Synergistic control of oscillations in the NF-κB signalling pathway

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

Abstract: In previous work, we studied the behaviour of a model of part of the NF-κB signalling pathway. The model displayed oscillations that varied both in number, amplitude and frequency when its parameters were varied. Sensitivity analysis showed that just nine of the 64 reaction parameters were mainly responsible for the control of the oscillations when these parameters were varied individually. However, the control of the properties of any complex system is distributed, and, as many of these reactions are highly non-linear, we expect that their interactions will be too. Pairwise modulation of these nine parameters gives a search space some 50 times smaller (81 against 4096) than that required for the pairwise modulation of all 64 reactions, and this permitted their study (which would otherwise have been effectively intractable). Strikingly synergistic effects were observed, in which the effect of one of the parameters was strongly (and even qualitatively) dependent on the values of another parameter. Regions of parameter space could be found in which the amplitude, but not the frequency (timing), of oscillations varied, and *vice versa.* Such modelling will permit the design and performance of experiments aimed at disentangling the role of the dynamics of oscillations, rather than simply their amplitude, in determining cell fate. Overall, the analyses reveal a level of complexity in these dynamic models that is not apparent from study of their individual parameters alone and point to the value of manipulating multiple elements of complex networks to achieve desired physiological effects.

1 Introduction

Given the speed with which the elements of signal transduction pathways and genetic circuits are being unravelled, it has become increasingly important to attain a theoretical understanding of their often rather complex dynamics. Consequently, as the proportion of the identified components involved in any of these networks continues to increase, the daunting challenge of developing useful models, both conceptual and mathematical, for how they work is attracting interest (see, for example, [1–8]).

The architecture of signal transduction networks is often highly complex owing to the large number of participating protein complexes, cross-interactions between pathways and the very non-linear functioning of regulatory circuits [9]. It is this complexity that makes the understanding of cellular signalling a challenging task [7, 10–12].

Networks of genetic interactions represent interconnected dynamic systems that generally can exhibit oscillating instabilities. These oscillations can be characterised by their amplitude and their phase, where the amplitude is the maximum value the variable attains during a particular period, and the phase is the state of the oscillation relative to the beginning of the period.

Typical signals, such as those detected by a radio [13], can be encoded in terms of their amplitude (amplitude-modulated (AM)) or frequency (FM). Although the encoding of biological signals in terms of frequency is well established in areas such as neurophysiology or Ca^{2+} signalling [14] and is potentially more accurate [15], only recently has experimental evidence come forward for the importance of frequency encoding in protein signalling pathways. Such evidence mainly comes from observations in single cells of the spatio-temporal dynamics of signalling molecules tagged with GFP and its derivatives, where substantial and sustained oscillations in their concentration can be detected. This requirement for single-cell measurements arises because such oscillations are typically out of phase with each other [16–18], and pooling observations from hundreds or thousands of cells by sampling them simultaneously means that such oscillations are damped, often to the point of invisibility. We ourselves have detected such oscillations in a variety of components of the NF-κB signalling pathway [19, 20] (and see [21]), and indicated that the frequency components have functional significance for downstream events, i.e. the signal is not simply in the amplitude (and see [22]). Experimental observations of oscillations have also been made for the p53 [23, 24] and MAP kinase [25] signalling pathways and can also be seen in mathematical models of such processes [23, 25–28].

The source of oscillations in many of the pathways exhibiting them is a coupled transcription–translation system with delayed feedback [29, 30] (although we note that, in at least one circadian system, oscillatory behaviour is based on a phosphorylation/dephosphorylation cycle [31, 32]), and this coupled transcription–translation system

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with delayed feedback is the essential basis for the oscillations we observe in the NF-κB system.

Indeed, such transcription factor pathways typically contain a network motif [33] in the form of a negative feedback loop composed of one transcription arm and one protein-interaction arm [23]. Oscillators based on auto-inhibition with time delay possess the striking property that the oscillation period is mainly determined by the time delay and depends only weakly on the average protein expression rates [34].

A prototypical signalling scheme is that represented by the nuclear transcription factor NF-κB system [35–37], which both plays an important role in the immune system [38] and regulates the expression of cytokines, growth factors, effector enzymes and of many genes not thought to be directly pertinent to the immune system. It is normally held inactive in the cytoplasm by being bound to an inhibitory factor IκB [39] (which has several isoforms such as α, β, ε). Following receptor activation by a suitable ligand such as TNF-α, a downstream kinase IκB kinase (IKK) phosphorylates IκB, leading to its ultimate degradation and thus the release of free NF-κB, which can enter the nucleus and effect the transcription of a variety of genes [40]. NF-κB stimulation also triggers negative feedback pathways in some cells that go on to terminate the NF-κB response by increasing the transcription/stability of newly synthesised IκBα [41, 42]; IκBα becomes a transcriptional target for NF-κB, creating a negative feedback loop [43]. This feedback loop operates by increasing the rate of synthesis or activation of the inhibitor (IκBα) when the pathway is activated, thereby down-regulating its own activity (see Fig. 1). The time required to synthesise or activate the inhibitor can generate a delay in the system [34, 44]. The result of this can be a series of oscillations that can be observed both in single cells [43] and in mathematical models [21, 43, 45, 46]. As the downstream events affected by changes in the transcription of NF-κB are multifarious (and include both apoptosis and proliferation), it is an open question as to exactly what features of the dynamics are responsible for cell fate decision making.

In previous work [21], based on an earlier model [45], we reconstructed a mathematical model of IκB-NF-κB, integrating the IKK-induced activation of NF-κB and resynthesis of IκBα. Sensitivity analysis allowed the identification of the most significant reactions following stimulation and showed that just nine (out of 64) of these dominated the response in terms of their effects on the amplitude or frequency of the oscillations. Further, the dynamics of just two molecular species (free [IKK] and free [IκBα]) were intimately coupled to the oscillations in the free nuclear NF-κB levels (see Fig. 9 of [21]). In addition, this mathematical model was useful for the design of new experiments [43], and the results therefrom were at least broadly consistent with this comparatively simple model.

However, we know from the theory underlying metabolic control analysis (which is closely related to the type of sensitivity analysis that we used) [47–50], as well as from experiment [51], that the control exerted by individual reaction parameters depends not only on their own magnitude but on that of all the others. As well as the sensitivity analyses, we also studied the effects of changing some of the individual parameters over two orders of magnitude either side of their 'basal' value (41 runs per parameter) [21, 43]. To study the interactions between these reaction parameters, the next level of complexity is clearly to do this in a pairwise manner. However, analysing the interactions between *each* of the 64 reactions in this way pairwise would be computationally prohibitive using a standard PC. This is because such a study would involve $41 \times 41 \times 63 \times 64/2 = 3\,388\,896$ runs, and even at 1 s per run, it would require about 6 weeks just to acquire the data, before the analysis ever started. In addition, each model requires ~20 kbytes and each dataset derived therefrom requires ~200 kbytes of storage, and so even the storage requirements, ~750 Gbytes, would be considerable. However, the recognition that only nine of the reactions exert major control when studied alone allowed a more realistic study of the pairwise interactions between just these nine reactions (giving a 50-fold decrease in the computational size of the task and thus making it tractable). This paper therefore discusses the outcome of this study of the interactions between the critical parameters observed to influence the system's dynamics. The

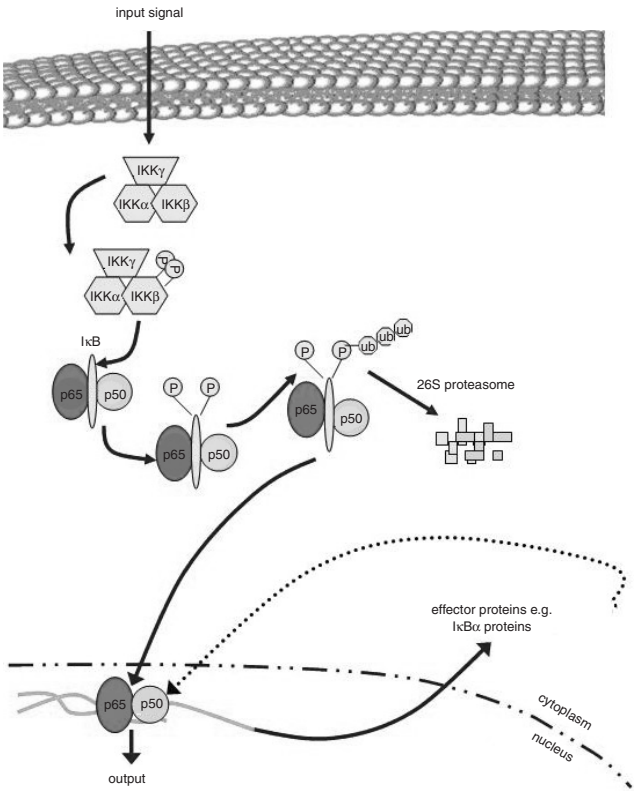

Fig. 1 *Schematic representation of NF-κB auto-regulatory network*

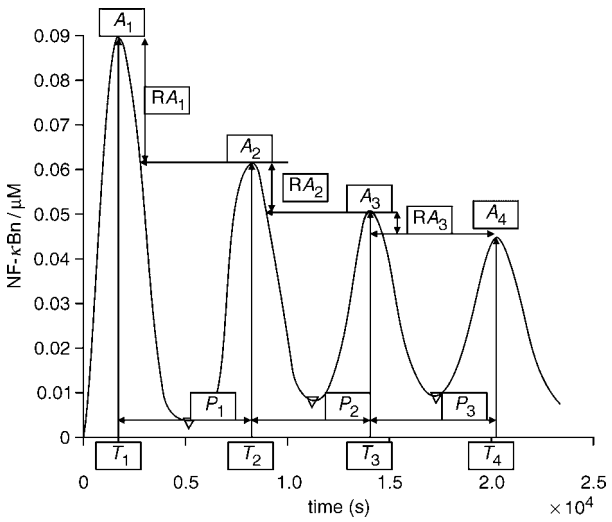

Fig. 2 *Time course of nuclear NF-κB in 'base' model as implemented herein, showing definitions of amplitudes A, times T and periods P used in subsequent analysis [21]*

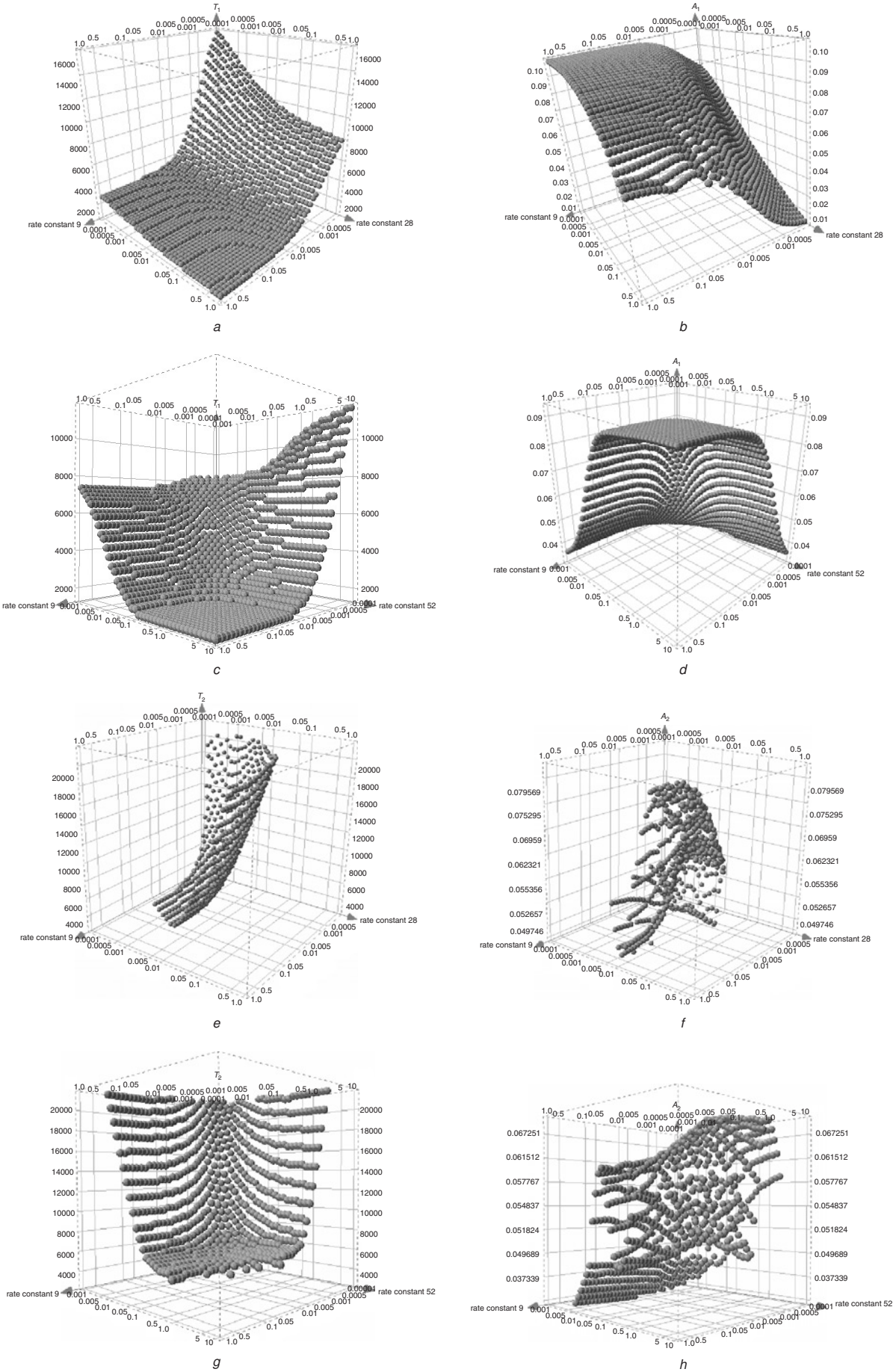

Fig. 3 *Dual modulation of critical reaction parameters over two orders of magnitude*

a, *b*, *e*, *f* Parameters 9 (IKKIκBα-NF-κB catalytic rate constant) and 28 (IκBα (IκBα-t) inducible mRNA synthesis rate constant)
c, *d*, *g*, *h* Parameters 9 and 52 (IKKIκBα-NF-κB association rate constant)
Plots of timing: *a*, *c* T_1; *e*, *g* T_2; and amplitude: *b*, *d* A_1; *f*, *h* A_2 at first and second oscillatory peaks of dual-modulated reactions

chief findings are that indeed these parameters interact in a non-linear, synergistic manner, and that specific perturbations that vary the period, the amplitude or both features of the time series can be observed.

2 Methods

For the analysis and better understanding of this signalling network, a kinetic pathway model was constructed using the modelling system Gepasi 3.3 [52–54]. The model of the IκBα-NF-κB signal transduction network [21, 45] was thus translated into a system of coupled ordinary differential equations (ODEs), whose solution produced a time series of the nuclear NF-κB concentration and indeed of all the other system variables [21]. The 'base' parameters of the model were those given previously and were varied logarithmically by two orders either side of their 'basal' value [21]. The output data (in ascii format) were imported into MS-Excel and/or Matlab to allow calculation of the periods and amplitudes of the relevant time series. Occasionally, the automated procedure devised for this did not work, leading to the occasional missing value and to a certain type of quantisation in the values obtained. These did not affect the overall conclusions and were ignored.

2.1 Control analysis

To quantify the amount of control exerted by a step in a pathway, Kacser and Burns [55] and Heinrich and Rapoport [56] introduced the concept of control strength. The control strength C_i^{Xj} of a step in a metabolic pathway, for example, quantifies the extent to which a reaction parameter i (e.g. a rate or rate constant) controls a steady state or other variable Xj [55, 57] and is numerically equal to its sensitivity [58, 59]:

$$C_i^{Xj} = \frac{\delta Xj/Xj}{\delta vi/vi} = \frac{\delta \ln Xj}{\delta \ln[vi]} \tag{1}$$

The essence of this approach is the substitution of the inadequate abstraction of a 'rate-limiting step' in metabolic or signalling pathways by a quantitative measure, the control coefficient. In other words, the sensitivity of the changes in the variable (metabolite concentration and fluxes) of an 'existing' metabolic system due to changes in the parameter (external factors) is quantified by control coefficients [55, 56, 60]. In the present case, we used 'large' changes (see [47, 48]) in the activity of pairs of reactions to seek areas of the parameter space that were 'interesting'. In addition, the sensitivities were modified to recognise that we were modulating more than one reaction, here two designated i and p, although this was done by the same amount z in each case (10% or 100%, see below):

$$C_{i,p,z}^{Xj} = \frac{\delta Xj/Xj}{\delta v(i,p,z)/v(i,p)} = \frac{\delta \ln Xj}{\delta \ln[vi,p,z]} \tag{2}$$

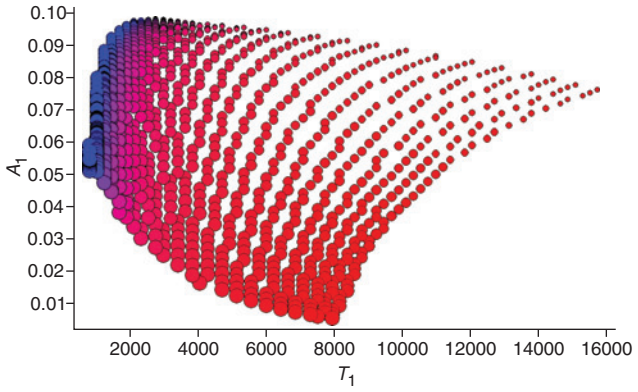

Fig. 4 *Peak amplitude A_1 against timing T_1 of first simulated peak for dual-modulated reaction parameter 9 (IKKIκBα-NF-κB catalytic rate constant) and 28 (IκBα (IκBα-t) inducible mRNA synthesis rate constant)*

Parameters were varied in range shown in Figs. 3A and C, parameters being encoded by colour from red to blue with increasing rate constant 9 and by size by increasing rate constant 28

3 Results and discussion

As previously, we characterise the time course of the changes in nuclear NF-κB according to their period and amplitude (Fig. 2), and these represent the Xj values in (2).

As described above, we narrowed the search space of 'possible' models to those involving just the nine reactions with the greatest sensitivities and studied their interactions in two ways

- The nine critical parameters were modulated pairwise ('dual modulated') over two orders of magnitude either side of their 'basal' values to establish the degree to which they interacted.
- The nine critical parameters were varied pairwise by either 10% or 100%, and sensitivity analysis was applied to the system.

3.1 Impact of dual modulation of critical parameters over two orders of magnitude on signalling mechanism

Each of the proposed parameter pairs of the nine 'critical' parameters of this IκBα-NF-κB model were varied over

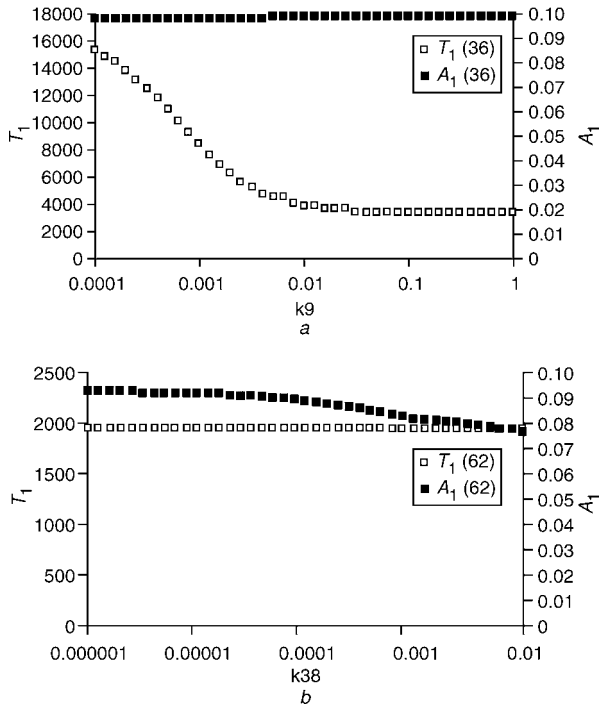

Fig. 5 *Peak timing T_1 and amplitude A_1 of first simulated peak for certain critical reaction parameters*

Peak variables T_1 and A_1 at varied rate constant *a* k9 and *b* k38, modulated by two orders of magnitude on either side of 'standard' rate constant at fixed k36 (1×10^{-5} s^{-1}) (*a*) or k62 (1×10^{-5} s^{-1}) (*b*)

two orders of magnitude on either side of the standard 'basal' rate constants. Variation of the rate constants was carried out using the scan utility of *Gepasi*, and, for this investigation, 41 values per parameter were used, from each of which a time course was created. The variable values time T, amplitude A and period P (see Fig. 2) were subsequently measured for each of the resulting 1681 (41^2) scans and used to construct scatter plots relating each of these to the values of the chosen parameters. We here consider results from the dual modulation of reactions 9 and 28 and 9 and 52 (Fig. 3).

Fig. 3 is a plot of the peak timing (T_1, Fig. 3*a*) and amplitude (A_1, Fig. 3*b*) for the first simulated peak for the different rate constant values of the dual-modulated reactions 9 (IKKIκBα-NF-κB catalytic rate constant) and 28 (IκBα (IκBα-t) inducible mRNA synthesis rate constant). Figs 3*c* and *d* show similar results for the effect on T_1 and A_1, respectively, of the dual modulation of reaction 9 with reaction 52 (IKKIκBα-NF-κB association rate constant). It can first be noted that the overall model was only capable of supporting sustained oscillations over a restricted set of values of the critical parameters [21, 43] (refer to Figs. 3*e–h*), and so it makes sense to start with the initial oscillation. Figs. 3*e* (T_2) and *f* (A_2) similarly show the timing and amplitude of the second peak for the different rate constant values of the dual-modulated reactions 9 and 28. Figs 3*g* and *h* show similar results for the effect on T_2 and A_2, respectively, of the dual-modulated reactions 9 and 52.

Impact of the rate constant variation of reactions 9 and 28 and 9 and 52 on the time observed for peak 1(Figs. 3a and c): A number of points emerge from this analysis. First, there are substantial non-linear interactions between the paired rate constants. This is observed through the non-linearity in the shapes of these plots. Although, as we would expect, the time of the peak of the first oscillation tended to increase as any of the rate constants was decreased, this was not at all the case when the partner of reaction 9 was changed from reaction 28 (Fig. 3*a*) to

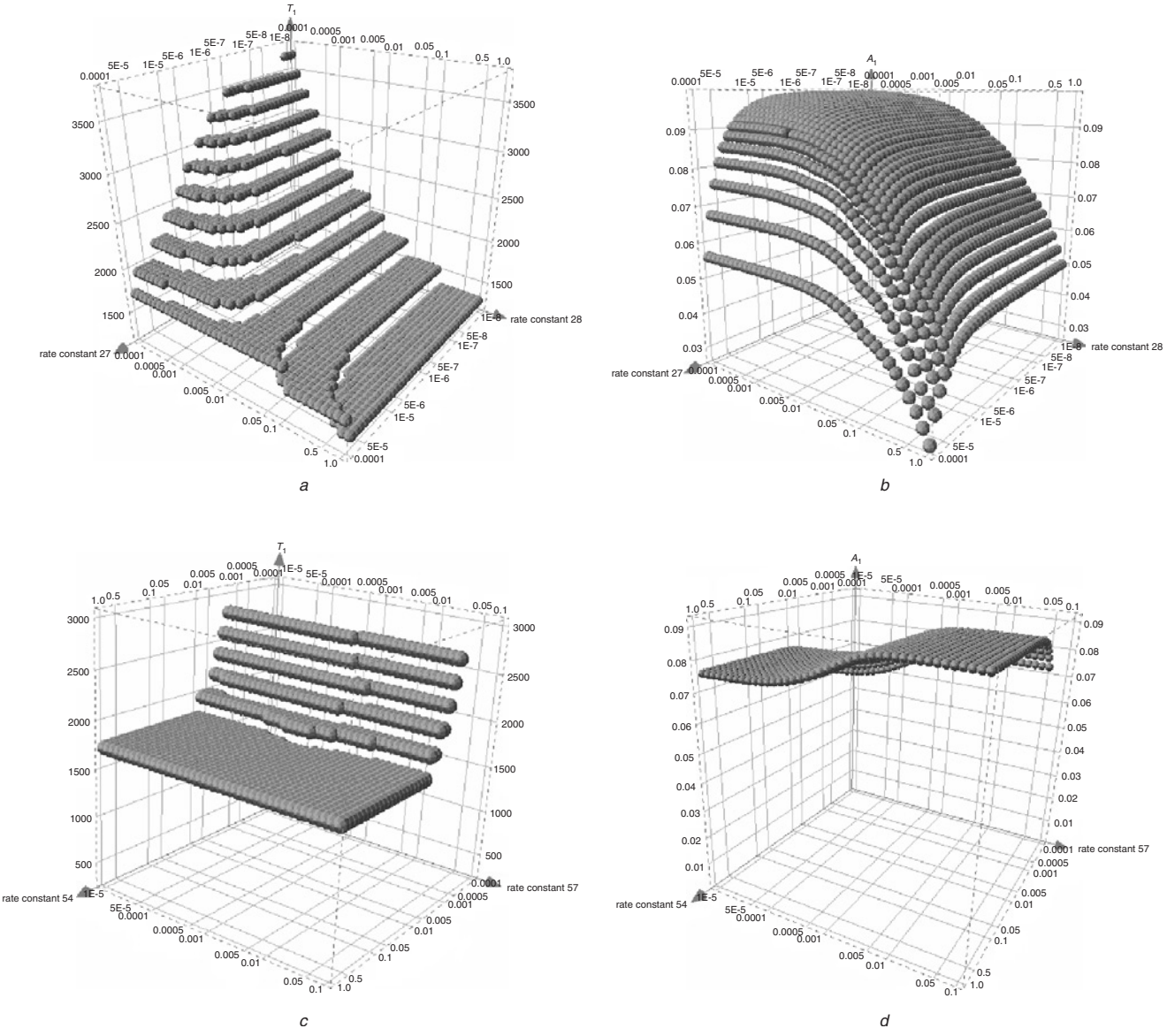

Fig. 6 *Lack of synergistic effects of non-critical parameters on behaviour of model*

Dual modulation of reaction parameters over two orders of magnitude
a, *b* Non-critical reaction parameter 27 (IκBα (IκBα-t) constitutive mRNA synthesis rate constant) and critical reaction parameter 28 (IκBα (IκBα-t) inducible mRNA synthesis rate constant)
c, *d* Non-critical parameters 54 (IκBα_n-NF-κB$_n$ nuclear export rate constant) and 57 (IκBβ_n-NF-κB$_n$ nuclear export rate constant)
a, *c* Timing T_1 at first oscillatory peak of dual-modulated reactions
b, *d* Amplitude A_1 at first oscillatory peak

reaction 52 (Fig. 3*c*). Thus even the form of the non-linearity depends strikingly on the nature of the modulations: although the data from k9/k28 are monotonic (decreasing either always causes T_1 to increase), a decrease in k52 causes T_1 to increase when k9 is high but to decrease when k9 is low. A variety of types of non-linearity were observed for the effects of the rest of the nine paired rate constants on T_1 (data not shown), suggesting that such synergy was the norm. In this case, the amplitude data (Figs. 3*b* and *d*) are somewhat more robust to these changes than are the timings, although synergistic behaviour is also observed here. Another way of looking at such data is to look at the covariation of T_1 and A_1 (Fig. 4).

Inspection of these kinds of plot suggested that some reaction combinations, when dual modulated, would influence A_1 and not T_1, and *vice versa* (Fig. 5). Thus the parameter combination 9 and 36 (Fig. 5*a*) impacted the timing observed for the first simulated peak but not the amplitude, and *vice versa* for 38 and 62 (Fig. 5*b*). This is of significance when we are seeking to modulate specific reactions to disentangle the importance of frequency and amplitude to downstream cellular events [22, 43].

Having established substantial non-linear interactions between paired 'critical' rate constants and shown how the kind of non-linearity depended on the nature of the modulations, it was of interest to know whether interactions between one 'critical' and one 'non-critical' reaction led to significant synergy. We therefore studied (for a small number of these) the effect of combining non-critical and critical parameter modulations on the model. As NF-κB stimulation induces its own inhibitor (IκBα) to regulate cellular activation, we examined the effect of dual modulations of reactions 27 (IκBα (IκBα-t) constitutive mRNA synthesis rate constant) and 28 (IκBα (IκBα-t) inducible mRNA synthesis rate constant). We found that reaction 27 had little effect on the values of T_1 at a given value of k28 (Fig. 6*a*), although it resulted in the appearance of quantised plots as a consequence of the software used when peak picking. There were slight interactions between k27 and k28, as observed in A_1 when both rate constants were large, but overall these effects were small compared with those interactions seen when pairs of 'critical' parameters were modulated.

We next determine further the impact of the rate constant variation of non-critical reactions 54 (IκBα_n-NF-κB$_n$ nuclear export rate constant) and 57 (IκBβ_n-NF-κB$_n$ nuclear export rate constant) on the frequency and amplitude of the oscillations. There were no synergistic interactions between the parameters, and only at extreme values (well away from the basal values) did they influence the model at all (Figs 6*c* and *d*).

3.2 Sensitivity analysis of dual modulated critical parameters

Using the parameter scan utility of *Gepasi*, three scans per parameter were executed (initial value and then initial value increased and decreased by 10% for the pairs of parameters of interest). We here focus on the translocation of nuclear NF-κB. For the individual plots obtained for the parameter combinations, the values for time ($T_1 - T_4$) and amplitude

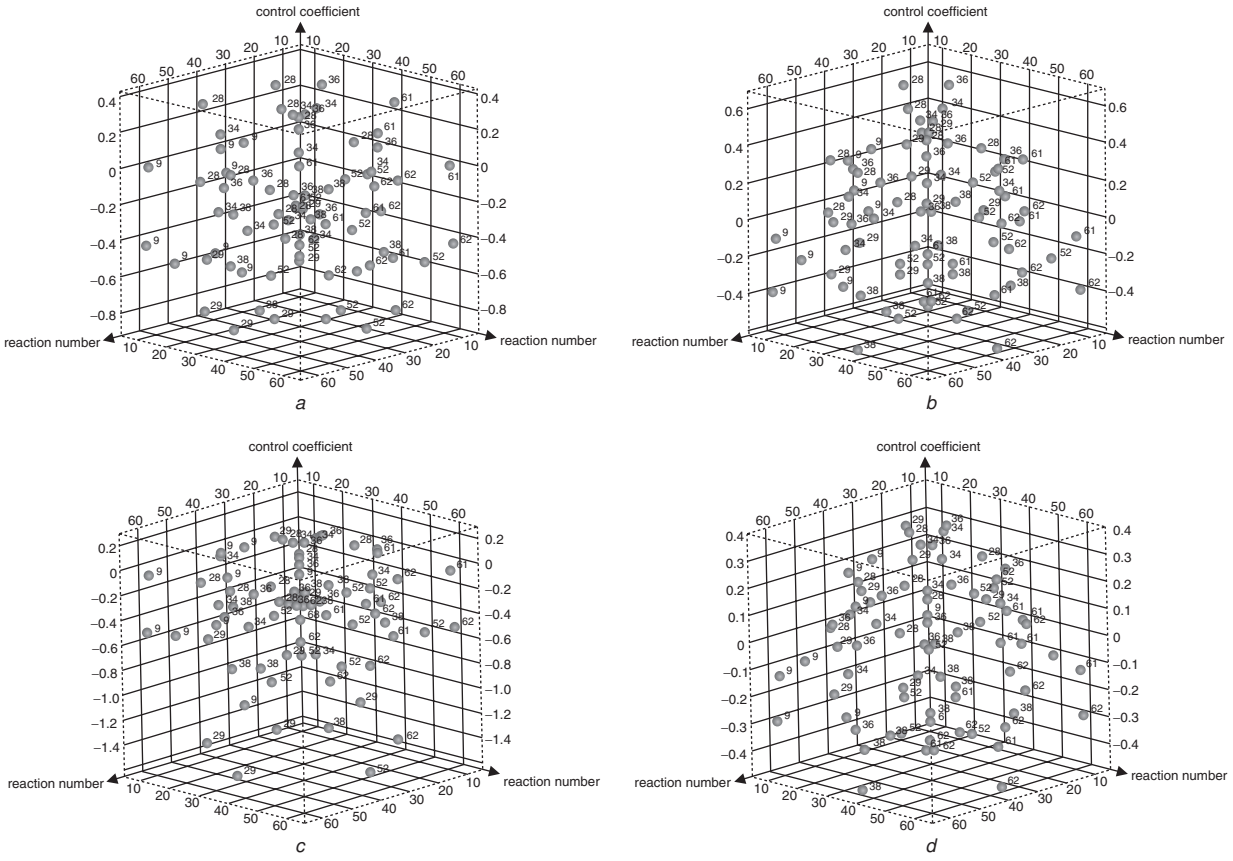

Fig. 7 *Sensitivity coefficients with respect to dual-modulated critical parameters of peak timing and amplitude for second simulated oscillation when changed by 10% or 100%*

a, *c* Timing
b, *d* Amplitude
a, *b* 10% change
c, *d* 100% change
Plots are symmetric, in that labelled spots on left represent image of equivalent labelled spot on right along same plane

(A_1–A_4) of each oscillatory peak and the periods (P_1–P_3, data not shown) between each peak were measured. These scans were also carried out for all the paired 'critical' parameter combinations, with the sensitivities determined as described in Section 2, using the averaged value for the paired up and down modulations. Figs. 7*a* and *b* are sensitivity analysis plots thereby obtained for the average timing and amplitude at the second oscillation as a function of 'reaction number' combination (where each of the labelled reaction combinations represents one of the critical parameters in a pair). A second study was also carried out where the critical parameters were either doubled or halved (termed 100% variation) (Figs. 7*c* and *d*).

We previously reported the maximum sensitivity coefficients obtained when all of the different parameters affecting nuclear NF-κB oscillations were considered [21] and found reactions 28 and 38 to have the highest either positive or negative sensitivity coefficient for modulations of both 10% and 100% when varied alone. However, when 28 and 38 are dual modulated, their combined sensitivity value is reduced relative to that of 28 interacting with 36. In addition, Fig. 7*a*, for example, shows that when 52 partners 29, as opposed to 62, their combination gives a more negative sensitivity value. In that the combinations differ significantly depending on what is combined with a given reaction, these findings overall demonstrate again that synergy was the norm.

4 Conclusions

Investigation of the dynamics of complex systems has shown that the interplay of many components in some systems can lead readily to oscillatory behaviour. The source of the emergence of oscillations in such complex systems can be subtle as it depends crucially on the dynamic properties of the interacting components and their collective behaviour. Given, in particular, that even simple non-linear systems can display very complicated and unexpected dynamics (e.g. [61–63]), mathematical modelling is an essential tool for their study (e.g. [64, 65]).

Even this comparatively small model of part of the NF-κB system exhibits quite remarkably complex dynamics when its parameters are varied significantly one at a time [21, 43, 66]. Varying them all in pairs is computationally close to intractable on a standard desktop machine, but by concentrating on the subset of nine reactions previously determined to exert the greatest control on the oscillations, we have been able to observe complex and synergistic interactions between these pairs of 'critical' parameters. In some cases, the effects are even qualitative, in that a decrease in k52 causes T_1 to increase when k9 is high but to decrease when k9 is low (Fig. 3).

Overall, the analyses reveal a level of complexity in these dynamic models that is not determined by their individual parameters alone. In terms of the search for novel drug targets, we might expect that modulation of more than one reaction alone will be able to give physiological effects that cannot be obtained by modulating reactions singly. Interestingly enough, this is exactly what is found both in theory [67–70] and in practice [71–73].

5 Acknowledgments

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