Comprehensive Single and Paired Drug Target Identification in Healthy and Disease Models of NF-kB Pathway

by

Sahar Ashraf Alkhairy

Submitted to the Department of Electrical Engineering and Computer Science
in partial fulfillment of the requirements for the degree of

Masters of Engineering in Computer Science and Molecular Biology

at the

MASSACHUSETTS INSTITUTE OF TECHNOLOGY

June 2016

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Abstract

Nuclear NF-κB ("NF-κBn") is a transcription factor responsible for regulating many genes that play important roles in inter- and intra-cellular signaling, cellular stress responses, cell growth, survival and apoptosis. Defective levels of NF-κBn has been associated with cancer, inflammatory, and autoimmune diseases. Therefore, it is important to identify species in the NF-κB pathway that if inhibited/ targeted by added drugs normalize the levels of NF-κBn. Also, since there is a limit to how effective single drug targets are in complicated diseases, identification of combinations of targets is very important. Existing experimental and computational work are disease specific, not comprehensive as they only analyze specific drug targets, and rarely analyze combinations of targets. In this thesis, we computationally modify a healthy model of the pathway to reflect what is defective in different classes of diseases. Then for each of the healthy and disease models, we score individual targets based on how much change in concentration of NF-κBn they induce when inhibited with an added inhibitor. Furthermore, we explain (1) why certain species score better than others, (2) why the inhibition profile of the output (NF-κBn) is not linear, and (3) why some isoforms of the same species score better than others. We also classify pairs of targets based on whether they are synergistic, additive, or antagonistic and obtain general trends for synergism of target combinations. Finally, we provide a comparison of the identified best targets with previously identified ones. One of our main findings is that very few species are effective at low levels of inhibition but many are effective at extremely high levels of inhibition. We also find that the scores of the species greatly depend on their steady state concentrations, the relative reaction parameters such as nuclear import/export rates and synthesis rates, and the relative concentrations of IKK and NF-κB. Furthermore, we find that all species that have some effect on the output are synergistic with one another. In departure from other approaches we have computationally targeted both individual proteins and protein complexes, rather than just individual proteins. Although protein complexes score better they are more difficult to inhibit physically.

Thesis Supervisor: Bruce Tidor
Title: Professor of Biological Engineering and Computer Science

Acknowledgments

I would like to thank my direct supervisor David Flowers for his invaluable supervision, Nirmala Paudel for her great help as this thesis is based on her PhD thesis work, the rest of the Tidor lab for producing a great work environment, Prof. Bruce Tidor for his supervision, and my parents and siblings for their eternal love and support.

Contents

Chapter 1

Introduction

1.1 Background and Motivation

Nuclear NF-κB (NF-κBn) is a transcription factor that is responsible for regulating many genes that play important roles in inter- and intra-cellular signaling, cellular stress responses, cell growth, survival and apoptosis [1]. Studies have found that many diseases are associated with a defective level of NF-κBn such as chronic inflammatory and autoimmune diseases (eg.asthma, COPD, and Celiac disease) [2, 3].

Inhibiting the NF-kκB pathway by inhibiting its species (proteins/ protein complexes) would normalize the output of NF-κBn. However, inhibition of the various species in the pathway do not all have the same level of effect on NF-κBn's output level. A few species might greatly increase/ decrease NF-κBn's output when inhibited by an added drug (inhibitor), while other species might have negligible effects. Therefore, identification of the best individual species (drug targets) to target is very important.

Furthermore, in many complex diseases like cancer there is a limit to how effective a single drug target is in normalizing the output either because of drug resistance or due to narrow therapeutic windows. Combination therapy, in which two or more drug targets are inhibited simultaneously, has emerged as a potential way to circumvent some of these shortcomings. The drug combinations may be additive, synergistic, or antagonistic. An additive pairs is one in which a linear combination of the two drug concentrations leads to the same overall effect on the output. A synergistic pair is one in which the addition

of one of the drugs reduces the amount required of the second drug to obtain an equal overall output effect compared to a linear combination of the two, requiring an overall lower amount of total drug intake. An antagonistic pair is one where we need a greater total amount of drug to have the same effect on the output than if only one of the drugs was given. Therefore, classification of target combinations in terms of synergism is another very important objective as we would like to simultaneously target species that are synergistic with one another and not antagonistic.

Screening drug targets in labs is expensive and time-consuming, thus comprehensively screening all the species in the pathway is extremely costly and technically infeasible. Computational comprehensive identification of drug targets, using computational models of the pathway, is faster, less expensive and provides a holistic view of the pathway. Thus this thesis takes a computational approach.

1.2 Previous Work

There have been great work towards the goal of identifying drug targets in the NF-κB pathway. Experimental work by Luron et al. [4], Trepicchio et al. [5], and Gohda et al. [6] focus on identifying drugs(s) for only a specific target and/ or analyzing if an existing drug that treats another disease would treat a disease associated with NF-κB pathway and then identifying the point of inhibition. Other more comprehensive experimental work like Miller et al.'s [7], start with many clinically approved drugs and screen them to identify the ones that inhibit the NF-κB signalling and then identify the points of inhibition.

As computational modeling can provide strong quantitative predictions, there have been computational work that also have the goal of identifying drug targets in the NF-κB pathway. Zhou et al. [8] analyzed the inhibition effects of four existing drugs and their targets individually and in combinations using a NF-κB pathway model with parameters obtained from cells with multiple myeloma.

These excellent works use cell lines or models that are disease specific. They are also not comprehensive and are limited to the analyses of previously identified drugs and their targets missing out on other potentially good targets. While there have been comprehensive

analyses of the NF-κB pathway such as the work of Kell et al. [9], they usually have the goal of analysing cellular signalling interactions by identifying the parameters that have the most control on the oscillatory output of NF-κBn which is different than the goal of identifying drug targets.

1.3 Objectives

There are five main objectives to this thesis. The first is to identify the points of defects in the pathway that are the cause for disease and modify the healthy model to reflect that in order to allow for a more robust way of discovering drug targets for different diseases. The second is to obtain the best individual drug targets in the healthy and disease models. The third is to categorize the target combinations as additive, synergistic, or antagonistic. The fourth is to provide explanations to why certain targets score better than others and explanations to key observations. The two key observations are (1) Why does the output, NF-κBn, not have a linear profile? (2) Why are alpha isoforms better drug targets than the beta and epsilon isoforms? The fifth is to provide a comparison of the identified best targets with previously identified ones.

1.4 Organization

This thesis consists of six main chapters. In Chapter 2, we describe the healthy and disease models of NF-κB. In Chapter 3, we describe the methodology used for scoring individual species and the categorization of combinations of species. In Chapter 4, we show how the output changes with the different disease models and determine if the objective is to increase or decrease the output. In Chapter 5, we present the scoring results of the individual species for the different models. In Chapter 6, we present the categorization results of the combinations of species. In Chapter 7, we provide explanations to the results obtained and compare the identified targets to previously identified ones.

Chapter 2

Models

2.1 Healthy Model

2.1.1 The System

The deterministic NF-κB pathway model by Kell et al [9] is periodic and contains species in both the nucleus and the cytoplasm. It contains 26 species (including the source which represents DNA and the sink which represents degradation) (table A.1 in Appendix A) and 64 mass actions reactions modeled by ordinary differential equations (table A.2 in Appendix A). There are 3 negative regulators for NF-κB: IκBα, IκBβ, and IκBϵ; and one kinase (IKK) that phosphorylates the inhibitors for degradation. All the IκBs are similar in structure but have different binding preferences. In general, NF-κB can either be p65/p50 or p50/p50. The p65/p50 leads to the transcription of a variety of genes, and the p50/p50 homodimer inhibits transcription [10]. The NF-κB that is in the model is the p65/p50 [9].

There are approximately seven surface receptors that are upstream of the NF-κB pathway and they are: BCR, TCR, TLRs, IL-1R, TNFR, GF-Rs, and (LTBR, CD40, BR3) [11]. In the model used, the pathway is activated by TNF-a. The activation of TNF-a leads to activation of IKK, which in turn phosphorylates the inhibitor-NF-κB complex (IκB-NF-κB), which leads to ubiquitination of the inhibitors and release of NF-κB, which then enters the nucleus and transcribes proteins including its inhibitors.

The model includes only the core of the NF-κB pathway and does not include the

surface receptors mentioned above. However, the stimulation of IKK by the TNF-a surface receptor and other means is captured by having the concentration of IKK to be initially at 0.1 μM and then having it gradually decrease (as the stimulation goes away). A very simplified version of the pathway is shown in figure 2-1. A more detailed pathway is found in figure B-1 in Appendix B.

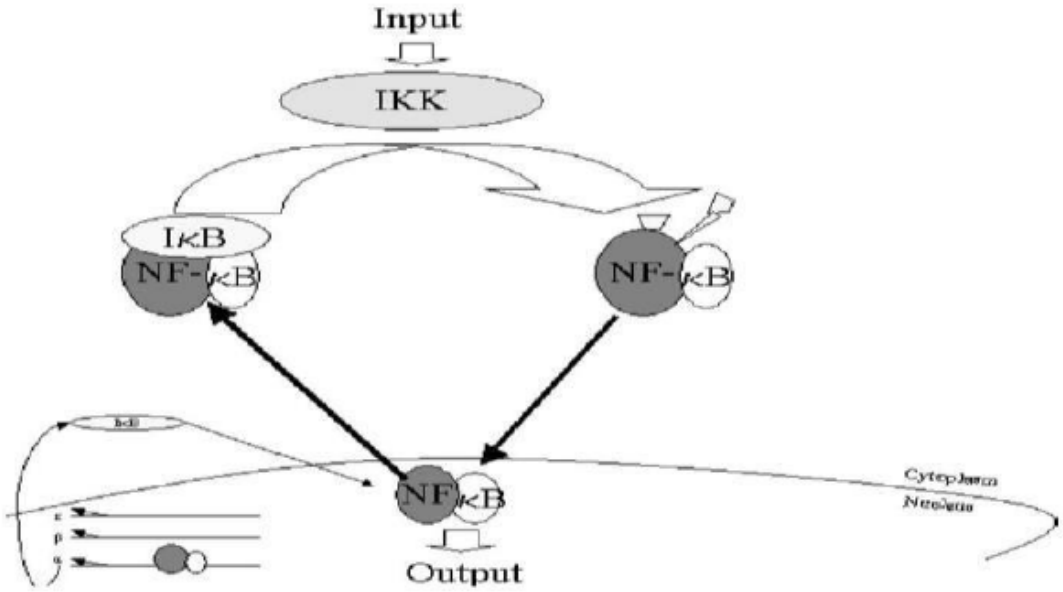


Figure 2-1: A simplistic overview of the NF-κB pathway. Source Kell et al. [9]

2.1.2 System's Behavior

The System at the Beginning and After Reaching Steady State

The system starts off with IκBx, IκBxn, NF-κB, NF-κBn, IκBx-NF-κB, IκBxn-NF-κBn, IKK, and IκBx-t where x refers to all three isophorms alpha, beta, and epsilon. All complexes with IKK start off with zero concentration. The species that get degraded are IKK, IκBx, IκBx-t. IκBx-t gets produced continuously, IκBα-t at a rate of $1.54 * 10^{-6} \mu M^{-1} s^{-1}$, I$\kappa$B$\beta$-t at a rate of $1.78 * 10^{-7} \mu M^{-1} s^{-1}$, and I$\kappa$B$\epsilon$-t at a rate of $1.27 * 10^{-7} \mu M^{-1} s^{-1}$.

After running the system to steady state, most of the species have a steady state concentration approximately the same as the starting concentrations except IKK which gets completely degraded. Complexes with NF-κB and NF-κBn have a steady state concentration slightly higher than the initial starting concentration. The output of concern, NF-κBn, reaches steady state after many oscillations. The area under the curve is 24.1600 μM with peak amplitude of 0.0002 μM and steady state concentration of $2.4 * 10^{-4} \mu M$.

2.2 Diseases Associated with Defective NF-κB Output

2.2.1 Associated Diseases

IKK is composed of 3 subunits: IKK1(IKKα), IKK2(IKKβ), and NEMO(IKKγ). IKK1 and IKK2 are catalytic subunits, whereas NEMO is a regulatory subunit. When ligands bind to the surface receptors, after a cascade of signals, NEMO either get activated or inhibited by different upstream species [10].

Diseases associated with NF-κB are caused by alterations to 'healthy' resulting from defects in (1) NF-κB itself, (2) species that directly regulate NF-κB but are not in our model, (3) IκBs, (4) IKK (especially NEMO), or (5) species upstream of IKK [12]. We only consider diseases caused by the last three types (3, 4, 5) of modification.

An example of a disease caused by modifications in IκBs is HL (common B-cell lymphoma). In this disease, the IκBs lose their function thereby leading to a constitutively active NF-κB [12].

Our model does not include species upstream of IKK and furthermore treats IKK1 (IKKα), IKK2 (IKKβ), and NEMO (IKKγ) as one species - IKK. Thus we can collapse the alterations (4) and (5) into ones with inactive IKK and ones with a constitutively active IKK. The first category (ones with inactive IKK) causes a decrease in NF-κB activation and the second category (ones with constitutively active IKK) causes an increase in NF-κB activation.

Examples of diseases that belong to the first category are incontinentia pigmenti (IP) [13], a genetic disease that causes altered pigmentation and developmental abnormalities,

and anhidrotic ectodermal dysplasia with immunodeficiency (EDA-ID) [12], a group of conditions characterized by abnormal development of ectodermal tissues including the skin, hair, teeth, and sweat glands. An example of a disease that belongs to the second category is Multiple Myeloma, cancer that forms in plasma cells [14]. It is important to note that there are extremely rare diseases that are caused by IκBα not getting phosphorylated by IKK such as EDA-ID(t), but they are not considered as they are very rare [12].

To summarize, the three disease models we will study are: (1) Model 1: Loss of IκBs function, (2) Model 2: inactive IKK, (3) Model 3: constitutively active IKK.

2.2.2 Disease Model 1: Loss of IκBs Function

HL (common B-cell lymphoma) is disease caused by modifications in IκBs such that they lose their functionality. In the original healthy model, we start off with initial amounts of IκBs that gets degraded and consumed by reactions but gets replenished at a constant rate. In order to alter the model to have it represent the disease state with dysfunctional IκBs, we change the concentrations of the three IκBs to be constant at zero. This is to show that even though IκBs are being produced, only dysfunctional ones are. Changing the concentration of transcriptional IκBs to be constant at zero produces the exact same results as when we modified the concentrations of IκBs. We will use the model in which the concentrations of the three transcriptional IκBs are set constant at zero.

2.2.3 Disease Model 2: Inactive IKK

Incontinentia pigmenti (IP) is a disease caused by an inactive IKK component NEMO. In the healthy model we have an initial amount of 0.1 μM of IKK which is consumed and degraded over time. In order to alter the model to have it represent the disease state with an inactive IKK, we make IKK be a constant input at 0 μM.

2.2.4 Disease Model 3: Constitutively Active IKK

Multiple Myeloma is a disease caused by having a constitutively active IKK. The initial amount of IKK (0.1 μM) gets consumed and degraded over time. In order to alter the model

to have it represent the disease state with a constitutively active IKK, we make IKK be a constant input at 0.1 μM.

Chapter 3

Methodology

Our methods are based on Nirmala's methodology [15] to target individual and pairs of species, score individual species, and categorize pairs of targets.

3.1 Targeting and Scoring of Individual Species

One of our main objectives is to exhaustively identify the best individual drug targets that either decrease the output in diseases that have an increased output or increase it in diseases that have a decreased output. In order to achieve that objective and identify the best individual targets, we systematically add a constant amount of reversible inhibitor to each species with an association rate constant of $10^{-3}\mu M^{-1}s^{-1}$ and a dissociation rate constant of $10^{-6}s^{-1}$ (inhibitor constant of $10^{-3}\mu M$) at 100 different logarithmically spaced concentrations between 10^{-4} and $10^{2}\mu M$. We maintain the inhibitor at a constant value to simulate having a large amount of drug in the system.

To quantify the effect of the inhibition on the output we obtain the target effect (TE) and output effect (OE) for each of the 100 different inhibitor concentrations after allowing the system to equilibrate with the inhibitor and reach steady state to get rid of transient fluctuations that are a result of initial concentrations. The target effect is the fraction of available target that is bound by inhibitors, which is inactive and cannot participate in reactions. The output effect is the percentage change in output from the unperturbed state; It is the percent decrease in diseases with a higher than normal output and a percent increase

in diseases with a lower than normal output. We then plot the 100 OE and TE pairs. There are three possible outcomes for the OE versus TE curves. One is that it is linear, where in order to have a 50 % OE we need to inhibit the species by 50 %. The second is a sublinear response, where in order to have an OE of 50 % we need to inhibit the species by more than 50 %. The third is superlinear, where inhibiting the species by less than 50 % would lead to an OE of 50 %.

To compare between species, we choose three TE values: 52%, 91%, and 100% and rank the OE for all the species. The reason we look at different degrees of inhibition of each species and not just when it is 100% inhibited is because it might be better to target a species that results in a higher OE at low levels of inhibition than to target a drug that results in a much higher OE but only when it is inhibited at very high levels. This is because in practice high doses of drug are likely to be toxic.

3.2 Targeting Pairs of Species and Categorizing Them

To identify which pairs of targets works well, we systematically inhibited each of the two species of the pair at 100 different logarithmically spaced concentrations between 10^{-4} and $10^{2}\mu M$ with a total of 10^{4} different combinations of concentrations. Similar to the targeting of individual species, we maintain a constant amount of the two reversible inhibitors introduced and allow the system to reach steady state with them before simulating the system and obtaining the OEs for all the combinations of the two drug levels.

We then looked at how pairs of drug targets effect the output compared to when each one of the drugs were given alone at equivalent concentrations. To that end, we plotted the first species' TEs, second species' TEs, and OEs for all the 10^{4} combinations in a heat plot and categorized the pairs as additive, synergistic, or antagonistic for seven contours of output effect levels of 30%, 50%, 70%, 90%, 99.0%, 99.99%, and 99.999%.

An additive pair means that the two drugs have a linear effect on the output. Graphically, these are the pairs where the overall OE is constant along lines where the total drug concentration is fixed. A synergistic pair is one in which the addition of one of the drugs reduces the amount required of the second drug to obtain an equal overall output effect

compared to a linear combination of the two, requiring an overall lower amount of total drug intake. Graphically, these are the pairs these are the pairs where a higher overall output effect is achieved at some combinations of drugs along some constant drug concentration curves. An antagonistic pair is one where we need a greater total amount of drug to have the same effect on the output than if only one of the drugs was given. Graphically, these are the pairs where a lower overall output effect is achieved at some combinations of drugs along some constant drug concentration curves.

3.3 Features

We scored the species based on the in integral of output concentration over time. This is because we saw that the change in amplitude is the same as the change in integral of output concentration over time as the species reached non-periodic steady state. We do not look at other features such as the number of peaks and the average frequency of oscillations as initial work showed that they highly rank species that do not make biological sense. For example, the highly scored negative regulators of NF-κB and inhibiting a negative regulator would lead to an increase in NF-κB and not a decrease in it which we want. Furthermore, the results using the number of peaks and average frequency of oscillations features are not consistent with the results obtained from the integral of output concentration over time and amplitude features which make biological sense.

Chapter 4

Model Results

4.1 Disease Model 1: Loss of IκBs Function

In diseases that are represented by this model, there are modifications in IκBs such that they lose their functionality. In the original healthy model, we start off with initial amounts of IκBs that are degraded and consumed by reactions but are also replenished at a constant rate. In order to alter the model to have it represent the disease state with dysfunctional IκBs, we change the concentrations of the three IκBs to be constant at zero. This is to show that even though IκBs are being produced, only dysfunctional ones are. Changing the concentration of transcriptional IκBs to be constant at zero produces the exact same results as when we modified the concentrations of IκBs. We will use the model in which the concentrations of the three transcriptional IκBs are set constant at zero μM.

To know what the effect of this modification of the model on the output nuclear NF-κB (NF-κBn) is, we obtained the area under the curve after running the system for 10^5 secs and saw a 41200 % increase in NF-κBn output, 24.16 μM to 9991.12 μM. This makes sense because we are disrupting the outputâs inhibitor. For this disease model, the objective is to find drugs that decrease the output of NF-κBn.

4.2 Disease Model 2: Inactive IKK

In diseases that are represented by this model, NEMO, a component of IKK, is inactive. In the healthy model we have an initial amount of 0.1 μM of IKK which gets consumed and degraded over time. In order to alter the model to have it represent the disease state with an inactive IKK, we make IKK be a constant input at 0 μM.

To know what the effect of this modification of the model on the output NF-κBn is, we obtained the area under the curve after running the system for 10^5 secs and saw no change in the output levels; In both the healthy model and this altered model the integral of the output is 24.1600 μM. This makes sense because at steady state, IKK and any species complex containing IKK have been completely consumed by degradation or reactions with other species so making them inactive and setting the concentration to zero makes no difference on the output.

There are two possible reasons to why we are not able to capture diseases with an inactive IKK: 1) the model used might not be accurate enough because it might need to represent components of IKK separately or it is not correctly modeling the concentration profile of IKK. 2) NEMO or IKK might be involved in another pathway and the disruption in the other pathway might be the cause of disease.

4.3 Disease Model 3: Constitutively Active IKK

In diseases represented by this model, IKK is constitutively active. In the healthy model we have an initial amount of 0.1 μM of IKK which gets used up and degraded over time. In order to alter the model to have it represent the disease state with a constitutively active IKK, we make IKK be a constant input at 0.1 μM.

To know what the effect of this modification of the model on the output NF-κBn is, we obtained the area under the curve after running the system for 10^5 secs and saw a 24000 $\%$ increase in the output (from 24.1600 μM in the healthy model to 5936.06 μM in this disease model).This makes sense biologically as having a constant input of the inhibitor to the inhibitors of NF-κB (IκBxs) is equivalent to activating NF-κB. For this disease model

the objective is to find drugs that decrease the output of NF-κBn.

4.4 Disease Models Used in Identifying Targets

Since only disease model 1 (Loss of IκBs function) and disease model 3 (Constitutively active IKK) have any effect on the output NF-κBn, we will identify individual and pair targets only for them and not disease model 2 (Inactive IKK). Also, since both disease model 1 and disease model 3 lead to an increase in output, we will identify targets that decrease the output.

Chapter 5

Single Target Results

5.1 Single Drug Targets in the Healthy Model

5.1.1 Output Effect - Inhibition Level Profiles for Each Target

The OE vs TE profiles for all species in the healthy model can be found in section B.2 in Appendix B. The species categorization can be seen in figure 5-1.

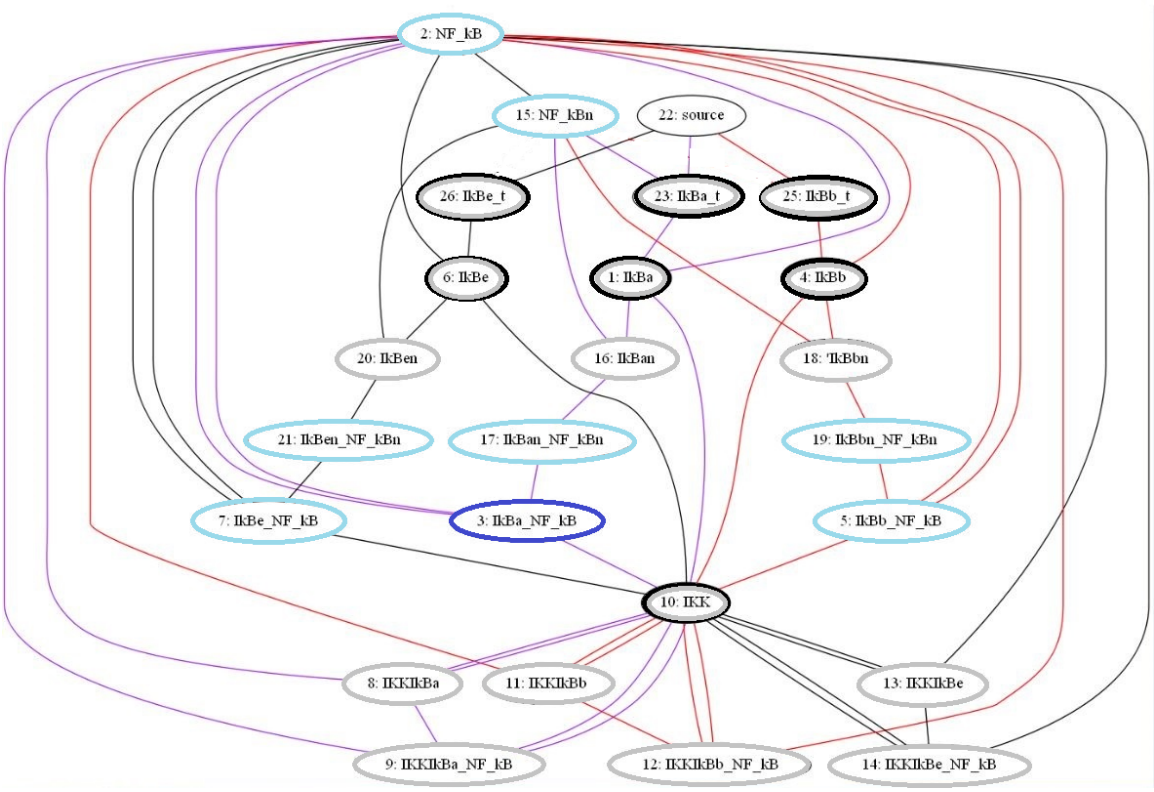


Figure 5-1: Classification of species in the healthy model based on their OE vs TE profiles. Species in dark blue circles have super-linear or linear profiles. Species in light blue circles have sub-linear profiles. Species in grey circles have no effect on the output. Species in black circles get degraded.

5.1.2 Optimal Target and TE level

Figure 5-2 shows the OE for various TEs for all species for the healthy model. The species that achieve an OE > 40

- TE > 30 %: IBα-NF-B (species 3)
- TE > 75 %: IBβ-NF-B (species 5)
- TE > 80 %: IBϵ-NF-B: (species 7)
- TE < 95 %: IBαn-NF-Bn (species 17)
- TE > 99 %: NF-B (species 2), NF-Bn (species 15), IBβn-NF-Bn (species 19) , and IBϵn-NF-Bn (species 21)

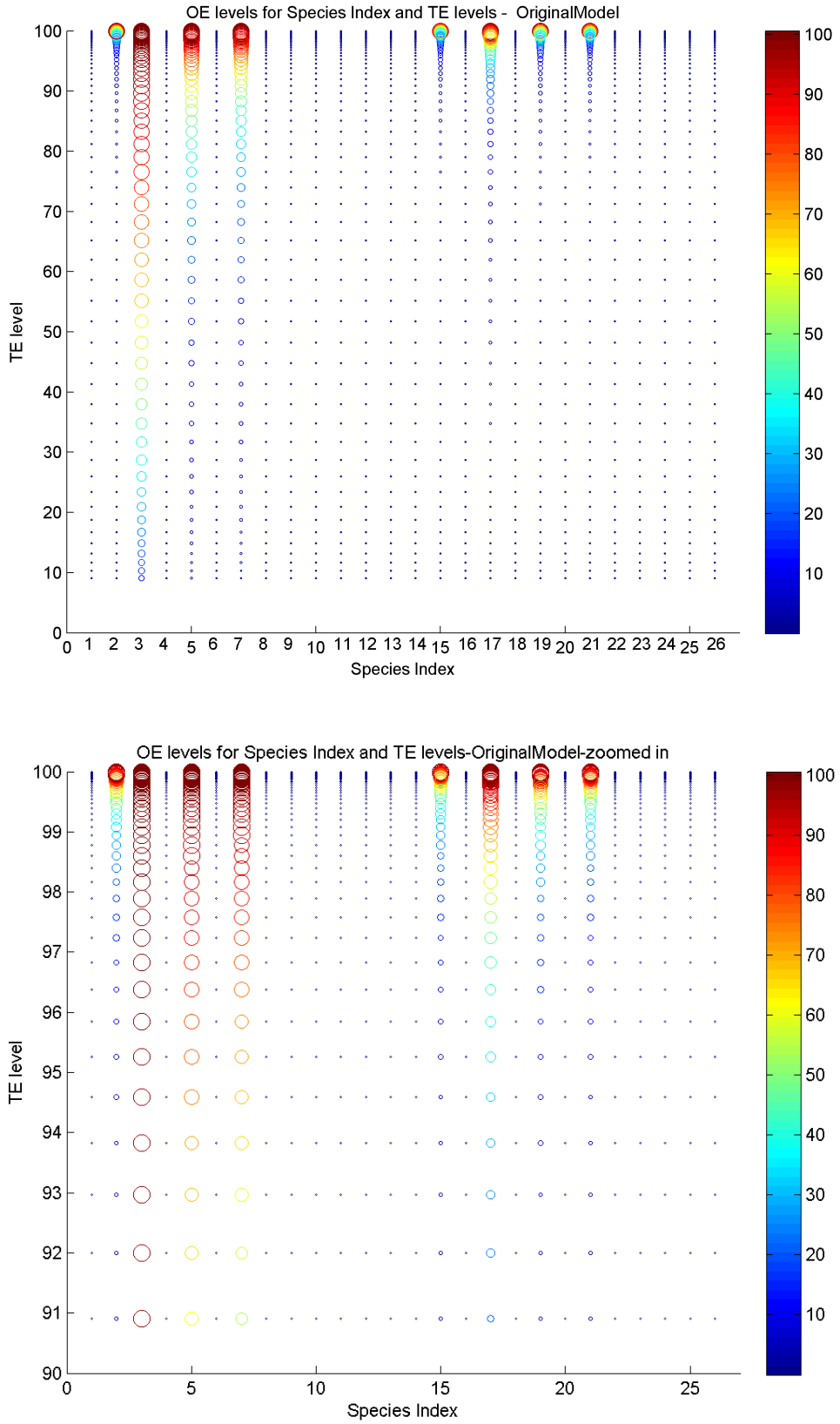


Figure 5-2: OE for various TEs for all species for the original healthy model. The size and color vary according to the OE. Top figure shows all TEs and the bottom figure shows TEs> 90 %.

5.1.3 Optimal Target

The species that achieve and OE level $>$ 40 % with TE $<=$ 80 are:

- IBα-NF-B (species 3)
- IBβ-NF-B (species 5)
- IBϵ-NF-B: (species 7)

5.1.4 TE Level Threshold

There is no level threshold that produces OE level > 40 % that works for all species.

5.2 Single Drug Targets in Disease Model 1

5.2.1 Output Effect - Inhibition Level Profiles for Each Target

The OE vs TE profiles for all species in disease model 1 can be found in section B.3 in Appendix B. The species categorization can be seen in figure 5-3.

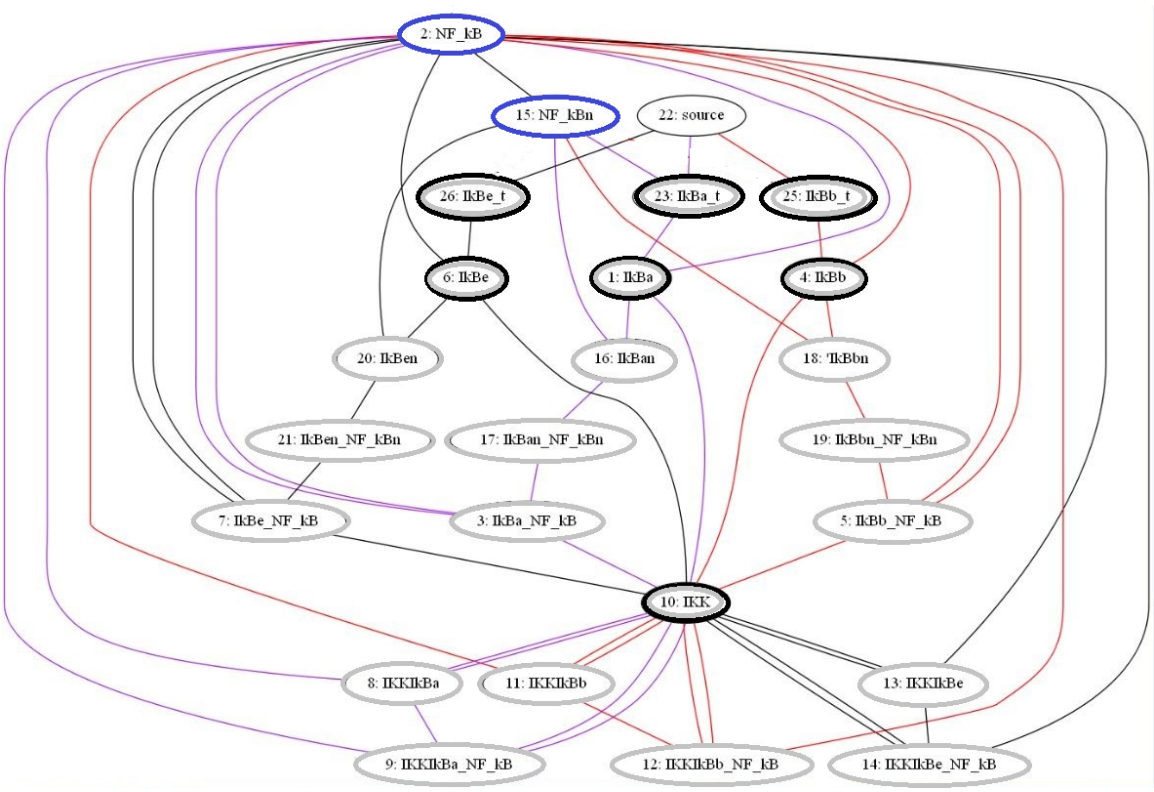


Figure 5-3: Classification of species in disease model 1 based on their OE vs TE profiles. Species in dark blue circles have super-linear or linear profiles. Species in light blue circles have sub-linear profiles. Species in grey circles have no effect on the output. Species in black circles get degraded.

5.2.2 Optimal Target and TE level

Figure 5-4 shows the OE for various TEs for all species for the healthy model. The species that achieve an OE $>$ 40 % are:

- TE $>$ 40 %: NF-Bn (species 15)
- TE very close to 100 %: NF-B (species 2)

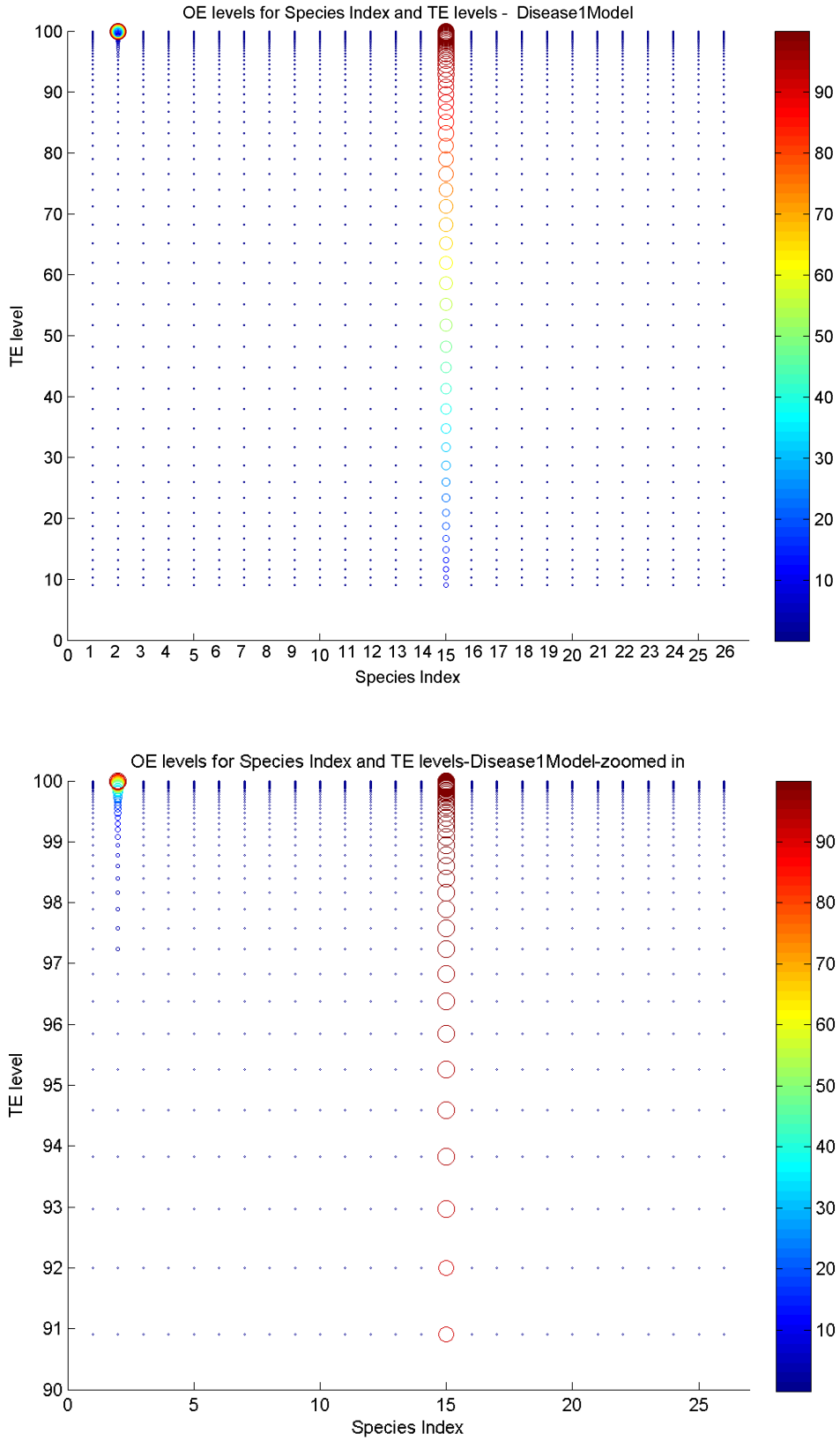


Figure 5-4: OE for various TEs for all species for disease model 1. The size and color vary according to the OE. Top figure shows all TEs and the bottom figure shows TEs> 90 %.

5.2.3 Optimal Target

The species that achieve and OE level $>$ 40 % with TE $<=$ 80:

- NF-Bn (species 15)

5.2.4 TE level Threshold

There is no level threshold that produces OE level > 40 that works for all species

5.3 Single Drug Targets in Disease Model 3

5.3.1 Output Effect - Inhibition Level profiles for each Target

The OE vs TE profiles for all species in the healthy model can be found in section B.4 in Appendix B. The species categorization can be seen in figure 5-5.

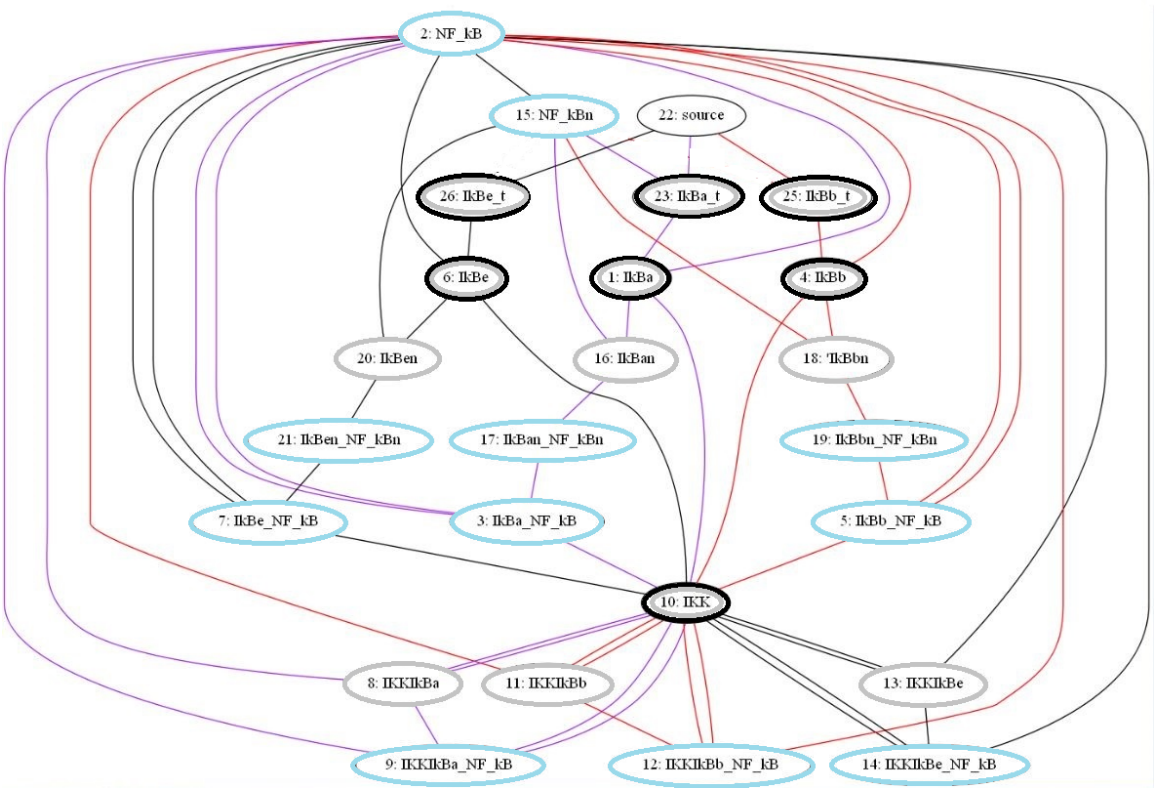


Figure 5-5: Classification of species in the disease model 3 based on their OE vs TE profiles. Species in dark blue circles have super-linear or linear profiles. Species in light blue circles have sub-linear profiles. Species in grey circles have no effect on the output. Species in black circles get degraded.

5.3.2 Optimal Target and TE level

Figure 5-6 shows the OE for various TEs for all species for the disease model 3. The species that achieve an OE $>$ 40 % are:

- TE $>$ 50 %: NF-Bn (species 15)
- TE $>$ 90 %: IBα-NF-B (species 3) and IKKIBα-NF-B (species 9)
- TE $>$ 94 %: IBαn-NF-Bn (species 17)
- TE $>$ 99 %: NF-B (species 2)
- Requires close to 100 % TE: IBβ-NF-B (species 5), IBϵ-NF-B (species 7), IKKIBβ-NF-B (species 12), IKKIBϵ-NF-B (species 14), IBβn-NF-Bn (species 19), and IBϵn-NF-Bn (species 21)

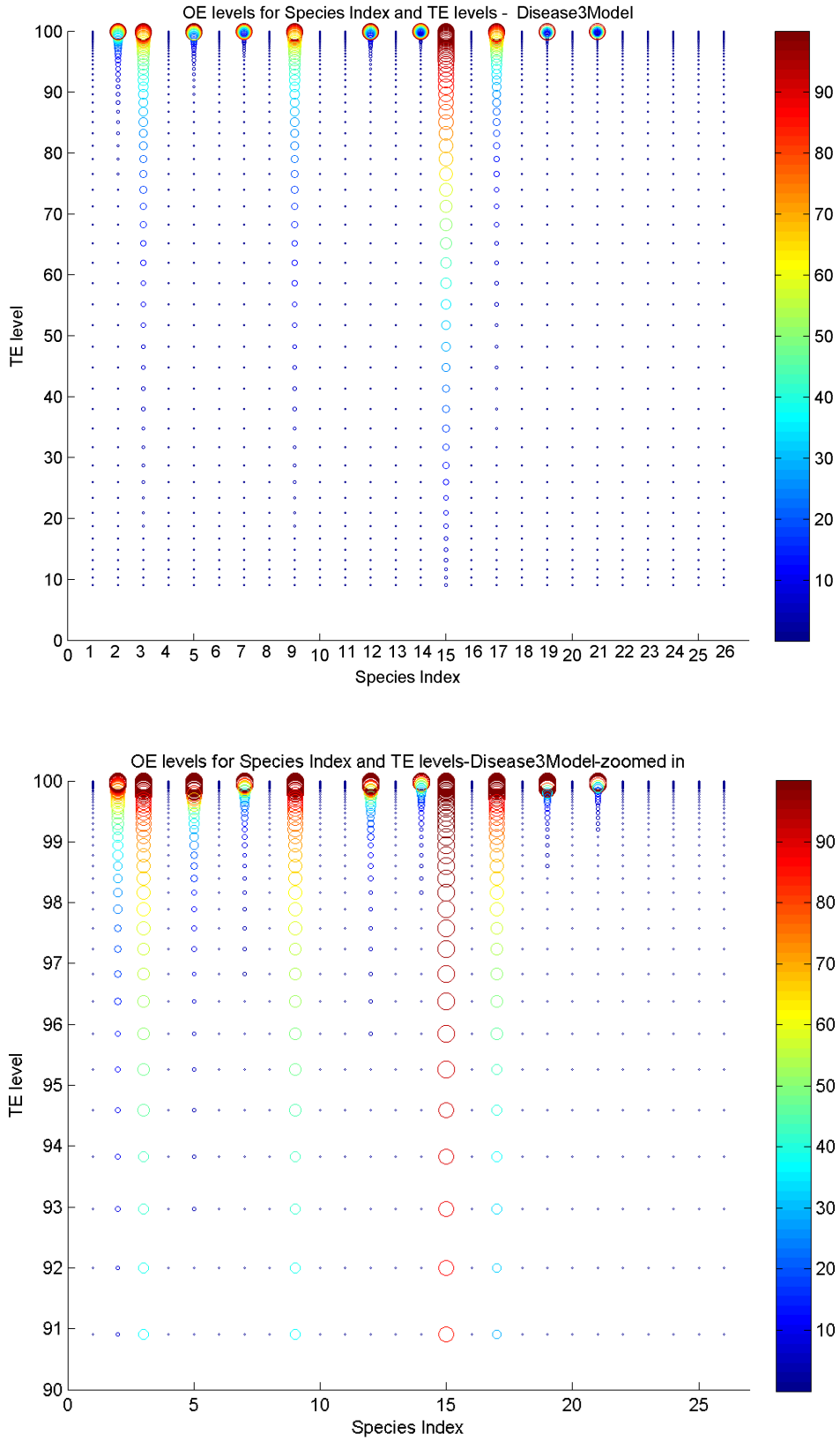


Figure 5-6: OE for various TEs for all species for disease model 3. The size and color vary according to the OE. Top figure shows all TEs and the bottom figure shows TEs> 90 %.

5.3.3 Optimal Target

The species that achieve and OE level $>$ 40 % with TE $<=$ 80:

- NF-Bn (species 15)

5.3.4 TE Level Threshold

There is no level threshold that produces OE level > 40 that works for all species

5.4 Comparison of Single drug targets across models

Figure 5-7 shows the OE for each target across models. Species that reach > 90 % OE regardless of how much inhibition is required that work for at least two of the three models (health model (0), disease 1 model (1), and disease 3 model (3)) are: NF-kB (species 2), IBα-NF-kB (species 3), IBβ-NF-kB (species 5), IBϵ-NF-kB (species 7), NF-kBn (species 15), IBαn-NF-kBn (species 17), IBβn-NF-kBn (species 19), and IBϵn-NF-kBn (species 21).

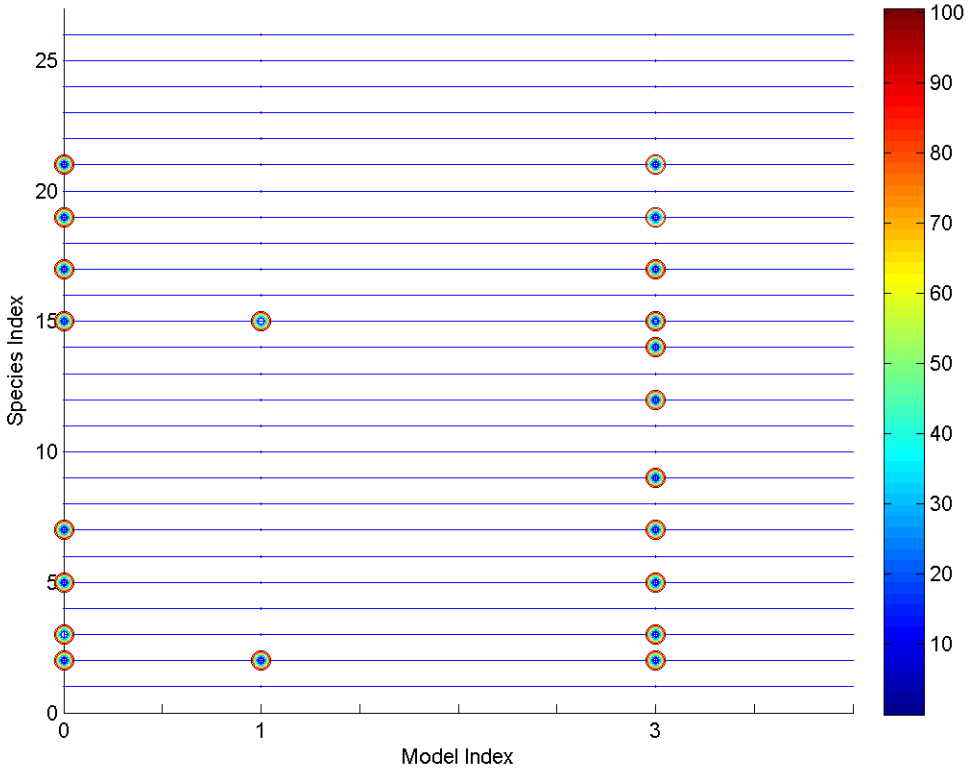


Figure 5-7: OE for each species across models. The size and color vary according to the OE.

Chapter 6

Paired Target Synergy Results

We looked at synergy of pairs at seven different OE levels: 30%, 50%, 70%, 90%, 99.0%, 99.99%, and 99.999% for each of the models.

6.1 Paired Drug Targets in the Healthy Model

Figure 6-1 shows the level of synergy between synergistic pairs at various OE levels. Figure B-5 in Appendix B.5 shows whether each combination of species is synergistic, additive, or antagonistic for the various OE levels. The pairs of species that are synergistic for at least one OE level are all pair combinations of the following species: NF-κB (2), IκBα-NF-κB (3), IκBβ-NF-κB (5), IκBϵ-NF-κB (7), NF-κBn (15), IκBαn-NF-κBn (17), IκBβn-NF-κBn (19), and IκBϵn-NF-κBn (21).

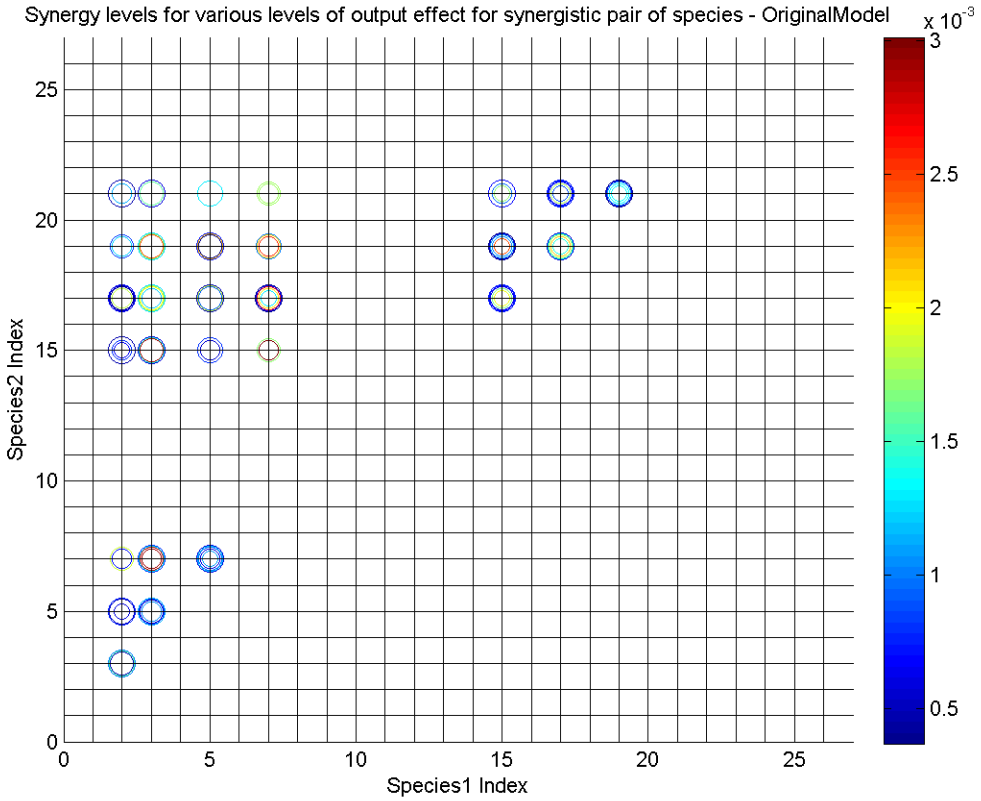


Figure 6-1: Synergy levels for various levels of OE for synergistic pairs of species for the healthy model. The size of the circles varies according to the OE level (but scaled to fit the grids) and the color varies according to the amount of synergism. To give a sense of scale, the pair (7,17) has the following levels of synergism for the seven OE levels: $0.0013, 0.0020, 0.0026, 6.616 * 10^{-4}, 4.017 * 10^{-4}, -1.772 * 10^{-5}$ (not shown), NaN (not shown).

6.2 Paired Drug Targets in Disease Model 1

Figure 6-2 shows the level of synergy between synergistic pairs at various OE levels. Figure B-6 in Appendix B.5 shows whether each combination of species is synergistic, additive, or antagonistic for the various OE levels. The pairs of species that are synergistic for at least one OE level is only (NF-κB (2), NF-κBn (15)).

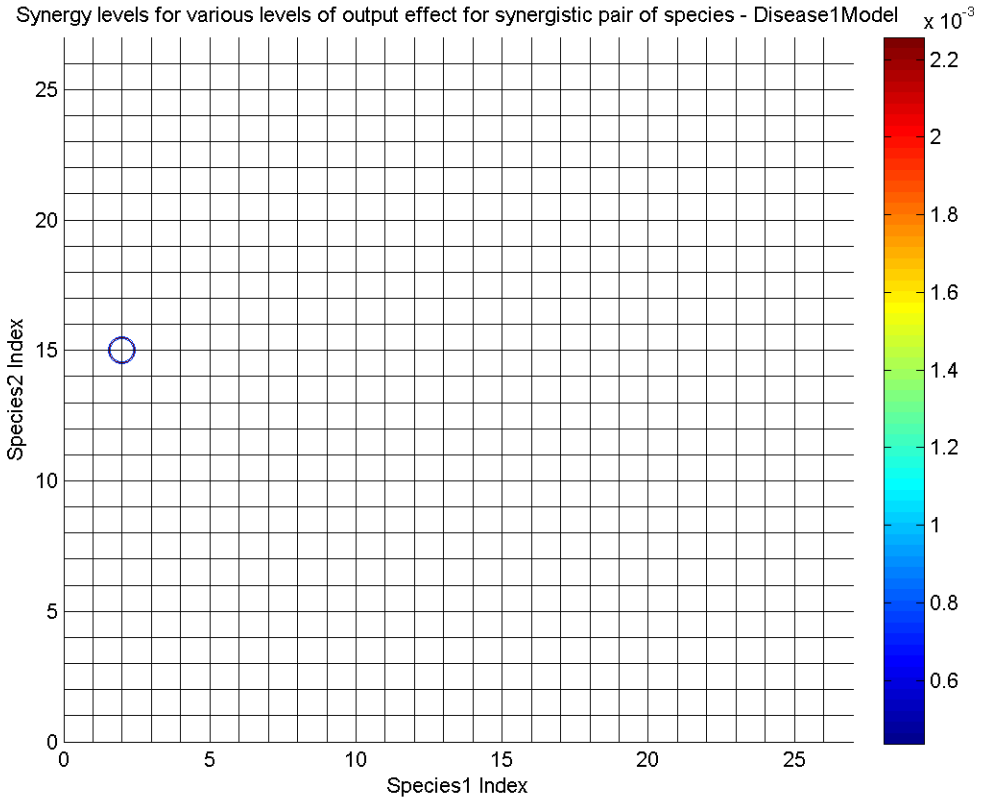


Figure 6-2: Synergy levels for various levels of OE for synergistic pairs of species for the disease model 1. The size of the circles varies according to the OE level and the color varies according to the amount of synergism.

6.3 Paired Drug Targets in Disease Model 3

Figure 6-3 shows the level of synergy between synergistic pairs at various OE levels. Figure B-7 in Appendix B.5 shows whether each combination of species is synergistic, additive, or antagonistic for the various OE levels. The pairs of species that are synergistic for at least one OE level are all pair combinations of NF-κB (2), IκBα-NF-κB (3), IκBβ-NF-κB (5), IκBϵ-NF-κB (7), IKKIκBα-NF-κB (9), IKKIκBβ-NF-κB(12), IKKIκBϵ-NF-κB(14), NF-κBn (15), IκBαn-NF-κBn (17), IκBβn-NF-κBn (19),and IκBϵn-NF-κBn (21).

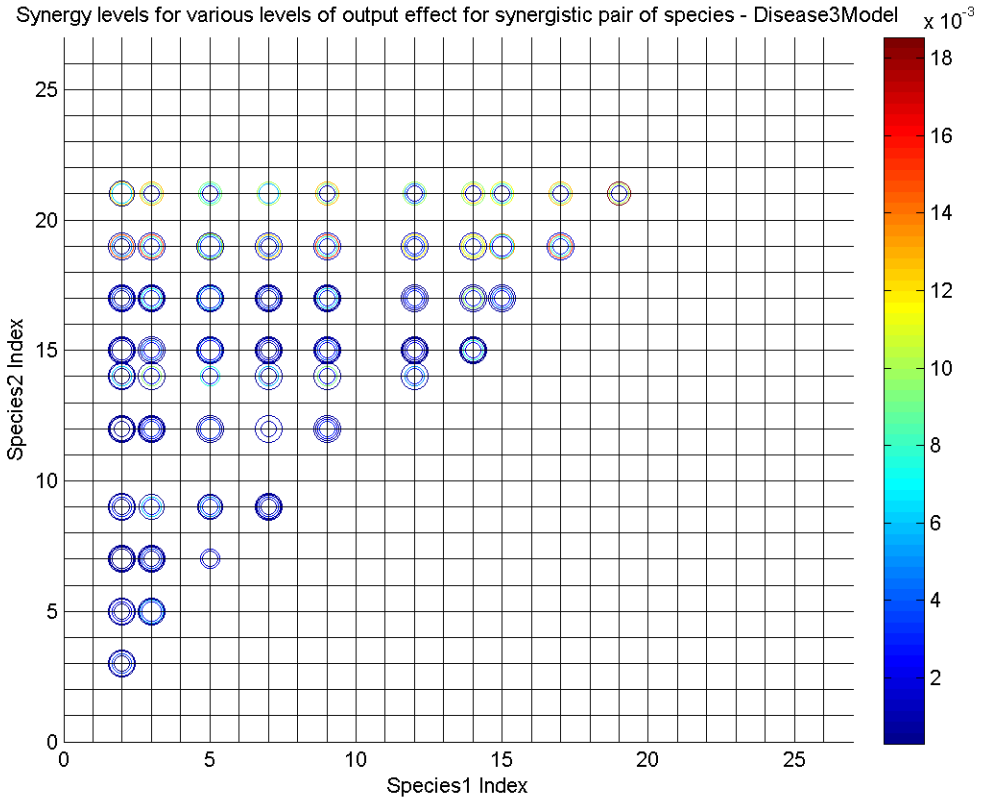


Figure 6-3: Synergy levels for various levels of OE for synergistic pairs of species for the disease model 3. The size of the circles varies according to the OE level and the color varies according to the amount of synergism.

6.4 Paired Drug Targets Across Models

The pairs of species that are synergetic along at least one OE level for at least two of the model are all pair combinations of the following species: NF-κB (2), IκBα-NF-κB (3), IκBβ-NF-κB (5), IκBϵ-NF-κB (7), NF-κBn (15), IκBαn-NF-κBn (17), IκBβn-NF-κBn (19), and IκBϵn-NF-κBn (21).

Chapter 7

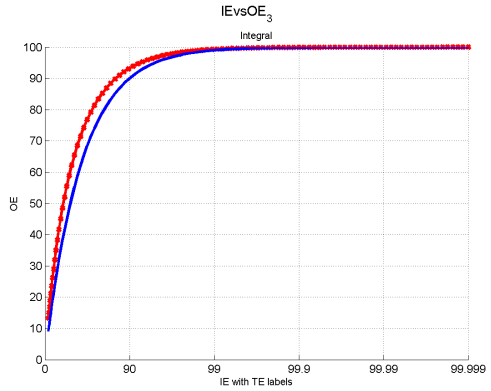
Discussion

7.1 Explanation of Top Scoring Single Targets

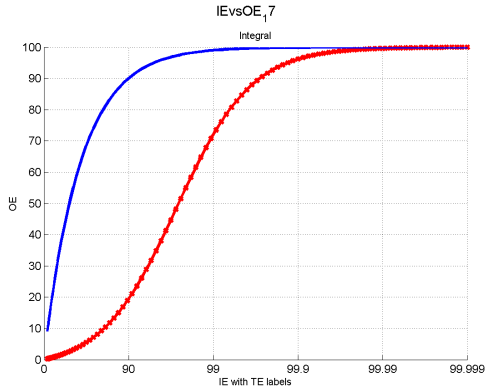
In the healthy model, at steady state, all the species are active except for species with IKK. This is because at steady state, all complexes with IKK are completely consumed/ degraded. Among the still active species, IκBx-t, IκBx, IκBxn have no effect on the output because no matter how much of it is inhibited, there is a constant amount of them being transcribed, thus there is no overall decrease in their amounts in the system when inhibited and thus do not induce any change in NF-κBn's output.

In this model, we see that IκBx-NF-κBs are the top scoring species and score much higher than their nuclear counterparts IκBxn-NF-κBns. This may be because there is only nuclear export of IκBxn-NF-κBns and no nuclear import resulting in a greater steady state concentration of IκBx-NF-κBs than of IκBxn-NF-κBns. To confirm, we added a nuclear import reaction of IκBα-NF-κB with equal reaction rate as the export. This addition resulted in IκBαn-NF-κBn having similar effectiveness as IκBα-NF-κB as can be seen in figure 7-1.

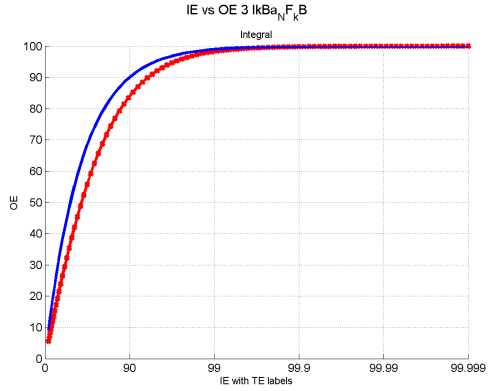
Also, in this model, NF-κB and NF-κBn are not as effective as IκBx-NF-κB and IκBxn-NF-κBn as can be seen in figure 7-2. This may be because cytoplasmic and nuclear NF-κB are limited in amount and most of them form complexes with IκBx and IκBxn. NF-κB gets consumed by IκBβ in reaction # 3, IκBb-NF-κB-association. NF-κBn gets consumed by IκBβn in reaction # 23, IκBb-NF-κB-nuclear-association. In both reactions, the forward



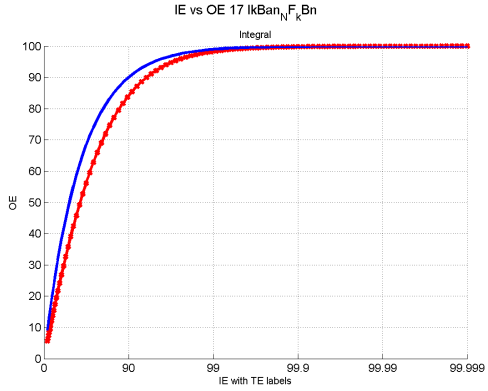
(a) OE vs TE profile for IκBα-NF-κB in the healthy model



(b) OE vs TE profile for IκBαn-NF-κBn in the healthy model



(c) OE vs TE profile for IκBα-NF-κB in model with added nuclear import

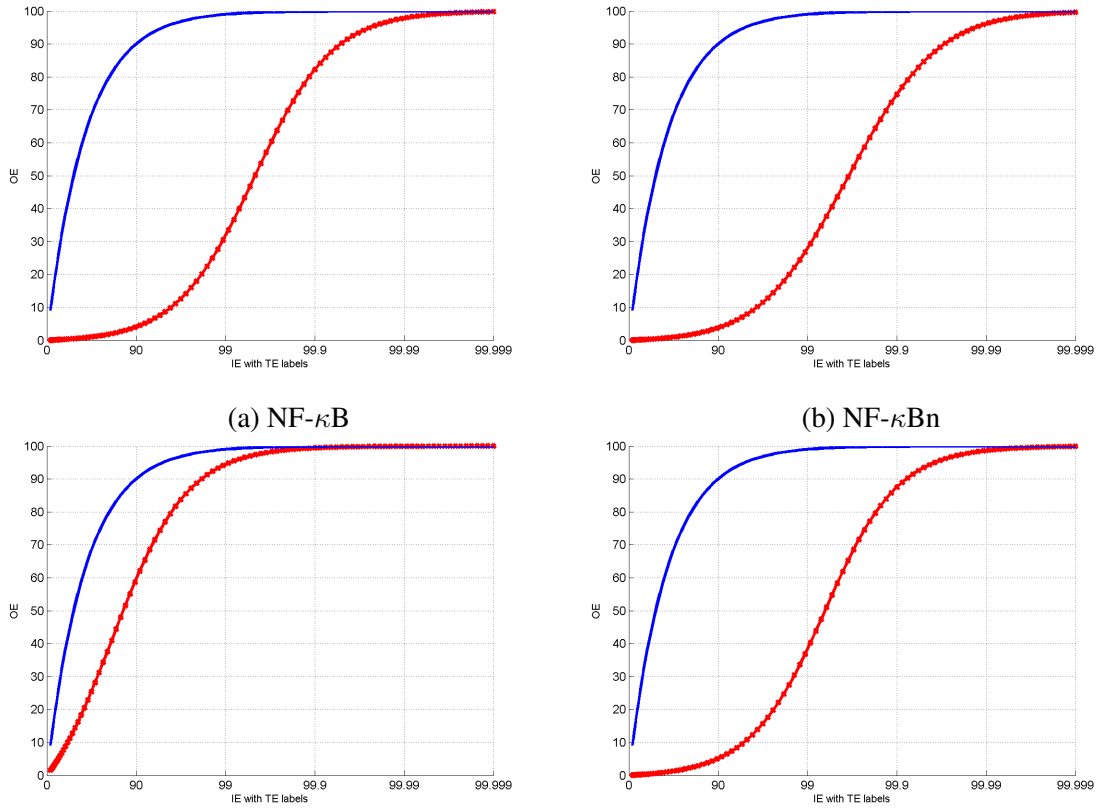


(d) OE vs TE profile for IκBαn-NF-κBn in model with added nuclear import

Figure 7-1: OE vs TE profiles for cytoplasmic and nuclear IκBα-NF-κB in the healthy model and model with added nuclear import. Adding nuclear import reaction with equal reaction rate as the export causes the nuclear complex to have similar effectiveness as its cytoplasmic counterpart.

reaction rate is greater than the reverse rate. Decreasing the forward rates to be equal to the reverse rates by altering the rate parameters decreases the effectiveness of IκBβ-NF-κB and IκBβn-NF-κBn as can be seen in figure 7-3.

In disease model 1, where IκBs are dysfunctional, the only two species that have any effect on the output are NF-κBn (species 15) and NF-κB (species 2). This is because all the other species are complexes with either IKK or IκBxs or both which are both non active/dysfunctional at steady state. Among the two active species, NF-κBn scores much higher than NF-κB, even though this is essentially a two-species linear pathway. The difference in scores is also the case in the other models. The reason for this is because the



(a) NF-κB

(b) NF-κBn

(c) IκBβ-NF-κB

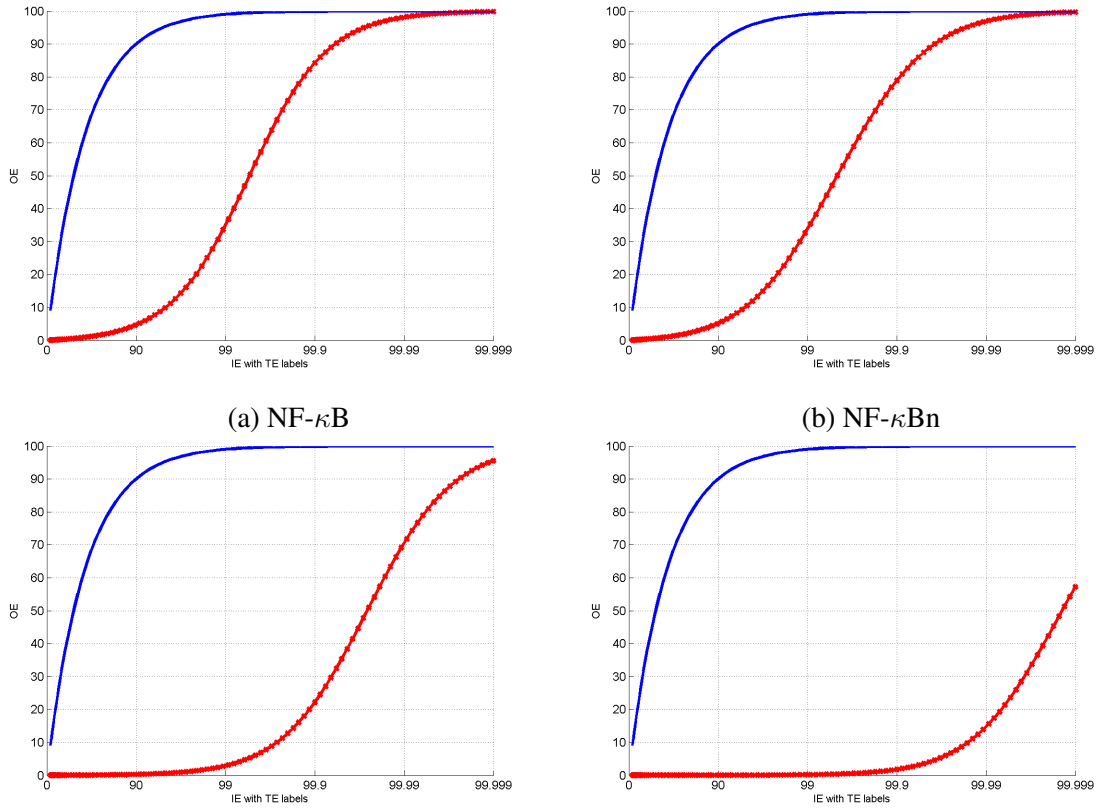
(d) IκBβn-NF-κBn

Figure 7-2: OE vs TE for cytoplasmic and nuclear NF-κB and cytoplasmic and nuclear IκBβ-NF-κB in the healthy model.

nuclear import rate of NF-κB is $9 * 10^{-2}s^{-1}$ and the nuclear export rate of NF-κBn is $8 * 10^{-5}s^{-1}$ resulting in NF-κBn having a much greater steady state concentration than NF-κB thus having a greater effect when inhibited. Making the import and export rates equal results in NF-κB and NF-κBn having equal effectiveness as can be seen in figure 7-4.

In disease model 3, where IKKs are constitutively active, all the species have some effect on the output except for IκBx, IκBxn, IκBx-t, IKK, and IKKIκBx. This is because there is a constant input of IKK and IκBx-t (which goes on to become IκBx and IκBxn).

In this model, IκBx-NF-κBs do not score as highly as they did in the healthy model. This is because most of the IκBxs are used up in forming IKKIκBxs with IKK instead of forming IκBx-NF-κB with NF-κB as the latter is limited by the amount of NF-κB and at steady state there is a lot more IKK than NF-κB. To confirm that the decrease in effectiveness of the IκBs between the healthy model and disease model 3 is due to having the



(a) NF-κB

(b) NF-κBn

(c) IκBβ-NF-κB

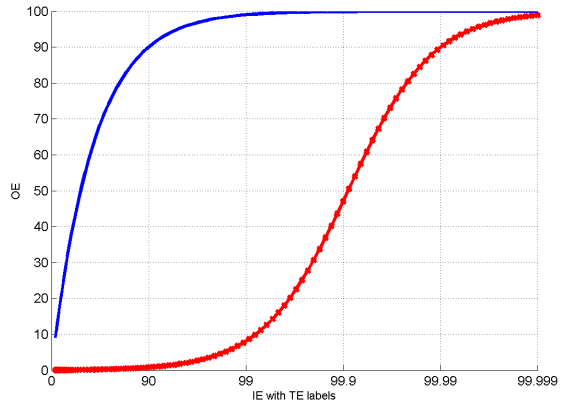
(d) IκBβn-NF-κBn

Figure 7-3: OE vs TE profiles for cytoplasmic and nuclear NF-κB and IκBβ-NF-κB in the model with equal forward and reverse rates for IκBβ-NF-κB-association and IκBβ-NF-κB-nuclear-association.

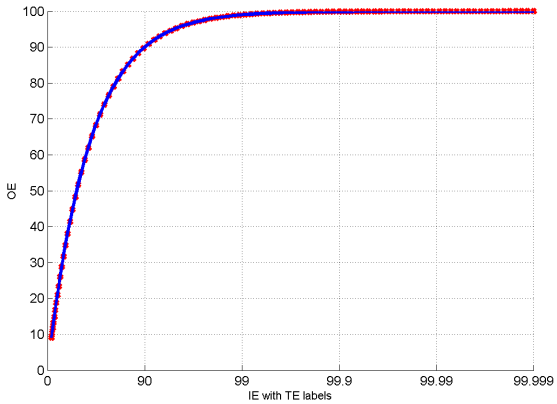
steady state concentration of IKK being much greater than that of NF-κB, we altered the model to have IKK be at a constant concentration of 0.0002 μM roughly equal to the initial concentration of NF-κB. Decreasing the concentration of IKK results in great increase in OEs of IκBx-NF-κBs (figure 7-5) confirming our hypothesis.

Also, in this model the OE of NF-κB is a lot greater than its effect in the healthy model. This could also be attributed to the same cause as before, namely, more of the IκBxs are forming complexes with IKK instead of with NF-κB. This causes there to be more free NF-κB/NF-κBn in the system to be inhibited. The same alteration of decreasing the concentration of IKK results in a large decrease in the OE of NF-κB as can be seen in figure 7-6.

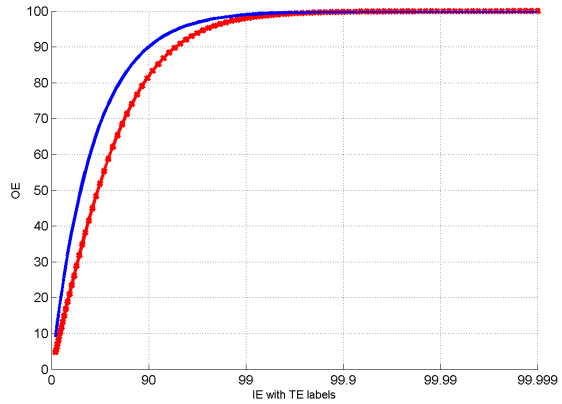
Overall, we can say that only species that are limited in production have any effect on



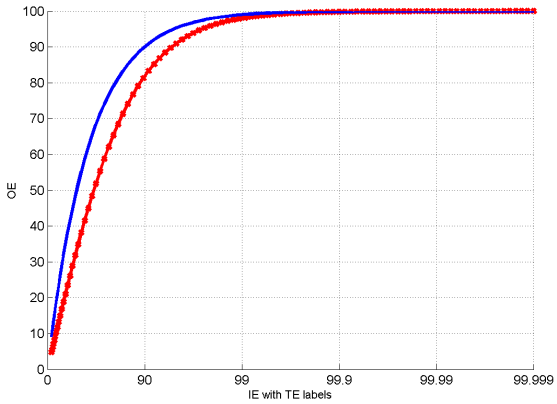
(a) NF-κB profile in disease model 1



(b) NF-κBn profile in disease model 1



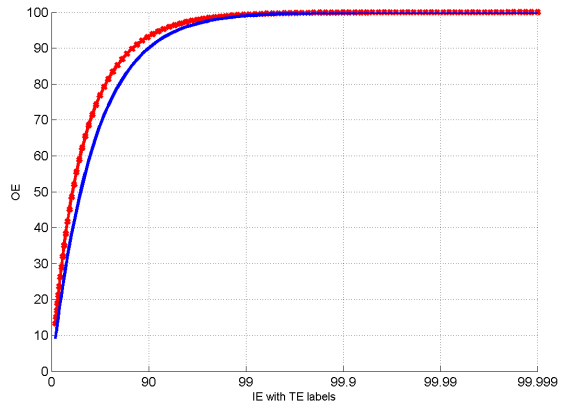
(c) NF-κB profile in model with equal nuclear import and export rates of NF-κB



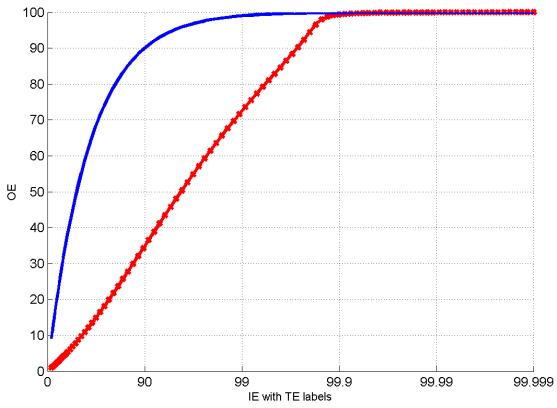
(d) NF-κBn profile in model with equal nuclear import and export rates of NF-κB

Figure 7-4: OE vs TE profiles of cytoplasmic and nuclear NF-κB in disease model 1 and in a model with equal nuclear import and export rates of NF-κB. Modifying the import and export rates to be equal causes the profiles of the cytoplasmic and nuclear NF-κB to be similar.

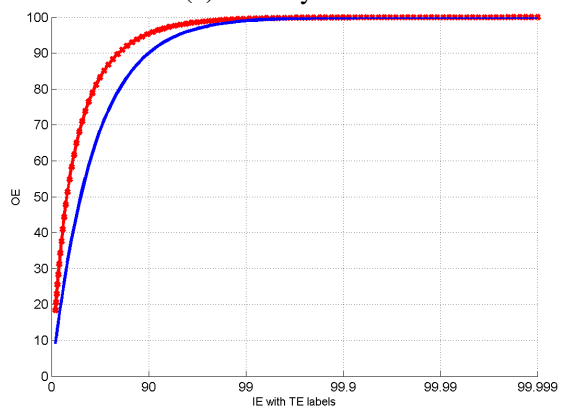
the output and any species that are constantly being produced have no effect. Also, among the species that are limited in production, the ones with greater steady state concentration have a greater effect.



(a) Healthy model

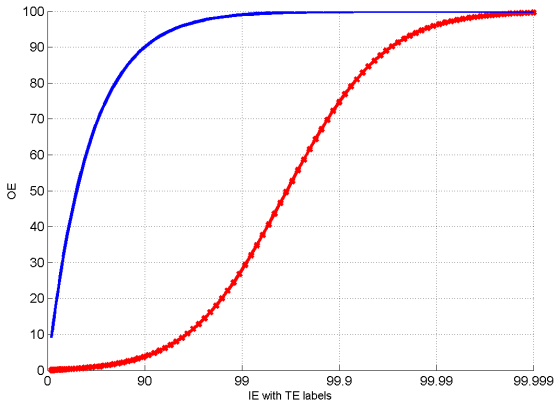


(b) Disease model 3

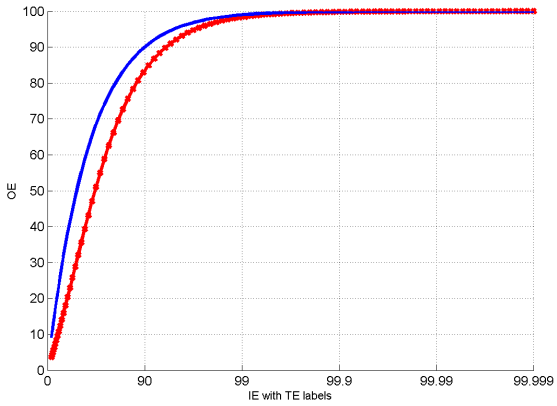


(c) Model with decreased concentration of IKK

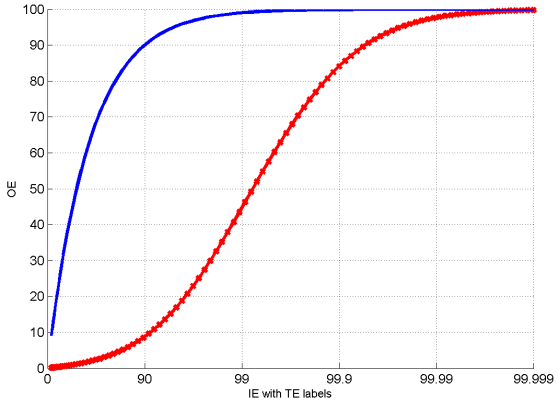
Figure 7-5: IκBα-NF-κB profile in the healthy model, disease model 3, and model with decreased IKK concentration. IκBα-NF-κB is less effective in disease model 3 than in the healthy model. Decreasing the concentration of IKK increases effectiveness of IκBα-NF-κB.



(a) NF-κBn profile in the healthy model



(b) NF-κBn profile in disease model 3



(c) NF-κBn profile in model with decreased concentration of IKK

Figure 7-6: NF-κBn profiles in the healthy model, disease model 3, and and model with decreased IKK concentration. The effectiveness of NF-κBn is greater in disease model 3 than it is in the healthy model. Decreasing the concentration of IKK decreases the effectiveness of NF-κBn

7.2 Why does the output, NF-κBn, not have a linear profile?

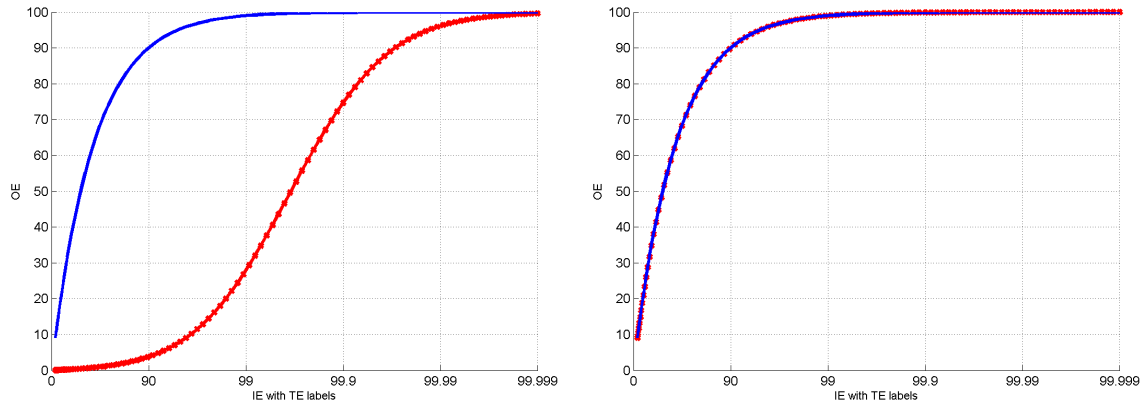
One key observation we see is that the OE vs TE profile for the output, nuclear NF-κB (NF-κBn, species # 15), is not linear. We expect a linear profile for the output because we are inhibiting and measuring the change for the same species. Understanding the reactions and the species NF-κBn is directly involved in led us to come up with three possible explanations to to this observation. These are: (1) there are competing reactions with the added inhibitor, (2) NF-κBn is involved in the synthesis of one of its inhibitors, IκBα, (3) there is a limitless amount of its inhibitors in the system and only a limited amount of NF-κBn.

NF-κBn is directly involved in the following sets of reactions: (1) NF-κB-nuclear-import/export ($k_i = 1.125 * 10^3$), (2) IκBx-NF-κB nuclear-association/dissociation ($k_i = 1 * 10^{-3} \mu M$), (3) IκBα-Inducible-mRNA-synthesis ($k = 0.0165 \mu M^{-1} s^{-1}$). The inhibition constant that we used was $10^{-3} \mu M$. The smaller the dissociation constant the stronger the reaction is and the less free NF-κBn there is. The inhibition constant we used is greater or equal in strength to its competitors. To truly confirm whether competition is the reason for this observation, we used a stronger inhibitor with an inhibition constant of $10^{-6} \mu M$ and saw that using a much stronger inhibitor did not make the NF-κBn OE vs TE profile any more linear. Thus, it is not competition that caused this non-linearity.

To see if the reason for this non-linearity is because NF-κBn is involved in the synthesis of IκBα (one of its inhibitors)via reaction # 28 (NF-κBn + NF-κBn -> IκBα-t + NF-κBn + NF-κBn), we removed the reaction from the model. This did not make the profile linear.

The last possible explanation for why the output itself has a nonlinear profile is that there is a limitless amount of its inhibitors (IκBα, IκBβ, and IκBϵ) but only a limited amount of NF-κBn. To test this hypothesis, we modified the system and removed the constant input of inhibitor by setting the source to zero. In this modification the output did have a linear profile. Therefore, the constant amount of IκBs is the reason for the non-linearity. This reasoning is reinforced by the fact that in disease model 1, where the IκBs are dysfunctional, result in a linear profile, whereas in the healthy model and disease model 3 do not result in a linear profile. Figure 7-7 shows the OE vs TE profile for the output in

disease model 1 and the healthy model.



(a) OE vs TE profile of NF-κkBn in the healthy model

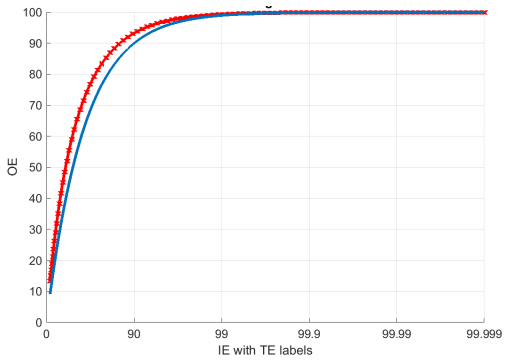
(b) OE vs TE profile of NF-κkBn in disease model 1

Figure 7-7: OE vs TE profile for the output NF-κB in the healthy model and disease model 1. The profile is sub-linear in the healthy model and linear in disease model 1.

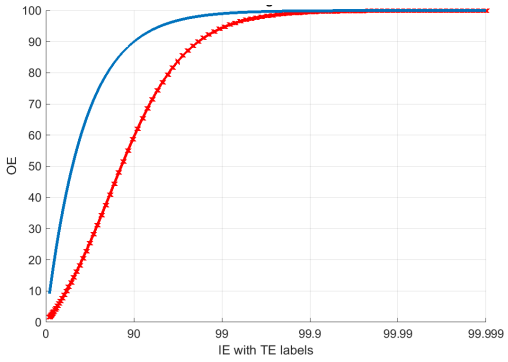
7.3 Why are alpha isoforms better drug targets than the beta and epsilon isoforms?

Another key observation we see is that alpha isoforms are better drug targets than the beta and epsilon isoforms. This is true in both the healthy model and disease model 3, and is not applicable to disease model 1 because the IκBs are dysfunctional causing all species with IκBs to be dysfunctional. The biggest differences in scores can be observed in the IκBx-NF-κB species.

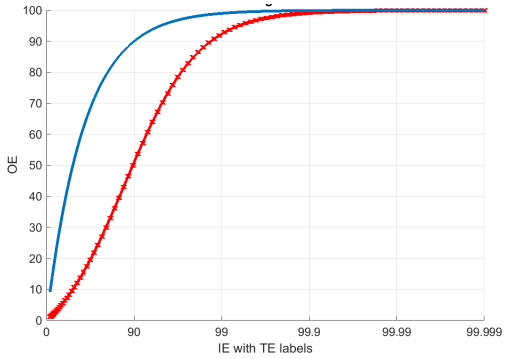
Figures 7-8 and 7-9 show the OE vs TE profiles for the three isoforms in the healthy model and disease model 3 respectively. In the healthy model, IκBα-NF-κB (species 3) only requires a TE of about 30 % to achieve an OE level of 40 %; IκBβ-NF-κB (species 5) and IκBϵ-NF-κB (species 7) require TEs greater than 75 % to achieve the same OE level. Similarly, in disease model 3, IκBα-NF-κB can achieve an OE level of 40 % with a TE of 90 %, whereas both IκBβ-NF-κB and IκBϵ-NF-κB require a TE close to 100 % to achieve the same OE level.



(a) IκBα-NF-κB

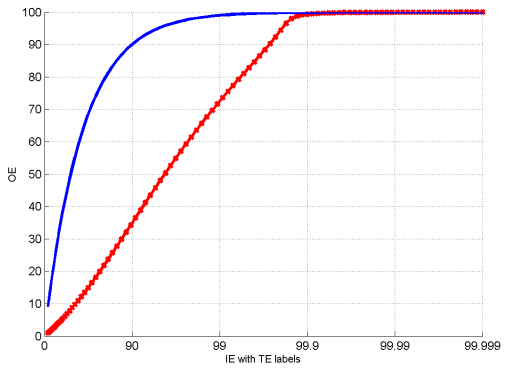


(b) IκBβ-NF-κB

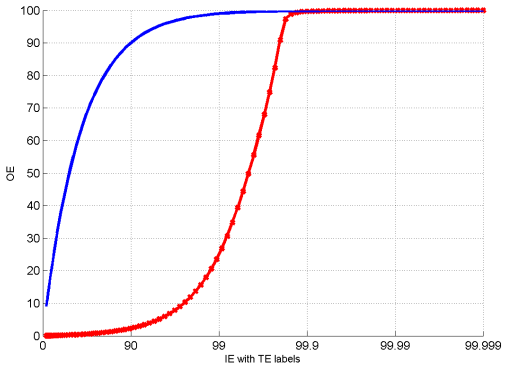


(c) IκBϵ-NF-κB

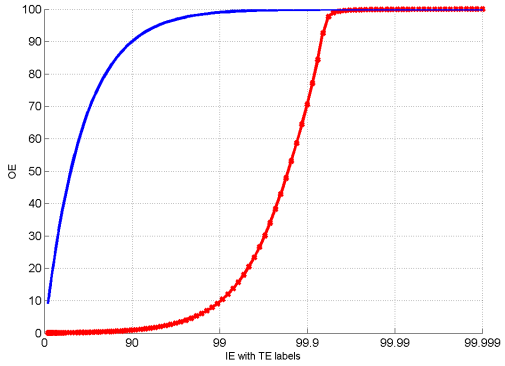
Figure 7-8: OE vs TE profiles for IκBα-NF-κB, IκBβ-NF-κB, and IκBϵ-NF-κB using the original healthy model.



(a) IκBα-NF-κB



(b) IκBβ-NF-κB



(c) IκBϵ-NF-κB

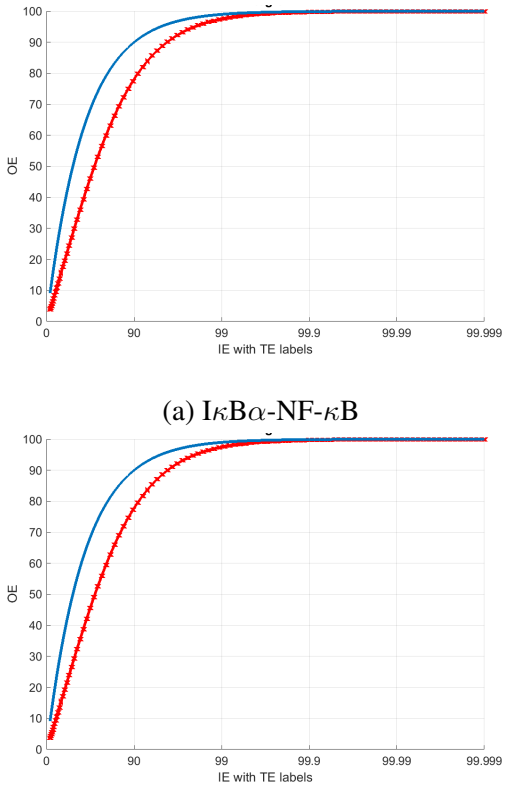
Figure 7-9: OE vs TE profiles for IκBα-NF-κB, IκBβ-NF-κB, and IκBϵ-NF-κB using disease model 3.

There are two major differences between the alpha and the beta/epsilon isoforms in the pathway. The first is that the alpha isoform of IκBx-t is the only isoform that gets synthesized inducibly by NF-κBn (reaction # 28). The second is that IκBα-t is synthesized at a greater rate than either IκBβ-t or IκBϵ-t. In order to know which of the two explains the gap in scores the best, we made two alterations to the healthy model. In the first, we eliminated the IκBα inducible mRNA synthesis reaction. In the second, we altered the model so that all the isoforms are synthesized at the same rate. We chose a rate of $1.54 * 10^{-6} \mu M^{-1} s^{-1}$ as the synthesis rate of all three isoforms (the same as the original syntheses rate of IκBα-t).

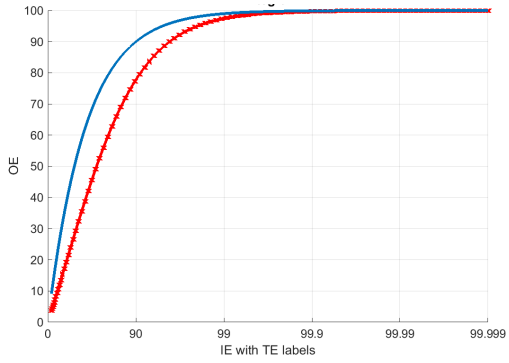
Eliminating the IκBα inducible mRNA synthesis reaction does not reduce the gap between the three different isoforms and hence is not the reason for the differences in scoring. This makes sense because the majority of IκBα-t is synthesized constituitively via the "con-

stitutive IκBα- mRNA synthesis" reaction and not from the inducible synthesis NF-κBn + NF-κBn -> IκB$\alpha - t + NF-\kappa$Bn + NF-κBn as the latter depends on the amount of NF-κBn, which is limited, while there is constant input of IκBα-t from the source. Also, it would not have explained why beta isoforms score better than the epsilon ones.

Modifying the synthesis rates of IκBα-t, IκBβ-t, and IκBϵ-t to be the same completely eliminated the gap between the three isoforms of IκBx-NF-κB as can be seen in figure 7-10. This confirms the hypothesis that the difference in synthesis rates of the three isoforms is the main reason for the differences in their scores.



(a) IκBα-NF-κB



(b) IκBβ-NF-κB

(c) IκBϵ-NF-κB

Figure 7-10: OE vs TE profiles for IκBα-NF-κB, IκBβ-NF-κB, and IκBϵ-NF-κB using model with equal transcription rates of IκBα-t, IκBβ-t, and IκBϵ-t .

The varying rates of transcription could be attributed to the sizes of the proteins. The molecular weight of the alpha, beta, and epsilon isoforms of IκBx are 35,609 Da, 37,771 Da, and 52,864 Da [16], respectively. The synthesis rates of these three proteins decrease with the increase in molecular weight. IκBϵ, the heaviest, is the slowest to get transcribed

and IκBα, the lightest, is the fastest to get transcribed.

Having the synthesis rates of all three isoforms equal made all three IκBx-NF-κB isoforms have the same effect on the output, however, at the same time it greatly decreased the impact of IκBα-NF-κB (the TE required to achieve an OE of 40% increased from $\approx$ 30% to $\approx$ 60%). A possible way of looking at the pathway which will explain the decrease in IκBα-NF-κB's efficacy is looking at it as having three parallel paths with each path's capacity being proportional to the transcription rate of that isoform. This simplified explanatory model is illustrated in figure 7-11.

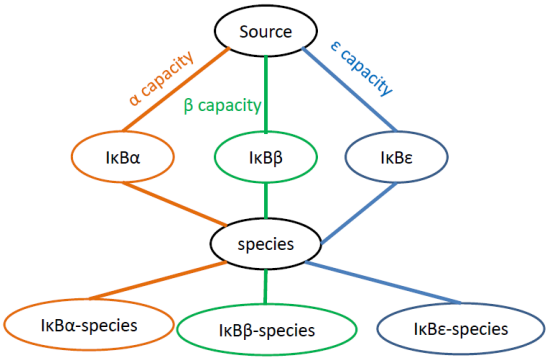
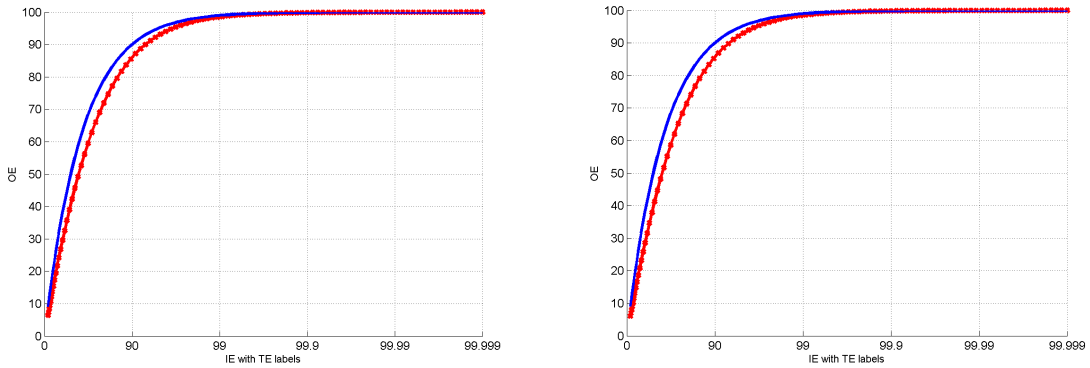


Figure 7-11: A simplified view of the NF-κB pathway where there are three parallel paths corresponding to the three isoforms each with its own capacity.

To confirm that this view of the pathway is reasonable, we asserted that the model exhibits the following two behaviors: (1)increasing the capacity of the other paths would decrease the effectiveness of the path in question, (2) decreasing the number of parallel paths would increase the effectiveness of the path in question.

We already saw when comparing figures 7-8 and 7-10 that increasing the transcription rate (the "capacity") of the beta, and epsilon isoforms of IκBx, decreases the effectiveness of IκBα. To confirm that the number of parallel paths effects the impact of the path being examined, we altered the pathway such that the epsilon path is removed and we only have the alpha and beta paths with the same capacity (by having the synthesis rates equal). If our hypothesis is true we would expect to see an increase in the effectiveness of both the alpha and beta targets. Figure 7-12 shows the OE vs TE of both IκBα-NF-κB and IκBβ-NF-κB with the alterations specified and we indeed see an increase in effectiveness.

Having confirmed that the view of parallel paths is a reasonable one, we can now see



(a) IκBα-NF-κB (b) IκBβ-NF-κB

Figure 7-12: OE vs TE profiles for IκBα-NF-κB and IκBβ-NF-κB using model with equal transcription rates of IκBα-t and IκBβ-t but without IκBϵ-t.

that the reason the impact of IκBα-NF-κB decreased when we increased the transcription rate of IκBβ-t and IκBϵ-t is because even though we are restricting the α path the same percentage as before there is now more capacity in the β and ϵ paths to compensate for that requiring a greater restriction to achieve the same OE.

7.4 Combination of Targets Classification

We classified all combinations of targets as additive, synergistic, or antagonistic. An additive pair is one in which a linear combination of the two drug concentrations leads to the same overall effect on the output. A synergistic pair is one in which the addition of one of the drugs reduces the amount required of the second drug to obtain an equal overall output effect compared to a linear combination of the two, requiring an overall lower amount of total drug intake. An antagonistic pair is one where we need a greater total amount of drug to have the same effect on the output than if only one of the drugs was given. Figures 6-1, 6-2, and 6-3 in section 6.1 show only the synergistic pairs in the healthy model, disease model 1, and disease model 3 respectively. Figures B-5, B-6, and B-7 in section B.5 show the classification of all pairs.

The combination of targets that are categorized as synergistic are combinations of targets that individually have some effect on the output when inhibited. Furthermore, all effective targets (super-linear, linear, and sub-linear) are synergistic with each other for most

of the OE contours. This is regardless of whether the two targets are isoforms of the same species such as IκBα-NF-κB and IκBβ-NF-κB, or different species such as IκBα-NF-κB and IKKIκBα-NF-κB. Furthermore, there does not seem to be a trend in the level of synergy between targets. Targets that are isoforms of the same species are no more synergistic than other pairs and combinations of targets that are both super-linear or linear are no more synergistic than pairs between super-linear/linear and sub-linear targets or sub-linear and sub-linear targets. Also, the level of synergism does not seem to depend on the OE contour levels.

The combinations of pairs that are categorized as antagonistic and additive are the combinations of targets with one of them having some effect on the output and the other having no effect. The ones that are categorized as antagonistic are the ones in which the first target has some effect on the output when inhibited but the second has no effect. An example of an antagonistic pair is NF-κB and IκBϵ-NF-κBn (in disease model 1). NF-κB has sub-linear effect on the output but IκBϵ-NF-κBn has no effect. The ones that are categorized as additive are ones in which the first target has no effect on the output and the second has some effect, very similar to pairs categorized as antagonistic but reversed. An example of an additive pair is IκBα and NF-κB (in disease model 1).

The remaining combinations of targets that do not fall into any of the three categories are the ones with both species not having any effect on the output. An example is the pair IKKIκBα and IKKIκBϵ-NF-κB (in the healthy model).

In the thesis work of Nirmala Paudel [15], she had categorized combinations of targets in the EGFR pathway and found a general trend for the synergistic combinations, which is that synergism happens between binding partners. Our finding that synergism happens when both species have some effect on the output includes binding partners but also include non-binding partners. The thesis work also found a general trend for antagonistic pairs. Namely, she found that generally antagonism occurs between one of the binding partners and the downstream complex it forms. Our findings do not support this trend as we found that if the downstream complex and one of the binding partners both have some effect on the output then they are synergistic. The difference in the trends found may be because they are pathway/model dependent.

7.5 Comparison of Identified Drug Targets to Existing Ones

Overall, we can categorize the species in the pathway as either having some effect on the output (nuclear NF-κB or "NF-κBn") or not having any. Specifically, any species that is not constantly being produced and is active at steady state has an effect on the output.

In disease model 1, where IκBs are dysfunctional, only two species are effective targets: cytoplasmic NF-κB, or "NF-κB", and nuclear NF-κB, or "NF-κBn". The effective targets in the healthy model include the two effective species in disease model 1 but also include cytoplasmic IκBs complexes with cytoplasmic NF-κB (IκBα-NF-κB, IκBβ-NF-κB, and IκBϵ-NF-κB) and nuclear IκBs with nuclear NF-κB (IκBαn-NF-κBn, IκBβn-NF-κBn, and IκBϵn-NF-κBn). The effective targets in disease model 3, where IKK is constitutively active, include all the effective targets in the healthy model in addition to complexes of IκB-NF-κB with IKK (IKKIκBαn-NF-κBn, IKKIκBβn-NF-κBn, and IKKIκBϵn-NF-κBn). The optimal drug targets depend on the model used. For the healthy model, we found it to be IκBα-NF-κB, IκBβ-NF-κB, and IκBϵ-NF-κB; for disease model 1 and 3 it is NF-κBn.

There have been many species and reactions in the NF-κB pathway that have been identified as NF-κB inhibitors. Most of them are upstream of IKK and NF-κB and are not included in our model. Examples of such targets are TNF-Receptors [6], TRAF6 binding [17], activation of NF-κB [18], etc. Among the ones identified and are included in our model are IκBα [19], IκBβ [5], IKK [4], and NF-κB [20]. Specifically, it has been found that upregulation of IκBα and IκBβ and the downregulation of IKK and NF-κB by added drugs lead to the inhibition of NF-κBn.

NF-κB being an effective drug target is consistent with our findings where in all the models inhibiting cytoplasmic NF-κB leads to the inhibition of nuclear NF-κB. However, our findings are not consistent with previous ones for IκBα, IκBβ, and IKK. Specifically, we have found that IκBα, IκBβ, and IKK have no effect on the output NF-κBn. This inconsistency is because in our models whenever a species gets transcribed, as in the case of IκBα and IκBβ, it is modeled as having an infinite supply where in reality it doesn't. In

the case of IKK, in the healthy model and disease model 1, it is modeled as only having an initial amount that dies out when in reality it might be produced at other times. And in disease model 3, it is modeled as having a constant value and in reality that might not always be the case.

There are targets that we found to be effective but have not been previously identified. These include IκBα-NF-κB, IκBβ-NF-κB, IκBϵ-NF-κB, IκBαn-NF-κBn, IκBβn-NF-κBn, and IκBϵn-NF-κBn; and specifically in disease model 3, IKKIκBαn-NF-κBn, IKKIκBβn-NF-κBn, and IKKIκBϵn-NF-κBn. One reason they might not have been experimentally identified is because protein complexes are harder to physically inhibit than individual ones.

Chapter 8

Conclusion

In this thesis, we altered the healthy NF-kB model by Kell et al. [9] to reflect what happens in three different disease states. We restricted the alterations to species/reactions that are included in the model used and gave examples of diseases represented by each of the disease models. The different disease models were ones with loss of IkBs function, inactive IKK, and constitutively active IKK.

For the disease models that increased or decreased the amount of nuclear NF-κB (NF-κBn) relative to what is produced in the healthy model, we exhaustively scored the species based on how much change in the amount of NF-kBn produced they caused when inhibited. We performed the same analysis on the healthy model in order to get an overall picture of which species have control over the amount of NF-κBn produced. We found that in all the models analysed, all the species either decreased the output when inhibited or had no effect. We also found that very few species are effective at low levels of inhibition but many are effective at extremely high levels of inhibition. The exception is in the model with loss of IkBs function in which we found that only one species other than the output, NF-κBn, had any effect and that is the cytoplasmic NF-κB. The species that are effective at low levels of inhibition in the healthy model are the three cytoplasmic IκB-NF-κB complexes; and in disease model 1 and 3 it is the nuclear NF-κB.

We then provided explanations to why certain species score better than others and explanations to two key observations: 1) why does the output, NF-kBn, not have a linear profile? 2) why are alpha isoforms better drug targets than the beta and epsilon isoforms?

We also categorized all combinations of targets as synergistic, antagonistic, or additive and found that all species that have some effect on the output are synergistic with one another. Antagonistic pairs were found to be ones where one of the targets is effective and the other target has no effect when inhibited individually.

Finally, we compared the targets we found to be effective to previously identified drug targets. We found that existing drug targets are restricted to individual proteins and not complexes, though we found that complexes generally scored better than others. The inconsistency is because we only considered signal propagation and not structural limitations when scoring species.

Also, our model only includes the interactions between the species most downstream of the pathway and does not include upstream species and reactions starting with the surface receptors. Expanding the model to include upstream species and their interactions would allow one to model many other different diseases that are caused by alterations in those upstream species and reactions.

Appendix A

Tables

A.1 Healthy NF-κB Model Species and Their Initial Concentrations.

Species index	Species name	Initial concentration (μM)
1	IκBα	0.189664
2	NF-κB	0.000251
3	IκBα-NF-κB	0.083333
4	IκBβ	0.021635
5	IκBβ-NF-κB	0.008305
6	IκBϵ	0.015436
7	IκBϵ-NF-κB	0.005925
8	IKKIκBα	0
9	IKKIκBα-NF-κB	0
10	IKK	0.1
11	IKKIκBβ	0
12	IKKIκBβ-NF-κB	0
13	IKKIκBϵ	0
14	IKKIκBϵ-NF-κB	0

15	NF-κBn	0.000217
16	IκBαn	1.89E-01
17	IκBαn-NF-κBn	1.43E-03
18	IκBβn	1.65E-02
19	IκBβn-NF-κBn	0.000314
20	IκBϵn	1.18E-02
21	IκBϵn-NF-κBn	0.000224
22	source	1
23	IκBα-t	0.005503
24	sink	0
25	IκBβ-t	0.000636
26	IκBϵ-t	0.000454

A.2 Healthy NF-κB model reactions and their rates

Reaction Name	Reaction	Symbol	Rate
IκBαNF-κB-association	IκBα + NF-κB -> IκBα-NF-κB	k-1	0.5
IκBα-NF-κB-dissociation	IκBα-NF-κB -> NF-κB + IκBα	k-2	0.0005
IκBβ-NF-κB-association	IκBβ + NF-κB -> IκBβ-NF-κB	k-3	0.5
IκBβ-NF-κB-dissociation	IκBβ-NF-κB -> NF-κB + IκBβ	k-4	0.0005
IκBϵ-NF-κB-association	IκBϵ + NF-κB -> IκBϵ-NF-κB	k-5	0.5
IκBϵ-NF-κB-dissociation	IκBϵ-NF-κB -> NF-κB + IκBϵ	k-6	0.0005
IKKIκBα-NF-κB-association	IKKIκBα + NF-κB -> IKKIκBα-NF-κB	k-7	0.5
IKKIκBα-NF-κB-dissociation	IKKIκBα-NF-κB -> NF-κB + IKKIκBα	k-8	0.0005
IKKIκBα-NF-κB-catalysis	IKKIκBα-NF-κB -> IKK + NF-κB	k-9	0.0204
IKKIκBβ-NF-κB-association	IKKIκBβ + NF-κB -> IKKIκBβ-NF-κB	k-10	0.5
IKKIκBβ-NF-κB-dissociation	IKKIκBβ-NF-κB -> NF-κB + IKKIκBβ	k-11	0.0005
IKKIκBβ-NF-κB-catalysis	IKKIκBβ-NF-κB -> IKK + NF-κB	k-12	0.0075
IKKIκBϵ-NF-κB-association	IKKIκBϵ + NF-κB -> IKKIκBϵ-NF-κB	k-13	0.5
IKKIκBϵ-NF-κB-dissociation	IKKIκBϵ-NF-κB -> NF-κB + IKKIκBϵ	k-14	0.0005
IKKIκBϵ-NF-κB-catalysis	IKKIκBϵ-NF-κB -> IKK + NF-κB	k-15	0.011
IκBα-constituitive-degradation–complexed-to-NF-κB	IκBα-NF-κB -> NF-κB	k-16	2.25E-05

IκBβ-constituitive-degradation–complexed-to-NF-κB	IκBβ-NF-κB -> NF-κB	k-17	2.25E-05
IκBϵ-constituitive-degradation–complexed-to-NF-κB	IκBϵ-NF-κB -> NF-κB	k-18	2.25E-05
NF-κB-nuclear-import	NF-κB -> NF-κBn	k-19	0.09
NF-κB-nuclear-export	NF-κBn -> NF-κB	k-20	8.00E-05
IκBα-NF-κB-nuclear-association	IκBαn + NF-κBn -> IκBαn-NF-κBn	k-21	0.5
IκBα-NF-κB-nuclear-dissociation	IκBαn-NF-κBn -> NF-κBn + IκBαn	k-22	0.0005
IκBβ-NF-κB-nuclear-association	IκBβn + NF-κBn -> IκBβn-NF-κBn	k-23	0.5
IκBβ-NF-κB-nuclear-dissociation	IκBβn-NF-κBn -> NF-κBn + IκBβn	k-24	0.0005
IκBϵ-NF-κB-nuclear-association	IκBϵn + NF-κBn -> IκBϵn-NF-κBn	k-25	0.5
IκBϵ-NF-κB-nuclear-dissociation	IκBϵn-NF-κBn -> NF-κBn + IκBϵn	k-26	0.0005
constituitive-IκBα-mRNA-synthesis	source -> IκBα-t	k-27	1.54E-06
IκBα-Inducible-mRNA-synthesis	NF-κBn + NF-κBn -> IκBαt + NF-κBn + NF-κBn	k-28	0.0165
IκBα-mRNA-degradation	IκBα-t -> sink	k-29	0.00028
constituitive-IκBβ-mRNA-synthesis	source -> IκBβ-t	k-30	1.78E-07
IκBβ-mRNA-degradation	IκBβ-t -> sink	k-31	0.00028
constituitive-IκBϵ-mRNA-synthesis	source -> IκBϵ-t	k-32	1.27E-07
IκBϵ-mRNA-degradation	IκBϵ-t -> sink	k-33	0.00028
IKKIκBα-association	IKK + IκBα -> IKKIκBα	k-34	0.0225
IKKIκBα-dissociation	IKKIκBα -> IKK + IκBα	k-35	0.00125

IκBα-translation-rate	IκBα-t -> IκBα + IκBα-t	k-36	0.00408
constitutive-IκBα-degradation–free	IκBα -> sink	k-37	0.000113
IκBα-nuclear-import	IκBα -> IκBαn (Import)	k-38	0.0003
IκBα-nuclear-export	IκBαn -> IκBα (Export)	k-39	0.0002
IKKIκBβ-association	IKK + IκBβ -> IKKIκBβ	k-40	0.006
IKKIκBβ-dissociation	IKKIκBβ -> IKK + IκBβ	k-41	0.00175
IκBβ-translation-rate	IκBβ-t -> IκBβ + IκBβ-t	k-42	0.00408
constitutive-IκBβ-degradation–free	IκBβ -> sink	k-43	0.000113
IκBβ-nuclear-import	IκBβ -> IκBβn (Import)	k-44	0.00015
IκBβ-nuclear-export	IκBβn -> IκBβ (Export)	k-45	0.0001
IKKIκBϵ-association	IKK + IκBϵ -> IKKIkBk	k-46	0.009
IKKIκBϵ-dissociation	IKKIκBϵ -> IKK + IκBϵ	k-47	0.00175
IκBϵ-translation-rate	IκBϵ-t -> IκBϵ + IκBϵ-t	k-48	0.00408
constitutive-IκBϵ-degradation–free	IκBϵ -> sink	k-49	0.000113
IκBϵ-nuclear-import	IκBϵ -> IκBϵn (Import)	k-50	0.00015
IκBϵ-nuclear-export	IκBϵn -> IκBϵ (Export)	k-51	0.0001
IKK-IκBαNF-κB-association	IKK + IκBα-NF-κB -> IKKIκBα-NF-κB	k-52	0.185
IKK-IκBαNF-κB-dissociation	IKKIκBα-NF-κB -> IKK + IκBα-NF-κB	k-53	0.00125
IκBα-NF-κB-nuclear-export	IκBαn-NF-κBn -> IκBα-NF-κB (Export)	k-54	0.0138

IKK-IκBβNF-κB-association	KK + IκBβ-NF-κB -> IKKIκBβ-NF-κB	k-55	0.048
IKK-IκBβNF-κB-dissociation	IKKIκBβ-NF-κB -> IKK + IκBβ-NF-κB	k-56	0.00175
IκBβ-NF-κB-nuclear-export	IκBβn-NF-κBn -> IκBβ-NF-κB (Export)	k-57	0.0052
IKK-IκBϵNF-κB-association	IKK + IκBϵ-NF-κB -> IKKIκBϵ-NF-κB	k-58	0.07
IKK-IκBϵNF-κB-dissociation	IKKIκBϵ-NF-κB -> IKK + IκBϵ-NF-κB	k-59	0.00175
IκBϵ-NF-κB-nuclear-export	IκBϵn-NF-κBn -> IκBϵ-NF-κB (Export)	k-60	0.0052
slow-adaptation	IKK -> sink	k-61	0.00012
IKKIκBα-catalysis	IKKIκBα -> IKK	k-62	0.00407
IKKIκBβ-catalysis	IKKIκBβ -> IKK	k-63	0.0015
IKKIκBϵ-catalysis	IKKIκBϵ -> IKK	k-64	0.0022

Appendix B

Figures

B.1 NF-κB Model

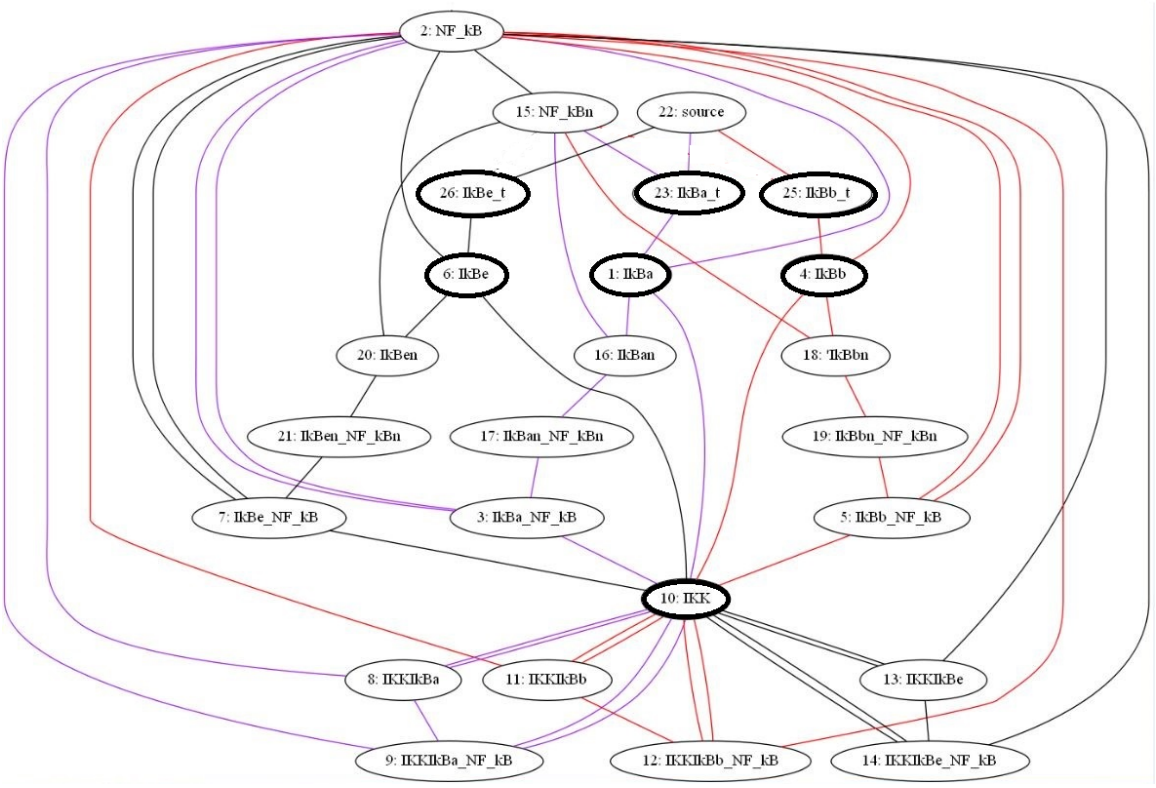
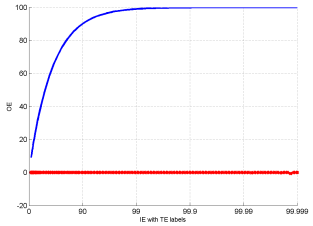
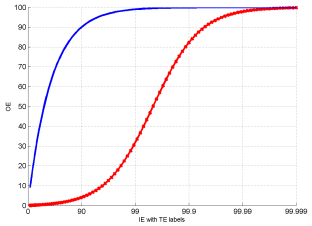


Figure B-1: NF-κB model. Species encircled in darker boundaries get degraded

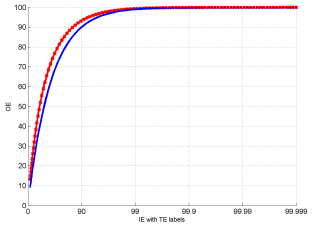
B.2 OE vs TE Species Profiles for the Healthy Model



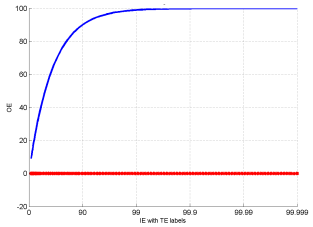
(a) 1: IκBα



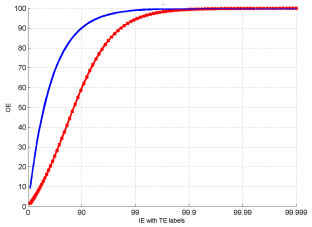
(b) 2: NF-κB



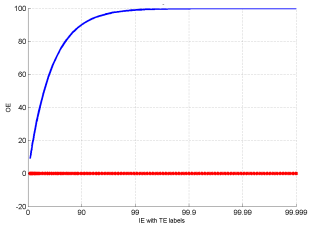
(c) 3: IκBα-NF-κB



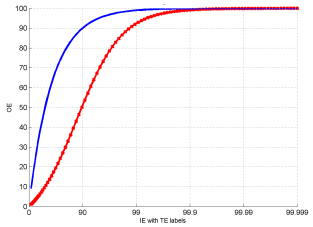
(d) 4: IκBβ



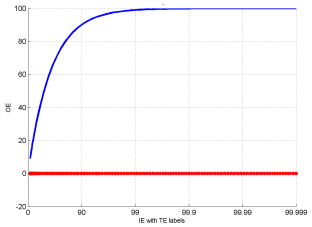
(e) 5: IκBβ-NF-κB



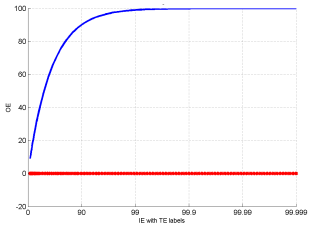
(f) 6: IκBϵ



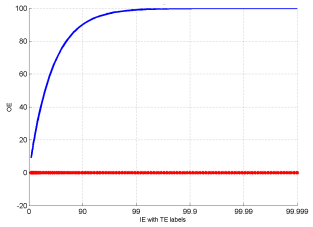
(g) 7: IκBϵ-NF-κB



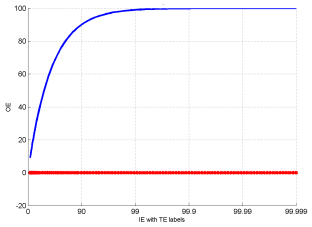
(h) 8: IKKIκBα



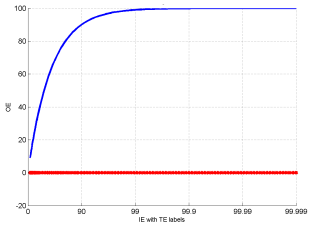
(i) 9: IKKIκBα-NF-κB



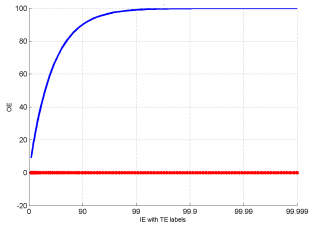
(j) 10: IKK



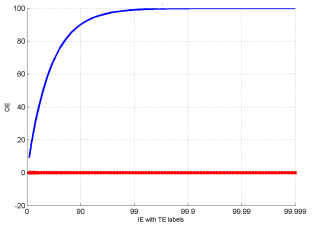
(k) 11: IKKIκBβ



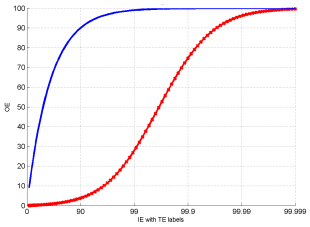
(l) 12: IKKIκBβ-NF-κB



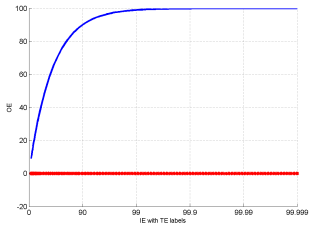
(m) 13: IKKIκBϵ



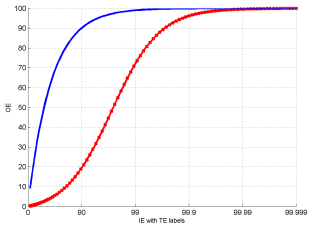
(n) 14: IKKIκBϵ-NF-κB



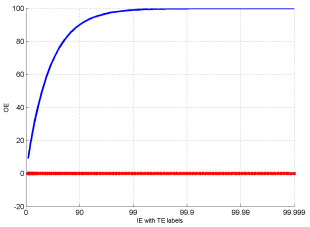
(o) 15: NF-κBn



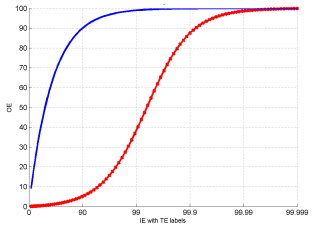
(p) 16: IκBαn



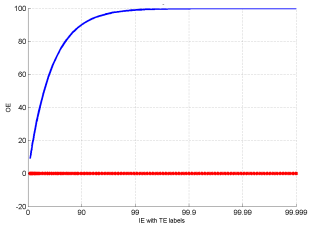
(q) 17: IκBαn-NF-κBn



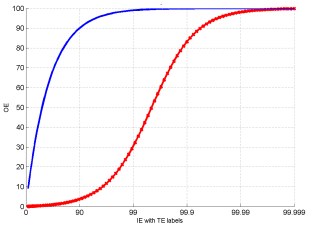
(r) 18: IκBβn



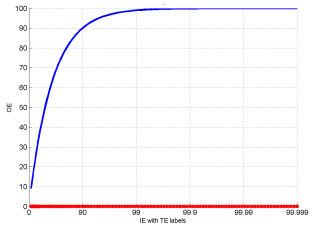
(s) 19: IκBβn-NF-κBn



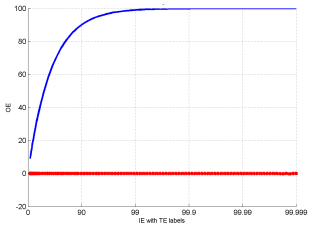
(t) 20: IκBϵn



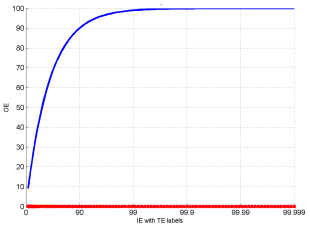
(u) 21: IκBϵn-NF-κBn



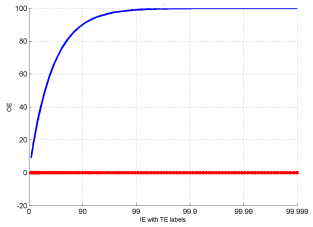
(v) 22: Source



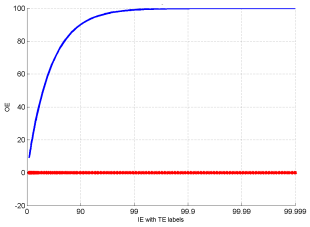
(w) 23: IκBα-t



(x) 24: Sink

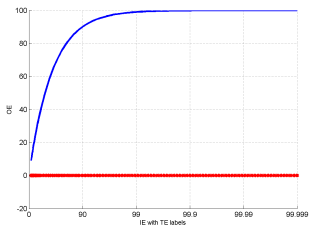


(y) 25: IκBβ-t

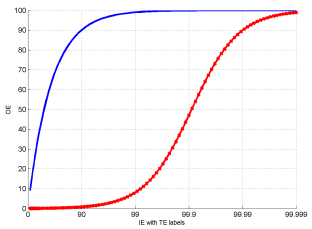


(z) 26: IκBϵ-t

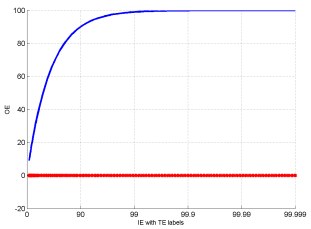
B.3 OE vs TE Species Profiles for Disease Model 1



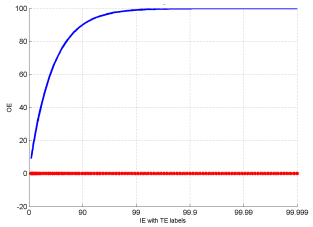
(a) 1: IκBα



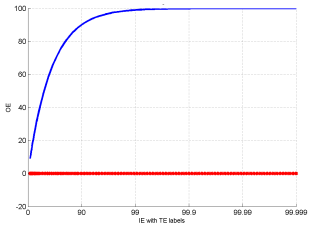
(b) 2: NF-κB



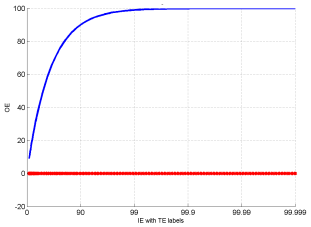
(c) 3: IκBα-NF-κB



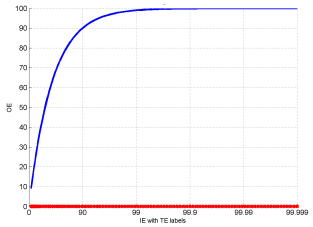
(d) 4: IκBβ



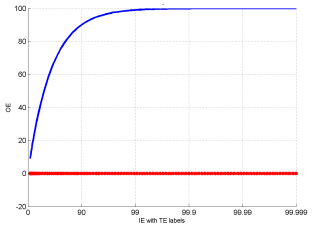
(e) 5: IκBβ-NF-κB



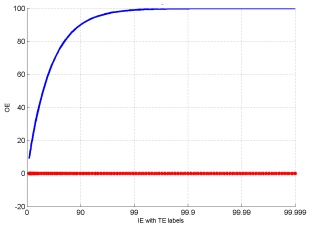
(f) 6: IκBϵ



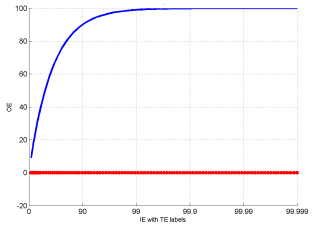
(g) 7: IκBϵ-NF-κB



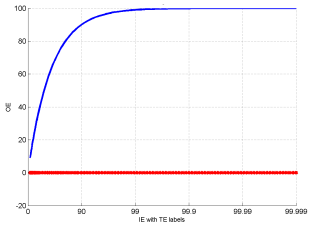
(h) 8: IKKIκBα



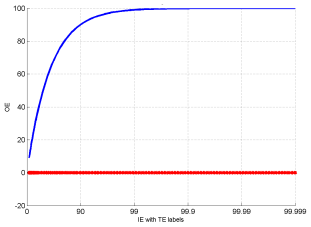
(i) 9: IKKIκBα-NF-κB



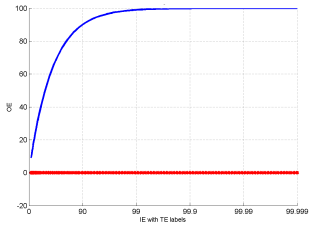
(j) 10: IKK



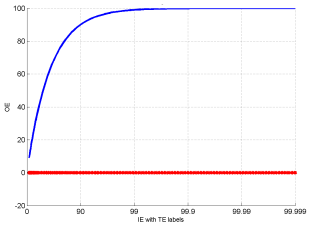
(k) 11: IKKIκBβ



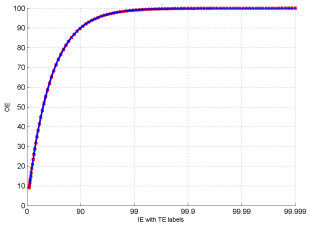
(l) 12: IKKIκBβ-NF-κB



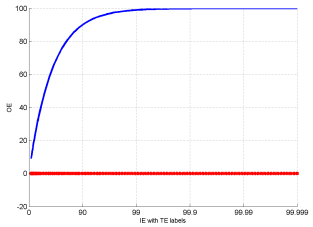
(m) 13: IKKIκBϵ



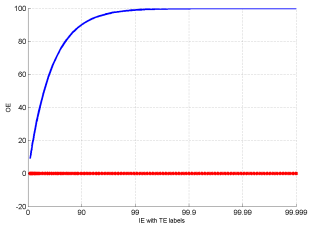
(n) 14: IKKIκBϵ-NF-κB



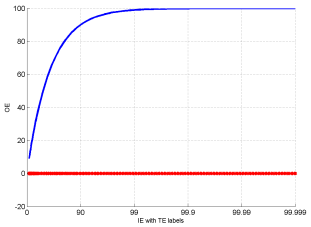
(o) 15: NF-κBn



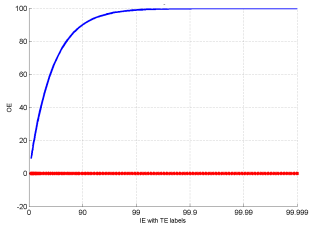
(p) 16: IκBαn



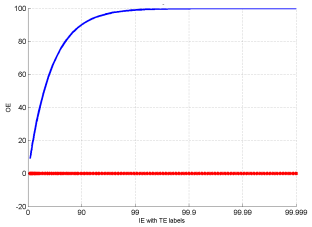
(q) 17: IκBαn-NF-κBn



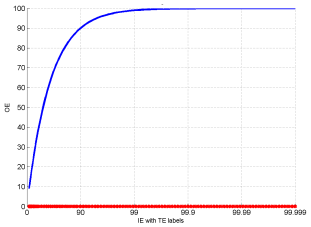
(r) 18: IκBβn



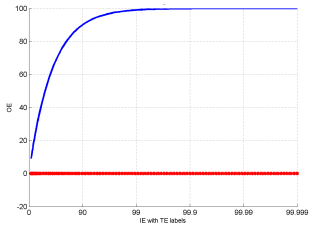
(s) 19: IκBβn-NF-κBn



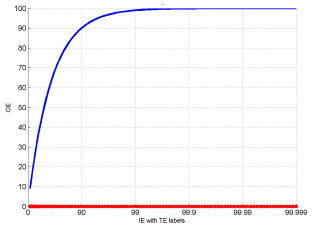
(t) 20: IκBϵn



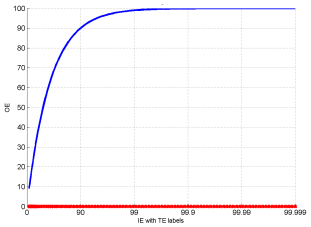
(u) 21: IκBϵn-NF-κBn



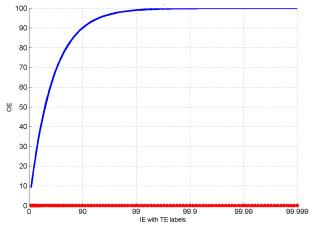
(v) 22: Source



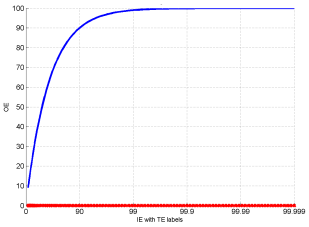
(w) 23: IκBα-t



(x) 24: Sink

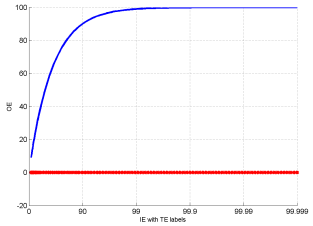


(y) 25: IκBβ-t

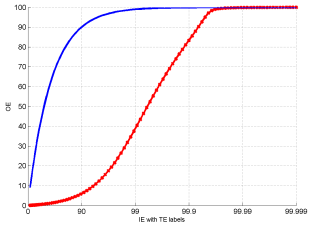


(z) 26: IκBϵ-t

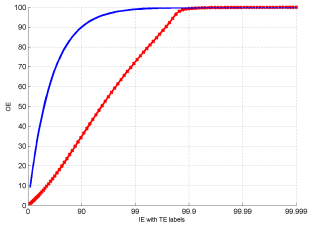
B.4 OE vs TE Species Profiles for Disease Model 3



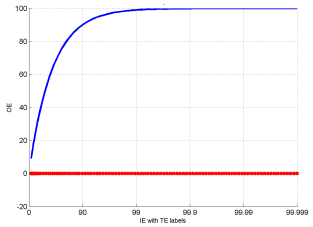
(a) 1: IκBα



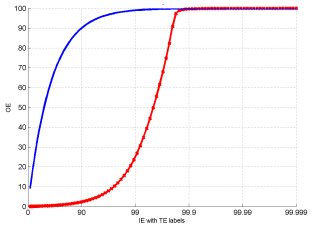
(b) 2: NF-κB



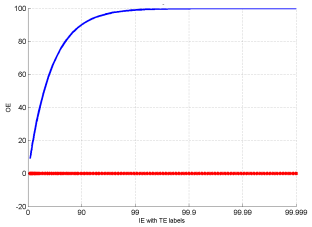
(c) 3: IκBα-NF-κB



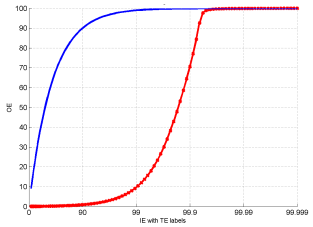
(d) 4: IκBβ



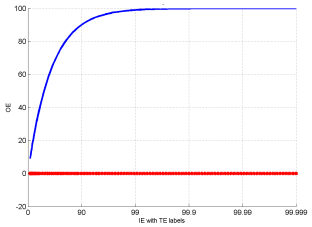
(e) 5: IκBβ-NF-κB



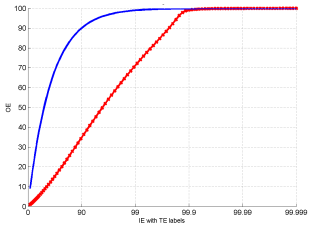
(f) 6: IκBϵ



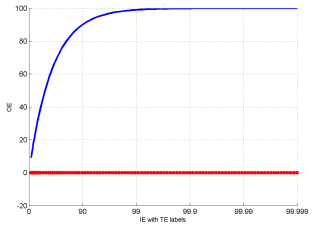
(g) 7: IκBϵ-NF-κB



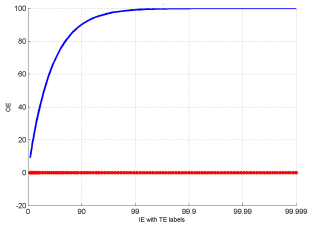
(h) 8: IKKIκBα



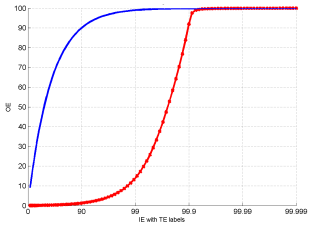
(i) 9: IKKIκBα-NF-κB



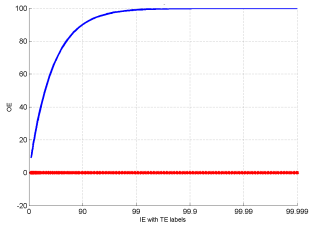
(j) 10: IKK



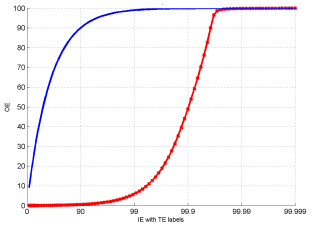
(k) 11: IKKIκBβ



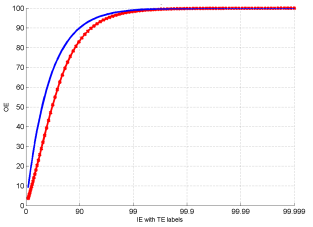
(l) 12: IKKIκBβ-NF-κB



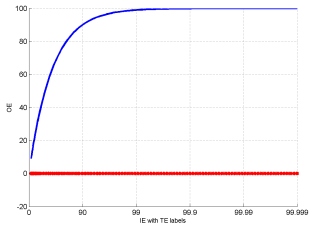
(m) 13: IKKIκBϵ



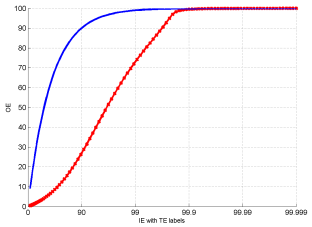
(n) 14: IKKIκBϵ-NF-κB



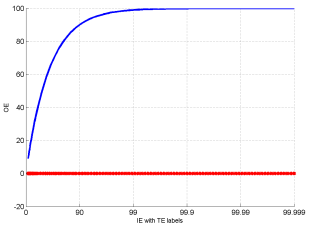
(o) 15: NF-κBn



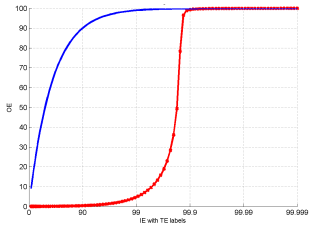
(p) 16: IκBαn



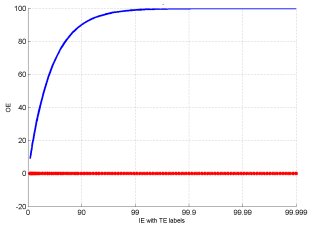
(q) 17: IκBαn-NF-κBn



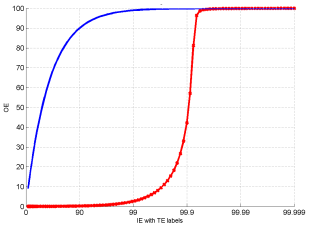
(r) 18: IκBβn



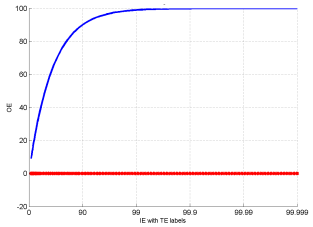
(s) 19: IκBβn-NF-κBn



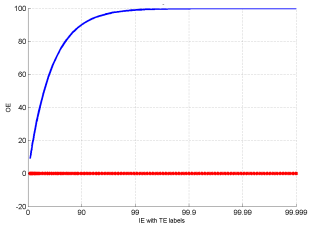
(t) 20: IκBϵn



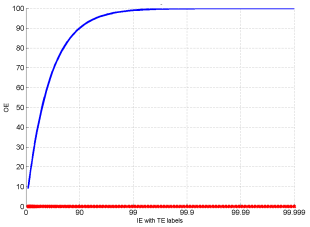
(u) 21: IκBϵn-NF-κBn



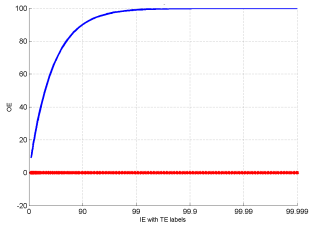
(v) 22: Source



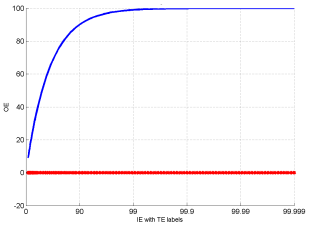
(w) 23: IκBα-t



(x) 24: Sink



(y) 25: IκBβ-t



(z) 26: IκBϵ-t

B.5 Synergism Classifications

Plots of synergism classification of each combination of pairs at various OE levels for various model. Circle size varies according to OE levels and circle colors correspond to different classes. Red circles are synergistic, green circles are additive, and blue circles are antagonistic. The pairs that do not have any associated circles do not achieve any of the following seven OE levels: 30 %, 50 %, 70 %, 90 %, 99.0 %, 99.99 %, and 99.999 %.

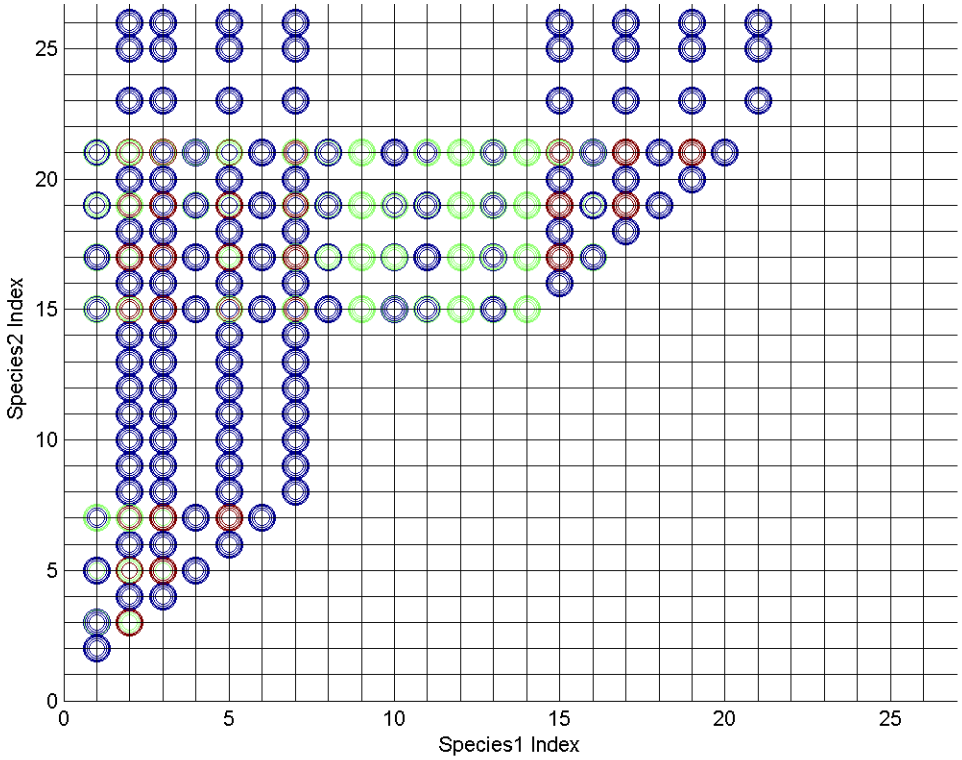


Figure B-5: Synergism classification for all combinations of species in the healthy model.

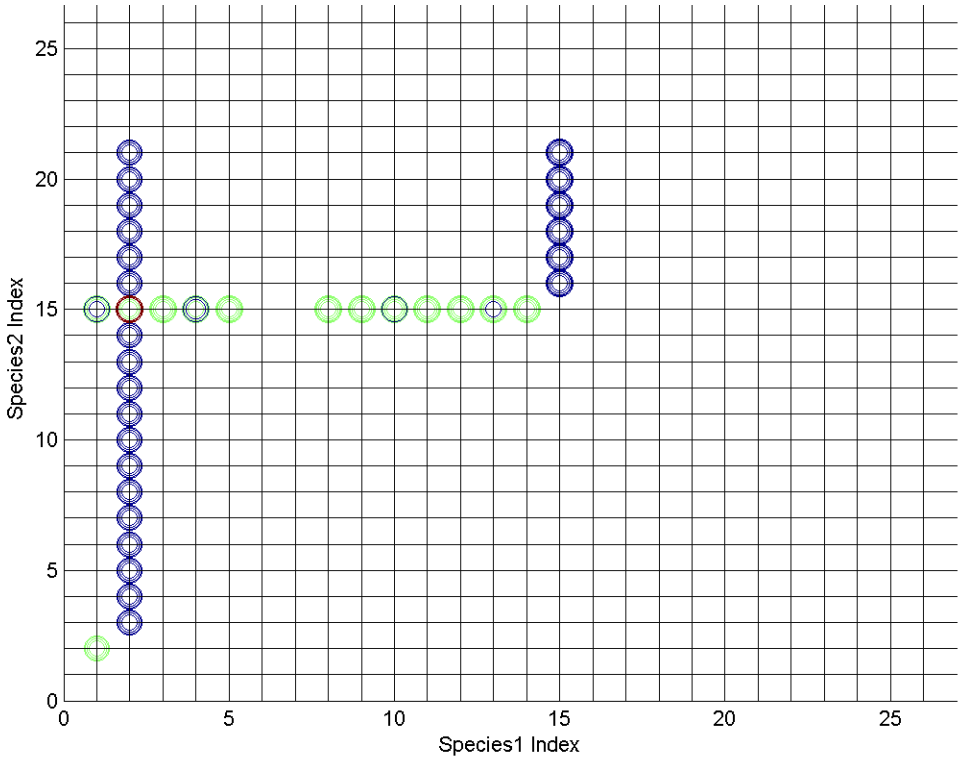


Figure B-6: Synergism classification for all combinations of species in disease model 1.

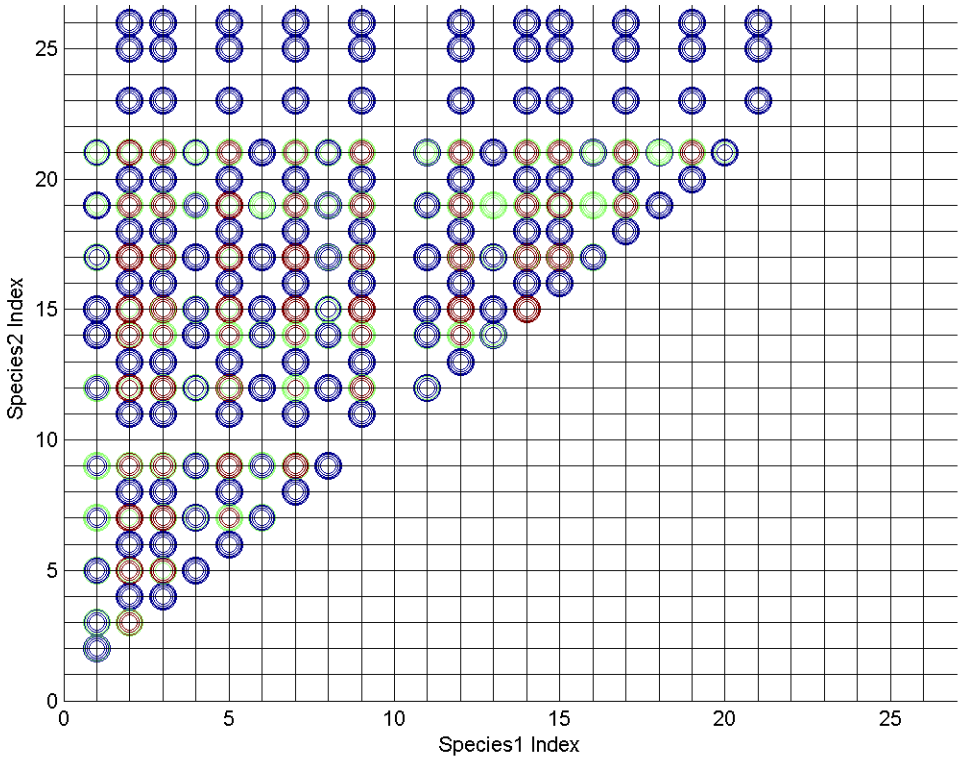


Figure B-7: Synergism classification for all combinations of species in disease model 3.

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