

Appendix 1: Generalization of the Method for the Case of Several Communicating Intermediates in a Module

Our method is readily generalized for the case when modules have more than one communicating intermediate as the output. In this case, the number of independent perturbations applied to a module should be more than one and equal to the number of communicating intermediates in that module. The resulting expression for the network interaction map becomes slightly more complicated. Instead of the diagonal matrix $[dg(\mathbf{R}_p^{-1})]^{-1}$, the block-diagonal matrix, $\mathbf{B} = [bldg(\mathbf{R}_p^{-1})]^{-1}$, is determined by using the elements of the inverse matrix, \mathbf{R}_p^{-1} . For a network with m modules, the matrix \mathbf{B} has m central minors (square blocks) with nonzero elements, whereas all other elements, which do not enter these blocks, are equal to zero. The dimension of each block corresponds to the number of communicating intermediates in the corresponding module. Assuming k such intermediates in module i , the i th principal minor of the block-diagonal matrix \mathbf{B} is determined as follows:

$$\begin{bmatrix} B_{ii} & B_{i,i+1} & \cdots & B_{i,i+k} \\ B_{i+1,i} & B_{i+1,i+1} & \cdots & B_{i+1,i+k} \\ \cdots & \cdots & \cdots & \cdots \\ B_{i+k,i} & B_{i+k,i+1} & \cdots & B_{i+k,i+k} \end{bmatrix} = \begin{bmatrix} R_{ii}^{-1} & R_{i,i+1}^{-1} & \cdots & R_{i,i+k}^{-1} \\ R_{i+1,i}^{-1} & R_{i+1,i+1}^{-1} & \cdots & R_{i+1,i+k}^{-1} \\ \cdots & \cdots & \cdots & \cdots \\ R_{i+k,i}^{-1} & R_{i+k,i+1}^{-1} & \cdots & R_{i+k,i+k}^{-1} \end{bmatrix}^{-1} \quad [1]$$

For the case a single communicating intermediate in each module, the matrix \mathbf{B} is identical to the diagonal matrix $[dg(\mathbf{R}_p^{-1})]^{-1}$ in Eq. 8 of the main text. In the general case, the network interaction map, \mathbf{r} , can be obtained by using measured values of system responses (matrix \mathbf{R}_p) as follows:

$$\mathbf{r} = -\mathbf{B} \cdot \mathbf{R}_p^{-1} \quad [2]$$

Note that the analysis also can be conducted in terms of nonnormalized responses ($\partial x_i / \partial x_j$) defined as the quotients of absolute rather than fractional changes.

In summary, we present an experimental strategy that provides a systematic approach to analyzing functional interactions in complex signaling or gene network systems in a quantitative manner. It offers the potential of generating a complete description of the relevant network interactions and can even identify the contribution of those components that may have escaped identification thus far. It also offers the flexibility of being applicable at different levels of organization by redefining the modules of which the interactions are being explored. For instance, whereas the examples analyzed for illustrative purposes were focused on intracellular signaling and gene expression pathways, the method can be adapted to the analysis of cell–cell interactions (or beyond) by taking whole cells or tissues as single modules and considering hormone and cytokine

signals or similar mediators as communicating intermediates. Hence, it becomes possible to generate a quantitative analysis of a complex biological system at progressively deeper levels by applying the analysis at sequential levels of modular organization.