

***Inferring dynamic architecture of cellular networks using time series of gene expression, protein and metabolite data***

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**Supplementary Information**

## Supplementary Proof 1

This supplement discusses the fact that, in contrast to the stationary case, for time-varying responses, the rank of the matrix  $\mathbf{R}(t, \mathbf{P}_i)$  generically equals  $n$  at any given time, for any  $n$  independent perturbations selected according to Eq. 2 (that is, if  $\partial f_i / \partial p_j(\mathbf{x}, \mathbf{p}) = 0$  for all  $p_j \in \mathbf{P}_i$ ). Moreover, we show that, in a precise mathematical sense, this rank generically equals  $n$  *even when only a single network node* is directly affected by  $n$  experimental interventions, each of which changes an independent parameter influencing that particular node. We will pick the single network node which is directly affected by  $n$  experiments to be the first one, and we will drop the subscript  $i$  in  $\mathbf{P}_i$  (since we are only interested in  $i = 1$ ).

Since we are interested in properties of the Jacobian of  $f$ , the genericity statement is expressed in terms of linearizations of  $f$ . For simplicity, we study the case of time-invariant linear systems  $\dot{x} = \mathcal{F}x$  (dot indicates time derivative) whose matrix  $\mathcal{F}$  has the following form:

$$\mathcal{F} = \begin{pmatrix} F_{11} + p_1 & F_{12} + p_2 & \cdots & F_{1n} + p_n \\ F_{21} & F_{22} & \cdots & F_{2n} \\ \vdots & \vdots & \vdots & \vdots \\ F_{n1} & F_{n2} & \cdots & F_{nn} \end{pmatrix}$$

where the  $F_{ij}$ 's are fixed coefficients and the  $p_j$ 's are the parameters being perturbed. (We view such a matrix as a possible linearization of  $f$  around a particular state. A result may also be proved for time-dependent matrices, corresponding to linearizations along trajectories, but the present approach is sufficient in order to show that one gets a full rank even when perturbations only directly affect one of the variables.) We will show that for this system, and for generic values of the  $F_{ij}$ 's, the parameters  $p_j$ 's, the initial condition  $x(0)$ , and the time  $T$ , the sensitivity matrix  $\mathbf{R}(t, \mathbf{P}_i)$  with respect to the parameters  $p_j$  at time  $T$  has full rank.

Let us first define everything precisely.

For any given  $n \times n$  matrix  $\mathbf{F} = (F_{ij})$  and  $n$ -vector  $\mathbf{P} = (p_1, \dots, p_n)$ , we write:

$$\mathcal{F}(\mathbf{F}, \mathbf{P}) = \begin{pmatrix} F_{11} + p_1 & F_{12} + p_2 & \cdots & F_{1n} + p_n \\ F_{21} & F_{22} & \cdots & F_{2n} \\ \vdots & \vdots & \vdots & \vdots \\ F_{n1} & F_{n2} & \cdots & F_{nn} \end{pmatrix}.$$

Observe that, for any  $n$ -vector  $x = \text{col}(x_1, \dots, x_n)$ , we have that:

$$\frac{\partial \mathcal{F}(\mathbf{F}, p)x}{\partial p_j} = b_j(x) = \begin{pmatrix} x_j \\ 0 \\ \vdots \\ 0 \end{pmatrix}$$

for each  $j = 1, \dots, n$ . We next introduce the sensitivity matrices with respect to the parameter vector  $\mathbf{P}$  along solutions of  $\dot{x} = \mathcal{F}(\mathbf{F}, \mathbf{P})x$ . Pick any  $n$ -vector  $\xi$  and consider the following initial value problem, a system of  $(n+1)n$  differential equations:

$$\begin{aligned} \dot{x} &= \mathcal{F}(\mathbf{F}, \mathbf{P})x, & x(0) &= \xi \\ \dot{z}_1 &= \mathcal{F}(\mathbf{F}, \mathbf{P})z_1 + b_1(x), & z_1(0) &= 0 \\ \dot{z}_2 &= \mathcal{F}(\mathbf{F}, \mathbf{P})z_2 + b_2(x), & z_2(0) &= 0 \\ &\vdots & & \\ \dot{z}_n &= \mathcal{F}(\mathbf{F}, \mathbf{P})z_n + b_n(x), & z_n(0) &= 0. \end{aligned}$$

We define the *sensitivity matrix* as follows

$$\mathbf{R}(T, \mathbf{F}, \xi, \mathbf{P}) = (z_1(T), \dots, z_n(T))$$

for any positive time  $T$ . This is the same as “ $\mathbf{R}(t, \mathbf{P}_i)$ ” in the main text, except that we are showing the dependence on initial states and constants defining the system.

**Theorem.** For generic values of  $T, \mathbf{F}, \xi, \mathbf{P}$ ,  $\det \mathbf{R}(T, \mathbf{F}, \xi, \mathbf{P}) \neq 0$ .

The meaning of “generic” is: consider the set of vectors of size  $1 + n^2 + n + n$  which list  $T$  and the entries of  $\mathbf{F}, \xi, \mathbf{P}$ ; for all such vectors except for a set of measure zero, the matrix is nonsingular.

**Proof.** Solutions of the shown initial value problem are real-analytic functions of time and of the parameters defining the system (see for instance [2]). Therefore, to show that the determinant is generically nonzero, it is enough to show that  $\mathbf{R}(T, \mathbf{F}, \xi, \mathbf{P}) \neq 0$  for just *one* choice of  $(T, \mathbf{F}, \xi, \mathbf{P})$ . We pick  $T = 1, \mathbf{P} = 0$  (zero vector),

$$\xi = \begin{pmatrix} 1 \\ 0 \\ 0 \\ \vdots \\ 0 \end{pmatrix},$$

and:

$$\mathbf{F} = \begin{pmatrix} 0 & 0 & \cdots & 0 & 0 \\ 1 & 0 & \cdots & 0 & 0 \\ 0 & 1 & \cdots & 0 & 0 \\ \vdots & \vdots & \vdots & \vdots & \vdots \\ 0 & 0 & \cdots & 0 & 0 \\ 0 & 0 & \cdots & 1 & 0 \end{pmatrix}.$$

Notice that  $\mathcal{F}(\mathbf{F}, \mathbf{P}) = \mathbf{F}$ , because  $\mathbf{P} = 0$ .

We have that the  $j$ th coordinate of the solution of  $\dot{x} = \mathbf{F}x$  with initial condition  $\xi$  is:

$$x_j(t) = t^{j-1}, \quad j = 1, \dots, n.$$

From this, one verifies induction that:  $z_j(t) = \text{col}(z_{1j}(t), \dots, z_{nj}(t))$  where:

$$z_{ij}(t) = \frac{(i-1)!(j-1)!}{(j+i-1)!} t^{i+j-1}.$$

We pick, in particular,  $t = 1$ . Therefore,  $\mathbf{R}(1, \mathbf{F}, 0, 0) = Z$ , where  $Z$  is the following  $n \times n$  matrix:

$$\left( \frac{(i-1)!(j-1)!}{(i+j-1)!} \right).$$

To show that  $Z$  is nonsingular, we first multiply the  $i$ -th row by  $(i-1)!$  and the  $j$ -th column by  $(j-1)!$ , so we may without loss of generality assume that  $Z$  is the  $n \times n$  matrix with entries:

$$\left( \frac{1}{(i+j-1)!} \right).$$

This is the  $n$ -th Hankel matrix  $H_n$  of the expansion of the exponential. The matrix  $H_n$  has full rank, since its determinant is nonzero:

$$\det H_n = \prod_{p=1}^n \prod_{q=2}^p -\frac{1}{4(2q-1)(2q-3)}$$

(this well-known formula can be derived using the computer-algebra system CLD, developed by Doron Zeilberger, and can be verified by application of “Dodgson’s rule” for determinants, cf. [1]).

## References

- [1] Zeilberger, D. (2003) Liebe Opa Paul, Ich Bin Auch Ein Experimental Scientist, *Adv. Appl. Math.*, 31, 532-543.
- [2] E.D. Sontag (1998) *Mathematical Control Theory: Deterministic Finite Dimensional Systems, Second Edition*, Springer, New York.

**Supplementary Table 1. Rate expressions, differential equations and parameter values of the gene network model of Fig. 2.**

Concentrations ( $[mRNA_i]$ ,  $i=1-4$ ) and Michaelis constants ( $K_i^a$ ,  $K_i^l$ ;  $K_i^d$ ) are given in nM. Maximal enzyme rates ( $V_i^s$ ,  $V_i^d$ ) are expressed in nM/hr.

Rate equation	Parameter values
$V_1^{\text{synth}} = \frac{V_1^s \cdot \left(1 + A_{14} \cdot \left(\frac{[mRNA_4]}{K_{14}^a}\right)^{n_{14}}\right)}{\left(1 + \left(\frac{[mRNA_4]}{K_{14}^a}\right)^{n_{14}}\right) \cdot \left(1 + \left(\frac{[mRNA_2]}{K_{12}^l}\right)^{n_{12}}\right)}$	$V_1^s=1$ ; $A_{14}=4$ ; $K_{14}^a=1.6$ ; $n_{14}=2$ ; $K_{12}^l=0.5$ ; $n_{12}=1$
$V_2^{\text{synth}} = \frac{V_2^s \cdot \left(1 + A_{24} \cdot \left(\frac{[mRNA_4]}{K_{24}^a}\right)^{n_{24}}\right)}{1 + \left(\frac{[mRNA_4]}{K_{24}^a}\right)^{n_{24}}}$	$V_2^s=0.7$ ; $A_{24}=4$ ; $K_{24}^a=1.6$ ; $n_{24}=2$
$V_3^{\text{synth}} = \frac{V_3^s \cdot \left(1 + A_{32} \cdot \left(\frac{[mRNA_2]}{K_{32}^a}\right)^{n_{32}}\right)}{\left(1 + \left(\frac{[mRNA_2]}{K_{32}^a}\right)^{n_{32}}\right) \cdot \left(1 + \left(\frac{[mRNA_1]}{K_{31}^l}\right)^{n_{31}}\right)}$	$V_3^s=0.6$ ; $A_{32}=5$ ; $K_{32}^a=1.5$ ; $n_{32}=2$ ; $K_{31}^l=0.7$ ; $n_{31}=1$
$V_4^{\text{synth}} = \frac{V_4^s \cdot \left(1 + A_{43} \cdot \left(\frac{[mRNA_3]}{K_{43}^a}\right)^{n_{43}}\right)}{1 + \left(\frac{[mRNA_3]}{K_{43}^a}\right)^{n_{43}}}$	$V_4^s=0.8$ ; $A_{43}=2$ ; $K_{43}^a=0.15$ ; $n_{43}=2$
$V_1^{\text{degr}} = V_1^d \cdot \frac{[mRNA_1]}{K_1^d + [mRNA_1]}$	$V_1^d=40$ ; $K_1^d=30$
$V_2^{\text{degr}} = V_2^d \cdot \frac{[mRNA_2]}{K_2^d + [mRNA_2]}$	$V_2^d=100$ ; $K_2^d=60$
$V_3^{\text{degr}} = V_3^d \cdot \frac{[mRNA_3]}{K_3^d + [mRNA_3]}$	$V_3^d=30$ ; $K_3^d=10$
$V_4^{\text{degr}} = V_4^d \cdot \frac{[mRNA_4]}{K_4^d + [mRNA_4]}$	$V_4^d=100$ ; $K_4^d=50$
System of differential equations: $d[mRNA_i]/dt = V_i^{\text{synth}} - V_i^{\text{degr}}$ , $i=1,2,3,4$ .	

*Initial conditions.* We assume that at  $t=0$ , all four genes were inactive (the catalytic constants of transcription rates and  $[mRNA_i]$  equaled zero). When  $t = +0$ , the constants were assigned the values given in the Supplementary Table 1 above, and the transition to an active state began.

*Perturbations.* The following parameters were perturbed in order to calculate the appropriate row of Jacobian elements:

- row 1:  $V_2^s$ ,  $V_2^d$ ,  $V_3^s$ ,  $V_3^d$
- row 2:  $V_1^s$ ,  $V_1^d$ ,  $V_3^s$ ,  $V_3^d$
- row 3:  $V_1^s$ ,  $V_1^d$ ,  $V_4^s$ ,  $V_4^d$
- row 4:  $V_1^s$ ,  $V_1^d$ ,  $V_2^s$ ,  $V_2^d$

The finite differences between the control and perturbed transitions were calculated according to Eq. 7 of the main text.

**Supplementary Table 2. Rate expressions, differential equations and parameter values for the MAPK cascade model of Fig. 4.**

Concentrations and the Michaelis constants ( $K_{ij}$ ,  $i=1, 3, 5, 7, 9, 11$ ;  $j=1, 2, 3$ ;  $K_{mp}$ ;  $K_i$ ) are given in nM. The catalytic rate constants ( $k_i^{cat}$ ,  $i=1, 2, 5, 6, 9, 10$ ) and the maximal enzyme rates ( $V_i^{max}$ ,  $i=3, 4, 7, 8, 11, 12$ ) are expressed in  $s^{-1}$  and  $nM \cdot s^{-1}$ , respectively.

Rate equation	Parameter values
$v_1 = \frac{k_1^{cat} \cdot [RasGTP] \cdot [MKKK]}{(K_{11} + [MKKK] + [MKKKP] \cdot K_{11}/K_{12}) \cdot (1 + [MAPKPP]/K_i)}$	$k_1^{cat}=1$ ; $[RasGTP]=20$ ; $K_{11}=300$ ; $K_{12}=20$ ; $K_i=100$
$v_2 = \frac{k_2^{cat} \cdot [RasGTP] \cdot [MKKKP]}{(K_{11} + [MKKK] + [MKKKP] \cdot K_{11}/K_{12}) \cdot (1 + [MAPKPP]/K_i)}$	$k_2^{cat}=15$ ; $[MKKK]_{total}=200$
$v_3 = \frac{V_3^{max} \cdot [MKKKP]}{(K_{31} + [MKKKP] + [MKKKP] \cdot K_{31}/K_{32} + [MKKK] \cdot K_{31}/K_{33})}$	$V_3^{max}=18.8$ ; $K_{31}=22$ ; $K_{32}=18$ ; $K_{33}=80$
$v_4 = \frac{V_4^{max} \cdot [MKKKP]}{(K_{31} + [MKKKP] + [MKKKP] \cdot K_{31}/K_{32} + [MKKK] \cdot K_{31}/K_{33})}$	$V_4^{max}=16.4$
$v_5 = \frac{k_5^{cat} \cdot [MKK] \cdot [MKKKP]}{(K_{51} + [MKK] + [MKKP] \cdot K_{51}/K_{52})}$	$k_5^{cat}=1$ ; $K_{51}=300$ ; $K_{52}=20$
$v_6 = \frac{k_6^{cat} \cdot [MKKP] \cdot [MKKKP]}{(K_{51} + [MKK] + [MKKP] \cdot K_{51}/K_{52})}$	$k_6^{cat}=15$ ; $[MKK]_{total}=180$
$v_7 = \frac{V_7^{max} \cdot [MKKP] \cdot (1 + A \cdot [MAPKPP]/K_{mp})}{(K_{71} + [MKKKP] + [MKKP] \cdot K_{71}/K_{72} + [MKK] \cdot K_{71}/K_{73}) \cdot (1 + [MAPKPP]/K_{mp})}$	$V_7^{max}=18.8$ ; $K_{71}=22$ ; $K_{72}=18$ ; $K_{73}=80$ ; $A=5$ ; $K_{mp}=100$
$v_8 = \frac{V_8^{max} \cdot [MKKP] \cdot (1 + A \cdot [MAPKPP]/K_{mp})}{(K_{71} + [MKKKP] + [MKKP] \cdot K_{71}/K_{72} + [MKK] \cdot K_{71}/K_{73}) \cdot (1 + [MAPKPP]/K_{mp})}$	$V_8^{max}=16.4$
$v_9 = \frac{k_9^{cat} \cdot [MKKP] \cdot [MAPK]}{(K_{91} + [MAPK] + [MAPKP] \cdot K_{91}/K_{92})}$	$k_9^{cat}=1$ ; $K_{91}=300$ ; $K_{92}=20$
$v_{10} = \frac{k_{10}^{cat} \cdot [MKKP] \cdot [MAPKP]}{(K_{91} + [MAPK] + [MAPKP] \cdot K_{91}/K_{92})}$	$k_{10}^{cat}=15$ ; $[MAPK]_{total}=360$
$v_{11} = \frac{V_{11}^{max} \cdot [MAPKPP]}{(K_{111} + [MAPKPP] + [MAPKP] \cdot K_{111}/K_{112} + [MAPK] \cdot K_{111}/K_{113})}$	$V_{11}^{max}=8.4$ ; $K_{111}=22$ ; $K_{112}=18$ ; $K_{113}=80$
$v_{12} = \frac{V_{12}^{max} \cdot [MAPKP]}{(K_{111} + [MAPKPP] + [MAPKP] \cdot K_{111}/K_{112} + [MAPK] \cdot K_{111}/K_{113})}$	$V_{12}^{max}=7.3$

Supplementary Table 2 continued

Differential equation system:	Concentrations of unphosphorylated forms:
$d[MK K K P]/dt = v_1 - v_2 + v_3 - v_4$	$[MK K K] = [MK K K]_{total} - [MK K K P] - [MK K K P P]$
$d[MK K K P P]/dt = v_2 - v_3$	
$d[MK K P]/dt = v_5 - v_6 + v_7 - v_8$	$[MK K] = [MK K]_{total} - [MK K P] - [MK K P P]$
$d[MK K P P]/dt = v_6 - v_7$	
$d[MAP K P]/dt = v_9 - v_{10} + v_{11} - v_{12}$	$[MAP K] = [MAP K]_{total} - [MAP K P] - [MAP K P P]$
$d[MAP K P P]/dt = v_{10} - v_{11}$	

*Initial conditions.* In all simulations, the initial condition (t=0) corresponded to the steady state of the MAPK pathway with a low Ras activity, [RasGTP] = 0.3 nM, [MK K K P] = 0.6177 nM, [MK K K P P] = 0.0334 nM, [MK K P] = 0.1683 nM, [MK K P P] = 0.0091 nM, [MAP K P] = 1.6407 nM, [MAP K P P] = 0.1519 nM. When t = +0, the [RasGTP] level increased to a new high value of 20 nM, and the transition from the steady state with a low activity to a high activity state was considered. Note that the responses  $R_{ij}(0) = 0$  at time zero, since both perturbed and unperturbed solutions have the same initial condition.

*Perturbations.* The six following parameters were perturbed in order to calculate the appropriate row of Jacobian elements:

- row 1:  $k_5^{cat}$ ,  $k_6^{cat}$ ,  $V_7^{max}$ ,  $V_8^{max}$ ,  $k_9^{cat}$ ,  $k_{10}^{cat}$
- row 2:  $k_1^{cat}$ ,  $V_4^{max}$ ,  $k_5^{cat}$ ,  $k_6^{cat}$ ,  $V_7^{max}$ ,  $V_8^{max}$
- row 3:  $k_1^{cat}$ ,  $k_2^{cat}$ ,  $k_9^{cat}$ ,  $k_{10}^{cat}$ ,  $V_{11}^{max}$ ,  $V_{12}^{max}$
- row 4:  $k_1^{cat}$ ,  $k_2^{cat}$ ,  $V_3^{max}$ ,  $V_4^{max}$ ,  $k_5^{cat}$ ,  $V_8^{max}$
- row 5:  $V_3^{max}$ ,  $V_4^{max}$ ,  $k_5^{cat}$ ,  $k_6^{cat}$ ,  $V_7^{max}$ ,  $V_8^{max}$
- row 6:  $k_5^{cat}$ ,  $k_6^{cat}$ ,  $V_7^{max}$ ,  $V_8^{max}$ ,  $k_9^{cat}$ ,  $V_{12}^{max}$

The finite differences between the control and perturbed transitions were calculated for perturbation magnitudes of 5, 25 and 50% according to Eq. 7 of the main text.

**Supplementary Table 3. A snapshot of the retrieved “experimental” (superscript a) and known “theoretical” (b) interaction strengths for the MAPK pathway model.** The Jacobian elements,  $F_{ij}$ , are calculated using 5% perturbation of the parameters indicated in Supplementary Table 1, and correspond to 0.75 min after a transition of the cascade from a resting state to an active state began.

$F_{11} =$ -7.4 <sup>a</sup> -21.2 <sup>b</sup>	$F_{12} =$ 2.1 <sup>a</sup> 9.3 <sup>b</sup>	$F_{13} =$ 0.0 <sup>a</sup> 0.0 <sup>b</sup>	$F_{14} =$ -0.1 <sup>a</sup> 0.0 <sup>b</sup>	$F_{15} =$ 0.0 <sup>a</sup> 0.0 <sup>b</sup>	$F_{16} =$ -0.1 <sup>a</sup> 0.3 <sup>b</sup>
$F_{21} =$ 10.8 <sup>a</sup> 9.9 <sup>b</sup>	$F_{22} =$ -9.7 <sup>a</sup> -8.9 <sup>b</sup>	$F_{23} =$ 0.0 <sup>a</sup> 0.0 <sup>b</sup>	$F_{24} =$ 0.1 <sup>a</sup> 0.0 <sup>b</sup>	$F_{25} =$ 0.0 <sup>a</sup> 0.0 <sup>b</sup>	$F_{26} =$ -1.0 <sup>a</sup> -0.9 <sup>b</sup>
$F_{31} =$ 0.0 <sup>a</sup> 0.0 <sup>b</sup>	$F_{32} =$ 5.6 <sup>a</sup> 2.5 <sup>b</sup>	$F_{33} =$ -27.7 <sup>a</sup> -67.5 <sup>b</sup>	$F_{34} =$ 9.4 <sup>a</sup> 40.1 <sup>b</sup>	$F_{35} =$ 0.0 <sup>a</sup> 0.0 <sup>b</sup>	$F_{36} =$ -0.3 <sup>a</sup> -0.2 <sup>b</sup>
$F_{41} =$ 0.0 <sup>a</sup> 0.0 <sup>b</sup>	$F_{42} =$ 10.8 <sup>a</sup> 13.8 <sup>b</sup>	$F_{43} =$ 20.2 <sup>a</sup> 25.8 <sup>b</sup>	$F_{44} =$ -30.8 <sup>a</sup> -38.6 <sup>b</sup>	$F_{45} =$ 0.1 <sup>a</sup> 0.0 <sup>b</sup>	$F_{46} =$ -0.6 <sup>a</sup> -0.8 <sup>b</sup>
$F_{51} =$ 0.1 <sup>a</sup> 0.0 <sup>b</sup>	$F_{52} =$ -0.2 <sup>a</sup> 0.0 <sup>b</sup>	$F_{53} =$ -0.1 <sup>a</sup> 0.0 <sup>b</sup>	$F_{54} =$ -19.5 <sup>a</sup> -19.7 <sup>b</sup>	$F_{55} =$ -7.5 <sup>a</sup> -6.8 <sup>b</sup>	$F_{56} =$ 1.1 <sup>a</sup> 1.1 <sup>b</sup>
$F_{61} =$ 0.0 <sup>a</sup> 0.0 <sup>b</sup>	$F_{62} =$ 0.1 <sup>a</sup> 0.0 <sup>b</sup>	$F_{63} =$ 0.1 <sup>a</sup> 0.0 <sup>b</sup>	$F_{64} =$ 29.9 <sup>a</sup> 31.4 <sup>b</sup>	$F_{65} =$ 3.6 <sup>a</sup> 3.9 <sup>b</sup>	$F_{66} =$ -1.1 <sup>a</sup> -1.2 <sup>b</sup>

**Supplementary Table 4. Rate expressions, differential equations and parameter values for the oscillating MAPK cascade model.**

A MAPK cascade model, where the phosphorylation levels of component proteins exhibit sustained oscillations, was reported previously [3]. It possesses a single negative feedback from MAPK-PP to MKKK-P. A rigorous study of the emergence of oscillations in this model was carried out in [4,5]. All concentrations and the Michaelis constants ( $K_1 - K_{10}$ ) are given below in nM. The catalytic rate constants ( $k_3, k_4, k_7, k_8$ ) and the maximal enzyme rates ( $V_1, V_2, V_5, V_6, V_9, V_{10}$ ) are expressed in  $\text{min}^{-1}$  and  $\text{nM}\cdot\text{min}^{-1}$ , respectively.

Reaction number	Rate equation	Parameter values
1*	$V_1 \cdot [\text{MKKK}] / ((1 + ([\text{MAPK-PP}] / K_I)^n) \cdot (K_1 + [\text{MKKK}]))$	$V_1=150; n=1; K_I=9; K_1=10;$
2	$V_2 \cdot [\text{MKKK-P}] / (K_2 + [\text{MKKK-P}])$	$V_2 = 15; K_2 = 8;$
3	$k_3 \cdot [\text{MKKK-P}] \cdot [\text{MKK}] / (K_3 + [\text{MKK}])$	$k_3 = 1.5; K_3 = 15;$
4	$k_4 \cdot [\text{MKKK-P}] \cdot [\text{MKK-P}] / (K_4 + [\text{MKK-P}])$	$k_4 = 1.5; K_4 = 15;$
5	$V_5 \cdot [\text{MKK-PP}] / (K_5 + [\text{MKK-PP}])$	$V_5=45; K_5 = 15;$
6	$V_6 \cdot [\text{MKK-P}] / (K_6 + [\text{MKK-P}])$	$V_6=45; K_6 = 15;$
7	$k_7 \cdot [\text{MKK-PP}] \cdot [\text{MAPK}] / (K_7 + [\text{MAPK}])$	$k_7 = 1.5; K_7 = 15;$
8	$k_8 \cdot [\text{MKK-PP}] \cdot [\text{MAPK-P}] / (K_8 + [\text{MAPK-P}])$	$k_8 = 1.5; K_8 = 15;$
9	$V_9 \cdot [\text{MAPK-PP}] / (K_9 + [\text{MAPK-PP}])$	$V_9=30; K_9 = 15;$
10	$V_{10} \cdot [\text{MAPK-P}] / (K_{10} + [\text{MAPK-P}])$	$V_{10}=30; K_{10} = 15;$
Total concentrations: $[\text{MKKK}]_{\text{total}} = 100; [\text{MKK}]_{\text{total}} = 300; [\text{MAPK}]_{\text{total}} = 300$		
System of differential equations:		Moiety conservation relations:
$d[\text{MKKK-P}]/dt = v_1 - v_2$		$[\text{MKKK}]_{\text{total}} = [\text{MKKK}] + [\text{MKKK-P}]$
$d[\text{MKK-P}]/dt = v_3 + v_5 - v_4 - v_6$		
$d[\text{MKK-PP}]/dt = v_4 - v_5$		$[\text{MKK}]_{\text{total}} = [\text{MKK}] + [\text{MKK-P}] + [\text{MKK-PP}]$
$d[\text{MAPK-P}]/dt = v_7 + v_9 - v_8 - v_{10}$		
$d[\text{MAPK-PP}]/dt = v_8 - v_9$		$[\text{MAPK}]_{\text{total}} = [\text{MAPK}] + [\text{MAPK-P}] + [\text{MAPK-PP}]$

*Perturbations.* The following parameters were perturbed in order to calculate the appropriate row of Jacobian elements:

- row 1:  $k_3, k_4, V_5, V_6, k_7$
- row 2:  $V_1, V_2, k_8, V_9, V_{10}$
- row 3:  $V_1, V_2, k_3, V_6, k_7$
- row 4:  $V_1, V_2, k_3, k_4, V_5$
- row 5:  $k_3, k_4, V_5, V_6, V_{10}$

There are additional requirements on the experimental protocol applied to sustained (limit cycle) oscillations. In fact, we have shown elsewhere that because of the phase differences between the original and perturbed trajectories, the sensitivities to parameter change tend to infinity when the time after perturbation infinitely increases [6]. Therefore, experimental setup should allow to effectively restart perturbation responses following oscillatory behavior, e.g., applying perturbing agents to aliquots of unperturbed cells at the selected time points.

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