Simulation study of the TNFα mediated NF-κB signaling pathway

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Abstract. Tumor necrosis factor α (TNFα) is a potent pro-inflammatory cytokine that plays an important role in immunity and inflammation, in the control of cell proliferation, differentiation and apoptosis. This paper presents a simulation study of the TNFα mediated NF-κB signaling pathway based on a system-theoretic approach. Up to the present, there have been numerous approaches to analyze and model cellular dynamics. The most prominent one is utilizing nonlinear differential equations to establish a mathematical model based on reaction kinetics. This approach can provide us with mathematically well-founded and tractable interpretations regarding pathways, especially those best described by enzyme reactions. This paper introduces not only an intuitive graphical model but also a quantitative mathematical model for the TNFα mediated NF-κB signaling pathway. Throughout simulation study, we can qualitatively validate the developed mathematical model compared with experimental results along this pathway. Further investigations into the TNFα mediated NF-κB signaling pathway are in progress to include an inhibitor kinase functioning in this pathway.

1 Introduction

Tumor necrosis factor α (TNFα) is a potent proinflammatory cytokine that plays an important role in immunity and inflammation, in the control of cell proliferation, differentiation and apoptosis. Binding of TNFα to its two receptors, TNFR1 and TNFR2, results in recruitment of signal transducers that activate at least three distinct effectors. Through complicated signaling cascades and networks, these effectors lead to the activation of caspases and two transcription factors, AP-1 and NF-κB [1]-[4].

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This paper is to propose a system-theoretic approach for the analysis and quantitative modeling of the TNFα mediated NF-κB signaling pathway.

We can find numerous approaches in the literature for the modeling of dynamics of cellular dynamics [5]-[12]. The most prominent one is to use ordinary differential equations (ODEs) to model biochemical reactions. This approach can provide us with mathematically well-founded and tractable interpretations for biological pathways, especially those best described by enzyme reactions. This paper first introduces a graphical method to intuitively represent the TNFα mediated NF-κB signaling pathway and then utilizes ODEs to quantitatively model the pathway dynamics. For this purpose, we complement the enzyme kinetics to incorporate the restoring process into the steady-state concentration of substrates. We then model the pathway step-by-step, based on these complemented enzyme kinetics leading to a quantitative model of the signaling pathway. The simulation study shows qualitative validation of the proposed model based on current experimental results. Each parameter value for the computer simulation is derived via inference based on the experimental reaction time.
The paper is organized as follows. Section II briefly introduces some preliminaries on mathematical modeling of reaction kinetics. Section III describes the TNFα mediated NF-κB signaling pathway. Section IV presents the proposed graphical and mathematical modeling of the TNFα mediated NF-κB signaling pathway based on ODEs. Section V illustrates the simulation studies. Finally, conclusions are made in Section VI.

2 Preliminaries

$$E + S \underset{k_2}{\overset{k_1}{\rightleftharpoons}} ES \xrightarrow{k_3} E + P \qquad (1)$$

Equation (1) is a very well known simple model for enzyme kinetics. It is often used to account for typical kinetic properties of various kinds of enzymes. In eq. (1), E is the concentration of an enzyme which combines with a substrate S to form an enzyme-substrate complex ES with a rate constant k_1. The complex ES holds two possible outcomes in the next step. It can be dissociated into E and S with a rate constant k_2 or it can further proceed to form a product P with a rate constant k_3. It is assumed that none of the products reverts to the initial substrate. It is required to express the relations between the rate of catalysis and the change of concentration for the substrate, the enzyme, the complex, and the product. These relations are usually represented by the following set of ODEs. The reaction kinetics we develop in the remainder of this paper are based on these ODEs.
Figure 1 illustrates the reaction profile of the basic enzyme kinetics in eq. (1) evolving in time. Figure 1 illustrates that the formation of an enzyme-substrate complex is the first step in enzyme catalysis. Initially, both, the concentration of the substrate and the enzyme decrease until after about 25 seconds, the concentration of the enzyme increases before settling to its steady state. On the other hand, the concentration of the complex reaches its maximum at about 25 seconds and then decreases exponentially. The curve for the concentration of the product increases and reaches its steady state long after these transitional changes. In reaction kinetics, the change of each concentration implies a signal transfer, passing information to other agonists. The trail of this

signal transfer constitutes a signaling pathway. Some signals in a signaling pathway can be merged and amplified, or reduced due to interference among signals.

$$\begin{aligned}
\frac{dE(t)}{dt} &= -k_1 \cdot E(t) \cdot S(t) + (k_2 + k_3) \cdot ES(t) \\
\frac{dS(t)}{dt} &= -k_1 \cdot E(t) \cdot S(t) + k_2 \cdot ES(t) \\
\frac{dES(t)}{dt} &= k_1 \cdot E(t) \cdot S(t) - (k_2 + k_3) \cdot ES(t) \\
\frac{dP(t)}{dt} &= k_3 \cdot ES(t)
\end{aligned} \tag{2}$$

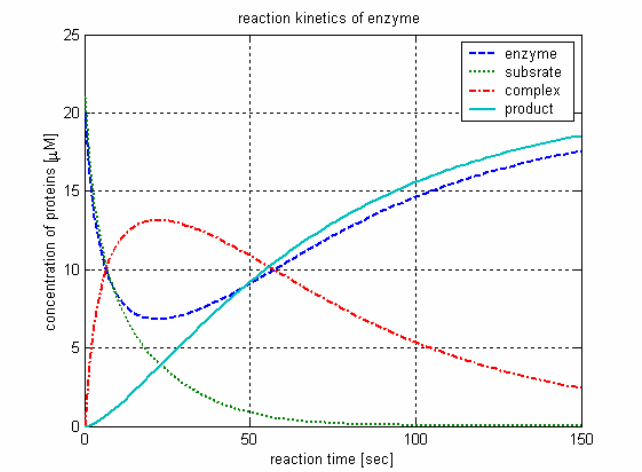

Fig. 1. Reaction profile of the basic enzyme kinetic reaction in eq. (2).

A popular way to intuitively describe a signaling pathway utilizes a graphical representation, akin to diagrams familiar to or popular with biologists. These cartoons are usually insufficiently descriptive to explain molecular dynamics quantitatively but they provide an intuition of the overall dynamics and information processing in cellular systems. Here we propose a graphical method that is associated unambiguously with a mathematical model based on ODEs. This model is then used to investigate the TNFα mediated NF-κB signaling pathway.

The graphical tool is based on a bipartite directed multigraph, where structure of the bipartite graph consists of two types of nodes and directed arcs: a circle ○ represents a state for the concentration of a protein, a bar ▯ represents a rate of reaction, and directed arcs (arrows) connect the circles and the bar. The signal flow in a signaling pathway can then be qualitatively described by this graphical tool.

Equations (2) show the differential equation model of the reaction kinetics outlined in eq. (1) and Figure 2 illustrates the graphical representation for the kinetics in eq. (1)

and (2). In Figure 2, the bar represents the rate of formation and breakdown of the complex or the product.

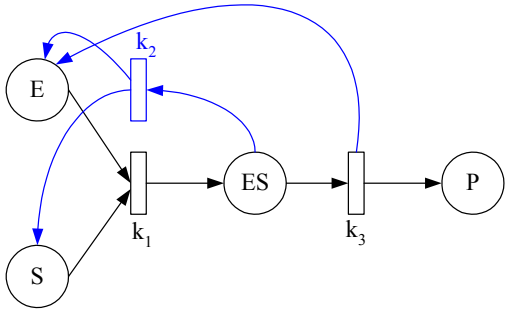

Fig. 2. Graphical representation of the reaction kinetics.

Since the dynamics regarding generation and degradation of the enzyme is not included in the conventional representation of eq. (1), (2), we complement the enzyme kinetic model (2) by considering these dynamics. The main idea is based on then observation that the signal transduction system usually behaves as a slowly time-varying nonlinear system during the reaction period. Figure 3 illustrates the complemented model of an enzyme (or protein) including the generation and degradation, which role is to maintain the steady state concentration of the enzyme (or protein). In Figure 3, the two bars for $k_f(t)$ and $k_r(t)$ represent the rates of generation and degradation, respectively. If we consider the fact that the steady state of enzymes or proteins depends upon the local environment in the cell, it is reasonable to model the rate as a function of time.

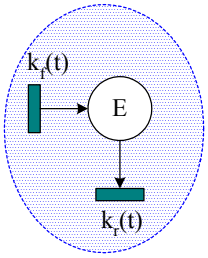

Fig. 3. The complemented graphical model of an enzyme.

Hence, the concentration of an enzyme (or protein) can be modeled as follows.

$$\frac{dE(t)}{dt} = k_f(t) - k_r(t) \cdot E(t) \qquad (3)$$

Based on the assumption of slowly time-varying processes, we can approximate the rates for the generation and degradation as constant during the reaction period, which means that the steady state concentration of the enzyme (or protein) is independent of time. Thus,

$$k_f(t) \cong k_f(t_0), \quad k_r(t) \cong k_r(t_0). \tag{4}$$

Substituting (4) into equation (3) and solving the resultant differential equation, we have

$$E(t) = \frac{k_f(t_0)}{k_r(t_0)} - \left(\frac{k_f(t_0)}{k_r(t_0)} - E_0 \right) \cdot e^{-k_r(t_0) \cdot t}. \tag{5}$$

Taking the limit in equation (5), we obtain the following steady-state value for E(t),

$$\lim_{t \to \infty} E(t) = \frac{k_f(t_0)}{k_r(t_0)}. \tag{6}$$

Finally, we have obtained the following complemented reaction kinetics model based on ODEs including the rate of generation and degradation for an enzyme (or protein):

$$\begin{aligned}
\frac{dE(t)}{dt} &= \boxed{k_f(t) - k_r(t) \cdot E(t)} - k_1 \cdot E(t) \cdot S(t) + (k_2 + k_3) \cdot ES(t) \\
\frac{dS(t)}{dt} &= -k_1 \cdot E(t) \cdot S(t) + k_2 \cdot ES(t) \\
\frac{dES(t)}{dt} &= k_1 \cdot E(t) \cdot S(t) - (k_2 + k_3) \cdot ES(t) \\
\frac{dP(t)}{dt} &= k_3 \cdot ES(t)
\end{aligned} \tag{7}$$

In the extreme, if $k_r(t_0) >> k_f(t_0)$ then $E(t) \approx 0$. This implies a case when the signal flow is blocked since there is no more signaling protein involved in its signaling pathway. The case depends wholly upon the cell environment.

Fig. 4 and 5 provide a graphical representation of the complemented model for the reaction kinetics and the corresponding simulation results for eq. (7).

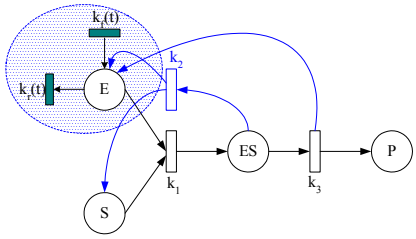

Fig. 4. Graphical representation of the complemented model of the reaction kinetics.

Fig. 5 shows a similar behavior to Figure 1, based on eqs. (2). Note that the curve for the enzyme returns to its steady state more quickly in Fig. 5. If we reduce the rate of generation or raise the rate of degradation for the enzyme, the response would be slowed down. This implies that the steady state of an enzyme (or protein) can modulate the response of the signal.

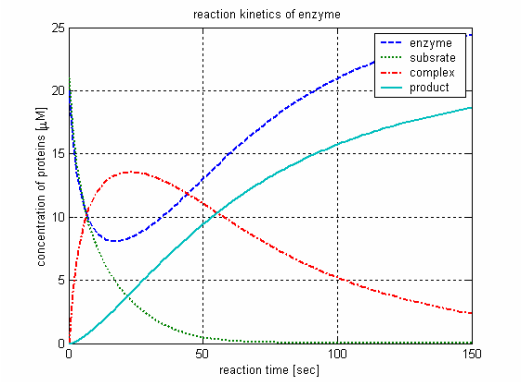

Fig. 5. Simulation results of the complemented reaction kinetics .

3 TNFα mediated NF-kB signaling pathway

Tumor necrosis factor (TNFα) is a potent cytokine often produced by various cell types including macrophage, monocytes, lymphocytes, keratinocytes, and fibroblasts in response to inflammation, infection, injury, and other environmental challenges [1]. Exposure of cells to TNFα can recruit an activation of a caspase cascade which leads to apoptosis. However, more commonly, the binding of TNFα to its receptor causes an activation of two major transcriptional factors, AP-1 and NF-kB, these in turn induce genes involved in chronic and acute inflammatory responses. NF-kB is regulated primarily by phosphorylation of inhibitory proteins, the IkBs which are retained in the cytoplasm of non-stimulated cells. In response to TNFα and other agonists, the IkBs are phosphorylated by the IkB kinase (IKK) complex, resulting in their ubiquitination, degradation, and the nuclear translocation of the freed NF-kB. Generally, TNFα exerts its effects through two distinct receptors, TNFR1 and TNFR2. Binding of the inherently trimeric TNFα to TNFR1 induces receptor trimerization and recruitment of several signaling proteins to the cytoplasmic domains of the receptors as shown in Fig. 6. The first protein recruited to TNFR1 is TNFR1-associated death domain protein (TRADD) which serves as a platform to recruit at least three additional mediators, a receptor-interacting protein (RIP), a Fas-associated death domain protein (FADD) and a TNF-receptor-associated factor 2 (TRAF2). TRAF2 plays a central role in early events, common to TNFR1 and TNFR2, which leads to the IKK activation [1].
Figure 6 shows the graphical model of the TNFα mediated FN-κB signaling pathway.

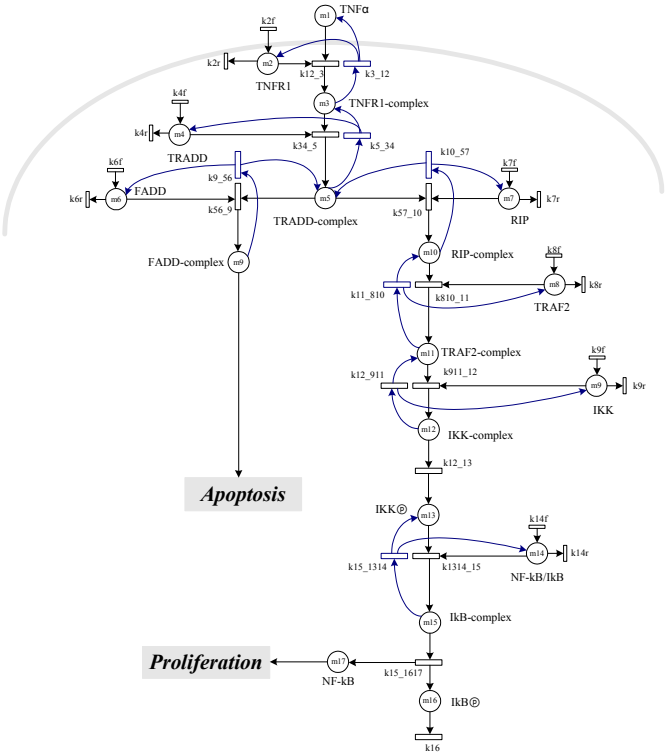

Fig. 6. Graphical model of the TNFα mediated NF-κB signaling pathway.

4 Mathematical modeling of TNFα mediated NF-kB signaling pathway

Applying the previous complemented model (7) of the reaction kinetics to each pathway step-by-step and integrating each model into a whole, the final mathematical model of the TNFα mediated NF-kB signaling pathway, based on a set of ODEs, is summarized in the Appendix. Moreover, all of the relevant definition of variables and parameters appearing in the ODE model, together with nominal values for simulation studies in Section V, are given in Table 1 and 2. The terminology of the dependent variables and their acronyms follows the conventional biological description and for which the symbols are based on common use of mathematical notation. It is assumed that the cell keeps the concentration of each signaling protein constant before and after each signaling, i.e., the concentration of these proteins returns to a steady state after the reaction. Note that this assumption reflects the biological observation. In addition, we assume that the signaling pathway behaves as a slowly-time varying system as described in Section 2.

The exact value of parameters such as the concentration of each signaling protein, the rate constants for the generation and degradation, *etc.* are difficult to obtain since their numerical value not only depends on the species and tissue but also on the physiological state of the cells/organism. Hence we derive the nominal value for each parameter from the reaction time. For instance, it is well known that it takes several seconds for two proteins to interact in the signaling pathway. We therefore make a reasonable quantitative inference from the reaction time. We summarize the in this way derived nominal value for each parameter in Table 1 and Table 2. Note that whenever we can get more specific information regarding the reaction time in a particular experimental condition, e.g., the measured time for TNFα returning to the steady state after a reaction from its initial state, we can then modify more exact parameter values for the rate of generation, the rate of degradation, and so forth based on new information about the reaction time.

Table 1. Definition of variables and nominal values for signaling proteins at steady state.

Dependent variables	Acronym	Symbol	Concentration [μM]	Independent variables	[μMsec^{-1}]/ [sec^{-1}]
TNF Necrosis Factor	TNF	m1	20	-	-
TNF Receptor 1	TNFR1	m2	25	k2f/ k2r	0.139/ 0.00556
TNFR1-complex		m3	0	-	-
TNFR1-associated death domain protein	TRADD	m4	25	k4f/k4r	0.139/ 0.00556
TRADD-complex	-	m5	0	-	-
Fas-associated death domain	FADD	m6	0	k6f/ k6r	0.139/ 0.00556
FADD-complex	-	m9	0	-	-
Receptor-interacting protein	RIP	m7	25	k7f/ k7r	0.139/ 0.00556
RIP-complex	-	m10	0	-	-
TNF-Receptor-associated factor 2	TRAF2	m8	12	k8f/ k8r	0.139/ 0.00556
TRAF2-complex	-	m11	0	-	-
IkB kinase	IKK	m9	25	k9f/ k9r	0.139/ 0.00556
NF-kB Inhibitor /Nuclear factor kB	IkB/NF-kB	m14	25	k14f/ k14r	0.139/ 0.00556
IKK-complex	-	m12	0	-	-
NF-kB Inhibitor	IκB	m16	0	-	
Nuclear factor kB	NF-κB	m17	0	-	-

Table 2. Definition of variables and nominal values for parameters in reaction kinetics.

Independent variables	Symbol	$[\mu M^{-1}sec^{-1}]/[sec^{-1}]$
TNF/TNFR1 ratio	k12_3/ k3_12	0.00096/ 0.004
TNFR1/TRADD ratio	k34_5/ k5_34	0.00096/ 0.004
TRADD/FADD ratio	k56_9/ k9_56	0.00096/ 0.004
TRADD/RIP ratio	k57_10/ k10_57	0.00096/ 0.004
RIP/TRAF2	k810_11/ k11_810	0.00096/ 0.004
TRAF2/IKK ratio	k911_12/ k12_911	0.00096/ 0.004
IKK activation ratio	k12_13	0.1
IKK_act/(IkB/NF-kB) ratio	k1314_15/ k15_1314	0.00096/ 0.004
NF-kB activated ratio	k15_1617	0.00096/ 0.004
Degradation ratio	k16	0.1

In Table 2, the rate of formation for the complex is assumed to be 0.00096 $[\mu M^{-1}sec^{-1}]$, the rate of dissociation from the complex is assumed to be 0.004 $[sec^{-1}]$, and the rate of production is assumed to be 0.1 $[sec^{-1}]$.

5 Simulation studies

The computer simulation is carried out with a 2GHz Pentium 4 PC. The differential equations in the model of the signaling pathway are solved by utilizing MATLAB library functions (ode45 etc.). The results can be classified into two groups: one group for protein interactions and the other group for three dimensional profiles along with time and the concentration for TNFα. The later group is to be called the sensitivity to TNFα.

As in Section II and IV, it is assumed that the cell keeps the concentration of each signaling protein constant before and after signaling takes place. Figure 7 illustrates the formation process for the complex from TNFα and TNFR1. The concentration of the complex reaches its maximum in about 10[sec] and then decreases exponentially to the initial condition zero. TNFα reduces to about 8[μM], and then increases gradually. After a while, it returns to its steady state. Here we consider the change of concentration of the complex as a signal. This signal is transferred to the next stage in Figure 8 where TRADD is recruited and bound to the TNFR1-complex forming a TRADD-complex. The concentration change of the TRADD-complex can be considered as another biochemical signal transferred from the previous stage. Similarly, the signal is transferred to IKK in Figure 11 (for intermediate procedures refer to Fig. 8-11). The signal activates IKK and then the activated IKK finally phosphorylates IκB which in turn releases NF-κB. Thereupon the concentration of released NF-κB becomes increased up to its maximum value as illustrated in Figure 12. After all, the released NF-κB translocates into the nucleus and it triggers the corresponding gene to start transcription to make responses to the variation of the ligand TNFα.

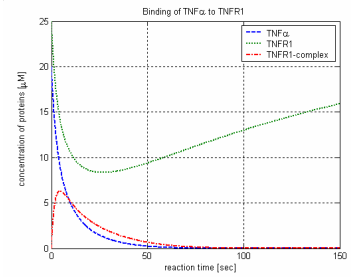

Fig. 7. Binding of TNFα to TNFR1.

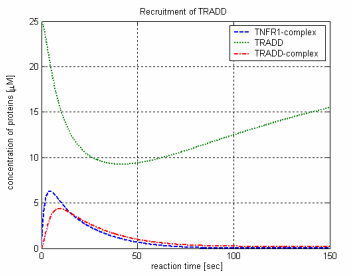

Fig. 8. Recruitment of TRADD.

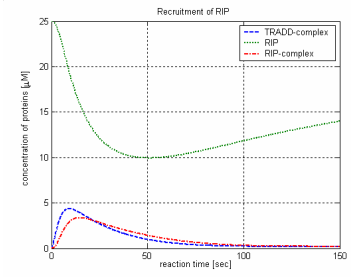

Fig. 9. Recruitment of RIP.

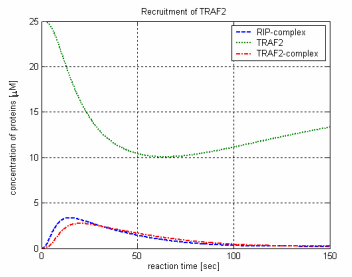

Fig. 10. Recruitment of TRAF2.

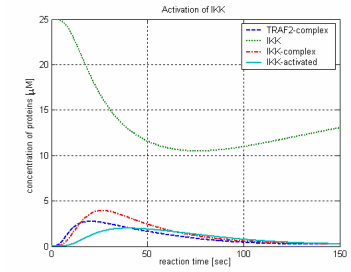

Fig. 11. Activation of IKK.

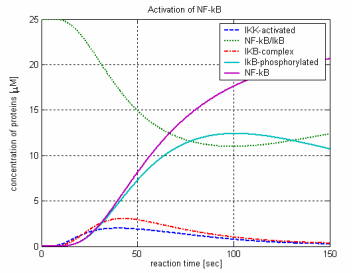

Fig. 12. Activation of NF-κB

The second group of the results is the 3D graphical representation which illustrates the profile as function time and the concentration of TNFα. The concentration varies form 0 to 35[μM]. In Figure 14, TNFR1 is almost occupied by TNFα at its highest density; however, after a while TNFR1 turns to its steady state. Figure 15 shows the profile of TNFR1-complex, where the concentration increases along with time and the concentration of TNFα. The TNFα-complex recruits TRADD in Figure 16 and its concentration decreases during the recruiting, while the concentration of the TRADD-complex increases along with time and the concentration of TNFα. Similarly, the signal propagates into NF-κB through variation of the concentration of each complex (for intermediate procedures refer to Fig. 17-27).

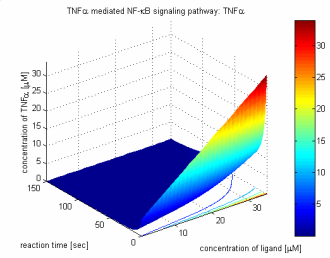

Fig. 13. TNFα

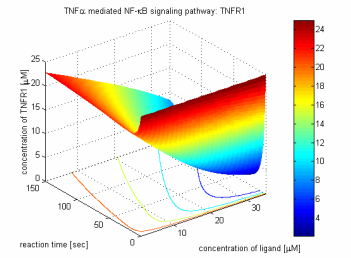

Fig. 14. TNFR1.

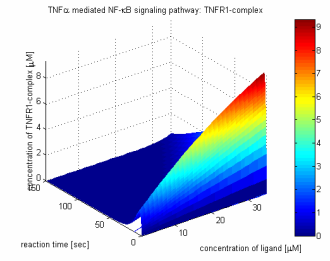

Fig. 15. TNFR1-complex.

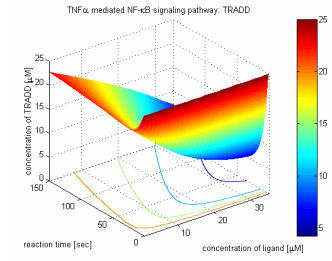

Fig. 16. TRADD.

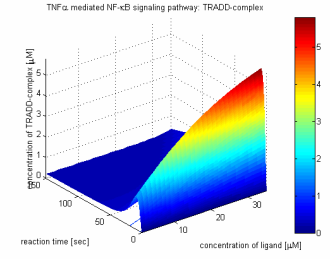

Fig. 17. TRADD-complex

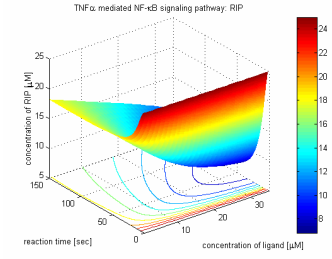

Fig. 18. RIP.

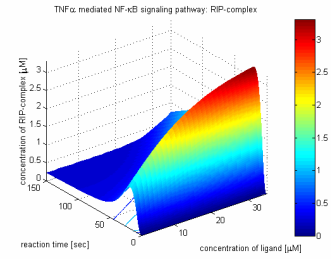

Fig. 19. RIP-complex.

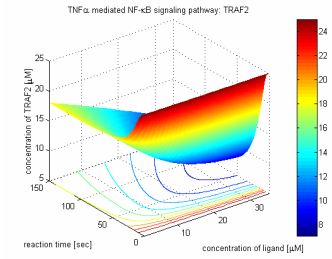

Fig. 20. TRAF2.

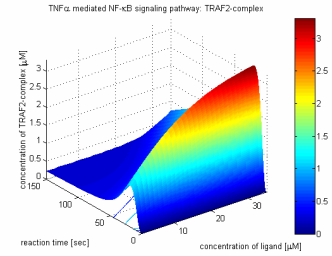

Fig. 21. TRAF2-complex

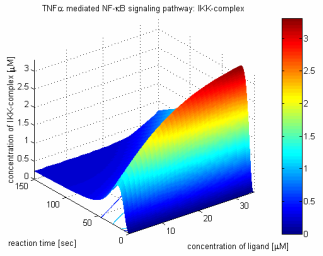

Fig. 22. IKK-complex.

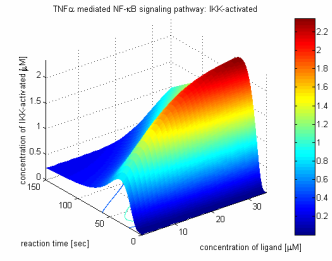

Fig. 23. Activated IKK.

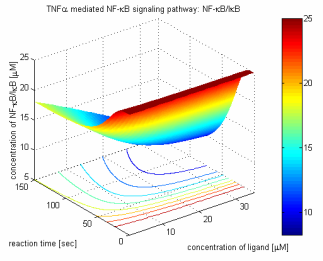

Fig. 24. NFκB/IkB.

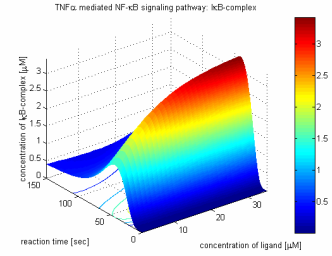

Fig. 25. IκB-complex.

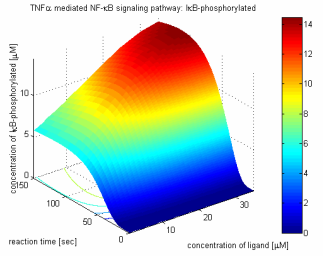

Fig. 26. Phosphorylated IκB.

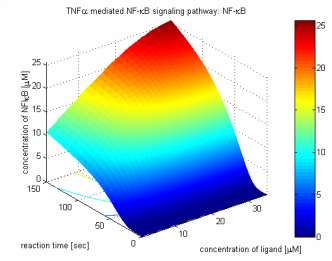

Fig. 27. NF-κB.

6 Concluding Remarks and Further Studies

In this paper, we investigated a system-theoretic approach to the analysis and quantitative modeling of the TNFα mediated NF-κB signaling pathway. The quantitative model was complemented with a qualitative model. We proposed an intuitive graphical model of the signal pathway based on a bipartite directed multigraph and then presented the mathematical model of the signal pathway via a set of ODEs based on complemented reaction kinetics. For simulation studies, we have derived the nominal value for each parameter through inference based upon the reaction time. The simulation studies have illustrated the process of variation of each protein concentration along with the TNFα mediated NF-κB signaling pathway as the concentration of the ligand TNFα varies. Moreover, the computer simulation based on the proposed quantitative model reveals the transient behavior of the signaling pathway. The proposed signaling pathway model based on a system-theoretic approach can be extended and applicable to other signaling pathways in the same manner. As a next step, it will be useful to include more detailed elements of the pathway excluded in the current study such as an inhibitor kinase functioning. This may lead to hybrid systems, combining discrete event systems with continuous dynamics in order to model the switching or decision making in signaling pathways. Feedback control and time delays are further challenges for an extended model. As yet, the model produces hypotheses and can guide the biologist in experimental design. In order to predict quantitative behavior from the proposed model and to apply the predicted results in biotechnological application, the proposed model needs to be supplemented by more accurate nominal values for each parameter based on specific reaction time under in particular *in vivo* or *in vitro* experiments.

Acknowledgement

This work was supported by the Post-doctoral Fellowship Program of Korea Science & Engineering Foundation (KOSEF).

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Appendix: The mathematical model of TNFα mediated NF-kB signaling pathway based on the complemented reaction kinetics

This appendix summarizes the mathematical model of the TNFα mediated NF-κB signaling pathway based on the complemented reaction kinetics in Section II. For the nominal value of each parameter, refer to Tables 1 and 2 in Section IV.

$$\frac{dm_1(t)}{dt} = -k_{12\_3}\cdot m_1(t)\cdot m_2(t) + k_{3\_12}\cdot m_3(t)$$

$$\frac{dm_2(t)}{dt} = k_{2f} - k_{2r}\cdot m_2(t) - k_{12\_3}\cdot m_1(t)\cdot m_2(t) + k_{3\_12}\cdot m_3(t)$$

$$\frac{dm_3(t)}{dt} = k_{12\_3}\cdot m_1(t)\cdot m_2(t) - \left[k_{3\_12} + k_{34\_5}\cdot m_4(t)\right]\cdot m_3(t) + k_{5\_34}\cdot m_5(t)$$

$$\frac{dm_4(t)}{dt} = k_{5\_34}\cdot m5(t) - \left[k_{4r} + k_{34\_5}\cdot m_3(t)\right]\cdot m_4(t) + k_{4f}$$

$$\frac{dm_5(t)}{dt} = k_{34\_5}\cdot m_3(t)\cdot m_4(t) - [k_{56\_9}\cdot m_6(t) + k_{57\_10}\cdot m_7(t) + k_{5\_34}]\cdot m_5(t) + k_{9\_56}\cdot m_9(t) + k_{10\_57}\cdot m_{10}(t)$$

$$\frac{dm_6(t)}{dt} = -\left[k_{6r} + k_{56\_9}\cdot m_5(t)\right]\cdot m_6(t) + k_{9\_56}\cdot m_9(t) + k_{6f}$$

$$\frac{dm_7(t)}{dt} = k_{7f} - \left[k_{7r} + k_{57\_10}\cdot m_5(t)\right]\cdot m_7(t) + k_{10\_57}\cdot m_{10}(t)$$

$$\frac{dm_8(t)}{dt} = -\left[k_{8r} + k_{810\_11}\cdot m_{10}(t)\right]\cdot m_8(t) + k_{11\_810}\cdot m_{11}(t) + k_{8f}$$

$$\frac{dm_9(t)}{dt} = -\left[k_{9r} + k_{911\_12}\cdot m_{11}(t)\right]\cdot m_9(t) + k_{12\_911}\cdot m_{12}(t) + k_{9f}$$

$$\frac{dm_{10}(t)}{dt} = k_{57\_10} \cdot m_5(t) \cdot m_7(t) - k_{810\_11} m_8(t) \cdot m_{10}(t) + k_{11\_810} \cdot m_{11}(t)$$

$$\frac{dm_{11}(t)}{dt} = k_{810\_11} \cdot m_8(t) \cdot m_{10}(t) + k_{12\_911} \cdot m_{12}(t) - [k_{911\_12} \cdot m_9(t) + k_{11\_810}] \cdot m_{11}(t)$$

$$\frac{dm_{12}(t)}{dt} = k_{911\_12} \cdot m_9(t) \cdot m_{11}(t) - \left[k_{12\_13} + k_{12\_911}\right] \cdot m_{12}(t)$$

$$\frac{dm_{13}(t)}{dt} = k_{15\_1314} \cdot m_{15}(t) + k_{12\_13} \cdot m_{12}(t) - k_{1314\_15} \cdot m_{13}(t) \cdot m_{14}(t)$$

$$\frac{dm_{14}(t)}{dt} = k_{14f} + k_{15\_1314} \cdot m_{15}(t) - \left[k_{14r} + k_{1314\_15} \cdot m_{13}(t)\right] \cdot m_{14}(t)$$

$$\frac{dm_{15}(t)}{dt} = k_{1314\_15} \cdot m_{13}(t) \cdot m_{14}(t) - \left[k_{15\_1314} + k_{15\_1617}\right] \cdot m_{15}(t)$$

$$\frac{dm_{16}(t)}{dt} = k_{15\_1617} \cdot m_{15}(t) - k_{16} \cdot m_{16}(t)$$

$$\frac{dm_{17}(t)}{dt} = k_{15\_1617} \cdot m_{15}(t)$$