

Mathematical modelling of canonical and non-canonical NF- κ B pathways in TNF stimulation

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ABSTRACT

Background and Objective: NF- κ B can be activated by the canonical and non-canonical pathways. These two pathways interplay via the TRAF1|NIK complex after stimulation by TNF. However existing mathematical models of two pathways are inadequate. In this context, an improved mathematical model is constructed to simulate these two pathways and their coupling stimulated by TNF.

Methods: A schematic description of two NF- κ B pathways and their relation after TNF stimulation is constructed at first. Then twenty-eight ordinary differential equations are utilized to build the mathematical model. Model equations are solved via the ordinary differential equation solver (ode23).

Results: The proposed model firstly reconstructs the changes in concentrations of NF- κ B pathway related biochemical factors with time, and further investigates the underlying mechanism of interaction between two pathways through the TRAF1|NIK complex after stimulation.

Conclusions: The model is validated through good agreement between simulation results and published experimental observations. This study helps to well understand the canonical and non-canonical pathways and their interaction. It also provides a potential tool to investigate how the dysregulated pathways act in pathological conditions.

KEY WORDS: NF- κ B; Canonical pathway; Non-canonical pathway; Coupling; Mathematical modelling

1. INTRODUCTION

The mammalian nuclear factor-kappa B (NF- κ B) includes five transcription factors: NF- κ B1(p50/p105), NF- κ B2(p52/p100), RelA(p65), RelB and cRel [1-3]. These five factors are able to form homo-or heterodimers with each other [4]. Specially, p50 and p65 form p50|p65 heterodimers acting in the canonical pathway, while p52|RelB heterodimers formed by p52 and RelB, take part in the non-canonical pathway [5, 6].

NF- κ B always stays in un-stimulated state in the cytoplasm by inhibitors, such as I κ B α in resting cells [7]. NF- κ B can be activated via the canonical and non-canonical signalling pathways [8-10]. The canonical pathway mainly involves I κ B α being targeted for degradation by the cytoplasmic I κ B α kinase (IKK). IKK can phosphorylate, ubiquitinate and thereby target I κ B α for degradation, consequently decomposing the complex of I κ B α |NF- κ B and further releasing NF- κ B (p50|p65) [11,12]. The key point in the non-canonical pathway is p100 transformed into p52 under mediation of NF- κ B-inducing kinase (NIK), which then forms p52|RelB heterodimers with RelB [13, 14]. As an essential part of the NF- κ B pathway, the non-canonical pathway acts important functions in many biological processes and its abnormalities is reported to linked to several malignancies [5].

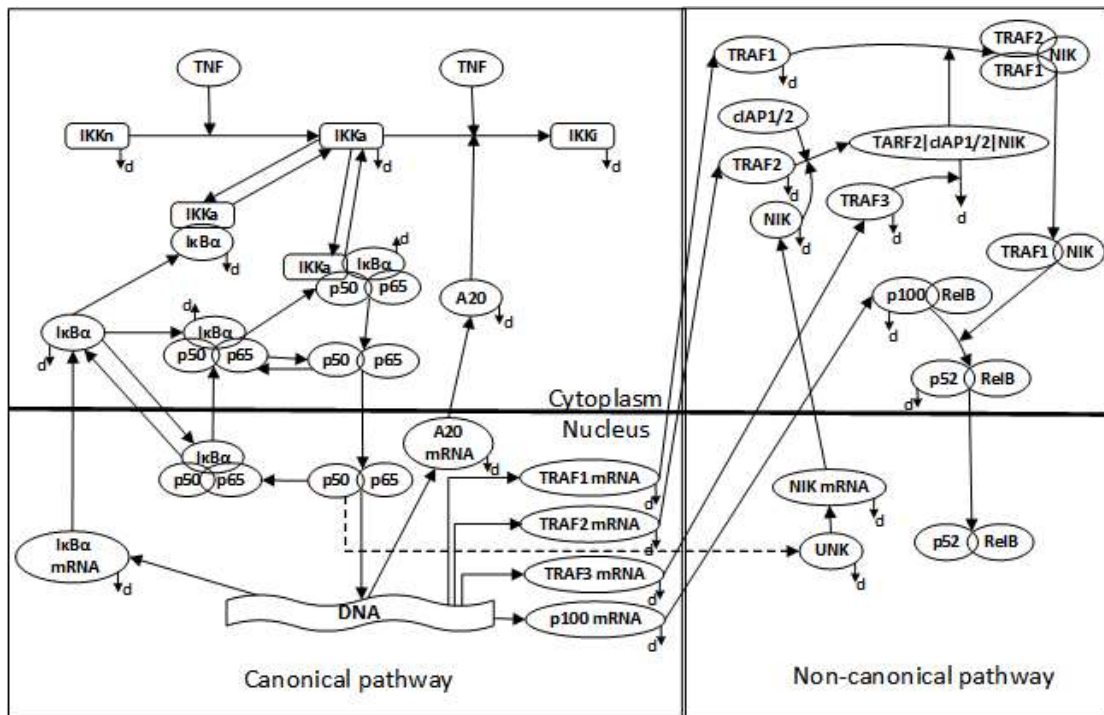
Despite NF- κ B pathway being such an important biological process, it has not been completely elucidated. Hence mathematical models, proven to be an auxiliary method for studying complicated biological processes [15-18], have been proposed to mimic NF- κ B signalling pathways [1, 6, 16, 19-22]. However, as far as we know, these previous models mainly focused on the canonical pathway, but did not consider the non-canonical pathway, with the exception of the work of Choudhary et al. [23]. Although the study of [23] simulated both NF- κ B pathways, we noted several significant inconsistencies exists. The details are included in Appendix A. In this

1 context, an extended mathematical model is constructed in this paper to mimic the
2 canonical as well as non-canonical pathway, further investigate the underlying coupling
3 mechanism of two pathways.

4 2. METHODS

5 Figure 1 shows a schematic description of two NF- κ B pathways and the relation
6 between them after TNF stimulation. As demonstrated in Figure 1, the left part of Figure
7 1 presents functions of the canonical pathway. p50|p65 always stays in un-stimulated
8 state in the cytoplasm by several inhibitors in resting cells [1, 4, 24]. These NF- κ B
9 inhibitors named I κ Bs, include I κ B α , I κ B β , I κ B γ and I κ B ϵ , are able to bind to p50|p65,
10 and then suppress its activities [24]. Considering p50|p65 is primarily suppressed by
11 I κ B α , this model only considers I κ B α for simplicity. IKK serves as an activator for the
12 canonical pathway and usually stays in neutral state (denoted by IKKn) in resting cells
13 [25]. TNF can trigger the transformation of IKKn into its active state (denoted by IKKa).
14 IKKa is able to phosphorylate and ubiquitinate I κ B α , targeting it for destruction,
15 consequently releasing free p50|p65 heterodimers. The released p50|p65 can quickly
16 move into the nucleus from the cytoplasm and bind to gene promoters on DNA to
17 express genes such as I κ B α , A20, TRAF1, TRAF2, TRAF3 and p100 [26]. Among
18 these newly produced proteins, I κ B α and A20 serve as two inhibitors of the canonical
19 pathway [1, 4]. They can limit the canonical pathway in different ways and thus trigger
20 a negative feedback loop. Thus, I κ B α moves into the nucleus from the cytoplasm to
21 inactivate p50|p65 heterodimers and initiate their translocation back to the cytoplasm.
22 In addition, A20 can promote the transformation of IKKa into its un-activated state
23 (denoted as IKKi in our model). IKKi cannot phosphorylate I κ B α [1, 27]. It should be
24 noted that although IKK are transformed among three states (IKKn, IKKa and IKKi),
25 the total amount of IKK keeps nearly constant.

1 The right part of Figure 1 demonstrates underlying mechanisms of the activation
 2 of the non-canonical pathway as well as coupling between two pathways [23]. NIK acts
 3 as an important biochemical factor in activating the non-canonical signalling, Its
 4 concentration is always limited at a rather low level by the TNF receptor-associated
 5 factor3 (TRAF3) in the TRAF2|cIAP1/2 complex in resting cells [4]. Newly
 6 synthesized TRAF1 in the canonical pathway can disturb the ubiquitination of NIK by
 7 TRAF3 through forming the TRAF1|NIK complex. The TRAF1|NIK complex can then
 8 promote p100 transformation into p52, which is necessary for the non-canonical
 9 pathway. p52 forms p52|RelB heterodimers with RelB and moves into the nucleus from
 10 the cytoplasm to regulate gene expression.



11
 12 Figure 1: The schematic description of two NF-κB pathways for TNF stimulation. (Note that
 13 “d” represents the degradation of biochemical factors).

14 According to biological fundamentals of two pathways as demonstrated in Figure
 15 1, an extended mathematical models is built to simulate two pathways as well as their
 16 interaction. The model totally consists of twenty-eight ordinary differential equations

1 example, $\frac{d}{dt}TRAF1_t(t)$ is the variation of $TRAF1_t$ with time as demonstrated in
2 equation (1). n_{1e} represents the synthesis of constitutive TRAF1 mRNA. n_{1a}
3 represents the synthesis of TRAF1 mRNA induced by p50_n. n_{1c} represents the
4 degradation rate of TRAF1 mRNA. Equations (1), (3), and (10) all involve p50_n. Since
5 p50_n is produced in the canonical pathway, the definition of the variation in p50_n
6 concentration is included in equation (B.7) in Appendix B. Considering that factors
7 which induce the synthesis of NIK mRNA are currently not completely understood, for
8 simplicity, it is assumed here that NIK gene transcription is regulated by an unknown
9 promoter denoted by UNK in equation (5) as also suggested by Choudhary et al. [23].
10 As shown in equation (14), the amount of UNK is assumed to be regulated by p50_n.
11 The definitions of variables used in the model are listed in Table 1. The details of model
12 parameters are listed in Table 2. In these tables, experimental data of n_{1a} and n_{1c} are not
13 available, and thus their values are fitted via a Genetic Algorithm. n_{1a} and n_{1c} are set as
14 inputs of GA, while the increasing fold changes in TRAF1 mRNA and TRAF2 mRNA
15 after TNF stimulation are set as outputs. However, more biological research is require
16 to identify the exact levels of the fold change. Here, the fold change is set as 36. More
17 detailed information of GA is included in Appendix C.

Variable	Description
$TRAF1_t$	The amount of TRAF1 mRNA
$TRAF1$	The amount of TRAF1 protein
$TRAF2_t$	The amount of TRAF 2 mRNA
$TRAF2$	The amount of TRAF2 protein

18 Table 1: Definitions of variables used in the model.
19

Variable	Description
NIK_t	The amount of NIK mRNA
NIK	The amount of NIK protein
TRAF2 NIK	The amount of complexes of TRAF2 and NIK
TRAF1 TRAF2 NIK	The amount of complexes of TRAF1, TRAF2 and NIK
TRAF1 NIK	The amount of complexes of TRAF1 and NIK
$p100_t$	The amount of p100 mRNA
$p100$	The amount of cytoplasmic p100 protein
$p52$	The amount of p52 in the cytoplasm
$p52_n$	The amount of p52 in the nucleus
$p50_n$	the nuclear concentration of p50 protein produced in the canonical NF- κ B pathway
UNK	the amount of UNK in the nucleus

1 Table 1 (cont): Definitions of variables used in the model.
2

Symbol	Values	Units	Description	Comments
n_{1a}	7×10^{-8}	s^{-1}	TRAF1-inducible mRNA synthesis	Fitted
n_{1b}	0.5	s^{-1}	TRAF1 translation rate	[23]
n_{1c}	4.62×10^{-5}	s^{-1}	TRAF1 mRNA degradation	Fitted
n_{1d}	0.0003	s^{-1}	TRAF1 degradation rate	[23]

3 Table 2: Definitions and values of model parameters.
4

Symbol	Values	Units	Description	Comments
n_{1e}	0.0	μMs^{-1}	TRAF1-constitutive mRNA synthesis	[23]
n_{2a}	5×10^{-7}	s^{-1}	TRAF2-inducible mRNA synthesis	[23]
n_{2b}	0.5	s^{-1}	TRAF2 translation rate	[23]
n_{2c}	0.0004	s^{-1}	TRAF2 mRNA degradation	[23]
n_{2d}	0.0003	s^{-1}	TRAF2 degradation rate	[23]
n_{2e}	0.0	μMs^{-1}	TRAF2-constitutive mRNA synthesis	[23]
n_{3a}	3.75×10^{-8}	s^{-1}	NIK-inducible mRNA synthesis	Assumption
n_{3b}	0.5	s^{-1}	NIK translation rate	[23]
n_{3c}	0.0004	s^{-1}	NIK mRNA degradation	[23]
n_{3d}	0.0	μMs^{-1}	NIK-constitutive mRNA synthesis	[23]
n_{4a}	1.2	s^{-1}	UNK-inducible synthesis	Assumption
n_{4d}	0.0003	s^{-1}	NIK degradation rate	Assumption
n_{4c}	1.0	s^{-1}	UNK degradation	Assumption
n_{4e}	0.0	μMs^{-1}	UNK-constitutive synthesis	Assumption
b_1	1.0	s^{-1}	TRAF2-NIK association	[23]
b_2	6.42×10^{-5}	s^{-1}	NIK and TRAF1 degradation from TRAF2-NIK complex	[23]

1 Table 2 (cont): Definitions and values of model parameters.

2

Symbol	Values	Units	Description	Comments
b_3	0.5	s^{-1}	TRAF1 association with TRAF2- NIK complex	[23]
b_4	0.25	s^{-1}	formation of TRAF1-NIK complex by displacing TRAF2 from TRAF2- NIK complex	[23]
nc_1	2.5×10^{-8}	s^{-1}	p100-inducible mRNA synthesis	[33]
nc_2	0.5	s^{-1}	p100 translation rate	[23]
nc_3	3.2×10^{-5}	s^{-1}	p100 mRNA degradation	[33]
nc_4	0.0004	s^{-1}	p100 degradation rate	[23]
nc_5	0.002	s^{-1}	TRAF1-NIK and p100 association	[23]
nc_6	0.0	μMs^{-1}	p100-constitutive mRNA synthesis	[23]
nc_7	7.5×10^{-4}	s^{-1}	p52 nuclear import	[33]
nc_8	0.0002	s^{-1}	p52 nuclear export	[33]
nc_9	3.2×10^{-5}	s^{-1}	p52 degradation rate	Assumption

1 Table 2 (cont): Definitions and values of model parameters.

2 3. RESULTS

3 The proposed model equations above are solved by the ordinary differential equation
4 solver (ode23) with the initial values of model variables as shown in Table 3. The initial
5 values in Table 3 refer to steady values of the biochemical factors with regard to the
6 NF- κ B pathway without TNF stimulation. These values are derived following such way:

1 given a group of randomly chosen values of state variations (representing
2 concentrations of the biochemical factors), the concentrations of related biochemical
3 factors will vary and finally achieve a steady state without TNF stimulation. The code
4 implementation was performed via the Matlab language (R2018b, The MathWorks, Inc,
5 Natick, USA). The corresponding MATLAB codes are included in supplementary data.
6 Considering that model simulation of the canonical NF- κ B pathway has been
7 performed as discussed in section 2 [1], thereby the corresponding simulation results
8 are not presented in this section, but demonstrated in Appendix B. This section mainly
9 demonstrates simulation results of the non-canonical pathway and the interaction
10 between two pathways.

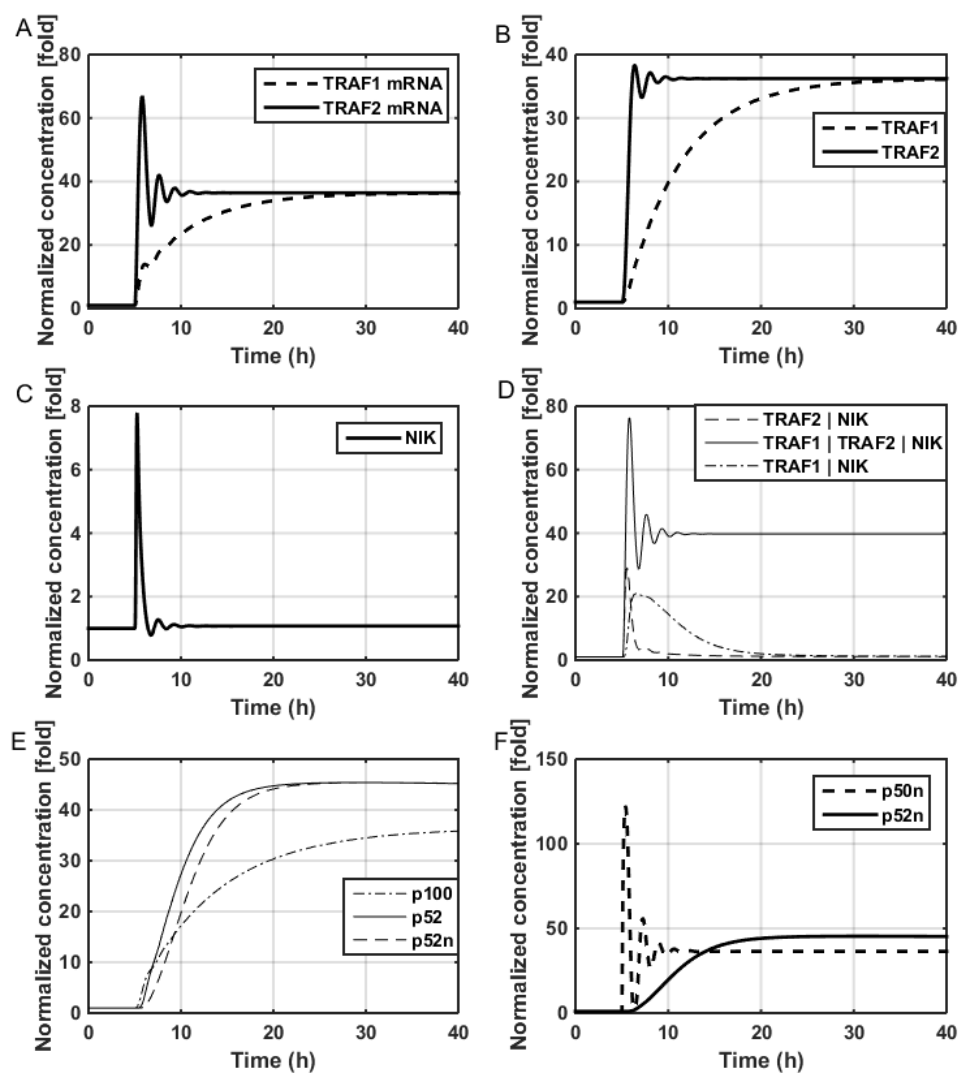
Variable	Initial value	Units
TRAF1 mRNA	3.48×10^{-6}	μM
TRAF2 mRNA	2.87×10^{-6}	μM
TRAF1	0.0054	μM
TRAF2	0.0044	μM
NIK mRNA	2.58×10^{-7}	μM
NIK	2.76×10^{-5}	μM
TRAF2 NIK	4.37×10^{-5}	μM
TRAF1 TRAF2 NIK	4.72×10^{-7}	μM
TRAF1 NIK	1.15×10^{-6}	μM
p100 mRNA	1.79×10^{-6}	μM
p100	0.0022	μM

11 Table 3: Initial values for the model variables.

Variable	Initial value	Units
p52	1.61×10^{-7}	μM
p52n	6.04×10^{-7}	μM
p50n	0.0023	μM
UNK	0.0028	μM

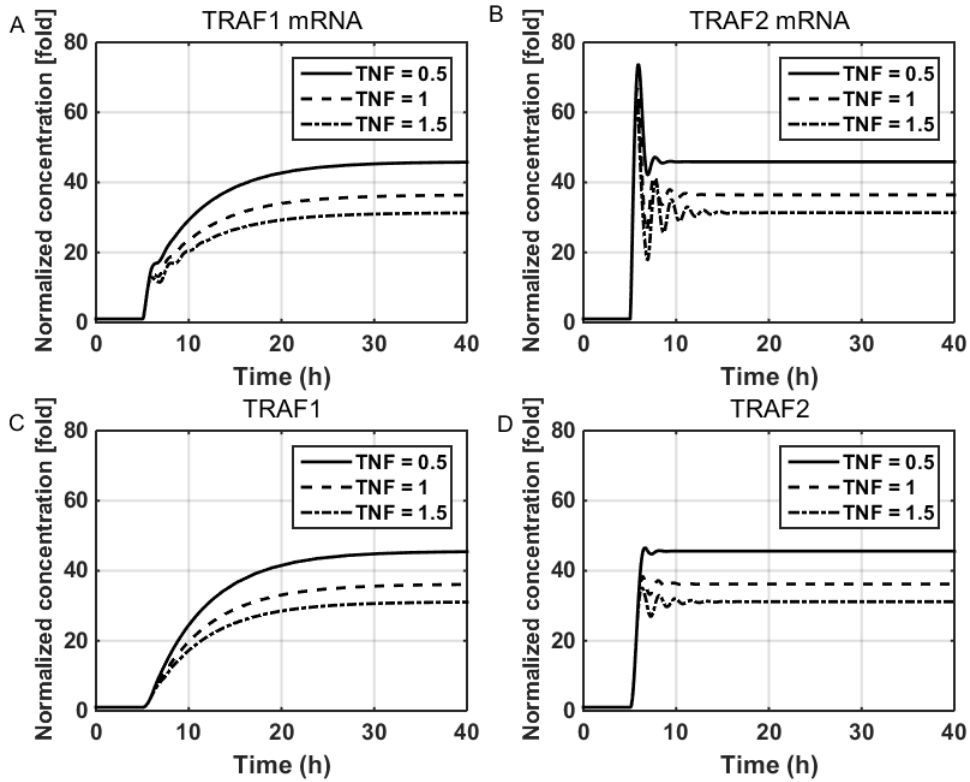
1 Table 3 (cont): Initial values for the model variables.

2 Figure 2 describe the variations of biochemical factors involved the non-canonical
3 pathway with time and their concentrations are normalized by their initial values (as
4 demonstrated in Table 3 and TNF concentration was set as 1 μM following the work of
5 Lipniacki et al. [1]). Specially, Figure 2A and Figure 2B illustrate the variations in the
6 concentrations of TRAF1 mRNA, TRAF2 mRNA and their proteins with time,
7 respectively. Figure 2C and Figure 2D describes the variations in the concentrations of
8 NIK, TRAF1|NIK complex, TRAF2|NIK complex and TRAF1|TRAF2|NIK complex
9 with time. Figure 2E demonstrates the variations in concentrations of cytoplasmic p100,
10 p52 and the nuclear p52 (p52n) with time. Figure 2F presents the temporal changes in
11 the concentrations of p50n and p52n. Figure 3 to Figure 5 demonstrate influences of
12 TNF variations on the biochemical factors related to the NF- κB pathway.



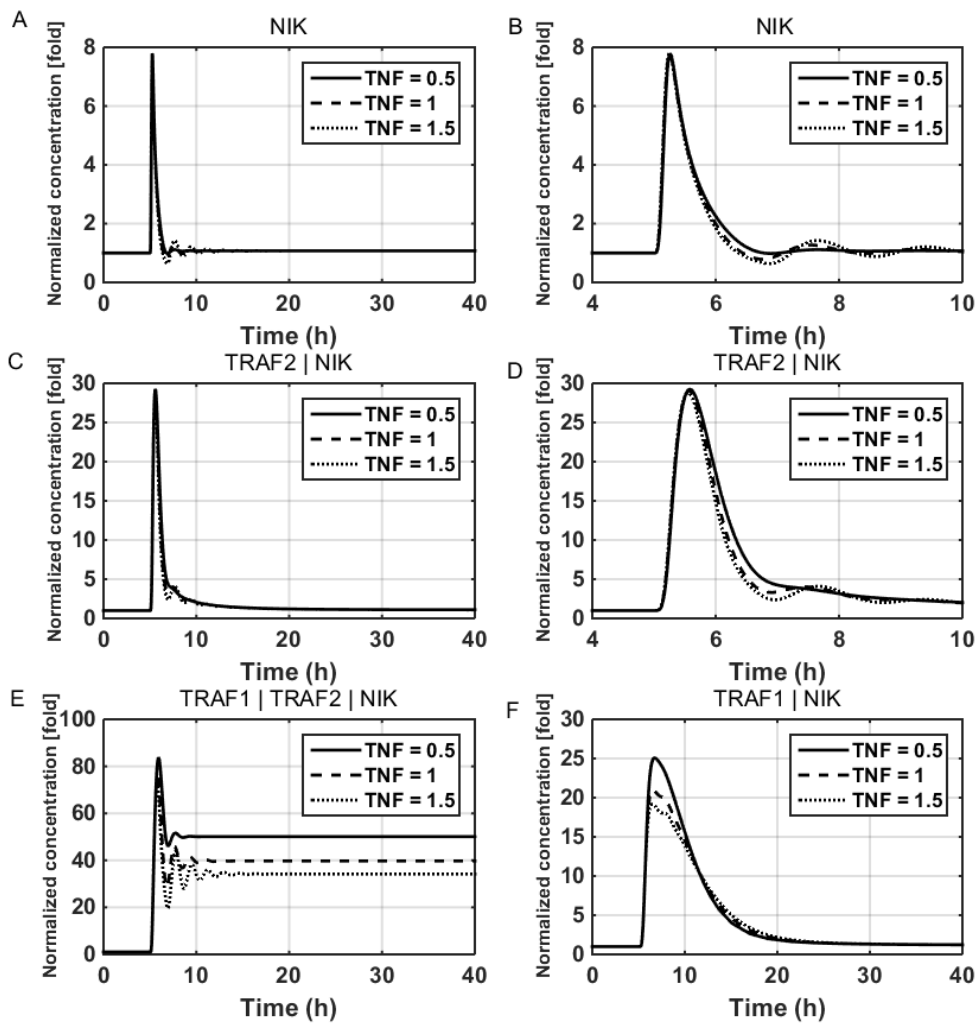
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2 Figure 2: (A) Mimicked changes in the concentrations of TRAF1 mRNA and TRAF2 mRNA with
 3 time. (B) Mimicked changes of the variation in the concentrations of TRAF1 and TRAF2
 4 proteins with time. (C) Mimicked changes of variation in the concentration of NIK with
 5 time. (D) Mimicked changes of variation in the concentrations of TARF2|NIK,
 6 TARF1|NIK and TARF1|TARF2|NIK with time. (E) Mimicked changes of the variation
 7 in the concentrations of p100, p52 and p52n with respect to their initial values with time.
 8 (TNF stimulation starts at 5th hour). (F) Mimicked changes of the variation in the
 9 concentrations of p50n and p52n with time. (TNF stimulation starts at 5th hour).



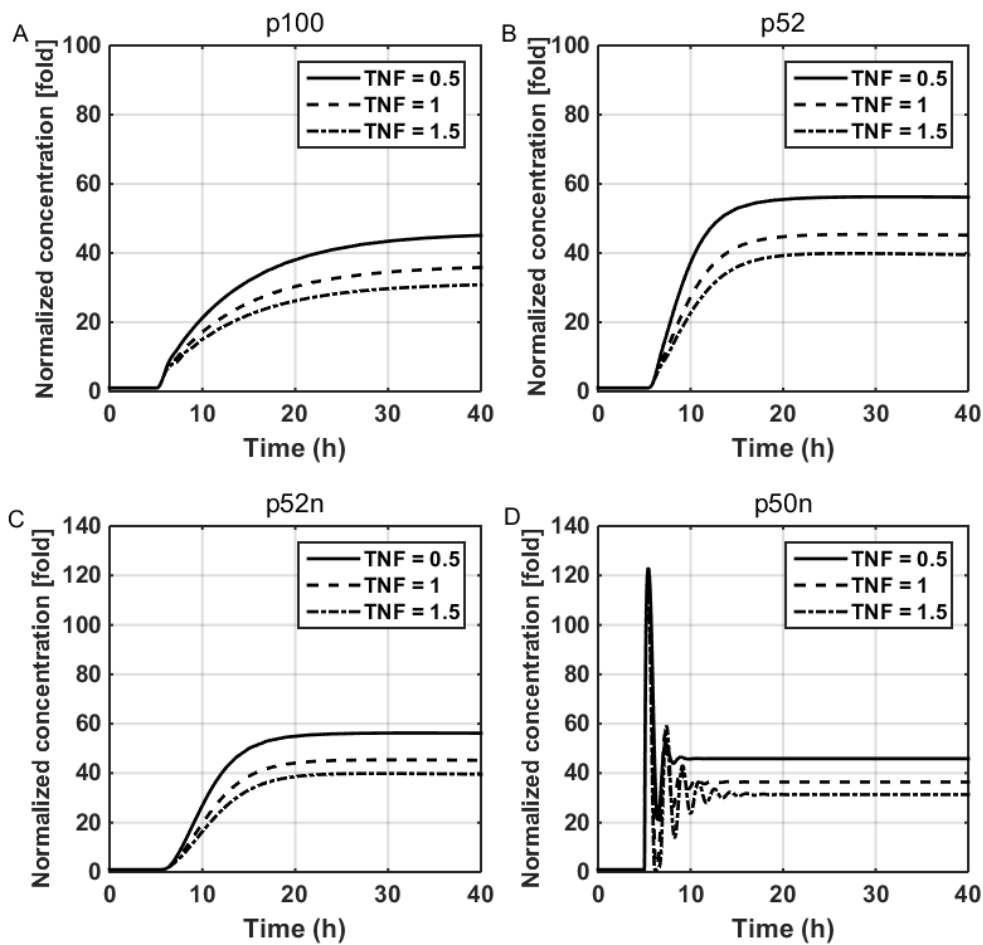
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2 Figure 3: (A) Mimicked changes in the concentration of TRAF1 mRNA with time when TNF
 3 are set as 0.5, 1 and 1.5 μM respectively. (B) Mimicked changes in the concentration
 4 of TRAF2 mRNA when TNF are set as 0.5, 1 and 1.5 μM respectively. (C)
 5 Mimicked changes in the concentration of TRAF1 protein with time when TNF are
 6 set as 0.5, 1 and 1.5 μM respectively. (D) Mimicked changes in the concentration of
 7 TRAF2 protein with time when TNF are set as 0.5, 1 and 1.5 μM respectively. (TNF
 8 stimulation starts at 5th hour).



1

2 Figure 4: (A) Mimicked changes in the concentration of NIK with time when TNF are set as 0.5,
 3 1 and 1.5 μM respectively. (B) Simulation results demonstrated in (A), but with the
 4 short range of the abscissa. (C) Mimicked changes in the concentration of TRAF2|NIK
 5 with time when TNF are set as 0.5, 1 and 1.5 μM respectively. (D) Simulation results
 6 demonstrated in (C), but with the short range of the abscissa. (E) Mimicked changes
 7 in the concentration of TRAF1|TRAF2|NIK with time when TNF are set as 0.5, 1 and
 8 1.5 μM respectively. (F) Mimicked changes in the concentration of TRAF1|NIK
 9 with time when TNF are set as 0.5, 1 and 1.5 μM respectively. (TNF stimulation
 10 starts at 5th hour).



1

2 Figure 5: (A) Mimicked changes in the concentration of p100 with respect to its initial value
 3 with time when TNF are set as 0.5, 1 and 1.5 μM respectively. (B) Mimicked changes
 4 in the concentration of p52 with respect to its initial value with time when TNF are set
 5 as 0.5, 1 and 1.5 μM respectively. (C) Mimicked changes in the concentration of
 6 p52n with respect to its initial value with time when TNF are set as 0.5, 1 and 1.5 μM
 7 respectively. (D) Mimicked changes in the concentration of p50n with time when
 8 TNF are set as 0.5, 1 and 1.5 μM respectively. (TNF stimulation starts at 5th hour).

9

4. DISCUSSION

10 The simulation results in Figure 2A show that the concentrations of TRAF1 mRNA and
 11 TRAF2 mRNA are stable, and stayed at a low level without TNF stimulation during the
 12 first four hours. The amounts of TRAF1 mRNA and TRAF2 mRNA begin to increase,
 13 and finally reach a new stable state after TNF stimulation starting from 5th hour. These
 14 simulation results not only agree with the fact that free p50 released in the canonical

1 pathway enters the nucleus and then promotes the production of TRAF1 mRNA and
2 TRAF2 mRNA, but are also partially validated by experimental findings of Wang et al.
3 [28], where the experimental observations indicate that TRAF1 mRNA and TRAF2
4 mRNA both increase during 4 hours after TNF stimulation (the experiment only
5 provided 4 hours' data). As demonstrated in Figure 2B, the stable states of TRAF1 and
6 TRAF2 are disturbed after TNF stimulation (from 5th hour) with a sharp rise in TRAF2
7 and a gradual rise in TRAF1, which is partially validated by the observations in work
8 of [29] that TRAF1 is upregulated upon TNF stimulation.

9 As shown in Figure 2C and Figure 2D, the stimulation of TNF disturbs the stable
10 state and triggers a rapid growth of concentrations of NIK, TRAF1|NIK complex,
11 TRAF2|NIK complex and TRAF1|TRAF2|NIK complex. These simulations match the
12 corresponding experimental observations. To be specific, experimental findings
13 indicate that the steady level of NIK protein in resting cells is very low [5]. TNF
14 stimulation promotes the synthesis of NIK by 4 fold initially followed a decrease at 2
15 hours [30], broadly confirming a 7.5 fold increase and then a decrease in simulation
16 results. Figure 2D shows that TRAF2|NIK undergoes an initial increase and then begins
17 to decrease, while TRAF1|NIK keeps increasing before 6.45 hours. This agrees with
18 the biological fact that NIK is usually held by TRAF2 in the complex, and the increase
19 of NIK can lead to increase in the amount of TRAF2|NIK complex [4]. TRAF1
20 competes for NIK binding with TRAF2, and consequently TRAF1 produced in the
21 canonical NF- κ B pathway leads to a decrease in TRAF2|NIK followed by TRAF1|NIK
22 as shown by the experimental data [23].

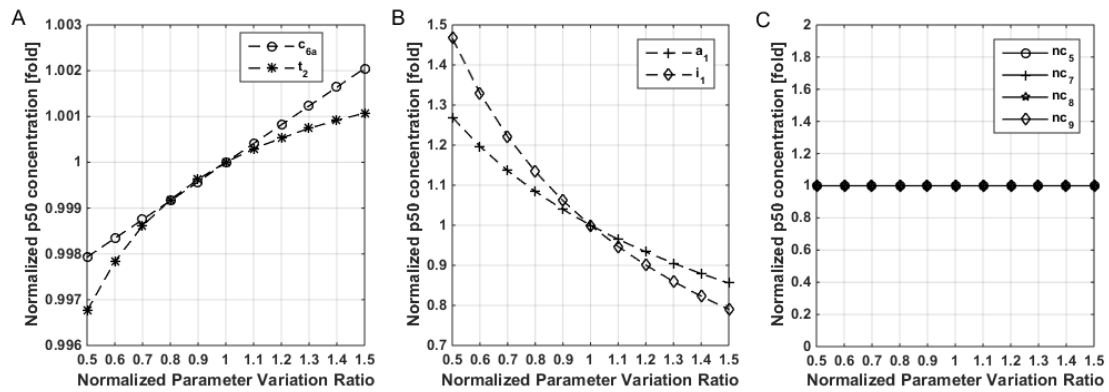
23 The simulation results in Figure 2E demonstrate that p100, p52 and p52n
24 successively increase after TNF stimulation, which agrees with the biological fact that
25 p100 up-regulated by the canonical pathway, is transformed into p52 induced by

1 TRAF1|NIK complexes and then transported into the nucleus [23]. These simulation
2 results are further confirmed by experimental observations that TNF stimulation can
3 lead to the increase of p100, p52 and p52n [23, 31]. It should be noted that the higher
4 level of p100 than p52 cannot be observed directly in Figure 2E, due to concentrations
5 of p100 and p52 normalized by their initial values respectively.

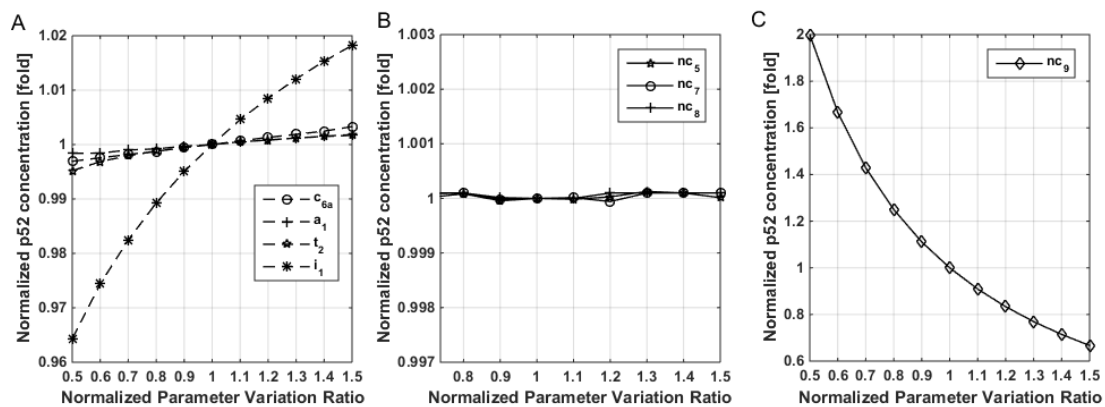
6 As shown in Figure 2F, concentrations of p50n and p52n both initially maintain a
7 stable state, and then undergo an increase due to TNF stimulation. But p50n undergoes
8 an obvious oscillation before achieving a steady state. These model simulations are
9 broadly validated by the experimental observations [6, 23]. In particular, the work of
10 [6] and [23] indicates that p50n increases and achieves a peak value after TNF
11 stimulation at first. Afterwards p50n decreases to a lower value and then switches to
12 increase again. On the other hand, p52n rises and then reaches a steady state, and no
13 obvious oscillations occur. In addition, the model simulations show that p50n increases
14 and reaches a stable state earlier than that of p52n, which agrees with the biological
15 observation that the non-canonical pathway is activated after the canonical NF- κ B
16 pathway [23].

17 Figure 3 to Figure 5 demonstrate influences of TNF variations on the biochemical
18 factors related to the NF- κ B pathway. The increase or decrease in TNF leads to the
19 decrease or increase in concentrations of these biochemical factors. However, effects
20 of TNF changes on NIK, TRAF2|NIK and TRAF1|NIK are relatively smaller than the
21 other factors. The sensitivity study of eight of the key model parameters (including c_{6a} ,
22 a_1 , t_2 , i_1 , nc_5 , nc_7 , nc_8 and nc_9) is performed, aiming to investigate the further information
23 of the NF- κ B pathway. When the values of parameters are changed between 0.5 and 1.5
24 with regard to their base values as defined in Table 2, the influences on steady values
25 (normalized to their corresponding values under the condition that TNF is set as 1 μ M)

1 of p50 and p52 are observed. Figure 6 shows how the variations in eight parameters
 2 affect p50 concentration. As shown in Figure 6, growths of c_{6a} and t_2 both trigger the
 3 increase of p50, whereas the opposite influence is observed with parameters (a_1 and i_1).
 4 In addition, the changes of nc_5 , nc_7 , nc_8 and nc_9 have no effect on p50. Figure 7 indicates
 5 the effect of variations in eight parameters on p52 concentration. It can be seen that the
 6 increases in c_{6a} , a_1 , t_2 and i_1 lead to the increase in p52, while the growth in nc_9 causes
 7 the drop of p52. However the changes of nc_5 , nc_7 and nc_8 have a negligible effect on
 8 p52.



9
 10 Figure 6: (A) The effect of independently varying each model parameter (c_{6a} and t_2) on the steady
 11 concentration of p50. (B) The effect of independently varying each model parameter (a_1
 12 and i_1) on the steady concentration of p50. (C) The effect of independently varying each
 13 model parameter (nc_5 , nc_7 , nc_8 and nc_9) on the steady concentration of p50. p50
 14 concentration is normalized to the value of the base case.



15
 16 Figure 7: (A) The effect of independently varying each model parameter (c_{6a} , a_1 , t_2 and i_1) on the
 17 steady concentration of p52. (B) The effect of independently varying each model
 18 parameter (nc_5 , nc_7 and nc_8) on the steady concentration of p52. (C) The effect of

1 independently varying each model parameter (nc_9) on the steady concentration of p52.
2 p52 concentration is normalized to the value of the base case.

3 NF- κ B can be activated through the canonical and non-canonical pathways. The
4 non-canonical pathway serves as an essential role in many biological processes, and its
5 dysregulation is related to several malignancies. But the non-canonical pathway was
6 usually ignored in the previous models. As discussed above, the model proposed in this
7 paper mimicked the underlying mechanism of the canonical and the non-canonical
8 pathways, as well as the interaction between them. Then such model was utilized to
9 analyze the experimental observations regarding the canonical and non-canonical
10 pathways.

11 5. CONCLUSIONS

12 The non-canonical pathway serving as an important component of the NF- κ B pathway,
13 is usually ignored in previously developed mathematical models. Hence the proposed
14 model in this paper extends the work of Lipniacki et al. [1], which only considered the
15 canonical pathway, by adding another fourteen ODEs to include the non-canonical
16 pathway. It should be noted that the specific factors regulating the synthesis of NIK
17 mRNA are not included in the current model as they are still not completely understood.
18 As knowledge of the NF- κ B pathway grows, the model can be further refined and
19 optimized to include new observations to produce a more complete simulation and more
20 accurate results.

21 The NF- κ B pathway is un-activated and its associated factors remain in a stable
22 state in resting cells. TNF stimulation can activate the NF- κ B pathway and disturb such
23 a stable state with TRAF1 clearly playing a central role in the relation between two
24 pathways. The proposed model not only demonstrates how TNF stimulation triggers
25 the NF- κ B pathway and the resultant variation in its associated biochemical factors, but

1 also investigates the coupling mechanism of two pathways. We believe that this model
2 provides a useful tool to improve our knowledge of the NF- κ B pathway and, in
3 particular, how its different components can be differentially deployed in differing
4 biological contexts. For example, the non-canonical NF- κ B pathway is reported to be
5 involved in enhanced osteoclastogenesis occurred in inflammatory arthritis and
6 metastatic bone cancer [4, 32]. This proposed model can be potentially utilized to
7 investigate the role of the non-canonical pathway in the pathogenesis of such diseases.

8 **ACKNOWLEDGEMENT**

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12 number 201907064]. We declare that we have no competing interests. The MATLAB
13 codes for the mathematical model is included in attachment.

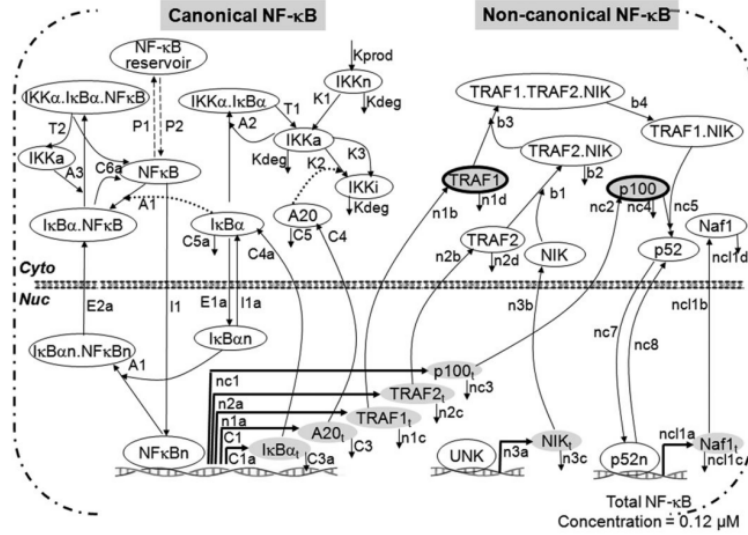
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15 **APPENDIX A**

16 **A1 Apparent inconsistencies in the model developed by Choudhary et al. (2013)**

17 **A1.1 Model equations do not agree with the schematic description of the model**

18 Figure 4A in Choudhary et al. (2013) describe the mechanism of both the canonical
19 pathway and non-canonical pathway coupled by TRAF1 dynamics. However, the
20 model built by Choudhary et al. (2013) does not agree with figure 4A. The details are
21 listed as follows:



1

2 Figure 4A. Schematic description of canonical and non-canonical pathways coupled by TRAF1
3 [23].

$$4 \quad \frac{d}{dt} TRAF1(t) = n_{1b} TRAF1_t(t) - n_{1d} TRAF1(t) \quad (2)$$

5 Figure 4A shows that TRAF1 binding to TRAF2|NIK is involved in the variation
6 of TRAF1, however it is not considered in Equation (2).

$$7 \quad \frac{d}{dt} TRAF2(t) = n_{2b} TRAF2_t(t) - n_{2d} TRAF2(t) \quad (4)$$

8 Figure 4A shows that TRAF2 binding to NIK involves in the variation of TRAF2,
9 however again it is not considered in Equation (4).

$$10 \quad \frac{d}{dt} NIK(t) = n_{3b} NIK_t(t) - b_2 (TRAF2|NIK)(t) + b_4 (TRAF1|NIK)(t) \quad (6)$$

11 Figure 4A shows that the degradation of TRAF2|NIK and the formation of
12 TRAF1|NIK are not involved in the variation of NIK directly. However, it is included
13 in Equation (6). On the other hand, the association of NIK and TRAF2 is related to NIK,
14 but is not considered in Equation (6).

$$15 \quad \frac{d}{dt} (TRAF2|NIK)(t) = b_1 TRAF2(t) NIK(t) - b_2 (TRAF2|NIK)(t) \quad (7)$$

16 Figure 4A shows that TRAF2 | NIK binding to TRAF1 is involved in the variation
17 of TRAF2 | NIK, however it is not considered in Equation (7).

$$\frac{d}{dt}(TRAF1|TRAF2|NIK)(t) = b_3TRAF1(t)(TRAF2|NIK)(t) - b_4(TRAF1|NIK)(t) \quad (8)$$

According to figure 4A, TRAF1|NIK in Equation (8) should be replaced by TRAF1 | TRAF2 |NIK.

$$\frac{d}{dt}p100(t) = nc_2p100_t(t) - nc_4p100(t) \quad (10)$$

Figure 4A shows that p100 can transformed into p52 under the regulation of TRAF1 | NIK, but it is not considered in Equation (10).

$$\frac{d}{dt}p52(t) = nc_5p100(t)(TRAF1|NIK)(t) \quad (11)$$

Figure 4A demonstrates that p52 concentration is influenced by transportation of p52 into and out of the cytoplasm, which is not considered in Equation (11).

In addition, TRAF1 | NIK is involved in Equations (6) and (11). However Choudhary, Kalita et al. (2013) does not define its temporal variation in their model.

A1.2 Model simulation results are inconsistent with model equations

Figure 4B of Choudhary et al. (2013) describes the temporal variation of TRAF1 and TRAF2 after TNF stimulation. However, the simulation results in their figure 4B cannot be produced based on the proposed model of Choudhary et al. (2013). To be specific, as we can see in figure 4B, TRAF1 concentration keeps around zero in the first two hours and then undergoes a rapid decline around the 6th hour. These simulation results do not agree with the model equations.

APPENDIX B

B1 ODEs simulating the canonical NF-κB pathway from Lipniacki et al. (2004)

$$\frac{d}{dt}IKKn(t) = prod - degIKKn(t) - T_{R_1}IKKn(t) \quad (B.1)$$

$$\frac{d}{dt}IKK(t) = T_{R_1}IKKn(t) - {}_3IKK(t) - T_{R_2}IKK(t) \cdot A20(t) - degIKK(t)$$

$$- {}_2IKK(t) \cdot I(t) + t_1(IKK | I)(t)$$

$$\begin{aligned}
& - {}_3IKK(t) \cdot (I|NF)(t) \\
& + t_2(IKK|I|NF)(t)
\end{aligned} \tag{B.2}$$

$$\frac{d}{dt}IKK(t) = {}_3IKK(t) + T_R {}_2IKK(t) \cdot A20(t) - {}_{deg}IKK(t) \tag{B.3}$$

$$\frac{d}{dt}(IKK|I)(t) = {}_2IKK(t) \cdot I(t) - t_1(IKK|I)(t) \tag{B.4}$$

$$\begin{aligned}
\frac{d}{dt}(IKK|I|NF)(t) &= {}_3IKK(t) \cdot (I|NF)(t) \\
& - t_2(IKK|I|NF)(t)
\end{aligned} \tag{B.5}$$

$$\begin{aligned}
\frac{d}{dt}NF(t) &= c_{6a}(I|NF)(t) - {}_1NF(t) \cdot I(t) \\
& + t_2(IKK|I|NF)(t) - {}_1NF(t)
\end{aligned} \tag{B.6}$$

$$\frac{d}{dt}NF_n(t) = {}_1{}_vNF(t) - {}_1I_n(t) \cdot NF_n(t) \tag{B.7}$$

$$\frac{d}{dt}A20(t) = c_4A20_t(t) - c_5A20(t) \tag{B.8}$$

$$\frac{d}{dt}A20_t(t) = c_2 + c_1NF_n(t) - c_3A20_t(t) \tag{B.9}$$

$$\begin{aligned}
\frac{d}{dt}I(t) &= - {}_2IKK(t) \cdot I(t) - {}_1I(t) \cdot NF(t) + c_{4a}I_t(t) \\
& - c_{5a}I(t) - {}_1aI(t) + {}_1aI_n(t)
\end{aligned} \tag{B.10}$$

$$\frac{d}{dt}I_n(t) = - {}_1I_n(t) \cdot NF_n(t) + {}_1a{}_vI(t) - {}_1a{}_vI_n(t) \tag{B.11}$$

$$\frac{d}{dt}I_t(t) = c_{2a} + c_{1a}NF_n(t) - c_{3a}I_t(t) \tag{B.12}$$

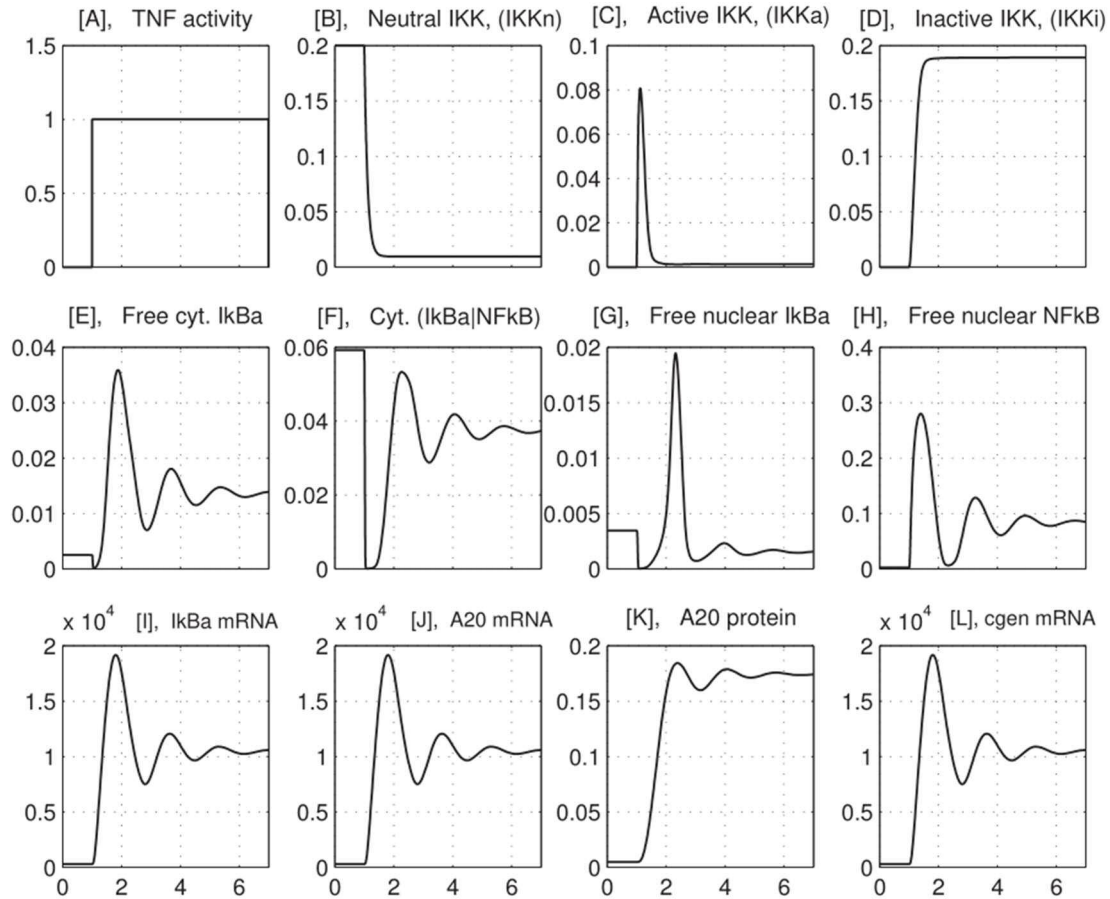
$$\begin{aligned}
\frac{d}{dt}(I|NF)(t) &= {}_1I(t) \cdot NF(t) - c_{6a}(I|NF)(t) \\
& - {}_3IKK(t) \cdot (I|NF)(t) \\
& + {}_2a(I_n|NF_n)(t)
\end{aligned} \tag{B.13}$$

$$\frac{d}{dt}(I_n|NF_n)(t) = {}_1I_n(t) \cdot NF_n(t) - {}_2a{}_v(I_n|NF_n)(t) \tag{B.14}$$

21

22 **B2 Simulation results of the canonical NF-κB pathway from Lipniacki et al. (2004)**

23



1

2 Figure B.1 Numerical Model simulations of the variation in concentrations of biochemical factors
 3 regarding the canonical pathway [1].

4

APPENDIX C

5 C1 Calculation of model parameters based on GA

6 The equations of Genetic Algorithm are described as follows:

$$7 \quad F(\mathbf{X}) = \sum_{i=1:3} b((\mathbf{X})_i - \mathbf{y}_i) \quad (C.1)$$

$$8 \quad = \quad (C.2)$$

9 Where $\mathbf{X} = [n_{1a}, n_{1c}]$ is a vector that consist of two parameters the value is difficult to

10 know in the model equations. $(\mathbf{X})_i (i = 1, 2, 3, \dots)$ presents the model outputs

11 based on the vector \mathbf{X} corresponding to each point in the parameter space. $E_i (i =$

1 1, 2, 3, ...) presents the target outputs that we want to get corresponding to each point
2 in the parameter space. Our purpose is to minimize $F(\)$ which is to make model
3 outputs approach to target outputs as much as possible.

4

5

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1 **FIGURE LEGENDS**

2 Figure 1: The schematic description of two NF- κ B pathways for TNF stimulation. (Note that
3 “d” represents the degradation of biochemical factors).

4 Figure 2: (A) Mimicked changes in the concentrations of TRAF1 mRNA and TRAF2 mRNA
5 with time. (B) Mimicked changes of the variation in the concentrations of TRAF1 and
6 TRAF2 proteins with time. (C) Mimicked changes of variation in the concentration
7 of NIK with time. (D) Mimicked changes of variation in the concentrations of
8 TARF2|NIK, TARF1|NIK and TARF1|TARF2|NIK with time. (E) Mimicked changes
9 of the variation in the concentrations of p100, p52 and p52n with respect to their initial
10 values with time. (F) Mimicked changes of the variation in the concentrations of p50n
11 and p52n with time. (TNF stimulation starts at 5th hour).

12 Figure 3: (A) Mimicked changes in the concentration of TRAF1 mRNA with time when TNF
13 are set as 0.5, 1 and 1.5 μ M respectively. (B) Mimicked changes in the concentration
14 of TRAF2 mRNA when TNF are set as 0.5, 1 and 1.5 μ M respectively. (C)
15 Mimicked changes in the concentration of TRAF1 protein with time when TNF are
16 set as 0.5, 1 and 1.5 μ M respectively. (D) Mimicked changes in the concentration of
17 TRAF2 protein with time when TNF are set as 0.5, 1 and 1.5 μ M respectively. (TNF
18 stimulation starts at 5th hour).

19 Figure 4: (A) Mimicked changes in the concentration of NIK with time when TNF are set as 0.5,
20 1 and 1.5 μ M respectively. (B) Simulation results demonstrated in (A), but with the
21 short range of the abscissa. (C) Mimicked changes in the concentration of TARF2|NIK
22 with time when TNF are set as 0.5, 1 and 1.5 μ M respectively. (D) Simulation results
23 demonstrated in (C), but with the short range of the abscissa. (E) Mimicked changes
24 in the concentration of TRAF1|TARF2|NIK with time when TNF are set as 0.5, 1 and
25 1.5 μ M respectively. (F) Mimicked changes in the concentration of TARF1|NIK
26 with time when TNF are set as 0.5, 1 and 1.5 μ M respectively. (TNF stimulation
27 starts at 5th hour).

28 Figure 5: (A) Mimicked changes in the concentration of p100 with respect to its initial value
29 with time when TNF are set as 0.5, 1 and 1.5 μ M respectively. (B) Mimicked
30 changes in the concentration of p52 with respect to its initial value with time when
31 TNF are set as 0.5, 1 and 1.5 μ M respectively. (C) Mimicked changes in the
32 concentration of p52n with respect to its initial value with time when TNF are set as
33 0.5, 1 and 1.5 μ M respectively. (D) Mimicked changes in the concentration of p50n
34 with time when TNF are set as 0.5, 1 and 1.5 μ M respectively. (TNF stimulation
35 starts at 5th hour).

36 Figure 6: (A) The effect of independently varying each model parameter (c_{6a} and t_2) on the steady
37 concentration of p50. (B) The effect of independently varying each model parameter (a_1
38 and i_1) on the steady concentration of p50. (C) The effect of independently varying each
39 model parameter (nc_5 , nc_7 , nc_8 and nc_9) on the steady concentration of p50. p50
40 concentration is normalized to the value of the base case.

1 Figure 7: (A) The effect of independently varying each model parameter (c_{6a} , a_1 , t_2 and i_1) on the
2 steady concentration of p52. (B) The effect of independently varying each model
3 parameter (nc_5 , nc_7 and nc_8) on the steady concentration of p52. (C) The effect of
4 independently varying each model parameter (nc_9) on the steady concentration of p52.
5 p52 concentration is normalized to the value of the base case.
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7