Supporting information for Kholodenko *et al.* (2002) *Proc. Natl. Acad. Sci. USA*, 10.1073/pnas.192442699

Appendix 2: Mathematical Description

In this Appendix we provide an abstract mathematical derivation of the method presented in the paper. This presentation uses the implicit function theorem and matrix notation. A more general version that relaxes the assumption that perturbations considered affect only single modules is published elsewhere (1).

The problem studied in this paper can be reformulated, in an abstract mathematical way, as follows. We consider a dynamic system,

$$dx/dt = f(x,p), \quad x = x_1,...,x_n, \quad p = p_1,...,p_n, \qquad \textbf{[1]}$$

where the vector of variables $x = (x_1,\ldots, x_n)$ and the vector of parameters $p = (p_1,\ldots, p_m)$ belong to open subsets of Euclidean spaces. It is assumed that the system has a stable steady state (x^0, p^0),

$$f(x,p) = 0, \qquad \textbf{[2]}$$

where the Jacobian matrix, **J**, is nonsingular,

$$\mathbf{J} = (\partial f/\partial x). \qquad \textbf{[3]}$$

According to the implicit function theorem, there is a unique vector $x(p)$ solving the set of Eq. **2** in some neighborhood of a particular value p^0. One objective would be to determine the Jacobian (**J**), assuming that one can determine the global response matrix ($\mathbf{R_p}$) experimentally given by Eq. **4** of the main text (hereafter nonnormalized derivatives are used),

$$\mathbf{R_p} = (\partial x/\partial p). \qquad \textbf{[4]}$$

Unfortunately, such an objective is impossible to achieve: the equation $f(x,p) = 0$ is equivalent to the equation $2f(x,p) = 0$, and thus there will be no way to distinguish between $(\partial f/\partial x)$ and $2(\partial f/\partial x)$. Thus, we will restate our objective as that of finding the matrix **r** of the local response coefficients (r_{ij}). Eq. **1** of the main text defines r_{ij} in terms of the fractional changes in x_i brought about by a change in x_j, provided that all other variables (x_k, $k \neq i,j$) remain unperturbed. The coefficients r_{ij} correspond to the elements $(\partial f_i/\partial x_j)$ of the Jacobian matrix (**J**) "normalized" by the diagonal elements, $(\partial f_i/\partial x_i)$, i.e., $r_{ij} = \partial x_i/\partial x_j = -(\partial f_i/\partial x_j)/(\partial f_i/\partial x_i)$, as calculated by the differentiation of the equation, $f_i(x, p) = 0$. In matrix notations,

$$\mathbf{r} = -(dg\mathbf{J})^{-1}\cdot\mathbf{J}\,. \qquad \textbf{[5]}$$

Eq. **2** allows us to relate the global response matrix ($\mathbf{R_p}$), the Jacobian matrix (**J**), and the matrix of the partial derivatives of functions *f* with respect to the vector of parameter (*p*),

$$\mathbf{R_p} = (\partial x/\partial p) = -(\partial f/\partial x)^{-1}\cdot(\partial f/\partial p) = -(\mathbf{J})^{-1}\cdot(\partial f/\partial p). \qquad \textbf{[6]}$$

It is assumed that the matrix $(\partial f/\partial p)$ is nonsingular in the vicinity of the state (x^0, p^0). As explained in the main text, we perturb specific parameters (p_i) that affect only single modules (*i*), which makes the matrix $(\partial f/\partial p)$ diagonal, $(\partial f/\partial p) = dg\mathbf{f_p}$. It is related to the local response matrix, $dg\mathbf{r_p}$, which is defined by Eq. **3** of the main text, as follows,

$$dg\mathbf{r_p} = -(dg\mathbf{J})^{-1}\cdot dg\mathbf{f_p}. \qquad \textbf{[7]}$$

[Eq. **7** is obtained by the differentiation of the equation, $f_i(x,p) = 0$, with respect to p_i assuming that all other variables except x_i (x_k, $k \neq i$) remain fixed]. Using Eqs. **5**–**7**, we find

$$\mathbf{R_p} = -(\mathbf{J})^{-1}\cdot dg\mathbf{f_p} = -(\mathbf{J})^{-1}\cdot(dg\mathbf{J})\cdot(dg\mathbf{J})^{-1}\cdot dg\mathbf{f_p} = -\mathbf{r}^{-1}\cdot dg\mathbf{r_p}. \qquad \textbf{[8]}$$

From Eq. **8**, the matrix **r** is expressed as follows,

$$\mathbf{r} = -dg\mathbf{r_p}\cdot\mathbf{R_p}^{-1}. \qquad \textbf{[9]}$$

Because all the diagonal elements of the matrix, **r,** are equal to –1 (see Eq. **5**), we can write

$$\mathbf{I} = dg\mathbf{r_p}\cdot dg(\mathbf{R_p}^{-1}), \qquad \textbf{[10]}$$

where **I** is the identity matrix, and $dg(\mathbf{R_p}^{-1})$ is the diagonal matrix with diagonal elements $(\mathbf{R_p}^{-1})_{ii}$ and all off-diagonal elements equal to zero. By expressing $dg\mathbf{r_p}$ from this equation, we obtain final Eq. **8** of the main text,

$$\mathbf{r} = -[dg(\mathbf{R_p}^{-1})]^{-1}\cdot\mathbf{R_p}^{-1}. \qquad \textbf{[11]}$$

1. Kholodenko, B. N. & Sontag, E. D. (2002) arXiv: physics/0205003.