Sensitivity analysis of parameters controlling oscillatory signalling in the NF-κB pathway: the roles of IKK and IκBα

A.E.C. Ihekwaba, D.S. Broomhead, R.L. Grimley, N. Benson and D.B. Kell

Abstract: Analysis of cellular signalling interactions is expected to create an enormous informatics challenge, perhaps even greater than that of analysing the genome. A key step in the evolution towards a more quantitative understanding of signalling is to specify explicitly the kinetics of all chemical reaction steps in a pathway. We have reconstructed a model of the nuclear factor, κB (NF-κB) signalling pathway, containing 64 parameters and 26 variables, including steps in which the activation of the NF-κB transcription factor is intimately associated with the phosphorylation and ubiquitination of its inhibitor κB by a membrane-associated kinase, and its translocation from the cytoplasm to the nucleus. We apply sensitivity analysis to the model. This identifies those parameters in this (IκB)/NF-κB signalling system (containing only induced IκBα isoform) that most affect the oscillatory concentration of nuclear NF-κB (in terms of both period and amplitude). The intention is to provide guidance on which proteins are likely to be most significant as drug targets or should be exploited for further, more detailed experiments. The sensitivity coefficients were found to be strongly dependent upon the magnitude of the parameter change studied, indicating the highly non-linear nature of the system. Of the 64 parameters in the model, only eight to nine exerted a major control on nuclear NF-κB oscillations, and each of these involved as reaction participants either the IκB kinase (IKK) or IκBα, directly. This means that the dominant dynamics of the pathway can be reflected, in addition to that of nuclear NF-κB itself, by just two of the other pathway variables. This is conveniently observed in a phase-plane plot.

1 Introduction

A principal challenge for the life sciences is to understand the 'organisation' and 'dynamics' of those components that make up a living system, specifically, to investigate the spatio-temporal relationships between macromolecules, cells, and tissues in living systems. A major problem is that networks of cellular processes are regulated through complex (nonlinear) interactions among a large number of genes, proteins and other molecules. Therefore, an important goal is to understand the nature of this regulation in order to gain greater insight into the mechanisms that determine the organisation and functions of cells and ultimately their behaviour at the physiological or phenotypic levels [1]. This typically involves an iterative interplay between 'wet' (experimental) and 'dry' (modelling) strategies [2].

Typical models include both metabolic models, in which the understanding of the control of metabolic fluxes is paramount (e.g. [3–5]), and signalling models in which there is no real metabolic flux as such, and what is transferred is essentially information.

An important cellular signalling pathway, of which protein phosphorylation is a major factor for the activation of further downstream events, is the nuclear factor-κB (NF-κB) signalling pathway. The NF-κB proteins are small groups of closely related transcription factors which in mammals consist of five members: *Rel* (also known as c-Rel), *RelA* (also known as p65 and NF-κB3), *RelB*, *NF-κB*1 (p50), and *NF-κB*2 (p52) [6]. These related members are critical regulators in the development and maintenance of the immune system and in the coordinated response to infections [6, 7]. All five proteins have a Rel homology domain (RHD), which serves in their dimerisation, in DNA binding, and is the principal regulatory domain [8]. The RHD contains at its C-terminus a nuclear localisation sequence (NLS), which is rendered inactive in non-stimulated cells through binding of specific NF-κB inhibitors, known as Inhibitor-κB (IκB) proteins [8].

The transcription factor NF-κB is responsible for regulating numerous genes that play important roles in inter- and intra-cellular signalling, cellular stress responses, cell growth, survival and apoptosis and as such, the specificity and temporal control of gene expression are of crucial physiological interest [9]. Furthermore, the realisation of the potential of the NF-κB as a drug target for chronic inflammatory and autoimmune diseases is dependent on the understanding of the specificity mechanisms that govern NF-κB-responsive gene expression [6, 9].

Activation of most forms of NF-κB, especially the most common form – the p50-RelA dimer – depends on phosphorylation-induced ubiquitination of the IκB proteins.

Systems Biology online no. 20045009

doi: 10.1049/sb:20045009

Paper first received 7th April and in revised form 11th May 2004

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This sequential modification depends on two protein complexes: the IκB kinase (IKK) complex and the $E3^{IκB}$ ubiquitin ligase complex [10]. Once poly-ubiquitinated, the IκBs undergo rapid degradation through the 26S proteasome and the liberated NF-κB dimers translocate to the nucleus, where they participate in transcriptional activation of specific target gene [6]. IκBα synthesis is controlled by a highly NF-κB-responsive promoter generating autoregulation of NF-κB signalling [9, 11]. In this model there are significant oscillations in the concentration of NF-κB in the nucleus [9], a feature also observed in the p53 system [12]. Cho *et al.* [1, 13] have recently produced a slightly smaller model of the TNFα-mediated activation of the NF-κB pathway [1, 13], and have used it to point up the importance of designing experiments in which the most significant parameters are modulated preferentially [14] (for this see also [15–17]).

This paper therefore analyses a model of the (TNF-α mediated) NF-κB signal transduction pathway, and uses sensitivity analysis to identify those parameters that exert the greatest control on the oscillatory concentrations of NF-κB in the nucleus. In order to do this, we begin with the model created by Hoffmann *et al.* [9]. Based on sensitivity analysis we find that, most interestingly, all the most important parameters control the concentrations of just two molecules (other than NF-κB): IKK and IκBα.

2 Methods

There are several modelling environments that are now available which can be used to develop kinetic simulations of signalling pathways and networks. We have chosen to use `Gepasi` 3.30 (GEneral PAthway SImulator – http://www.gepasi.org or http://dbk.ch.umist.ac.uk/softw/gepasi.html). This is a modelling platform that allows the simulation of biochemical pathways [18]. `Gepasi` 3.30 runs under the MS-Windows operating system and is able to carry out time-course and steady-state simulations. One feature of GEPASI that we exploited here is its parameter scan capability [18]. The user is able to select the parameters that will be varied, the range and the extent of the variation and develop a set of simulations that can be compared with experimentally observed data. This feature can then be used to optimise, fit or even estimate unknown parameters; this might allow one to simulate experimentally observed input-output relationships [19].

2.1 Sensitivity analysis

Sensitivity analysis is an important tool in the studies of the dependence of a system on external parameters [20], and sensitivity considerations often play an important role in the design of control systems [21]. It is also widely used within metabolic control analysis (MCA), where the dimensionless control coefficients of MCA are effectively sensitivities (e.g. [20, 22–27]). Sensitivity analysis is therefore a general technique for establishing the contribution of individual parameter values to the overall performance of a complex system. This concept can be extended to non-linear systems such as the cellular signal transduction pathway by introducing sensitivity functions and sensitivity equations [14, 20]. Hence, without loss of generality, the sensitivity gain can be written (for finite changes δ) as

$$S_P^M = \frac{\delta M/M}{\delta P/P} \qquad (1)$$

where P represents the parameter that may be varied and M the response of the overall system [21]. δM denotes the incremental change in M due to the incremental change in P. In the limit of infinitesimal changes, where the sensitivity coefficient $= \frac{d \ln M}{d \ln P}$ there are useful summation theorems relating individual sensitivities to the overall system behaviour (e.g. [28, 29]). Parameter sensitivity analysis can also be utilised to validate a model's response and iteratively, to design experiments that support the estimation of parameters [14].

Modelling, simulation and sensitivity analysis are as a result perfectly positioned for integration into the experimental cycle of cell biology. In addition to demystifying non-intuitive phenomena, an area in which mathematical modelling and simulation is seen as vital is the inter- and intra-dynamics of cell signalling. Once a reasonable mathematical model for a small part of the system has been built, the potential benefits become quite considerable, in that such a sub-model serves to support experimental design, generate hypotheses, and potentially reduce experimental costs [14, 30].

2.2 Design and implementation

2.2.1 Brief summary of ODE modelling: Chemical kinetic simulations are usually performed by converting chemical equations to systems of ordinary differential equations (ODEs) of the following form:

$$\begin{aligned} d[S]/dt &= -k_1[S][E] + k_2[ES] \\ d[E]/dt &= -k_1[S][E] + k_2[ES] + k_3[ES] \\ d[ES]/dt &= k_1[S][E] - k_2[ES] - k_3[ES] \\ d[P]/dt &= k_3[ES] \end{aligned}$$

and applying standard numerical integration methods to calculate the time evolution of these reactions. Spatially heterogeneous systems require the use of partial differential equations, which are computationally much more intensive, but coarse-graining can assist here [31]. Figure 1 illustrates a basic graphical 'template model' of a signal transduction pathway [13, 14]. These components account for one step in the signal transduction of a signalling cascade. In Fig. 1, an enzyme (E) combines with substrate (S) to form an enzyme

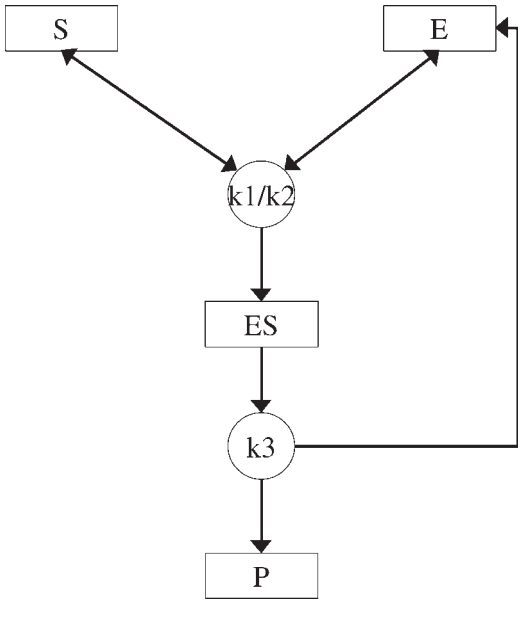

Fig. 1 *A graphical basic template model of a step in the signal transduction pathway where a rectangle (S = Substrate, E = Enzyme, ES = Enzyme substrate complex and P = Products) represents a state variable (protein concentration) and a circle (k) represents the relevant kinetic parameter (s)*

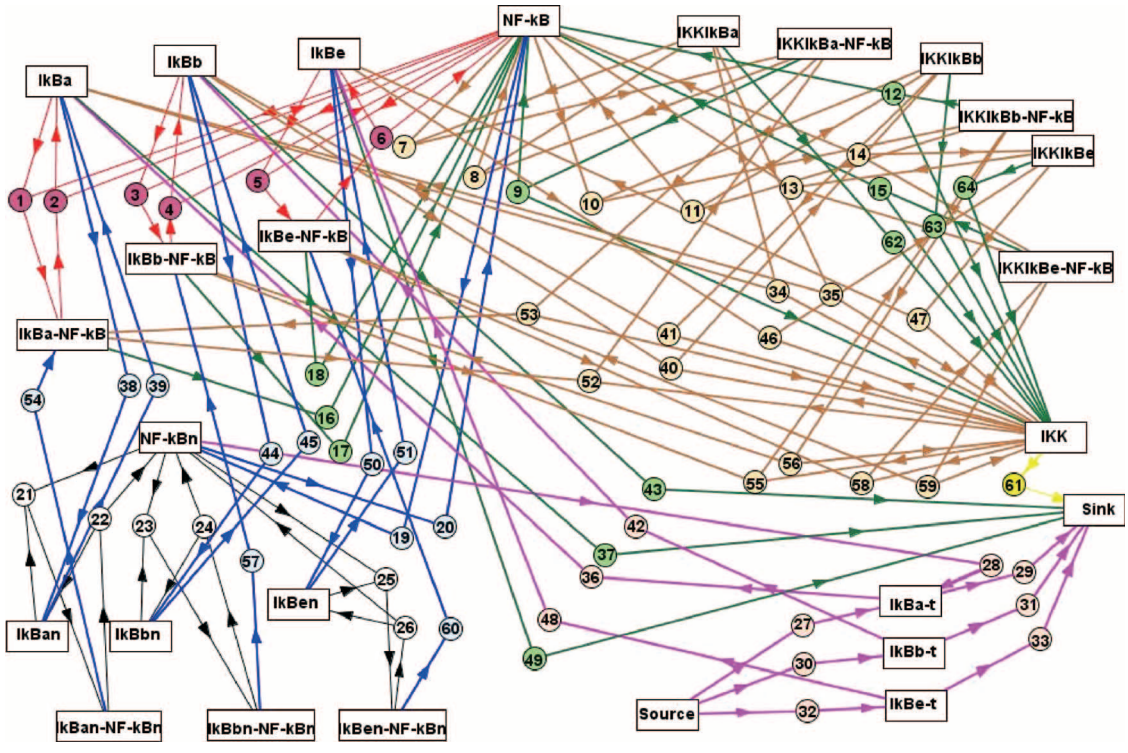

Fig. 2 *Connection of the reactions of the NF-κB model analysed in the present work. Red arrows and violet red circles = IκB-NF-κB cytoplasmic reactions; blue arrows and circles = nuclear transport; magenta arrows and pink circles = IκB mRNA synthesis (including transcription, translation and degradation); black arrows and white circles = IκB-NF-κB nuclear reactions; light green arrows and circles = IκB phosphorylation and degradation reactions; brown arrows and brown circles = Bimolecular IKK-IκB and tri-molecular IKK-IκB-NF-κB; yellow arrows and circles = IKK slow adaptation coefficient*

substrate (ES) complex with an association coefficient k_1. The complex can proceed to dissociate into E and S with a dissociation coefficient k_2, or it can further proceed to form a product P with a production rate coefficient k_3. This basic template model will be used to exemplify how we employ the multi-parametric sensitivity analysis to study the IκB-NF-κB signalling pathway.

In general, enzymes, substrates and products of individual reactions can be shared among multiple reactions giving rise to more complex differential equations for the corresponding concentrations. However, in order to describe changes in the concentration of a reaction component completely, all reactions that the component participates in, including possible transport, degradation and complex formation rates, must be fully considered [19]. Typically, these models can be written as connection maps and are qualitative in nature. The identity of the components and their interactions are defined, but quantitative information about both the components and interactions is needed to develop predictive models [19].

Once the map has been set up, the next step is to collect parameter information needed for each of the components and their interactions. This involves knowing the initial concentrations of each component, and the binding and kinetic rate constants for interactions and enzymatic reactions and diffusion [19].

2.2.2 *The NF-κB model:*

The connection map for this IκB-NF-κB model is given in Fig. 2 This depicts the IκB-NF-κB signalling pathway as described by Hoffmann *et al.*[32], which seems to model the experimental data quite effectively. The supplementary information to the paper [32] gives all the relevant parameters.

This model, which is effectively the central signalling module of the NF-κB pathway, acts to transduce all the

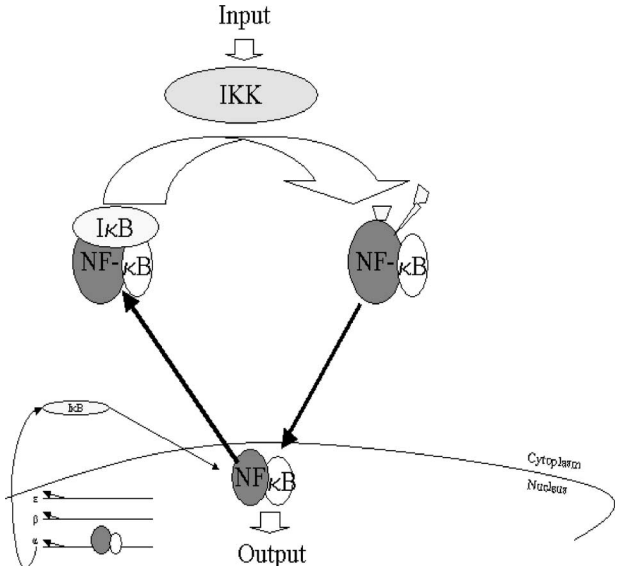

Fig. 3 *Basic IκB-NF-κB signalling model. NF-κB is held inactive in the cytoplasm of non-stimulated cell by three IκB isoforms. During cell stimulation, IKK complex is activated, leading to phosphorylation and ubiquitination of the IκB proteins. Free NF-κB translocates to the nucleus, activating genes including IκBα. IκBβ& -ε are synthesised at steady rate, allowing for complex temporal control of NF-κB activation involving negative feedback [9]*

NF-κB response from the activation of Inhibitor-κB kinase (IKK) to the transport rates into and out of the nucleus of each of the components (IκBα, -β, -ε; NF-κB and derived complexes). IKK is represented here as a single entity (without separate descriptions for the IKKα/β heterodimer and its scaffold protein IKK γ). NF-κB heterodimer isoforms

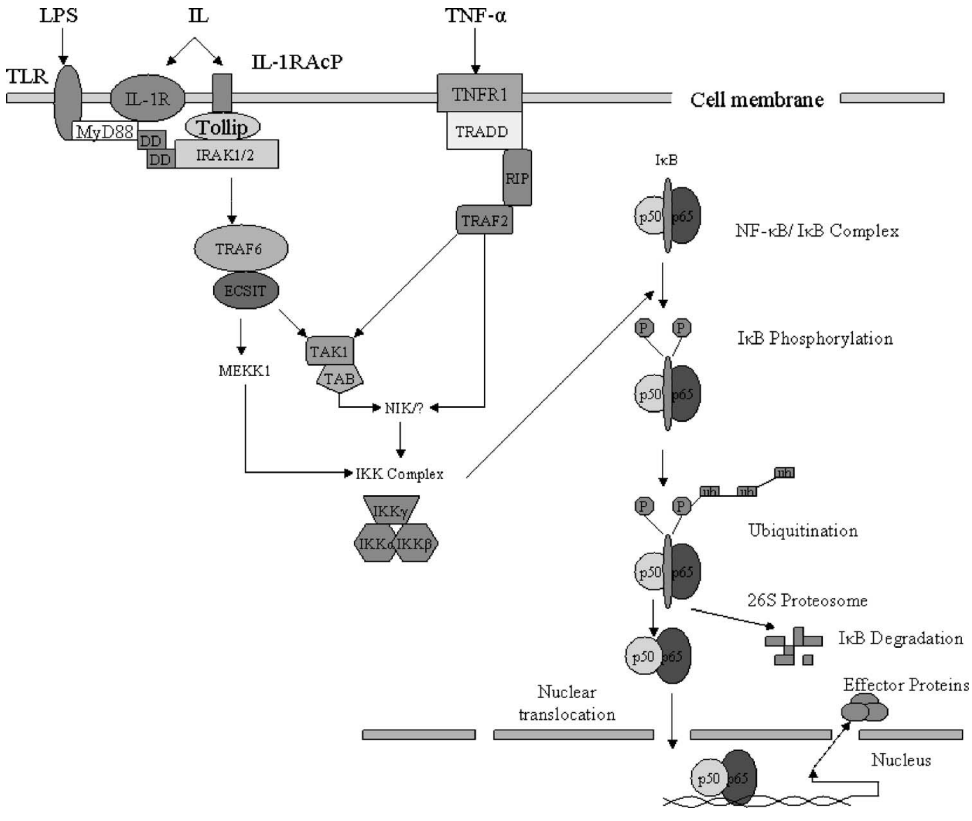

Fig. 4 *A schematic representation of signalling cascades for LPS, IL and TNF-α stimulation and activation of NF-κB (p50/p65)*

are also not specified in this model; this is because a single NF-κB isoform (p50/RelA) with transcriptional activation predominates in many cells [32]. Reactions were modelled as unidirectional 'primitives', with the back reaction where appropriate being modelled as a separate unidirectional reaction.

The model consists of 26 participant species, specifically nuclear NF-κB, bimolecular IKK-IκB and IκB-NF-κB, and trimolecular IKK-IκB-NF-κB complexes for each IκB isoform (IκBα, NF-κB, IκBα-NF-κB, IκBβ, IκBβ-NF-κB, IκBε, IκBε-NF-κB, IKKIκBα, IKKIκBα-NF-κB, IKK, IKKIκBβ, IKKIκBβ-NF-κB, IKKIκBε, IKKIκBε-NF-κB, NF-κB$_n$, IκBα$_n$, IκBα$_n$-NF-κB$_n$, IκBβ$_n$, IκBβ$_n$-NF-κB$_n$, IκBε$_n$, IκBε$_n$-NF-κB$_n$, *Source*, IκBα-t, *Sink*, IκBβ-t, IκBε-t). The participating molecular species translocate between two sub-cellular compartments, the cytoplasm and the nucleus, thus necessitating inclusion of the transportation rates in addition to binding constants and reaction rates.

The IκB-NF-κB signalling model of Fig. 3 demonstrates that IκBα is responsible for strong negative feedback that allows for a fast turn-off of the NF-κB response. The regulation of the TNFα mediated NF-κB signal transduction pathway is depicted in Fig. 4. The kinetic equations summarised in Table A2 of the Appendix describe this mathematical model explicitly. The values for each parameter (e.g. binding and kinetic constants) and the initial value of each signalling protein concentration for simulation are also summarised in Tables A1 and A2 of the Appendix.

3 Results and discussion

While attempting to implement the published model, we came across some discrepancies between supplementary material published by Hoffmann *et al.* [9]. To resolve these discrepancies we contacted the authors, who kindly provided various materials including a version of their model in the form of a Mathematica Notebook. After reviewing the contents of the Mathematica Notebook and implementing the model we obtained results similar to those published. Details of the parameters that differ from those originally published are provided below (Table 1 and the full model is reproduced in the Appendix, Section 7, Tables, 2, 3 and 4) (NB the present online version at Science also uses these values):

In the representation of ODEs (pages 3 to 5 of the supplement) we also replaced the terms in the following ODEs as described below:

- For IκBβ: (a) read 0.5 × tp1 as tp1
  (b) read 0.5 × tp2 as tp2
- For IκBε: (a) read 0.5 × tp1 as tp1
  (b) read 0.5 × tp2 as tp2
- For IκBβ$_n$: (a) read 0.5 × tp1 as tp1
  (b) read 0.5 × tp2 as tp2
- For IκBε$_n$: (a) read 0.5 × tp1 as tp1
  (b) read 0.5 × tp2 as tp2
- For IκBβ-NF-κB: read 0.4 × k2 as 0.5 × k2
- For IκBε-NF-κB: read 0.4 × k2 as 0.5 × k2
- Also deg4 should be read as deg2 wherever it appears.

Hoffmann *et al.* [9] (and ourselves) considered active IKK concentrations and started all the simulation with the IKK concentration equal to zero. Following equilibration for 2000 minutes, IKK was raised as a step function to 0.1 μM (to simulate its stimulation by TNFα or indeed by any other means). Hoffmann *et al.* assumed that following the signal onset there was a slow adaptation that gradually reduced the active IKK concentration (by mathematical means). These processes were also implemented in the present model, and similar results to those published were obtained, as shown in Fig. 5, which also illustrates the amplitudes and periods of the oscillations whose variance we analyse below.

The proposed parametric sensitivity analysis was performed for all of the system's parameters. This was carried out in a stepwise form. To begin with, the association rate constant k_a of IκBα-NF-κB as the parameter to be analysed by sensitivity analysis was selected and the range for

Table 1: Summary of the altered parameter values

Interactions	Symbol	Values	Units
IKKIκBα → IKK	k_{r1}	4.07×10^{-3}	s^{-1}
IKKIκBβ → IKK	k_{r2}	1.5×10^{-3}	s^{-1}
IKKIκBε → IKK	k_{r3}	2.2×10^{-3}	s^{-1}
IKKIκBα-NF-κB → IKK + NF-κB	k_{r4}	2.04×10^{-2}	s^{-1}
IKKIκBβ-NF-κB → IKK + NF-κB	k_{r5}	7.5×10^{-3}	s^{-1}
IKKIκBε-NF-κB → IKK + NF-κB	k_{r6}	1.1×10^{-2}	s^{-1}
IKK + IκBβ-NF-κB → IKKIκBβ-NF-κB	k_{a8}	4.8×10^{-2}	$\mu M^{-1}\ s^{-1}$
IKK + IκBε-NF-κB → IKKIκBε-NF-κB	k_{a9}	7.0×10^{-2}	$\mu M^{-1}\ s^{-1}$
IκBβ constitutive mRNA synthesis	k_{tr2b}	1.07×10^{-5}	$\mu M^{-1}\ m^{-1}$
IκBε constitutive mRNA synthesis	k_{tr2e}	7.644×10^{-6}	$\mu M^{-1}\ m^{-1}$

parameter variation assumed to be 'Min: $0.45\,\mu M^{-1}\ s^{-1}$' and 'Max: $0.55\,\mu M^{-1}\ s^{-1}$' (a 10% variation change). Using the parameter scan facility three scans were executed which corresponded to three simulations at k_a $0.45\,\mu M^{-1}\ s^{-1}$, $0.5\,\mu M^{-1}\ s^{-1}$ and $0.55\,\mu M^{-1}\ s^{-1}$, sequentially. This produced three separate graph patterns for each parameter.

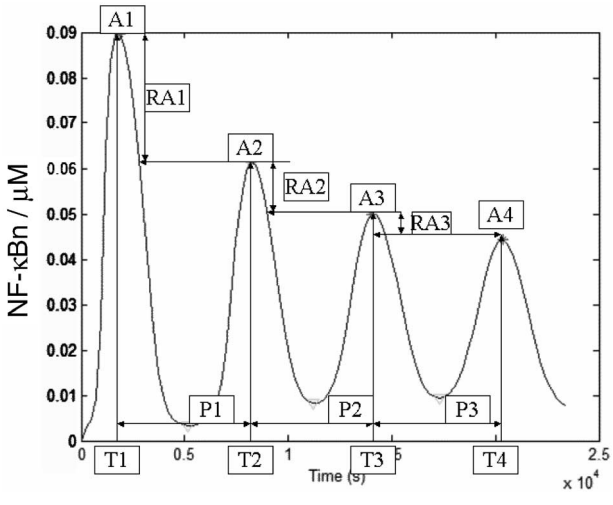

Fig. 5 *Time course of nuclear NF-κB in the 'base' Hoffman model as implemented herein. This illustrates the NF-κB signalling pathway in knockout cells lacking two IκB isoforms (IκBβ and IκBε). Activation of the NF-κB signalling pathway by TNF reduces IκBα-mediated inhibition of NF-κB. Also illustrated are the definitions of the amplitudes (A), times (T) and periods (P) used in the subsequent analysis*

We consider here only the behaviour of NF-κB in the nucleus ($NF\text{-}\kappa B_n$) (Fig. 5). For each of the three graph patterns obtained for the $NF\text{-}\kappa B_n$, we obtained the values (see Fig. 5) of: (i) the time at first, second, third and fourth oscillations; (ii) the amplitudes at the first, second, third and fourth oscillations; (iii) the periods between the oscillations.

This process was subsequently carried out for all 64 parameters in the model. The information generated was used to construct a table from which the sensitivity coefficient values for the above variables (time (T), amplitude (A) and period (P)) were calculated by averaging the values obtained when the parameters were decreased and increased by 10%. Figure 6 is a plot of the sensitivity coefficients thereby obtained for the average time at the third oscillation as a function of the 'reaction number' (where each reaction number represents the reaction parameters in the Appendix). A second study was also done in which the same scan process was carried out for the parameters but where the parameters were doubled or halved (referred to as '100% change', see Fig. 6). A similar plot for the amplitude of the third oscillation is given in Fig. 7. Similar phenomena were observed for the data on the periods P (data not shown).

It is evident that of the 64 parameters with their values as in the present model, only a small number (nine) have a significant effect on the oscillations in nuclear NF-κB, i.e. with a sensitivity coefficient $< minus\,0.2$ or $> +0.2$, and these were in fact the same reactions for the other amplitude and time variables defined in Fig. 5 (raw data not shown). As mentioned above, the sensitivity coefficients are usually defined in the limit of an infinitesimal change in the parameter [28], and their magnitude and even their sign can

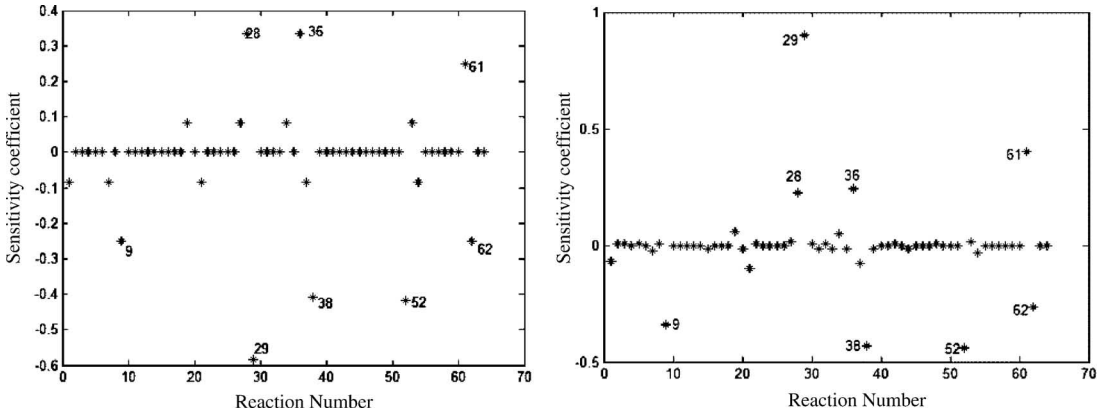

Fig. 6 *The sensitivity coefficients with respect to the 64 reactions of the time at the third oscillation (T3) when the model parameters are changed by 10% (left) and 100% (right)*

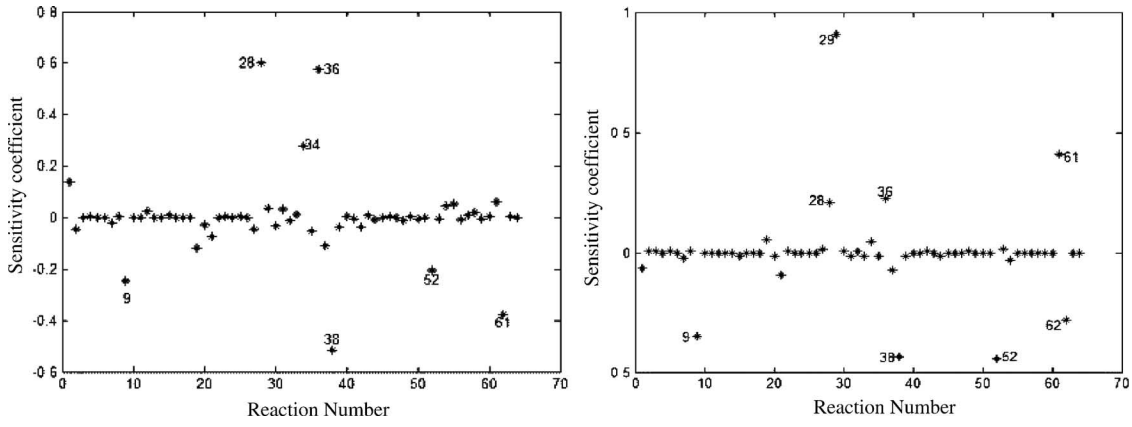

Fig. 7 *The sensitivity coefficients with respect to the 64 reactions of the amplitude of the third oscillation (A3) when the model parameters are changed by 10% (left) and 100% (right)*

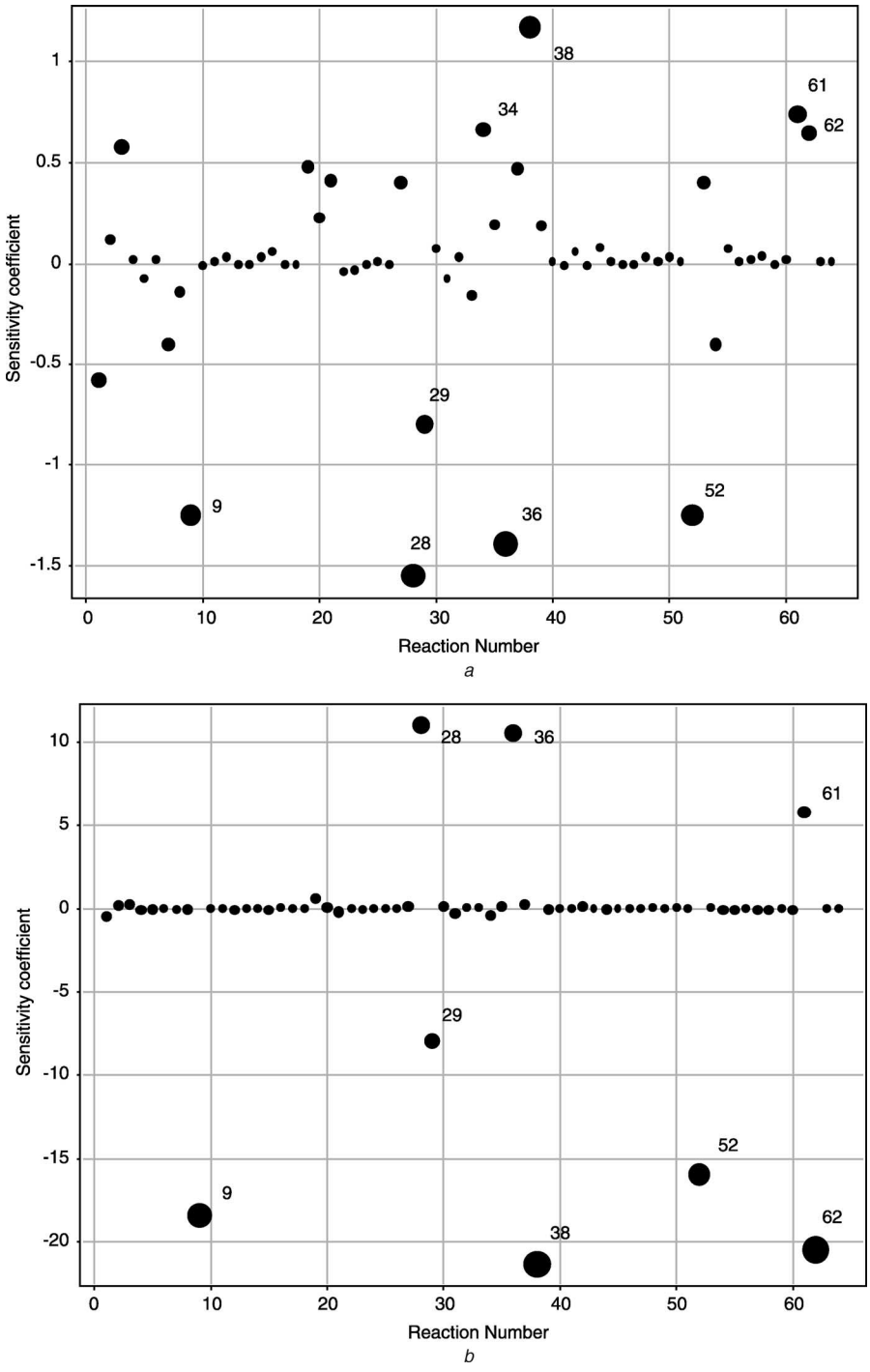

Fig. 8 *Plot of maximum sensitivity coefficient data against reaction number for (a) 10% and (b) 100% variation. The size of the symbols reflects the modulus of the sensitivity coefficients*

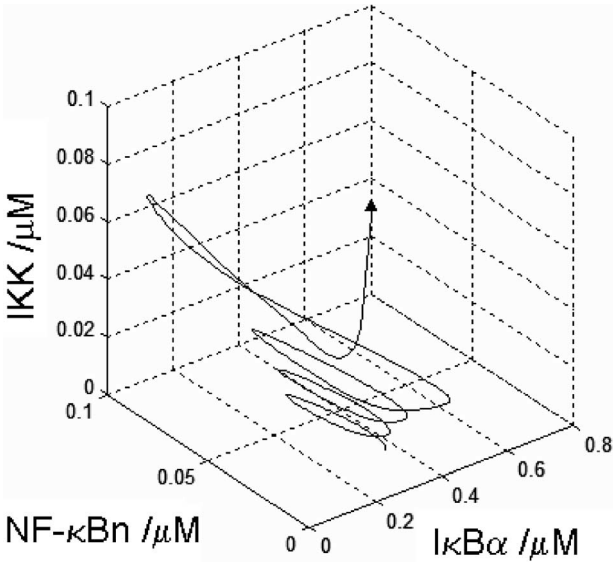

Fig. 9 *Phase plane plot of the time-dependent relationship between the concentrations of IKK, IκBα and nuclear NF-κB in the model of Fig. 5. In this representation, time is implicit and we plot the values of the three stated variables against each other as their time-dependent values as the 'base' model of Fig. 5 is run*

and will change with larger parameter changes in non-linear systems. We note that the sensitivity coefficients themselves were indeed a significant function of the magnitude of the parameter changes (e.g. as in Figs. 6 and 7 where, for example, the large sensitivity coefficient for reaction 61 in Fig. 7 actually changes its sign), indicating the very strong non-linearity of the system. Some of the values of the sensitivity could be very large, especially for parameter changes of 100%. These nine most important reactions/parameters were:

9: IKKIκBα-NF-κB catalytic rate constant
28: IκBα (IκBα-$_t$) inducible mRNA synthesis rate constant
29: IκBα (IκBα-$_t$) mRNA degradation rate constant
34: IKKIκBα association rate constant
36: Constitutive IκBα translation rate constant
38: IκBα$_n$ nuclear Import Rate constant
52: IKKIκBα-NF-κB association rate constant
61: IKK signal onset slow adaptation coefficient
62: IKKIκBα catalysis rate constant

The maximum sensitivity coefficients obtained when all of the different variables pertaining to nuclear NF-κB oscillations (i.e. all of the amplitudes, periods and times) were considered are shown in Fig. 8*a* (for 10% changes) and Fig. 8*b* (for 100% changes). Obviously these results depend on the chosen specific range of parameter variations and should not be extrapolated beyond them for this signaling pathway.

Two specific features bear comment. The first concerns the relative importances of the different reactions. Hoffman *et al*. [32] mentioned that both the IκBα transcription rate (reaction 28) and the rate of IκB-NF-κB nuclear export (reactions 54, 57 and 60) affected both the frequency and degree of damping of the oscillations. Our analysis agrees with the former but our list of the most important parameters (reactions) do not lend support to the significance of the latter.

The most interesting and striking feature, however, comes from an analysis of the variables (i.e. signalling molecules) that are involved in these nine 'most controlling' reactions. Each of them turns out either to produce or consume one of just two molecules, viz. Free IKK and IκBα. This prompted us to look at the co-variation between NF-κB, IKK and IκBα in the form of a phase plane plot (Fig. 9).

The restricted set of reactions with significant sensitivities and the data in Fig. 9 illustrate rather strikingly the intimate involvement of these mediators in the oscillations of nuclear factor NF-κB. This leads to the interesting prospect of finding a much lower dimensional system of equations that will represent, qualitatively, oscillatory signalling in this pathway. Such a reduction, while not representing the biology *per se*, would provide insight through a simple mechanistic picture. It would also suggest, and limit, the range of possible instabilities that the oscillatory signalling can exhibit. For example, should it turn out that everything can be represented qualitatively by a system of two autonomous non-linear ordinary differential equations in the parameter range described herein, this would preclude the possibility of chaotic dynamics.

4 Conclusion and summary

We have analysed a model of the NF-κB signalling pathway containing 26 species in terms of the sensitivity of the oscillating nuclear NF-κB concentration to each of the 64 parameters (reactions) of the model. Interestingly, only nine of the parameters exerted significant influence, and each of these was involved in reactions which directly affected the concentrations of just two other reactants in the model. These molecules were IKK and IκBα. A phase plane analysis of the model showed that these molecules were indeed intimately involved in the oscillations, and the sensitivity analysis showed which reactions might arguably best be modulated by those seeking to intervene therapeutically in this signalling pathway. However, the extreme non-linearity of this system means that quite small changes in such modulations could have unexpected (and therapeutically undesirable) downstream consequences if detailed experimental and modelling studies are not performed.

5 Acknowledgments

We thank Pfizer (UK) Ltd and the BBSRC for the award of a CASE studentship to AECI and the DTI (under the terms of the Beacon project) for financial support. We thank Alexander Hoffmann and Andre Levchenko for assistance with their NF-κB model, and we thank Marie Brown, Alberto de la Fuente, Glyn Nelson, Pedro Mendes, Hailin Shen, Dave Spiller and Mike White for many useful discussions.

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7 Appendix

Table 2: A summary of parameter values used in the literature

	Symbol	Values	Units	Reference
Two component reaction:				
IκBα-NF-κB association	k_{a4}	0.5	$\mu M^{-1} s^{-1}$	[33]
IκBα-NF-κB dissociation	k_{d4}	0.5×10^{-3}	s^{-1}	[34]
IκBβ-NF-κB association	k_{a5}	0.5	$\mu M^{-1} s^{-1}$	[33]
IκBβ-NF-κB dissociation	k_{d5}	0.5×10^{-3}	s^{-1}	[34]
IκBε-NF-κB association	k_{a6}	0.5	$\mu M^{-1} s^{-1}$	[33]
IκBε-NF-κB dissociation	k_{d6}	0.5×10^{-3}	s^{-1}	[34]
IKK-IκBα association	k_{a1}	22.5×10^{-3}	$\mu M^{-1} s^{-1}$	[35]
IKK-IκBα dissociation	k_{d1}	1.25×10^{-3}	s^{-1}	[35]
IKK-IκBα catalysis	k_{r1}	4.07×10^{-3}	s^{-1}	[35]
IKK-IκBβ association	k_{a2}	6.0×10^{-3}	$\mu M^{-1} s^{-1}$	[35]
IKK-IκBβ dissociation	k_{d2}	1.75×10^{-3}	s^{-1}	[35]
IKK-IκBβ catalysis	k_{r2}	1.5×10^{-3}	s^{-1}	[35]
IKK-IκBε association	k_{a3}	9.0×10^{-3}	$\mu M^{-1} s^{-1}$	[35]
IKK-IκBε dissociation	k_{d3}	1.75×10^{-3}	s^{-1}	[35]
IKK-IκBε catalysis	k_{r3}	2.2×10^{-3}	s^{-1}	[35]
Three component interactions:				
IKK-IκBαNF-κB association	k_{a7}	0.185	$\mu M^{-1} s^{-1}$	[36]
IKK-IκBαNF-κB dissociation	k_{a1}	1.25×10^{-3}	s^{-1}	[36]

(*continued*)

Table 2: *continued*

	Symbol	Values	Units	Reference
IKKIκBα-NF-κB association	k_{a4}	0.5	$\mu M^{-1} s^{-1}$	[36]
IKKIκBα-NF-κB dissociation	k_{d4}	0.5×10^{-3}	s^{-1}	[36]
IKKIκBα-NF-κB catalysis	k_{r4}	2.04×10^{-2}	s^{-1}	[36, 37]
IKK-IκBβNF-κB association	k_{a8}	4.8×10^{-2}	$\mu M^{-1} s^{-1}$	[36]
IKK-IκBβNF-κB dissociation	k_{d2}	1.75×10^{-3}	s^{-1}	[36]
IKKIκBβ-NF-κB association	k_{a5}	0.5	$\mu M^{-1} s^{-1}$	[36]
IKKIκBβ-NF-κB dissociation	k_{d5}	0.5×10^{-3}	s^{-1}	[36]
IKKIκBβNF-κB catalysis	k_{r5}	7.5×10^{-3}	s^{-1}	[36, 37]
IKK-IκBεNF-κB association	k_{a9}	7.0×10^{-2}	$\mu M^{-1} s^{-1}$	[36]
IKK-IκBεNF-κB dissociation	k_{d3}	1.75×10^{-3}	s^{-1}	[36]
IKKIκBε-NF-κB association	k_{a6}	0.5	$\mu M^{-1} s^{-1}$	[36]
IKKIκBε-NF-κB dissociation	k_{d6}	0.5×10^{-3}	s^{-1}	[36]
IKKIκBεNF-κB catalysis	k_{r6}	1.1×10^{-2}	s^{-1}	[36, 37]
Synthesis and Degradation:				
IκBα inducible mRNA synthesis	k_{tr2}	1.65×10^{-2}	$\mu M^{-1} s^{-1}$	[9]
IκBα constitutive mRNA synthesis	k_{tr2a}	1.54×10^{-6}	$\mu M\, s^{-1}$	[9]
IκBβ constitutive mRNA synthesis	k_{tr2b}	1.78×10^{-7}	$\mu M\, s^{-1}$	[9]
IκBε constitutive mRNA synthesis	k_{tr2e}	1.27×10^{-7}	$\mu M\, s^{-1}$	[9]
IκB mRNA degradation	k_{tr3}	2.8×10^{-4}	s^{-1}	[38]
constitutive IκB translation rate	k_{tr1}	4.08×10^{-4}	s^{-1}	[9]
constitutive IκB degradation (free)	k_{deg1}	1.13×10^{-4}	s^{-1}	[39]
constitutive IκB degradation (complexed to NF-κB)	k_{deg4}	2.25×10^{-5}	s^{-1}	[39]
Nucleo-cytoplasmic transport:				
IκBα nuclear import	k_{tp1}	3×10^{-4}	s^{-1}	[34]
IκBα nuclear export	k_{tp2}	2×10^{-4}	s^{-1}	[34]
IκBβ nuclear import	$0.5\, k_{tp1}$	1.5×10^{-4}	s^{-1}	[40]
IκBβ nuclear export	$0.5\, k_{tp2}$	1×10^{-4}	s^{-1}	[40, 41]
IκBε nuclear import	$0.5\, k_{tp1}$	1.5×10^{-4}	s^{-1}	[40]
IκBε nuclear export	$0.5\, k_{tp2}$	1×10^{-4}	s^{-1}	[40]
NF-κB nuclear import	k_1	0.9×10^{-1}	s^{-1}	[34]
NF-κB nuclear export	k_{01}	0.8×10^{-4}	s^{-1}	[34]
IκBα-NF-κB nuclear export	k_2	1.38×10^{-3}	s^{-1}	[34, 42]
IκBβ-NF-κB nuclear export	$0.4\, k_2$	5.2×10^{-3}	s^{-1}	[41]
IκBε-NF-κB nuclear export	$0.4\, k_2$	5.2×10^{-3}	s^{-1}	[34, 42]

Table 3: Summary of the parameter values in form of reactions

	Reactions	Symbol	Values	Units
1	IκBα + NF-κB → IκBα-NF-κB	k_{a4}	0.5_10^0	$\mu M^{-1} s^{-1}$
2	IκBα-NF-κB → NF-κB + IκBα	k_{d4}	0.5×10^{-3}	s^{-1}
3	IκBβ + NF-κB → IκBβ-NF-κB	k_{a5}	0.5_10^0	$\mu M^{-1} s^{-1}$
4	IκBβ-NF-κB → NF-κB + IκBβ	k_{d5}	0.5×10^{-3}	s^{-1}
5	IκBε + NF-κB → IκBε-NF-κB	k_{a6}	0.5_10^0	$\mu M^{-1} s^{-1}$
6	IκBε-NF-κB → NF-κB + IκBε	k_{d6}	0.5×10^{-3}	s^{-1}
7	IKKIκBα + NF-κB → IKKIκBα-NF-κB	k_{a4}	0.5_10^0	$\mu M^{-1} s^{-1}$
8	IKKIκBα-NF-κB → NF-κB + IKKIκBα	k_{d4}	0.5×10^{-3}	s^{-1}
9	IKKIκBα-NF-κB → IKK + NF-κB	k_{r4}	2.04×10^{-2}	s^{-1}
10	IKKIκBβ + NF-κB → IKKIκBβ-NF-κB	k_{a5}	0.5_10^0	$\mu M^{-1} s^{-1}$
11	IKKIκBβ-NF-κB → NF-κB + IKKIκBβ	k_{d5}	0.5×10^{-3}	s^{-1}
12	IKKIκBβ-NF-κB → IKK + NF-κB	k_{r5}	7.5×10^{-3}	s^{-1}
13	IKKIκBε + NF-κB → IKKIκBε-NF-κB	k_{a6}	0.5_10^0	$\mu M^{-1} s^{-1}$

(*continued*)

Table 3: ***continued***

	Reactions	Symbol	Values	Units
14	IKKIκBε-NF-κB → NF-κB + IKKIκBε	k_{d6}	0.5×10^{-3}	s^{-1}
15	IKKIκBε-NF-κB → IKK + NF-κB	k_{r6}	1.1×10^{-2}	s^{-1}
16	IκBα-NF-κB → NF-κB	k_{deg4}	2.25×10^{-5}	s^{-1}
17	IκBβ-NF-κB → NF-κB	k_{deg4}	2.25×10^{-5}	s^{-1}
18	IκBε-NF-κB → NF-κB	k_{deg4}	2.25×10^{-5}	s^{-1}
19	NF-κB → $NF\text{-}\kappa B_n$	k_1	0.9×10^{-1}	s^{-1}
20	$NF\text{-}\kappa B_n$ → NF-κB	k_{01}	0.8×10^{-4}	s^{-1}
21	$I\kappa B\alpha_n + NF\text{-}\kappa B_n \rightarrow I\kappa B\alpha_n\text{-}NF\text{-}\kappa B_n$	k_{a4}	0.5_10^0	$\mu M^{-1}\, s^{-1}$
22	$I\kappa B\alpha_n\text{-}NF\text{-}\kappa B_n \rightarrow NF\text{-}\kappa B_n + I\kappa B\alpha_n$	k_{d4}	0.5×10^{-3}	s^{-1}
23	$I\kappa B\beta_n + NF\text{-}\kappa B_n \rightarrow I\kappa B\beta_n\text{-}NF\text{-}\kappa B_n$	k_{a5}	0.5_10^0	$\mu M^{-1}\, s^{-1}$
24	$I\kappa B\beta_n\text{-}NF\text{-}\kappa B_n \rightarrow NF\text{-}\kappa B_n + I\kappa B\beta_n$	k_{d5}	0.5×10^{-3}	s^{-1}
25	$I\kappa B\varepsilon_n + NF\text{-}\kappa B_n \rightarrow I\kappa B\varepsilon_n\text{-}NF\text{-}\kappa B_n$	k_{a6}	0.5_10^0	$\mu M^{-1}\, s^{-1}$
26	$I\kappa B\varepsilon_n\text{-}NF\text{-}\kappa B_n \rightarrow NF\text{-}\kappa B_n + I\kappa B\varepsilon_n$	k_{d6}	0.5×10^{-3}	s^{-1}
27	source → $I\kappa B\alpha\text{-}_t$	k_{tr2a}	1.54×10^{-6}	$\mu M^{-1}\, s^{-1}$
28	$NF\text{-}\kappa B_n + NF\text{-}\kappa B_n \rightarrow I\kappa B\alpha_t + NF\text{-}\kappa B_n + NF\text{-}\kappa B_n$	k_{tr2}	1.65×10^{-2}	$\mu M^{-1}\, s^{-1}$
29	$I\kappa B\alpha\text{-}_t$ → sink	k_{tr3}	2.8×10^{-4}	s^{-1}
30	source → $I\kappa B\beta\text{-}_t$	k_{tr2b}	1.78×10^{-7}	$\mu M^{-1}\, s^{-1}$
31	$I\kappa B\beta\text{-}_t$ → sink	k_{tr3}	2.8×10^{-4}	s^{-1}
32	source → $I\kappa B\varepsilon\text{-}_t$	k_{tr2e}	1.27×10^{-7}	$\mu M^{-1}\, s^{-1}$
33	$I\kappa B\varepsilon\text{-}_t$ → sink	k_{tr3}	2.8×10^{-4}	s^{-1}
34	IKK + IκBα → IKKIκBα	k_{a1}	22.5×10^{-3}	$\mu M^{-1}\, s^{-1}$
35	IKKIκBα → IKK + IκBα	k_{d1}	1.25×10^{-3}	s^{-1}
36	$I\kappa B\alpha\text{-}_t \rightarrow I\kappa B\alpha + I\kappa B\alpha\text{-}_t$	k_{tr1}	4.08×10^{-3}	s^{-1}
37	IκBα → sink	k_{deg1}	1.13×10^{-4}	s^{-1}
38	$I\kappa B\alpha \rightarrow I\kappa B\alpha_n$ (Import)	k_{tp1}	3×10^{-4}	s^{-1}
39	$I\kappa B\alpha_n \rightarrow I\kappa B\alpha$ (Export)	k_{tp2}	2×10^{-4}	s^{-1}
40	IKK + IκBβ → IKKIκBβ	k_{a2}	6.0×10^{-3}	$\mu M^{-1}\, s^{-1}$
41	IKKIκBβ → IKK + IκBβ	k_{d2}	1.75×10^{-3}	s^{-1}
42	$I\kappa B\beta\text{-}_t \rightarrow I\kappa B\beta + I\kappa B\beta\text{-}_t$	k_{tr1}	4.08×10^{-3}	s^{-1}
43	IκBβ → sink	k_{deg1}	1.13×10^{-4}	s^{-1}
44	$I\kappa B\beta \rightarrow I\kappa B\beta_n$ (Import)	$0.5\, k_{tp1}$	1.5×10^{-4}	s^{-1}
45	$I\kappa B\beta_n \rightarrow I\kappa B\beta$ (Export)	$0.5\, k_{tp2}$	1×10^{-4}	s^{-1}
46	IKK + IκBε → IKKIκBκ	k_{a3}	9.0×10^{-3}	$\mu M^{-1}\, s^{-1}$
47	IKKIκBε → IKK + IκBε	k_{d3}	1.75×10^{-3}	s^{-1}
48	$I\kappa B\varepsilon\text{-}_t \rightarrow I\kappa B\varepsilon + I\kappa B\varepsilon\text{-}_t$	k_{tr1}	4.08×10^{-3}	s^{-1}
49	IκBε → sink	k_{deg1}	1.13×10^{-4}	s^{-1}
50	$I\kappa B\varepsilon \rightarrow I\kappa B\varepsilon_n$ (Import)	$0.5\, k_{tp1}$	1.5×10^{-4}	s^{-1}
51	$I\kappa B\varepsilon_n \rightarrow I\kappa B\varepsilon$ (Export)	$0.5\, k_{tp2}$	1×10^{-4}	s^{-1}
52	$IKK + I\kappa B\alpha\text{-}NF\text{-}\kappa B \rightarrow IKKI\kappa B\alpha\text{-}NF\text{-}\kappa B$	k_{a7}	1.85×10^{-1}	$\mu M^{-1}\, s^{-1}$
53	$IKKI\kappa B\alpha\text{-}NF\text{-}\kappa B \rightarrow IKK + I\kappa B\alpha\text{-}NF\text{-}\kappa B$	k_{d1}	1.25×10^{-3}	s^{-1}
54	$I\kappa B\alpha_n\text{-}NF\text{-}\kappa B_n \rightarrow I\kappa B\alpha\text{-}NF\text{-}\kappa B$ (Export)	k_2	1.38×10^{-2}	s^{-1}
55	$IKK + I\kappa B\beta\text{-}NF\text{-}\kappa B \rightarrow IKKI\kappa B\beta\text{-}NF\text{-}\kappa B$	k_{a8}	4.8×10^{-2}	$\mu M^{-1}\, s^{-1}$
56	$IKKI\kappa B\beta\text{-}NF\text{-}\kappa B \rightarrow IKK + I\kappa B\beta\text{-}NF\text{-}\kappa B$	k_{d2}	1.75×10^{-3}	s^{-1}
57	$I\kappa B\beta_n\text{-}NF\text{-}\kappa B_n \rightarrow I\kappa B\beta\text{-}NF\text{-}\kappa B$ (Export)	$0.4\, k_2$	5.2×10^{-3}	s^{-1}
58	$IKK + I\kappa B\varepsilon\text{-}NF\text{-}\kappa B \rightarrow IKKI\kappa B\varepsilon\text{-}NF\text{-}\kappa B$	k_{a9}	7.0×10^{-2}	$\mu M^{-1}\, s^{-1}$
59	$IKKI\kappa B\varepsilon\text{-}NF\text{-}\kappa B \rightarrow IKK + I\kappa B\varepsilon\text{-}NF\text{-}\kappa B$	k_{d3}	1.75×10^{-3}	s^{-1}
60	$I\kappa B\varepsilon_n\text{-}NF\text{-}\kappa B_n \rightarrow I\kappa B\varepsilon\text{-}NF\text{-}\kappa B$ (Export)	$0.4\, k_2$	5.2×10^{-3}	s^{-1}
61	IKK → sink	k_{02}	1.2×10^{-4}	s^{-1}
62	IKKIκBα → IKK	k_{r1}	4.07×10^{-3}	s^{-1}
63	IKKIκBβ → IKK	k_{r2}	1.5×10^{-3}	s^{-1}
64	IKKIκBε → IKK	k_{r3}	2.2×10^{-3}	s^{-1}

Table 4: Summary of the initial values for the 26 participant species

Participant Specie	Initial Value (μM)
IκBα	0
NF-κB	0.1
IκBα-NF-κB	0
IκBβ	0
IκBβ-NF-κB	0
IκBε	0
IκBε-NF-κB	0
IKKIκBα	0
IKKIκBα-NF-κB	0
IKK	0
IKKIκBβ	0
IKKIκBβ-NF-κB	0
IKKIκBε	0
IKKIκBε-NF-κB	0
$NF\text{-}\kappa B_n$	0
$I\kappa B\alpha_n$	0
$I\kappa B\alpha_n\text{-}NF\text{-}\kappa B_n$	0
$I\kappa B\beta_n$	0
$I\kappa B\beta_n\text{-}NF\text{-}\kappa B_n$	0
$I\kappa B\varepsilon_n$	0
$I\kappa B\varepsilon_n\text{-}NF\text{-}\kappa B_n$	0
Source	1
IκBα-t	0
Sink	0
IκBβ-t	0
IκBε-t	0