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Mathematical modeling in understanding NFκB signaling pathway

Huanling Liu

Thesis submitted to the

College of Engineering and Mineral Resources

at West Virginia University

in partial fulfillment of the requirements

for the degree of

Master of Science

in

Engineering

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Morgantown, West Virginia

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ABSTRACT

Mathematical modeling in understanding NF κ B signaling pathway

Huanling Liu

Chronic diseases, cancers and diabetes are associated with dysregulation of many biochemical cues. These biochemical cues are proteins that regulate cellular activity migration and death. The synthesis of these proteins is regulated by nuclear transcription factors. One of the most studied transcription factor is nuclear factor kappa B (NF κ B). Many different proteins have been identified that regulate the activity of NF κ B. Yet, how these proteins regulate NF κ B is still unclear.

Understanding the regulation of NF κ B is important for developing drugs to treat these diseases. Our long term goal is to understand the mechanisms that regulate NF κ B activity. The goal of this research is to identify how NF κ B activity is regulated. As a model system, we will use LPS to stimulate macrophage cells with or without 3, 4-dichloropropionanilide (DCPA) treatment. DCPA is a post-emergent herbicide used for controlling weeds in rice crops. Exposure to DCPA causes increases in liver and spleen weight demonstrated by toxicity study on rats. Previous study in our lab showed that DCPA could modulate NF κ B activity. Our central hypothesis is that a mathematical model can be used to infer the regulation steps that are altered following DCPA treatment. To test our central hypothesis, we performed the following specific aim:

Establish that NF κ B is differentially regulated by I κ B α and I κ B β and that these proteins are in turn differentially regulated by DCPA. Moreover, a mathematical model was used to establish observed dynamics of NF κ B activities. Our working hypothesis is that an ordinary differential equation (ODE)-based model that includes NF κ B regulation by I κ B α proteins can capture the observed dynamics. Furthermore, we used an empirical Bayesian approach to establish confidence in model parameters. Then, we included I κ B β in the model to more realistically describe the regulation of NF κ B activity in macrophages.

We expect that the results of this research will lead to greater understanding of the regulatory mechanism of NF κ B signaling pathway in macrophages and have important implication for human health. This improved understanding may also inspire new ideas to treat these diseases.

DEDICATION

To my husband, Mingyang Gong, and my parents

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CHAPTER 1

LITERATURE REVIEW

1.1 Nuclear factor kappa B

Chronic diseases, cancers and diabetes are associated with dysregulation of many biochemical cues [1, 2]. These biochemical cues are proteins that regulate cellular activity migration and death. The synthesis of these proteins is regulated by nuclear transcription factors. One of the most studied transcription factors is nuclear factor kappa B (NF κ B), which plays an important role in regulating the expression of various inflammatory mediators [3,4,5]. Inflammatory mediators are released by immune cells during times when harmful agents invade our body. Immune cells like macrophages can recognize the bacterial cell wall components, such as lipopolysaccharide (LPS), and secrete inflammatory mediators like TNF α and IL-1. In rheumatoid arthritis patients, inflammatory mediators like TNF α and IL-1 are secreted by primarily macrophages and also upregulated by NF κ B. Then TNF α and IL-1 activate NF κ B in a wide variety of cells to produce more inflammatory mediators inducing cytokines [41]. Roy L and co-workers [42] observed that pro-arthritis mice had significant increase in breast cancer-associated secondary metastasis compared to non-arthritis mice. Increased inflammatory mediators like IL-6, TNF α and IL-17 were measured on arthritis mice and were suggested as the underlying factors that are responsible for the increased metastasis in the arthritis mice. NF κ B was also observed constitutively active in mononuclear cells from patients with type 1 diabetes and some cancers [1, 4]. For inflammation-associated cancers, activity of NF κ B was proposed to enhance tumor development by promoting inflammation. Therefore, understanding the regulatory mechanisms for NF κ B activity is important for treating these diseases [6].

3,4-dichloropropionanilide (DCPA), which is also known as propanil, is a common herbicide for control of weeds on commercial rice crops worldwide. DCPA has been demonstrated suppressing inflammatory mediator TNF α production by macrophage following the LPS stimulation on both mRNA and protein levels [27]. Further study on

the mechanism of DCPA suppressing macrophage functions by Frost and coworkers [29] demonstrated that DCPA treatment decreases NF κ B nuclear localization and DNA binding in IC-21 cells following 10 μ g/ml LPS stimulation. A simple mathematical model was built to display the effect of DCPA on NF κ B activity [28]. DCPA treatment resulted in a potentiation of early LPS-induced NF κ B activation and could be a tool to probe the fundamental aspects of NF κ B signaling. However, the mechanism of DCPA modulating NF κ B is still not clear. Our current research aims to expand this model to incorporate mechanisms by which NF κ B is regulated in macrophages. Furthermore, an empirical Bayesian approach will be used to establish confidence in model parameters and the special aspects affected by DCPA treatment.

1.2 Regulation of NF κ B activity

Many different proteins have been identified having ability to regulate NF κ B activity. Besides those proteins in the signaling process identified as inhibitors of NF κ B, a lot of other compounds like small molecules are found to modulate NF κ B activity [25,26,29]. DCPA has also been shown to alter NF κ B activity [28]. Yet, how these proteins and compounds regulate NF κ B is still unclear [7, 8]. Moreover, the regulatory mechanisms may be different in different cell types. In Figure 1 which is summarized Klinker [28], we can see that NF κ B is activated more rapidly in macrophages than in Fibroblasts following LPS stimulations.[28] The different dynamics of NF κ B activity may be caused by different signaling pathways. Different cells have different functions in the immune system. Macrophages are important and essential to the regulation of immune response and the development of inflammation. Therefore, it is meaningful to figure out the mechanism of NF κ B activity in macrophages.

I κ B proteins is a family of similar proteins, including I κ B α , I κ B β and I κ B ϵ that has been identified as major inhibitors of NF κ B activity in different kinds of cells [5,7]. I κ B α , I κ B β and I κ B ϵ proteins have several structural motifs in common, including six ankyrin repeats and N-terminal regulatory regions (shown as Figure 2). The similarity in structure also correlates to similar functions: they bind to NF κ B, and mask the nuclear localization sequence (NLS) of NF κ B. In addition, phosphorylation of I κ B proteins by I κ B kinase

(IKK) liberates NF κ B [9]. The slight differences in structure lead to differential functions or mechanisms in regulating NF κ B [10, 11, 34]. For instance, different dynamics of NF κ B activity in embryonic fibroblasts were observed in α -/- β -/- cells, α -/- ϵ -/- cells, and β -/- ϵ -/- cells [11]. Mathematical models have been used to explain the discrete functions of I κ B α , I κ B β and I κ B ϵ proteins in regulation NF κ B via negative feedbacks. I κ B α proteins are rapidly synthesized in response to NF κ B and are suggested to provide strong negative feedback leading to oscillations in NF κ B activity. In contrast, I κ B β and I κ B ϵ proteins dampen the long-term oscillatory activity of NF κ B. Sparked by this work, several other models has been built to help understanding the mechanism of different proteins regulating NF κ B activity following extracellular stimulations as reviewed in [12].

Figure 1 The dynamic activation of NF- κ B in macrophages compared against prior studies using fibroblasts. Nuclear NF- κ B was assayed by EMSA at the indicated times after persistent stimulation with 1 μ g/ml LPS in macrophages (in Klinke 2008, $n = 8$, values reported as average $\pm$ 95% confidence interval), 0.5 μ g/ml LPS in fibroblasts demonstrated by Covert et al. (30) ($\circ$), and 0.1 μ g/ml LPS in fibroblasts demonstrated by Werner et al. (46) ($\times$).

Figure 2 The IκB family. Schematic diagram showing different IκB proteins that contain several ankyrin repeats (ANK). Phosphorylation and ubiquitination at specific amino acid residues are indicated. Proline (P), glutamic acid (E), serine (S), and threonine (T) domains are indicated as PEST.

Besides IκBα, IκBβ and IκBε proteins, A20 is suggested as an important inhibitor of NFκB activation by deactivating IKK [13] or by blocking the upstream activator of IKK [14]. Prolonged NFκB activity was observed not only in A20-deficient fibroblasts following TNF stimulation [15] but also in macrophages following LPS stimulation [16]. Persistent phosphorylation of IκBα proteins in A20-deficient cells following the stimulation suggests that prolonged IKK activity results in persistent NFκB activity. Although A20 is essential in regulating NFκB activity following extracellular stimuli, the molecular mechanism of this regulation is still unknown. Ubiquitination (or ubiquitylation) refers to the post-translational modification of a protein by the covalent attachment (via an isopeptide bond) of one or more ubiquitin monomers. A20 has both de-ubiquitination and ubiquitin ligase domains which label proteins for proteasomal degradation. Wert and coworkers established receptor interacting protein (RIP) as an A20 substrate by analyzing possible ubiquitination in the TNF-induced NFκB signaling pathway and found that A20 directly ubiquitinated RIP, which is essential for TNF-induced signaling [17].

The expression of most of these important regulators of NF κ B is also regulated by NF κ B activity [18]. For instance, NF κ B directly promotes I κ B α transcription [19]. NF κ B binding with I κ B α results in a slower bound I κ B α degradation rate compared to free I κ B α [20, 21], but increases phosphorylation of I κ B α by IKK [22]. Thereby, NF κ B is not only responsible for the production but also stability of I κ B α . For I κ B β and I κ B ϵ , less is known about their regulation by NF κ B. Kearns and coworkers studied the gene expression of I κ B α , I κ B β and I κ B ϵ in wild-type murine embryonic fibroblasts (MEFs) and NF κ B-deficient MEFs following TNF stimulation [23]. Gene expression of I κ B α , I κ B β and I κ B ϵ was observed increasing in wild-type cells following the stimulation, but not in NF κ B-deficient cells. Therefore, NF κ B is essential for all of I κ B α , I κ B β and I κ B ϵ gene expression in MEFs following TNF stimulation. However, the dynamics of expression in response to NF κ B for these species are different. In particular, I κ B β and I κ B ϵ had a 45 minutes transcription delay comparing to I κ B α in wild-type MEFs following TNF stimulation [23]. Although some intermediate transcription factors, like Foxj1, which was demonstrated to have the ability to regulate I κ B β by Lin [24], may cause the time delay, the mechanisms of these different promotions by NF κ B are still unknown. Similar to I κ B α , rapid A20 gene expression was observed following NF κ B activation in wild-type MEFs and also in bone marrow-derived macrophages (BMDMs) [15, 16]. NF κ B controls gene expression of its inhibitors which forms auto-regulatory feedback loops to terminate the NF κ B response.

As illustrated by the regulation of A20 and I κ Bs on NF κ B, these auto-feedback loops play different roles in regulating NF κ B dynamics. Given the reciprocal nature of NF κ B/I κ B regulation, mathematical models integrated with biochemical studies have been an instrumental tool to understand the regulation of NF κ B [31]. Most of the models focused on TNF-induced or LPS-induced NF κ B activity in MEFs [13, 23, 30]. Biological events were assembled into a biochemical reaction network and modeled using non-linear ordinary differential equations. Parameters were refit to reproduce experimental data under different conditions and assumptions [12], as shown in Figure 3. Figure 3-A reproduced NF κ B activity measured in wild-type MEFs following during persistent 6 h-

long TNF stimulation, while Figure 3-C reproduced that after 15 min-long TNF stimulation. Through this mathematical model which could reproduce some

Figure 3 Schematic depiction of Lipniack's model (B) and Model predictions versus Hoffmann et al. (2002) measurements on wild-type cells (down) (A) NF-kB during persistent 6 h-long TNF stimulation [13]; (C) NF-kB at and after 15 min-long TNF stimulation.

experimental data measured under different conditions, the mechanism of NFκB regulation by A20 and IκBα was proposed that A20 regulates NFκB activity through inhibiting IKK activity while IκBα through binding with NFκB and keeping it in the cytoplasm. Through mathematical modeling, the distinct function of other inhibitors in controlling NFκB activity was also demonstrated [23, 32].

1.3 Mathematical modeling

Mathematical modeling has been demonstrated as an instrumental tool in understanding signaling mechanisms in biological system [11-13]. Common modeling approaches give the maximum likelihood estimate of some unknown parameters by comparing the simulation results and experimental data[11,13, 45]. However, in biological systems, a signaling network involves tens of or even hundreds of biological events like protein-protein interaction, mRNA transcription and translation. Not all of these events are currently measurable. Most of the time, only some of the parameters associated with certain events can be measured. For other parameters, a slight change in value may not influence the production or affect system results in ways that are difficult to separate from other parameters. These parameters are called unidentifiable or inestimable. Like experimental studies, we need to establish a level of confidence associate with how well the mathematical model describes a system.

1.3.1 Values for Parameter Identification

Parameters associated with biological events can be measured directly by experiment or estimated by analysis data using a mathematical method. For parameters that can't be measured currently, *A priori* identifiability approach was developed by Jacquez [52]. *A priori* identifiability is used to check whether the values of the parameters at a point in parameter space can be estimated independently for models described by systems of ordinary differential equations. Klinke [28] used the approach to demonstrate that estimates of the strength parameter of effect DCPA on NFκB activation could be uniquely determined from the data. The sensitivity function is defined as

$$S_{ij}(t) = (\partial y_j(t) / \partial k_i) \cdot k_i / \text{MAX}(y_j(t)) \quad (1)$$

where y_j represents model variables, k_i denotes model parameters around a local optimum, and the partial derivative values are scaled by the parameter value and the maximum value of the model variable during the simulation. The sensitivity function is practically approximated by obtaining the sensitivity measure S_{ij} at a set of discrete time points, t_k , and experimental conditions, $[Cn]_n$. Then a reduced sensitivity matrix (M) is constructed as

$$M = [S_{ij}(t_1, Cn_0), \dots, S_{ij}(t_k, Cn_0), \dots, S_{ij}(t_1, Cn_n), \dots, S_{ij}(t_k, Cn_n)]^T \quad (2)$$

A set of correlation coefficients between model parameters is calculated from M. Parameters that are locally identifiable have correlations with all other parameters between in practical -0.99 and +0.99. Parameters that are not locally identifiable, termed a *proiori* unidentifiable, have correlations of greater than 0.95 and less than -0.95 with at least one other parameter.

1.3.2 Simulated Annealing

Simulated Annealing (SA) is a random searching technique, developed in 1983 to deal with highly nonlinear problems. The principle and advantages of SA was reviewed by Busetti[56]. SA exploits an analogy between the way in which a metal cools and freezes into a minimum energy crystalline structure (the annealing process) and the search for a minimum in a more general system. The principle of SA approaching the global maximization is similarly to using a bouncing ball which can bounce over mountains from valley to valley. At the beginning, it starts from a “high temperature” bounce with high energy to access any valley, and then finally get into a small range of valleys. The SA method needs a generating distribution that generates possible valleys or states to be explored and an acceptance distribution which depends on the difference between the function value of the present generated valley to be explored and the last saved lowest valley. The acceptance distribution decides probabilistically whether to stay in a new lower valley or to bounce out of it. All generating and acceptance distributions depend on the temperature. The structure of the simulated annealing algorithm is shown as Figure 4.

Figure 4 The structure of the simulated annealing algorithm.[56]

The major advantage SA over other methods is an ability to avoid becoming trapped in local minima. It is flexible and able to approach global optimality. Carefully controlling the rate of cooling of the temperature, SA can find the global optimum. However, this

requires infinite time. Also, the evaluation of the problem functions is essentially a “black box” operation as far as the optimization algorithm is concerned, because the SA algorithm does not require or deduce derivative information. However, for many applications, the computational efficiency is important. The standard implementation of SA algorithm is one in which a collection Markov chain of finite length are generated at decreasing temperatures.

1.3.3 Markov Chain Monte Carlo Methods

A Markov chain is a mathematical tool for statistical modeling in modern applied mathematics. In a Markov chain, the next state depends only on the current state, with the state changing randomly between steps. After sufficient amount of steps, the chain may reach a stationary distribution where the probability values are independent of the actual starting value. Since the system changes randomly, it is generally impossible to predict the exact state of the system in the future. However, the statistical properties of the system’s future can be predicted. In many applications, these statistical properties are important.

Markov chain Monte Carlo (MCMC) methods are a class of algorithms for empirically creating a probability distribution by constructing a Markov chain that has the desired distribution as its equilibrium distribution. MCMC techniques provide random walks in parameter space whereby successive steps are weighted by the likelihood of observing data given the corresponding parameter values. They are widely used in the field of Bayesian statistics where they provide an attractive option for assessing the uncertainty in the model parameters given the calibration data.[51,53] One of the big challenge in the application of Bayesian approach to more realistic problems, such as modeling biological system networks, is computational efficiency. The computational efficiency of a MCMC algorithm depends highly on the structure of the proposal distribution. One recent advance in MCMC algorithms has been to improve the computational efficiency by dynamically adjusting the proposal distribution from a non-informative prior distribution at the start of the simulation to a proposal distribution that reflects the structure in the cumulative Markov chain. These are called Adaptive MCMC methods. Klinke developed

and applied an empirical Bayesian approach to establish the confidence that one particular mathematical model can describe signal transduction mechanisms in biological signaling networks, given the available data [37]. In the study, an Adaptive Markov Chain Monte Carlo (MCMC) techniques was used to assess the uncertainty in the model parameters given the calibration data for the mathematical model by providing random walks in parameter space whereby successive steps are weighted by the likelihood of observing data given the corresponding parameter values. Bayesian approach has been used to infer confidence of models for transcription factor activity and cellular signaling networks. Monte Carlo integration was used to approximate posterior distribution required for a Bayesian analysis. A Markov chain was generated, using the previous sample values to randomly generate the next sample. Metropolis-Hasting algorithm can be used to generate a Markov chain by random sampling. Typically the first 100000 to 500000 elements are in the burn-in or “learning” period. A poor choice of starting values can greatly increase the required burn-in time. The values obtained by simulated annealing were used as a starting point to generate Markov chains.

Sequential steps in a generated Markov Chain exhibit autocorrelation. To minimize the effect of autocorrelation, a technique called “thinning” was used by selecting values from the Markov Chain at every n th iteration [37]. Recursive calculation of the proposal covariance of the Markov Chain during the MCMC run is improved by thinning. The expected value for some property of a model can be calculated from the following integral:

$$E[f(\Theta)|M, Y] = \int_{\Theta_{Min}}^{\Theta_{Max}} f(\Theta) \cdot P(\Theta|M, Y) d\Theta, \quad (3)$$

where $f(\Theta)$ is a generic function of the model parameters. In this case $f(\Theta)$ is a deterministic function that provides a prediction of the dynamic trajectory of the system in response to a stimuli, given a set of parameter values. This equation used in conjunction with the posterior distribution in the model parameter provides an estimate of the uncertainty in the model prediction, given a model of particular calibration data.

The expected value is dependent on the particular formulation of the model, M , and the data used in calibrating the model, Y . As not all combinations of parameters provide realistic simulations, values for $f(\Theta)$ are weighted by distribution of parameters given M and Y (i.e., the posterior distribution $P(\Theta|M, Y)$). A Bayesian estimate of $P(\Theta|M, Y)$ could be provided by computer-intensive methods like Monte Carlo algorithms. Markov chain represents a random walk within parameter space. Recently developed AMCMC can dynamically adjust the structure of the proposal distribution based upon the prior steps of an evolving Markov chain. The prior distribution used in this study was the same for all parameters, proper, normally distributed, and used to specify the initial proposal distribution. Following a specified “learning” period, the proposal distribution was adjusted to reflect the structure in the cumulative Markov chain.

However, deciding when the cumulative Markov chain is a representative sample drawn from the underlying stationary distribution is still a big challenge with implementing a MCMC approach for Bayesian inference. Convergence is a criteria used to evaluate how long of a chain is necessary to traverse a representative sample of parameter space. Most of algorithms developed diagnose the convergence of a Markov chain by focusing on the model parameters. Klinke instead focused on the the predictions of the model to assess convergence of Markov chains using Gelman-Rubin method. The Gelman-Rubin method is based upon the concept that convergence has been achieved when the variance among chains is less than within single chains.

CHAPTER 2

RESEARCH METHODS

2.1 Experimental Aspects

All biological experiments were done by Irina V. Ustyugova who was a graduate student in John Barnet's lab, Department of Microbiology, Immunology & Cell Biology, West Virginia University, Morgantown, WV, USA

Cell culture, stimulation and DCPA treatment The murine peritoneal macrophage cell line, IC-21, was cultured to 80% confluency in complete RPMI (cRPMI) Cells were treated with 99% pure DCPA and simultaneously stimulated with 1 μ g/ml LPS phenol extracted for various times. 100 μ M of DCPA were dissolved in 100% ethanol and added to cells. The final ethanol concentration added to all cultures was 0.1%; control cultures received equal concentrations of ethanol.

Nuclear Extracts

Cells were then treated with either 0.1% ethanol or 100 μ M DCPA, then stimulated with 1 μ g/ml LPS. Cells were washed. A total protein concentration for nuclear extracts was determined with Coomassie Plus Protein Assay Reagent, as described by manufacturer (Pierce, Rockford, IL).

Electrophoretic mobility shift assay (EMSA)

NF- κ B consensus oligonucleotides were labeled with γ -³²P-ATP using Ready-To-Go™ T4 polynucleotide kinase kit. Nuclear extracts, 5 μ g per sample, were incubated with 50,000 cpm of labeled probe and 1 μ g/ml dI:dC to allow formation of band shift complexes and electrophoresed. The EMSA experimental results are reported as the ratio of the intensity measured for a particular condition (i.e. time > 0) relative to the intensity of nuclear NF- κ B prior to treatment (i.e. time = 0).

Western Blotting of I κ B α and I κ B β

After the nuclear extracts, IC-21 cells were treated as indicated, collected by centrifugation, and washed two times with cold phosphate-buffered saline. Western blot analysis was performed using the indicated antibodies of I κ B α and I κ B β .

2.2 Mathematical Modeling Aspects

The application of mathematical models to describe NF κ B-I κ Bs signaling mechanism was pioneered by Hoffmann A [11], and extended by many others [13, 23]. This model builds upon prior modeling studies by Lipniacki [13] but incorporates two key changes. First, the reactions were grouped into reaction classes that are defined based upon peptide motif-motif interactions [35]. Equal parameters are assigned to reactions in the same class. Additional effect factors to some complexes reactions in the presence of NF κ B are considered, according published experimental data [36]. Second, dynamic equilibrium is considered between protein-protein interactions. NF κ B is considered as a single protein. The interaction between IKK and I κ B α as well as I κ B α NF κ B complex in the cytoplasm effectively proceeded as a single enzymatic degradation reaction scheme:

where E is the enzyme, IKK α (IKK in active status), S is the substrate (I κ B α or I κ B α NF κ B) and ES is the enzyme-substrate complex (IKK α I κ B α or IKK α I κ B α NF κ B complex). Three parameters are used to describe this process, including association rate, a_2 , dissociation rate, d_2 and catalytic rate, k_{cat} . After this enzymatic process, IKK α is regained but I κ B α is lost while NF κ B is released. The interaction between I κ B α and NF κ B also consists in the forward and reverse reactions with rate a_1 and d_1 . Dynamics of IKK is represented as a module, where details of upstream signaling interaction are not represented in detail. The description of all reactions is shown in supplement material 1.

2.3 Model Calibration

The reactions between all components associated with parameters were converted to a set of non-linear ordinary differential equations. For the values of the parameters, some of

them are from literature published before, while a subset of parameters will be determined from the experimental data. As many of the parameters are correlated, a parameter identifiability analysis described by Klinker [28] was used to establish the parameters which can be uniquely determined, given unlimited information about the model. Parameters that are not locally identifiable, termed *a priori* unidentifiable, have correlation values less than -0.985 or greater than 0.985 with at least one other parameter.

Two models were built. Model 1 was built to describe the system by fitting experimental data from $\text{I}\kappa\text{B}\alpha$ and $\text{NF}\kappa\text{B}$ measured in IC-21 cells with and without DCPA treatment following LPS stimulation. To further study the signaling mechanism, model 2 was built to include also fitting $\text{I}\kappa\text{B}\beta$ experimental data. Initial concentrations of the components are needed to simulate the response. The initial concentration of $\text{NF}\kappa\text{B}$ is assumed as $0.06\text{ }\mu\text{M}$ in the cytoplasm [36]. All of $\text{NF}\kappa\text{B}$ is assumed to exist as $\text{I}\kappa\text{B}\alpha\text{NF}\kappa\text{B}$ for model 1 in the cytoplasm. For model 2, the initial concentration of $\text{NF}\kappa\text{B}$ is assumed as $0.03\text{ }\mu\text{M}$ $\text{I}\kappa\text{B}\alpha\text{NF}\kappa\text{B}$ and $0.03\text{ }\mu\text{M}$ $\text{I}\kappa\text{B}\beta\text{NF}\kappa\text{B}$ in the cytoplasm; the initial concentration of $\text{NF}\chi$ is assumed as $0.02\text{ }\mu\text{M}$ in the cytoplasm. All other components are assumed as zero. A 2000 minute period is simulated to get the equilibrium concentrations of all the variables before the stimulation and DCPA treatment.

Unknown parameters obtained from the first model were used as a starting point to simulate the second model. Experimental data of $\text{NF}\kappa\text{B}$ in the nucleus, $\text{I}\kappa\text{B}\alpha$ in the cytoplasm and $\text{I}\kappa\text{B}\beta$ in the cytoplasm, measured previously in our lab, were used to calibrate the model. More unknown parameters were identified by the simulated annealing program. Simulated annealing is a robust and general technique to deal with highly nonlinear models. The greatest advantage of this technique is the ability to approach global optimality. The model equations are encoded and evaluated in MATLAB V7.1 (The MathWorks, Natick, MA). Summed squared error between experimental data measured previously in our lab and simulated measurements was used to determine goodness-of-fit. The optimum values obtained from the simulated annealing were used as a starting point for the Markov Chain [37].

2.4 Bayesian Approach

After parameter identifiability analysis, Simulated Annealing was used to find the optimum fitness of the given experimental data for of NF κ B in the nucleus and I κ B α in the cytoplasm. Metropolis-Hasting algorithm was used to generate possible states to be explored in the parameter space. An acceptance distribution depends on the difference between the function value of the present state to be explored and the last saved lowest state. The acceptance distribution decides probability whether to stay in a new state or to bounce out of it. The Simulated annealing program was used to produce the initial values for the AMCMC algorithm, shown as Figure 5.

Using the results from Simulated Annealing program as the start point to generate a Markov chain by An empirical Bayesian approach using Adaptive Markov Chain Monte Carlo (AMCMC) algorithms, as described by Klinker [37]. The generated Chain by Metropolis-Hasting algorithm is autocorrelative. To minimize the effect of autocorrelation, a technique called “thinning” was used by selecting values from the Markov Chain at every n th iteration. Recursive calculation of the proposal covariance of the Markov Chain during the MCMC run was improved by thinning. The thinning value used to estimate the covariance recursively from the evolving Markov chain is 40. To obtain $P(\Theta|Y,M)$ from the final Markov chains, a thinning value of 20 was used.

The prior distribution used in this study was the same for all parameters, proper, normally distributed, and used to specify the initial proposal distribution. Following a specified “learning” period, the proposal distribution was adjusted to reflect the structure in the cumulative Markov chain. How a Monte Carlo Markov chain is generated is as same as description by Klinker [37]. The Gelman-Rubin method is then used to analyze the convergence of the model using two parallel chains. The first N steps, called “learning period” or “burn-in period”, are discarded as they are assumed to be drawn from tails of the stationary distributions. The remainder of the parallel chains was used to estimate the convergence of the predictions to a stationary distribution. Two parallel Markov chains were calculated each containing 900,000 steps. The simulation of each chain took

approximately 500 hours on a single core of a 2.66 GHz Dual-Core Intel Xeon 64-bit processor with 8 GB RAM.

Figure 5 The AMCMC algorithm structure for our model

The parameter values obtained using simulated annealing provided the starting point for these chains. A “learning” (a.k.a. “burn-in”) period of 250,000 steps was specified a *priori* to provide an initial estimate for the proposal covariance.

To estimate convergence, the prediction PY_{ij} obtained from a single draw from J parallel MCMC samples of length N , where $j \in N$ and $i \in N$. The overall variance of prediction, $\text{Var}(PY)$, derived from the target distribution is estimated from the between-sequence (B) and the within-sequence (W) variances. The Gelman-Rubin Method diagnoses convergence from the potential scale reduction factor is calculated as follows:

$$R = \text{Var}(PY) / W$$

where parallel chains of length N should be increased until R is less than 1.2.

CHAPTER 3

RESULTS AND DISCUSSION

3.1 Analysis of the experimental data

Experimental results (measured previously in our lab by Irina V. Ustyugova)

Figure 6 Initial experimental results. (A) NFκB in the nucleus measured by Electrophoretic mobility Shift Assay (EMSA); (B) IκBα, IκBβ and β-actin which was used to normalize IκBα and IκBβ, in the cytoplasm measured by western blotting. E represents ethanol treatment while D represents DCPA treatment.

The changes of NFκB concentration in the nucleus of IC-21 macrophages were measured following LPS stimulation or LPS stimulation and DCPA treatment for period of 6 hours using electrophoretic mobility shift assay, shown as in Figure 6 (A). The

concentration change of I κ B α and I κ B β proteins in the cytoplasm of IC-21 cells were also measured by western blot, shown as B in Figure 6. Ethanol treatment following LPS stimulation was used as control experiments. 100 μ L DCPA was used to test the effect of DCPA on NF κ B activity in macrophages.

Differential dynamics of I κ B α and I κ B β were observed following LPS stimulation for both ethanol and DCPA treated IC-21 cells. From Figure 6, we can see that following LPS stimulation with or without DCPA treatment (both of LPS and DCPA or ethanol were added at time 0), I κ B α (middle band) concentration decreased and then rebounded more rapid than I κ B β (top band). All these experimental data are numerically summarized as Figure 7. From Figure 7, we can see clearly that the increase of free nuclear NF κ B concentration starts after 5 minutes, which is following the decrease in I κ B α . The cytoplasmic I κ B α reaches the lowest concentration earlier than free nuclear NF κ B reaches its highest concentration. Following free nuclear NF κ B, which is thought to be the active form with the ability to promote the productions of other proteins, cytoplasmic I κ B α concentration recovers to a level close to that before the LPS stimulation. Following the resynthesis of cytoplasmic I κ B α , the active NF κ B concentration decreases. Whether the cells were treated with or without DCPA, the nuclear NF κ B concentration shows oscillation and reaches a high level of constant activity after about 3 hours. This higher level of NF κ B activity is about 8 times as that before the stimulation. This oscillation behavior of nuclear NF κ B was also observed by other groups in other kind of cells.[10,11,15,18] Hoffman's study demonstrates that, in fibroblasts, the negative feedback effect of I κ B α on the NF κ B activity signaling network cause the oscillation while I κ B β and I κ B ϵ dampen the oscillations and stabilize NF κ B during longer stimulations.[11]

We also measured cytoplasmic I κ B β concentration following LPS stimulation in IC-21 cells shown in Figure 7 C. Unlike I κ B α , the decrease of I κ B β concentration following LPS stimulation is much slower. After about 90 minutes following the stimulation, the concentration of I κ B β begins to rise, at a rate that is much slower than the rising of I κ B α concentration. I κ B β concentration keeps rising to a level higher than that before LPS

Figure 7 Summary of experimental data and normalized by the concentration before LPS stimulation (A) Nuclear NFκB (NFκBn); (B) Cytoplasm IκBα; (C) Cytoplasm IκBβ. Red squares represent experimental results with DCPA treatment while black dots represent ethanol treatment.

stimulation, while I κ B α and the active NF κ B concentration oscillate slightly around the constant level. Active NF κ B in the nucleus induces I κ B α and I κ B β transcription. Re-synthesized I κ B α proteins bind to free NF κ B in the cytoplasm to keep it in the cytoplasm. Re-synthesized I κ B α proteins can also go to the nucleus to export free NF κ B to the cytoplasm.[11,19] The negative feedback effect of I κ B α causes the oscillation of NF κ B activity. I κ B α and I κ B β have distinct functions in regulating NF κ B activity. [10] However, the mechanisms of I κ B α and I κ B β regulation and how they regulate NF κ B activity are still not clear.

The different dynamic profiles of I κ B α and I κ B β following an external stimulation were also observed by other groups.[10,11,23] According to Kearns's study,[23] NF κ B is essential both for I κ B α and I κ B β mRNA transcription. In the wild-type cells, the I κ B β mRNA level remains constant for about 45 minutes after chronic TNF α stimulation, while I κ B α mRNA level rises immediately. This is consistent with our observations on I κ B β protein level in the cytoplasm. We observed a 60 minute delay in resynthesis compared with I κ B α following the LPS stimulation.

The profile of nuclear NF κ B in the cells exposed to DCPA looks similar but a little ahead of time compared to cells without DCPA treatment. In [28], it was demonstrated that DCPA has the ability to potentiate an early NF κ B activity. Moreover, Klinke and coworkers demonstrate that an EMSA assay lacks the sensitivity to detect change in early NF κ B activity. However, how the DCPA affects NF κ B activity is still unknown.[27] Not surprisingly our EMSA results suggest that free NF κ B concentration was not significantly decreased by DCPA treatment following LPS stimulation. As shown in Figure 7 B, I κ B α concentration decreased quickly following the stimulation and increased immediately after NF κ B activated. Differences in I κ B α expression upon DCPA treatment do not become apparent until 3 hours following LPS stimulation. But in the first three hours of the LPS stimulation and DCPA treatment, the decrease is not that obvious. Compared with I κ B α , I κ B β concentration decreased relatively slowly and following a delay, increased more slowly, shown as Figure 7 C. After 6 hours' stimulation, I κ B β concentration in the cytoplasm increased to a level greater than before the stimulation. The dynamics profile for I κ B β is different from the I κ B α dynamics profile, whereby a 60

minute delay was observed in cytoplasmic I κ B β protein level following NF κ B activity. From the western blot image, two form of I κ B β with different dynamics can be recognized, as shown in Figure 6 B, but are summed together as the total cytoplasmic I κ B β . Furthermore, DCPA treatment also decreased cytoplasmic I κ B β concentration following LPS stimulation. The significant decrease by DCPA treatment started from 90 minutes, a little earlier than I κ B α . We also noticed that the resynthesis of cytoplasmic I κ B β concentration starts at 90 minutes following the LPS stimulation with or without DCPA treatment and is about 60 minutes later than cytoplasmic I κ B α concentration resynthesis. The slower decrease and delayed resynthesis of cytoplasmic I κ B β suggest that the contribution of this negative feedback mechanism is not a major contributor to the observed dynamics of NF κ B activation during the first 90 minutes following LPS stimulation. In the first 90 minutes, I κ B α appeared to be more important in regulating NF κ B activity than I κ B β at the beginning. Therefore, in IC-21 cells, I κ B α protein is a key early regulator of NF κ B activity. However, the mechanism of how I κ B α regulating NF κ B is still unclear. To help understand the process, we developed a mathematical model to describe the first 90 minutes following extracellular stimulation. I κ B α is more important as a negative feedback to regulate NF κ B activity at the beginning period following LPS stimulation. Therefore, our initial effort to expand the mathematical model of NF κ B activity in IC-21 cells included the contribution of I κ B α alone. Using this model we focused on the dynamics of NF κ B and I κ B α in the first 90 minutes with and without DCPA treatment and used the model to predict the rest of the stimulation time (Model 1). I κ B α was used to represent the functions of inhibition of NF κ B activity. We also compared the difference of the two sets of parameter values that fit experimental data of NF κ B and I κ B α to identify difference induced by DCPA treatment. Finally we extended the model to incorporate both I κ B α and I κ B β and their distinct functions and dynamics. As suggested experimentally, I κ B β starts to play an important role on regulating NF κ B activity after 90 minutes.

3.2 Model topology

3.2.1 Mathematical Models development

Following from experimental observation, the $\text{IkB}\beta$ resynthesis occurred after 90 minutes following LPS stimulation; therefore, we built two models.

Model 1

The first model was developed based on the simple model published by Klinke[28], but more proteins and mRNAs were added to describe the signaling network of NF κ B activity. In model 1, we focused on the first 90 minutes following LPS stimulation and postulate that $\text{IkB}\alpha$ represents the major regulator of IkB proteins at this period; for model 2, we included both $\text{IkB}\beta$ and $\text{IkB}\alpha$ as regulatory elements for NF κ B. The schematic diagram of model 1 is shown in Figure 8. Activation of NF κ B following LPS stimulation of the cells is depicted as a binary on/off stimulus. Before the stimulation, the activity of TLR4 is set to zero (off). During the whole LPS simulation period, TLR4 is kept as 1. Following the stimulation, LPS binding to TLR4 activates the signaling network, transferring IKK from a neutral form, IKK_n, to the active form, IKK_a. Active IKK phosphorylates free $\text{IkB}\alpha$, bound $\text{IkB}\alpha$, and $\text{IkB}\beta$. The presence of NF κ B makes the phosphorylation more efficient.[21] Free NF κ B dynamically shuttles between the cytoplasm and the nucleus; within the nucleus, free NF κ B binds to DNA, promoting the transcription and translation of numerous proteins including $\text{IkB}\alpha$, $\text{IkB}\beta$ and A20. Newly synthesized $\text{IkB}\alpha$ goes to the cytoplasm, binding to NF κ B to prevent its nuclear localization or shuttles to the nucleus to inhibit its binding to DNA and exporting it out of the nucleus. Newly synthesized A20 inhibits IKK activity.

The first model consists of 14 components, as listed in Table A.1 in APPENDIX: three status of IKK including IKK_n(neutral), IKK_a(active), IKK_i(inactive), NF κ B, $\text{IkB}\alpha$, A20 and their complexes, IKK_a $\text{IkB}\alpha$, $\text{IkB}\alpha$ NF κ B, IKK_a $\text{IkB}\alpha$ NF κ B, $\text{IkB}\alpha$ _n, $\text{IkB}\alpha$ _nNF κ B_n and NF κ B_n, (n means in the nucleus), and the following mRNAs: $\text{IkB}\alpha$ _m and A20_m(m denotes mRNA). IKK_n(neutral) can be activated to IKK_a(active), or deactivated to IKK_i(inactive), which are not reversible. Biological events in this model are converted to

25 groups of reactions listed in Table A.2 in APPENDIX; and concentration changes of these components are calculated using differential equations as shown in Table A.3 in the APPENDIX.

Model 2

Based on the first model, the second model including the regulation of $\text{I}\kappa\text{B}\beta$ was built to capture experimental data in the whole stimulation period. Since less is known about $\text{I}\kappa\text{B}\beta$, most of its properties are assumed based on $\text{I}\kappa\text{B}\alpha$. But one major difference with $\text{I}\kappa\text{B}\alpha$ is proposed. The delay in $\text{I}\kappa\text{B}\beta$ mRNA synthesis was due to an unknown intermediate transcription factor (NFX), whose synthesis was dependent on $\text{NF}\kappa\text{B}$ activation. Therefore, we assume that NFX can shuttle between the nucleus and the cytoplasm. NFX is $\text{NF}\kappa\text{B}$ inducible and promotes the transcription and translation of $\text{I}\kappa\text{B}\beta$ responsible. The schematic demonstration of the second model is shown in Figure 9. Before the stimulation, $\text{I}\kappa\text{B}\beta$ exists in the cytoplasm and similar to $\text{I}\kappa\text{B}\alpha$ is bond to $\text{NF}\kappa\text{B}$. Active IKK phosphorylates $\text{I}\kappa\text{B}\beta$ to release $\text{NF}\kappa\text{B}$. In contrast to $\text{I}\kappa\text{B}\alpha$ and, $\text{I}\kappa\text{B}\beta$ is induced through an intermediate nuclear factor NFX. New synthesized $\text{I}\kappa\text{B}\beta$ proteins enter the cytoplasm, binding with free $\text{NF}\kappa\text{B}$ to inhibit its activity. In this model, we assume that $\text{I}\kappa\text{B}\beta\text{NF}\kappa\text{B}$ can shuttle between the cytoplasm and the nucleus, while $\text{I}\kappa\text{B}\beta$ could shuttle between the cytoplasm and the nucleus, and bind to nucleus $\text{NF}\kappa\text{B}$ and promote the export of this complex from the nucleus.

After classifying biochemical reactions, 46 groups of reactions are converted to describe the concentration change of 24 components, as shown in table 1 and 2. Differential equations being used to describe the rates of change of the concentrations of these components are shown in Table A.4 in APPENDIX.

For the big model, because of too many unknown parameters and most of them may be correlated, the simulation annealing program couldn't get a good fit for all the three data sets, free $\text{NF}\kappa\text{B}$, $\text{I}\kappa\text{B}\alpha$ and $\text{I}\kappa\text{B}\beta$. Otherwise, more steps are needed to get the best fit efficiently. MCMC sampling may help in this way.

Figure 8 Schematic demonstration of the model 1 for NFκB signaling following the LPS stimulation. A schematic diagram of the biochemical events represented in the mathematical model represented using Cell Designer 4.1. The model represents synthesis of IκBα and A20, association and dissociation between IκBα + IKKα, IκBα + NFκB, and IKKα + IκBα + NFκB, and the degradations of IKK, IκBα and A20 proteins, and IκBα and A20 mRNAs, and transcriptions of IκBα and A20 mRNAs and transportations of IκBα and NFκB between the nucleus and the cytoplasm.

3.2.2 Model Calibration

The mathematical models, shown schematically in Figure 6 and 7, were calibrated against values obtained from experimental data measured previously in our lab. The values were obtained with IC-21 cells (macrophage cell line) in response to 1 $\mu\text{g/ml}$ LPS with 100 μM ethanol (as control experiment) or with 100 μM DCPA. Nucleus NF κ B was measured by EMSA while total I κ B α and I κ B β in the cytoplasm were measured by western blot. Initial data is shown in Figure 6, while the numerical summary is shown in Figure 7. The mathematical models were implemented in MATLAB as detailed in supplement 2. Maximum likelihood values for the parameter were determined using simulated annealing. The specific parameter values are shown in Table 3.2. An empirical Bayesian approach was used to estimate the uncertainty in the model parameters given the available calibration data.

3.2.3 “*A priori*” identifiability analysis

Sensitivity analysis (SA) is the study of how the variation (uncertainty) in the output of a mathematical model can be apportioned, qualitatively or quantitatively, to different sources of variation in the parameters, either rate constants or initial conditions associated with a model. Based upon how the model is constructed, different parameters influence the output of the model in the same way. These parameters that cannot be estimated separately are called unidentifiable parameters.

Correlation of the unknown parameters in model 1 was analyzed and the results are shown in Table 3.1. This symmetric table represents a correlation matrix where each element in the table represents the correlation between the two parameters in the row and in the column. If the absolute value greater than 0.95, we will consider these two parameters are nonidentifiable. From the table, we can see that some parameters are not correlated to any other parameters. For instance, k_2 which associates with IKK inactivation via A20, has correlation values with other parameters all smaller than 0.95. Therefore, it is not correlated to any other parameters in this model and can be identified. On the other side, some of parameters are correlated, which are unidentifiable. For

instance, s_1 , which represents the inducible production of $I\kappa B\alpha$ mRNA, and t_1 , which represents the translation rate of $I\kappa B\alpha$, are completely correlated (0.999). For instance, the effect of slow $I\kappa B\alpha$ mRNA production on the observed cytoplasmic $I\kappa B\alpha$ and nuclear NF κ B concentration could be compensated by fast translation of $I\kappa B\alpha$ mRNA to $I\kappa B\alpha$ protein. Whether the production is slow and the translation is fast or the translation is slow and the production is fast will have the same effect on the observed cytoplasmic $I\kappa B\alpha$ protein concentration. Therefore, we cannot uniquely determine s_1 and t_1 given the mathematical structure of the model. However, as we don't have enough information about these parameters, we allow the simulation annealing algorithms to estimate them and optimize the fitness of the model to experimental data. Then AMCMC will be used to estimate the uncertainty in the model parameters.

Table 3.1 Correlation analysis of unknown parameters in Model 1

	s_1	cs_1	t_1	s_2	cs_2	dm_2	t_2	k_1	k_2	k_3	k_{prod}	k_{deg}	e_{1a}	i_1
s_1	1	0.991506	0.998573	-0.94237	-0.83454	0.944598	-0.94257	0.773763	-0.1271	-0.6263	0.869673	-0.77143	0.770211	-0.41547
cs_1	0.991506	1	0.994022	-0.95219	-0.83374	0.954541	-0.95205	0.770174	-0.15005	-0.6162	0.865416	-0.76845	0.749965	-0.45422
t_1	0.998573	0.994022	1	-0.95247	-0.83742	0.954586	-0.95247	0.772709	-0.12826	-0.6291	0.873774	-0.77396	0.756215	-0.44376
s_2	-0.94237	-0.95219	-0.95247	1	0.878846	-0.99991	0.999889	-0.79676	0.204923	0.748428	-0.92145	0.85095	-0.74421	0.692386
cs_2	-0.83454	-0.83374	-0.83742	0.878846	1	-0.88042	0.885843	-0.97106	-0.07714	0.894959	-0.99137	0.984112	-0.60369	0.59231
dm_2	0.944598	0.954541	0.954586	-0.99991	-0.88042	1	-0.99986	0.800166	-0.20322	-0.74475	0.922563	-0.85066	0.745892	-0.68663
t_2	-0.94257	-0.95205	-0.95247	0.999889	0.885843	-0.99986	1	-0.80534	0.1974	0.755471	-0.92706	0.85816	-0.74271	0.691574
k_1	0.773763	0.770174	0.772709	-0.79676	-0.97106	0.800166	-0.80534	1	0.0251	-0.82711	0.955502	-0.94862	0.557999	-0.49789
k_2	-0.1271	-0.15005	-0.12826	0.204923	-0.07714	-0.20322	0.1974	0.0251	1	-0.09162	-0.00301	-0.04058	-0.24967	0.316873
k_3	-0.6263	-0.6162	-0.6291	0.748428	0.894959	-0.74475	0.755471	-0.82711	-0.09162	1	-0.88683	0.955517	-0.45453	0.710773
k_{prod}	0.869673	0.865416	0.873774	-0.92145	-0.99137	0.922563	-0.92706	0.955502	-0.00301	-0.88683	1	-0.97721	0.653539	-0.6351
k_{deg}	-0.77143	-0.76845	-0.77396	0.85095	0.984112	-0.85066	0.85816	-0.94862	-0.04058	0.955517	-0.97721	1	-0.56518	0.663359
e_{1a}	0.770211	0.749965	0.756215	-0.74421	-0.60369	0.745892	-0.74271	0.557999	-0.24967	-0.45453	0.653539	-0.56518	1	-0.33463
i_1	-0.41547	-0.45422	-0.44376	0.692386	0.59231	-0.68663	0.691574	-0.49789	0.316873	0.710773	-0.6351	0.663359	-0.33463	1

Table 3.2 Comparison of the parameter values fit to the two experimental conditions. There values represent the maximum likelihood values as determined using simulated annealing.

Parameters	Definition	Without DCPA	With DCPA
s1	IκBα inducible synthesis rate	3.80e-3	2.95e-5
cs1	IκBα constitutive synthesis rate	3.00e-12	2.57e-13
t1	IκBα translation rate	3.00e-4	8.23e-1
s2	A20 inducible synthesis rate	314.22	0.24
cs2	A20 constitutive synthesis rate	3.13e-4	1.65e-11
dm2	A20 mRNA degradation rate	28.23	0.16
t2	A20 translation rate	3.10	0.59
k1	IKK activation rate	0.93	2.71e-4
k2	IKK inactivation rate by A20	0.59	0.37
k3	IKK spontaneous inactivation rate	3.00e-7	1.33e-18
kprod	IKKα production rate	0.16	6.94e-4
kdeg	IKKα, IKKα and IKKα degradation	0.10	8.7e-4
e1a	IκBα nuclear import rate	4.48e-4	1.21e-4
i1	IκBα nuclear export rate	3.10e-3	5.04e-4
e2a	IκBαNFκB nuclear export rate	1.10e-3	6.80e-3

3.2.4 Results of Simulated Annealing

The simulation results for model 1 are compared against the experimental data without DCPA are shown in Figure 10. The first 90 minutes of total I κ B α in the cytoplasm and free NF κ B in the nucleus following the LPS stimulation were used to select parameter values using simulated annealing. The resulting simulation is compared against the whole 6 hours observation period in Figure 10.

As shown in Figure 10, the simulation response was based on the first 90 minutes experimental data, can still predict NF κ B activity well for the rest of 6 hrs after the stimulation. However, the model predicts higher levels of cytoplasmic I κ B α data after 90 minutes than was experimentally observed. In other words, additional inhibitors besides I κ B α are needed to keep NF κ B activity as measured.

Figure 10 Simulation results for model 1 compared with experimental data without DCPA treatment using simulated annealing program. Line curves are the simulation results respectively for total I κ B α in the cytoplasm and free NF κ B in the nucleus. The + symbols are the experimental data of total cytoplasmic I κ B α , while the o symbols are the experimental data of free nuclear NF κ B.

The simulation results for the experiment with DCPA treatment is shown in Figure 11. The simulation results for the experimental data with DCPA treatment suggest an enhanced dampening of the NF κ B oscillations as compared to without DCPA treatment, while the overall agreement between model and data is not particularly good.

Figure 11 Simulation results for model 1 compared with experimental data with DCPA treatment by simulated annealing program. Line curves are the simulation results respectively for total I κ B α in the cytoplasm and free NF κ B in the nucleus. The + symbols are the experimental data of total cytoplasmic I κ B α , while the o symbols are the experimental data of free nuclear NF κ B.

From table 3.2, we can see most of the parameter values are much different for experiments without DCPA and with DCPA. Except k_2 , e_{1a} and e_{2a} are in the same level, all other parameter are much different. It is difficult to say which parameters are changed by DCPA. Even values for some parameters are much different, the simulated results against the two experimental conditions are still in the same level. For example, the transcription of I κ B α mRNA, s_1 and the translation of I κ B α mRNA should be correlated. For experimental data without DCPA treatment, s_1 is $3.8e-3$ and t_1 is $3.0e-4$, while for experimental data with DCPA treatment, s_1 is $2.947e-5$ and t_1 is $8.229e-1$. A small production rate needs a big translation rate to get the similar the protein production level. To further decide which parameters are identifiable, *a priori* identifiability analysis was used to determine which parameter could be structurally identified.

3.3 Results of AMCMC

A series of Markov chains generated using Metropolis-Hasting algorithm were used to estimate the conditional uncertainty in the model parameters given the experimental data with and without DCPA treatment. The proposal distribution is scaled dynamically to achieve an acceptance fraction of 0.2. The trace of the acceptance fraction is shown as a function of AMCMC step, in Figure 12 (A, C). The trace of the covariance scaling factor is shown as a function of AMCMC step, in Figure 12 (B, D). The trace of the acceptance fraction demonstrates that the scaling factor was adjusted at regular intervals to maintain the acceptance fraction around 0.2.

The trace of the conditional probability for each of the two chains against experimental data with and without DCPA treatment, shown in Figure 13, the better the fitness is, the greater the $P(Y|\Theta, M)$ is. From the results, chain 2 fits the results better than Chain 1.

The Gelman-Rubin potential scale reduction factor (PSRF) was applied to the model predictions to assess the convergence of the cumulative Markov Chains.[37] The Gelman-Rubin PSRF statistics were calculated for the species observed experimentally as a function of time and AMCMC step and shown graphically as a contour plot in Figure 14.

Figure 12 Evolution of the performance of the AMCMC algorithm. AMCMC model fits for DCPA treated (panels C and D) and untreated cells (panels A and B) are shown separately. The proposal covariance scaling factor (B, D) and the acceptance fraction (A, C) are shown as a function of AMCMC step against experimental data. The results for each of the two parallel chains are shown in different colors: chain 1 (Red) and chain 2 (Blue).

Figure 13 Evolution in the likelihood, $P(Y|\Theta, M)$, as a function of AMCMC step. The normalized likelihood value is shown as a function of AMCMC step for both two parallel chains against experimental data without DCPA treatment. The results for each of the two chains are shown in different colors: chain 1(Blue), chain 2(Red).

The colored contours correspond to values of the PSRF. The model predictions exhibited a potential scale reduction factor of below 1.2 immediately after the “learning” period. The variability among chains, as represented by an increase in PSFR, increased as a function of simulation time, existed for both NF κ B and I κ B α . This behavior was due to under-sampling at longer times relative to earlier times. For comparison, the 95th

percentile, 5th percentile, and median responses for each of the two chains are overlaid upon the experimental data without DCPA treatment in Figure 15. Traces for each of the parameters are shown in Figure 16. Density distribution for the model parameters is shown in Figure 17. Covariance structure of $P(\Theta|Y)$ is shown in Figure 18.

Figure 14 Gelman-Rubin assessment of the Convergence of AMCMC . A contour plot of the Gelman-Rubin statistic applied to the model predictions is shown as a function of time (i.e., the y-axis) and as a function of the cumulative chain up to a specific AMCMC step (i.e., the x-axis). Two parallel chains were used to calculate the Gelman-Rubin statistics for the simulated (panels A and C) Free NFκB expression in the nucleus and (panels B and D) total IκBα in the cytoplasm. Values less than 1.2 suggest convergence of the chains.

Figure 15 The mathematical model 1 for early signaling events reproduces the early dynamics for activation of NFkB. Simulated results (lines) are compared against the experimental observations without DCPA treatment (symbols). The uncertainty in the model predictions obtained from each chain is represented by three lines of the same color: the most likely prediction is represented by the solid lines and the dashed lines represent the 95th and 5th percentile of the predicted response. The results for each of the three parallel chains are shown in different colors: chain 1 (Red), chain 2 (Blue). (A, C) Simulated response (lines) versus measured free NFkB (squares) in the nucleus; (B, D) Simulated response (lines) versus measured total IκBα (squares) in the cytoplasm.

In Figure 14, the Gelman Rubin statistic suggests convergence between the two chains at the initial start point. At longer times the two predictions from the two chains diverge. In Figure 16, the trace of the parameters suggests that the accepted parameter values are very different for the two chains.

The posterior distribution in the predictions (see in Figure 15) shows that even both chains (red and blue) reproduce most the experimental data in the first 90 min, but their oscillation frequencies are much different. The blue chain fits the NF κ B data better while the red chain fits the I κ B α data better. It seems that the frequencies of these two sets of data are different. One chain tries to catch the frequency of one set data while the other tries to catch the frequency of the other one. Even after 1 million steps, these two chains still can't find a set of parameter values to fit both frequencies. According to Hyyot's work[54] and Nelson's study[55], the external stimulations can cause the frequency of NF κ B to vary a lot. Because NF κ B and I κ B α were measured on different dates, a slight of difference of the LPS stimulation maybe cause the variance of signaling frequency.

From the normalized error of the model prediction, shown as Figure 17, we can see that, the ranges of parameters in the blue chains are much bigger than those of the red one. The blue chain has a difficult time to find a best fitness for both of the data. This is also because of the correlations among parameters in the signaling network. From the Covariance structure of $P(\Theta|Y)$, shown as Figure 18, we can also see that most of the parameters are correlated, which is consistent with the results from the *a priori* identifiability analysis.

Figure 16 AMCMC summary plots for each of the model parameters for model 1 without DCPA treatment. The trace of each of the model parameters is shown as a function of MCMC step. The traces for two parallel chains are shown in different colors: chain 1 (Red) and chain 2 (Blue).

Figure 17 Normalized Distributions in Parameters for model 1 without DCPA treatment. The distribution in the sum of the normalized error between the calibration data and the model predictions is shown for each study used in the analysis. Distribution for the two parallel chains against experimental data without DCPA treatment is shown in different colors: chain 1(Blue) and chain 2(Red).

Figure 18 Covariance structure of $P(\Theta|Y)$ for model 1 without DCPA treatment. The pairwise correlation coefficients of the parameters derived from all two thinned ($K_{thin} = 200$) Markov chains are shown above the diagonal. A high value for the correlation coefficient suggests that the parameters are unidentifiable given the calibration data. Below the diagonal, pairwise projections of the marginalized probability density for $P(\Theta|Y)$ are shown. The parameter names are shown on the diagonal. Each scatter plot axis spans a range of 1012 (i.e., $(\log\text{-mean}-6.0 \text{ to } \log\text{-mean}+6.0)$). The values in each scatter plot are centered at the log-mean values (i.e., the expectation maximum) determined from two parallel Markov Chains each containing 900,000 MCMC steps.

Figure 19 AMCMC summary plots for each of the model parameters for experimental data with DCPA treatment. The trace of each of the model parameters is shown as a function of MCMC step. The traces for two parallel chains are shown in different colors: chain 1 (Red) and chain 2 (Blue)

For the experimental data with DCPA treatment, we also did MCMC sampling to compare with the result for experimental data with DCPA treatment. From the convergence plot, shown as Figure 14, we can see that some of points are converged while some of them are not. Combined with the prediction of the simulation results, shown as Figure 15, although both chains could fit most of the data points, the frequency vary so much that they are not converged at other time points. From that we can also expect the parameter values also vary a lot, shown as in Figure 19 and Figure 20.

Figure 20 Normalized Distributions in Parameters for experimental data with DCPA treatment. The distribution in the sum of the normalized error between the calibration data and the model predictions is shown for each study used in the analysis. Distribution for the two parallel chains are shown in different colors: chain 1(Blue) and chain 2(Red)

Using the first 90 minutes' experimental data to estimate the values of the parameter and the rest of 6 hours experimental period has some limits like the oscillation frequency of NFkB activity and the prediction vary much. To further test the model, we applied the AMCMC algorithm to the data of the whole 6 hours experimental period. From the results for with and without DCPA treatment, we can see that the model predictions are

more consistent compared with that only using the first 90 minutes of data, shown as Figure 21 and Figure 22. However, even the model are more converged, but from the trace of all the parameters, shown as Figure 27, the parameters sampling by two chains are in two different valleys.

Figure 21 - Convergence of AMCMC . A contour plot of the Gelman-Rubin statistic of the model predictions as a function of time (i.e., the y-axis) calculated as a function of the cumulative chain up to a specific AMCMC step (i.e., the x-axis). Two parallel chains were used to calculate the Gelman-Rubin statistics for the simulated Free NFκB(panels A, C) expression in the nucleus and total IκBα (panels B,D) in the cytoplasm without and with DCPA treatment. Values less than 1.2 suggest convergence of the chains. The models were fit to the entire six hour time period following ;PS stimulation without and with DCPA treatment.

Figure 22 The mathematical model 1 for early signaling events reproduces the early dynamics for activation of free NFκB expression (A, C) in the nucleus and IκBα (B, D) in the cytoplasm. Simulated results (lines) are compared against the experimental observations (symbols) used to calibrate the mathematical model for the entire 6 hours experimental data without DCPA treatment (panels A and B) and with DCPA treatment (panels C and D). The uncertainty in the model predictions obtained from each chain is represented by three lines of the same color: the most likely prediction is represented by the solid lines and the dashed lines represent the 95th and 5th percentile of the predicted response. The results for each of the three parallel chains are shown in different colors: chain 1 (Red), chain 2 (Blue).

Figure 23 AMCMC summary plots for each of the model parameters for experimental data without DCPA treatment. The trace of each of the model parameters is shown as a function of MCMC step. The traces for two parallel chains are shown in different colors: chain 1 (Red) and chain 2 (Blue).

Figure 24 The mathematical model 2 for early signaling events reproduces the early dynamics for activation of NFκB. Simulated results (lines) are compared against the experimental observations (symbols) used to calibrate the mathematical model. The uncertainty in the model predictions obtained from each chain is represented by three lines of the same color: the most likely prediction is represented by the solid lines and the dashed lines represent the 95th and 5th percentile of the predicted response. The results for each of the three parallel chains are shown in different colors: chain 1 (Red), (A) Simulated response (lines) versus measured free NFκB (squares) in the nucleus without DCPA treatment; (B) Simulated response (lines) versus measured total IκBα (squares) in the cytoplasm without DCPA treatment; (C) Simulated response (lines) versus measured total IκBβ (squares) in the cytoplasm without DCPA treatment.

According Peng group's work, Foxj1 is essential to IκBβ transcription.[24]

Combine the measured cytoplasmic I κ B β data, which re-increased after 90 minutes following the LPS stimulation, I κ B β should play an important role to regulate NF κ B activity in IC-21 cells after 90 minutes following the LPS stimulation. Therefore, to better understand the regulation of NF κ B activity in macrophages, I κ B β should be included into the signaling network.

For the big model, to involve I κ B β in the signaling network, more parameters are introduced, which causes more calculation in the modeling progress. From our current MCMC prediction, we can see that the big model couldn't catch the second peak of NF κ B. However, it gives a good simulation of the trend of all NF κ B, I κ B α and I κ B β following LPS stimulation in macrophages. More experimental data are needed to estimate some parameters to reduce the number of being-fitted parameters.

CHAPTER 4

Conclusion

4.1 Regulation of NF κ B activity following LPS stimulation

Macrophages play an important role in the immune system. NF κ B is an essential nuclear transcription factor in many kinds of cells including macrophages. NF κ B induces or promotes the productions of lots of proteins. Conversely, the activity of NF κ B is also regulated by other proteins. From our experimental data, I κ B α proteins play a major role in regulating NF κ B activity. Our mathematical Model 1 works well by using the first 90 minutes data to predict the rest of the 6 hours' experiment measured free NF κ B in the nucleus and total I κ B α in the cytoplasm. Further, the stimulation results show that additional proteins besides I κ B α are needed to keep the NF κ B activity in the level as measured after 90 minutes following the LPS stimulation. This result is consistent with our experimental results which showed that I κ B β proteins are produced 90 minutes after LPS stimulation and eventually rise to a higher level than that before the LPS stimulation. Therefore, we conclude that I κ B α proteins are playing a major role in regulating the NF κ B activity in the beginning of the LPS stimulation and then I κ B β proteins become more important as the time increases.

4.2 Mathematical Modeling of NF κ B signaling network

Based on our conclusion from experiment measured NF κ B, I κ B α and I κ B β , we developed two mathematical models, model 1 and model 2. In model 1, I κ B α represent the role of inhibitors of NF κ B and is also induced by NF κ B. A20 is used to regulate IKK activity and also induced by NF κ B. Two negative feedbacks involve 14 components and 25 groups of reactions. Twenty seven parameters are associated with these reactions between the 14 components. From simulation annealing results, model 1 works well for

the NF κ B signaling. Furthermore, more I κ B α proteins are predicted than measured, which is consistent with the increased I κ B β proteins measured by the experiment. Model 2 uses an unknown nuclear factor NFX to introduce I κ B β mRNA and also cause the delay of I κ B β mRNA transcription, comparing with I κ B α mRNA. Model 2 includes 46 groups of reactions, translations and transportations. The simulation annealing program was not able to select appropriate simulate this high dimensional model. We used MCMC sampling to fit the model and could simulate for all NF κ B, I κ B α and I κ B β , as measured by the experiment. However, the fit was not good enough to catch most of the observed dynamics. More information about the parameters in the signaling network and the unknown nuclear factor NFX is needed to further improve Model 2.

From the *a priori* identifiability analysis, some of these parameters in Model 1 are correlated to each other. Comparing the parameter values fitted by the simulation annealing program, they are much different for the experimental data without DCPA and with DCPA treatment. It is difficult to say whether these differences are because of the DCPA effect or just a result of the correlation between parameters. Similarly, we can expect parameters are correlated in the Model 2, too.

4.3 AMCMC to estimate the conditional uncertainty in the model parameters

Two parallel Markov chains generated using Metropolis-Hasting algorithm were used to estimate the conditional uncertainty in the model parameters against experimental data with and without DCPA treatment. Using data of the first 90 minutes following the LPS stimulation, the model doesn't converge, although it could catch most of the data points. The frequency of the predicted oscillations varies a lot between different chains. This may be because of the frequency of the two data sets NF κ B and I κ B α were measured under slightly different conditions. Slight difference in the external stimulation by LPS may cause the difference of frequency of these two data sets.

However, when we include all the data from the whole 6 hours experiments, the convergence of the model is improved. The predictions of the two chains for experimental data with and without DCPA are much more consistent. However, the

improved fit may be due to inclusion of additional parameters into the model. Model discrimination criteria should be used to compare these two models. Overall more information about parameters will help to estimate the unknown parameters and predict the system.

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APPENDIX

Table A.1 List of Models Variables

Variable Name	Definition	Units
TLR4	Toll-like receptor 4	1
R0	LPS Stimulation signal	-
IKK _n	Free neutral cytoplasmic IKK proteins	μM
IKK _n	Free active cytoplasmic IKK proteins	μM
IKK _n	Free inactive cytoplasmic IKK proteins	μM
IκB _a	Free cytoplasmic IκB _a proteins	μM
NFκB	Free cytoplasmic NFκB	μM
IKK _a IκB _a	Cytoplasmic IKK _a -IκB _a complexes	μM
IKK _a IκB _a NFκB	Cytoplasmic IKK _a -IκB _a NFκB complexes	μM
A20	Cytoplasmic A20 proteins	μM
A20 _m	A20 mRNA	μM
IκB _a _m	IκB _a mRNA	μM
IκB _a _n	Free Nuclear IκB _a proteins	μM
NFκB _n	Free Nuclear NFκB	μM
IκB _a NFκB	Cytoplasmic IκB _a NFκB complexes	μM
IκB _a _n NFκB _n	Nuclear IκB _a -NFκB complexes	μM
IκB _b (#)	Free cytoplasmic IκB _b proteins	μM
IκB _b NFκB (#)	Cytoplasmic IκB _b NFκB complexes	μM
IKK _a IκB _b (#)	Cytoplasmic IKK _a -IκB _b complexes	μM
IKK _a IκB _b NFκB (#)	Cytoplasmic IKK _a -IκB _a NFκB complexes	μM
IκB _b _m (#)	IκB _b mRNA	μM
IκB _b _n (#)	Free Nuclear IκB _b proteins	μM
IκB _b _n NFκB _n (#)	Nuclear IκB _b -NFκB complexes	μM
NFX (#)	Unknown transcription factor X	μM
NFX _n (#)	Nuclear unknown transcription factor X	μM
NFX _m (#)	Unknown transcription factor X mRNA	μM

(#) represents the components which are involved only in Model 2.

Table A.2 Reaction rate equations of Model 1 and Model 2 for NFkB signaling pathway

Reaction	Rate Relationship
Reaction class 1: IkBa-NFkB Association	
$R1a = a1 * NFkB * IkBa$	
$R1b = a1 * NFkBn * IkBan$	
$R1c = a1 * NFkB * IKKaIkBa$	
Reaction class 2: IKKa-IkBa Association	
$R2a = a2 * IKKa * IkBa$	
$R2b = a2 * f1 * IKKa * IkBaNFkB$	
Reaction class 3: IkBa-NFkB dissociation	
$R3a = d1 * IkBaNFkB$	
$R3b = d1 * IkBanNFkBn$	
$R3c = d1 * IKKaIkBaNFkB$	
Reaction class 4: IKKa-IkBa dissociation	
$R4a = d2 * IKKaIkBa$	
$R4b = d2 * f1 * IKKaIkBaNFkB$	
Reaction class 5: IKKa-IkBa Catalytic dissociation	
$R5a = kcat * IKKaIkBa$	
$R5b = kcat * f2 * IKKaIkBaNFkB$	
Reaction class 6: Degradation of IKK	
$R6a = kdeg * IKKn$	
$R6b = kdeg * IKKa$	
$R6c = kdeg * IKKi$	
Reaction class 7: Degradation of free IkBa proteins	

$R7 = de1 * IkBa$

Reaction class 8: Degradation of IkBa complex to NFkB

$R8 = de3 * IkBaNFkB$

Reaction class 9: Degradation of A20 proteins

$R9 = de2 * A20$

Reaction class 10: Degradation of IkBa mRNA

$R10 = dm1 * IkBam$

Reaction class 11: Degradation of A20 mRNA

$R11 = dm2 * A20m$

Reaction class 12: Synthesis of IKKn

$R12 = kprod$

Reaction class 13: Inducible synthesis of IkBa mRNA

$R13 = s1 * NFkBn$

Reaction class 14: Inducible synthesis of A20 mRNA

$R14 = s2 * NFkBn$

Reaction class 15: Constitutive synthesis of IkBa mRNA

$R15 = cs1$

Reaction class 16: Constitutive synthesis of A20 mRNA

$R16 = cs2$

Reaction class 17: Translation from IkBa mRNA to IkBa proteins

$R17 = t1 * IkBam$

Reaction class 18: Translation from A20 mRNA to A20 proteins

$$R18 = t2 * A20m$$

Reaction class 19: Activation of IKK_n to IKK_a

$$R19 = k1 * R0 * IKK_n * TLR4$$

Reaction class 20: Inactivation of IKK_a because of A20

$$R20 = k2 * R0 * A20 * IKK_a$$

Reaction class 21: Spontaneous Inactivation of IKK_n to IKK_i

$$R21 = k3 * IKK_a$$

Reaction class 22: IkB_a Import to the nucleus

$$R22 = i1a * IkB_a$$

Reaction class 23: IkB_a export from the nucleus to cytoplasm

$$R23 = e1a * IkB_n$$

Reaction class 24: IkB_nNFkB_n export from the nucleus to cytoplasm

$$R24 = e2a * IkB_nNFkB_n$$

Reaction class 25: NFkB import to the nucleus from cytoplasm

$$R25 = i1 * NFkB$$

Reaction class 26: IkB_b-NFkB Association (#)

$$R26a = a3 * NFkB * IkB_b$$

$$R26b = a3 * NFkB * IKK_aIkB_b$$

$$R26c = a3 * NFkB_n * IkB_bn$$

Reaction class 27: IKK_a-IkB_b Association (#)

$$R27a = a4 * IKKa * IkBb$$

$$R27b = a4 * f3 * IKKa * IkBbNFkB$$

Reaction class 28: IkBb-NFkB dissociation (#)

$$R28a = d3 * IkBbNFkB$$

$$R28b = d3 * IKKaIkBbNFkB$$

$$R28c = d3 * IkBbnNFkBn$$

Reaction class 29: IKKa-IkBb dissociation (#)

$$R29a = d4 * IKKaIkBb$$

$$R29b = d4 * IKKaIkBbNFkB$$

Reaction class 30: IKKa-IkBbCatalytic dissociation (#)

$$R30a = kcatb * IKKaIkBa$$

$$R30b = kcatb * f2 * IKKaIkBaNFkB$$

Reaction class 31: Degradation of free IkBb proteins (#)

$$R31 = de4 * IkBb$$

Reaction class 32: Degradation of IkBb complex to NFkB (#)

$$R32 = de5 * IkBbNFkB$$

Reaction class 33: Degradation of NFX mRNA (#)

$$R33 = dm3 * NFXm$$

Reaction class 34: Degradation of IkBb mRNA (#)

$$R34 = dm4 * IkBbm$$

Reaction class 35: Inducible synthesis of NFX mRNA (#)

$$R35 = s3 * NFkBn$$

Reaction class 36: Inducible synthesis of IkBb mRNA (#)

$R36 = s4 * NFXn$

Reaction class 37: Constitutive synthesis of NFX mRNA (#)

$R37 = cs3$

Reaction class 38: Constitutive synthesis of IkBb mRNA (#)

$R38 = cs4$

Reaction class 39: Translation from NFX mRNA to NFX proteins (#)

$R39 = t3 * NFXm$

Reaction class 40: Translation from IkBb mRNA to IkBb proteins (#)

$R40 = t4 * IkBbm$

Reaction class 41: NFX Import to the nucleus (#)

$R41 = i3 * NFX$

Reaction class 42: NFX export from the nucleus to cytoplasm (#)

$R42 = e3 * NFXn$

Reaction class 43: IkBb Import to the nucleus (#)

$R43 = i1b * IkBb$

Reaction class 44: IkBb export from the nucleus to cytoplasm (#)

$R44 = e1b * IkBbn$

Reaction class 45: IkBbnNFkBn export from the nucleus to cytoplasm (#)

$R45 = e2b * IkBbnNFkBn$

Reaction class 46: Degradation of NFX Proteins (#)

$$R46 = de6 * NFX$$

(#) represents the components which are involved only in Model 2.

Table A.3 Differential equations that define the mathematical model 1 for NFkB signaling pathway

$dIKK_n/dt$	$= R12 - R19 - R6a$
$dIKK_a/dt$	$= R19 - R20 - R21 - R6b - R2a - R2b + R4a + R4b + R5a + R5b$
$dIKK_i/dt$	$= R20 + R21 - R6c$
$dIKK_aIkBa/dt$	$= R2a - R4a - R5a - R1c + R3c$
$dIKK_aIkBaNFkB/dt$	$= R1c + R2b - R3c - R4b - R5b$
$dNFkB/dt$	$= R3a + R3c - R1a - R1c + R5b + R8 - R25$
$dNFkB_n/dt$	$= R3b + kv * R25 - R1b$
$dA20/dt$	$= R18 - R9 - R20$
$dA20_m/dt$	$= R16 + R14 - R11$
$dIkBa/dt$	$= R3a - R1a + R4a - R2a - R22 + R23/kv + R17 - R7$
$dIkBa_n/dt$	$= R3b - R1b + kv * R22 - R23$
$dIkBa_m/dt$	$= R13 + R15 - R10$
$dIkBaNFkB/dt$	$= R1a - R3a - R2b + R4b + R24 / kv - R8$
$dIkBa_nNFkB_n/dt$	$= R1b - R3b - R24$

Table A.4 Differential equations that define the mathematical model 2 for NFkB signaling pathway

$dIKKn/dt$	$= R12 - R19 - R6a$
$dIKKa/dt$	$= R19 - R20 - R21 - R6b - R2a - R2b + R4a + R4b + R5a + R5b - R27a - R27b + R29a + R29b + R30a + R30b$
$dIKKi/dt$	$= R20 + R21 - R6c$
$dIKKaIkBa/dt$	$= R2a - R4a - R5a - R1c + R3c$
$dIKKaIkBaNFkB/dt$	$= R1c + R2b - R3c - R4b - R5b$
$dNFkB/dt$	$= R3a + R3c - R1a - R1c + R5b + R8 - R25 + R28a + R28b - R26a - R26b + R30b + R32$
$dNFkBn/dt$	$= R3b + kv * R25 - R1b - R26c + R28c$
$dA20/dt$	$= R18 - R9 - R20$
$dA20m/dt$	$= R16 + R14 - R11$
$dIkBa/dt$	$= R3a - R1a + R4a - R2a - R22 + R23/kv + R17 - R7$
$dIkBan/dt$	$= R3b - R1b + kv * R22 - R23$
$dIkBam/dt$	$= R13 + R15 - R10$
$dIkBaNFkB/dt$	$= R1a - R3a - R2b + R4b + R24 / kv - R8$
$dIkBanNFkBn/dt$	$= R1b - R3b - R24$
$dIkBb/dt$	$= R40 - R26a - R27a + R28a + R29a - R31 - R43 + R44/kv$
$dIkBbNFkB/dt$	$= R26a - R27b - R28a + R29b - R32 + R45/kv$
$dIKKaIkBb/dt$	$= R27a - R29a - R26b - R30a + R28b$
$dIKKaIkBbNFkB/dt$	$= R26b + R27b - R28b - R29b - R30b$
$dIkBbm/dt$	$= R36 + R38 - R34$
$dNFX/dt$	$= R39 - R41 + R42/kv - R46$
$dNFXn/dt$	$= kv * R41 - R42$
$dNFXm/dt$	$= R37 + R35 - R33$
$dIkBbn/dt$	$= R43/kv - R44 - R26c + R28c$
$dIkBbnNFkBn/dt$	$= R26c - R28c - R45$

Table A.5 List of parameters and corresponding values model 1 and model 2. The values for the reaction rate parameters correspond to the maximum likelihood values Numerical subscripts indicate the reaction class to which the parameters correspond. Values determined during model calibration are indicated by an asterisk (*).

Symbol	Definition	Value	Units	Refs
a1	IkB _a -NF κ B association rate	0.5	s ⁻¹	11,13
a2	IKK _a -IkB _a association rate		$\mu\text{M}^{-1} \text{s}^{-1}$	*
f1	The factor of NF κ B on IKK _a -IkB _a association	0.2	-	11,22
kcat	KK _a -IkB _a catalytic dissociation rate		s ⁻¹	*
f2	The factor of NF- κ B on IKK _a -IkB _a catalysis	5	-	11,22
d1	IkB _a -NF κ B reversible dissociation rate	1.25e-3	s ⁻¹	11
d2	IKK _a -IkB _a reversible dissociation rate	0.5e-3	s ⁻¹	11
s1	IkB _a -inducible mRNA synthesis		s ⁻¹	*
s2	A20-inducible mRNA synthesis		s ⁻¹	*
cs1	IkB _a -constitutive mRNA synthesis		$\mu\text{M} \text{s}^{-1}$	*
cs2	A20-constitutive mRNA synthesis		$\mu\text{M} \text{s}^{-1}$	*
de1	Spontaneous, free IkB _a protein degradation	0.0001	s ⁻¹	20
de2	A20 protein degradation	0.0003	s ⁻¹	13
de3	IkB _a degradation (complexed to NF- κ B)	2e-5	s ⁻¹	20
dm1	IkB _a mRNA degradation	0.0004	s ⁻¹	43
dm2	A20 mRNA degradation		s ⁻¹	*
t1	IkB _a translation rate	0.5	s ⁻¹	13
t2	A20 translation rate		s ⁻¹	*
k1	IKK activation rate	0.0025	s ⁻¹	13
k2	IKK inactivation rate by A20	0.1	s ⁻¹	13
k3	IKKspontaneous inactivation rate	0.0015	s ⁻¹	13
kprod	IKK _n production rate	2.5e-5	s ⁻¹	13
kdeg	IKK _a , IKK _n and IKK _i degradation	1.25e-4	s ⁻¹	13
kv	Cytoplasmic to nuclear volume	7	-	45
i1	NF- κ B nuclear import rate	0.09	s ⁻¹	11
i1a	IkB _a nuclear import rate	0.0003	s ⁻¹	11

e1a	IkB α nuclear export rate	0.0002	s ⁻¹	11
e2a	IkB α -NF κ B nuclear export rate	0.014	s ⁻¹	*
dcpa	DCPA treatment concentration	0 or 100	μ M	-
The following parameters are only in Model 2				
a3	IkB β -NF κ B association rate	0.5	s ⁻¹	11,13
a4	IKK α -IkB β association rate		μ M ⁻¹ s ⁻¹	*
f3	The factor of NF κ B on IKK α -IkB β association	5	-	11,22
kcatb	IKK α -IkB α catalytic dissociation rate		s ⁻¹	*
f4	The factor of NF- κ B on IKK α -IkB β catalysis	5	-	11,22
d3	IkB β -NF κ B reversible dissociation rate	1.25e-3	s ⁻¹	11
d4	IKK α -IkB β reversible dissociation rate	0.5e-3	s ⁻¹	11
s3	NFX-inducible mRNA synthesis		s ⁻¹	*
s4	IkB β -inducible mRNA synthesis		s ⁻¹	*
cs3	NFX-constitutive mRNA synthesis		μ M s ⁻¹	*
cs4	IkB β -constitutive mRNA synthesis		μ M s ⁻¹	*
de4	Spontaneous, free IkB β protein degradation	0.0001	s ⁻¹	20
de5	IkB β degradation (complexed to NF- κ B)	2e-5	s ⁻¹	20
dm4	IkB β mRNA degradation	0.0004	s ⁻¹	43
dm3	NFX mRNA degradation		s ⁻¹	*
t4	IkB β translation rate	0.5	s ⁻¹	13
t3	NFX translation rate		s ⁻¹	*
i3	NFX nuclear import rate	0.09	s ⁻¹	*
e3	NFX export rate from nucleus	0.0003	s ⁻¹	*

Supplement 1: Description of biochemical reactions and converting them to ordinary differential equations

IKKn: IKK in neutral state

The first term describes IKKn synthesis; the second one describes the depletion of IKKn due to the TLR4 signal-induced transformation into active form IKKa, while the last one describes the spontaneous degradation.

$$dIKKn / dt = k_{prod} - k_1 * R0 * IKKn * TLR4 - k_{deg} * IKKn \quad (1)$$

IKKa: IKK in active state

The first line represents the activation of IKK by TLR4 signal, the deactivation of IKK induced by A20 and spontaneous deactivation of active IKK; The second line and the third line represent spontaneous degradation of IKKa, depletion of free IKKa due to formation of complexes of IKKaIkbBa and IKKaIkbBaNFkB, and concentration change of IKKa due to dissociation of IKKaIkbBa and IKKaIkbBaNFkB complexes. The presence of NFkB enhances the efficiency of IKKa and IkbBa interaction by a factor f1[9]. The final line represents the concentration change due to catalysis. IKKa is still active after catalytic phosphorylation.

$$\begin{aligned} dIKKa/dt = & k_1 * IKKn * TLR4 - k_2 * R0 * A20 * IKKa - k_3 * IKKa \\ & - k_{deg} * IKKa - a_2 * IKKa * IkbBa - a_2 * f_1 * IKKa * IkbBaNFkB \quad (2) \\ & + d_2 * IKKaIkbBa + d_2 * f_1 * IKKaIkbBaNFkB \\ & + k_{cat} * IKKaIkbBa + k_{cat} * f_2 * IKKaIkbBaNFkB \end{aligned}$$

IKKi: inactive state of IKK

First term describes inactive IKK forming due to A20, the second describes the concentration increase of IKKi due to IKKa spontaneous deactivation, while the third one represents the degradation of IKKi. IKKi is assumed has the same spontaneous degradation rate as IKKn and IKKa.

$$dIKKi/dt = k_2 * A20 * IKKa + k_3 * IKKa - k_{deg} * IKKi \quad (3)$$

IKKaIkbBa: complex of IKKa and IkbBa

The first line describes the concentration change of IKK α I κ B α due to I κ B α associates ,dissociates with IKK α and catalytic by IKK α ; The second line represents association and dissociation between IKK α I κ B α and NF κ B.

$$\begin{aligned} d\text{IKK}\alpha\text{I}\kappa\text{B}\alpha/dt &= a_2 * \text{IKK}\alpha * \text{I}\kappa\text{B}\alpha - d_2 * \text{IKK}\alpha\text{I}\kappa\text{B}\alpha - k_{cat} * \text{IKK}\alpha\text{I}\kappa\text{B}\alpha \\ &\quad - a_1 * \text{NF}\kappa\text{B} * \text{IKK}\alpha\text{I}\kappa\text{B}\alpha + d_1 * \text{IKK}\alpha\text{I}\kappa\text{B}\alpha\text{NF}\kappa\text{B} \end{aligned} \quad (4)$$

IKK α I κ B α NF κ B: complex of IKK α , I κ B α and NF κ B

The first line represents the association and dissociation between IKK α I κ B α and NF κ B; the second line represents the association and dissociation between IKK α and I κ B α NF κ B; The final line describes the depletion of IKK α I κ B α NF κ B due to the catalysis process.

$$\begin{aligned} d\text{IKK}\alpha\text{I}\kappa\text{B}\alpha\text{NF}\kappa\text{B}/dt &= a_1 * \text{NF}\kappa\text{B} * \text{IKK}\alpha\text{I}\kappa\text{B}\alpha - d_1 * \text{IKK}\alpha\text{I}\kappa\text{B}\alpha\text{NF}\kappa\text{B} \\ &\quad + a_2 * f_1 * \text{IKK}\alpha * \text{I}\kappa\text{B}\alpha\text{NF}\kappa\text{B} - d_2 * f_1 * \text{IKK}\alpha\text{I}\kappa\text{B}\alpha\text{NF}\kappa\text{B} \quad (5) \\ &\quad - k_{cat} * f_2 * \text{IKK}\alpha\text{I}\kappa\text{B}\alpha\text{NF}\kappa\text{B} \end{aligned}$$

NF κ B: Free cytoplasmic NF κ B

The first line describes free NF κ B concentration increase due to dissociation of I κ B α NF κ B and IKK α I κ B α NF κ B; the second line describes the decrease of free NF κ B due to association with I κ B α , IKK α I κ B α and shuttling into the nucleus. The final line represents the concentration increase due to IKK α catalytic degrading I κ B α and spontaneous degradation of bound I κ B α . The factor f_2 represents the effect of the presence of NF κ B on IKK α phosphorylating I κ B α [9].

$$\begin{aligned} d\text{NF}\kappa\text{B}/dt &= d_1 * \text{I}\kappa\text{B}\alpha\text{NF}\kappa\text{B} + d_1 * \text{IKK}\alpha\text{I}\kappa\text{B}\alpha\text{NF}\kappa\text{B} \\ &\quad - a_1 * \text{NF}\kappa\text{B} * \text{I}\kappa\text{B}\alpha - a_1 * \text{NF}\kappa\text{B} * \text{IKK}\alpha\text{I}\kappa\text{B}\alpha - i_1 * \text{NF}\kappa\text{B} \quad (6) \\ &\quad + k_{cat} * f_2 * \text{IKK}\alpha\text{I}\kappa\text{B}\alpha\text{NF}\kappa\text{B} + d_{e3} * \text{I}\kappa\text{B}\alpha\text{NF}\kappa\text{B} \end{aligned}$$

NF κ B $_n$: Free NF κ B in the nucleus

First term and second represent free NF κ B $_n$ changes due to dissociation and association between I κ B α and NF κ B in the nucleus; and the last term represents that NF κ B imports to the nucleus from the cytoplasm. Because of the volume of cytoplasm is larger than the nucleus, k_v = the volume of the cytoplasm / the volume of the nucleus, the concentration change due to importation is k_v times of that in the cytoplasm.

$$d\text{NF}\kappa\text{B}_n/dt = d_1 * \text{I}\kappa\text{B}\alpha_n\text{NF}\kappa\text{B}_n - a_1 * \text{NF}\kappa\text{B}_n * \text{I}\kappa\text{B}\alpha_n + k_v * i_1 * \text{NF}\kappa\text{B} \quad (7)$$

A20: A20 protein in the cytoplasm

First term describes A20 protein production rate due to the translation from A20 mRNA; the second term represents degradation of A20 protein; and the final term represents that free A20 decreases because of inhibiting IKK activity. We assume that TLR4 conductive signaling is required to activate A20.

$$dA20/dt = t2 * A20m - de2 * A20 - k2 * TLR4 * A20 * IKKa \quad (8)$$

A20m: A20 messenger RNA

We separate the synthesis of A20 mRNA to two parts. One is constitutive and the other is first-order dependent on free NFkB concentration in the nucleus. The last term represents first order degradation of A20 mRNA.

$$dA20m/dt = cs2 + s2 * NFkBn - dm2 * A20m \quad (9)$$

IkBa: Free IkBa in the cytoplasm

The first and second lines represent the concentration change of free IkBa due to the dissociation and association between IkBa and NFkB, and between IKKa and IkBa; the last line represents free IkBa protein importing to, exporting from the nucleus, translation from IkBa mRNA and spontaneous degradation.

$$\begin{aligned} dIkBa/dt = & d1 * IkBanNFkB - a1 * NFkB * IkBa \\ & + d2 * IKKaIkBa - a2 * IKKa * IkBa \\ & - i1a * IkBa + e1a * IkBan /kv + t1 * IkBam - de1 * IkBa \end{aligned} \quad (10)$$

IkBan: Free IkBa in the nucleus

First line represents IkBa concentration change due to the dissociation and association between IkBa and NFkB; the second line represents IkBa concentration change caused by free IkBa shuttling between the cytoplasm and the nucleus.

$$\begin{aligned} dIkBan/dt = & d1 * IkBanNFkBn - a1 * NFkBn * IkBan \\ & + kv * i1a * IkBa - e1a * IkBan \end{aligned} \quad (11)$$

IkBam: IkBa messenger RNA

We separate the synthesis of IkBa mRNA to two parts. One is constitutive and the other is first-order dependent on free NFkB concentration in the nucleus. The last term represents first order degradation of IkBa mRNA.

$$dIkBam/dt = cs1 + s1 * NFkBn - dm1 * IkBam \quad (12)$$

IkBaNFkB: cytoplasmic IkBaNFkB complex

First line represents the concentration change of IkBaNFkB due to the association and dissociation between IkBa and NFkB; second line describes the the concentration change of IkBaNFkB due to the association and dissociation between IKKa and IkBa NFkB. NFkB increases the association rate of IKKa and IkBa according experimental data[9]. Based on the motif-motif interaction principle, the affinity of IKKa and IkBa doesn't change, so the dissociation between them also is increased by f1 times. The final line represents the exporting of IkBaNFkB from the nucleus and the degradation of IkBaNFkB.

$$\begin{aligned} dIkBaNFkB/dt &= a1 * NFkB * IkBa - d1 * IkBaNFkB \\ &\quad - a2 * f1 * IKKa * IkBaNFkB + d2 * f1 * IKKaIkBaNFkB \quad (13) \\ &\quad + e2a * IkBanNFkBn / kv - de3 * IkBaNFkB \end{aligned}$$

IkBanNFkBn: IkBaNFkB complex in the nucleus

First line represents the concentration change due to the association and dissociation between NFkBn and IkBan; the second line represents the exporting of IkBan NFkBn from the nucleus.

$$\begin{aligned} dIkBanNFkBn/dt &= a1 * NFkBn * IkBan - d1 * IkBanNFkBn \\ &\quad - e2a * IkBanNFkBn \end{aligned} \quad (14)$$

The second model will add IkBb into the signaling network. IkBb can reacte with IKKa and NFkB in the cytoplasm and can also bind with NFkB in the nucleus. The different of IkBb and IkBa is that IkBb is induced by an unknow nuclear factor X which is induced by NFkB. 10 more equations are added, described as below. However, the differential equations of IKK, NFkB and NFkBn will be changed due to their interaction with IkBb.

IKKa: IKK in active state

The first line represents the activation of IKK by TLR4 signal, the deactivation of IKK induced by A20 and spontaneous deactivation of active IKK; The second line and the third line reprent spontaneous degradation of IKKa, depletion of free IKKa due to formation of complexes of IKKaIkBa and IKKaIkBaNFkB, and concentration change of IKKa due to dissociation of IKKaIkBa and IKKaIkBaNFkB complexes . The presence of NFkB enhances the effecient of IKKa and IkBa interaction by a factor f1[9]. The fourth line represents the concentration change due to catalysis. IKKa is still active after

catalytic phosphorylation. IKK α can also phosphorylate I κ B β . The last three lines represent the association between IKK α and I κ B β , IKK α and I κ B β NF κ B, and catalysis of IKK α I κ B β and IKK α I κ B β NF κ B. Factors f3 and f4 represent the effects of NF κ B on IKK α associating and catalyzing I κ B β .

$$\begin{aligned}
 dIKK\alpha/dt = & k_1 * IKK_n * TLR4 - k_2 * R0 * A20 * IKK\alpha - k_3 * IKK\alpha \\
 & - k_{deg} * IKK\alpha - a_2 * IKK\alpha * I\kappa B\alpha - a_2 * f_1 * IKK\alpha * I\kappa B\alpha NF\kappa B \quad (2*) \\
 & + d_2 * IKK\alpha I\kappa B\alpha + d_2 * f_1 * IKK\alpha I\kappa B\alpha NF\kappa B \\
 & + k_{cat} * IKK\alpha I\kappa B\alpha + k_{cat} * f_2 * IKK\alpha I\kappa B\alpha NF\kappa B \\
 & - a_4 * IKK\alpha * I\kappa B\beta - a_4 * f_3 * IKK\alpha * I\kappa B\beta NF\kappa B \\
 & + d_4 * IKK\alpha I\kappa B\beta + d_4 * f_3 * IKK\alpha I\kappa B\beta NF\kappa B \\
 & + k_{cat} * IKK\alpha I\kappa B\beta + k_{cat} * f_4 * IKK\alpha I\kappa B\beta NF\kappa B
 \end{aligned}$$

NF κ B: Free cytoplasmic NF κ B

The first line describes free NF κ B concentration increase due to dissociation of I κ B α NF κ B and IKK α I κ B α NF κ B; the second line describes the decrease of free NF κ B due to association with I κ B α , IKK α I κ B α and shuttling into the nucleus. The Third line represents the concentration increase due to IKK α catalytic degrading I κ B α and spontaneous degradation of bound I κ B α . The factor f2 represents the effect of the presence of NF κ B on IKK α phosphorylating I κ B α [9]. The last three lines represent the concentration of NF κ B due to its dissociation and association with I κ B β , IKK α I κ B β , and degradation of I κ B β due to IKK α phosphorylation and spontaneous degradation.

$$\begin{aligned}
 dNF\kappa B/dt = & d_1 * I\kappa B\alpha NF\kappa B + d_1 * IKK\alpha I\kappa B\alpha NF\kappa B \\
 & - a_1 * NF\kappa B * I\kappa B\alpha - a_1 * NF\kappa B * IKK\alpha I\kappa B\alpha - i_1 * NF\kappa B \quad (6*) \\
 & + k_{cat} * f_2 * IKK\alpha I\kappa B\alpha NF\kappa B + de_3 * I\kappa B\alpha NF\kappa B \\
 & + d_3 * I\kappa B\beta NF\kappa B + d_3 * IKK\alpha I\kappa B\beta NF\kappa B \\
 & - a_3 * NF\kappa B * I\kappa B\beta - a_3 * NF\kappa B * IKK\alpha I\kappa B\beta \\
 & + k_{cat} * f_4 * IKK\alpha I\kappa B\beta NF\kappa B + de_5 * I\kappa B\beta NF\kappa B
 \end{aligned}$$

NFkBn: Free NFkB in the nucleus

First term and second represent free NFkBn changes due to dissociation and association between IkBa and NFkB in the nucleus; and the third term represents that NFkB imports to the nucleus from the cytoplasm. Because of the volume of cytoplasm is larger than the nucleus, k_v = the volume of the cytoplasm / the volume of the nucleus, the concentration change due to importation is k_v times of that in the cytoplasm. The last line represents the dissociation and association between NFkB and IkBb in the nucleus.

$$\begin{aligned} dNFkBn/dt = & d1 * IkBanNFkBn - a1 * NFkBn * IkBan + k_v * i1 * NFkB \\ & + d3 * IkBbnNFkBn - a3 * NFkBn * IkBbn \end{aligned} \quad (7^*)$$

IkBb: Free IkBb in the cytoplasm

The first and second lines represent the concentration change of free IkBb due to the dissociation and association between IkBb and NFkB, and between IKKa and IkBb; the last line represents free IkBb protein importing to, exporting from the nucleus, translation from IkBb mRNA and spontaneous degradation.

$$\begin{aligned} dIkBb/dt = & d3 * IkBbNFkB - a3 * NFkB * IkBb \\ & + d4 * IKKaIkBb - a4 * IKKa * IkBb \\ & - i1b * IkBb + e1b * IkBbn / k_v + t4 * IkBbm - de4 * IkBb \end{aligned} \quad (15)$$

IkBbNFkB: cytoplasmic IkBbNFkB complex

First line represents the concentration change of IkBbNFkB due to the association and dissociation between IkBb and NFkB; second line describes the the concentration change of IkBbNFkB due to the association and dissociation between IKKa and IkBb NFkB. NFkB increases the association rate of IKKa and IkBb according experimental data[9]. Based on the motif-motif interaction principle, the affinity of IKKa and IkBb doesn't change, so the dissociation between them also is increased by $f3$ times. The final line represents the exporting of IkBbNFkB from the nucleus and the degradation of IkBbNFkB.

$$\begin{aligned} dIkBanNFkB/dt = & a3 * NFkB * IkBa - d3 * IkBbNFkB \\ & - a4 * f3 * IKKa * IkBbNFkB + d4 * f3 * IKKaIkBbNFkB \end{aligned}$$

$$+ e2b * IkBbnNFkBn / kv - de5 * IkBbNFkB \quad (16)$$

IKKaIkBb: complex of IKKa and IkBb

The first line describes the concentration change of IKKaIkBb due to IkBb associates ,dissociates with IKKa and catalytic by IKKa; The second line represents association and dissociation between IKKaIkBb and NFkB.

$$\begin{aligned} dIKKaIkBa/dt &= a4 * IKKa * IkBb - d4 * IKKaIkBb - kcatb * IKKaIkBb \\ &\quad - a3 * NFkB * IKKaIkBb + d3 * IKKaIkBbNFkB \end{aligned} \quad (17)$$

IKKaIkBbNFkB: complex of IKKa, IkBb and NFkB

The first line represents the association and dissociation between IKKaIkBb and NFkB; the second line represents the association and dissociation between IKKa and IkBbNFkB; The final line describel the depletion of IKKaIkBbNFkB due to the catalysis process.

$$\begin{aligned} dIKKaIkBbNFkB/dt &= a3 * NFkB * IKKaIkBb - d3 * IKKaIkBbNFkB \\ &\quad + a4 * f3 * IKKa * IkBbNFkB - d4 * f3 * IKKaIkBbNFkB \\ &\quad - kcatb * f4 * IKKaIkBbNFkB \end{aligned} \quad (18)$$

IkBbm: IkBb messenger RNA

We separe the synthesis of IkBb mRNA to two parts. One is constuctive and the other is first-order dependent on free NFkB concentration in the nucleus. The last term represents first order degradation of IkBb mRNA.

$$dIkBbm/dt = cs4 + s4 * NFkBn - dm4 * IkBbm \quad (19)$$

NFX: unknown transcription factor

There is a time delay for IkBb transcription following NFkB activity.[23] This time delay may be caused by an intermidate transcription factor, like foxj1[24] . But we don't know much about this intermidate transcription factor. In the following equation, the first term represents the production of NFX translation from RNA, and the second and the third terms describe the concentration change of NFX due to shuttling between the cytoplasm and the nucleus; the final term represents the degradation of NFX.

$$dNFX/dt = t3 * NFXm - i3 * NFX + e3 * NFXn / kv - de6 * NFX \quad (20)$$

NFXn: NFX in the nucleus

First term represents NFX imports to the nucleus while the second term represents its exporting from the nucleus.

$$dNFXn/dt = kv * i3 * NFX - e3 * NFXn \quad (21)$$

NFXm: NFX messenger RNA

We assume that NFX is produced by two parts, one is constitutive synthesis rate $cs3$, and another part is induced by NFkB. The first term represents the constitutive synthesis of NFX mRNA and the second represents the inducible synthesis, while the final one represents the degradation of NFX mRNA.

$$dNFXm/dt = cs3 + s3 * NFkBn - dm3 * NFXm \quad (22)$$

IkBbn: Free IkBb in the nucleus

First line represents IkBb concentration change due to the dissociation and association between IkBb and NFkB; the second line represents IkBb concentration change caused by free IkBb shuttling between the cytoplasm and the nucleus.

$$dIkBbn/dt = d3 * IkBbnNFkBn - a3 * NFkBn * IkBbn + kv * i1b * IkBb - e1b * IkBbn \quad (23)$$

IkBbnNFkBn: IkBbNFkB complex in the nucleus

First line represents the concentration change due to the association and dissociation between NFkBn and IkBbn; the second line represents the exporting of IkBbnNFkBn from the nucleus.

$$dIkBbnNFkBn/dt = a3 * NFkBn * IkBbn - d3 * IkBbnNFkBn - e2b * IkBbnNFkBn \quad (24)$$

Supplement 2: Mathatical models coded in Matlab

% calc_Yhat_nfkb_01.m

```
function Y_hat = calc_Yhat_nfkb_01(k, X_pred);

%Dimension declarations:
n_diffeqn = 14;
n_algeb = 0;
n_tot = n_diffeqn + n_algeb;
id = ones(n_diffeqn,1);
id(n_diffeqn+1:n_tot) = zeros(n_algeb,1);

%Initial conditions:
[y0, data] = loadParams01(k, n_tot);
t0 = 0;
% set up options
%
options = CNodeSetOptions('RelTol',1.e-6,'AbsTol',1.e-6,...
                          'NonlinearSolver','Newton','MaxNumSteps',6000);
CNodeMalloc(@nfkb_core01,t0,y0,options,data);
tp0 = X_pred(1,1)-60*10;
[status,t,y] = CNode(tp0,'Normal');
if (status == 0)
    yp0 = y;
else
    yp0 = eps.*ones(n_tot,1);
end;
iout = 1;
Y = zeros(1,n_tot);
Y(1,:) = yp0';
nout = size(X_pred,1);
```

```

while iout < nout
    % update output time
    tout = X_pred(iout+1);
    [status,t,y] = CVode(tout,'Normal');
    if (status == 0)
        yout = y;
    else
        yout = eps.*ones(n_tot,1);
    end;
    Y = [Y; yout'];
    iout = iout+1;
end
CVodeFree;
%-----
% post-processing to compare to experimental data- free nuclear NFkB
%-----

%free nuclear NFkB
Free_NFkBn = zeros(size(Y,1),1);
Free_NFkBn = Y(:,7);
detect = yp0(7);
detect2= yp0(4)+yp0(5)+yp0(10)+yp0(13);
Yhat1 = Free_NFkBn ./ detect;
% total cytoplasmic Ikb protein and complex
TotalIkb = zeros(size(Y,1),1);
TotalIkb = Y(:,4)+ Y(:,5)+ Y(:,10) + Y(:,13);
Yhat2 = TotalIkb./ detect2;
%extract response predictions
Y_hat = [Yhat1 Yhat2];

return;

```

```

function[dydt, FLAG, NEW_DATA]=nfkb_core01(t,y_nfkb,data)
%dydt=zeros(size(y_nfkb));
%
%unpack initial concentrations
%
params      = data.params;
tstart      = data.tstart;
IKKn        = y_nfkb(1); % Free unactivated cytoplasmic IKK protein
IKKa        = y_nfkb(2); % Free activated cytoplasmic IKK protein
IKKi        = y_nfkb(3); % Free deactivated cytoplasmic IKK protein
IKKaIkBa    = y_nfkb(4); % Cytoplasmic IKKa-IkBa complexes
IKKaIkBaNFkB = y_nfkb(5); % Cytoplasmic IKKa-IkBa-NFkB protein
NFkB        = y_nfkb(6); % Free cytoplasmic NF-kB protein
NFkBn       = y_nfkb(7); % Free nuclear NF-kB protein
A20         = y_nfkb(8); % Cytoplasmic A20 protein
A20m        = y_nfkb(9); % A20 mRNA
IkBa        = y_nfkb(10); % Free cytoplasmic IkBa protein
IkBan       = y_nfkb(11); % Free nuclear IkBa protein
IkBam       = y_nfkb(12); % IkBa mRNA
IkBaNFkB    = y_nfkb(13); % Cytoplasmic IkBa-NFkB complexes
IkBanNFkBn  = y_nfkb(14); % Nuclear IkBa-NFkB complexes
%
%unpack times
%
start1 = tstart(1,1);
start2 = tstart(1,2);
start3 = tstart(1,3);
%
%=====
% Phase specific alterations to parameters

```

```

%=====
%unpack parameters
%
a1 = 10^params(1,1); %0.5 % microM-1 sec- IKBa2NFkB association Hoffmann et al.
a2 = 10^params(2,1); % mM-1 sec-1 IKKa IkBa association fitting
f1 = 10^params(3,1); %0.2 the factor of NFkB on IKKa-IkBa association Hoffmann et al.
kcat= 10^params(4,1); % sec-1 IKKajIkBa catalysis fitting
f2 = 10^params(5,1); %5% the factor of NFkB on IKKa-IkBa catalysis Hoffmann et al
d1 = 10^params(6,1); %IkBa-NFkB reversible dissociation rate    1.25e-3      s-1
    (2)
d2 = 10^params(7,1); %IKK-IkBa reversible dissociation rate    0.5e-3 s-1    (2)
s1 = 10^params(8,1); % 5.0e7 % sec-1 IkBa-inducible mRNA synthesis fitting
cs1 = 10^params(9,1); % 0.0 % mM sec-1 IkBa-constitutive mRNA synthesis
Assumption
dm1 = 10^params(10,1); % 0.0004 % sec-1 IkBa mRNA degradation Fitted, Blattner et al.
t1 = 10^params(11,1); % 0.5 % sec-1 IkBa translation rate Fitted lipniak 2004
de1 = 10^params(12,1); % 0.0001 % sec-1 Spontaneous, free IkBa protein degradation
Pando and Verma (2000)
de3 = 10^params(13,1); % 0.00002 % sec-1 IkBa degradation (complexed to NF-kB)
Pando and Verma (2000)
s2 = 10^params(14,1); % sec-1 A20-inducible mRNA synthesis fitting
cs2 = 10^params(15,1); % 0.0 % mM sec-1 A20-constitutive mRNA synthesis
Assumption
dm2 = 10^params(16,1); % sec-1 A20 mRNA degradation fitting
t2 = 10^params(17,1); % 0.5 % sec-1 A20 translation rate fitting
de2 = 10^params(18,1); % 0.0003 % sec-1 A20 protein degradation Fitted
k1 = 10^params(19,1); % 0.0025 % sec-1 IKK activation rate caused by TNF Fitted
k2 = 10^params(20,1); % 0.1 % sec-1 IKK inactivation rate caused by A20 Fitted
k3 = 10^params(21,1); % 0.0015 % sec-1 IKK spontaneous inactivation rate Fitted
kprod = 10^params(22,1); % 0.000025 % mM sec-1 IKKn production rate Fitted
kdeg = 10^params(23,1); % 0.000125 % sec-1 IKKa, IKKn and IKKi degradation Fitted

```



```

%NF 0:06 V mM V Total amount of free and complexed NF-kB
Assumption, Carlotti
kv = 10^params(24,1); % 5 % Cytoplasmic to nuclear volume Assumption
i1 = 10^params(25,1); % 0.0025 % sec-1 NF-kB nuclear import Fitted
i1a = 10^params(26,1); % 0.001 % sec-1 IkBa nuclear import Fitted
e1a = 10^params(27,1); % 0.0005 % sec-1 IkBa nuclear export Assumption
e2a = 10^params(28,1); % 0.01 % sec-1 (IkBaNf-kBp nuclear export Fitted
kd1 = 10^params(29,1); % 0.1 % mM Dissociation constant for NF-kB to kB1 sites
kd2 = 10^params(30,1); % 0.1 % mM Dissociation constant for NF-kB to kB2 sites
inhibited by DCPA
alpha1 = 10^params(31,1);% effect of DCPA on activation of IKK
alpha2 = 10^params(32,1);% effect of DCPA on inducible production of IkBat
dcpa = 10^params(33,1); %DCPA concentration
%
% Rate equations
%
R0 = 1.0 * (erf(2*(t-start2))-erf(2*(t-start3)));
R0a = dcpa * (erf(2*(t-start2))-erf(2*(t-start3)));
DCPA = R0a;
TLR4 = 1;
%Reaction class 1: IkBa-NFkB Association
R1a = a1 * NFkB * IkBa;
R1b = a1 * NFkBn * IkBan;
R1c = a1 * NFkB * IKKaIkBa;

%Reaction class 2: IKKa-IkBa Association
R2a = a2 * IKKa * IkBa;
R2b = a2 * f1 * IKKa * IkBaNFkB;

%Reaction class 3: IkBa-NFkB dissociation
R3a = d1 * IkBaNFkB;

```

$$R3b = d1 * IkBanNFkBn;$$

$$R3c = d1 * IKKaIkBanNFkB;$$

%Reaction class 4: IKKa-IkBa dissociation

$$R4a = d2 * IKKaIkBa;$$

$$R4b = d2 * f1 * IKKaIkBanNFkB;$$

%Reaction class 5: IKKa-IkBa Catalytic dissociation

$$R5a = kcat * IKKaIkBa; \% * (1 + \alpha1 * DCPA / (DCPA + kd1));$$

$$R5b = kcat * f2 * IKKaIkBanNFkB; \% * (1 + \alpha1 * DCPA / (DCPA + kd1));$$

%Reaction class 6: Degradation of IKK

$$R6a = kdeg * IKKn;$$

$$R6b = kdeg * IKKa;$$

$$R6c = kdeg * IKKi;$$

%Reaction class 7: Degradation of free IkBa proteins

$$R7 = de1 * IkBa;$$

%Reaction class 8: Degradation of IkBa complex to NFkB

$$R8 = de3 * IkBanNFkB;$$

%Reaction class 9: Degradation of A20 proteins

$$R9 = de2 * A20;$$

%Reaction class 10: Degradation of IkBa mRNA

$$R10 = dm1 * IkBam;$$

%Reaction class 11: Degradation of A20 mRNA

$$R11 = dm2 * A20m;$$

%Reaction class 12: Synthesis of IKK α

$R_{12} = k_{prod};$

%Reaction class 13: Inducible synthesis of IkBa mRNA

$R_{13} = s_1 * NFkBn * (1 - \alpha_2 * DCPA / (DCPA + k_{d2}));$

%Reaction class 14: Inducible synthesis of A20 mRNA

$R_{14} = s_2 * NFkBn * (1 - \alpha_2 * DCPA / (DCPA + k_{d2}));$

%Reaction class 15: Constitutive synthesis of IkBa mRNA

$R_{15} = cs_1;$

%Reaction class 16: Constitutive synthesis of A20 mRNA

$R_{16} = cs_2;$

%Reaction class 17: Translation from IkBa mRNA to IkBa proteins

$R_{17} = t_1 * IkBam;$

%Reaction class 18: Translation from A20 mRNA to A20 proteins

$R_{18} = t_2 * A20m;$

%Reaction class 19: Activation of IKK α to IKK γ

$R_{19} = k_1 * R_0 * IKK\alpha * TLR4 * (1 + \alpha_1 * DCPA / (DCPA + k_{d1}));$

%Reaction class 20: Inactivation of IKK γ because of A20

$R_{20} = k_2 * R_0 * A20 * IKK\gamma;$

%Reaction class 21: Spontaneous Inactivation of IKK γ to IKK δ

$R_{21} = k_3 * IKK\gamma;$

%Reaction class 22: IkBa Import to the nucleus

R22= i1a * IkBa;

%Reaction class 23: IkBa export from the nucleus to cytoplasm

R23 = e1a * IkBan;

%Reaction class 24: IkBanNFkBn export from the nucleus to cytoplasm

R24 = e2a * IkBanNFkBn;

%Reaction class 25: NFkB import to the nucleus from cytoplasm

R25 = i1 * NFkB * (1 + alpha2*DCPA/(DCPA+kd1));

%Differential equations that define the mathematical model for NFkB signaling pathway.

dydt(1) = R12 - R19 - R6a; % IKKn
dydt(2) = R19 - R20 - R21- R6b - R2a - R2b + R4a + R4b + R5a + R5b; %IKKa
dydt(3) = R20 + R21 - R6c; %IKKi
dydt(4) = R2a - R4a - R5a - R1c + R3c; %IKKaIkBa/dt
dydt(5) = R1c + R2b- R3c - R4b - R5b; %IKKaIkBaNFkB/dt
dydt(6) = R3a + R3c - R1a- R1c + R5b + R8 - R25; %NFkB/dt
dydt(7) = R3b + kv * R25 - R1b; %NFkBn/dt
dydt(8) = R18 - R9 - R20; %dA20/dt
dydt(9) = R16 + R14 -R11; %dA20m/dt
dydt(10) = R3a -R1a + R4a -R2a -R22+R23/kv +R17-R7; %dIkBa/dt
dydt(11) = R3b - R1b + kv * R22 - R23; %dIkBan/dt
dydt(12) = R13+ R15 - R10; %dIkBam/dt
dydt(13) = R1a - R3a - R2b +R4b + R24 / kv - R8; %dIkBaNFkB/dt
dydt(14) = R1b - R3b - R24; %dIkBanNFkBn/dt

FLAG = 0;

NEW_DATA = [];

```

return;

function [y0, data] = loadParams01(k, n_tot)

% Initial conditions:
y0 = zeros(n_tot,1);
y0(1,1) = 0.02;      % mico M IKKn
%y0(10,1) = 0.06;    % Rice and Ernst(1993)
y0(13,1) = 0.06;     % mico M IkBa-NFkB cheong 2006

%-----
% PARAMETERS
%-----

params = zeros(33,1);
params(1,1) = log10(0.5); % 0.5 a1 microM-1 sec-1 IkBa-NFkB association
Hoffmann et al. (2002)
params(2,1) = log10(0.0225); % a2 microM-1 sec-1 IKKa-IkBa association
Assumption
params(3,1) = log10(7); %5 f1 the factor of NFkB on IKKa-IkBa association
Hoffmann et al.
params(4,1) = log10(0.00407); % kcat % sec-1 IKKajIkBa catalysis fitting
params(5,1) = log10(5); %f2% the factor of NFkB on IKKa-IkBa catalysis
Hoffmann et al
params(6,1) = log10(0.0005); %d1 %IkBa-NFkB reversible dissociation rate
1.25e-3 s-1 (2)
params(7,1) = log10(1.25e-3); % d2 = 10^params(7,1); %IKK-IkBa reversible
dissociation rate 0.5e-3 s-1 (2)
params(8,1) = k(1,1); % s1 = 10^params(8,1); % 5.0e7 % sec-1 IkBa-inducible
mRNA synthesis fitting
params(9,1) = log10(1.0256e-11); % cs1 = 10^params(9,1); % 0.0 % mM sec-1 IkBa-
constitutive mRNA synthesis Assumption

```

$\text{params}(10,1) = \log_{10}(0.0004);$ % $\text{dm1} = 10^{\text{params}(10,1)};$ % 0.0004 % sec-1 IkBa
 mRNA degradation Fitted, Blattner et al.
 $\text{params}(11,1) = k(2,1);$ % $\text{t1} = 10^{\text{params}(11,1)};$ % 0.5 % sec-1 IkBa translation rate
 Fitted lipniak 2004
 $\text{params}(12,1) = \log_{10}(0.000113);$ % $\text{de1} = 10^{\text{params}(12,1)};$ % 0.0001 % sec-1
 Spontaneous, free IkBa protein degradation Pando and Verma (2000)
 $\text{params}(13,1) = \log_{10}(0.0000225);$ % $\text{de3} = 10^{\text{params}(13,1)};$ % 0.00002 % sec-1 IkBa
 degradation (complexed to NF-kB) Pando and Verma (2000)
 $\text{params}(14,1) = k(3,1);$ % $\text{s2} = 10^{\text{params}(14,1)};$ % sec-1 A20-inducible mRNA
 synthesis fitting
 $\text{params}(15,1) = k(4,1);$ % $\text{cs2} = 10^{\text{params}(15,1)};$ % 0.0 % mM sec-1 A20-constitutive
 mRNA synthesis Assumption
 $\text{params}(16,1) = k(5,1);$ % $\text{dm2} = 10^{\text{params}(16,1)};$ % sec-1 A20 mRNA
 degradation fitting
 $\text{params}(17,1) = k(6,1);$ % $\text{t2} = 10^{\text{params}(17,1)};$ % 0.5 % sec-1 A20 translation rate
 fitting
 $\text{params}(18,1) = \log_{10}(0.0003);$ % $\text{de2} = 10^{\text{params}(18,1)};$ % 0.0003 % sec-1 A20
 protein degradation Fitted
 $\text{params}(19,1) = k(7,1);$ % $k1$ sec-1 0.0025 IKK activation rate caused by TNF
 Fitted
 $\text{params}(20,1) = k(8,1);$ % $k2$ sec-1 IKK inactivation rate caused by A20 Fitted
 $\text{params}(21,1) = \log_{10}(3.2374e-10);$ % $k3$ sec-1 IKK spontaneous inactivation rate
 Fitted
 $\text{params}(22,1) = k(9,1);$ % k_{prod} IKKn synthesis rate
 $\text{params}(23,1) = k(10,1);$ % k_{deg} sec-1 IKKa, IKKn and IKKi degradation Fitted
 $\text{params}(24,1) = \log_{10}(7);$ % k_v 7 Cytoplasmic to nuclear volume Assumption
 7 Cheong 2006, 5 Lipniacki 2004, 2006
 $\text{params}(25,1) = k(12,1);$ % $\log_{10}(0.0013);$ % $i1$ sec-1 NF-kB nuclear import Fitted
 %0.0025 Lipniacki 2004, 2006; 0.09 Werner 2005
 $\text{params}(26,1) = k(11,1) + \log_{10}(2);$ % $i1a$ sec-1 IkBa nuclear import Fitted
 %0.001 Lipniacki 2004, 0.002 Lipniacki 2006, 0.0003 Werner 2005

```

params(27,1) = k(11,1); % e1a sec-1 IkBa nuclear export Assumption
                        %0.0005 Lipniacki 2004, 0.005 Lipniacki 2006, 0.0002 Werner 2005
params(28,1) = k(13,1); % e2a sec-1 IkBa-NF-kB nuclear export Fitted
                        %0.01 Lipniacki 2004, 0.05 Lipniacki 2006, 0.014 Werner 2005

params(29,1) = log10(5); % kd1 mM Half-maximum DCPA concentration
params(30,1) = log10(5); % kd2 mM Half-maximum DCPA concentration
params(31,1) = log10(0.48); % alpha1 Effect of DCPA on activating IKK
params(32,1) = log10(0.013); % alpha2 effect of DCPA on NFkB inducible
                        producing IkBa mRNA
params(33,1) = log10(1.0e-20 ); %DCPA concentration is 100 uM

if size(params,1) == 1
    params = params'; % convert K to a column vector instead of a row vector
end;

% start and stop times of LPS bolus in nfkb_core01 file
tstart = [0 2000*60 6*60*60+2000*60];
%pack parameters into data struct
data.params = params;
data.tstart = tstart;

return;
function [kin] = InitParams01()
% Load paramaters - mean values from MCMC run
kin = zeros(13,1);

kin(1,1) = log10(4.2615e-5); % s1 = 10^params(8,1); % 5.0e7 % sec-1 IkBa-inducible
mRNA synthesis fitting
%kin(2,1) = log10(1.0256e-11) ; % cs1 = 10^params(9,1); % 0.0 % mM sec-1 IkBa-
constitutive mRNA synthesis Assumption

```

```

kin(2,1) = log10(0.6592); % t1 IkBa translation rate
kin(3,1) = log10(0.0967); % s2 = 10^params(14,1); % sec-1 A20-inducible mRNA
synthesis fitting
kin(4,1) = log10(8.634e-7); % cs2 = 10^params(15,1); % 0.0 % mM sec-1 A20-
constitutive mRNA synthesis Assumption
kin(5,1) = log10(0.0716); % dm2 = 10^params(16,1); % sec-1 A20 mRNA
degradation fitting
kin(6,1) = log10(0.4138); %t2 = 10^params(17,1); % 0.5 % sec-1 A20 translation rate
fitting
kin(7,1) = log10(0.0016); % k1 sec-1 0.0025 IKK activation rate caused by TNF
Fitted
kin(8,1) = log10(0.2462); % k2 sec-1 IKK inactivation rate caused by A20 Fitted
%kin(10,1) = log10(3.2374e-10); % k3 sec-1 IKK spontaneous inactivation rate
Fitted
kin(9,1) = log10(4.707e-4); % kprod microM sec-1 IKKn production rate Fitted
kin(10,1) = log10(0.0031); % kdeg sec-1 IKKa, IKKn and IKKi degradation Fitted
kin(11,1) = log10(1.3858e-4); % e1a sec-1 IkBa nuclear export Assumption
%0.0005 Lipniacki 2004, 0.005 Lipniacki 2006, 0.0002 Werner 2005
kin(12,1) = log10(4.3958e-4); % i1 sec-1 NF-kB nuclear import Fitted
%0.0025 Lipniacki 2004, 2006; 0.09 Werner 2005
kin(13,1) = log10(0.002); % e2a sec-1 IkBa-NF-kB nuclear export Fitted
if size(kin,1) == 1
    kin = kin'; % convert k to a column vector instead of a row vector
end;

return;
function [data] = data_nfkb01
% Populate Propanil data array
% Initialization
data=struct('ppn',{ },'tdata',{ },'values',{ });

```



```

% Load data arrays
% tdata = [[0];[5];[7];[10];[11];[12];[15];[13];[16];[19];[22];[30-
4.5];[28];[31];[33];[35];[39];[45-4.5];[42];[43];[45];[47];[49];[51];[53];[60-
4.5];[57];[59];[61];[63];[65];[67];[69];[75-4.5];[73];[75];[77];[79];[81];[83];[85];[90-
4.5];[87];[89];[91];[93];[95];[97];[99];[105-
4.5];[103];[105];[107];[109];[111];[113];[115];[120-
4.5];[116];[117];[119];[121];[123];[125];[127];[130];[132];[134];[136];[138];[140];[142]
];[150-
4.5];[147];[149];[152];[155];[157];[159];[161];[163];[165];[167];[169];[171];[173];[175]
];[180-
4.5];[177];[179];[182];[185];[187];[189];[192];[195];[197];[199];[201];[203];[204];[205]
];[210-4.5];[207];[210];[215];[220];[225];[230];[233];[240-
4.5];[237];[240];[245];[250];[255];[260];[263];[270-
4.5];[267];[270];[275];[280];[285];[290];[293];[300-
4.5];[297];[300];[305];[310];[315];[320];[325];[330-
4.5];[327];[330];[335];[340];[345];[350];[355];[360-4.5]];
% Experiment 1
% data(1).ppn = 0;
% data(1).tdata = tdata .* 60 + 2000 * 60;
% data(1).values = [[1 1];[1.47 1];[3 0];[6.51 1];[7.5 0];[10 0];[9.71 1];[13 0];[19 0];[22
0];[16 0];[13.22 1];[28 0];[31 0];[33 0];[34.5 0];[39 0];[6.43 1];[42 0];[43 0];[45 0];[47
0];[49 0];[51 0];[53 0];[5.64 1];[57 0];[59 0];[61 0];[63 0];[65 0];[67 0];[69 0];[5.27
1];[73 0];[75 0];[77 0];[79 0];[81 0];[83 0];[85 0];[10.08 1];[73 0];[75 0];[77 0];[79
0];[81 0];[83 0];[85 0];[9.28 1];[73 0];[75 0];[77 0];[79 0];[81 0];[83 0];[85 0];[6.95
1];[73 0];[75 0];[77 0];[79 0];[81 0];[83 0];[85 0];[73 0];[75 0];[77 0];[79 0];[81 0];[83
0];[85 0];[5.67 1];[73 0];[75 0];[77 0];[79 0];[81 0];[83 0];[85 0];[73 0];[75 0];[77 0];[79
0];[81 0];[83 0];[85 0];[7.31 1];[73 0];[75 0];[77 0];[79 0];[81 0];[83 0];[85 0];[73 0];[75
0];[77 0];[79 0];[81 0];[83 0];[85 0];[7.51 0];[73 0];[75 0];[77 0];[79 0];[81 0];[83 0];[85
0];[6.88 0];[73 0];[75 0];[77 0];[79 0];[81 0];[83 0];[85 0];[6.77 0];[73 0];[75 0];[77
0];[79 0];[81 0];[83 0];[85 0];[7.84 0];[73 0];[75 0];[77 0];[79 0];[81 0];[83 0];[85
0];[7.47 0];[73 0];[75 0];[77 0];[79 0];[81 0];[83 0];[85 0];[8.23 0]];

```

% total cytoplasmic IκB

```
%data(1).values = [[1 1];[1.47 1];[3 0];[6.51 1];[7.5 0];[10 0];[9.71 1];[13 0];[19 0];[22  
0];[16 0];[13.22 1];[28 0];[31 0];[33 0];[34.5 0];[39 0];[6.43 1];[42 0];[43 0];[45 0];[47  
0];[49 0];[51 0];[53 0];[5.64 1];[57 0];[59 0];[61 0];[63 0];[65 0];[67 0];[69 0];[5.27  
1];[73 0];[75 0];[77 0];[79 0];[81 0];[83 0];[85 0];[10.08 1];[73 0];[75 0];[77 0];[79  
0];[81 0];[83 0];[85 0];[9.28 1];[73 0];[75 0];[77 0];[79 0];[81 0];[83 0];[85 0];[6.95  
1];[73 0];[75 0];[77 0];[79 0];[81 0];[83 0];[85 0];[73 0];[75 0];[77 0];[79 0];[81 0];[83  
0];[85 0];[5.67 1];[73 0];[75 0];[77 0];[79 0];[81 0];[83 0];[85 0];[73 0];[75 0];[77 0];[79  
0];[81 0];[83 0];[85 0];[7.31 1];[73 0];[75 0];[77 0];[79 0];[81 0];[83 0];[85 0];[73 0];[75  
0];[77 0];[79 0];[81 0];[83 0];[85 0];[7.51 1];[73 0];[75 0];[77 0];[79 0];[81 0];[83 0];[85  
0];[6.88 1];[73 0];[75 0];[77 0];[79 0];[81 0];[83 0];[85 0];[6.77 1];[73 0];[75 0];[77  
0];[79 0];[81 0];[83 0];[85 0];[7.84 1];[73 0];[75 0];[77 0];[79 0];[81 0];[83 0];[85  
0];[7.47 1];[73 0];[75 0];[77 0];[79 0];[81 0];[83 0];[85 0];[8.23 1]];
```

% Cyto. IκBa

```
%data(3).ppn = 0;
```

```
%data(3).tdata = tdata .*60+ 2000*60;
```

```
%data(3).values = [[1 1];[0.97 1];[3 0];[0.567 1];[7.5 0];[10 0];[0.255 1];[13 0];[19 0];[22  
0];[16 0];[0.482 1];[28 0];[31 0];[33 0];[34.5 0];[39 0];[0.950 1];[42 0];[43 0];[45 0];[47  
0];[49 0];[51 0];[53 0];[0.764 1];[57 0];[59 0];[61 0];[63 0];[65 0];[67 0];[69 0];[0.794  
1];[73 0];[75 0];[77 0];[79 0];[81 0];[83 0];[85 0];[0.769 1];[73 0];[75 0];[77 0];[79  
0];[81 0];[83 0];[85 0];[0.592 1];[73 0];[75 0];[77 0];[79 0];[81 0];[83 0];[85 0];[0.587  
1];[73 0];[75 0];[77 0];[79 0];[81 0];[83 0];[85 0];[73 0];[75 0];[77 0];[79 0];[81 0];[83  
0];[85 0];[0.663 1];[73 0];[75 0];[77 0];[79 0];[81 0];[83 0];[85 0];[73 0];[75 0];[77  
0];[79 0];[81 0];[83 0];[85 0];[0.608 1];[73 0];[75 0];[77 0];[79 0];[81 0];[83 0];[85  
0];[73 0];[75 0];[77 0];[79 0];[81 0];[83 0];[85 0];[0.733 0];[73 0];[75 0];[77 0];[79  
0];[81 0];[83 0];[85 0];[0.688 0];[73 0];[75 0];[77 0];[79 0];[81 0];[83 0];[85 0];[0.849  
0];[73 0];[75 0];[77 0];[79 0];[81 0];[83 0];[85 0];[0.693 0];[73 0];[75 0];[77 0];[79  
0];[81 0];[83 0];[85 0];[0.869 0];[73 0];[75 0];[77 0];[79 0];[81 0];[83 0];[85 0];[0.608 0]];  
%data(3).values = [[1 1];[0.97 1];[3 0];[0.567 1];[7.5 0];[10 0];[0.255 1];[13 0];[19 0];[22  
0];[16 0];[0.482 1];[28 0];[31 0];[33 0];[34.5 0];[39 0];[0.950 1];[42 0];[43 0];[45 0];[47  
0];[49 0];[51 0];[53 0];[0.764 1];[57 0];[59 0];[61 0];[63 0];[65 0];[67 0];[69 0];[0.794
```

```

1];[73 0];[75 0];[77 0];[79 0];[81 0];[83 0];[85 0];[0.769 1];[73 0];[75 0];[77 0];[79
0];[81 0];[83 0];[85 0];[0.592 1];[73 0];[75 0];[77 0];[79 0];[81 0];[83 0];[85 0];[0.587
1];[73 0];[75 0];[77 0];[79 0];[81 0];[83 0];[85 0];[73 0];[75 0];[77 0];[79 0];[81 0];[83
0];[85 0];[0.663 1];[73 0];[75 0];[77 0];[79 0];[81 0];[83 0];[85 0];[73 0];[75 0];[77
0];[79 0];[81 0];[83 0];[85 0];[0.608 1];[73 0];[75 0];[77 0];[79 0];[81 0];[83 0];[85
0];[73 0];[75 0];[77 0];[79 0];[81 0];[83 0];[85 0];[0.733 1];[73 0];[75 0];[77 0];[79
0];[81 0];[83 0];[85 0];[0.688 1];[73 0];[75 0];[77 0];[79 0];[81 0];[83 0];[85 0];[0.849
1];[73 0];[75 0];[77 0];[79 0];[81 0];[83 0];[85 0];[0.693 1];[73 0];[75 0];[77 0];[79
0];[81 0];[83 0];[85 0];[0.869 1];[73 0];[75 0];[77 0];[79 0];[81 0];[83 0];[85 0];[0.608 1]];

```

```

%Experiment 2

```

```

%data(2).ppn = 100;

```

```

%data(2).tdata = tdata.*60+2000*60;

```

```

%data(2).values = [[1 1];[1.41 1];[3 0];[5.60 1];[7.5 0];[10 0];[11.11 1];[13 0];[19 0];[22
0];[16 0];[10.77 1];[28 0];[31 0];[33 0];[34.5 0];[39 0];[6.01 1];[42 0];[43 0];[45 0];[47
0];[49 0];[51 0];[53 0];[6.21 1];[57 0];[59 0];[61 0];[63 0];[65 0];[67 0];[69 0];[7.93
1];[73 0];[75 0];[77 0];[79 0];[81 0];[83 0];[85 0];[9.29 1];[73 0];[75 0];[77 0];[79 0];[81
0];[83 0];[85 0];[7.49 1];[73 0];[75 0];[77 0];[79 0];[81 0];[83 0];[85 0];[5.72 1];[73
0];[75 0];[77 0];[79 0];[81 0];[83 0];[85 0];[73 0];[75 0];[77 0];[79 0];[81 0];[83 0];[85
0];[5.75 1];[73 0];[75 0];[77 0];[79 0];[81 0];[83 0];[85 0];[73 0];[75 0];[77 0];[79 0];[81
0];[83 0];[85 0];[8.80 1];[73 0];[75 0];[77 0];[79 0];[81 0];[83 0];[85 0];[73 0];[75 0];[77
0];[79 0];[81 0];[83 0];[85 0];[7.58 0];[73 0];[75 0];[77 0];[79 0];[81 0];[83 0];[85
0];[6.84 0];[73 0];[75 0];[77 0];[79 0];[81 0];[83 0];[85 0];[6.57 0];[73 0];[75 0];[77
0];[79 0];[81 0];[83 0];[85 0];[8.18 0];[73 0];[75 0];[77 0];[79 0];[81 0];[83 0];[85
0];[7.78 0];[73 0];[75 0];[77 0];[79 0];[81 0];[83 0];[85 0];[9.20 0]];

```

```

%data(2).values = [[1 1];[1.41 1];[3 0];[5.60 1];[7.5 0];[10 0];[11.11 1];[13 0];[19 0];[22
0];[16 0];[10.77 1];[28 0];[31 0];[33 0];[34.5 0];[39 0];[6.01 1];[42 0];[43 0];[45 0];[47
0];[49 0];[51 0];[53 0];[6.21 1];[57 0];[59 0];[61 0];[63 0];[65 0];[67 0];[69 0];[7.93
1];[73 0];[75 0];[77 0];[79 0];[81 0];[83 0];[85 0];[9.29 1];[73 0];[75 0];[77 0];[79 0];[81
0];[83 0];[85 0];[7.49 1];[73 0];[75 0];[77 0];[79 0];[81 0];[83 0];[85 0];[5.72 1];[73
0];[75 0];[77 0];[79 0];[81 0];[83 0];[85 0];[73 0];[75 0];[77 0];[79 0];[81 0];[83 0];[85

```

```

0];[5.75 1];[73 0];[75 0];[77 0];[79 0];[81 0];[83 0];[85 0];[73 0];[75 0];[77 0];[79 0];[81
0];[83 0];[85 0];[8.80 1];[73 0];[75 0];[77 0];[79 0];[81 0];[83 0];[85 0];[73 0];[75 0];[77
0];[79 0];[81 0];[83 0];[85 0];[7.58 1];[73 0];[75 0];[77 0];[79 0];[81 0];[83 0];[85
0];[6.84 1];[73 0];[75 0];[77 0];[79 0];[81 0];[83 0];[85 0];[6.57 1];[73 0];[75 0];[77
0];[79 0];[81 0];[83 0];[85 0];[8.18 1];[73 0];[75 0];[77 0];[79 0];[81 0];[83 0];[85
0];[7.78 1];[73 0];[75 0];[77 0];[79 0];[81 0];[83 0];[85 0];[9.20 1]];
%Cyto.IkB

```

```

% total cytoplasmic IkB

```

```

%data(4).ppn = 100;
%data(4).tdata = tdata .*60+ 2000*60;
%data(4).values = [[0.949 1];[0.864 1];[3 0];[0.376 1];[7.5 0];[10 0];[0.235 1];[13 0];[19
0];[22 0];[16 0];[0.430 1];[28 0];[31 0];[33 0];[34.5 0];[39 0];[0.928 1];[42 0];[43 0];[45
0];[47 0];[49 0];[51 0];[53 0];[0.726 1];[57 0];[59 0];[61 0];[63 0];[65 0];[67 0];[69
0];[0.897 1];[73 0];[75 0];[77 0];[79 0];[81 0];[83 0];[85 0];[0.630 1];[73 0];[75 0];[77
0];[79 0];[81 0];[83 0];[85 0];[0.559 1];[73 0];[75 0];[77 0];[79 0];[81 0];[83 0];[85
0];[0.544 1];[73 0];[75 0];[77 0];[79 0];[81 0];[83 0];[85 0];[73 0];[75 0];[77 0];[79
0];[81 0];[83 0];[85 0];[0.623 1];[73 0];[75 0];[77 0];[79 0];[81 0];[83 0];[85 0];[73
0];[75 0];[77 0];[79 0];[81 0];[83 0];[85 0];[0.552 1];[73 0];[75 0];[77 0];[79 0];[81
0];[83 0];[85 0];[73 0];[75 0];[77 0];[79 0];[81 0];[83 0];[85 0];[0.596 0];[73 0];[75
0];[77 0];[79 0];[81 0];[83 0];[85 0];[0.545 0];[73 0];[75 0];[77 0];[79 0];[81 0];[83
0];[85 0];[0.771 0];[73 0];[75 0];[77 0];[79 0];[81 0];[83 0];[85 0];[0.589 0];[73 0];[75
0];[77 0];[79 0];[81 0];[83 0];[85 0];[0.558 0];[73 0];[75 0];[77 0];[79 0];[81 0];[83
0];[85 0];[0.532 0]];
%data(4).values = [[0.949 1];[0.864 1];[3 0];[0.376 1];[7.5 0];[10 0];[0.235 1];[13 0];[19
0];[22 0];[16 0];[0.430 1];[28 0];[31 0];[33 0];[34.5 0];[39 0];[0.928 1];[42 0];[43 0];[45
0];[47 0];[49 0];[51 0];[53 0];[0.726 1];[57 0];[59 0];[61 0];[63 0];[65 0];[67 0];[69
0];[0.897 1];[73 0];[75 0];[77 0];[79 0];[81 0];[83 0];[85 0];[0.630 1];[73 0];[75 0];[77
0];[79 0];[81 0];[83 0];[85 0];[0.559 1];[73 0];[75 0];[77 0];[79 0];[81 0];[83 0];[85
0];[0.544 1];[73 0];[75 0];[77 0];[79 0];[81 0];[83 0];[85 0];[73 0];[75 0];[77 0];[79
0];[81 0];[83 0];[85 0];[0.623 1];[73 0];[75 0];[77 0];[79 0];[81 0];[83 0];[85 0];[73
0];[75 0];[77 0];[79 0];[81 0];[83 0];[85 0];[0.552 1];[73 0];[75 0];[77 0];[79 0];[81
0];[83 0];[85 0];[73 0];[75 0];[77 0];[79 0];[81 0];[83 0];[85 0];[0.596 0];[73 0];[75
0];[77 0];[79 0];[81 0];[83 0];[85 0];[0.545 0];[73 0];[75 0];[77 0];[79 0];[81 0];[83
0];[85 0];[0.771 0];[73 0];[75 0];[77 0];[79 0];[81 0];[83 0];[85 0];[0.589 0];[73 0];[75
0];[77 0];[79 0];[81 0];[83 0];[85 0];[0.558 0];[73 0];[75 0];[77 0];[79 0];[81 0];[83
0];[85 0];[0.532 0]];

```

```

0];[75 0];[77 0];[79 0];[81 0];[83 0];[85 0];[0.552 1];[73 0];[75 0];[77 0];[79 0];[81
0];[83 0];[85 0];[73 0];[75 0];[77 0];[79 0];[81 0];[83 0];[85 0];[0.596 1];[73 0];[75
0];[77 0];[79 0];[81 0];[83 0];[85 0];[0.545 1];[73 0];[75 0];[77 0];[79 0];[81 0];[83
0];[85 0];[0.771 1];[73 0];[75 0];[77 0];[79 0];[81 0];[83 0];[85 0];[0.589 1];[73 0];[75
0];[77 0];[79 0];[81 0];[83 0];[85 0];[0.558 1];[73 0];[75 0];[77 0];[79 0];[81 0];[83
0];[85 0];[0.532 1]];

```

```

tdata = [[0];[1];[2];[4];[5];[7];[9];[10];[12];[14];[15];[17];[20];[24];[27];[30];
[33];[36];[40];[42];[45];[47];[50];[53];[57];[60];[63];[67];[70];[72];[75];[77];[80];[83];[8
7];[90];[93];[97];[100];[102];[105];[108];[110];[113];[117];[119];[120];[125];[130];[135]
;[140];[145];[147];[150];[155];[160];[165];[170];[175];
[180];[185];[190];[195];[200];[205];[210];[215];[220];[225];[230];[235];[240];[245];[25
0];[255];[260];[265];[270];[275];[280];[285];[290];[295];[300];[305];[310];[315];[320];[
323];[327];[329];[330];[335];[340];[343];[347];[350];[355];[360]];

```

```

data(1).ppn = 0;

```

```

data(1).tdata = tdata .*60+ 2000*60;

```

```

%NFkB in the nucleus

```

```

data(1).values = [[1 1];[1 0];[1 0];[1 0];[1.47 1];[1 0];[1 0];[6.51 1];[1 0];[1 0];[9.71 1];[1
0];[1 0];[1 0];[1 0];[13.22 1];[1 0];[1 0];[1 0];[1 0];[6.43 1];[1 0];[1 0];[1 0];[1 0];[5.64
1];[1 0];[1 0];[1 0];[1 0];[5.27 1];[1 0];[1 0];[1 0];[1 0];[10.08 1];[1 0];[1 0];[1 0];[1
0];[9.28 0];[1 0];[1 0];[1 0];[1 0];[1 0];[6.95 0];[1 0];[1 0];[1 0];[1 0];[1 0];[5.67
0];[1 0];[1 0];[1 0];[1 0];[1 0];[7.31 0];[1 0];[1 0];[1 0];[1 0];[1 0];[7.51 0];[1 0];[1 0];[1
0];[1 0];[1 0];[6.88 0];[1 0];[1 0];[1 0];[1 0];[1 0];[6.77 0];[1 0];[1 0];[1 0];[1 0];[1
0];[7.84 0];[1 0];[1 0];[1 0];[1 0];[1 0];[1 0];[1 0];[7.47 0];[1 0];[1 0];[1 0];[1 0];[1 0];[1
0];[8.23 0]];

```

```

% total IkBa in the cytoplasm

```

```

data(3).ppn = 0;

```

```

data(3).tdata = tdata .*60+ 2000*60;

```



```

0];[1 0];[8.18 0];[1 0];[1 0];[1 0];[1 0];[1 0];[1 0];[7.78 0];[1 0];[1 0];[1 0];[1 0];[1
0];[1 0];[9.20 0]];
% total IkBa in the cytoplasm
% total IkBa in the cytoplasm
data(4).ppn = 100;
data(4).tdata = tdata .*60+ 2000*60;
data(4).values = [[1.0 1];[1 0];[0.96 1];[1 0];[0.87 1];[1 0];[1 0];[0.38 1];[1 0];[1 0];[0.25
1];[1 0];[1 0];[1 0];[1 0];[0.44 1];[1 0];[1 0];[1 0];[1 0];[0.93 1];[1 0];[1 0];[1 0];[1
0];[0.73 1];[1 0];[1 0];[1 0];[1 0];[0.90 1];[1 0];[1 0];[1 0];[1 0];[0.64 1];[1 0];[1 0];[1
0];[1 0];[0.57 0];[1 0];[1 0];[1 0];[1 0];[1 0];[0.56 0];[1 0];[1 0];[1 0];[1 0];[1 0];[1
0];[0.63 0];[1 0];[1 0];[1 0];[1 0];[1 0];[0.57 0];[1 0];[1 0];[1 0];[1 0];[1 0];[0.62 0];[1
0];[1 0];[1 0];[1 0];[1 0];[0.55 0];[1 0];[1 0];[1 0];[1 0];[1 0];[0.79 0];[1 0];[1 0];[1 0];[1
0];[1 0];[0.60 0];[1 0];[1 0];[1 0];[1 0];[1 0];[1 0];[1 0];[0.58 0];[1 0];[1 0];[1 0];[1 0];[1
0];[1 0];[0.55 0]];
% total IkBb in the cytoplasm
% total IkBb in the cytoplasm
data(6).ppn = 0;
data(6).tdata = tdata .*60+ 2000*60;
data(6).values = [[0.73 1];[1 0];[0.56 1];[1 0];[0.54 1];[1 0];[1 0];[0.43 1];[1 0];[1
0];[0.28 1];[1 0];[1 0];[1 0];[1 0];[0.23 1];[1 0];[1 0];[1 0];[1 0];[0.22 1];[1 0];[1 0];[1
0];[1 0];[0.20 1];[1 0];[1 0];[1 0];[1 0];[0.21 1];[1 0];[1 0];[1 0];[1 0];[0.23 1];[1 0];[1
0];[1 0];[1 0];[0.34 0];[1 0];[1 0];[1 0];[1 0];[1 0];[0.34 0];[1 0];[1 0];[1 0];[1 0];[1 0];[1
0];[0.43 0];[1 0];[1 0];[1 0];[1 0];[1 0];[0.43 0];[1 0];[1 0];[1 0];[1 0];[1 0];[0.47 0];[1
0];[1 0];[1 0];[1 0];[1 0];[0.45 0];[1 0];[1 0];[1 0];[1 0];[1 0];[0.72 0];[1 0];[1 0];[1 0];[1
0];[1 0];[0.65 0];[1 0];[1 0];[1 0];[1 0];[1 0];[1 0];[1 0];[1 0];[0.62 0];[1 0];[1 0];[1 0];[1
0];[1 0];[0.63 0]];
return;

```

Supplement 3.1 : Gel image for I κ B α in the cytoplasm

Supplement 3.2 : Gel image for β -actin

Supplement 3.3 : Gel image for IκBβ in the cytoplasm

Supplement 3.4 : Gel image for Free NFκB in the nucleus

10-13-05

p65 Nuclear
longer
exposure

KODH: 910900 LIGHT 0701 0 0423002500006711305271

Huanling Liu

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huanliu@gmail.com

QUALIFICATIONS

Polymers evaluation, Solid catalyst development and characterization ability and mathematic modeling

EDUCATION

M.S., Chemical Engineering, West Virginia University, Morgantown, WV, **2008-2011** GPA: 3.6
Thesis: "Modulating Temporal Control of NF- κ B Activation: Implications for Therapeutic Selection"
Advisor: Dr. David J. Klinke II

M.S., Catalysis, Dalian Institute of Chemical Physics, CAS, Dalian, China, **2004-2007** GPA: 3.3
Thesis: "Studies on Ce Promoted Pt/ γ -Al₂O₃ Catalysts for Preferential CO Oxidation"
Advisor: Associate Professor Lei Ma

B.S., Applied Chemistry, Dalian University of Technology, Dalian, China, **2000-2004** GPA: 3.2
Graduate Project: "Catalytically Selective Hydrogenation of C=O bond by Raney Ruthenium"
Advisor: Dr. Xinqin Gao

INDUSTRIAL EXPERIENCE

Chemical Lab CO-OP, Cummins Filtration, Cookeville, TN 2010.05-2010.12

- Tested and evaluated polymer materials (including thermoset & thermoplastic polymers, elastomers like gasket, seals, cellulose) by DSC and TGA
- Developed standard calibration procedure to keep DSC accurate and reliable
- Developed standard operating procedures for DSC and TGA
- Studied Filter Media Cellulose isotherm curing phenomena and kinetic theory
- Develop new and enhanced existing thermo analysis procedures for polymer characterization
- Cooperated with Research engineers in Cummins filtration and Coolant chemical groups
- Enhanced existing GC methods for glycol quantization
- Investigated filter media contaminant analysis by GC headspace
- Received 6 sigma overview training
- Supported VPI projects by DSC and TGA analysis

RESEARCH EXPERIENCE

Research Assistant, West Virginia University, Morgantown, WV **2008-2009**

- Establish the confidence of parameters by Empirical Bayesian approach and Markov Chain Monte Carlo techniques
- Analyzed the correlation of parameters by Sensitivity Analysis and optimized them by Simulation Annealing program using MATLAB
- Developed an Ordinary Differential Equations-based mathematical model with 28 parameters based on biochemical reaction engineering to study dynamics of 14 variables by MATLAB
- Cooperated and coordinated with team workers from multiple disciplines including chemical engineering, immunology, medicine and clinic departments

Research Assistant, Dalian Institute of Chemical Physics, CAS, Dalian, China **2004-2007**

- Designed a novel catalyst with high CO oxidation and selectivity for preferential CO oxidation in H₂ rich atmosphere, lowering the conversional temperature from 160 °C to 60 °C
- Prepared and screened mixed and supported metal oxide with the methods of incipient wetness impregnation, co-precipitation and vacuum multi-step impregnation

- Investigated the catalytic deactivation process of Pt/ γ -Al₂O₃ based catalysts by GC, ICP, BET, XRD, H₂-TPR and In situ DRIFTS
- Illustrated the catalyst structure and reaction mechanism and observed that addition of Cerium changes one active site adsorption mechanism to double active sites adsorption mechanism
- Synthesized Mesoporous Molecular Sieves MCM-41 by hydrothermal methods and Characterized by XRD and SEM

Research Assistant, Dalian University of Technology, Dalian, China

2003-2004

- Optimized the selection of Raney Ruthenium Catalysts to C=O hydrogenation in different solvents and at differential reaction temperatures
- Analyzed CH₃, CH₂, C=C and C=O organic functional groups by H-NMR, IR, and TLC
- Separated the products from batch reactions through extraction and rotary evaporation

PUBLICATIONS and PRESENTATIONS

1. **Liu, Huanling**, Irina V. Ustyugova, John B. Barnett, and Klinke, David J. II. Inferring the Impact of 3, 4-dichloropropionaniline on the Regulation of nuclear factor- κ B Activation by I κ B α and I κ B β . American Chemical Society Chemistry Symposium, Morgantown, WV, 2009 April.
2. **Liu, Huanling**, Irina V. Ustyugova, John B. Barnett, and Klinke, David J. II. Inferring the Impact of 3, 4-dichloropropionaniline on the Regulation of nuclear factor- κ B Activation by I κ B α and I κ B β . HSC Research Day & Van Liere Convocation, Morgantown, WV, 2009 April.
3. **Liu, Huanling**, Ma, Lei, Shao, Saibing, Li, Zenghe, Wang, Aiqin, Huang, Yanqiang, and Zhang, Tao. Preferential CO Oxidation on Ce-Promoted Pt/ γ -Al₂O₃ Catalysts under H₂-Rich Atmosphere. CHINESE JOURNAL OF CATALYSIS, 2007 Vol.28, No.12, P.1077-1082.
4. **Liu, Huanling**, Gao, Xinqin. Catalytically Selective Hydrogenation of C=O bond by Raney Ruthenium. Graduate project competition. Dalian, China, 2004.

COMPUTER SKILLS

- **High level :** MATLAB, Microsoft Word, Excel, PowerPoint
- **Middle level:** Origin, CHEMDRAW, CHEMCAD, R

LANGUAGES

- **Good in English**
- **Excellent in Chinese**