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Chapter 38

Studying NF-κB Signaling with Mathematical Models

Simon Mitchell, Rachel Tsui, and Alexander Hoffmann

Abstract

Mathematical modeling of NF-κB signaling can be employed to understand how the network of molecular interactions leads to signaling phenomena observed experimentally. Model construction is a challenging process; however, existing models can be utilized and can provide a great deal of insight quickly and inexpensively. The simulation of various inputs and the identification of potential therapeutic targets using the mathematical model are detailed here.

Key words Computational, Modeling, Simulation, Mathematical, Systems, Dynamical, Theoretical

1 Introduction

Mathematical models can be used as a virtual laboratory, to perform in silico experimentation and to replace, complement, and improve experimental approaches in the wet lab. The NF-κB signaling network is complex, and understanding the individual components and interactions in isolation only provides limited progress towards the goal of understanding its functions and enabling effective clinical intervention. Combining knowledge of network components with knowledge of their interactions through simulations allows emergent behavior to be predicted and explained in a rigorous manner.

There are many different modeling approaches and software packages available (including MATLAB, Mathematica, and COPASI); however, the principles used in constructing a model to ensure utility are consistent, regardless of the framework used. In this chapter we detail how a model of the NF-κB signaling networks can be constructed, validated, and utilized to gain the best insight from experimental studies and inform future experimentation. There is much knowledge to be gained from existing models, and constructing a new model should only be considered after attempting to utilize existing models. Therefore, in Section 3.2, a published model [1] is used to gain insight into the NF-κB signaling network.

2 Materials

1. A computer with access to appropriate software (detailed below).

2. Experimentally derived parameters, often based on extensive searches or expert understanding of the literature.

3. Experimental or clinical findings, which the model is required to reproduce or account for.

3 Methods

Here we describe a computational approach to improve understanding of NF-κB signaling. The general approach to model construction, validation, and application can be applied to any biological system; however, we illustrate this general approach with the specific steps to simulate NF-κB signaling (*see* **Note 1**).

3.1 Model Construction (see Notes 2 and 3)

1. *Define the question to be addressed by the model.* Modeling can provide insight into a wide variety of biological questions. The type of question that the model is required to answer must be identified first as this informs the model-building process. For example, a model designed to investigate physiological scale processes such as the control of fever may not provide insight into the importance of dimerization of Rel proteins.

    NF-κB signaling models [2] can provide insight into many of the important open questions in immune signaling. Some phenomena that can be investigated using such models are dose–response relationships [3, 4], dynamic control as revealed by time-course studies [1, 5, 6], the impact of cross talk [7, 8], analysis of sensitivities, and identification of points of control [9, 10], which may provide viable therapeutic targets. Questions beyond the scope of these models include the control of gene expression profiles, the physiological functions of cytokines, or the cellular decisions to divide or die.

    When the required scope, detail, and type of model (*see* below **steps 2–4**) have been identified, and an appropriate model has been found or constructed, a fundamental question that can be addressed is whether the known molecular mechanisms represented in the model are sufficient to account for cell biological or physiological level phenomena. When the answer is yes, the model can be used to explore the emergent system properties. When the answer is no, the model can direct experimentation to identify additional molecular mechanisms that render it sufficient.

2. *Define the required scope of the model.* The scope of an appropriate model is largely determined by the question of interest.

The scope determines what the input(s) and output(s) of the model are. Inputs and outputs are generally measureable quantities of metabolites ("molecular species"). All molecular species upstream of the input and downstream of the output are outside the scope of the model. The scope of a model could extend to a whole cell or organism or be restricted to small regulatory circuits networks. While it is tempting to choose an ambitious scope, this can lead to a poor model that is insufficiently determined by insufficient data. It is best practice to ensure that the initial scope of modeling work is the minimal required to provide insight and only expand the model's scope once initial models have been shown to be predictive. The practice of generating preliminary data before embarking on extensive work has been common in wet lab studies and remains important with computational approaches.

NF-κB signaling is a highly complex biological system with a wide variety of potentially important cross-talking pathways. To simulate every system that could potentially control NF-κB, via the IKK hub, is unfeasible as this would extend to a large proportion of cellular pathways. As a result, IKK activity profiles were chosen as inputs to the NF-κB simulation. Upstream signaling from tumor necrosis factor (TNF) receptor-mediated IKK activation was not included in initial models was later added as an additional regulatory module [4], thereby expanding the scope of the resulting model. NF-κB levels were identified as appropriate output from the model as this was highly informative without introducing the challenges associated with simulating the extensive NF-κB-induced gene expression profiles.

3. *Define the model detail required.* Within the identified scope of the model, the level of detail included must be decided; this can be considered the "graininess" of the model. This is a measure of how closely the underlying biological mechanisms identify to the mathematical representation constructed. It is often necessary to group a multiple-step process into a single-compound reaction. This is straightforward when one reaction has the highest control over the rate (the rate-limiting step) in all relevant conditions.

Components required to recreate the behavior being investigated, and answer the question of interest, should be included without adding poorly understood components that add unnecessary complexity or reliance on under-determined parameters. The key components of NF-κB signaling were identified as:

(a) NF-κB and its localization

(b) Multiple IκB dimers

(c) IKK activity curves

(d) mRNA levels for each protein

The scope chosen permits detailed investigation into the effect of temporal features of IKK activity on NF-κB activation. By including mRNAs we were able to investigate transcriptional regulation and utilize the large amount of mRNA data for model parameterization and validation. The multiple monomers, which combine to form the family of NF-κB dimers, were not included in initial models, as understanding general temporal NF-κB profiles was the priority.

IKK was modeled as an enzyme that degrades IκB directly, whereas the underlying process actually involves ubiquitination and proteasomal degradation [11]. By assuming that IKK-mediated phosphorylation of IκB is the rate-limiting step of IκB degradation, the intricate ubiquitin-dependent proteasomal degradation pathway could be represented by a single reaction.

4. *Decide on the type of model required*. We distinguish here between three types of mechanistic models. (We do not consider statistical models here.) (1) Logical modeling does not rely on kinetic parameters and may be most appropriate if the system is poorly characterized, but can provide insights about steady-state control. (2) A differential equation-based approach will make best use of kinetic parameter information and can provide insights on the dynamic time evolution of quantitative concentrations and fluxes. (3) A stochastic model accounts for the stochastic nature of individual molecular reactions and is the most detailed. While the primary consideration in choosing the appropriate model strategy is the biological question of interest, the trade-off between the need for high-quality, highly detailed data and accuracy of output contributes to choosing the right modeling strategy [12].

The outputs from differential equation-based models are deterministic representations of a system's average behavior. This deterministic result may differ from any single small-scale (e.g., single cell) experiment or simulation due to the effect of the noise present in all biological systems. This limitation of deterministic modeling is usually unimportant as most commonly used experimental techniques also produce data of this type. For simulations of systems with small numbers of molecules, stochastic modeling techniques are most appropriate as they capture the importance of noise in these systems. Recent studies of newly divided cells (sibling analysis) have shown that cell-to-cell variability is mainly attributed to extrinsic variability such as initial conditions and rate constants rather than intrinsic noise [13]. This type of cell-to-cell variability as a result of noise can be incorporated into an ordinary differential equation (ODE) modeling framework through sampling initial conditions.

For modeling of NF-κB signaling, an ODE-based approach is possible due to many of the individual components of the networks being well characterized and a number of relevant knockout experimental systems being available for model validation.

5. *Investigate if an appropriate model already exists.* This can be done through a standard literature search or through querying a repository of models such as the BioModels Database (www. Biomodels.net) [14]. If an appropriate model exists (i.e., one that answers the question of interest), then this should be utilized and Section 3.2 details this process. For investigation of the temporal control of NF-κB activation provided by the isoforms of IκBs, models from our laboratory are appropriate and employed in Section 3.2.

6. *Identify data available for model construction.* For the construction of a new model, its quality, and therefore the quality of the insight it provides, improves with the amount and quality of data available. Data useful for modeling can fall into two categories: (a) physicochemical results, which are used in model parameterization, and (b) emergent properties/physiological results, which are used in parameter fitting and model validation.

   There are multiple sources of parameters for modeling including existing literature, databases of reaction kinetics (such as SABIO-RK [15]), and quantitative experimental techniques performed in the wet lab.

   If more high-quality data than expected are available, then the scope and graininess of the proposed model (**steps 3** and **4**) can be increased to make best use of these data. Similarly if the data required to parameterize a model of the scope/graininess desired are not found, then these should be decreased. Constructing a smaller, good quality model that can later be expanded is preferable to attempting an over ambitious simulation that may not be feasible.

   For NF-κB signaling models, a wide variety of experimentally derived parameters were identified. Protein and mRNA half-lives, steady-state concentrations, and binding affinities were all measured for many of the interactions to be modeled. NF-κB activity time courses in IκB knockout systems were generated for the purpose of parameter fitting and model validation.

7. *Construct a network diagram of the system.* A network diagram of the system being modeled is a useful first step for model construction. An accurate network diagram represents a consensus of the current understanding and ensures that the mathematical formulation being constructed closely represents the underlying biochemical interactions. Care should be taken that each metabolite and reaction to be modeled is represented by a single shape or line in the diagram. Identifying diagrammatic entities with mathematical entities (equations or terms within equations) allows the model to be used as a protocol for model construction. While any representation that accurately represents both the biological and mathematical systems is appropriate, Systems Biology Graphical Notation (SBGN) [16] provides a standardized visual language that may aid communication.
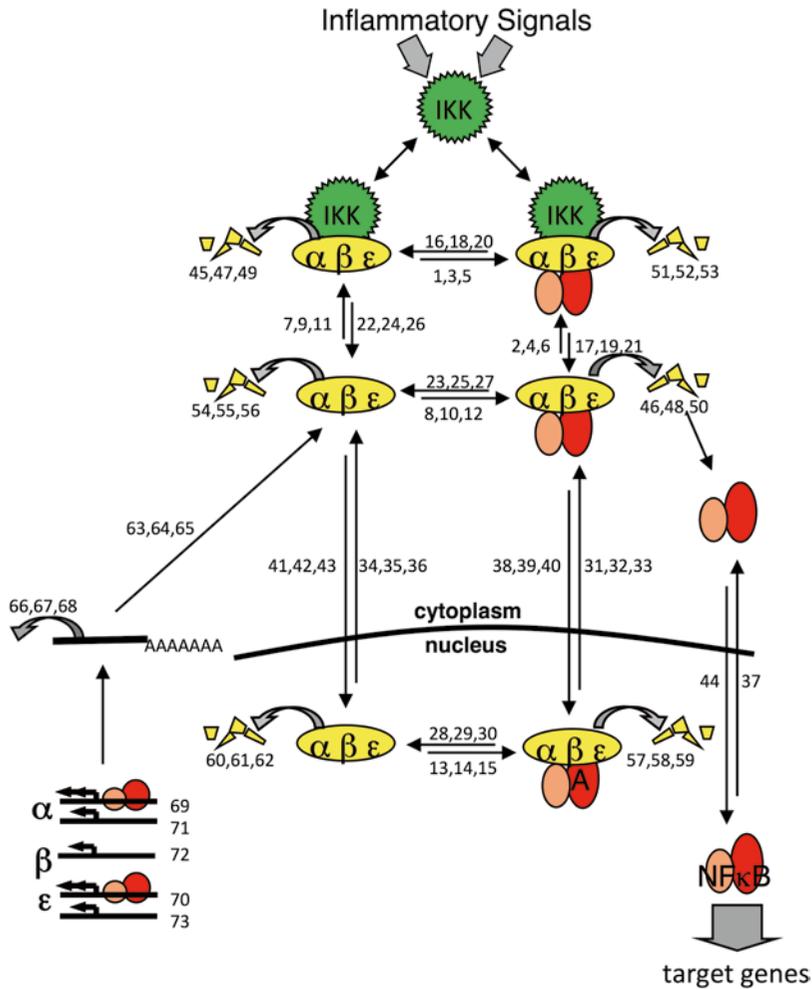
Fig. 1 Diagram representing NF-κB signaling used in the creation of computational simulations. Each colored shape is a metabolite (also known as "molecular species") in the system and an ODE in the mathematical representation. Each *arrow* is a reaction in the system, mathematically represented by terms in the ODEs of metabolites involved in the reaction. The inputs to the system are IKK activation curves, and the outputs are concentrations of free NF-κB, which is capable of binding DNA

A diagrammatic representation of the NF-κB signaling network is given in Fig. 1. This representation of the biochemical network was used to construct the computational model we described in 2002 [3].

8. *Identify an appropriate software framework.* A number of different computational environments and software packages exist for model construction and analysis including MATLAB, COPASI [17], Berkeley Madonna [18], and the SimBiology MATLAB toolbox. Choosing appropriate tools is key to efficient model construction and maximizing the models utility and reusability. Broadly speaking, constructing a model

directly in a programming language such as MATLAB, C++, or Python provides the most versatility for advanced analysis and construction of models that do not conform to common biochemical behavior. Constructing models in such an environment requires more specialized technical expertise and can restrict the models' utility for those without specific programming knowledge. COPASI and the SimBiology MATLAB toolbox provide a more accessible model-building environment, while also providing some checks to ensure that the model constructed is mathematically sensible and biochemically valid. These environments allow those without programming experience to construct and utilize models, but may restrict the model and its analysis to commonly used methods. Berkeley Madonna provides a powerful ODE solving graphical user interface without specifically limiting the system to biochemical simulations.

MATLAB was chosen as the environment for modeling of NF-κB signaling due to the complexity of some of the behavior and inputs required to recreate accurately experimentally observed NF-κB responses. There are well-characterized delays in transcriptional activation of IκBs in response to NF-κB activation; some modeling environments do not support explicit delays, but these can be implemented in MATLAB. To make best use of the experimentally derived IKK activity time courses as inputs to the model, interpolated input curves were required, and this was most easily implemented in MATLAB. To ensure that the complex model constructed for NF-κB signaling was accessible to those without programming experience, a web-based version of the model, which provided an intuitive interface for performing simulations, was published (http://signalingsystems.org/webmodel/).

9. *Construct the mathematical representation.* Using the previously constructed network diagram as a guide, the mathematical representation of the biochemical network should now be input into the software. For ODE-based modeling, as used for NF-κB signaling, this requires construction of an ODE for each metabolite in the system. The terms of each metabolite's ODE represent the reactions that alter the concentration of that metabolite.

For example, the terms of the ODE representing the change in concentration of free IκB as a result of its binding to NF-κB are given in Fig. 2. Mass action kinetics were used for most reactions in the NF-κB signaling network and should be used unless there are experimentally derived data suggesting a more intricate mechanism. Terms should be added to the appropriate ODE for each reaction it is involved in using the parameters identified.
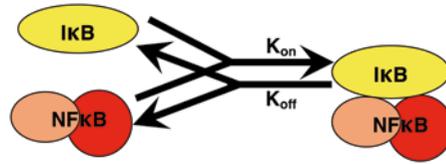
ODE: $\quad \dfrac{d[\text{I}\kappa\text{B}]}{dt} = -\text{K}_{\text{on}}[\text{I}\kappa\text{B}][\text{NF}\kappa\text{B}] + \text{K}_{\text{off}}[\text{I}\kappa\text{B-NF}\kappa\text{B}]$

MATLAB: delta(IkB) = - kon * IkBa * NFkB + koff * IkBaNFkB

Fig. 2 A diagrammatic representation of a complex binding reaction within the NF-κB signaling system. The reaction is reversible, permitting binding and release of reaction's. An ODE for free IκB was written using the diagram as a guide; binding depletes free IκB, while decomplexation increases free IκB. The MATLAB code representation of the ODE is given

10. *Estimate any parameters that were not experimentally determined.* The lack of accurate, experimentally derived, kinetic parameters is a common challenge for the construction of computational models. Any parameter that could not be identified from a search of the literature, or from a database, or through experimentation, must be estimated or derived.

    The most basic form of parameter deduction is used when there are parameters available for all but one of the reactions in which a metabolite partakes. For example, if a metabolite's half-life and steady-state concentration are known, then the expression rate constant can be deduced. This is done by setting the rate of change to zero to represent the steady state, substituting the known parameters, and solving the equation below for the remaining unknown parameter:

$$\frac{\mathrm{d}[\mathrm{X}]}{\mathrm{d}t} = -k_{deg}[\mathrm{X}] + k_{exp} = 0$$

    Some parameters can be estimated using knowledge of similar systems; for example, parameters from homologous proteins can often provide a guide for parameter estimation.

    If some parameters still remain unidentified, then they must be fitted to their most likely value. Many parameters can be constrained within ranges using knowledge from similar systems and common biochemical limits.

    If some parameters still remain unknown or broadly constrained, then a parameter-fitting methodology should be employed to find their most appropriate values. There exist a number of algorithms such as Hooke and Jeeves, particle swarm, gradient descent, etc. Many of these algorithms are incorporated into software packages such as COPASI [19].

    Despite being complex, the NF-κB signaling model was already highly constrained by previously published parameters. Multiple IκB isoform knockout cell lines were used to provide

data for multiple conditions that the model could be fitted to; this ensured that the parameters chosen were valid in a variety of conditions [3]. A number of fitting techniques were trialed to assess which gave the best fit when measured as standard deviation from the experimental results. A random search method was found to perform well, and the parameters identified by this search were subsequently adjusted to improve the qualitative fit regarding frequency and amplitude of oscillations. Standard fitting methods, based around a distance metric (such as root mean square), do not perform well with the NF-κB signaling simulation. The NF-κB response to stimuli is often highly dynamic with some oscillatory behavior; common fitting algorithms can fail to find good fits that are slightly offset in initial time or frequency of oscillation. Feature-based fitting techniques have been found to perform well for the NF-κB signaling network [4, 8]. These methods prioritize features such as a maximal peak at a specified time point that matches experimental observations.

3.2   Utilizing Models

3.2.1   Predicting Response to Stimuli

Once a model has been constructed that closely matches experimental data, it can be used as a tool to provide insight, to make testable predictions, and to target better experimental studies.

Here we demonstrate how the model of Werner et al. [1] can be used to make predictions on how temporally different IKK activity profiles in various genotypes affect the stimulus-specific gene expression program:

1. Select the TNF input curve, by choosing curve 1, from the array of defined input curves. These input values have been quantitated and normalized from IKK immunoprecipitation kinase assays.

   ```
   ikk_curves      = {'TNFp15' 'TNFc' 'LPSp45'};
   ikk_curve_num   = 1;
   ```

2. Run the simulation.

3. Select the lipopolysaccharide (LPS) input curve (curve 3) from the array.

   ```
   ikk_curve_num   = 3;
   ```

4. Run the simulation and compare the results.

The input curves chosen using the above method can be seen in Fig. 3a; the resulting simulated NF-κB profile can be seen in Fig. 3b. This predicted response could inform experimental protocol to ensure that the correct time points are chosen to observe the dynamics. The simulated response prediction was closely matched in mouse embryonic fibroblasts (MEFs) exposed to TNF and LPS by Werner et al. [1].
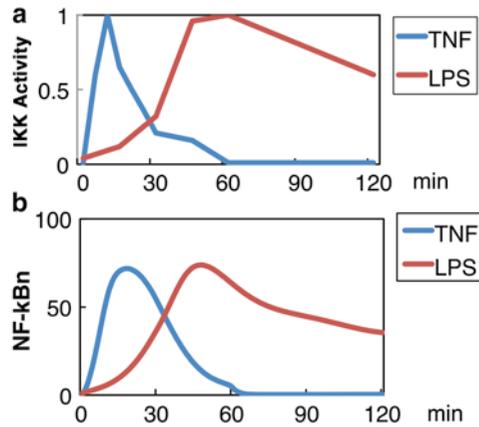
Fig. 3 (**a**) IKK activity input curves for TNF and LPS, quantitated and normalized from IKK immunoprecipitation kinase assays. (**b**) The simulated free NF-κB resulting from each input curve

3.2.2 Investigating Sensitivity to Identify Therapeutic Targets

The simulation can identify potential therapeutic targets for most effectively controlling the systems response to stimuli. A good therapeutic target is able to make large predictable changes to the system with only a small perturbation applied. A target with these properties leads to lower drug dosages and fewer side effects. The effect of perturbations that simulate therapeutic intervention can be easily tested in the simulation by adjusting parameters and measuring the output:

1. Specify the amount you wish to vary the parameters by in a variable. For an order of magnitude variation in a parameter,

```
delta = 10;
```

2. Multiply each parameter you wish to investigate by the previously defined variable. Often multiple parameters are changed simultaneously as the same process affects various isoforms. To change the rate of IKK-mediated degradation of all IκB isoforms,

```
params(78)=params(78)*(delta);
params(79)=params(79)*(delta);
params(80)=params(80)*(delta);
```

3. Run the simulation with the increased parameter; this will also plot the NF-κB response.

4. Divide each parameter by the perturbation variable.

```
params(78)=params(78)/(delta);
params(79)=params(79)/(delta);
params(80)=params(80)/(delta);
```

5. Run the simulation again to plot the response with the decreased parameter.
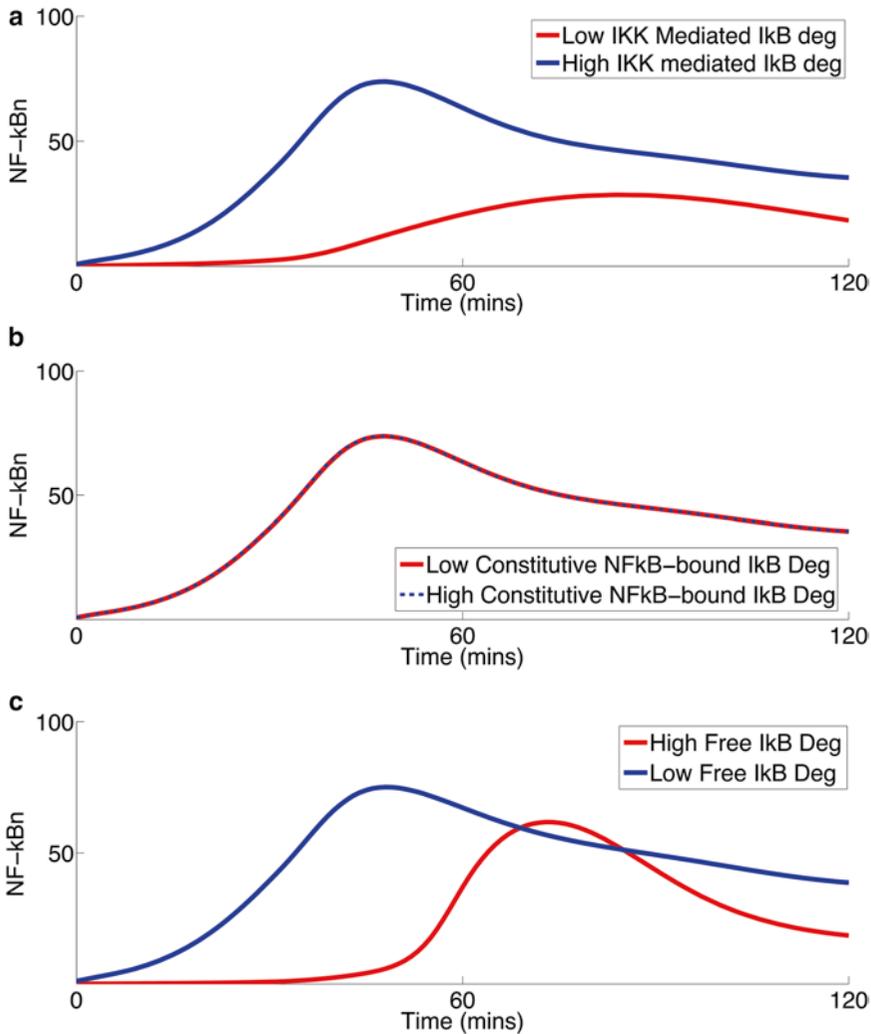
Fig. 4 Simulated NF-κB in response to LPS input curves with reaction rates increased an order of magnitude above and below basal for (**a**) IKK-mediated IκB degradation, (**b**) IKK-independent NF-κB-bound IκB degradation, and (**c**) free IκB degradation

Repeating this process for three sets of parameters representing the basal and IKK-mediated rate of IκB-NF-κB degradation and the rate of free IκB degradation gives the plots in Fig. 4.

As expected, increasing the rate of IKK-mediated degradation has a strong effect on the response of NF-κB (Fig. 4a). Also as expected, increased IKK-mediated IκB degradation results in an increase in free NF-κB. Interestingly, the amplitude of the response is not only reduced, but the time at which the response is maximal is shifted later with a lower degradation rate. As a result of the strong control provided by the rate of IKK activity, this process has been highly studied.

Figure 4b shows the basal (not IKK mediated) rate of NF-κB-IκB degradation. These parameters had no effect on the simulated NF-κB response. Therefore, attempting to use this process as a therapeutic intervention is unlikely to be successful.

Figure 4c shows perturbation of free IκB degradation; this was found to have a strong effect on the response. Decreasing free IκB degradation rate resulted in a slightly decreased peak of NF-κB response, but also a delayed response. The peak NF-κB response was found to be narrower and returned to lower levels more quickly when this parameter was decreased. Free IκB degradation rate has been much less actively studied, as it is a less intuitive point of intervention; however, through simulations we are able to identify this reaction as a point of strong control that should be investigated further.

3.2.3 Comparing Single-Cell Results with Population Level Results

The simulation of NF-κB signaling is a detailed representation of the intracellular environment; however, to draw conclusions relevant at a physiological level, then cell-to-cell variability must be considered. Here we create a simple physiologically relevant model by repeated simulations with variable delays applied to the output:

1. Define a mean and variance for the delay.

   ```
   meanValue=65;
   variance=1200;
   ```

2. Calculate μ and σ for a log-normal distribution.

   ```
   mu=log((meanValue^2)/sqrt(variance+meanValue^2));
   sigma = sqrt(log(variance/(meanValue^2)+1));
   ```

3. Define the range of repeated simulations you wish to run. Here we use a logarithmic range

   ```
   logspaceVals=logspace(0,2,6);
   ```

4. For each value chosen, run the simulation that number of times. Also create a vector consisting of a concatenation of the basal NF-κB level until the delayed time point, the simulation output, and the basal NF-κB level for the remainder of the vector. Store this vector in a variable and plot the average of these vectors to get the population level dynamics. Plot each of these averages on the same figure.

   ```
   for i=logspaceVals
       for j=1:i
           delay=floor(lognrnd(mu,sigma));
           plot(time+(delay),nfkb_timecourse);
           concatVector=[ones(delay,1) …
           *nfkb_timecourse(1,1);…
            nfkb_timecourse;…
   ```
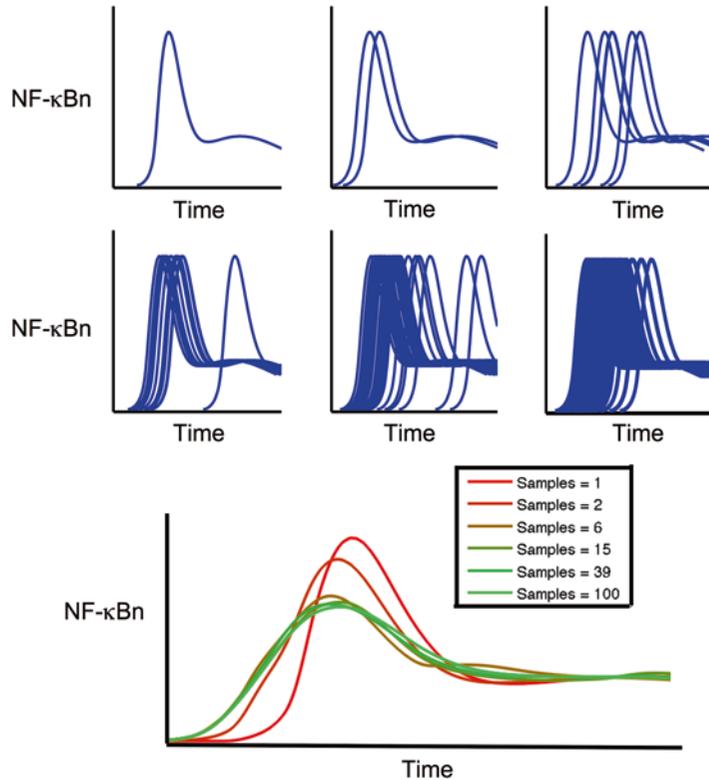
Fig. 5 NF-κB in response to LPS in repeated simulations sampled with a log-normally distributed delay. *Top*: Each curve represents a single simulation. *Bottom*: Average NF-κB concentration of multiple individual simulations

```
          ones(500-(SIM_TIME...
          +delay),1)*nfkb_timecourse(1,1)];
       averageMatrix=[averageMatrix,...
       concatVector];
   end
   figure(meanCurve);
   hold on;
   meanPlot=mean(averageMatrix,2);
   plot(meanPlot);
end
```

Figure 5 demonstrates that the average behavior of multiple single-cell simulations tends toward a less dynamic time course. This is in agreement with experimental results and represents a simple way of making the mechanistic cellular-scale model applicable at larger scales.

4   Notes

1. The web-based NF-κB signaling model available at http://
signalingsystems.org/webmodel/ can provide a convenient
way to produce preliminary results. Figures can be generated
without any modeling expertise and presented to modeling
experts in order to motivate more in-depth investigations or
model expansion.

2. While the goal is generally to produce models that reproduce
experimental findings and generate predictions that extend the
experimental work, it is important to note that an important
function of the model is to provide a sufficiency test. Akin to
the in vitro reconstituted biochemical systems that led to the
discovery and characterization of a multitude of replication
and transcription or translation factors and mechanisms, math-
ematical models of signaling allow one to ask whether the
known factors and molecular mechanisms are sufficient to
account for cell biological or physiological phenomena. In other
words, the model determines whether mechanistic knowledge
"adds up." If it does not, the model can direct the experimen-
tal discovery and characterization of the missing factor or
mechanism. This utility of modeling is often overlooked in the
field of purely theoretical computational biology, but is often
a major contribution in work that combines both experimen-
tal and modeling approaches.

3. To integrate computational studies into an interdisciplinary
team effort requires careful consideration of phasing the dif-
ferent aspects of a project. The ideal situation, with model
building and experimentation being conducted simultane-
ously, and iteratively informing each other, is often difficult to
realize successfully within the time frame available for projects
and relies on very close collaboration between researchers
from different backgrounds.

   Modeling work undertaken prior to wet lab work can be a
powerful tool for making predictions and generating hypoth-
eses. This type of exploratory modeling produces the most
exciting findings, but it can subsequently take many years for
the experimental work validating the findings to be completed.
Satisfying the needs of computational biologists may require
the publication of exclusively computational studies, though
they often lack the impact of studies combining theoretical
and experimental work.

   The alternative approach is to perform modeling work
after key experimental results have been obtained. The model
can then be used to provide mechanistic insights about the
experimental findings and direct additional, highly quantitative

experimentation that improves the quality of the work. This approach results in combined experimental and theoretical publications, which can have high impact; however, the full predictive potential of modeling is not explored, unless model predictions also lead to extensions of the scope of experimentation.

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