Developing Objective Sensitivity Analysis of Periodic Systems: Case Studies of Biological Oscillators

LU Bao-Yun[1, 2] YUE Hong[1, 3]

Abstract Sensitivity analysis is a powerful tool in investigating the impact of parameter variations on the change of system behaviours quantitatively. For a periodic system, sensitivity analysis is a challenging problem since the standard sensitivity metrics grow unbounded when time tends to infinity. Objective sensitivity analyses using various oscillation features such as period, phase, amplitude, etc. are therefore needed to circumvent this problem. In this work, a new concept of basal state sensitivity is proposed based on which a phase sensitivity calculation is derived. The improved period sensitivity calculation following an existing algorithm using singular value decomposition (SVD) is also presented, which provides a simple calculation for the basal state sensitivity. These new sensitivity calculations are developed with the purpose to analyse biological oscillators since there is an increasing interest in understanding how oscillations occur and what the main controlling factors are following a growing experimental and computational evidence of oscillations in biological systems. The improved calculation of period sensitivity is shown to be consistent with the previous methods through a well studied circadian rhythm model. The calculation of new objective sensitivities are also testified by the same circadian rhythm model as well as an oscillatory signal transduction pathway model, which further illustrates the efficiency of this approach in handling complex biological oscillators in the presence of reaction conservations.

Key words Sensitivity analysis, periodic systems, phase sensitivity, period sensitivity, biological oscillator

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Oscillation is one of the most important nonlinear behaviors which are widely observed in living cells such as circadian rhythm, bacterial cell division cycle, mammalian cell cycle, glycolytic oscillations, calcium signalling pathways[1−3], etc. Cellular oscillations are crucial for biological functions, for example, circadian rhythms are observed at all cellular levels since oscillations in enzymes and hormones affect cell function, cell division, and cell growth[4]. The rhythm is determined by the regulation of some key genes which produce endogenous oscillations of the mRNA and protein levels with a period of nearly 24 hours. Given the importance of oscillatory phenomena in biology, it is imperative to study the functioning of periodic processes comprehensively in a system manner.

Model-based analysis of complex networks using systems methods is a major topic of current systems biology[5−6]. Among the systematic methods, sensitivity analysis is a powerful approach originated from engineering and has been extensively applied in many areas including modeling and analysis of bio-chemical processes[7]. It investigates the effect of parameter variations or changes in initial conditions on system behaviors, including the system output and the derived functions (accordingly called output sensitivity and objective sensitivity, respectively). For a biological network that often involves a large number of parameters and variables, sensitivity analysis can be used to identify the most important interactions in the network, and help to understand the core features as well as assess the robustness of the system.

Due to the fact that the raw state sensitivity coefficients of a periodic system are growing unbounded as time tends to infinity[8−10], the sensitivity calculation for such a system is much more complicated compared with that of general non-periodic systems with stable steady states. In a quantitative study, period, phase and the limit cycles on the state-plane are normally used to characterize the features of oscillatory systems (we discuss limit cycle oscillations in this paper). Objective sensitivities to period, frequency, phase, extrema and amplitude, etc. are accordingly developed to understand the decisive mechanisms of oscillatory biochemical systems[9−17]. Ingalls carried out general sensitivity analysis which addressed period and extrema of oscillating biochemical systems[11]. Bagheri et al. introduced a set of performance sensitivity metrics based on different phase-measures[12]. Wilkins et al. developed methods for sensitivity analysis of oscillatory systems by solving a boundary value problem (BVP)[13]. Sensitivity and control analysis for forced periodically reaction networks was developed using the general Greens function in [14]. For a periodic system, the perturbations in the parameters will generally lead to different limit cycles from the nominal orbit. In phase sensitivity analysis, small variations to nominal parameters are given to obtain perturbed system features measured by different indices such as the parametric impulse phase response curve[15] and the isochron-based phase response[16]. Owing to the complex nature of oscillatory systems, many existing methods for phase sensitivity analysis are computationally complicated in applications especially to biological oscillators with high dimensions. This motivates us to derive easy-to-implement and easy-to-interpret methods on objective sensitivity calculation so as to facilitate systematic investigation of biological oscillators or other systems with limit-cycle oscillations.

For a periodic system, Fourier series can be employed to represent the states and the raw state sensitivity can then be decomposed into a combination of shape and period sensitivity measures[8−10]. This group of methods use state-based metrics involving calculation of ordinary differential equations (ODEs) for the raw state sensitivity, based on which period sensitivity can be readily calculated. Zak et al. proposed a method to calculate period sensitivity at a large time employing the singular value decomposition (SVD) technique[17]. This algorithm is easy to implement

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and has a good convergence property. While appreciating the advantages of this method in calculating period sensitivity, we aim to develop simple methods for phase sensitivity calculation employing the similar principle. To this end, a new concept of basal state sensitivity is proposed and the improvement of period sensitivity calculation is made to the existing SVD-based algorithm[17]. The phase sensitivity is formulated based on the basal state sensitivity, which can be computed using the largest SVD term produced from the improved period sensitivity calculation.

The rest of the paper is organized as follows. Development of methods is presented in Section 1. We first outline the state sensitivity for oscillatory systems in Subsection 1.1, and the new concept of basal state sensitivity is introduced. We then present the improved period sensitivity calculation in Subsection 1.2 and construct the basal state sensitivity calculation using the SVD technique. The phase sensitivity analysis algorithm is proposed next in Subsection 1.3. Case studies are undertaken on two important biological oscillators in Section 2. One is the well studied circadian rhythm model on Drosophila period protein, from which existing results on period sensitivities are available for comparison. The other is a simplified NF-κB signal pathway model, for which many previous studies have revealed its importance in cell processes and also its complexity nature in dynamic modeling. We use the second model to show how to handle conservations in a biological network for the purpose of sensitivity calculation. Conclusions are given in Section 3. Differential equation models of the two systems are presented in Appendixes A and B, respectively, with nominal model parameters given.

1 Method development

Consider the general form of an ODE model that can be used for many biological networks under certain conditions:

$$\dot{\boldsymbol{x}} = \boldsymbol{f}(\boldsymbol{x}(t), \boldsymbol{p}), \quad \boldsymbol{x}(t_0) = \boldsymbol{x}_0 \tag{1}$$

where $\boldsymbol{x} \in \mathbf{R}^n$ is the state vector. Each component of $\boldsymbol{x}$ is denoted as x_i, which normally stands for molecule concentration. $\boldsymbol{p} \in \mathbf{R}^m$ is the parameter vector, of which each component is denoted as p_i. $\boldsymbol{f}$ is the column vector function corresponding to the state time derivative with its ith component written as f_i. $\boldsymbol{x}_0$ is the initial condition of $\boldsymbol{x}$ at the initial point t_0. The solutions of system (1), $\boldsymbol{x}(t)$, are state time-series. For limit cycle oscillatory systems, $\boldsymbol{x}(t)$ is periodic in time, i.e., $\boldsymbol{x}(t+\tau) = \boldsymbol{x}(t)$ and τ is the period of the oscillation. Without loss of generality, it can be assumed that $\boldsymbol{f} \neq \boldsymbol{0}$ for a periodic system, i.e., all components of $\boldsymbol{f}$ will never be zero simultaneously.

1.1 State sensitivity and basal state sensitivity

The effect of a parameter change, $\Delta\boldsymbol{p}$, on a state can be approximated by a first-order Taylor series expression:

$$x_i(t, \boldsymbol{p} + \Delta\boldsymbol{p}) = x_i(t, \boldsymbol{p}) + \sum_{j=1}^{m} \frac{\partial x_i}{\partial p_j} \Delta p_j \tag{2}$$

In (2), the partial derivatives $\frac{\partial x_i}{\partial p_j}$ are called the first-order local concentration sensitivity coefficients. All the partial derivatives constitute the $n \times m$ state sensitivity matrix $S = \frac{\partial \boldsymbol{x}}{\partial \boldsymbol{p}}$, which represents a linear approximation of the dependence of the states on parameter changes[18].

Differentiation of (1) with respect to $\boldsymbol{p}$ yields the following sensitivity differential equations:

$$\dot{S} = AS + B \tag{3}$$

where $A = \frac{\partial \boldsymbol{f}}{\partial \boldsymbol{x}}$ is the Jacobian matrix, $B = \frac{\partial \boldsymbol{f}}{\partial \boldsymbol{p}}$ is the parameter Jacobian matrix. Sensitivity matrix S can be calculated by solving (1) and (3) simultaneously, which involves $n \times (m+1)$ dimension ODEs. The initial conditions of (3), S_0 are typically zeros unless the system initial conditions depend on parameters. This method is called direct differential method[7]. For oscillatory systems, S is a full information sensitivity matrix that contains the change in system behaviours including message on limit cycle shape, amplitude, period, phase, etc. Larter et al. derived a general expression for the state sensitivity of periodic systems using Fourier series expansions[9−10] of states. The periodic $x_i(t)$ can be represented by a Fourier series as

$$x_i(t) = \sum_{n=0}^{\infty} \left(a_n^i \cos \frac{2n\pi t}{\tau} + b_n^i \sin \frac{2n\pi t}{\tau} \right) \tag{4}$$

where a_n^i and b_n^i are Fourier coefficients for the i-th state, and they are functions of the system parameters $\boldsymbol{p}$. Accordingly,

$$\dot{x}_i(t) = \frac{2\pi}{\tau} \sum_{n=0}^{\infty} \left(-n a_n^i \sin \frac{2n\pi t}{\tau} + n b_n^i \cos \frac{2n\pi t}{\tau} \right) = f_i \tag{5}$$

Differentiating x_i with respect to parameter p_j yields an expression for the state sensitivity:

$$s_{ij} = -\frac{t}{\tau} \frac{\partial \tau}{\partial p_j} f_i + \left(\frac{\partial x_i}{\partial p_j} \right)_{\tau} \tag{6}$$

As a result, the state sensitivity for oscillatory systems can be decomposed into two terms[8]:

$$S = -\frac{t}{\tau} \boldsymbol{f} \boldsymbol{s}_\tau + S_c \tag{7}$$

where $\boldsymbol{s}_\tau$ is the period sensitivity vector defined as

$$\boldsymbol{s}_\tau = \frac{\partial \tau}{\partial \boldsymbol{p}} = \left[\begin{array}{cccc} \frac{\partial \tau}{\partial p_1} & \frac{\partial \tau}{\partial p_2} & \cdots & \frac{\partial \tau}{\partial p_m} \end{array} \right] \tag{8}$$

and $S_c = \left(\frac{\partial \boldsymbol{x}}{\partial \boldsymbol{p}} \right)_{\tau}$ is called the cleaned-out sensitivity or shape sensitivity, which is periodic in time[8]. The first term in the right-hand side of (7) contains the information of period change caused by parameter variations, and the second term S_c captures how variation in parameters affects the shape of state trajectory at the constant nominal period.

It is a valid assumption that $\boldsymbol{s}_\tau \neq \boldsymbol{0}$ for biophysical oscillatory systems, which means the period will be sensitive to at least one parameter of the system[10]. For a nonzero oscillatory system, the raw state sensitivity obtained by solving (3) will grow unbounded in time when $\boldsymbol{s}_\tau \neq \boldsymbol{0}$. It can be observed from (7) that the incremental rate of the state sensitivity in time is determined by the period sensitivity and the system function. The state sensitivity will increase an amount of $-\boldsymbol{f}\boldsymbol{s}_\tau$ after each period. With this in mind, we can also decompose the raw state sensitivity matrix into two terms:

$$S = S^* - l \cdot \boldsymbol{f} \boldsymbol{s}_\tau \tag{9}$$

where S^* is referred to as the basal state sensitivity and l is a truncated integer of t/τ representing l periods after the

basal period of time. Here, the basal period can be taken as the first period in calculation or any period that starts to show a stable oscillation. Without loss of generality, denote the basal period as $[0, \tau)$. At each time $t \in [0, \tau)$, the basal state sensitivity is uniquely determined by the position on the limit cycle orbit. Comparing (7) with (9), the basal term is constructed as

$$S^* = -\frac{\phi}{\tau}\boldsymbol{f}\boldsymbol{s}_\tau + S_c \tag{10}$$

where $\phi = \text{mod}\ (t, \tau)$ is defined as phase. It is obvious that the new concept S^* is also periodic in time. The definition of the basal state sensitivity not only provides an alternative state-based sensitivity metric, but also forms the basis for formulating the phase sensitivity to be discussed in Subsection 1.3.

1.2 Modified period sensitivity calculation using SVD

Period is an important feature to characterize oscillatory systems and calculation of period sensitivity receives frequent attention[9−11, 13, 17], among them an algorithm based on singular value decomposition developed by Zak et al. determines all the period sensitivities at once with no need to consider numerical stability as a separate issue[17]. Their work is based on the observation that at a large time $t\,(t \gg \tau)$, the first term in the right-hand side of (7) will dominate the periodic and bounded cleaned-out sensitivity term S_c, therefore

$$S \approx -\frac{t}{\tau}\boldsymbol{f}\boldsymbol{s}_\tau \tag{11}$$

Using matrix theory, the state sensitivity matrix can be decomposed into singular value terms as $S = U\Sigma V^{\mathrm{T}} = \sum_{i=1}^{r}\sigma_i\boldsymbol{u}_i\boldsymbol{v}_i^{\mathrm{T}}$, where Σ is a $n \times m$ diagonal matrix with rank r of non-negative singular value σ_i, U and V are unitary matrices containing the eigenvectors of SS^{T} and $S^{\mathrm{T}}S$, respectively (i.e., $\boldsymbol{u}_i$ and $\boldsymbol{v}_i$). Period sensitivity is approximated by[17]

$$\boldsymbol{s}_\tau \approx \pm\frac{\sigma_1\tau}{t\sqrt{\boldsymbol{f}^{\mathrm{T}}\boldsymbol{f}}}\boldsymbol{v}_1^{\mathrm{T}} \tag{12}$$

In this algorithm, the sign of the period sensitivity in (12) needs to be calibrated for each parameter individually by introducing a small perturbation to the parameter and determine if it increases or decreases the period. To circumvent this problem, we provided an alternative formulation and also justified the use of the largest SVD term of S in approximating $\boldsymbol{s}_\tau$ at a large time t.

Denote the SVD terms of S as

$$\widetilde{S}_i = \sigma_i\boldsymbol{u}_i\boldsymbol{v}_i^{\mathrm{T}},\ i = 1, \cdots, r \tag{13}$$

From the SVD theory, $\widetilde{S}_i$ are matrices of rank 1. Denoting $S_p = -(t/\tau)\boldsymbol{f}\boldsymbol{s}_\tau$, we argue that at a large time t, S_p can be described by the first SVD term of the state sensitivity matrix S, i.e.,

$$S_p = -\frac{t}{\tau}\boldsymbol{f}\boldsymbol{s}_\tau \approx \widetilde{S}_1 \tag{14}$$

The detailed proof can be found in [19] and we briefly describe the main idea as follows. S_p is a rank-1 matrix with its singular value linearly increasing with time by multiples of period. S_c is periodic in time with the period of τ. Furthermore, S_p and S_c are orthogonal in composing S. From the matrix knowledge that all the SVD terms, $\widetilde{S}_i = \sigma_i\boldsymbol{u}_i\boldsymbol{v}_i^{\mathrm{T}}$ $(i = 1, \cdots, r)$, are orthogonal and this decomposition is unique, it can then be concluded that S_p is one composing term among $\widetilde{S}_i$. At a large t, only S_p will be the most important rank-1 SVD component of S. As a result, the cleaned-out sensitivity is given by

$$S_c \approx \sum_{i=2}^{r}\sigma_i\boldsymbol{u}_i\boldsymbol{v}_i^{\mathrm{T}} \tag{15}$$

Since $\boldsymbol{f} \neq \boldsymbol{0}$, from (14), at a large time t, we can calculate the period sensitivity by the modified formulation

$$\boldsymbol{s}_\tau = -\frac{\tau}{t\boldsymbol{f}^{\mathrm{T}}\boldsymbol{f}}\boldsymbol{f}^{\mathrm{T}}\widetilde{S}_1 = -\frac{\sigma_1\tau}{t\boldsymbol{f}^{\mathrm{T}}\boldsymbol{f}}\boldsymbol{f}^{\mathrm{T}}\boldsymbol{u}_1\boldsymbol{v}_1^{\mathrm{T}} \tag{16}$$

By comparing the new calculation in (16) with the original one in (12), it can be seen that the new formulation maintains the advantages of easy calculation and good convergence property. The improved method does not need to introduce perturbations to determine the sign of period sensitivity for each parameter.

Substituting $\boldsymbol{s}_\tau$ in (16) into (10), we can get the basal state sensitivity S^* as

$$S^* = \frac{\phi}{t}\widetilde{S}_1 + S_c = S - \left(I - \frac{\phi\boldsymbol{f}\boldsymbol{f}^{\mathrm{T}}}{t\boldsymbol{f}^{\mathrm{T}}\boldsymbol{f}}\right)\widetilde{S}_1 \tag{17}$$

where I is the identity matrix. Equation (17) can be used to calculate the basal state sensitivity S^*. An alternative calculation is to substitute $\boldsymbol{f}\boldsymbol{s}_\tau = -(\tau/t)\widetilde{S}_1$ into (10), and S^* can then be obtained by

$$S^* = S - \left(1 - \frac{\phi}{t}\right)\widetilde{S}_1 \tag{18}$$

Using the special formulation of $\widetilde{S}_1$ as given in this paper, (17) and (18) are equivalent, but this may not always apply to general matrices. The computing costs of (18) is less than that of (17). The basal state sensitivity S^* plays an important role in deriving phase sensitivity as discussed in the next section.

1.3 Parametric phase sensitivity

The phase in a limit cycle refers to the relative position on the orbit as illustrated in Fig. 1, which is measured by the remainder of the elapsed time going from a reference point to the current position on the limit cycle modulo period, i.e., $\phi = \ \text{mod}\ (t, \tau)$. The curve in Fig. 1 is obtained from the circadian rhythm model[1] (see Appendix A). The phase is bounded in each period and is periodic in time as shown in Fig. 2, where the reference point is the initial time.

The cumulative phase sensitivity to parameters is derived as follows. Firstly, the roles of state $\boldsymbol{x}$ and time t as dependent and independent variables, respectively, must be reversed. Along any phase-space trajectory, a point may be described either by its coordinates $\boldsymbol{x}$, or time t at which the point is reached. There is, in fact, a one-to-one mapping between $\boldsymbol{x}$ and t along a given path[20]. The time t is usually treated as a variable of $\boldsymbol{x}$ for oscillatory systems described in (1). Correspondingly, time can be written as a function of position, i.e., $t = t(\boldsymbol{x}(\boldsymbol{p}))$, along a given path and the variation of parameters will affect t as well. It should be noted that time t here can be regarded as the cumulative phase for a phase-space trajectory. The parametric sensitivity of this function can then be developed.

Differentiating (1) with respect to time gives

$$\ddot{\boldsymbol{x}} = \frac{\mathrm{d}\boldsymbol{f}}{\mathrm{d}t} = \frac{\partial \boldsymbol{f}}{\partial \boldsymbol{x}} \frac{\mathrm{d}\boldsymbol{x}}{\mathrm{d}t} = A\boldsymbol{f} \tag{19}$$

The differential of $\boldsymbol{f}$ can be obtained as

$$\mathrm{d}\boldsymbol{f} = A\boldsymbol{f}\mathrm{d}t \tag{20}$$

Remember that local $\mathrm{d}\boldsymbol{f}$ can also be calculated by

$$\mathrm{d}\boldsymbol{f} = A\Delta\boldsymbol{x} + B\Delta\boldsymbol{p} \tag{21}$$

Therefore, the differential of $\mathrm{d}\boldsymbol{f}$ can be obtained as

$$A\boldsymbol{f}\mathrm{d}t = A\Delta\boldsymbol{x} + B\Delta\boldsymbol{p} \tag{22}$$

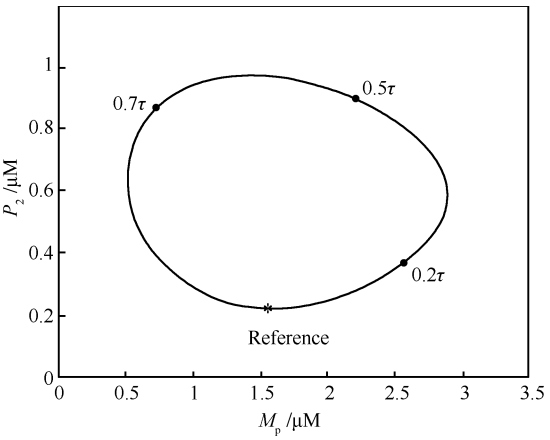


Fig. 1 A limit cycle on state plane with the circadian rhythm model in [1]

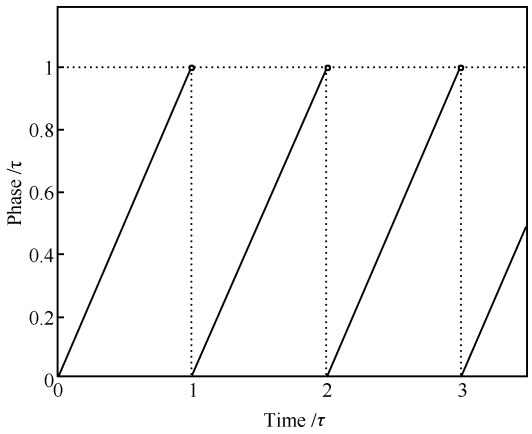


Fig. 2 Phase versus the elapsed time using the example of the circadian rhythm model in [1]

Taking derivative of (22) with respect to $\Delta\boldsymbol{p}$, we have

$$\frac{\mathrm{d}(A\boldsymbol{f}dt)}{\mathrm{d}\Delta\boldsymbol{p}} = \frac{\mathrm{d}(A\Delta\boldsymbol{x} + B\Delta\boldsymbol{p})}{\mathrm{d}\Delta\boldsymbol{p}} \tag{23}$$

that is

$$A\boldsymbol{f}\frac{\partial t}{\partial \boldsymbol{p}} = AS + B \tag{24}$$

For a periodic system, the Jacobian matrix A is not singular and $\boldsymbol{f} \neq \boldsymbol{0}$. Thus,

$$\frac{\partial t}{\partial \boldsymbol{p}} = \frac{1}{\boldsymbol{f}^{\mathrm{T}}\boldsymbol{f}}\boldsymbol{f}^{\mathrm{T}}(S + A^{-1}B) \tag{25}$$

The cumulative phase sensitivity is also unbounded with time increasing since it has a one-to-one mapping to the raw state sensitivity. It should be pointed out that the cumulative phase is also evaluated by the nominal system[20].

Since phase is limited in the basal period $[0, \tau)$, we can define the phase sensitivity in this period as

$$\frac{\partial \phi}{\partial \boldsymbol{p}} = \frac{1}{\boldsymbol{f}^{\mathrm{T}}\boldsymbol{f}}\boldsymbol{f}^{\mathrm{T}}(S^* + A^{-1}B) \tag{26}$$

Equation (26) will be used in calculating phase sensitivity following the phase definition of $\phi = \mathrm{mod}\ (t, \tau)$. The basal state sensitivity S^* is employed to calculate the phase sensitivity. Both S^* and the phase sensitivity are uniquely determined by the position on the limit cycle.

In addition to the phase definition of $\phi = \mathrm{mod}\ (t, \tau)$, phase can also be described by the time difference between two featured positions on the limit cycle orbit. For example, the time difference between two neighboring peaks of a state variable, or the time difference between the maximum and minimum values of a state variable. These alternative phases are called relative phase. In fact, period can be regarded as a relative phase since it describes the time difference between two nearby peaks of a state, that is

$$\boldsymbol{s}_\tau = \frac{\partial \tau}{\partial \boldsymbol{p}} = \frac{\partial t}{\partial \boldsymbol{p}} - \frac{\partial (t + \tau)}{\partial \boldsymbol{p}} \tag{27}$$

If the relative phase is defined as the time difference between the maximum and the minimum values of a state within a period, i.e., $\psi = \phi(t_{\min}) - \phi(t_{\max})$, where $t_{\min}$ and $t_{\max}$ are the time points at which the local minimum and maximum of the specified state occur, then the so-defined relative phase will characterize the shape of the limit cycle orbit using the amplitude of the state. Accordingly, the sensitivity can be calculated by

$$\boldsymbol{s}_\psi = \frac{\partial \psi}{\partial \boldsymbol{p}} = \frac{\partial \phi(t_{\min})}{\partial \boldsymbol{p}} - \frac{\phi(t_{\max})}{\partial \boldsymbol{p}} \tag{28}$$

It should be noted that the initial conditions for the sensitivity ODEs are not zeros for periodic systems though the initial value is on the nominal limit cycle orbit as argued in [13, 20]. The state sensitivity can be expressed in (7) and the basal state sensitivity is periodic, thus the initial condition is in general nonzero. However, in practice, the initial conditions of (3) are taken to be zeros because of the attraction property of a limit cycle, that is, at a large time t, the calculation of sensitivity matrix will converge to the true value.

Compared with the previous methods in computing the phase sensitivity that need to calculate the Green′s function[16, 20] or involve a boundary value problem[13], this approach is simple for implementation as it can be obtained from the basal state sensitivity S^*, and S^* can be calculated using the SVD technique. This is the main advantage of this algorithm.

2 Case studies

2.1 Sensitivity analysis of a circadian rhythm model

The proposed approach on phase sensitivity analysis is applied to Goldbeter′s model of Drosophila (fruit fly) circadian rhythm gene network[1]. The oscillator model is a

five-state system with 18 parameters (See Appendix A). The circadian period is 23.7 hours under nominal parameters. The sustained oscillation phenomenon is induced by the negative feedback of the transcriptional inhibition and the delay of the feedback by multiple phosphorylation. This model provides a molecular basis for circadian oscillations of the limit cycle type and was used in sensitivity analysis of periodic systems in previous works[11, 17].

The phase (ϕ) and relative phase (ψ) are depicted in Fig. 3. The phase is defined by taking the initial time as the reference point. The relative phase is defined as the time difference between the maximum and minimum of M_P (per mRNA)within a period. In the discussion, we take period (τ) as a relative phase.

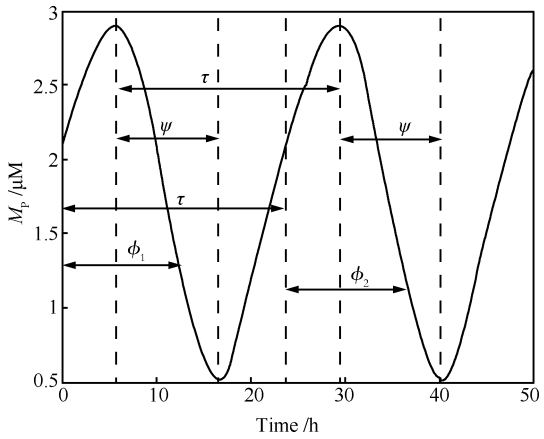


Fig. 3 The phase and relative phase of a limit cycle

The time profile for each component of state sensitivity in S is obtained by solving the sensitivity ODEs in (3) in parallel with the state ODEs in (1). As discussed in Subsection 1.1, the raw state sensitivities are unbounded (see Fig. 4). The proposed methods are used to compute the period sensitivity and the basal state sensitivity. Then the phase sensitivity is accordingly obtained. The basal state sensitivities are periodic in time as seen in Figs. 4 and 5. Period sensitivities are gradually converged to the true values as illustrated in Fig. 6. Using the same biological oscillator model, the calculation of period sensitivity provides the consistent results as those given in [17].

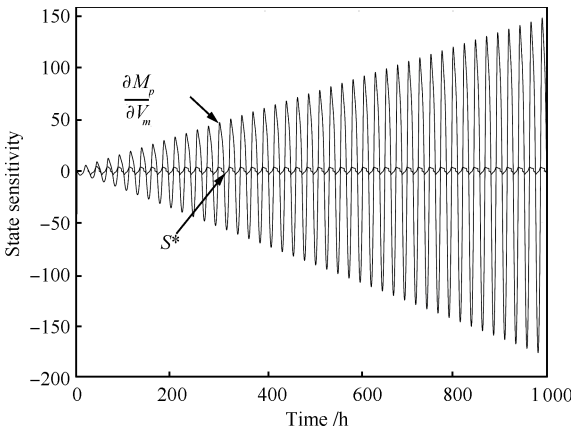


Fig. 4 State sensitivity (divergent) and basal state sensitivity (convergent)

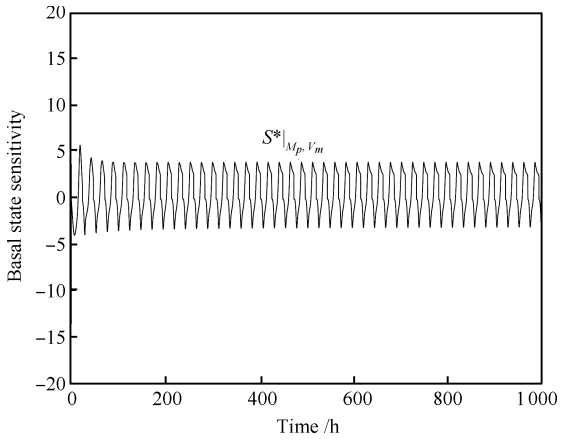


Fig. 5 Bounded basal state sensitivity

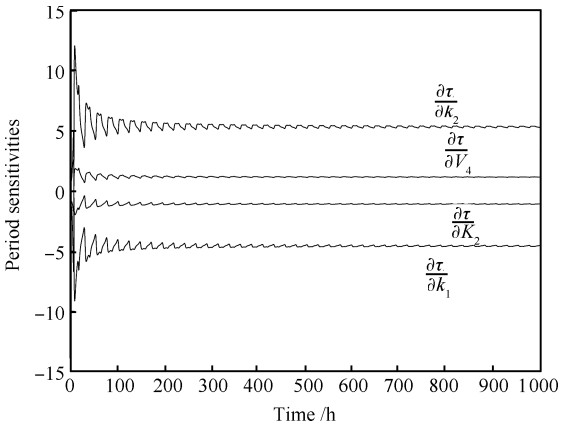


Fig. 6 Period sensitivities

If the phase as $\phi = \mod(t, \tau)$, then the phase sensitivity is periodic in time as shown in Fig. 7.

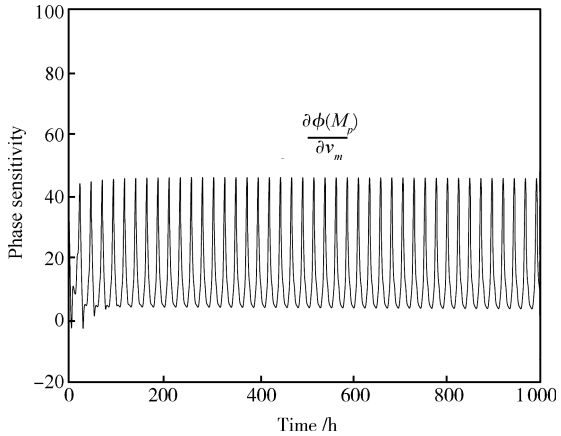


Fig. 7 A phase sensitivity time profile

Fig. 8 reflects the correlation between the relative phase sensitivity and period sensitivity which are given in normalized values (i.e., $\bar{\boldsymbol{s}}_\psi = \frac{\partial \ln \psi}{\partial \ln \boldsymbol{p}}$, $\bar{\boldsymbol{s}}_\tau = \frac{\partial \ln \tau}{\partial \ln \boldsymbol{p}}$). The relative phase used here is defined by the elapsed time between the maximum and minimum values of M_p within a period.

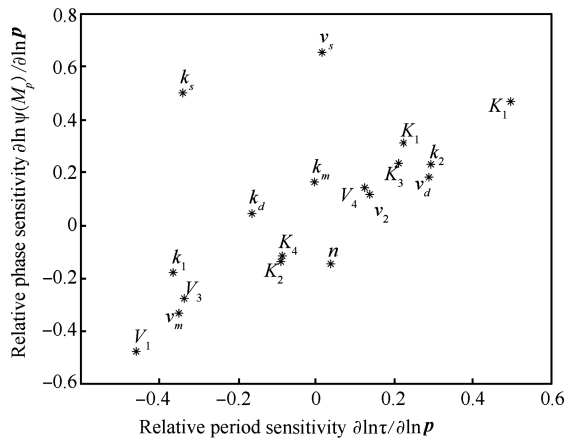


Fig. 8 Correlation between the relative phase sensitivity and period sensitivity

It is interesting to see that some parameters have similar effects on both phase ψ and period τ (see those parameters distributed alongside the diagonal in Fig. 8), but some do not. For example, v_s has a large effect on ψ but has a small effect on τ. The increase of k_s near the nominal value will lead to an increase in ψ but a decrease in τ. The numerical result indicates that different sensitivity metrics reflect different features of an oscillatory system. The relative phase and amplitude together will completely characterize the limit cycle orbit. It can be seen from Fig. 8 that V_1, K_1, k_s and v_s are the most sensitive parameters measured by both metrics. For this circadian rhythm system, V_1 and K_1 are associated with the reversible phosphorylation of PER; k_s and v_s characterize the rates of transcription and translation of per mRNA, respectively. They are within the category of global parameters according to the biochemical classification of model parameters suggested by Stelling et al.[21] and therefore have more impacts on the oscillator as expected.

2.2 Sensitivity analysis of a signal pathway model

2.2.1 Handling model singularity

It is common that conservation laws exist in biological systems. For such a system, if all ODEs are included in calculation without separating the independent and dependent ones, the Jacobian matrix A will be singular. To avoid this problem, the components of the state vector $\boldsymbol{x}$ in (1) are divided into independent dynamic state variables and dependent algebraic (state) variables. Rewrite the ODEs in the form of differential-algebraic equations (DAEs) as

$$\begin{cases} \dot{\boldsymbol{x}}_s = \boldsymbol{f}_s(\boldsymbol{x}_s, \boldsymbol{x}_a, \boldsymbol{p}) \\ \boldsymbol{0} \quad = \boldsymbol{f}_a(\boldsymbol{x}_s, \boldsymbol{x}_a, \boldsymbol{p}) \end{cases} \tag{29}$$

where $\boldsymbol{x}_s \in \mathbf{R}^{n_s}$ is the independent state vector and $\boldsymbol{x}_a \in \mathbf{R}^{n_a}$ is the dependent state vector, obviously, $n_s + n_a = n$, i.e., $\boldsymbol{x} = [\ \boldsymbol{x}_s^{\mathrm{T}} \quad \boldsymbol{x}_a^{\mathrm{T}}\ ]^{\mathrm{T}}$. $\boldsymbol{f}_s$ is the column vector function corresponding to the independent state time derivative and $\boldsymbol{f}_a$ is the column vector function that describes the conservation laws. If $\frac{\partial \boldsymbol{f}_a}{\partial \boldsymbol{x}_a}$ is not singular, then the algebraic constraint manifold is regular and there is a locally defined function $\boldsymbol{x}_a = \boldsymbol{g}(\boldsymbol{x}_s, \boldsymbol{p})$ which leads to $\boldsymbol{f}_a(\boldsymbol{x}_s, \boldsymbol{g}(\boldsymbol{x}_s, \boldsymbol{p})) = \boldsymbol{0}$. Then the system described by differential equations may be locally expressed by $\dot{\boldsymbol{x}}_s = \boldsymbol{f}_s(\boldsymbol{x}_s, \boldsymbol{g}(\boldsymbol{x}_s, \boldsymbol{p}), \boldsymbol{p})$. The Jacobian matrix is accordingly represented as

$$\bar{A} = \frac{\partial \boldsymbol{f}_s}{\partial \boldsymbol{x}_s} - \frac{\partial \boldsymbol{f}_s}{\partial \boldsymbol{x}_a}\left[\frac{\partial \boldsymbol{f}_a}{\partial \boldsymbol{x}_a}\right]^{-1}\frac{\partial \boldsymbol{f}_a}{\partial \boldsymbol{x}_s} \tag{30}$$

and the parameter Jacobian matrix is

$$\bar{B} = \frac{\partial \boldsymbol{f}_s}{\partial \boldsymbol{p}} - \frac{\partial \boldsymbol{f}_s}{\partial \boldsymbol{x}_a}\left[\frac{\partial \boldsymbol{f}_a}{\partial \boldsymbol{x}_a}\right]^{-1}\frac{\partial \boldsymbol{f}_a}{\partial \boldsymbol{p}} \tag{31}$$

With this reformulation, the Jacobian matrix $\bar{A}$ will not be singular and the phase sensitivity defined earlier can be calculated using the proposed algorithm.

2.2.2 Implementation to a signal pathway model

The transcription factor NF-κB is a regulator of expression of numerous genes and a large number of stimuli activate NF-κB, which makes NF-κB the subject of research[22]. Damped oscillations in the temporal response of NF-κB activity were firstly observed by electromobility shift assay (EMSA) in the studies of IκBβ and IκBε knockout mouse embryonic fibroblast cell populations and were simulated by a computational model[23]. Persistent oscillations of NF-κB and IκBα fluorescent fusion proteins were observed respectively in single living cells after continuous TNFα stimulation though the amplitude was slowly damping[24]. In our earlier bifurcation analysis of this model, it was found that limit cycle oscillations may exist under some conditions[25]. Sensitivity analysis of this system under conditions of non-periodic stable steady states was carried out in our previous studies[26]. In this work, we perform sensitivity analysis of this system under the conditions of limit cycle oscillations. The simplified IκBα-NF-κB model with IκBβ and IκBε knockout comes from the NF-κB full model with three IκB isoforms (IκBα, $-\beta$, $-\varepsilon$) developed by Hoffmann′s group[23, 27]. The model equations and nominal parameters are given in Appendix B. There are two conservation laws in the model, giving

$$\begin{aligned} &\frac{\mathrm{d}}{\mathrm{d}t}NF\text{-}\kappa B + \frac{\mathrm{d}}{\mathrm{d}t}I\kappa B\alpha\text{-}NF\text{-}\kappa B + \frac{\mathrm{d}}{\mathrm{d}t}NF\text{-}\kappa B_n + \\ &\quad \frac{\mathrm{d}}{\mathrm{d}t}I\kappa B\alpha_n\text{-}NF\text{-}\kappa B_n + \frac{\mathrm{d}}{\mathrm{d}t}IKKI\kappa B\alpha\text{-}NF\text{-}\kappa B = 0 \end{aligned} \tag{32}$$

$$\frac{\mathrm{d}}{\mathrm{d}t}IKKI\kappa B\alpha + \frac{\mathrm{d}}{\mathrm{d}t}IKKI\kappa B\alpha\text{-}NF\text{-}\kappa B + \frac{\mathrm{d}}{\mathrm{d}t}IKK = 0 \tag{33}$$

This leads to

$$\begin{aligned} &NF\text{-}\kappa B + I\kappa B\alpha\text{-}NF\text{-}\kappa B + NF\text{-}\kappa B_n + \\ &\quad I\kappa B\alpha_n\text{-}NF\text{-}\kappa B_n + IKKI\kappa B\alpha\text{-}NF\text{-}\kappa B = c_1 \end{aligned} \tag{34}$$

being the concentration of total cellular NF-κB contained proteins, and

$$IKKI\kappa B\alpha + IKKI\kappa B\alpha\text{-}NF\text{-}\kappa B + IKK = c_2 \tag{35}$$

being the concentration of total cellular IKK contained proteins. c_1 and c_2 are determined by initial conditions of ODEs. Based on the above discussions and our previous bifurcation analysis of this model[25], c_1, c_2 and the stimulus-related multiplier γ are identified to be critical in producing limit cycles under certain ranges. In the following simulation, $\gamma = 0.3$, $c_1 = 0.3$ and $c_2 = 0.3$ and all the other parameters take their nominal values given in the literature. Considering the two conservation conditions, $IKKI\kappa B\alpha$ and $I\kappa B\alpha_n$-NF-κB_n are taken as the algebraic variables.

A basal state sensitivity curve (concentration of NF-κB with respect to parameter k_4) is shown in Fig. 9, in which only the stable part is used for illustration since the

transient process in calculation is quite long. The period sensitivities of several crucial parameters are displayed in Fig. 10. It can be seen from Figs. 9 and 10 that the basal state sensitivity is periodic and bounded, and the period sensitivities gradually converge to fixed values. In this example, period sensitivities take a long time to converge to the vicinity of the final values. This is because the inaccurate initial conditions cause a transient process. Zero initial conditions are taken in solving the sensitivity ODEs, which is not exact for oscillatory systems. Wilkins et al. studied this problem by solving a boundary value problem[13]. However, considering the ability of attraction of a limit cycle, the computed values of sensitivity matrix will converge to the true values after a transient process. The length of the transient process is subject to the attraction ability of the oscillator.

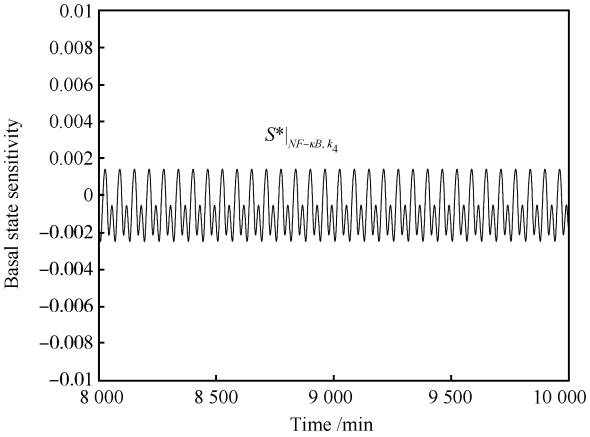


Fig. 9 A bounded basal state sensitivity

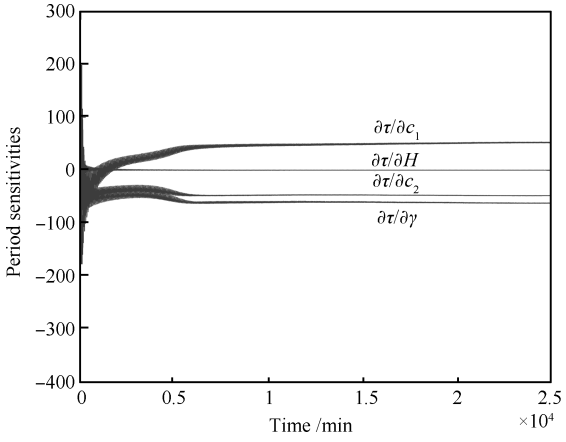
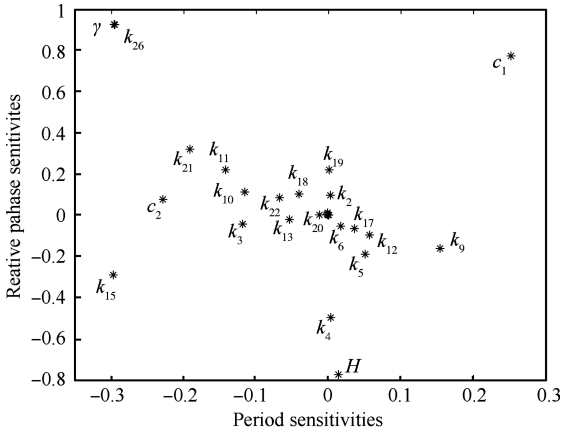
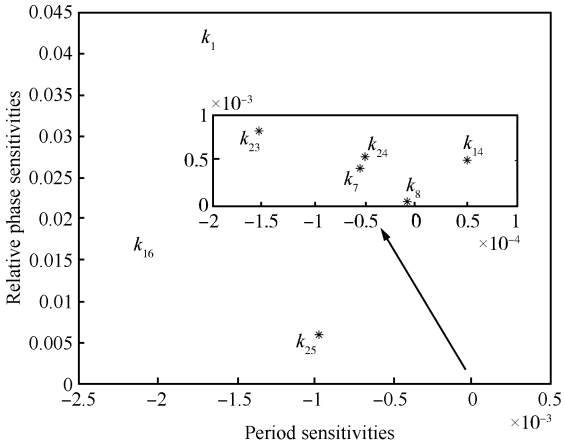


Fig. 10 Convergent period sensitivities

Fig. 11 reflects the correlation between the relative phase sensitivity ($\boldsymbol{s}_\psi$) and period sensitivity ($\boldsymbol{s}_\tau$). Similar to Case study 1 in Subsection 2.1, the normalized values are used for comparison. The relative phase is taken as the time difference between the maximum and the minimum values of $NF\text{-}\kappa B_n$. Correlation analysis between the relative phase sensitivities and period sensitivities indicates the different impacts of parameters when measured by different features of the oscillator. From those consistent results of the two metrics in Fig. 11, it can be seen that parameters γ, k_{26}, k_{15} and c_1 are the most sensitive parameters, whose variations cause larger changes in both period and phase. In this signal pathway, c_1 is the total concentration of NF-κB that is involved in most processes; k_{26} is associated with the IKK-mediated bound IκBα degradation; k_{15} is associated with IκBα nuclear import; γ is IKK multiplier relating to external stimulus, which is directly relevant to transcription. Following the parameter classifications in [21], k_{26}, γ and c_1 are within the group of global parameters, k_{15} belongs to the mixed group. This result is in agreement with the general opinion that oscillatory systems are more sensitive to global parameters than local parameters[21].



(a) Parameters with relatively large sensitivities



(b) Parameters with relatively small sensitivities

Fig. 11 Correlation between the relative phase sensitivity and period sensitivity (Sensitivities with relatively large values are shown in (a), those with smaller values are shown in (b) with a zoomed set to illustrate the details.)

3 Conclusions

In this paper, new methods of objective sensitivity analysis are proposed to explore parametric sensitivities to state, period and phase of biological oscillatory systems. In the modified period sensitivity analysis based on SVD, the calculation is straightforward with no need to calibrate the direction of parametric impact. A systematic phase sensitivity analysis method is established by analyzing the relationship between the phase sensitivity and the state sensitivity. To derive the phase sensitivity, a new concept of basal state sensitivity is proposed. These algorithms are applied to two carefully selected biological oscillatory networks. One is a Drosophila circadian rhythms model based on which we compared our calculation of period sensitivities with other methods, and obtained the same results.

The other is an IκBα-NF-κB signal transduction pathway model, which is more complicated than contains conservation laws in its original ODEs. The proposed objective sensitivity calculation is convenient for this model and those parameters that are sensitive to both period and phase are identified from the analysis.

The main contribution of this work is the simple formulation of phase sensitivity based on the new concept of basal state sensitivity. Numerical studies on the two biological oscillators demonstrate the main features of this algorithm. Both the basal state sensitivity and the derived phase sensitivity are bounded, periodic and uniquely determined by the position in a limit cycle orbit. Compared with some other methods for objective sensitivity calculation, the key advantage of this algorithm is that it has intuitive formulation and is easy for computation. We also checked the correlations between relative phase sensitivities and period sensitivities of the two biological oscillators, and found inconsistency in some cases. It is perhaps fair to say that for oscillatory systems, each objective sensitivity metric provides a unique perspective on the effect of parameters on system properties. This is different from the sensitivity analysis of general non-periodic systems with stable steady states. In the latter case, state sensitivity is normally adequate to evaluate the parameter contribution to the system output.

Appendix A

The model of circadian rhythm[1] is as follows:

$$\frac{\mathrm{d}M_p}{\mathrm{d}t} = v_s\frac{K_I^n}{K_I^n+P_N^n} - v_m\frac{M_p}{K_m+M_p}$$
$$\frac{\mathrm{d}P_0}{\mathrm{d}t} = k_sM_p - V_1\frac{P_0}{K_1+P_0} + V_2\frac{P_1}{K_2+P_1}$$
$$\frac{\mathrm{d}P_1}{\mathrm{d}t} = V_1\frac{P_0}{K_1+P_0} - V_2\frac{P_1}{K_2+P_1} - V_3\frac{P_1}{K_3+P_1} + V_4\frac{P_2}{K_4+P_2}$$
$$\frac{\mathrm{d}P_2}{\mathrm{d}t} = V_3\frac{P_1}{K_3+P_1} - V_4\frac{P_2}{K_4+P_2} - k_1P_2 + k_2P_N - v_d\frac{P_2}{K_d+P_2}$$
$$\frac{\mathrm{d}P_N}{\mathrm{d}t} = k_1P_2 - k_2P_N$$

where the nominal model parameters are: $k_1 = 1.9\mathrm{h}^{-1}$, $k_2 = 1.3\mathrm{h}^{-1}$, $V_1 = 3.2\mu\mathrm{Mh}^{-1}$, $V_2 = 1.58\mu\mathrm{Mh}^{-1}$, $V_3 = 5\mu\mathrm{Mh}^{-1}$, $V_4 = 2.5\mu\mathrm{Mh}^{-1}$, $v_s = 0.76\mu\mathrm{Mh}^{-1}$, $v_m = 0.65\mu\mathrm{Mh}^{-1}$, $K_m = 0.5\mu$M, $k_s = 0.38\mathrm{h}^{-1}$, $v_d = 0.95\mu\mathrm{Mh}^{-1}$, $K_d = 0.2\mu$M, $n = 4$, $K_1 = 2\mu$M, $K_2 = 2\mu$M, $K_3 = 2\mu$M, $K_4 = 2\mu$M, $K_I = 1\mu$M.

Appendix B

The IκBα-NF-κB signal transduction pathway model is a simplified version of the NF-κB model in [23, 27], in which only the IκBα isoform is maintained. Related reactions and kinetic parameters are illustrated in Table A1, and the nominal model parameters are also given. The complete reaction list including three IκB isoforms can be found in [25]. The time unit is minute and the concentration unit is μM. In the corresponding ODE model, uppercase letters denote concentrations of molecular species. All are proteins, except those subscripted with "$-t$", which are relevant messenger RNA transcripts. Subscript n indicates proteins inside nucleus.

The IκBα-NF-κB ODE model is as follows:

$$\frac{\mathrm{d}}{\mathrm{d}t}I\kappa B\alpha = -(k_{15}+k_{21})\times I\kappa B\alpha + k_7\times I\kappa B\alpha\text{-}NF\text{-}\kappa B + k_{10}\times IKKI\kappa B\alpha + k_{16}\times I\kappa B\alpha_n + k_4\times I\kappa B\alpha_{-t} - k_5\times I\kappa B\alpha\times NF\text{-}\kappa B - k_9\times I\kappa B\alpha\times IKK$$

$$\frac{\mathrm{d}}{\mathrm{d}t}NF\text{-}\kappa B = -k_{19}\times NF\text{-}\kappa B + (k_7+k_{23})\times I\kappa B\alpha\text{-}NF\text{-}\kappa B + k_{20}\times NF\text{-}\kappa B_n - k_5\times I\kappa B\alpha\times NF\text{-}\kappa B + (k_{14}+\gamma k_{26})\times IKKI\kappa B\alpha\text{-}NF\text{-}\kappa B - k_{13}\times NF\text{-}\kappa B\times IKKI\kappa B\alpha$$

$$\frac{\mathrm{d}}{\mathrm{d}t}IKKI\kappa B\alpha = -(k_{10}+\gamma k_{25})\times IKKI\kappa B\alpha + k_9\times IKK\times I\kappa B\alpha - k13\times NF\text{-}\kappa B\times IKKI\kappa B\alpha + k_{14}\times IKKI\kappa B\alpha\text{-}NF\text{-}\kappa B$$

$$\frac{\mathrm{d}}{\mathrm{d}t}I\kappa B\alpha\text{-}NF\text{-}\kappa B = -(k_7+k_{17}+k_{23})\times I\kappa B\alpha\text{-}NF\text{-}\kappa B + k_{12}\times IKKI\kappa B\alpha\text{-}NF\text{-}\kappa B + k_{18}\times I\kappa B\alpha_n\text{-}NF\text{-}\kappa B_n + k_5\times I\kappa B\alpha\times NF\text{-}\kappa B - k_{11}\times I\kappa B\alpha\text{-}NF\text{-}\kappa B\times IKK$$

Table A1 List of reactions, kinetic parameters, and the nominal values

Reactions	Parameters	Values
source $\rightarrow$ IκBα_{-t}	k_1	1.848E−4
NF-κB$_n$ + NF-κB$_n$ $\rightarrow$ IκBα_{-t}+ NF-κB$_n$ + NF-κB$_n$	k_2	7.92
IκBα_{-t} $\rightarrow$ sink	k_3	0.0168
IκBα_{-t} $\rightarrow$ IκBα + IκBα_{-t}	k_4	0.2448
IκBα + NF-κB $\rightarrow$ IκBα-NF-κB	k_5	30.0
IκBα_n + NF-κB$_n$ $\rightarrow$ IκBα_n-NF-κB$_n$	k_6	30.0
IκBα-NF-κB $\rightarrow$ IκBα + NF-κB	k_7	6E−5
IκBα_n-NF-κB$_n$ $\rightarrow$ IκBα_n + NF-κB$_n$	k_8	6E−5
IKK + IκBα $\rightarrow$ IKKIκBα	k_9	1.35
IKKIκBα $\rightarrow$ IKK + IκBα	k_{10}	0.075
IKK + IκBα-NF-κB $\rightarrow$ IKKIκBα-NF-κB	k_{11}	11.1
IKKIκBα-NF-κB $\rightarrow$ IKK + IκBα-NF-κB	k_{12}	0.075
IKKIκBα + NF-κB $\rightarrow$ IKKIκBα-NF-κB	k_{13}	30.0
IKKIκBα-NF-κB $\rightarrow$ IKKIκBα + NF-κB	k_{14}	6E−5
IκBα $\rightarrow$ $I\kappa$Bα_n	k_{15}	0.09
IκBα_n $\rightarrow$ $I\kappa$Bα	k_{16}	0.012
IκBα-NF-κB $\rightarrow$ IκBα_n-NF-κB$_n$	k_{17}	0.276
IκBα_n-NF-κB$_n$ $\rightarrow$ IκBα-NF-κB	k_{18}	0.828
NF-κB $\rightarrow$ NF-κB$_n$	k_{19}	5.4
NF-κB$_n$ $\rightarrow$ NF-κB	k_{20}	0.0048
IκBα $\rightarrow$ sink	k_{21}	0.12
IκBα_n $\rightarrow$ sink	k_{22}	0.12
IκBα-NF-κB $\rightarrow$ NF-κB	k_{23}	6E−5
IκBα_n-NF-κB$_n$ $\rightarrow$ NF-κB$_n$	k_{24}	6E−5
IKKIκBα $\rightarrow$ IKK	k_{25}	1.8E−3γ
IKKIκBα-NF-κB $\rightarrow$ IKK + NF-κB	k_{26}	0.36γ

$$\frac{\mathrm{d}}{\mathrm{d}t}IKKI\kappa B\alpha\text{-}NF\text{-}\kappa B = -(k_{12}+k_{14}+\gamma k_{26})\times IKKI\kappa B\alpha\text{-}NF\text{-}\kappa B + k_{13}\times NF\text{-}\kappa B\times IKKI\kappa B\alpha + k_{11}\times I\kappa B\alpha\text{-}NF\text{-}\kappa B\times IKK$$

$$\frac{\mathrm{d}}{\mathrm{d}t}IKK = -k_9\times IKK\times I\kappa B\alpha - k_{11}\times I\kappa B\alpha\text{-}NF\text{-}\kappa B\times IKK + (k_{12}+\gamma k_{26})\times IKKI\kappa B\alpha\text{-}NF\text{-}\kappa B + (k_{10}+\gamma k_{25})\times IKKI\kappa B\alpha$$

$$\frac{\mathrm{d}}{\mathrm{d}t}NF\text{-}\kappa B_n = k_{19}\times NF\text{-}\kappa B + (k_8+k_{24})\times I\kappa B\alpha_n\text{-}NF\text{-}\kappa B_n - k_{20}\times NF\text{-}\kappa B_n - k_6\times NF\text{-}\kappa B_n\times I\kappa B\alpha_n$$

$$\frac{\mathrm{d}}{\mathrm{d}t}I\kappa B\alpha_n = k_{15}\times I\kappa B\alpha - k_6\times NF\text{-}\kappa B_n\times I\kappa B\alpha_n + k_8\times I\kappa B\alpha_n\text{-}NF\text{-}\kappa B_n - (k_{16}+k_{22})\times I\kappa B\alpha_n$$

$$\frac{\mathrm{d}}{\mathrm{d}t} I\kappa B\alpha_n\text{-}NF\text{-}\kappa B_n = k_{17} \times I\kappa B\alpha\text{-}NF\text{-}\kappa B + k_6 \times NF\text{-}\kappa B_n \times I\kappa B\alpha_n - (k_8 + k_{18} + k_{24}) \times I\kappa B\alpha_n\text{-}NF\text{-}\kappa B_n$$

$$\frac{\mathrm{d}}{\mathrm{d}t} I\kappa B\alpha_{-t} = k_1 + k_2 \times (NF\text{-}\kappa B_n)^H - k_3 \times I\kappa B\alpha_{-t}$$

where γ is a stimulus-related multiplier whose value varies according to different stimulus. The Hill coefficient is taken to be $H = 3.0$.

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