature of modular networks is exploited to simplify the quantitative description of the responses of these networks to environmental changes, to a description solely in terms of the communicating intermediates. Such a strategy reduces the number of variables that need to be considered. It allows for the determination of the quantitative contribution of individual modular interactions to the regulation of concentrations of communicating intermediates. This is illustrated by a calculation for an example of a modular biochemical network.

Keywords: modularity, modelling, regulation, signal transduction, networks, metabolic control analysis, response analysis

1. Introduction

The mechanistic understanding of emergent properties of cellular networks is one of the objectives of contemporary cell biology. So far, molecular biology has focused on identifying the numerous interactions that occur in a cell (Kohn, 1999). To achieve the desired mechanistic understanding the contribution of individual interactions, loops and modules to the regulation of the states of particular intermediates, e.g. transcription factors, should be quantified.

Information flow between modules is mediated by communicating intermediates, e.g. through covalent modification, protein-protein interactions or small-metabolite binding. Modular interactions are considered regulatory if the amount of mass flow between modules associated with them is negligible. In such cases modules are referred to as levels (Westerhoff, 1989; Hofmeyr and Westerhoff, 2001). Modular response analysis (MRA) allows for a description of global responses of communicating intermediates, in terms of local responses between levels (Bruggeman, 2002). It defines the topology of the regulatory network quantitatively in terms of a (reduced) interaction map that has as its

entries local response coefficients of communicating intermediates of levels that interact directly (see Appendix). MRA is a generalization of the approach pioneered in Kholodenko et al. (1997 & 1998).

In MRA, the number of communicating intermediates depends on the number of levels and their biochemical composition. By decreasing the number of levels a gradual simplification can be accomplished, which may make cellular networks more comprehensible. Furthermore, modular interaction strengths are defined that quantify the sensitivity of certain communicating intermediates to changes in others.

Here, MRA will be applied to a particular modular network. The individual modular interaction strengths will be determined as function of a signal concentration. The resulting characteristics of the modular interaction strengths will be discussed in the light of a modular understanding of the example network.

2. Results

The modular interaction strengths were studied for the regulatory network that is depicted in Fig. 1A. This is a modular decomposition of the network that is shown in the Appendix (Fig. 2). The network was decomposed into 4 levels that are linked through 6 communicating intermediates. Hereby the number of intermediates to be considered was decreased from 11 to 6 and can be decreased even more if, for instance, level 3 and 4 were fused into a single supra-level. A quantitative representation of the network depicted in Fig. 1A in terms of the reduced interaction map and further information regarding the kinetic model is given in the Appendix.

Each graph in Fig. 1B portrays the modular interaction strengths for a particular module as function of the concentration of S. A modular interaction strength of X_i on a communicating intermediate of the j-th module, e.g. X_j, equals a regulatory strength if X_i affects only one rate in the j-th module, e.g. $R^{X_j}_{X_i} = C^{X_j}_{v_j} \cdot \epsilon^{v_j}_{X_i}$. Regulatory strengths were first defined by Kahn & Westerhoff (1993). If multiple rates are affected modular interaction strengths represent a sum of regulatory strengths, e.g. $R^{X_j}_{X_i} = \sum_k C^{X_j}_{v_{j,k}} \cdot \epsilon^{v_{j,k}}_{X_i}$ (where k runs over all rates in module j). The determination of the modular interaction strengths is achieved through inversion of the reduced interaction map (see Appendix). The reduced interaction resembles a quantitative representation of the interactions in a modular network (see Appendix).

The graphs in Fig. 1B indicate that all the modular interaction strengths change with S. The modular interaction strengths for module 1 indicate that X_2 is more sensitive to changes in E_2I than is X_3. The

sensitivity of X_3 to E_2I proves to be relatively constant and close to zero. The results for module 2 indicate that E_2I is most sensitive to $mRNA_2$ and much less sensitive to R_2 and R_2P. The communicating intermediate R_2 of the third module, is more sensitive to changes in X_2 and X_3 than is its partner R_2P. Additionally, the sensitivity to X_2 increases with S whereas the sensitivities to X_3 decrease with S. The last graph portrays the modular interaction strength between $mRNA_2$ and R_2 which proves to be a monotonically decreasing function of S.

Figure 1. A. A cellular network that is composed of 4 modules and constitutes 6 communicating intermediates. S is an intermediate that is metabolized in module 1. Arrows denote interaction between individual modules quantified through local response coefficients that are entries in the reduced interaction map (see Appendix). **B.** Scaled modular interaction strengths between communicating intermediates, e.g. R[R_2P,X_2] ($R_{X_2}^{R_2P}$) represents the strength by which R_2P affects X_2 through module 3 and 1, respectively.

Additionally, the relative importance of regulation through gene expression and metabolism can be assessed. As an example the genetic contribution to the response of E_2I upon a perturbation in S will be analyzed. A perturbation of module 1 through S is spread through the network by changed levels of the communicating intermediates, X_2 and X_3 and eventually affects the steady-state concentration of E_2I,

$$R_S^{E_2I} = R_{X_2}^{E_2I} \cdot r_S^{X_2} + R_{X_3}^{E_2I} \cdot r_S^{X_3} \tag{1}$$

The modular interaction strengths, $R_{X_2}^{E_2I}$ and $R_{X_3}^{E_2I}$, can be equated in terms of local response coefficients of communicating intermediates (entries of the reduced interaction map)(see Appendix),

$$\begin{aligned} R_S^{E_2I} &= \frac{(r_{R_2}^{E_2I} \cdot r_{X_2}^{R_2} + r_{R_2P}^{E_2I} \cdot r_{X_2}^{R_2P} + r_{X_2}^{E_2I})}{1 - \sum_i \mathrm{Loop}_i} \cdot r_S^{X_2} + \\ &\quad \frac{(r_{R_2}^{E_2I} \cdot r_{X_3}^{R_2} + r_{R_2P}^{E_2I} \cdot r_{X_3}^{R_2P})}{1 - \sum_i \mathrm{Loop}_i} \cdot r_S^{X_3} + \\ &\quad \frac{r_{mRNA_2}^{E_2I} \cdot r_{R_2}^{mRNA_2} \cdot r_{X_2}^{R_2}}{1 - \sum_i \mathrm{Loop}_i} \cdot r_S^{X_2} + \frac{r_{mRNA_2}^{E_2I} \cdot r_{R_2}^{mRNA_2} \cdot r_{X_3}^{R_2}}{1 - \sum_i \mathrm{Loop}_i} \cdot r_S^{X_3} \\ &\equiv {}^M R_{X_2}^{E_2I} \cdot r_S^{X_2} + {}^M R_{X_3}^{E_2I} \cdot r_S^{X_3} + {}^G R_{X_2}^{E_2I} \cdot r_S^{X_2} + {}^G R_{X_3}^{E_2I} \cdot r_S^{X_3} \\ &\equiv {}^M R_S^{E_2I} + {}^G R_S^{E_2I} \end{aligned} \tag{2}$$

where $\sum_i \mathrm{Loop}_i$ represent the sum of closed loops in the systems (closed loops are products of local response coefficients that start and end at

the same communicating intermediate, e.g. $r_{E_2I}^{X_3} \cdot r_{X_3}^{E_2I}$ (Bruggeman, 2002)) , ${}^{M}R_S^{E_2I}$ denotes the metabolic response of E_2I upon change in S and ${}^{G}R_S^{E_2I}$ denotes the genetic response of E_2I upon change in S. For instance the ratio, $\frac{{}^{G}R_S^{E_2I}}{{}^{M}R_S^{E_2I}}$, can be applied to determine the relative response of gene expression and metabolism of a cellular network to an environmental change.

3. Discussion

Studies aiming at the understanding and manipulation of metabolism profit greatly from conceptual tools such as modelling and metabolic control analysis (MCA). Attempts to apply MCA tools to networks that comprise signalling, metabolic, and genetic subnetworks have been scarce. This may be due to the complexity of such networks. Quantitative approaches that allow the construction of black-box modules might assist analysis of such networks.

Here we applied MRA to decompose a network into (black-box) levels that interact through communicating intermediates. This decomposition reduced the number of intermediates from 11 to 6. All modular interactions were computed as a function of the level of the signal (S). The determination of the modular regulatory strengths enabled the assessment of the relative importance of modular interactions. Hereby the functional properties of modules can be addressed. For example, module 4 can be envisioned as a strong signal attenuator, whereas module 2 resembles a moderate amplifier with respect to R_2. Furthermore, disentangling contributions of metabolic and genetic nature to particular global responses were illustrated. In conclusion, MRA appears to be a rational approach for analyzing complex networks by virtue of a stepwise and sequential decomposition of the network into an increasing number of modules.

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Appendix

Modular Response Analysis

Matrices and vectors are bold face and scalars in regular font. Reder (1988) found the concentration control matrix ($\mathbf{\Gamma_v^X}$) for a steady-state biochemical network with m intermediates (elements of vector $\mathbf{s}$), of which m_0 are independent (entries in vector $\mathbf{x}$), and r reactions, to be equal to,

$$\frac{\partial \mathbf{x}}{\partial \mathbf{p}} = -\left(\mathbf{M_{m_0,m_0}}\right)^{-1} \cdot \mathbf{N}^0_{m_0,r} \cdot \frac{\partial \mathbf{v}}{\partial \mathbf{p}} \equiv \left(\mathbf{\Gamma_v^X}\right)_{m_0,r} \cdot \frac{\partial \mathbf{v}}{\partial \mathbf{p}} \tag{3}$$

where $\mathbf{M}$ is the Jacobian matrix of the biochemical network, $\mathbf{N}^0 \cdot \frac{\partial \mathbf{v}}{\partial \mathbf{s}}_{r,m} \cdot \mathbf{L}_{m,m_0}$ (assumed to be nonsingular). Kahn (1993) extended this derivation explicitly for modular networks of the level-type, i.e. $\mathbf{N^0} = \mathbf{Dg}(\mathbf{N_i}^0)$ and $\mathbf{L} = \mathbf{Dg}(\mathbf{L_i})$ ($\mathbf{Dg}$ denotes a block-diagonal matrix and the subscript i refers to the i-th module),

$$\frac{\partial \mathbf{x}}{\partial \mathbf{p}} = -\left(-\mathbf{Dg}\left(\mathbf{M_i}\right)^{-1} \cdot \mathbf{M}\right)^{-1} \cdot -\mathbf{Dg}\left(\mathbf{M_i}\right)^{-1} \cdot \mathbf{Dg}(\mathbf{N_i}^0) \cdot \frac{\partial \mathbf{v}}{\partial \mathbf{p}} \tag{4}$$

$\mathbf{Dg}(\mathbf{M}_i)$ denotes a block-diagonal invertible matrix with the Jacobian matrix of the i-th module as its (i)-th diagonal entry with $\mathbf{M}_i = \mathbf{N_i^0} \cdot \frac{\partial \mathbf{v_i}}{\partial \mathbf{s_i}} \cdot \mathbf{L_i}$. This step in the derivation has similarities with the approach of Hofmeyr and Westerhoff (2001) based on the $\mathbf{C} \cdot \mathbf{E} = \mathbf{I}$ formalism of MCA. The matrix that determines the global response of all intermediates to a change in $\mathbf{p}$ ($\mathbf{R_p^X}$) can be expressed in terms of the interaction map ($\mathbf{r_X^X}$) and local external responses (Bruggeman, 2002),

$$\mathbf{R_p^X} = -\left(\mathbf{r_X^X}\right)^{-1} \cdot \mathbf{Dg}\left(\mathbf{r_{p_i}^{X_i}}\right) \tag{5}$$

The interaction map is a partitioned matrix that contains as its (i,j)-th (matrix) entry the sensitivity of the intermediates of module i to a change in the intermediates of module j, $\mathbf{r_{X_j}^{X_i}}$, and as its diagonal (matrix) entries the sensitivities of the intermediates of module i to a change in themselves, $\mathbf{r_{X_i}^{X_i}}$. The latter matrix represents the connectivity theorem for concentration control for the i-th module, i.e. $\mathbf{r_{X_i}^{X_i}} = -\mathbf{I}$. Bruggeman (2002) showed that irrespective of the exact modular decomposition, the responses of the communicating intermediates to parameter changes can always be expressed as,

$$\mathbf{R_p^{X^{com}}} = -\left(\mathbf{r_X^{X^{com}}}\right)^{-1} \cdot \mathbf{Dg}\left(\mathbf{r_{p_i}^{X_i^{com}}}\right) = \mathbf{R_X^{X^{com}}} \cdot \mathbf{Dg}\left(\mathbf{r_{p_i}^{X_i^{com}}}\right) \tag{6}$$

Minus the inverse of the reduced interaction map $\mathbf{r}^{\mathbf{X_{com}}}_{\mathbf{X_{com}}}$ equals the reduced matrix of modular interaction strengths, $\mathbf{R}^{\mathbf{X_{com}}}_{\mathbf{X_{com}}}$. The entries of $\mathbf{r}^{\mathbf{X_{com}}}_{\mathbf{X_{com}}}$ depict and quantify direct *local* interactions between individual modules mediated by communicating intermediates. The entries of $\mathbf{R}^{\mathbf{X_{com}}}_{\mathbf{X_{com}}}$ are the *global* modular interaction strengths between communicating intermediates as defined in the main text. In scaled format the reduced interaction map and reduced modular interaction strength matrices are $\mathbf{Dg}(X^{com}) \cdot \mathbf{r}^{\mathbf{X_{com}}}_{\mathbf{X_{com}}} \cdot \mathbf{Dg}(\frac{1}{X^{com}})$ and $\mathbf{Dg}(X^{com}) \cdot \mathbf{R}^{\mathbf{X_{com}}}_{\mathbf{X_{com}}} \cdot \mathbf{Dg}(\frac{1}{X^{com}})$, respectively. Further details on the computation of the reduced interaction map from the jacobian of the entire regulatory network can be found in Bruggeman (2002).

Kinetic Model of a Regulatory Network

Figure 2 depicts a biochemical scheme of the cellular network. This network does not represent an existing biochemical network but merely serves as an example. The first level is a metabolic subsystem that consists of 7 reactions and 3 intermediates of which 2 intermediates are communicating, i.e. X_2 and X_3. The second level contains the covalent modification cycle of enzyme E_2 that is active in the metabolic module. The inactive form E_2I communicates with module 1. Additionally, the translation of $mRNA_2$ into E_2 occurs in this subnetwork. The third level harbors the catalytic processes associated with phosphorylation and dephosphorylation of the regulatory protein R_2 (a dimer). Both monomers can be phosphorylated but only R_2 and R_2P are communicating. The fourth module contains the phosphorylation-dephosphorylation cycle of a transcription factor which in phosphorylated form activates transcription of the gene encoding E_2.

Table 1 contains the kinetic parameters of the constituent enzyme-catalyzed reactions of the regulatory network depicted in Fig. 2. All enzymes were either modelled with uni-uni (uu) or bi-uni (bu) kinetics, i.e.

$$v_{uu,i} = \frac{V_i \cdot \frac{S_1}{K_{mi,S_1}}(1 - \frac{P}{K_{eq,i}})}{1 + \frac{S_1}{K_{mi,S_1}} + \frac{P}{K_{mi,P}}} \tag{7}$$

$$v_{bu,i} = \frac{V_i \cdot \frac{S_1}{K_{mi,S_1}} \cdot \frac{S_2}{K_{mi,S_2}}(1 - \frac{P}{K_{eq,i}})}{(1 + \frac{S_1}{K_{mi,S_1}} + \frac{P}{K_{mi,P}}) \cdot (1 + \frac{S_2}{K_{mi,S_2}})} \tag{8}$$

Table 1. Kinetic parameters used in the calculations (all equilibrium constants ($K_{eq,i}$) were set to $1{\cdot}10^5$ and the moiety conservation relationships were $R_2 + R_2P + R_2P_2 = 1$ and $T + TP = 1$). All concentrations were in mM and time in minutes.

v_i	uu/bu	V_i	S_1	K_{mi,S_1}	S_2	K_{mi,S_2}	P	$K_{mi,P}$
1	bu	100	S	0.5	-	-	X_1	8
2	bu	$\frac{100}{1+(\frac{E_2I}{0.1})^3}$	S	0.1	X_1	0.1	X_2	0.5
3	uu	100	X_2	1	-	-	1	0.5
4	bu	100	X_2	$1 \cdot 10^{-2}$	X_3	0.1	X_1	0.1
5	uu	100	X_1	0.1	-	-	1	0.1
6	uu	50	1	0.1	-	-	X_3	0.1
7	uu	50	X_3	0.1	-	-	1	2
8	uu	$\frac{100}{1+(\frac{1\cdot10^{-4}}{X_2})^2}$	E_2	0.01	-	-	E_2I	0.01
9	uu	$\frac{100}{1+\frac{0.1}{X_7}+\frac{X_6}{0.1}}$	E_2I	0.01	-	-	E_2	0.01
10	uu	$\frac{90}{1+\frac{X_2}{0.1}}$	R_2	0.1	-	-	R_2P	1
11	uu	$\frac{90}{1+\frac{X_2}{0.1}}$	R_2P	0.1	-	-	R_2P_2	1
12	uu	$\frac{90}{1+\frac{0.1}{X_3}}$	R_2P	0.1	-	-	R_2	1
13	uu	$\frac{90}{1+\frac{0.1}{X_3}}$	R_2P_2	0.1	-	-	R_2P	1
14	uu	$\frac{10}{1+\frac{0.3}{R_2}}$	TP	0.1	-	-	T	1
15	uu	10	T	0.1	-	-	TP	1
16	uu	$\frac{1}{1+(\frac{0.1}{TP})^2}$	1	0.1	-	-	$mRNA_2$	0.1
17	uu	1	$mRNA_2$	0.1	-	-	E_2	1
18	uu	$mRNA_2$	1	0.1	-	-	E_2	1
19	uu	1	E_2	0.1	-	-	1	1

The modular interaction strength matrix was calculated in the different steady states as function of the signal concentration (S) through inversion of minus the reduced interaction map (Eq. 4) of the regulatory network. The reduced interaction for the modular representation of the network (Fig. 1A main text) can be read from its regulatory topology and equals,

$$\mathbf{r}^{\mathbf{X_{com}}}_{\mathbf{X_{com}}} = \begin{bmatrix} -1 & 0 & r^{X_2}_{E_2I} & 0 & 0 & 0 \\ 0 & -1 & r^{X_3}_{E_2I} & 0 & 0 & 0 \\ r^{E_2I}_{X_2} & 0 & -1 & r^{E_2I}_{R_2} & r^{E_2I}_{R_2P} & r^{E_2I}_{mRNA_2} \\ r^{R_2}_{X_2} & r^{R_2}_{X_3} & 0 & -1 & 0 & 0 \\ r^{R_2P}_{X_2} & r^{R_2P}_{X_3} & 0 & 0 & -1 & 0 \\ 0 & 0 & 0 & r^{mRNA_2}_{R_2} & 0 & -1 \end{bmatrix} \quad (9)$$

MRA for this network was programmed in Maple 7 (Waterloo Maple Inc.). The Maple scripts can be obtained from the author.

Figure 2. Biochemical scheme of the regulatory network. Reactions are denoted by arrows and modules by gray boxes. Intermediates that affect rates of processes through regulatory interactions (denoted by dashed arrows) outside their intrinsic module are communicating intermediates.

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