# DETERMINING MECHANISM OF ACTION OF ANTIVIRALS FOR RESPIRATORY INFECTIONS

by

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# Contents

# List of Figures

# List of Tables

# Glossary

- **Antisense.** Of or relating to a gene that is derived from RNA or complementary DNA, is inserted in reverse orientation into a strand of DNA, and is used in genetic engineering to regulate genetic expression of a trait.
- **Budding.** A form of fission in which the parent cell does not divide, but puts out a small budlike process (daughter cell) with its proportionate amount of chromatin; the daughter cell then separates to begin independent existence.
- **Cytotoxicity.** The degree to which an agent possesses a specific destructive action on certain cells or the possession of such action cytotoxic.
- **Endocytosis.** A process of cellular ingestion by which the plasma membrane folds inward to bring substances into the cell.
- **Host cell.** A living cell invaded by or capable of being invaded by an infectious agent (as a bacterium or a virus).
- **Lysis.** Destruction or decomposition, as of a cell or other substance, under influence of a specific agent.
- **MOI.** Multiplicity of infection is the number of viruses added per cell to initiate an infection.
- **Nucleocapsid.** A viral enclosure consisting of a capsid or protein coat and a nucleic acid that it surrounds. Some viruses consist solely of bare nucleocapsids; others have more complex enclosures.
- **Nucleoside.** Any of the class of compounds derived by the hydrolysis of nucleic acids or nucleotides, consisting typically of deoxyribose or ribose combined with adenine, guanine, cytosine, uracil, or thymine.
- **Nucleotide.** Any of a group of molecules that, when linked together, form the building blocks of DNA or RNA: composed of a phosphate group, the bases adenine, cytosine, guanine, and thymine, and a pentose sugar, in RNA the thymine base being replaced by uracil.
- **Pathogenicity.** The condition or quality of being pathogenic, or the ability to cause disease.

- **Plaque.** An area of clearing in a flat, confluent growth of bacteria or tissue cells.
- **Prophylactic.** Acting to defend against or prevent something, especially disease; protective.
- **Reverse transcriptase.** A DNA polymerase enzyme that catalyzes the process of reverse transcription.
- $TCID_{50}$**.** This is defined as the necessary quantity of virus to infect 50% of the susceptible population in the assay.
- **Zoonosis.** Any disease of animals communicable to humans.

# List of abbreviations

| | |
|---|---|
| **AIDS** | Acquired Immunodeficiency Syndrome |
| **AUC** | Area Under the Curve |
| $EC_{50}$ | half maximal Effective Concentration |
| **HA** | Hemagglutinin |
| **HF** | Hollow Fiber |
| $IC_{50}$ | half maximal Inhibitory Concentration |
| **LHS** | Latin hypercube sampling |
| **MCA** | Multiple cycle assay |
| **MOI** | Multiplicity of infection |
| **PFU** | Plaque forming units |
| **SCA** | Single cycle assay |

# Chapter 1

# Introduction

Respiratory virus infections are a leading cause of mortality world-wide (1). Treating these infections can be difficult since there are no known effective antivirals for many of the viruses, and for infections where antivirals have been developed, the virus can develop a drug-resistant mutation. For these reasons, different compounds are tested, in a constant search for antiviral activity against respiratory viruses. Most modern drugs were developed using large-scale random screening of compounds. For example, all the current approved drugs against human immunodeficiency virus (HIV) and the first generation of antibiotics (2) were discovered in this way. The disadvantage of this method is that the mechanism of action by which the antiviral blocks viral activity is unkown. This paper explores whether currently used experimental assay techniques can help discover the part of the life cycle targeted by an antiviral.

## 1.1 Antiviral drugs

Antivirals are chemical compounds developed to treat viral infections, equivalent to the function of antibiotics in bacterial infections. The development of antivirals is of high importance because drug therapy is one of our primary defenses against many infectious diseases. Around 40 antiviral drugs are permitted for human use, half of them just for human immunodeficiency virus (HIV). Most antivirals are effective against just one or a few viruses (3).

The development of antiviral drugs is difficult. One problem is that the therapeutic effect of antivirals is small; when the symptoms and clinical signs appear usually viral production has reached its peak. Sometimes it is better to treat people before they are infected, for example to prevent epidemics. Another problem with antiviral drugs is that viruses use the host cell, and cellular processes, to replicate, so the antivirals have to interfere with the virus without harming the host cells. Most powerful antiviral drugs, however, cannot discriminate between cellular or virus activity (3, 4).

### 1.1.1 Development of antiviral drugs

There are currently two primary methods for development of antivirals: rational design and random screening of compounds (5).

In the rational design of antiviral drugs, a drug is known to interfere with a particular virus activity. For example, drug designers choose a target protein (and a target site on this protein), such as a viral enzyme. The compounds are designed to bind to the target site and inhibit the activity of the protein. Examples of drugs developed in this way

are inhibitors for the enzyme protease in HIV (2, 6, 7) and inhibitors for the enzyme neuraminidase in the influenza virus (8, 9).

Random screening of compounds is a more traditional approach to develop an antiviral, this is done by screening compounds looking for antiviral activity. Here dissolutions of compounds are tested against a range of viruses growing in cell cultures (2). For each potentially useful compound, the half maximal inhibitory concentration, $IC_{50}$, is determined. To calculate $IC_{50}$, it is necessary to construct a dose-response curve and scan the effect of different concentrations of drugs on reversing the viral activity, (Fig. 1.1). $IC_{50}$, the half maximal inhibitory concentration is used for in vitro experiments, while for in vivo experiments researchers use $EC_{50}$, half maximal effective concentration, which is the plasma concentration required for obtaining 50% of maximum effect.

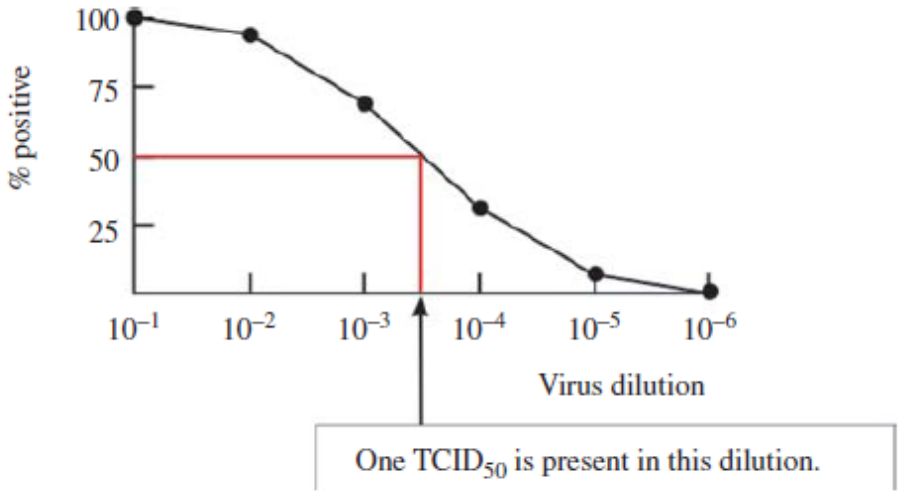


Figure 1.1: Dose response curve used to calculate $IC_{50}$ (4).

Many compounds are tested as antivirals using cell culture experiments, known as assays. The disadvantages of this method are that it is very time consuming and it

does not provide an explanation of how the antiviral works or its possible side effects. Also, it is not enough that a drug is proved to be useful with one assay; potential drugs have to be validated with alternative assays and also be improved in their efficacy and pharmacological properties (for example cytotoxicity) (5). Computational work is helpful in this method (10). Through computational tools it is possible to: manage the data acquired from the different assays, analyze this data, identify patterns, and present these patterns in a form that can be used to create a hypothesis about the mechanism of action.

### 1.1.2 Infectivity assays

Infectivity assays measure the concentration of infectious virus in a specimen. In virology experiments, this is important because this information can help determine: the virus pathogenicity, the activity of chemotherapeutic agents, or the neutralization of virus infectivity (4, 11). Some common terms in virology, which will help us understand the assays:

- **MOI:** Multiplicity of infection is the number of viruses added per cell to initiate an infection.
- **PFU:** Plaque forming units, in plaque assays, is the amount of virus needed to form one plaque ("hole" or dead cells) in a monolayer of susceptible cells. If in a plaque assay a virus is 100% successful then the number of PFU will be equal to the quantity of virus particles.
- $TCID_{50}$: This is defined as the necessary quantity of virus to infect 50% of the susceptible population in the assay.

## 1.2 Viral replication

The viral life cycle consists of several key steps.

1. **Attachment:** The first stage of the viral replication cycle is attachment. Viruses attach to cells through attachment proteins located on the surface of the virus (3, 4). These attachment proteins bind to cellular receptors on the surface of host cells. Attachment is an electrostatic phenomenon and it does not need cellular energy (12). Factors that can affect the efficiency of viral attachment are: density of attachment proteins or receptors, temperature and pH (12, 13). Viruses show preference for a particular cell type or species by binding only to specific cell receptors, a phenomenon known as tropism.

2. **Entry:** After attachment, the virus crosses the lipid bilayer of the plasma membrane or nuclear membrane (the type of membrane depends on the kind of virus or host cell). The modes of virus entry are:

    - *Ligand-mediated fusion.* This is a fusion between the cellular and viral membranes. The nucleocapsid material of the virus is deposited into the cell and the viral membrane is left as a patch in the cell membrane.

    - *Receptor-mediated endocytosis.* After the virus is attached to the membrane, the cell is stimulated to engulf the entire virus and forms an endocytotic vesicle.

    The mechanism of entry depends on the type of virus. For enveloped viruses, a type of virus that possesses an external envelope around the capsid (protein coat), the mechanism can be either ligand-mediated fusion or receptor-mediated endocytosis

(4, 12). For naked viruses, studies suggest that the mechanism of entry is via receptor-mediated endocytotis (12, 14).

3. **Uncoating:** Once the virus is inside the host cell the capsid is removed and the genetic material of the virus is released inside the cell. After uncoating, infectious particles cannot be detected in experiments, this is the beginning of the so-called eclipse phase.

4. **Genome replication:** In this stage of the viral replication cycle two events occur:

    - Replication of the viral genome, which is different for each type of genome. The genome of a virus is composed of single or double strands of DNA or RNA.
    - Production of the enzymes and protein structures that will be needed for the new virus particles.

5. **Assembly:** In this step, the components of the new virus particles are assembled to create a stable structure. Assembly is possible when the necessary viral proteins and genomic material are produced and localized at specific sites (this depends on the type of virus) inside of the infected cell.

6. **Maturation:** This is when the capsid proteins go through specific proteolytic cleavage. Proteolytic cleavage is a process where proteins are synthesized to become more stable.

7. **Release:** The newly matured virus particles are released to the outside of the infected cell. Possible mechanisms of release are through lysis, via budding, or via

secretion (12). This is the end of the eclipse phase and the start of the infectious phase.

## 1.3 Mechanism of action

A compound is considered an antiviral drug if it inhibits any stage of the virus replication cycle. Figure 1.2 is a schematic of the following antiviral mechanisms of action that can be observed (4, 15):

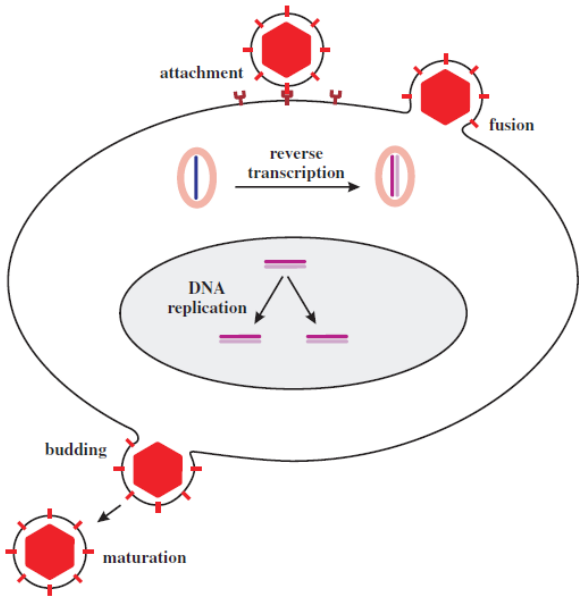


Figure 1.2: Examples of virus activities that are potential drug targets (4).

- **Preventing cell attachment:** Antivirals can inhibit the entry of virus into the cell using agents that bind to the cellular receptors or to the virus-associated protein (VAP) (fig. 1.2). Examples of these drugs are anti-Vap antibodies, receptor anti-idiotypic antibodies, extraneous receptor, and synthetic receptor mimics (16, 17).

- **Inhibiting entry:** Drugs developed to block the entry of virus into the cell. For example, a commercial drug named Fuzeon, used in treatment of HIV or TMC353121 which is a new RSV fusion inhibitor (18–21).

- **Uncoating Inhibitors:** These drugs inhibit the uncoating of viruses inside of the host cell. Examples of these drugs are Amantadine and Rimantadine used against influenza virus (22–24).

- **Inhibiting reverse transcriptase:** These drugs are analogues to the nucleotide or nucleoside; these analogues deactivate the enzymes that synthesize the RNA or DNA. Examples of these drugs are Acyclovir used against herpesvirus infections and Zidovudine for HIV (22, 25, 26).

- **Blocking integrase:** Integrase inhibitors are drugs designed to block integrase, which is an enzyme that inserts the DNA synthesized by the virus into the host cell genome. An example is the drug Fomivirsen, which is used to treat cytomegalovirus which causes eye infections in AIDS patients (22, 27).

- **Preventing transcription:** Blocking the action of transcription factors, which are the proteins responsible for the production of mRNA.

- **Preventing translation:** These are antisense molecules, segments of DNA and RNA, which attach to viral genomes and block their activity. Translation based antivirals have been designed to deal with Hepatitis C and HIV (28).

- **Blocking protease:** Some viruses have an enzyme known as a protease. Blocking protease inhibits cutting viral proteins to the configuration necessary for assembly. These drugs are being developed to treat and control HIV infection (29).

- **Impeding assembly:** Some antivirals interfere in the assembly phase of new virions. For example Rifampcin, which is an antibiotic, is used against vaccinia virus in this way (30).

- **Preventing release:** Blocking neuramidase activity, which is an enzyme that catalyzes the substance that gives mobility to the new virions. Examples of these drugs are Zanamivir (22, 31), named commercially as Relenza, and Oseltamivir, also known as Tamiflu, that treat influenza (22, 23).

- **Immune stimulants:** These are substances (drugs and nutrients) that stimulate the immune system, adaptive or innate responses, by producing an activation or increasing activity of any of its components. Immune response has an important role in how severe can an infection be (32).

Our aim is to determine if experimental assays can be used to help determine the mechanism of action of respiratory viral infections antiviral. Previously, Heldt et al (33) used a computational model, where they took into account intra- and extra-cellular processes of an influenza infection, to show which steps of viral replication are more susceptible to drugs. They found dynamical differences in the infection as drugs with different mechanisms were modeled. We would like to know whether these dynamical differences can be exploited to determine an unknown mechanism of action of potential antivirals. We

start with a model limited to virus-cell interactions during the infection, one that does not capture all the intra-cellular processe, but which can still be used to model different mechanisms of action. For example to model blocking of assembly, we reduce the production of virions in the mathematical model or by increasing the time where the virus is not infectious, we can model the mechanism of preventing release.

## 1.4 Respiratory Infections

This study focuses on a particular respiratory virus: influenza.

### 1.4.1 Influenza

Influenza is an infectious disease caused by influenza virus. In the last century, there have been three major pandemics caused by influenza: the 1918 Spanish Flu, the 1957 Asian Flu, and the 1968 Hong Kong Flu. These pandemics caused approximately 50 million, 2 million and 1 million deaths. Also, annual epidemics of influenza A viruses and influenza B virus affect 5–20% of the world's population and result in 250,000–500,000 deaths each year. It was estimated that the accumulated outcome of the annual influenza epidemics in the $20^{th}$ century exceeded those of three great world pandemics of plague recorded (Justinian Plague in 521 AD, Black Death in 1347 AD and Modern Plague 1894 AD) (4, 34).

Influenza viruses are RNA viruses and belong to the family of Orthomyxoviridae virus. There are three types of influenza viruses:

- **Influenza A:** This influenza virus causes influenza in birds and mammals. The main variants of this type of virus are bird flu, human flu, swine influenza, equine influenza, and canine influenza. This type is the most virulent and has caused devastating pandemics: Spanish Flu, Swine Flu (18500 deaths), Asian Flu, and Hong Kong Flu.

- **Influenza B:** Less common than influenza A, influenza B infects humans, seals, and ferrets. Its mutation rate is slower than the mutation rate for influenza A, which makes it less genetically diverse. Consequently, humans develop a degree of immunity to influenza B.

- **Influenza C:** Less common than the other types, this can infect pigs and humans, but still can be dangerous and cause local epidemics. Influenza C causes mortality mainly in kids, but after an individual is infected once, they develop antibodies against this type of influenza.

The structure of the viral particle is similar for must types of influenza. Their viral particles are 80–120 nm in diameter with spherical shape, although influenza C can have a filamentous form. The particles possess a lipid membrane envelope that contains the glycoproteins hemagglutinin (HA) and neuraminidase (NA). These proteins are responsible for attachment and release of the virus and determine the type of influenza A virus. These viruses exhibit a cell tropism for epithelial respiratory cells and are transmitted through host-virus interaction: in mammals via respiratory, in birds fecal-oral route from drinking contaminated water and in zoonosis in general through animal contact.

Influenza viruses have high mutation rates and frequently experience genetic reassortment, which causes a variability in HA and NA antigens. The mechanisms used by the virus to evade the host immune response are called antigenic variation. There are two types of antigenic variation (35, 36):

- **Antigenic drift:** Results from a change in a few amino acids. In this case, the virus changes slowly over time, which means antibodies stop recognizing the virus resulting in a loss of immunity against the virus. This is one reason that we need flu vaccines each year.

- **Antigenic shift:** It is the outcome of the acquisition of new proteins. This occurs when two different influenza strains infect the same cell and combine their genetic material. As a result of antigenic shift, new influenza subtypes are created, these viruses can cause epidemics as there is no immunity for them.

The antiviral drugs used against influenza include:

- **Amantadine, Rimantadine:** These are inhibitors of M2 ion channels and prevent the uncoating of influenza A virus (2).

- **Zanamivir, Oseltamivir, Peramivir:** These are neuraminidase inhibitors and prevent escape of new virions from the host cell prevent infections of other cells (Fig. 1.3) (22, 23, 31). These drugs are used for Influenza A and B.

- **Favipiravir:** It is an experimental drug used to inhibit viral replication and transcription of RNA virus (37).

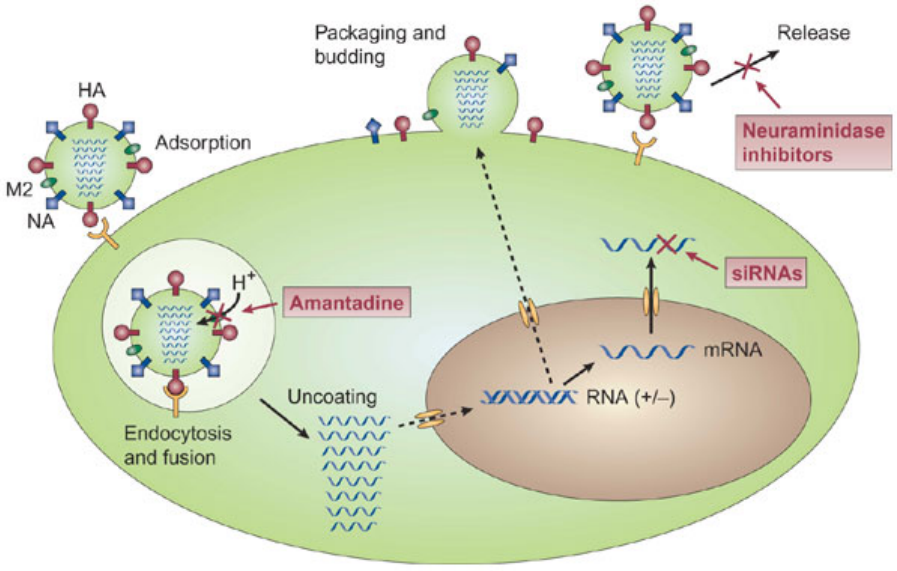


Figure 1.3: Influenza viral replication cycle. In this figure you can observe the mechanisms of action of Amantadine and Neuraminidase Inhibitors.

## 1.5 Background of Mathematical Models

Mathematical models have been used as a tool against epidemics, they can be used to compare strategies to plan for an anticipated epidemic or to deal with an actual disease outbreak. The first epidemic model was established by Daniel Bernoulli in 1766 (38). His model was used for the spread of smallpox in the 18th century. He considered two outcomes to the infection: death or recovery with immunity. As a result of his model, he estimated that three quarters of all the persons alive in the 18th century had been infected with smallpox.

The implementation of a mathematical model describing the infection cycle requires a detailed specification of the biological processes, for example:

- Infections have been observed to be a Poisson processes where the rate of infection is proportional to the virus concentration.

- Virus production by infectious cells can be assumed to be a constant rate.
- The infectivity of free virus is known to decrease exponentially in time.
- The distribution of the times between the states of the infection have a delay.
- Description of other factors, such as the environment where the infections spread, the kind of populations infected, and the dynamics between these populations.

To study respiratory infections we use compartmental models. Compartmental models are deterministic models, where it is assumed that the population is homogeneous and the subjects in the population pass through different compartments (states) (39). To derive the compartmental models, first we define the compartments based on assumptions about the nature and time rate of change from one compartment to other. Then the models are formulated in terms of the derivatives of the members within each compartment, where the time $t$ is considered as an independent variable. In this way, models are formulated as differential equations.

To perform computational simulations with these models, it is necessary to get parameter values from data or literature. We can also analyze these models mathematically to some extent: we can evaluate their equilibrium points and stability of these points; we can derive threshold conditions (for example the Basic Reproduction Number $R_0$); and we can perform bifurcation analysis. From the mathematical analysis and numerical simulations, we can get information about the properties that make the pathogen spread and manipulate the outcome of an infection.

### 1.5.1 Basic Viral Replication Model

The basic viral replication model is used to study the dynamics of viral infections. To represent the dynamics of these infections, we take into account the biological processes mentioned before (infectivity rate, viral production rate, etc.). The virus replication model (depicted in Fig. 1.4) starts with healthy target cells, $T$, which are infected by virions, $V$, and enter the eclipse phase, $E$. During the eclipse phase, the cell's replication machinery produces all the viral proteins and packages them into new virions. When the new virions are ready, the cell transitions to the infectious state, $I$, when virions are released from the cells to infect other cells. Eventually the infectious cells will die. To reproduce this cycle, the following system of differential equations was proposed in (40):

$$\dot{T} = -\beta TV \tag{1.1}$$

$$\dot{E} = \beta TV - \frac{E}{\tau_E} \tag{1.2}$$

$$\dot{I} = \frac{E}{\tau_E} - \frac{I}{\tau_I} \tag{1.3}$$

$$\dot{V} = pI - cV. \tag{1.4}$$

Where these are the parameters in the model:

- $\beta$**:** the rate at which virions infect target cells get infected.
- $p$**:** the production rate of virions by infected cells.
- $c$**:** the rate at which virions lose infectivity.
- $\tau_E$**:** Time in the eclipse phase.

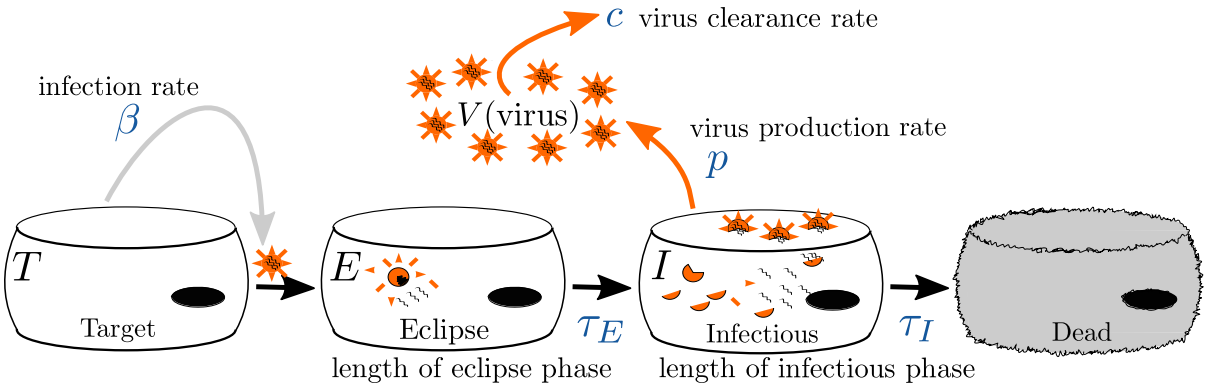


Figure 1.4: Virus replication model.

- $\tau_I$**:** Time in the infectious phase.

Note that in respiratory infections virus replication is faster than the recovering time for respiratory tissue, so there is no target cell replacement in this model.

## 1.6 Questions

In this paper, we will use mathematical models to investigate antiviral treatment of respiratory viruses. Specifically, we will address the following questions:

- What experiments can help us determine the mechanism of action?
- How do model parameters affect our predictions about the mechanism of action?
- Can mechanism of action be determined from dose-response curves?

# Chapter 2

# Methods

## 2.1 Mathematical Model

The model presented earlier assumes cells leave the eclipse and infectious compartments at a rate proportional to the number of cells in the compartment. This supposition produces an exponential distribution for the times spent in the eclipse and infectious phases. This is not biologically accurate, and leads to a large variance between the average period in the eclipse and infectious compartments meaning that cells produce virus immediately after getting infected and they are likely to die long before or after the mean life span $\tau_I$ (41). In Figure 2.1, we see different possible delay distributions in the evolution between states. To model the infection more accurately, we use a mathematical device known as "The Method of Stages", where a single compartment in the model is replaced by a set of $n$ subcompartments. The time spent in each subcompartment is exponentially distributed, so the life span of the cells is described by the sum of $n$

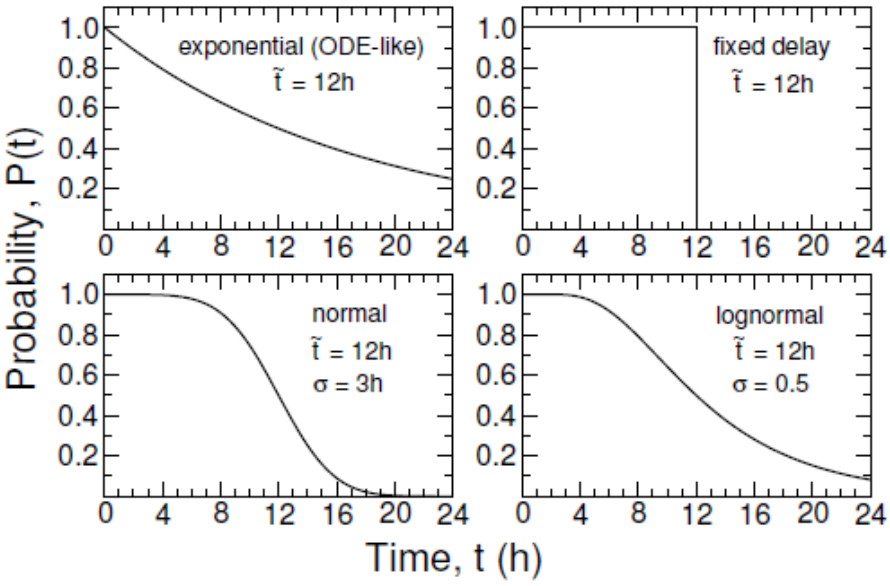


Figure 2.1: Examples of delay distributions. The function plotted, $P(t)$, is the probability that after a time $t$ the cell remains in a current state (42).

exponential distributions. This leads to a gamma distribution in the times for these stages (42, 43).

To apply "The Method of Stages" in the basic viral infection model, the eclipse and infectious compartments are divided into $n$ subcompartments ($n_E$ and $n_I$), with $n_E/\tau_E$ and $n_I/\tau_I$ giving the rates of sequential progression through the subcompartments. The advantage of this method is that when $n_E$ and $n_I$ are equal to one we are back to the exponentially distributed model (Fig. 2.2), while for $n_E \to \infty$ we get a normal distribution (41).

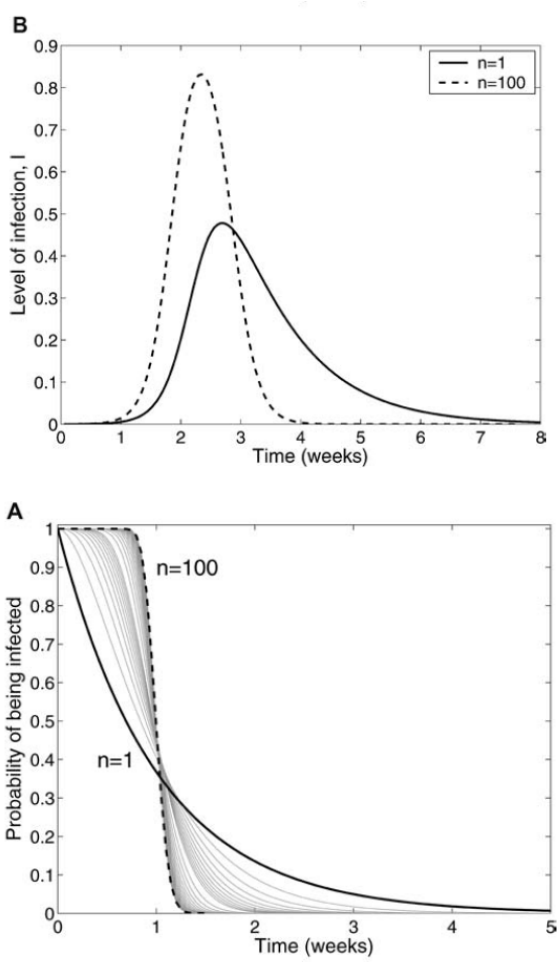


Figure 2.2: Effects of gamma distributed infectious period on the epidemic curve (43).

When implementing the stages,we use a system of differential equations for the gamma-distributed viral infection model:

$$\dot{T} = -\beta TV \tag{2.1}$$

$$\dot{E}_1 = \beta TV - \frac{n_E}{\tau_E} E_1 \tag{2.2}$$

$$\dot{E}_j = \frac{n_E}{\tau_E} E_{j-1} - \frac{n_E}{\tau_E} E_j \quad \text{for } j = 2, ..., n_E \tag{2.3}$$

$$\dot{I}_1 = \frac{n_E}{\tau_E} E_{n_E} - \frac{n_I}{\tau_I} I_1 \tag{2.4}$$

$$\dot{I}_j = \frac{n_I}{\tau_I} I_{j-1} - \frac{n_I}{\tau_I} I_j \quad \text{for } j = 2, ..., n_I \tag{2.5}$$

$$\dot{V} = p \sum_{j=1}^{n_I} I_j - cV, \tag{2.6}$$

where the target cells $T$ are infected by the virus $V$ at an infectivity rate $\beta$. Then the infected cells enter in a eclipse phase $E$ and pass through all the compartments $E_j$ before become infectious. Next all the cells in the infectious compartments, $I_j$, produce new virus particles at a rate $p$ and at the same time the virus loses infectivity at a rate $c$. We will use the gamma-distributed model for our studies of mechanism of action of antivirals. In this study, we specifically examine the effect of antivirals on influenza. The parameters used to describe the infection are given in Table 2.1, which are the only parameters available from the literature that model influenza infectios using the gamma model.

Table 2.1: Influenza parameters. Parameters for influenza taken from (44).

| Parameter | Flu Parameter |
|---|---|
| $T_0$ | $10^6$ cells |
| $\beta$ | $4.26 \times 10^{-4}$ $(\mathrm{h} \cdot \mathrm{TCID/mL})^{-1}$ |
| $p$ | 176 $(\mathrm{TCID/mL}) \cdot \mathrm{h}^{-1}$ |
| $c$ | 0.13 /h |
| $\tau_I$ | 49.0 h |
| $\tau_E$ | 6.6 h |
| $n_E$ | 30 |
| $n_I$ | 100 |


Figure 2.3: Multiple Cycle Assay (MCA)

## 2.2 Experimental Assays

Experimental assays are used for the titration of virus, which measures the number of infectious units per milliliter. The titers are determined by making dilutions of virus suspensions in cell culture or growth medium. There are two kind of in vitro assays for viral infections:

- **Multiple Cycle Assay (MCA):** In the MCA a small quantity of virus, low MOI, is applied to a cell culture. In this way just a few cells get infected and will produce more virus to infect more cells. The viral cycle is repeated until all the cells get infected.

- **Single Cycle Assay (SCA):** In the SCA a large quantity of virus, high MOI, is applied to a cell culture to infect all the cells in the culture at the same time. Therefore all the cells will be synchronized in one viral cycle.


Figure 2.4: Single Cycle Assay (SCA)

To simulate the MCA and SCA using the model, it is necessary to apply the proper initial conditions when solving the differential equations. In the MCA to simulate a low MOI, we set a small ratio of the total cells to initially be in the eclipse phase. For a SCA, we set all the cells to initially be in the eclipse phase.

## 2.3 Modeling the drug effect

To simulate different possible mechanisms of action, we apply the effect of a drug to different parameters in the model. The effect of a drug is modeled using the efficacy, $\varepsilon$. The efficacy is the ratio of successful responses of a drug, so this can vary between 0 and 1.

The mechanism of action for antiviral drugs that we modeled are:

- **Reducing the infection rate of new cells and allowing large amount of virus to enter cells before infection**. This is modeled by applying the efficacy to the parameter $\beta$:

$$\dot{T} = -\beta(1-\epsilon)TV \tag{2.7}$$

$$\dot{E}_1 = \beta(1-\epsilon)TV - \frac{n_E}{\tau_E}E_1. \tag{2.8}$$

In this way, the infection rate decreases as efficacy increases, this means that the number of target cells decreases slowly and the number of cells in the eclipse phase increases also slowly.

- **Reducing the infection rate of new cells without allowing large amounts of virus to enter the cells**. To do this we place the efficacy on the parameter $\beta$, but only in the equation of the first compartment in the eclipse phase:

$$\dot{T} = -\beta TV \tag{2.9}$$

$$\dot{E}_1 = \beta\big(1-\epsilon\big)TV - \frac{n_E}{\tau_E}E_1. \tag{2.10}$$

Here the infection rate just decreases for the eclipse phase equation, so we have that the cells entering in the eclipse are less than the loss of target cells.

- **Reducing the production of new virions**. For this mechanism, we put the efficacy on $p$:

$$\dot{V} = p\big(1-\epsilon\big)\sum_{j=1}^{n_I} I_j - cV. \tag{2.11}$$

This means that the production rate will decreases as the efficacy of the drug increases.

- **Increasing the rate of loss of virion infectivity**. In this case, the efficacy is applied to $c$:

$$\dot{V} = p\sum_{j=1}^{n_I} I_j - \frac{c}{1-\epsilon}V. \tag{2.12}$$

The virions become less infectious as the efficacy increases.

- **Increasing the length of the eclipse phase**. The parameter $\tau_E$ is affected by the efficacy.:

$$\dot{E}_1 = \beta TV - \frac{n_E}{\frac{\tau_E}{1-\epsilon}}E_1 \tag{2.13}$$

$$\dot{E}_j = \frac{n_E}{\frac{\tau_E}{1-\epsilon}}E_{j-1} - \frac{n_E}{\frac{\tau_E}{1-\epsilon}}E_j \tag{2.14}$$

$$\dot{I}_1 = \frac{n_E}{\frac{\tau_E}{1-\epsilon}}E_{n_E} - \frac{n_I}{\tau_I}I_1. \tag{2.15}$$

This represents an antiviral that blocks release of the virus.

- **Decreasing the lifespan of infectious cells**. The parameter $\tau_I$ is modified by the efficacy in the following way.

$$\dot{I}_1 = \frac{n_E}{\tau_E}E_{n_E} - \frac{n_I}{\tau_I\big(1-\epsilon\big)}I_1 \tag{2.16}$$

$$\dot{I}_j = \frac{n_I}{\tau_I\big(1-\epsilon\big)}I_{j-1} - \frac{n_I}{\tau_I\big(1-\epsilon\big)}I_j \quad \text{for } j = 2, ..., n_I \tag{2.17}$$

This could represent the effect of an immune stimulant that causes the immune response to kill infected cells more rapidly.

For each theoretical drug, we assume the drug concentration remains constant from the beginning of the experiment. Then we examine the time course of the virus and the time course of dead cells as the concentration of drug is varied.

## 2.4 Measurements

Experimentally, the viral titer and sometimes also the number of dead cells are measured over time. In this way, the viral titer and dead cell time course data is determined, Fig. 2.5. From the time course data is possible get quantities that are interesting:

- **Viral peak:** The maximum amount of virus produced. This represent how critical is an infection, higher viral peak harde symptoms.
- **Time peak:** Time when the viral peak is reached. It is related with the duration of the infection.
- **AUC:** Area under the viral titer curve, which assesses the severity of the infection and how infectious is.
- **Viral slopes:** Upslope for the growth rate of the viral titer and downslope for the viral decay rate.
- **Maximum amount of dead cells:** Used also to assess the efficacy of a drug.

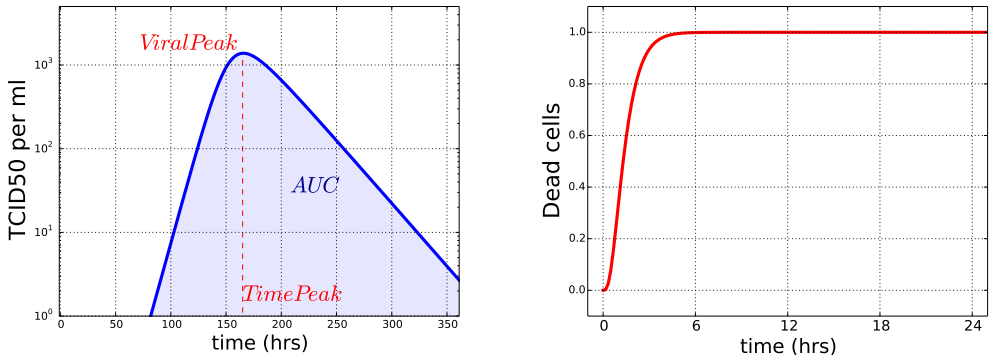


Figure 2.5: Examples of Viral titer and dead cells time courses curves for different infections, left curve is for Respiratory Syncytial Virus infection (RSV) and right curve for Influenza infection. Also are shown the measurements that can be extracted from these curves.

## 2.5 Latin hypercube sampling

After numerical simulations are performed, it is necessary to assess the results using a sensitivity analysis. Sensitivity analysis is the study of how the uncertainty in the inputs of the models can affect the output. To perform our analysis, we used Latin Hyercube Sampling.

Latin Hypercube Sampling (LHS) is a statistical method to produce a sample of probable collections of parameter values from a multidimensional distribution. This method is used to construct computer experiments where it is desired that the data of each simulation is constrained to match the input distribution very closely. To do this, the Latin Hypercube method takes random parameters within some fraction of the standard error and uses these parameters to produce data.

In this work, we examined how the changes in the parameters can change the course of the infection for the different theoretical drugs. The parameters that we want to analyze

are: $\beta$, $p$, $c$, $\tau_I$ and $\tau_E$. We took a random value, for each parameter, from a distribution constrained between:

$$\text{Experimental value} \qquad \pm 10\% \text{ Experimental value} \tag{2.18}$$

Where the best fit value comes from the experimental data available. We redid this 300 times, in this way we got a collection of different outputs. Then we plot these outputs to compare them.

## 2.6 Fitting data curve

Curve fitting is a procedure to construct a curve, or function, that has the best fit to a group of data points. This is a very useful tool in virology, because experimental data points are limited. To help us extract some of the measurements described in Section 2.4, we use the function,

$$V = \frac{2V_p}{e^{\lambda_g(t-t_p)} + e^{-\lambda_d(t-t_p)}}, \tag{2.19}$$

where $V_p$ is the viral peak, $t_p$ is the time of peak, $\lambda_g$ is the growth rate and $\lambda_d$ is the decay rate. This function is used because it shows two characteristics found in the experimental data; an exponential growth of the viral titer (after the eclipse phase) followed by an exponential decrease after the viral peak is reached (42).

To evaluate our fitting we use the "Reduced Chi-Square Goodness of Fit test". This test measures how close the fitted values are to the expected values. The test involves

calculating the chi-square statistic of the fitting,

$$\tilde{\chi}^2 = \sum_{k=1}^{n} \left( \frac{(F_k - E_k)}{\sigma_k} \right)^2, \tag{2.20}$$

where $F_k$ are the fitted values and $E_k$ are the expected value, the experimental data, and $\sigma_k$ are the Gaussian errors. The chi-square values will be one if there is no deviation (perfect fit).

# Chapter 3

# Results

## 3.1 Quantifying the effect of a drug

Experimentally, virologists use values of measurable quantities, like the viral peak, as a function of drug dose to determine the effect of a drug. For our models, we use efficacy, rather than drug dose, although we can relate efficacy to dose through the $E_{\max}$ model,

$$\varepsilon = \varepsilon_{max} \frac{D}{\mathrm{IC}_{50} + D} \tag{3.1}$$

where $D$ is the drug dose, $\mathrm{IC}_{50}$ is the drug concentration at which we have 50% of the maximum effect and $\varepsilon_{max}$ is the maximum effect of the drug. We generate efficacy curves with our model as follows. First, it is necessary get the time course curves for the amount of virus and dead cells for each value of the efficacy. Second, we got the values of different measurements (viral peak, time peak, AUC, viral slopes, and maximum amount of dead cells), as described in Section 2.4.

For example, in Fig. 3.1 we can observe the viral time courses for the MCA of influenza under the influence of drugs with different mechanisms, each figure showing the effect for several values of efficacy. For example, drugs with mechanisms *Protecting target cells* and p have the same effect: they keep the shape of the curve but the viral peak decreases as function of the efficacy. In drugs with mechanism *Slowing infection*, we don't observe effect of the drug in the viral course until the efficacy is 100%. For mechanisms *Increasing the length of the eclipse phase* and *Decreasing the lifespan of infectious cells* the viral peak is the same but the shape of the curve changes, for *Increasing the length of the eclipse phase* the time for viral peak increases as a function of the efficacy, while for *Decreasing the lifespan of infectious cells*, it decreases. For the mechanism *Increasing the rate of loss of virion infectivity*, the viral peak, the time when the viral peak is reached and the shape of the curve change as a function of the efficacy. in this way, if we study the effect of a drug by measuring different quantities, we would expect to get different types of curves for each mechanism. For example, measuring the drug effect on peak viral titer would yield a different result than measuring drug effect on viral decay rate. From these time courses, we can extract quantities that are possible to measure experimentally, such as viral peak, AUC, or amount of virus at different times post infection.

We plot the extracted quantities as a function of the drug efficacy to produce efficacy curves. In Fig. 3.2, we have plotted $V_{max}$ and AUC as functions of the efficacy for different theoretical drugs. For $V_{max}$, viral peak, we found that for mechanisms: *Protecting target cells*, *Reducing the production of new virions* and *Increasing the rate of loss of virion infectivity* the viral peak decreases linearly with the efficacy. For *Slowing infection*, the viral peak is independent of the efficacy and for *Increasing the length of the eclipse phase*

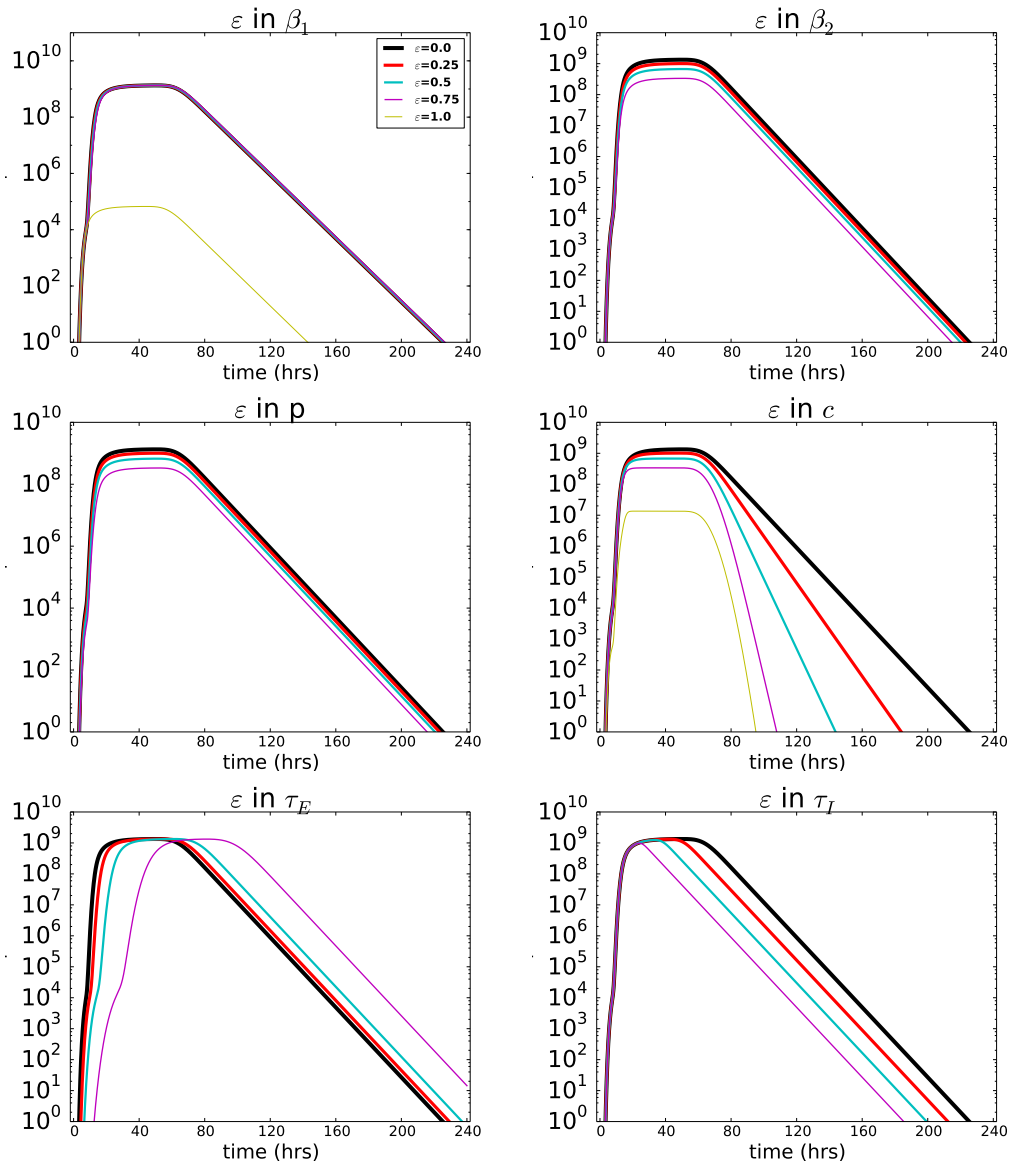


Figure 3.1: Viral time course for different mechanisms of action during the MCA for influenza infection. The top left figure shows the effect of a drug on *Slowing infection*, for this figure all the curves for efficacy values less than 1 follow the same trend; top right figure shows drug on *Protecting target cells*; center left figure shows drug on *Reducing the production of new virions*; center right drug on *Increasing the rate of loss of virion infectivity*; bottom left figure shows drug on *Increasing the length of the eclipse phase* and bottom right shows drug on *Decreasing the lifespan of infectious cells*.

and *Decreasing the lifespan of infectious cells* the viral peak decreases in a concave curve.

For AUC, all the drugs are independent of the efficacy except *Protecting target cells*, which

decreases linearly as function of efficacy, and *Increasing the length of the eclipse phase* which has sigmoidal curve.

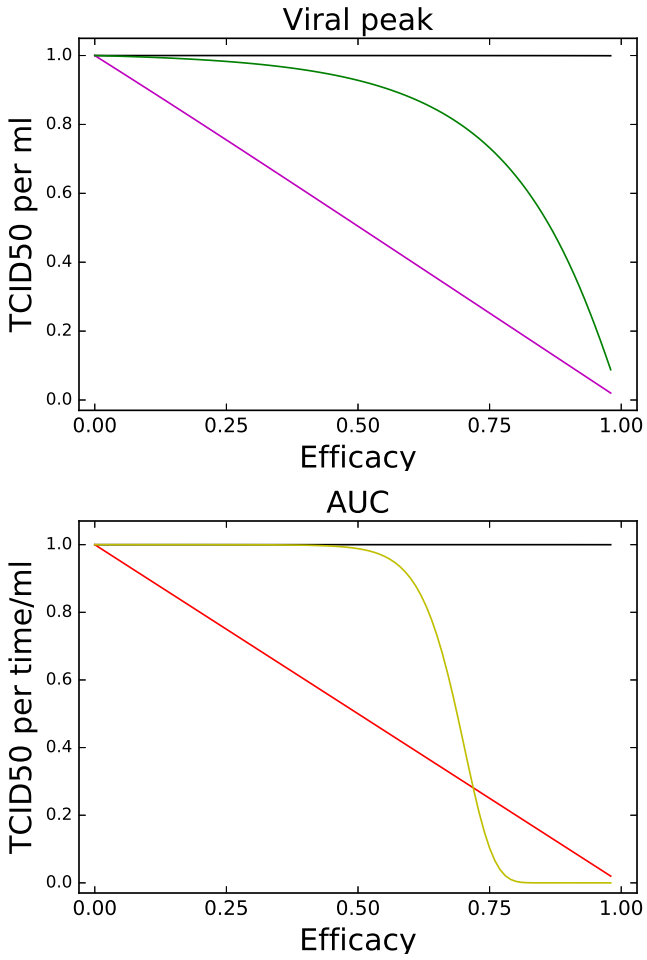


Figure 3.2: Efficacy curves for different mechanisms of action under the MCA for influenza infection. Top figure shows the maximum amount of virus as a function of efficacy; Bottom figure shows AUC as a function of efficacy. Black curve is for *Slowing infection*, red is for *Protecting target cells*, purple is for *Increasing the rate of loss of virion infectivity*, yellow is for *Increasing the length of the eclipse phase* and green is for *Decreasing the lifespan of infectious cells*.

A similar procedure is used to extract measurable quantities from the time course of dead cells, such as the maximum amount of dead cells. We do not examine eclipse or infectious cells in this study because experimentally we cannot detect which cells are in

the infectious or eclipse phase. We can detect the amount of virus in the dish, but it is not possible recognize the cells which produce it.

## 3.2 Efficacy curves

We first examined the effect of different hypothetical drugs on influenza. The parameters used to simulate a typical influenza infection are given in Table 2.1. We simulated both MCA and SCA experiments and found drug efficacy curves for our hypothetical drugs for the quantities described in Section 2.4.

The results for AUC are shown in Fig. 3.5 and Fig. 3.8. From the AUC graphs, we can observe that the most effective drugs for reducing AUC of the MCA are the ones with mechanisms acting on: *Protecting target cells* and *Increasing the length of the eclipse phase*, while for SCA the most efficient mechanisms are *Increasing the length of the eclipse phase*, *Reducing the production of new virions* and *Increasing the rate of loss of virion infectivity*. The results for Viral Peak are shown in Fig. 3.3 and Fig. 3.6. From the viral peak curve we can see that the mechanisms that do not affect peak viral titer are *Slowing infection* for MCA and *Slowing infection* and *Protecting target cells* for SCA. The results for maximum amount dead cells are shown in Fig. 3.4 and Fig. 3.7. For the SCA, a drug that reduces infection rate is not expected to have any effect since all the cells are already infected.

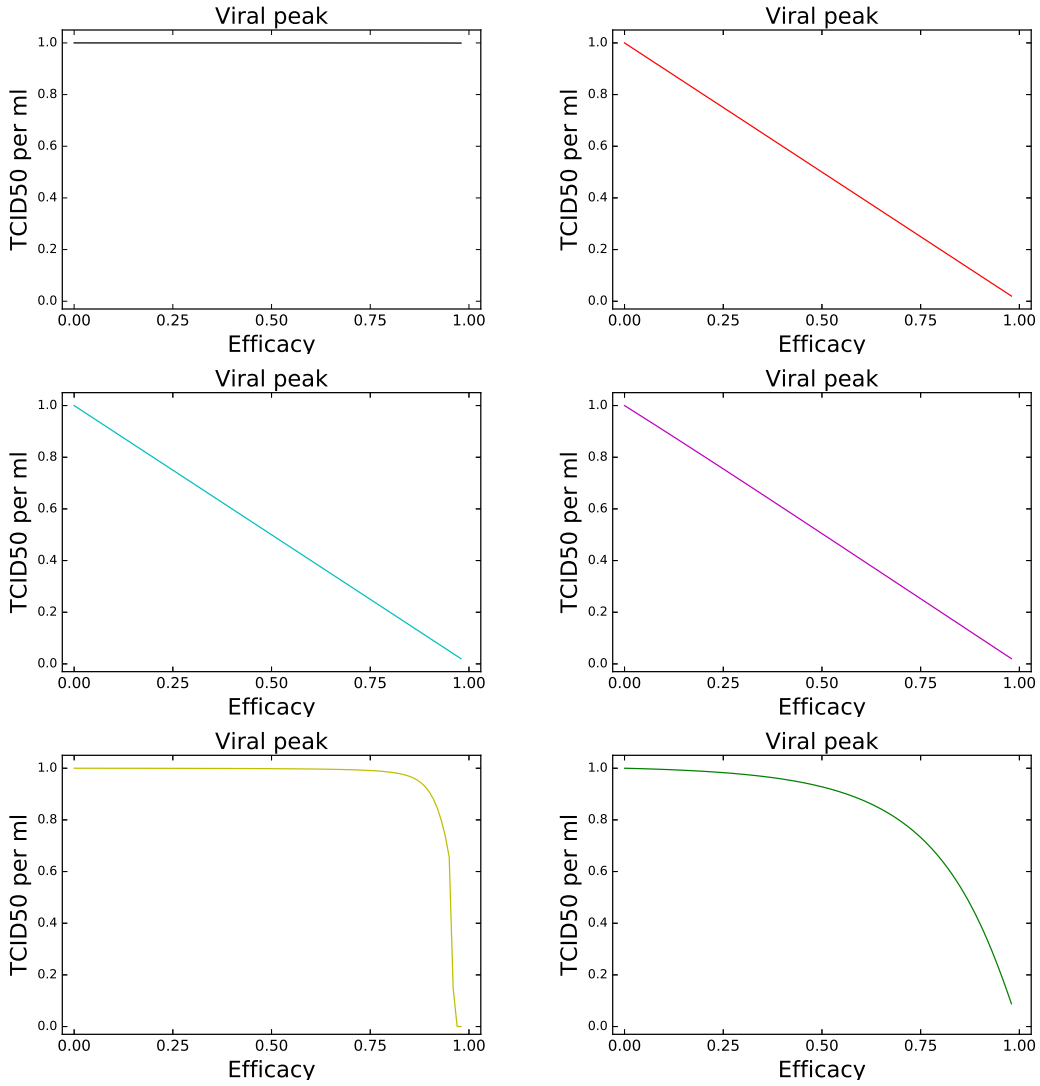


Figure 3.3: Viral peak efficacy curves for drugs with different mechanisms of action for influenza infection for MCA. Black curve is for *Slowing infection*, red is for *Protecting target cells*, cyan is for *Reducing the production of new virions*, purple is for *Increasing the rate of loss of virion infectivity*, yellow is for *Increasing the length of the eclipse phase* and green is for *Decreasing the lifespan of infectious cells*.

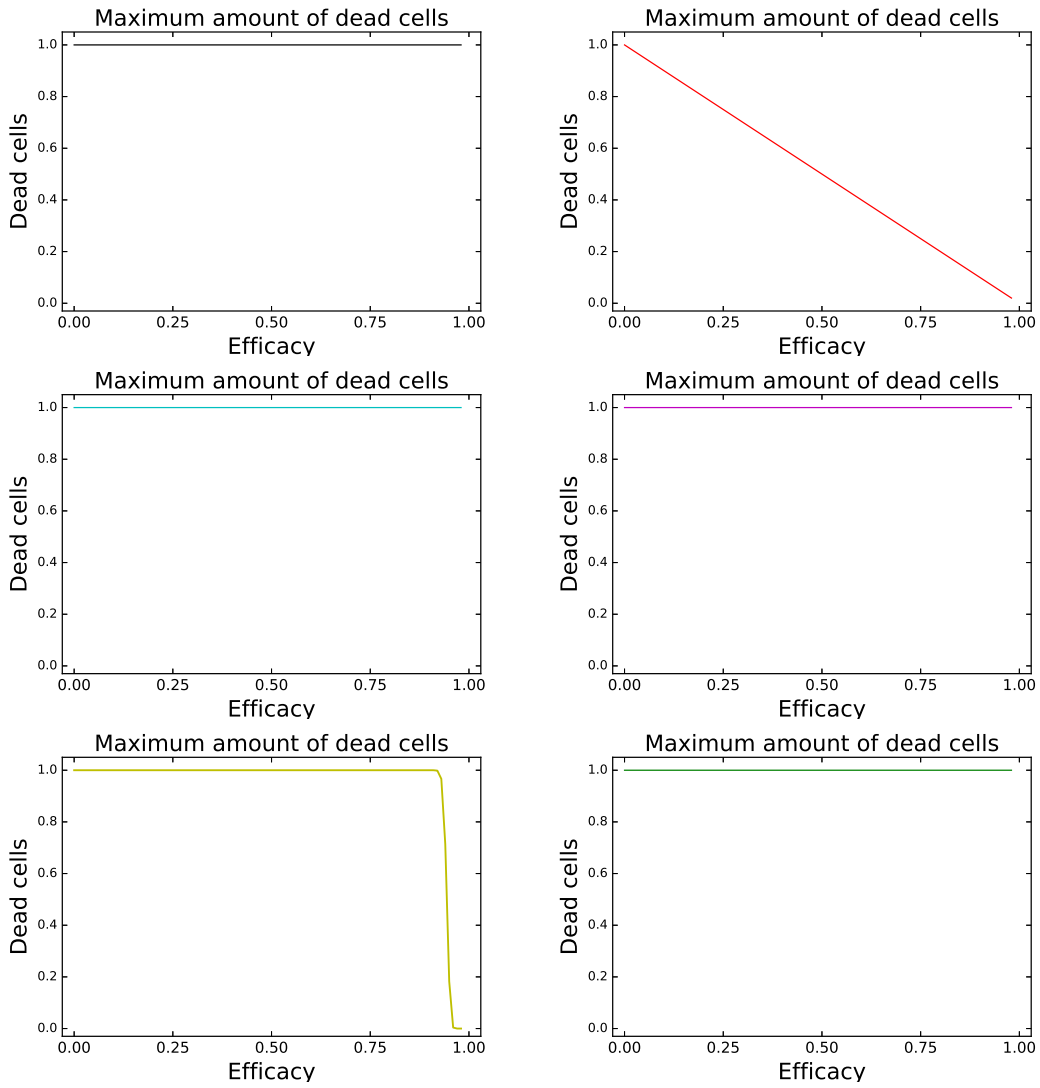


Figure 3.4: Maximum amount of dead cells efficacy curves for drugs with different mechanisms of action for influenza infection for MCA. Black curve is for *Slowing infection*, red is for *Protecting target cells*, cyan is for *Reducing the production of new virions*, purple is for *Increasing the rate of loss of virion infectivity*, yellow is for *Increasing the length of the eclipse phase* and green is for *Decreasing the lifespan of infectious cells*.

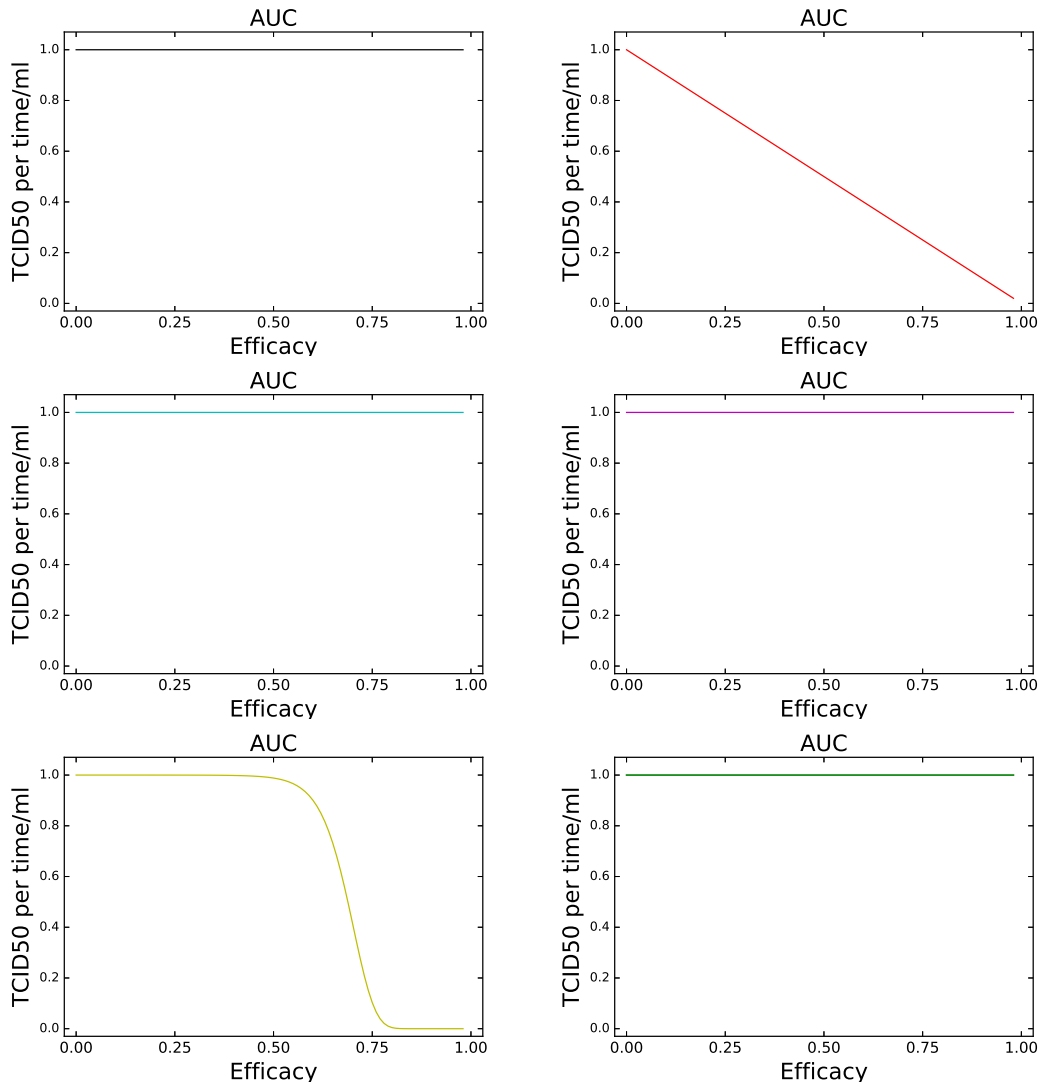


Figure 3.5: AUC efficacy curves for drugs with different mechanisms of action for influenza infection for MCA. Black curve is for *Slowing infection*, red is for *Protecting target cells*, cyan is for *Reducing the production of new virions*, purple is for *Increasing the rate of loss of virion infectivity*, yellow is for *Increasing the length of the eclipse phase* and green is for *Decreasing the lifespan of infectious cells*.

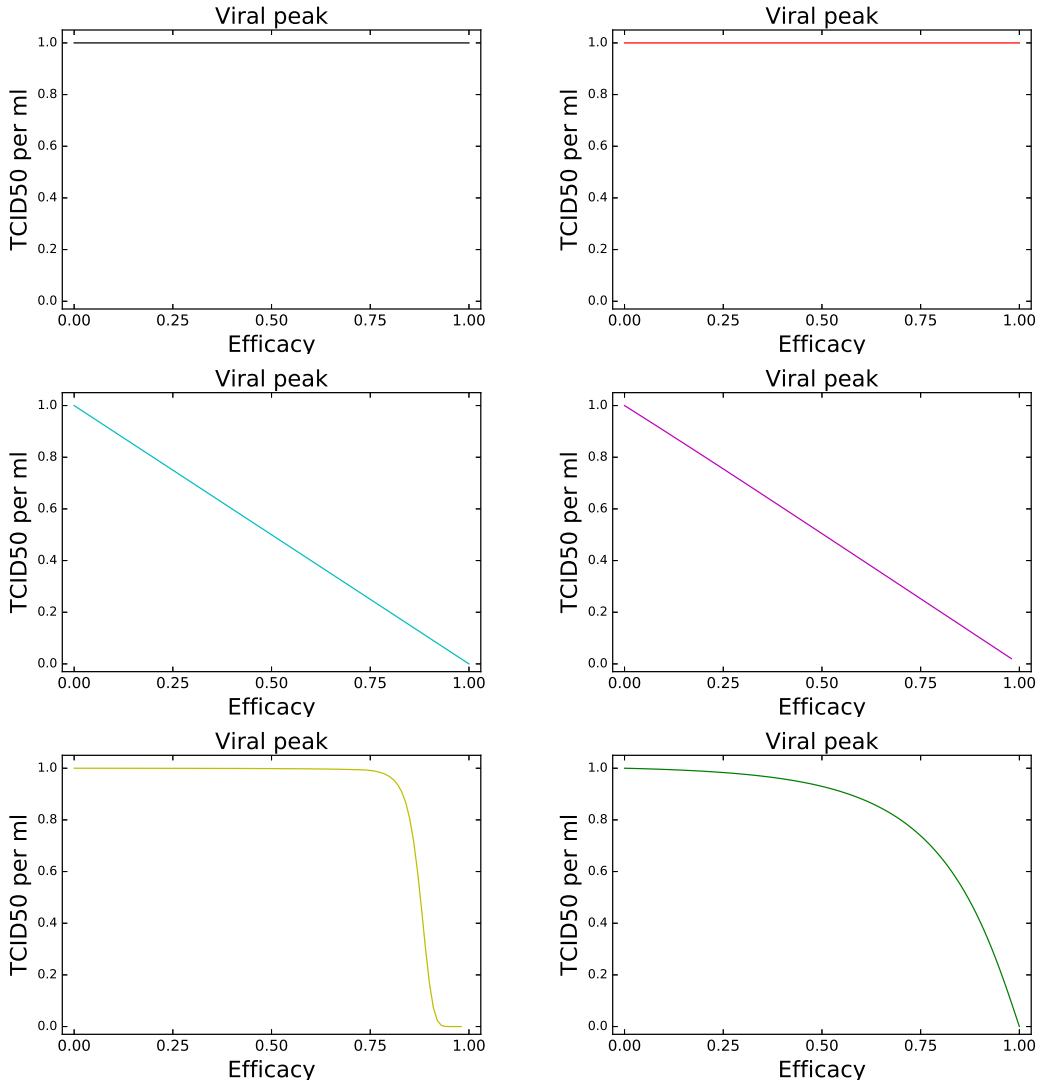


Figure 3.6: Viral peak efficacy curves for drugs with different mechanisms of action for influenza infection for SCA. Black curve is for *Slowing infection*, red is for *Protecting target cells*, cyan is for *Reducing the production of new virions*, purple is for *Increasing the rate of loss of virion infectivity*, yellow is for *Increasing the length of the eclipse phase* and green is for *Decreasing the lifespan of infectious cells*.

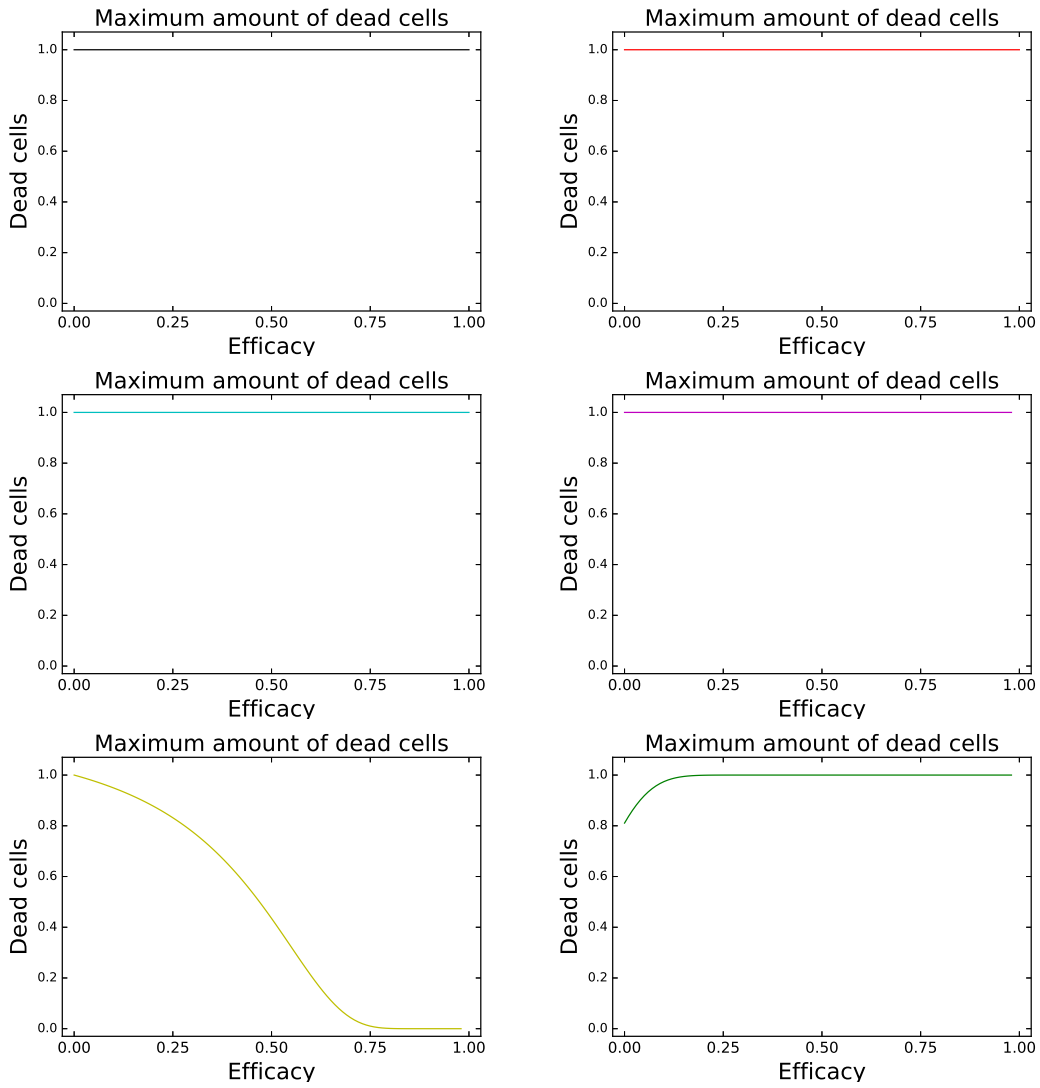


Figure 3.7: Maximum amount of dead cells efficacy curves for drugs with different mechanisms of action for influenza infection for SCA. Black curve is for *Slowing infection*, red is for *Protecting target cells*, cyan is for *Reducing the production of new virions*, purple is for *Increasing the rate of loss of virion infectivity*, yellow is for *Increasing the length of the eclipse phase* and green is for *Decreasing the lifespan of infectious cells*.

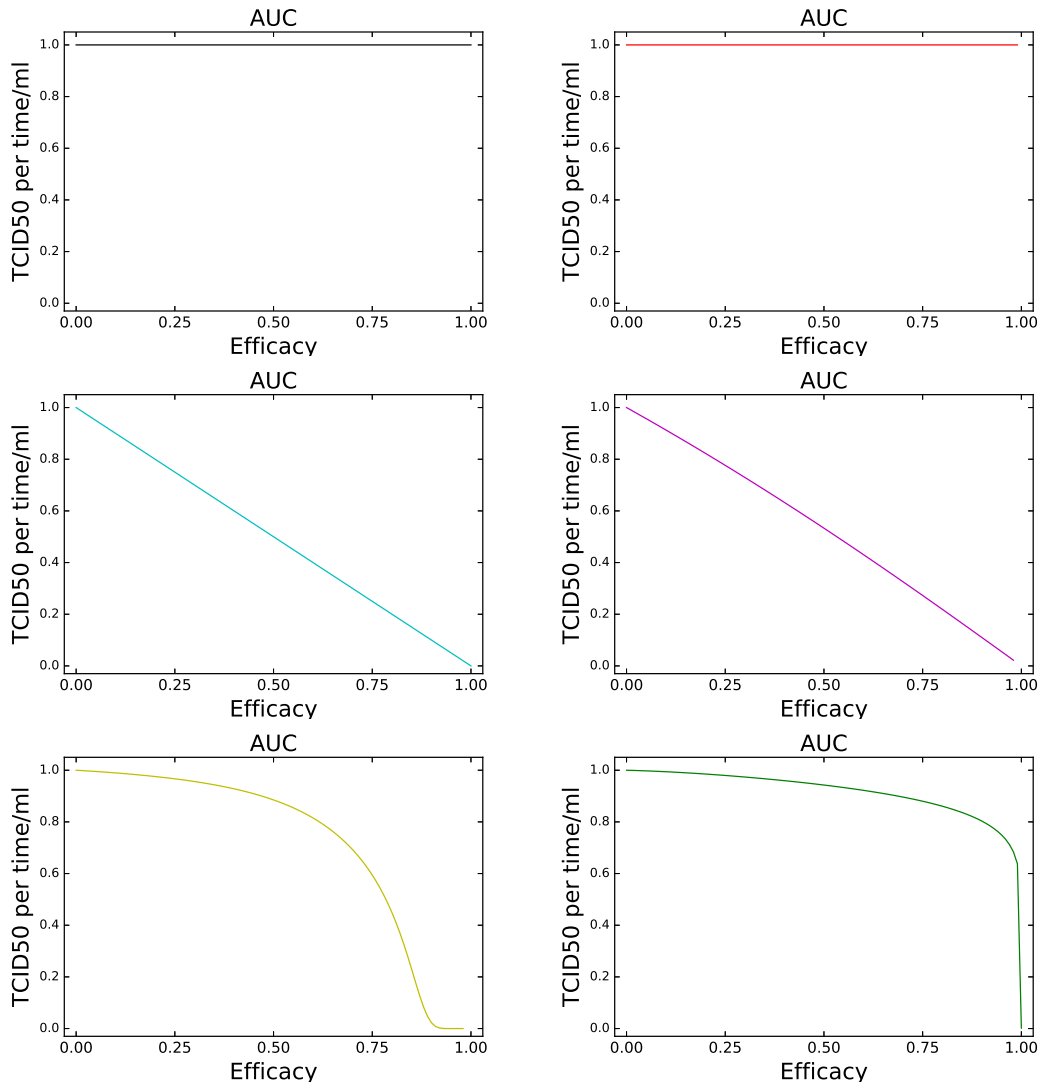


Figure 3.8: AUC efficacy curves for drugs with different mechanisms of action for influenza infection for SCA. Black curve is for *Slowing infection*, red is for *Protecting target cells*, cyan is for *Reducing the production of new virions*, purple is for *Increasing the rate of loss of virion infectivity*, yellow is for *Increasing the length of the eclipse phase* and green is for *Decreasing the lifespan of infectious cells*.

We note that some mechanisms of action had no effect on the time course of one of the assays. These are summed up in Table 3.1. For influenza during the MCA, a drug

inhibiting cell entry ( *Slowing infection*) didn't have any effect on either viral or dead cell time courses. For influenza during the SCA, both variations of the cell entry drug ( *Slowing infection* and *Protecting target cells*) had no impact on the time courses of either virus or dead cells.

Table 3.1: Mechanisms where the viral and dead course are not affected by the efficacy.

| | |
|---|---|
| Viral time course | |
| MCA | *Slowing infection* |
| SCA | *Slowing infection*, *Protecting target cells* |
| Dead cell time course | |
| MCA | *Slowing infection*, *Increasing the rate of loss of virion infectivity* *Reducing the production of new virions* |
| SCA | *Slowing infection*, *Protecting target cells* *Increasing the rate of loss of virion infectivity* *Reducing the production of new virions* |

We can identify most of the mechanisms of action through the measurements shown here. This is summarized in Table 3.2 where each column describes the types of efficacy curves expected for each mechanism of action. Note that there is a uniques combination of curves for all mechanisms except drugs reducing production (*Reducing the production of new virions*) and drugs increasing clearance (*Increasing the rate of loss of virion infectivity*). For example, we can determine mechanisms of action by measuring a particular property:

- *Slowing infection*: From the MCA if the viral peak is constant.
- *Protecting target cells*: From the MCA if AUC decreases linearly as the efficacy increases.

- *Increasing the length of the eclipse phase*: From the MCA if AUC curve has a sigmoidal shape.

- *Decreasing the lifespan of infectious cells*: From the MCA and SCA if the viral peak decreases as function of the efficacy, in a concave curve.

- *Reducing the production of new virions* and *Increasing the rate of loss of virion infectivity* have the same efficacy curves for most of the measurements, except for AUC in SCA where both decrease linearly but *Increasing the rate of loss of virion infectivity* seems to have a bigger rate of change.

Table 3.2: Results of efficacy curves for MCA and SCA.

| Measurement | *Slowing infection* | *Protecting target cells* | *Reducing the production* |
|---|---|---|---|
| MCA Viral peak | Constant | Linear | Linear |
| SCA Viral peak | Constant | Constant | Linear |
| MCA Max dead cells | Constant | Linear | Constant |
| SCA Max dead cells | Constant | Constant | Linear |
| MCA AUC | Constant | Linear | Constant |
| SCA AUC | Constant | Constant | Constant |
| Measurement | *Increasing the loss of virion infectivity* | *Increasing the eclipse phase* | *Decreasing the lifespan of infectious cells* |
| MCA Viral peak | Linear | Concave | Concave |
| SCA Viral peak | Linear | Concave | Concave |
| MCA Max dead cells | Constant | Sigmoid | Constant |
| SCA Max dead cells | Linear | Concave | Concave |
| MCA AUC | Constant | Constant | Constant |
| SCA AUC | Constant | Concave | Constant |

While our results suggest that at least some mechanisms could be identified using the SCA or MCA, in order for these results to be used experimentally, we need to ensure that the shape of the curves does not depend on the chosen underlying model parameters.

## 3.3 Parameter dependence of efficacy curves

To test the dependence of efficacy curves on model parameters, we use Latin Hypercube Sampling (LHS) as described in Methods. We use the LHS method to produce 300 efficacy

curves each based on slightly different model parameters. This allows us to evaluate how much the curves change when underlying parameters change. Results of our LHS simulations are shown in Fig. 3.9 and Fig. 3.10 for peak viral titer, Fig. 3.11 and Fig. 3.12 for maximum number of dead cells. and Fig. 3.13 and Fig. 3.14 for AUC. For viral peak, we observe that the change in parameters is irrelevant for the mechanisms: *Slowing infection*, *Protecting target cells*, *Reducing the production of new virions*, and *Increasing the rate of loss of virion infectivity.* For *Increasing the length of the eclipse phase* and *Decreasing the lifespan of infectious cells*, we observe a dependence on parameter selection, which for *Decreasing the lifespan of infectious cells* is more obvious for higher efficacies. For the maximum number of dead cells, the effect is the same, just *Increasing the length of the eclipse phase* and *Decreasing the lifespan of infectious cells* appear to be dependent on infection parameters, but on this occasion the distribution of the results appears to be bigger. For AUC curves, again the mechanisms *Increasing the length of the eclipse phase* and *Decreasing the lifespan of infectious cells* are dependent on the selected parameters, but we also see a parameter dependence in the mechanism *Increasing the rate of loss of virion infectivity* for SCA.

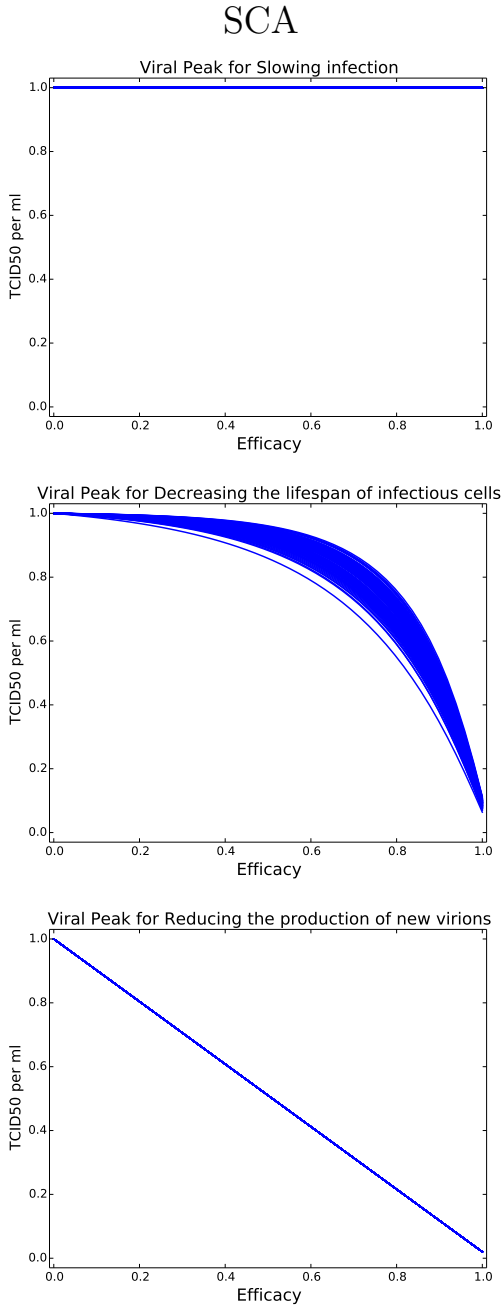


Figure 3.9: LHS for viral peak efficacy curves for influenza infection with different antiviral mechanisms of action. Here we observe the three different results (constant viral peak, linearly decreasing viral peak and sigmide curve). All the results can be found in Fig. 5.2. Plots are for SCA: the effect of a drug on *Slowing infection*: drug on *Decreasing the lifespan of infectious cells*; and drug on *Reducing the production of new virions*.

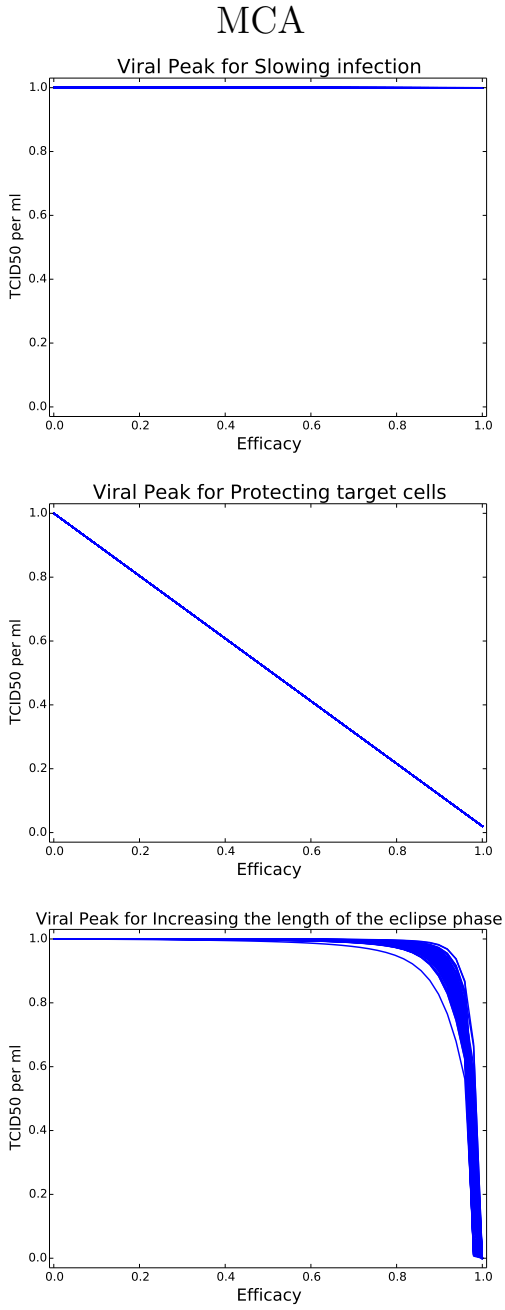


Figure 3.10: LHS for viral peak efficacy curves for influenza infection with different antiviral mechanisms of action. Here we observe the three different results (constant viral peak, linearly decreasing viral peak and sigmoide curve). All the results can be found in Fig. 5.2. Plots are for MCA: the effect of a drug on *Slowing infection*; drug on *Protecting target cells*; drug on *Increasing the length of the eclipse phase*.

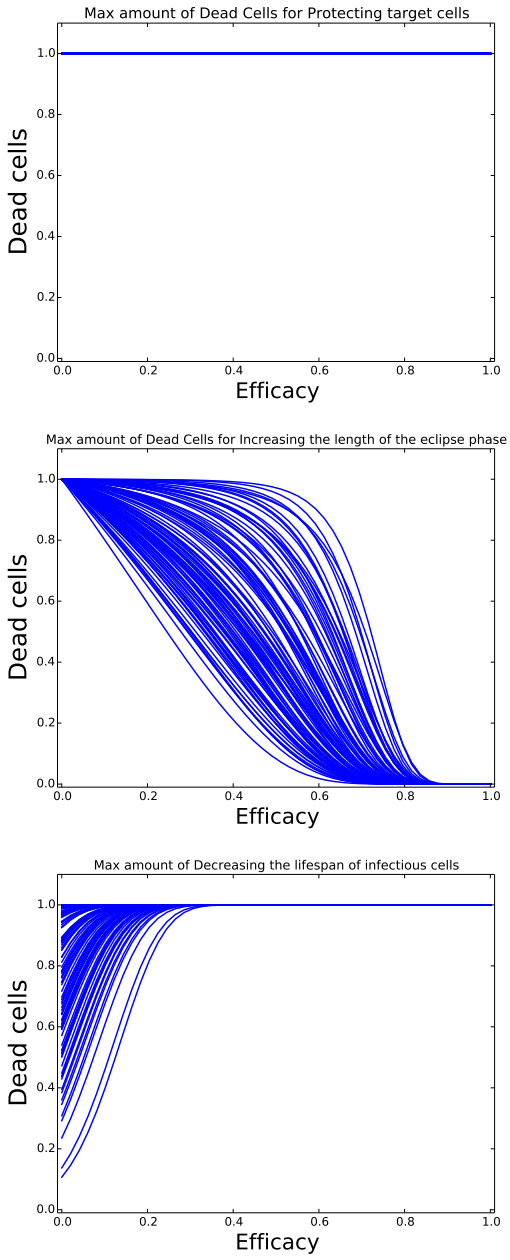


Figure 3.11: Maximum dead cells amount for drugs with different mechanisms used against of Influenza infection, the infection was modeled using parameters from the LHS. Here we observe the three different results (constant viral peak, linearly decreasing viral peak and sigmoide curve). All the results can be found in Fig. 5.4. Plots are for SCA. Figure shows the effect of a drug on *Protecting target cells*; drug *Increasing the length of the eclipse phase* and drug *Decreasing the lifespan of infectious cells.*

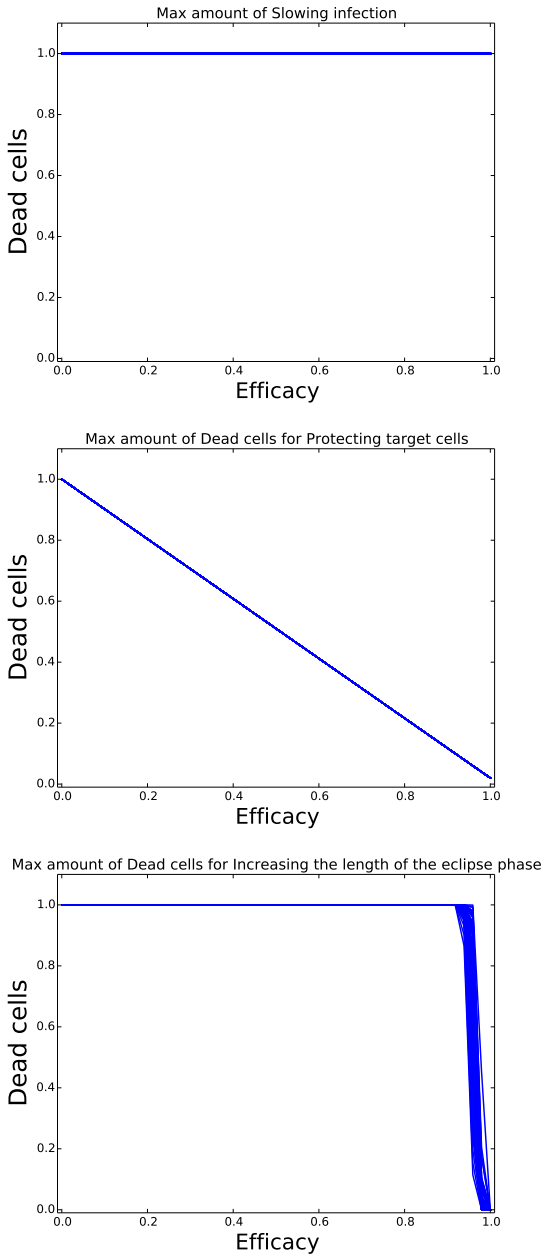


Figure 3.12: Maximum dead cells amount for drugs with different mechanisms used against of Influenza infection, the infection was modeled using parameters from the LHS. Here we observe the three different results (constant viral peak, linearly decreasing viral peak and sigmoide curve). All the results can be found in Fig. 5.4. Plots are for MCA. Figure shows the effect of a drug on *Slowing infection*; drug *Protecting target cells*; and drug *Increasing the length of the eclipse phase*.

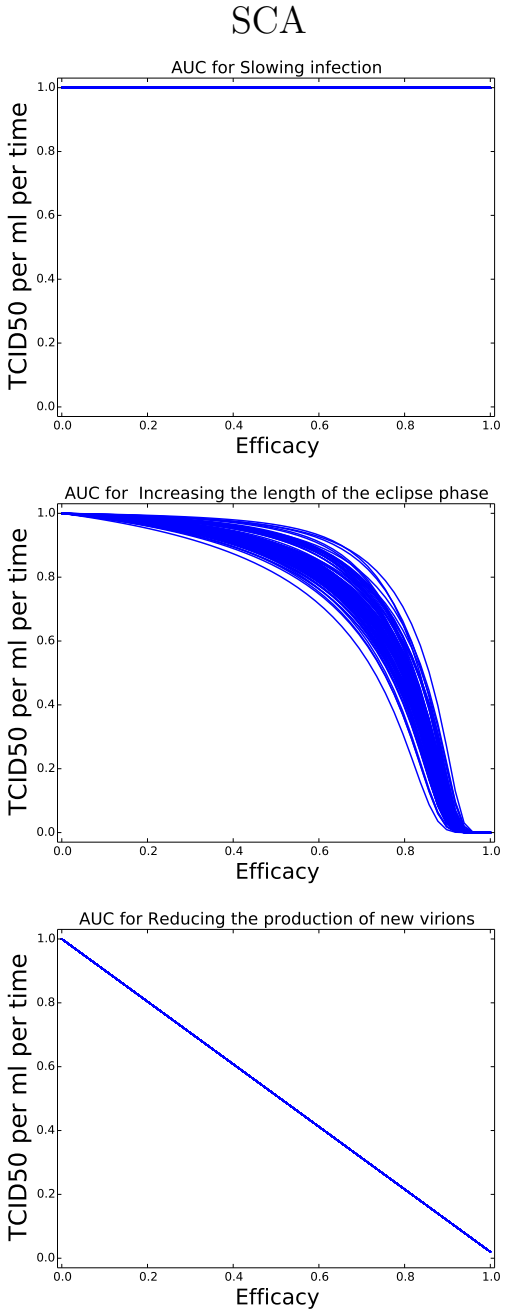


Figure 3.13: AUC for drugs with different mechanisms used against of Influenza infection, the infection was modeled using parameters from the LHS. Here we observe the three different results (constant viral peak, linearly decreasing viral peak and sigmoide curve). All the results can be found in Fig. 5.6 Plots are for SCA. Figure shows the effect of a drug on *Slowing infection*; drug *Increasing the length of the eclipse phase*; and drug *Reducing the production of new virions.*

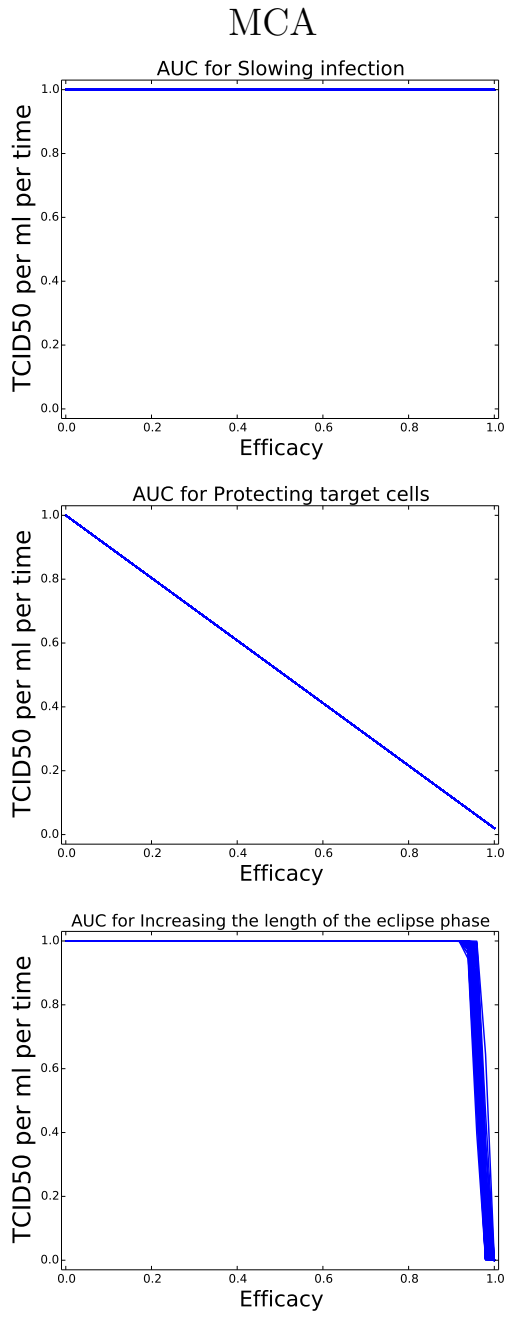


Figure 3.14: AUC for drugs with different mechanisms used against of Influenza infection, the infection was modeled using parameters from the LHS. Here we observe the three different results (constant viral peak, linearly decreasing viral peak and sigmoide curve). All the results can be found in Fig. 5.6 Plots are for MCA. Figure shows the effect of a drug on *Slowing infection*; drug *Protecting target cells*; drug *Increasing the length of the eclipse phase*.

## 3.4 Dose response curves

Most experiments do not measure complete time courses or plot the effect as a function of efficacy. Instead, they use dose response curves, which show the effect of various doses of a chemical. On the x-axis, we plot the drug dose, usually on a logarithmic scale and on the y-axis, we plot some outcome (typically obtained from in vitro experiments). Note that we can convert efficacy curves to dose-response curves using Eq. (3.1), as long as we know $\varepsilon_{\max}$ and $\mathrm{IC}_{50}$. These curves help us to understand the response to different levels of exposure to a drug after diverse exposure time. In Fig. 3.15 and Fig. 3.16, we show the viral peak for different mechanisms of action as function of the dose. But we can observe that the shape of the curves is very similar, so the mechanism of action is difficult to identify through these curves, since viral titer measurements tend to have large error (45).

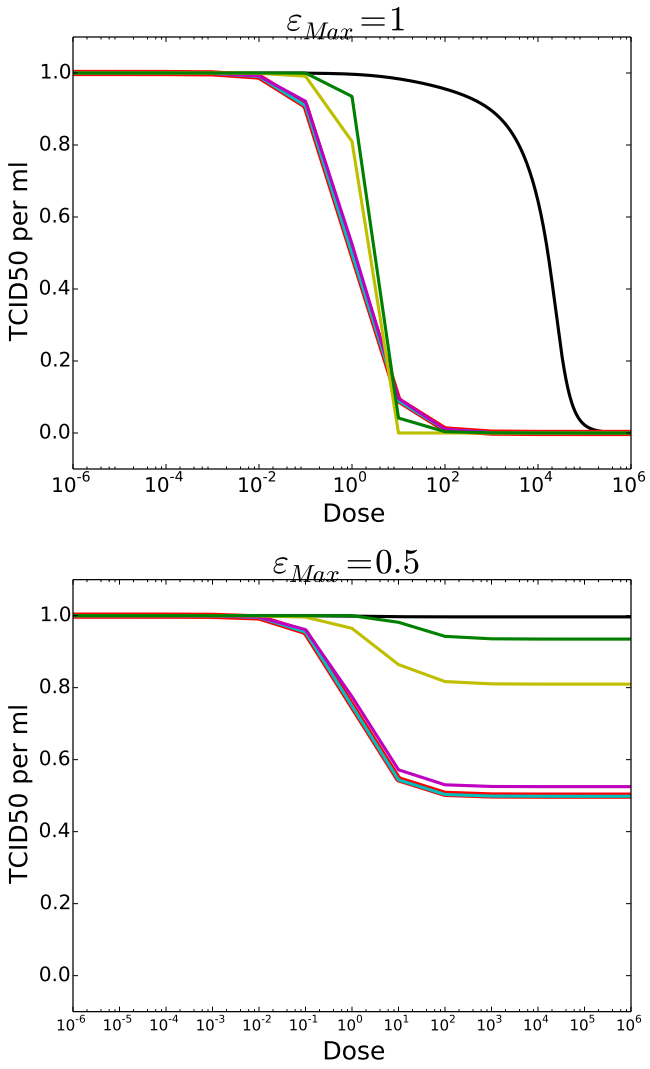


Figure 3.15: Dose response for drugs with different mechanisms of action for influenza infection. Figures are for MCA . The top row assumes an $\varepsilon_{\max}$ of 1, while the bottom row assumes an $\varepsilon_{\max}$ of 0.5. Black curve is for *Slowing infection*, red is for *Protecting target cells*, cyan is for *Reducing the production of new virions*, purple is for *Increasing the rate of loss of virion infectivity*, yellow is for *Increasing the length of the eclipse phase* and green is for *Decreasing the lifespan of infectious cells*.

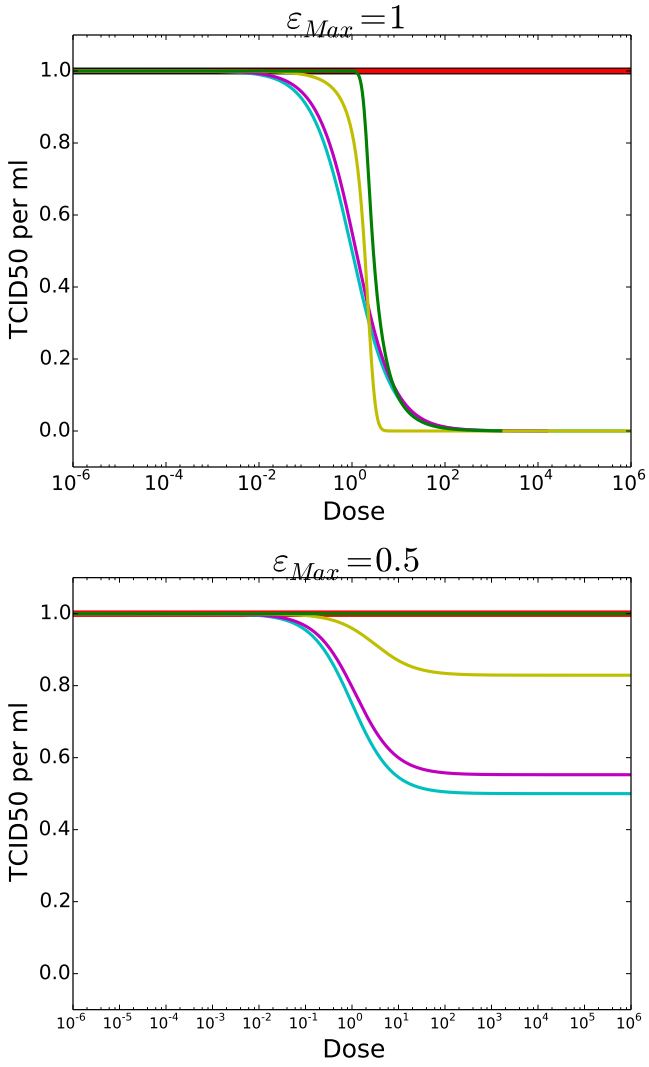


Figure 3.16: Dose response for drugs with different mechanisms of action for influenza infection. Figures are for SCA. The top row assumes an $\varepsilon_{\max}$ of 1, while the bottom row assumes an $\varepsilon_{\max}$ of 0.5. Black curve is for *Slowing infection*, red is for *Protecting target cells*, cyan is for *Reducing the production of new virions*, purple is for *Increasing the rate of loss of virion infectivity*, yellow is for *Increasing the length of the eclipse phase* and green is for *Decreasing the lifespan of infectious cells*.

## 3.5 Comparing to experimental data

We test our findings by examining experimental data for treated influenza infections.

The data used were obtained from MCA for influenza treated with amantadine (46), and for MCA for influenza treated with oseltamivir (unpublished data). The data is the only available data for viral courses of cells infected with influenza A treated with amantadine and oseltamivir.

In this experiment, they used a hollow fiber (HF) system, which possesses a cartridge where target cells are deposited. Pre-infected cells are added to initiate the viral infection. To measure the dose effect, the authors of (46) performed several experiments with different doses of oseltamivir or amantadine. Recall that amantadine is an antiviral that blocks uncoating of the virus once it has entered the cell (47) while oseltamivir is a neuraminidase inhibitor that blocks release of the virus (48, 49). The dose was held constant throughout each experiment. The viral titer of each HF experiment was measured at different times, 6 measurements for amantadine and 5 for oseltamivir.

We use the function described by Eq. (2.19) to perform the data fitting for different doses of amantadine and oseltamivir, Fig. 3.17 and Fig. 3.18. This function gives us measurements of different features of the viral time course as a function of drug concentration. This allows us to generate different efficacy curves for the drugs oseltamivir and amantadine.

We calculate "Reduced chi square" for each fitting. The chi square are shown in Table 3.3 and Table 3.4. The "reduced chi square" indicates that the fit has not fully captured the properties of the data, especially for oseltamivir doses of $10ng/ml$ where

$\tilde{\chi}^2 = 8.16530$, $1ng/ml$ where $\tilde{\chi}^2 = 17.93972$ , and $0ng/ml$ where $\tilde{\chi}^2 = 5.008397$. Using Eq. 3.1 we can convert our doses in efficacies, in term of eficacies this means that the data available for $\varepsilon = 0.90$, $\varepsilon = 0.50$ and $\varepsilon = 0.0$ are not precise. For amantadine in dose $53.3uM$ we have $\tilde{\chi}^2 = 3.86118$ , this affects the data for efficacy $\varepsilon = 0.98$, but as our efficacy curves are for values between 0 and 1 we can say that most of the data to compare are correct. As it is mentioned in the Section 2.6, the equation Eq. 2.19 has been used before (42) to fit data, and reflects the main characteristics of the viral course. So it might be due to the experimental error or the reduced chi square is not the appropriate method to evaluate the fitting for non linear models (50). When compared to our simulations for different possible mechanisms of action (Fig. 3.19, Fig. 3.20 and Fig. 3.21), the efficacy curves for viral peak, AUC, growth and decay rates correspond to the mechanism modeled as *Slowing infection* for oseltamivir and *Increasing the length of the eclipse phase* for amantadine, both of which disagree with previous results.

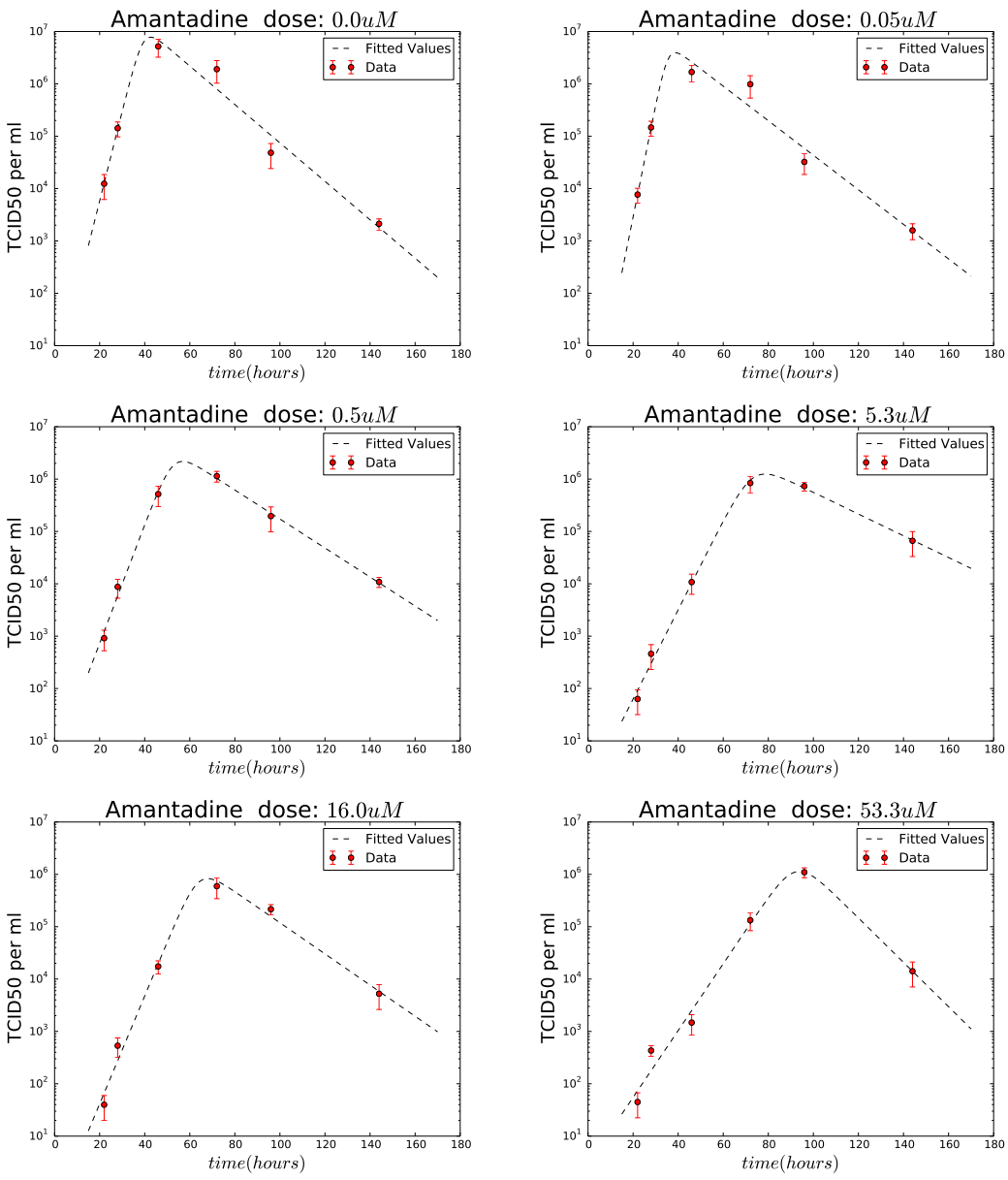


Figure 3.17: Fitting for different doses of amantadine

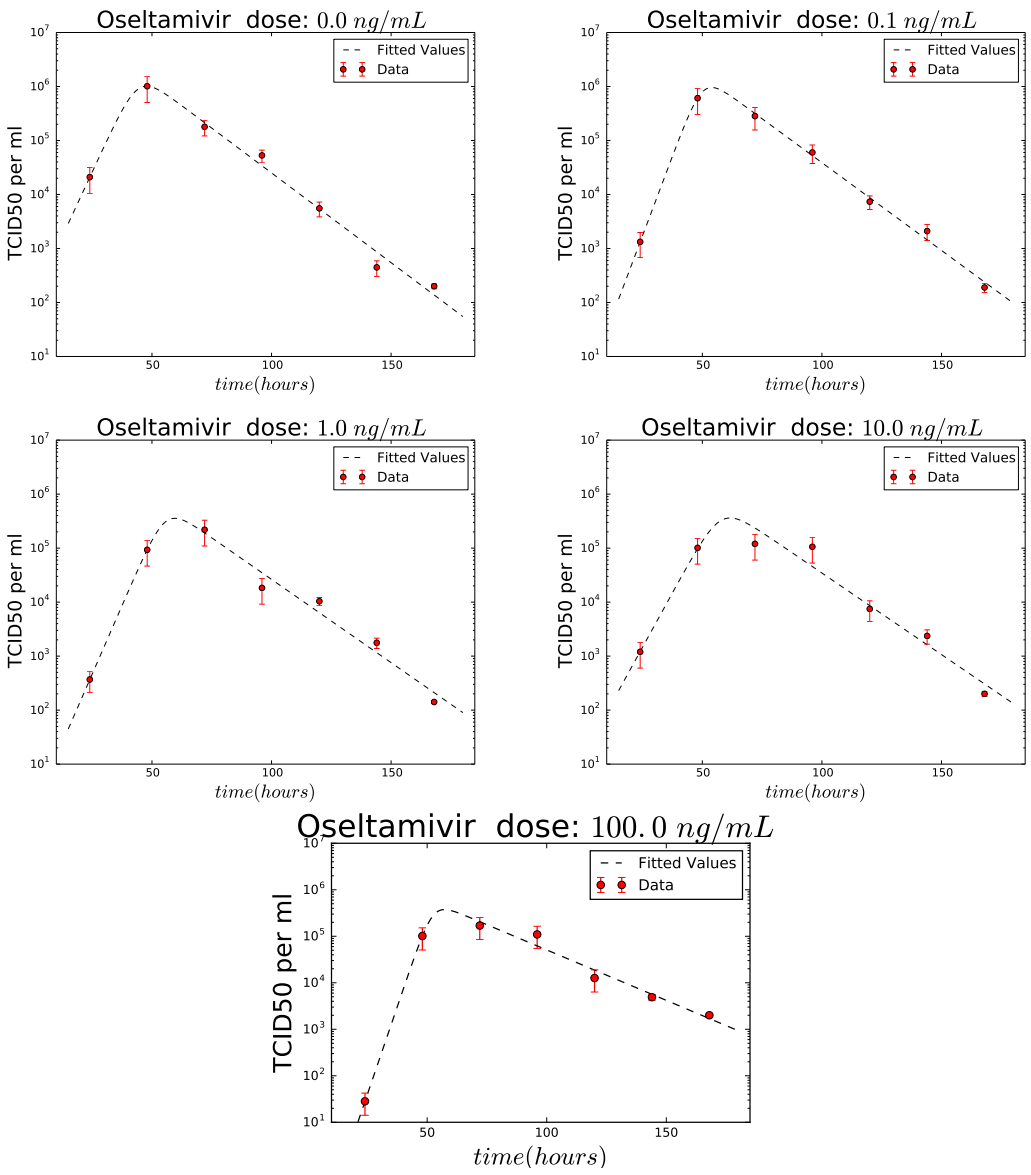


Figure 3.18: Fitting for different doses of oseltamivir

In (46), both *Slowing infection* and *Protecting target cells* models were tested for amantadine because amantadine prevents uncoating meaning that cells are not truly infected, suggesting a reduced infection rate. Our work suggests that a better interpretation would be that the uncoating increases the eclipse phase because the cells are infected

but viral replication is delayed, so the cells are infected as usual but become infectious after a longer period of time.

| | $\tilde{\chi}^2$ |
|---|---|
| $53.3uM$ | $\tilde{\chi}^2 = 3.86118$ |
| $16.0uM$ | $\tilde{\chi}^2 = 2.06818$ |
| $5.3uM$ | $\tilde{\chi}^2 = 0.69293$ |
| $0.5uM$ | $\tilde{\chi}^2 = 0.72771$ |
| $0.05uM$ | $\tilde{\chi}^2 = 2.48006$ |
| $0uM$ | $\tilde{\chi}^2 = 2.51936$ |

Table 3.3: $\tilde{\chi}^2$ for fitting of diferent Amantadine dose

| | $\tilde{\chi}^2$ |
|---|---|
| $100ng/ml$ | $\tilde{\chi}^2 = 1.30031$ |
| $10ng/ml$ | $\tilde{\chi}^2 = 8.16530$ |
| $1ng/ml$ | $\tilde{\chi}^2 = 17.93972$ |
| $0.1ng/ml$ | $\tilde{\chi}^2 = 0.81807$ |
| $0.0ng/ml$ | $\tilde{\chi}^2 = 5.008397$ |

Table 3.4: $\tilde{\chi}^2$ for fitting of diferent Oseltamivir dose

Oseltamivir was previously modeled as blocking production *Reducing the production of new virions* in (40, 51, 52), but our results suggest that a more accurate model is that it slows the infection by reducing the infection rate. This could be because our

model just considers cell free transmission. Virus can infect a host trough cell free transmission, virions moving freely in extracellular fluids until they enter in a host cell, or directly between infected and uninfected cells. Cell to cell transmission is faster and more efficient than cell free transmission, because it obviates early steps in the viral life cycle (53), for example attachment. Oseltamivir obstructs the mobility of new virions, hence affects cell free transmission only leaving cell to cell transmission as the primary mode of infection. So the result of modeling it as decreasing the infection rate, *Slowing infection*, makes sense.

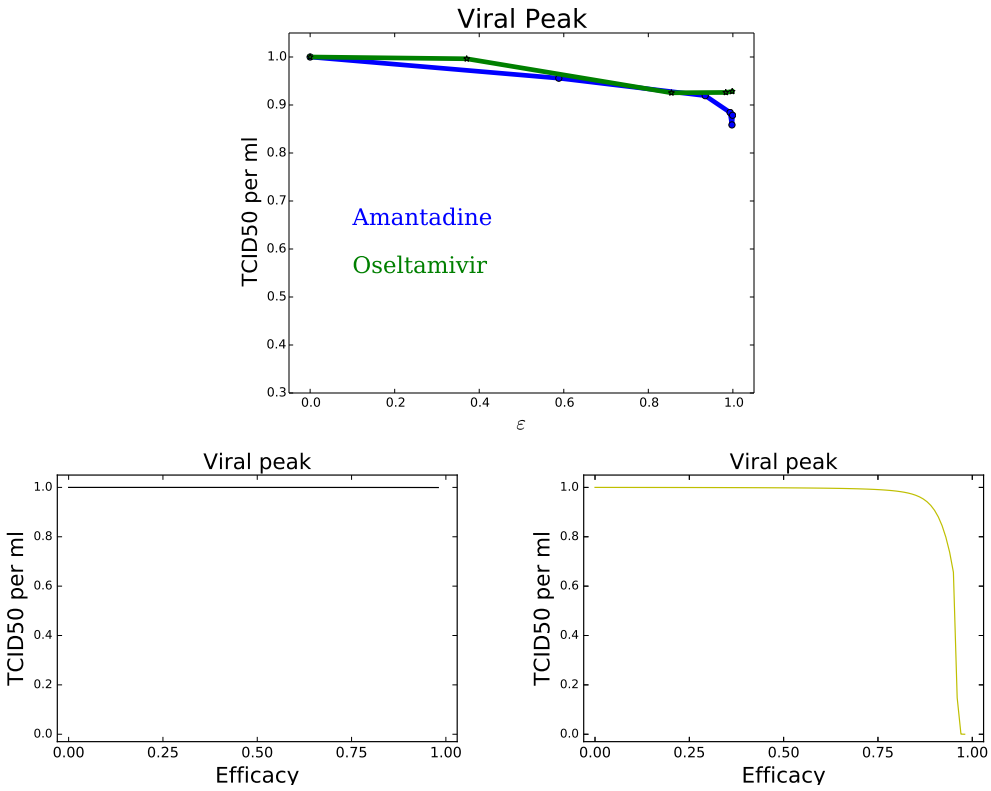


Figure 3.19: Efficacy curves for amantadine and oseltamivir, derived from experiment (top) and from our simulations (bottom) for Viral peak. In the right figures: black curve is for *Slowing infection*, red is for *Protecting target cells*, cyan is for *Reducing the production of new virions*, purple is for *Increasing the rate of loss of virion infectivity*, yellow is for *Increasing the length of the eclipse phase* and green is for *Decreasing the lifespan of infectious cells*.

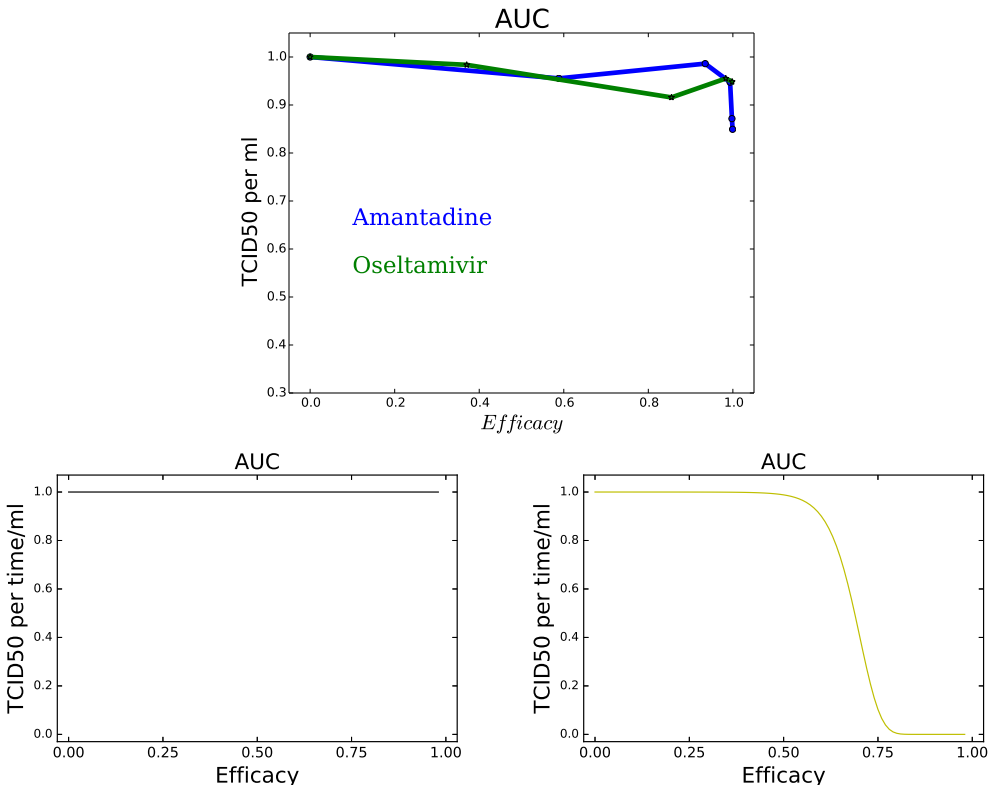


Figure 3.20: Efficacy curves for amantadine and oseltamivir, derived from experiment (top) and from our simulations (bottom) forAUC. In the right figures: black curve is for *Slowing infection*, red is for *Protecting target cells*, cyan is for *Reducing the production of new virions*, purple is for *Increasing the rate of loss of virion infectivity*, yellow is for *Increasing the length of the eclipse phase* and green is for *Decreasing the lifespan of infectious cells*.

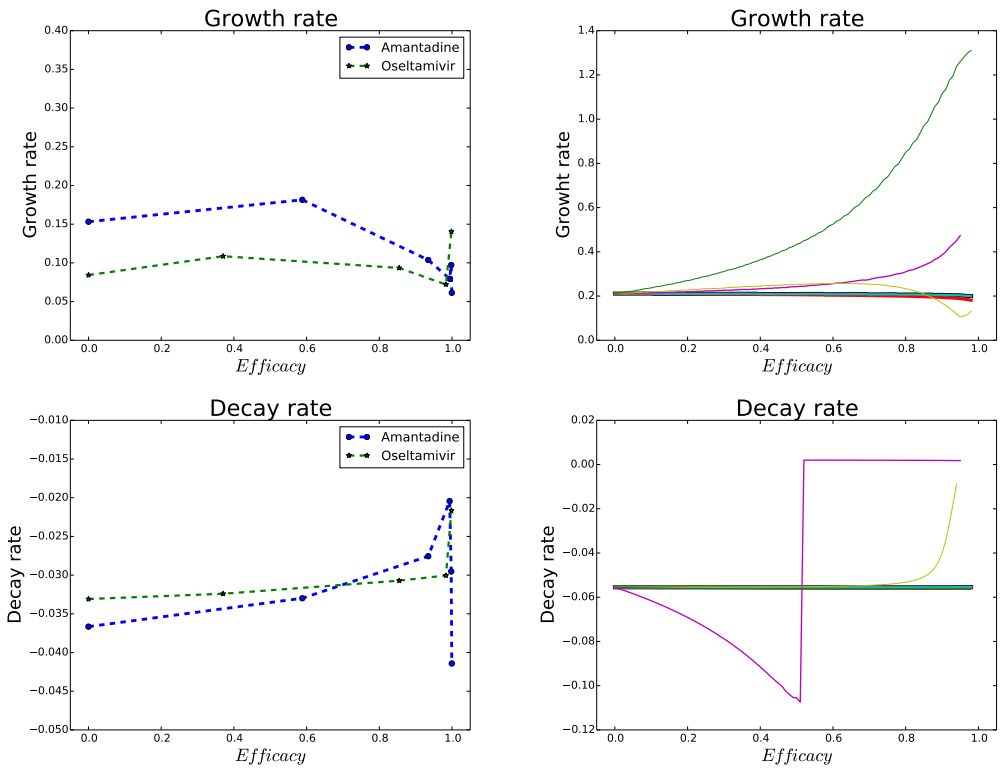


Figure 3.21: Efficacy curves for amantadine and oseltamivir, derived from experiment (left) and from our simulations (right) for viral growth and viral decay. In the right figures: black curve is for *Slowing infection*, red is for *Protecting target cells*, cyan is for *Reducing the production of new virions*, purple is for *Increasing the rate of loss of virion infectivity*, yellow is for *Increasing the length of the eclipse phase* and green is for *Decreasing the lifespan of infectious cells*.

# Chapter 4

# Discussion and Future Work

## 4.1 Questions Answered

**What experiments can help us determine the mechanism of action?**

We were able to identify possible measurements that could help identify, at least broadly, the mechanism of action of a new antiviral. For influenza, viral peak in the MCA can help us to identify the mechanism: *Protecting target cells*, which is a drug inhibiting cell entry. AUC in the MCA can identify *Protecting target cells*, inhibiting cell entry and *Increasing the length of the eclipse phase*, blocking release, while AUC in the SCA can identify *Reducing the production of new virions*, blocking production, *Increasing the rate of loss of virion infectivity*, increasing viral clearance, and *Decreasing the lifespan of infectious cells*, decreasing cell lifespan.

In the case of influenza, our study of experiments with amantadine and oseltamivir suggested alternative modeling mechanisms than those described in previous research (40, 46, 51, 52). We found that oseltamivir should be modeled as reducing infection

rate, *Protecting target cells*, as neuramidase inhibitor prevents cell-free transmission, but not cell-to-cell transmission, overall decreasing the infection rate. For amantadine the best modeling mechanism was found to be *Increasing the length of the eclipse phase*. Amantadine is an uncoating inhibitor, this means that virus enters the cells but viral production is not possible because the genetic material is not released to be copied, in this way the cells are infected but they do not become infectious, thus lengthening the eclipse phase.

**How do model parameters affect our predictions about the mechanism of action?**

Our sensitivity analysis suggested different parameter sensitivities depending on which model parameters were perturbed. For influenza, the most reliable results are for SCA, since they show the least amount of parameter sensitivity (see Table 4.1). Dependence on parameter values was seen most in *Increasing the length of the eclipse phase* and *Decreasing the lifespan of infectious cells*, with some small dependence on *Increasing the rate of loss of virion infectivity*.

**Can mechanism of action be determined from dose-response curves?**

No, the dose-response curves for different mechanisms have a very similar shape. This makes it difficult to identify different mechanisms.

| | Influenza |
|---|---|
| | **Vp:***Protecting target cells*,*Protecting target cells* *Increasing the rate of loss of virion infectivity* *Reducing the production of new virions* |
| MCA | **AUC:***Protecting target cells*,*Protecting target cells* *Increasing the rate of loss of virion infectivity* *Reducing the production of new virions* *Decreasing the lifespan of infectious cells* |
| | **Dc:** *Protecting target cells*,*Protecting target cells* *Increasing the rate of loss of virion infectivity* *Reducing the production of new virions* *Decreasing the lifespan of infectious cells* |
| | **Vp:***Protecting target cells*,*Protecting target cells*, *Increasing the rate of loss of virion infectivity* *Reducing the production of new virions* |
| SCA | **AUC:***Protecting target cells*,*Protecting target cells* *Reducing the production of new virions* |
| | **Dc:** *Protecting target cells*,*Protecting target cells* *Increasing the rate of loss of virion infectivity* *Reducing the production of new virions* |

Table 4.1: Mechanisms where results are independent of parameter selection in the LHS

## 4.2 Future Work

### 4.2.1 Towards an analytical solution

We would like to find an analytical expression which helps us predict the shapes of the curves. For example for the SCA, all cells are infected at the beginning, there are no target cells, and all the cells go through the viral cycle simultaneously. Since there are no target cells being infected, the equation for the first eclipse phase reduces to

$$\frac{\mathrm{d}E_1}{\mathrm{d}t} = -\frac{n_E}{\tau_E}E_1, \tag{4.1}$$

which is a simple exponential and has a solution of

$$E_1 = N \exp\left(-\frac{n_E t}{\tau_E}\right), \tag{4.2}$$

where $N$ is the total number of cells. To find the number of cells in any of the eclipse phase compartments we have to solve the series of differential equations. This gives a general solution

$$E_n = \frac{1}{(n-1)!}\left(\frac{n_E}{\tau_E}\right)^{n-1} N t^{n-1} \exp\left(-\frac{n_E t}{\tau_E}\right). \tag{4.3}$$

Now, using the general solution we can solve for the compartments in the infectious phase. Substituting the expression for the last compartment in the eclipse phase into the differential equation for the first compartment of the infectious phase,

$$\frac{\mathrm{d}I_1}{\mathrm{d}t} = \frac{1}{(n_E-1)!}\left(\frac{n_E}{\tau_E}\right)^{n_E} N t^{n_E-1} \exp\left(-\frac{n_E t}{\tau_E}\right) - \frac{n_I}{\tau_I} I_1. \tag{4.4}$$

In this way, we can solve for all the infectious compartments. Since the number of eclipse cells in the n$^{th}$ compartment is a series of exponentials and the transition from one infectious compartment to another is exponential, the number of cells in any infectious compartment will also be a series of exponentials. Notice that the number of infected cells in any of the eclipse or infectious compartments is independent of the amount of virus and independent of the infection rate.

To find the amount of virus as a function of time, we have to integrate the differential equation for $V$. Using the results for the infectious compartments and assuming that in the early times of the single cycle assay the viral clearance is negligible, the differential

equation for virus becomes

$$\frac{\mathrm{d}V}{\mathrm{d}t} = p \sum_{j=1}^{n_I} I_j. \tag{4.5}$$

Since the number of cells in any infectious compartment depends on time only, we can simply integrate this equation to get an expression for the virus as a function of time,

$$V = p \int_{s=0}^{s=t} \sum_{j=1}^{n_I} I_j(s) ds. \tag{4.6}$$

Here we find that the amount of virus is independent of the infection rate $\beta$, which explains why we do not observe any effect in the SCA for the mechanisms *Protecting target cells* and *Protecting target cells* in the viral course curves, viral peak and AUC. Using this expression we can determine the shape of the curves for viral courses, and all the measurements related with this.

For the number of dead cells we can integrate,

$$\frac{\mathrm{d}D}{\mathrm{d}t} = \frac{n_I}{\tau_I} I_{n_I}. \tag{4.7}$$

Again as $I_{n_I}$ is a function of time we can integrate to get,

$$D = \frac{n_I}{\tau_I} \int_{s=0}^{s=t} I_{n_I}(s) ds. \tag{4.8}$$

In this way, we can get the prediction for the shape of the dead cells course curves, and also for the maximum amount of dead cells.

Our next steps will be to derive expressions for experimentally measured quantities during the SCA so that we can understand the model parameter dependence of different efficacy curves. A similar analysis should also be feasible for the MCA by expanding the techniques of Smith et al. (54) who found approximations for the viral time course during an MCA using a simple exponential infection model.

We would also like to expand our analysis to examine combination therapy. Combination therapy is a type of therapy used against viral infections characterized by the use of more than one antiviral drug. Using the mathematical model and methods used to identify the drug mechanism, we can assess which combination of antiviral is more effective.

## 4.3 Conclusions

Using the gamma model we calculated the viral and dead cell time course curves for two types of assays, MCA and SCA, for influenza infections. From these, we measured drug efficacy curves for a variety of different drug mechanisms. These curves allow us to quantify the effect of different drugs on influenza infections. Using the efficacy curves, we were able to identify measurements that could identify the mechanism of action of novel antiviral drugs. We then used LHS to estimate the parameter dependence of the efficacy curves which helped us determine which measurements are most reliable. Finally, we compared our results to experimental data of influenza infections treated with amantadine and oseltamivir and found that the effects of these drugs are better simulated with mechanisms of action different than those previously used.

# Chapter 5

# Appendix: Parameter dependence of efficacy curves

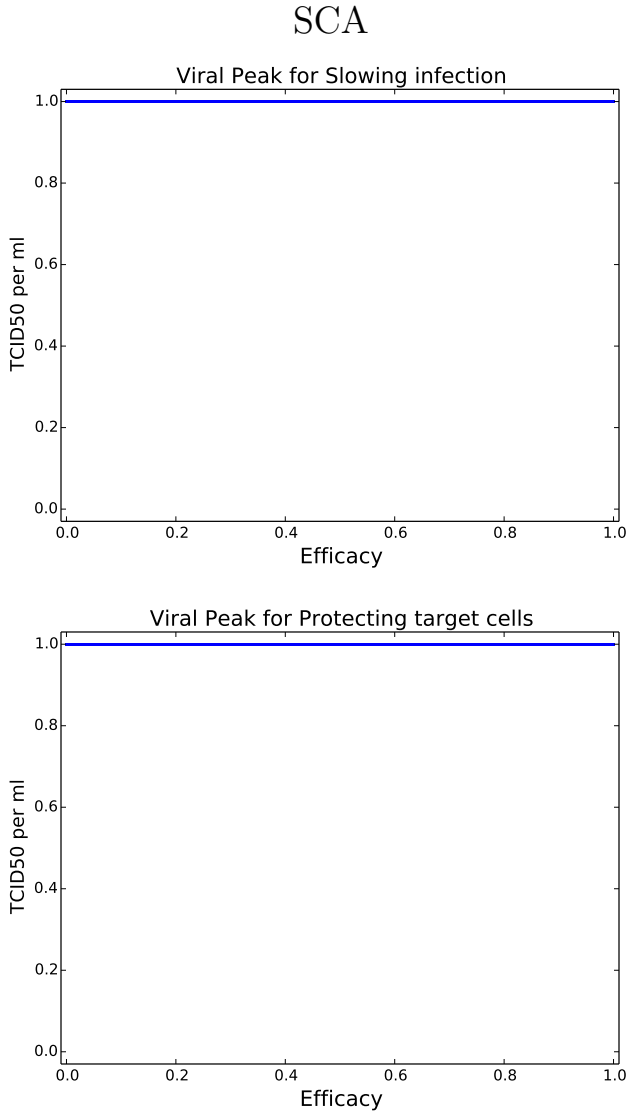


Figure 5.1: LHS for viral peak efficacy curves for influenza infection with different antiviral mechanisms of action. Plots for SCA, in the next order: The left figure shows the effect of a drug on *Slowing infection*; right figure shows drug on *Protecting target cells*.

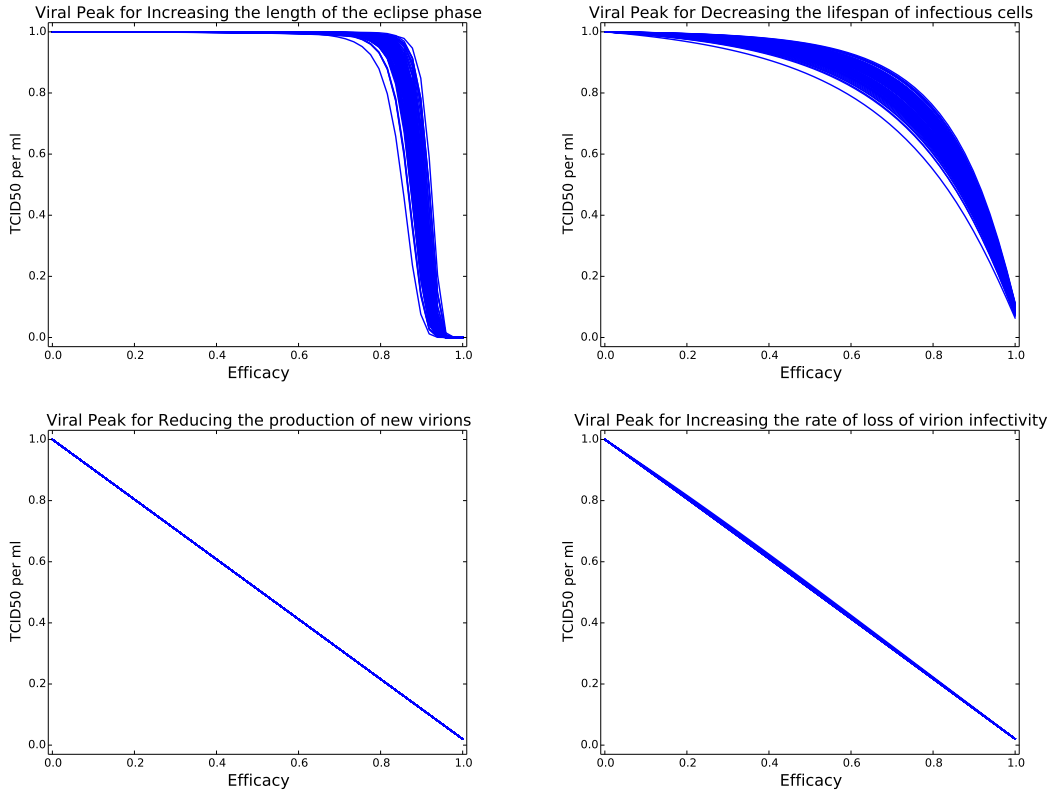


Figure 5.2: LHS for viral peak efficacy curves for influenza infection with different antiviral mechanisms of action. Plots for SCA, in the next order: The top right figure shows drug on *Increasing the length of the eclipse phase*; top left figure shows drug on *Decreasing the lifespan of infectious cells*; bottom left shows drug on *Reducing the production of new virions*; bottom right drug on *Increasing the rate of loss of virion infectivity.*

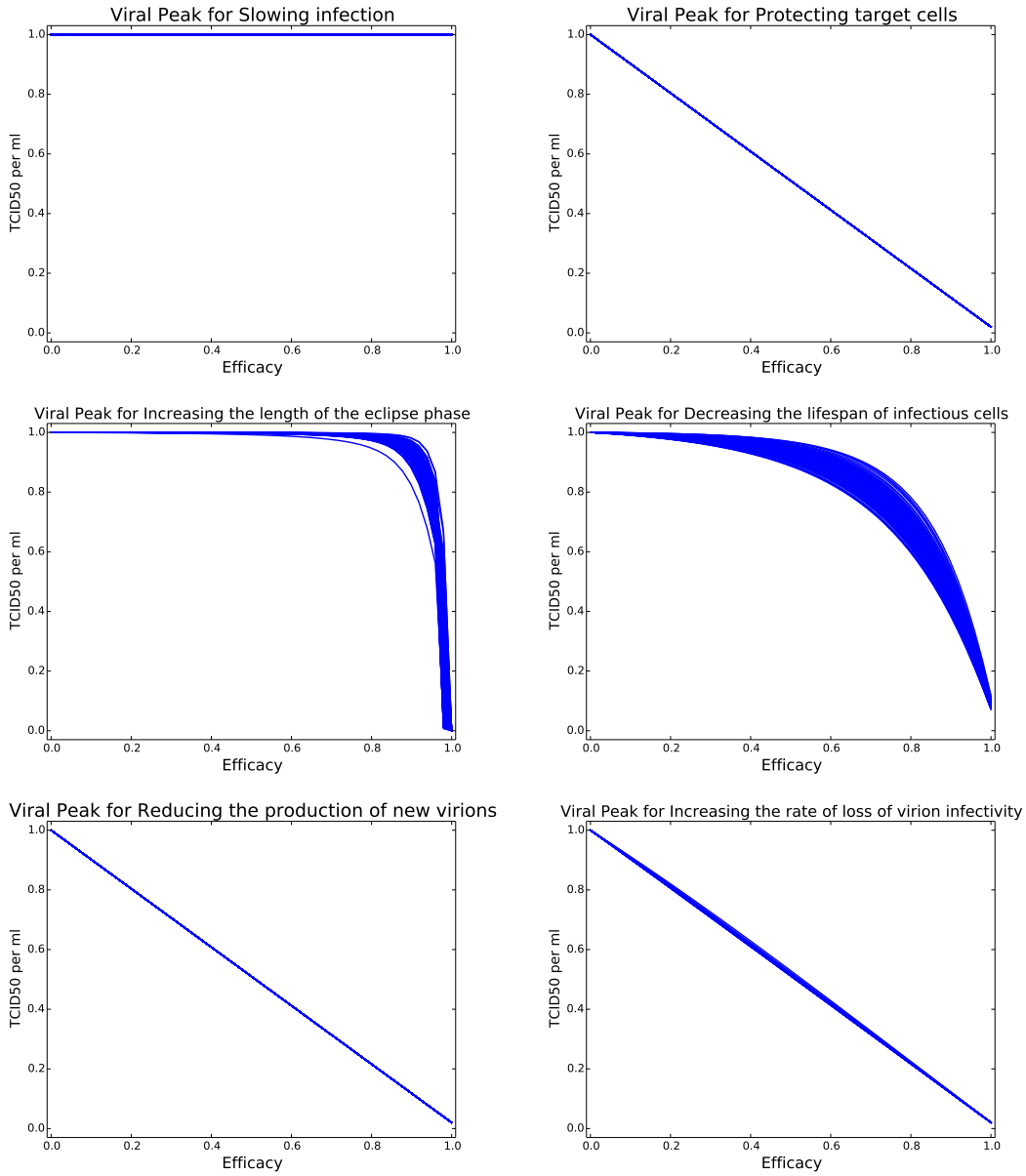


Figure 5.3: LHS for viral peak efficacy curves for influenza infection with different antiviral mechanisms of action. Plots are for MCA, in the next order: The top left figure shows the effect of a drug on *Slowing infection*; top right figure shows drug on *Protecting target cells*; middle left figure shows drug on *Increasing the length of the eclipse phase*;middle right figure shows drug on *Decreasing the lifespan of infectious cells*; bottom left shows drug on *Reducing the production of new virions*; bottom right drug on *Increasing the rate of loss of virion infectivity.*

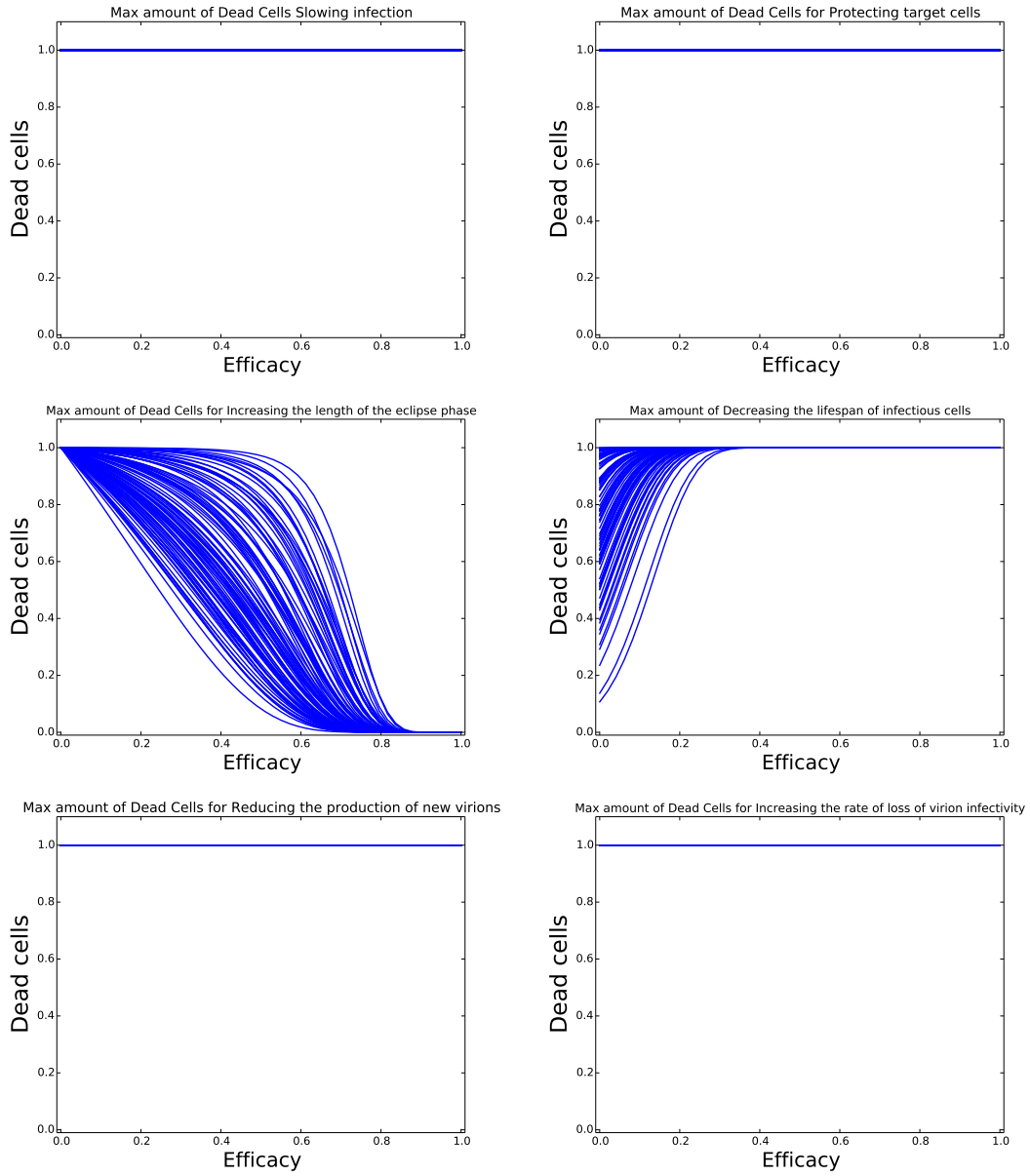


Figure 5.4: Maximum dead cells curves for influenza infection with different antiviral mechanisms of action. Plots are for MCA, in the next order: The top left figure shows the effect of a drug on *Slowing infection*; top right figure shows drug on *Protecting target cells*; middle left figure shows drug on *Increasing the length of the eclipse phase*;middle right figure shows drug on *Decreasing the lifespan of infectious cells*; bottom left shows drug on *Reducing the production of new virions*; bottom right drug on *Increasing the rate of loss of virion infectivityy.*

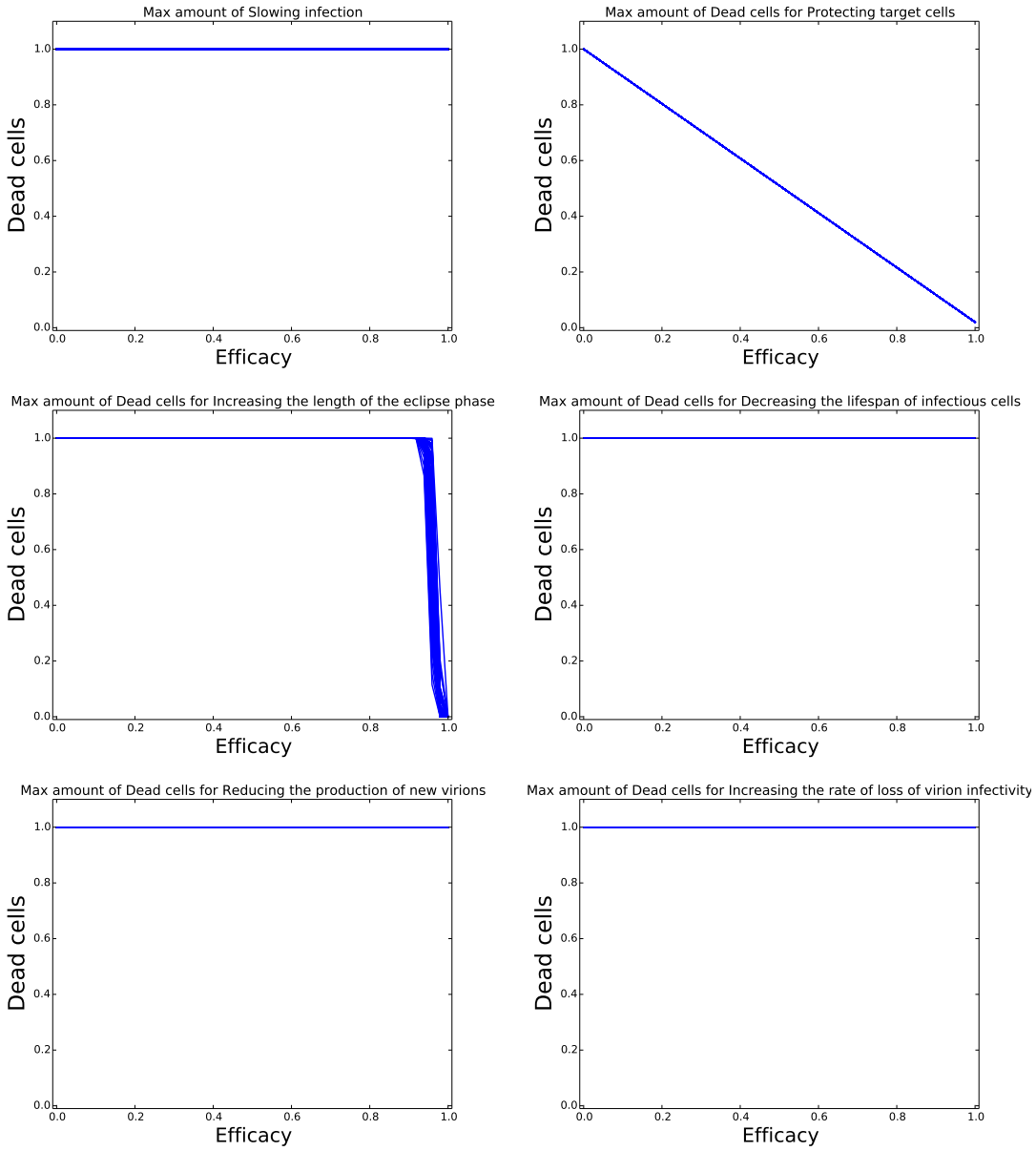


Figure 5.5: Maximum dead cells curves for influenza infection with different antiviral mechanisms of action. Plots are for MCA, in the next order: The top left figure shows the effect of a drug on *Slowing infection*; top right figure shows drug on *Protecting target cells*; middle left figure shows drug on *Increasing the length of the eclipse phase*;middle right figure shows drug on *Decreasing the lifespan of infectious cells*; bottom left shows drug on *Reducing the production of new virions*; bottom right drug on *Increasing the rate of loss of virion infectivity.*

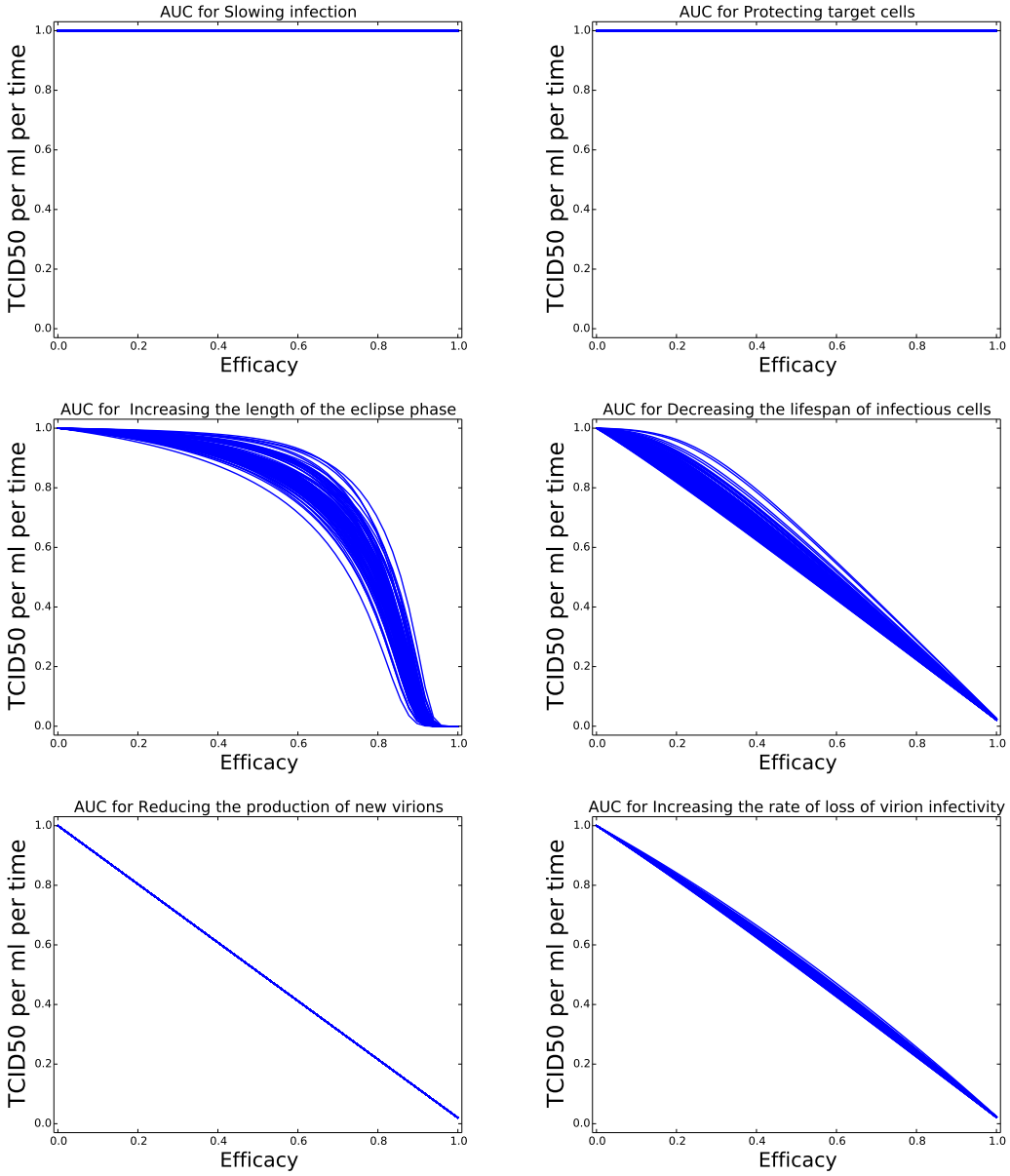


Figure 5.6: AUC curves for influenza infection with different antiviral mechanisms of action. Plots are for MCA, in the next order: The top left figure shows the effect of a drug on *Slowing infection*; top right figure shows drug on *Protecting target cells*; middle left figure shows drug on *Increasing the length of the eclipse phase*;middle right figure shows drug on *Decreasing the lifespan of infectious cells*; bottom left shows drug on *Reducing the production of new virions*; bottom right drug on *Increasing the rate of loss of virion infectivity.*

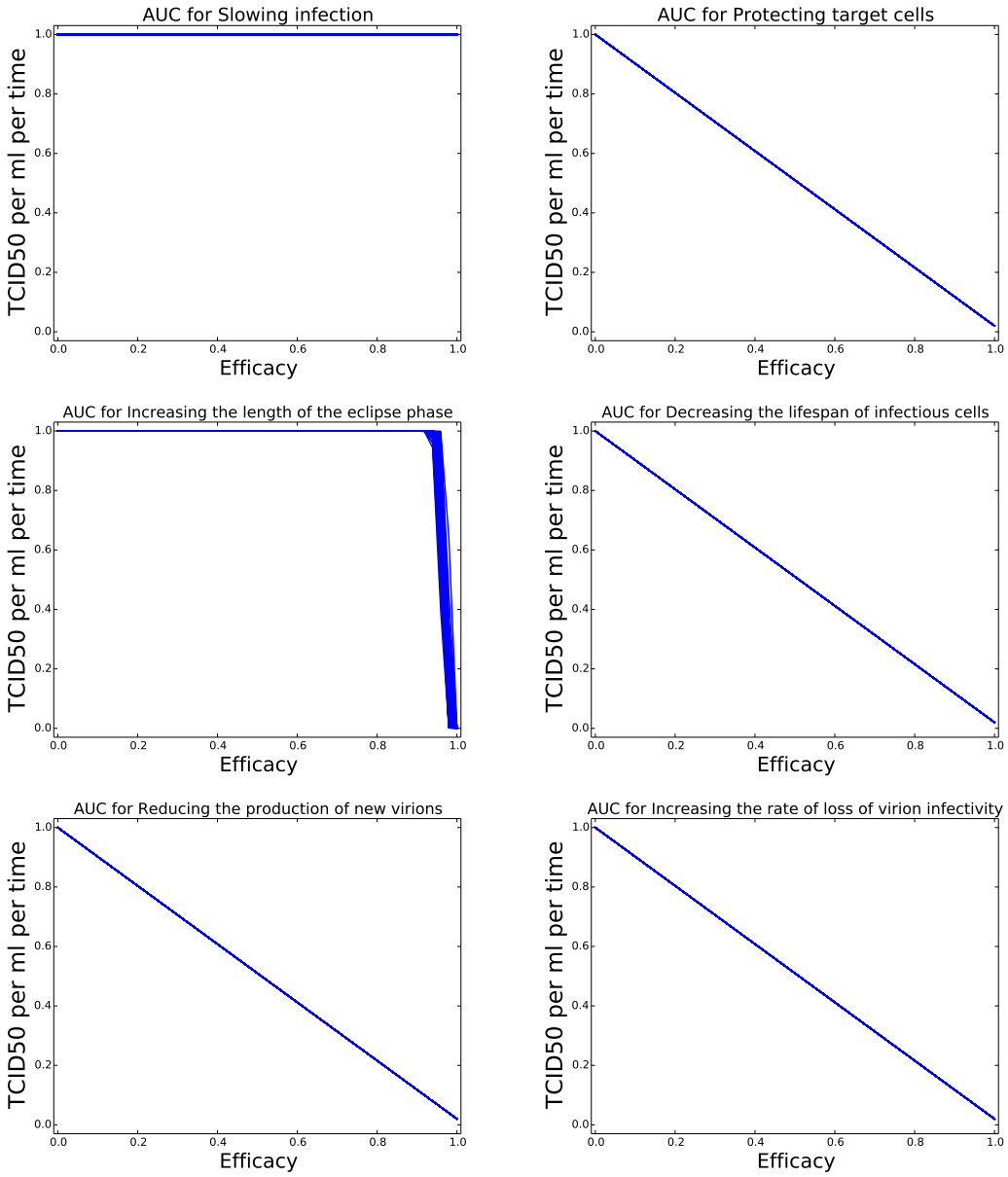


Figure 5.7: AUC curves for influenza infection with different antiviral mechanisms of action. Plots are for MCA, in the next order: T The top left figure shows the effect of a drug on *Slowing infection*; top right figure shows drug on *Protecting target cells*; middle left figure shows drug on *Increasing the length of the eclipse phase*;middle right figure shows drug on *Decreasing the lifespan of infectious cells*; bottom left shows drug on *Reducing the production of new virions*; bottom right drug on *Increasing the rate of loss of virion infectivity.*

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# ABSTRACT

## DETERMINING MECHANISM OF ACTION OF ANTIVIRALS FOR RESPIRATORY INFECTIONS

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Viral infections in the respiratory tract are common in humans and can cause serious illness and death. Drug treatment is the principal line of protection against many of these illnesses and many compounds are tested as antivirals. Often the efficacy of these antivirals are determined before a mechanism of action is understood. We use mathematical models to represent the evolution of influenza and establish which experiments can help determine the mechanism of action of antivirals. We find that curves describing the effect of a drug are dependent on the quantity being measured and that the predicted shape of the curves is dependent on model parameters.