Mitigating and exploiting stochasticity in the immune system

by

Peter Thill

Submitted to the Department of Chemistry in partial fulfillment of the requirements for the degree of Doctor of Philosophy at the

MASSACHUSETTS INSTITUTE OF TECHNOLOGY

June 2016

© Massachusetts Institute of Technology 2016. All rights reserved.

Signature redacted

Author .................................................................
Department of Chemistry
May 6, 2016

Signature redacted

Certified by ....................................................
Arup K. Chakraborty
Robert T. Haslam Professor of Chemical Engineering, Professor of Chemistry, Physics, Biological Engineering Thesis Supervisor

Signature redacted

Accepted by ...........................................
Robert W. Field
Haslam and Dewey Professor of Chemistry
This doctoral thesis has been examined by a Committee of the Department of Chemistry as follows:

Signature redacted

Professor Troy Van Voorhis... Chairman, Thesis Committee Haslam and Dewey Professor of Chemistry

Signature redacted

Professor Arup K. Chakraborty Thesis Supervisor Robert T. Haslam Professor of Chemical Engineering, Professor of Chemistry, Physics, Biological Engineering

Signature redacted

Professor Mehran Kardar Member, Thesis Committee Francis Friedman Professor of Physics
Mitigating and exploiting stochasticity in the immune system

by

Peter Thill

Submitted to the Department of Chemistry
on May 6, 2016, in partial fulfillment of the
requirements for the degree of
Doctor of Philosophy

Abstract

In the adaptive immune system of higher organisms, T cells are responsible for de-
tecting infections and mounting a response. It is of great importance that T cells
respond accurately to very small traces of pathogenic signal in a background sea of
healthy cells, to which mounting an immune response in the absence of viral infection
could prove fatal for the organism. T cells detect pathogenic signal through noisy
protein interaction networks. The goal of this work is to understand how the noise
intrinsic to signal transduction mechanisms is mitigated and in some cases exploited
to outperform corresponding deterministic mechanisms. Two broad areas of research
are presented in this work:

1). Due to fluctuating conformations of proteins, the rate constants of various chem-
ical reactions are not fixed but fluctuate stochastically throughout the course of a
signaling cascade. For modeling purposes, this implies that signal detection is based
on samples from a large, continuous-time Markov chain whose rate constants follow
their own stochastic process. We seek to understand how this behavior limits the
information that a network can transmit, and how these limitations can be mitigated
based on the specific network topology, or exploited in biological systems to limit au-
toimmunity. We develop algorithms to detect and characterize the distribution that
rate constants sample from.

2). The topology of very early stages in T cell signaling is critical for mounting an
accurate immune response. We explore a mechanism that contrasts with the con-
ventional signaling network topology, that outperforms the original by all metrics
considered and explains recent experimental results. We study the role that stochas-
ticity in the dwell time of a T cell at an APC plays in achieving a robust cellular
response, and explore models of sequential decision making in the immune system.

Thesis Supervisor: Arup K. Chakraborty
Title: Robert T. Haslam Professor of Chemical Engineering, Professor of Chemistry,
Physics, Biological Engineering
Acknowledgments

To Arup, for challenging me and teaching me to challenge myself and my ideas. To my committee members for intellectual guidance and support.

To the ‘signaling crew’, my office mates and close friends for the past 5 years, Maria and Hang. To all other members of the Chakraborty group who have pushed me intellectually and made this thesis better than it otherwise could be.

To Ming and Tom; our time in the group didn’t overlap by much, but I owe you both—your support and advice has been invaluable.

To Kevin for countless hours of interesting discussion, scientific and otherwise. To Dariusz for patience and support in all things computational and technical.

To my friends, David, Nihan and Tracy. Thanks for making the past five years in Boston an absolute blast.

To Mom, Dad, Matthew, David and Michael.

To my best friend and fiancé, Yoriko.
# Contents

1 Introduction 12

1.1 Background and motivation ........................................ 12
1.2 Multi-scale modeling in the immune system: A preliminary example ........................................ 14
1.3 Thesis outline .................................................. 16
  1.3.1 Dynamic disorder ........................................... 17
  1.3.2 Optimal decision making in immunological responses ........................................ 18

Chapter One References .................................................. 20

2 Dynamic disorder and the energetic costs of information transduction 21

2.1 Introduction .................................................. 21
2.2 Effects of limiting values of the switching rate ........................................ 22
2.3 Power consumption in Markov Chains ........................................ 24
2.4 Single enzyme, Two internal states ........................................ 26
2.5 Multiple Enzymes with three internal states ........................................ 28
  2.5.1 Information in a chemical network ........................................ 28
  2.5.2 Capacity Power Consumption ........................................ 29
2.6 Coherent Feedforward Type 1 network ........................................ 30
  2.6.1 Model and probability distributions ........................................ 30
  2.6.2 Channel capacity and rate variance ........................................ 32
  2.6.3 Power consumption and channel capacity ........................................ 32
2.7 Applications to Ras-SOS network ........................................ 35
2.8 Discussion .................................................. 38
2.9 Supplemental Information .................................................. 39
  2.9.1 The infinitely fast switching limit ........................................ 39
  2.9.2 System size expansion of CFF1 network ........................................ 40
  2.9.3 Marginal distribution of $P(Z)$ ........................................ 41
  2.9.4 Details of the Ras-SOS simulation ........................................ 42

Chapter Two References .................................................. 45
CONTENTS

3 Transitions in genetic toggle switches driven by dynamic disorder .............................................. 47
  3.1 Introduction .......................................................................................................................... 47
  3.2 Discrete space, discrete jumps .............................................................................................. 48
  3.3 Continuous Space Model ........................................................................................................ 49
    3.3.1 Discrete Jumps ................................................................................................................. 49
    3.3.2 Continuous Jumps ............................................................................................................ 52
  3.4 Discussion ............................................................................................................................ 54
  3.5 Supplemental Information ...................................................................................................... 55
    3.5.1 Time correlation functions for discrete and continuous models ..................................... 55
Chapter Three References .............................................................................................................. 58

4 Detecting latent enzyme states from time series data .................................................................... 60
  4.1 Introduction .......................................................................................................................... 60
  4.2 EM algorithm for protein interaction networks ....................................................................... 61
  4.3 Gibbs sampler for protein interaction networks ...................................................................... 67
  4.4 Continuous switch times ....................................................................................................... 68
  4.5 Chemical langevin limit: Kalman filter approach .................................................................... 75
  4.6 Supplemental Information ...................................................................................................... 82
    4.6.1 Kalman Filter .................................................................................................................... 82
    4.6.2 Posteriors for the continuous model .................................................................................. 85
Chapter Four References .............................................................................................................. 87

5 Phosphorylation of a Tyrosine residue on Zap70 by Lck and its subsequent binding via a SH2 domain may be a key gatekeeper of T cell receptor signaling in vivo ........................................................................... 88
  5.1 Introduction .......................................................................................................................... 88
  5.2 Motivation for the model ....................................................................................................... 89
  5.3 Proposed signaling model and methods ................................................................................. 90
  5.4 Results .................................................................................................................................. 92
    5.4.1 The new model recapitulates in vivo observations ............................................................. 92
    5.4.2 The new model is better at discriminating between self and agonist ligands .................... 93
    5.4.3 The new model is consistent with experiments showing that inhibiting Csk enhances the sensitivity of T cells to weak agonists, but not strong agonists ......................................................... 94
    5.4.4 The new model incurs lower metabolic costs for T cells .................................................. 94
CONTENTS

5.4.5 Further experimental tests of the new model .................................. 95
5.5 Discussion ................................................................. 95
5.6 Supplemental Information .................................................. 103
  5.6.1 Non-monotonicity in optimal parameters: T cell as a binary asymmetric channel .................................................. 103
  5.6.2 Stability of the signaling complex: mean first passage time for complex dissociation .............................................. 107
  5.6.3 A kinetic model for discrimination ...................................... 109
  5.6.4 Details of the stochastic simulation .................................... 111
Chapter Five References .......................................................... 121

6 Discrete and diffusion models for T cell decision making: Determining an optimal dwell time ........................................... 125
  6.1 Introduction ............................................................... 125
  6.2 Discrete time model ...................................................... 126
    6.2.1 Model details ....................................................... 126
    6.2.2 Cost function for a population of T cells ......................... 129
  6.3 Diffusion model for T cell decision makers .............................. 131
    6.3.1 Model description .................................................. 131
    6.3.2 Density of x after excursion n .................................... 139
    6.3.3 Comparison with CIR model ....................................... 144
  6.4 Discussion .................................................................. 148
Chapter Six References ............................................................. 149

7 T cell as an optimal learner ..................................................... 150
  7.1 Introduction ................................................................ 150
  7.2 Single APC Markov Decision Problem ................................... 151
  7.3 Multiple APC Markov Decision Problem ................................. 155
Chapter Seven References ........................................................ 161

8 Conclusion .................................................................. 162
Chapter 1

Introduction

1.1 Background and motivation

The human immune system has evolved to respond with precision to invasion by foreign pathogens. The primary cells that constitute the immune system recognize the presence of foreign pathogen through the binding of molecular signatures from the invading organism to receptors on the cell surface. As with many cases of signal transduction in biology, the stimulus results in a complicated protein signaling cascade which eventually leads the immune cells to mount a response and combat the invading organism.

T cells are adaptors of this immune response. When a host cell has been infected by an invading pathogen or virus, the host cell can internalize the foreign material and fragment it through a process known as phagocytosis or endocytosis. The resulting peptides are carried to the surface of the cell and bind to molecules called major histocompatibility complexes (MHC). A peptide associated with MHC is known as pMHC. These cells are called antigen presenting cells (APC). T cells scan across the surface of an APC and, using a T cell receptor (TCR) can recognize pMHC that is displaying peptide derived from a foreign, invading antigen.

How do T cells 'recognize' pMHC coming from a foreign, invading pathogen? The TCR can bind to a pMHC. As soon as the TCR-pMHC interaction is intact, a signaling cascade is initiated within the T cell. This means a series of protein modifications are made (known as a protein interaction network) ultimately leading to the T cell mounting an immune response, or mounting an attack against the cell that has been invaded. Importantly, though the binding of TCR to pMHC is stochastic, the dwell time is such that T cells will remain bound long enough to mount an immune response, with high probability. This story is complicated by the fact that APC can also display peptide derived from its own internal proteins, meaning that even if a cell
is not infected, it still displays pMHC on its surface. T cells that mount correct responses (in a high detection/low false alarm sense) must have a TCR which binds with high affinity (long dwell time) to pMHC derived from foreign antigen, and a TCR which binds with low affinity (very short dwell time) to pMHC derived from foreign antigen.

We have mentioned that as soon as TCR has bound to pMHC, a signaling cascade of protein modifications is set in motion. The exact sequence of events (e.g. protein A activates protein B which inhibits protein C etc.) are collectively known as a protein interaction network. Protein interaction networks are schematically represented by graphs in which proteins are drawn as nodes and interactions (stimulatory and inhibitory) are drawn as arcs connecting nodes. For instance, we may draw an arc from a kinase (one such example being Lck) to its substrate, ITAM, to indicate that Lck phosphorylates ITAM. Each arc in such a protein interaction network comes associated with a kinetic parameter known as a rate constant that determines how quickly these interactions occur. What does ‘quickly’ really mean?

If one appeals to a macroscopic picture, we can imagine the concentration of a certain molecule as some real valued number that continuously and smoothly evolves according to a set of ordinary differential equations. This is a valid description of the system in a macroscopic regime when copy number (the number of each protein) is present in abundant amounts. In a microscopic setting, one can use a detailed map of protein surfaces and model the actual quantum mechanical breaking/formation of chemical bonds due to protein collisions. In this framework, a rate constant is related to the probability that a collision between two molecules results in a chemical reaction.

For the work presented here, we will instead perform calculations in a ‘mesoscopic’ regime that is most applicable in the context of the immune system. Though we cannot assume that concentrations evolve deterministically according to mass action ODE’s, we also need not focus on the exact molecular details of the proteins in the networks we study. We instead use the well known framework of the master equation to map our protein interaction networks to very large, continuous time markov chains, in which the state space is a vector in \( \mathbb{N}^N \) (where we consider a system of \( N \) chemical species). Transitions are allowed between adjacent states according to allowed reactions.

As one very simple example, consider the chemical reaction \( A + B \rightarrow C \). A possible state in our markov chain can be \([10, 10, 0]\), indicating we have a system with 10 molecules of A, 10 molecules of B and 0 molecules of C. According to the reaction scheme, this state can transition to \([9, 9, 1]\), indicating that one molecule of A and one molecule of B have collided to form a molecule of C. The rate constant for this reaction, \( k \), indicates the probability per unit time that one molecule of A and B within some sub volume will collide and form a molecule of C. With multiple molecules of A and B present, how can we write down a transition probability
between adjacent states? This problem in general would require us to track the positions of each molecule and understand something about how that position evolves with time. If we make the simplifying assumption (valid for relatively small volume containers) that each A is equally likely to react with each B (known as the 'well-mixed' assumption), then we simply multiply $k$ by a combinatorial factor accounting for the number of ways the reaction can occur, $n_a \cdot n_b \cdot k$. If the volume in our simulation is too large to make the well-mixed assumption (such as for instance the 2D interface between a T cell and an APC) we can split the reaction volume into small sub volumes, each of which is effectively well-mixed, and model diffusion by a hopping reaction between adjacent sub volumes.

Though most of the work presented in this thesis will assume a well-mixed system, we will now present a small example based on work carried out in collaboration with researchers at the Whitehead Institute to demonstrate how a modeling approach to stochastic signaling networks with spatial resolution can elucidate structural details of cellular interfaces.

1.2 Multi-scale modeling in the immune system: A preliminary example

As noted above, noise and stochastic fluctuations influence protein interaction networks on a multitude of time and length scales. For example, molecules can be treated as brownian walkers, and in the 'diffusion limited' setting, the rate at which a reaction occurs (and therefore the rate constant $k$) is set by the time scales at which two brownian walkers meet each other. As such, the dimensionality of the system is important; are the two molecules diffusing on a 2D surface, or diffusing throughout the interior of a cell in a 3D volume? The diffusion-limited setting is not always appropriate though. Even if two molecules are in a very small volume (i.e. close enough for a reaction to occur without any considerable amount of time spent for the molecules to find each other), the occurrence of a reaction between the two is stochastic. Not all collisions occur with enough energy or in the proper orientation to facilitate necessary bond-breaking and bond-making that accompany a chemical reaction.

Both of these time and length scales contributed to an experimental result found by researchers at the Whitehead Institute [2]. Experimentalists took a B cell receptor (BCR, analogous to the TCR described above but in B cells) and created two synthetic peptides which bind to the BCR, a 17mer and 8mer. These synthetic peptides were named as such since they were created by cutting a small amount (either 17 base pairs or 8 base pairs) from a larger protein called Ovalbumin that is known to bind to the BCR.

It was seen that the 8mer binds with weaker affinity (a smaller dwell time) to the BCR than
17mer. However, oddly, when first flooding the system with 8mer, it was found that subsequently added 17mer was outcompeted at the BCR and could not bind. How can we model this system? Is it possible that even though the 8mer has a weaker affinity, it can rebind multiple times, finding its way back to the BCR before a 17mer has a chance to, thus outcompeting it in this way?

Consider an 8mer in a very small subvolume with a BCR. The 8mer can either bind to the BCR with a rate that equals $k_{on}$, or it can hop out of the subvolume with a rate equal to $k_{hop}$. The probability of binding before leaving the subvolume is $k_{on}/(k_{on} + k_{hop})$. Importantly, how to scale $k_{on}$, $k_{hop}$ depends on the dimension of the system. For a more detailed exposition, see the supplemental information in [3]. After an 8mer hops out of this subvolume, it goes on a brownian excursion, and potentially returns. If the 17mer that has been added is now uniformly distributed throughout the volume, we can ask whether or not the 8mer will return to the BCR before diffusing as far as the next 17mer (becoming effectively well-mixed). How far does the 8mer have to diffuse to reach the expected distance to the next 17mer? This depends as well on the dimensionality. Assuming a concentration $\rho$ of 17mer, let $R_+$ denote the expected inter particle distance in a spatial poisson process:

$$R_+(1D) = \frac{1}{\rho}$$
$$R_+(2D) = \frac{1}{2\sqrt{\rho}}$$
$$R_+(3D) \approx 0.55\rho^{-1/3}$$

(1.1)

To calculate the probability that an 8mer returns to the BCR before reaching as far away as the next 17mer and becoming effectively well-mixed, we calculate $P_{\text{return}}$ for a brownian walker diffusing between two concentric absorbing boundaries. We therefore ask: what is the probability for the particle that begins at a radius $r$ to return to an inner absorbing radius of $R_-$ before hitting an outer radius of $R_+$? The steady state probabilities satisfy Laplace’s equation: $\nabla^2 P_{\text{return}} = 0$. We use $R_+$ from above, and set $R_- = l/2$, where $l$ is a characteristic length scale that sets the size of the subvolume (typically set by the radius of gyration of a molecule). We set $r = l$. The solution again depends on dimension:

$$P_{\text{return}}(1D) = \frac{R_+ - l}{R_+ - R_-}$$
$$P_{\text{return}}(2D) = \frac{\ln(R_+/l)}{\ln(R_+/R_-)}$$
$$P_{\text{return}}(3D) = \frac{l - R_+}{\frac{1}{R_-} - \frac{1}{R_+}}$$

(1.2)
Summing over all possible ways for the 8mer to rebind before diffusing away gives

\[ P_{\text{rebind}} = P_{\text{on}} \sum_{k=0}^{\infty} (P_{\text{hop}}P_{\text{return}})^k = \frac{P_{\text{on}}}{1 - P_{\text{hop}}P_{\text{return}}} \] (1.3)

If it is true that the 8mer outcompetes 17mer due to multiple rebinding events simply since it was added first, we require that \( P_{\text{rebind}} \) is not negligibly small. Doing the calculation for multiple dimensions on a grid of \( D, \rho \) (\( D \) is the diffusion constant, related to the hopping rate), we see that the probability of rebinding is far less important in 1 or 2 dimensions (figure 1.1). We interpret this as evidence that the surface of the B cell is not perfectly spherical, but rather due to rugged folds in the cell membrane can behave more like a 2D or 1D surface if 8mer leaving a BCR is trapped in a fold or crevice.

![Contour plots showing the probability of 8mer rebinding as a function of 8mer diffusion coefficient (y-axis) and 17mer concentration (x-axis).](image)

Figure 1.1: Contour plots showing the probability of 8mer rebinding as a function of 8mer diffusion coefficient (y-axis) and 17mer concentration (x-axis).

### 1.3 Thesis outline

Two obvious questions that one can ask about stochastic protein interaction networks are, what is the importance of the connectivity of a certain graph, and what is the importance of the dynamics of a certain graph? The first question roughly asks why has nature designed a given signaling network with the connectivity we observe? The second question asks why the values of rate constants (which are directly related to the transition probabilities in the markov chain we use to model the system) have evolved to have the values they have?

This thesis outlines two broad areas of research which are representative of my scientific pursuits as a PhD student in the Department of Chemistry at MIT. The general goal of this work is to understand how biological systems mitigate and exploit stochasticity. Indeed in some cases, noise places constraints on biological systems. By understanding how noise limits the amount of information biological signals contain, one can begin to understand how nature has
designed biological systems optimally to mitigate this stochasticity. In other cases, biological systems can exploit this noise to outperform systems which behave deterministically.

1.3.1 Dynamic disorder

The first broad area of research presented in this thesis investigates the effect of fluctuating kinetic rate parameters. We have outlined above how rate constants are related to the transition rates between states in a very high dimensional markov chain that we use to describe biochemical networks, responsible for signal transduction, or ‘learning’ about their environment. However, due to stochastic fluctuation in the conformation of proteins involved in the networks, rate constants do not remain fixed, but instead fluctuate throughout the course of a signaling cascade.

In chapter 2, we seek to understand the implications of this stochasticity, known in statistical physics as ‘dynamic disorder’. We will treat the signaling network/markov chain as a black box signaling apparatus in which a particular chemical species represents the input or signal, and a downstream activated molecule corresponds to a ‘response’. If a certain markov chain is being used to learn information about the input signal based on readouts of the response, how can non-stationarity in the form of stochastic fluctuations in rate parameters influence the ability for this network to learn? In chapter 2, we explore how stationary distributions over chemical species change as a result of dynamic disorder. We analyze how the information a network can transmit is reduced by dynamic disorder, and how the particular topology of the network and location of the dynamic disorder has important implications in mitigating this loss of information content. We also explore a biological network in the immune system that displays this behavior, and offer a suggestion for how dynamic disorder is exploited in this network to achieve a lower false positive error rate.

In chapter 3, we study how dynamic disorder in bistable networks can be used to change the transition rate between stable basins. Moreover, by tuning the specific parameters of the dynamic disorder (in a way that is demonstrated in real biological systems, as explored in section 2.7), biological systems can tune the transition rates between stable states in response to extracellular signals.

In chapter 4, we reframe the question of dynamic disorder as one of statistical inference. Given access to a time series of chemical species throughout the course of a signaling cascade, can one detect dynamic disorder and make inferences about the particular distribution that a rate constant samples from? We develop frequentist and bayesian approaches based on incomplete data to either perform maximum likelihood estimation of parameters of the distribution, or to sample from the posterior of the parameters of these distributions.
1.3.2 Optimal decision making in immunological responses

As we outlined earlier, T cells scan APCs that present both peptide derived from self proteins (to which an immune response should not be mounted) and peptide derived from foreign, viral proteins (to which an immune response should be mounted). Respectively, we will refer to this as self and non-self. False positives in this context lead to autoimmunity, whereas false negatives lead to a viral infection. It is critical that T cells make the correct decision, but the 'measurement' apparatus used to detect the presence or absence of infection is the sort of noisy signaling network described above. To further complicate matters, each T cell may only see a handful (1-10) of non-self pMHC in a background of thousands [1].

In chapter 5, we investigate the actual biochemical reaction network at the earliest stages of T cell signaling. Recent experimental evidence suggests that the conventional model of this early protein interaction network may be incomplete. We propose a model that can account for the experimental results. By creating a detailed computational model of this network and by Monte Carlo simulation of the dynamics of signal transduction, we have determined that this new proposed model can not only account for experimentally observed results, but may be able to exploit a kinetic bottleneck to achieve greater signal discrimination than the original model.

The work in chapter 5 focuses on classification by a single T cell scanning the surface of a single APC. In the language of decision theory, this is known as a single-round decision problem. However, the actual T cell scans a single APC for some period of time after which it determines that the cell is not infected, and then jumps to another APC to scan. If we model the total decision making period $T$ as a constraint, then the amount of time that a T cell spends at each APC, $t_i$, is an important consideration in optimizing the simultaneous need to maximize detection probability and minimize false alarm probability. In chapter 6, we adapt a previously considered cost function for a population of decision making T cells in the context of a sequential or multi-round decision making process. We look at both discrete time models and diffusion models of populations of T cells. In particular, we investigate what happens when the T cell spends a stochastic amount of time $t_i$ at each APC instead of committing to a deterministic dwell time. What we find is that by tuning the shape of this dwell time distribution, a T cell can robustly choose to increase its detection probability (at the expense of a greater false alarm probability) or lower its false alarm probability (at the expense of a smaller detection probability) relative to what it could achieve with a deterministic dwell time. This result illustrates how noise can be used to achieve a wider range of cellular responses, and also suggests how biological systems can modulate this response to achieve a continuum of points along the PD,PF curve.

The work in chapter 6, while very coarse-grained, does not help us to understand or characterize what the 'optimal' decision rule for a T cell scanning multiple APCs is. Biological systems
operate under energetic constraints, an idea we explore throughout the thesis. In particular, storing measurements costs energy as biological systems have to maintain an activated form of a molecule. In chapter 6, we examine what amount of memory is optimal for the sorts of decision rules that can be implemented by biological networks. However, to fully understand how close to ‘optimal’ a network is, in chapter 7 we seek to characterize, for the discrete model considered in chapter 6, what the optimal decision rule would be. This of course will be a function of all ‘measurements’ the T cell makes throughout the course of the APC scanning period. For this analysis, we use more traditional concepts from decision theory such as Markov Decision Problems with Bayesian updates.
Chapter One References


Chapter 2

Dynamic disorder and the energetic costs of information transduction

2.1 Introduction

Single molecule experiments have given scientists unprecedented capabilities to look beyond the static picture of enzymology obtained from ensemble averaged experiments [1], [2]. Scientists have been able to demonstrate that enzymatic rates of catalysis sometimes fluctuate over several orders of magnitude, drawing from heavy-tailed distributions of catalytic activity [3],[5]. In the language of statistical physics, a stochastic process with a fluctuating rate is known as 'dynamic disorder' [5]. Much theoretical work has been done to examine models of dynamic disorder that involve discrete transitions between finitely many rates [3],[4],[7],[9]. A commonly used experimental tool to probe the internal states of single molecules and the transition rates between various states is single molecule FRET spectroscopy [10],[11],[12].

Chemical reaction networks serve a number of important roles in biological systems, including the problem of signal transduction. Given a signal or input, a biological network must convert this signal to a downstream response. The reaction network can be seen as transmitting mutual information between the signaling molecules that are stimulated first and a downstream response molecule. Due to the stochastic nature of chemical kinetics, information processing will always have some associated error, even without dynamic disorder. However, dynamic disorder can further change the statistics of downstream signaling molecules, and thus fundamentally alter the fidelity of information a network is capable of transmitting.

It is clear that dynamic disorder can drive a system out of equilibrium. A well known consequence of Landauer's principle is that irreversible computation requires energy consumption [13].
For chemical reaction networks, how does dynamic disorder influence the energetic requirements (metabolic costs) for signal transduction? In this work, we seek to understand the implications that dynamic disorder can have on the fundamental capacity for biochemical networks to convey information. We will show that there are energetic costs associated with the intrinsic 'switch rate' for an enzyme. In other words, when an enzyme transitions very rapidly between internal states thus 'self-averaging', the maximal information between signal and response is achieved. However, this rapid switching drives the chemical network very far from equilibrium, necessitating greater energy consumption, as measured by the entropy production. Importantly, this energy consumption cannot be explained merely by thermal energy necessary for the enzyme to make conformational transitions.

The chapter is organized as follows. In section 2, we analyze the limits of fast and slow switching between internal enzyme states. In section 3, we review entropy production in non equilibrium steady states (NESS) and analyze the entropy production for birth death processes in the presence of dynamic disorder. In section 4, we look at a model of a single enzyme with two internal states, for which entropy production and the PDF can be solved exactly to illustrate the properties in the first three sections. Finally, we study the information content in network motifs and define the capacity power consumption. We look at two specific examples. The first is a simple birth death network with multiple enzymes each of which has three internal states. The second is a more complicated network known as the Coherent Feedforward Type 1 network, which has been used as a model for the arabinose gene transcription network of E. coli [16]. Here, we allow the enzyme rates to draw from various continuous distributions. We show that for these networks, dynamic disorder leads to a trade-off in which maximal information capacity of the network is associated with the greatest energetic costs.

2.2 Effects of limiting values of the switching rate

Conventional stochastic chemical kinetics involves the use of a Gillespie algorithm to sample the master equation [19],[20],[21]. For a system of chemical reactions involving $K$ chemical species $\{X_i\}_{i=1}^K$, we let $X$ represent the vector of numbers of each molecular species. A reaction indexed by $\rho$ changes the vector $X$ by some stoichiometric vector $n_\rho$, via $X \xrightarrow{\rho} X + n_\rho$. We assign to each reaction $\rho$ a transition rate $W_\rho(X|X + n_\rho)$, also known in chemical kinetics as a reaction propensity. Transition rates for chemical reactions are given by the product of a fundamental rate constant (denoted by $k_\rho$) and a combinatorial factor accounting for all the possible ways the reaction can occur (assuming homogeneously mixed concentrations).

We will consider here a simplified picture of dynamic disorder. We will assume that some of the rate constants $k_\rho$ are allowed to fluctuate by drawing a value from a prescribed distribu-
CHAPTER 2. DYNAMIC DISORDER

tion, remaining at this value for an exponentially distributed amount of time as governed by a parameter we will denote $\lambda$ (the switch rate, with units of $s^{-1}$), and then redrawing a new value from the same distribution. We note that this is a special case of dynamic disorder in which the transition probability between internal states $i \rightarrow j$ depends only on state $j$. In general, one can consider that the transition probability for transitions between various internal states is a more complicated function of both the initial state and the final state. However, the qualitative results presented here are likely to hold for more complicated transition probabilities, as we only make arguments appealing to the stationary distribution of the internal enzyme state, or the rate at which the enzyme reaches steady state. (Though we consider the case in which all internal states of the enzyme are interconnected, many have studied how to infer more complicated topologies connecting internal enzyme states from single molecule studies. [21],[22],[23],[24])

As an explicit example, consider the following bimolecular reaction, which may represent phosphorylation of a species $Y$:

$$X + Y \xrightarrow{k} X + Y^*$$  \hspace{1cm} (2.1)

The transition rate for the above reaction is given by $k[X][Y]$. If the rate parameter $k$ changes due to fluctuating structural conformations of the species $X$, the transition rate will become a stochastic variable. At a particular instant in time, the transition rate will be given by $(\sum_i k_i)[Y]$, where the index $i$ counts the individual rate constants $k_i$ for each molecule of $X$ in the system. We will denote by $K$ the 'total rate', which is the sum of all individual rate constants $k_i$, such that the transition rate for the above reaction becomes $K[Y]$. We will assume in this work that each molecule will have an individual rate that is independent of the others (i.e. switching events are uncorrelated) but each individual rate draws from the same distribution.

To quantify how fast or slow the switch rate $\lambda$ is, we assume that there exists some intrinsic rate constant in the network (such as the decay or dephosphorylation rate of active species) that sets the time scales of reaching the stationary distribution. Throughout the paper, we will refer to this parameter as $\alpha$. To speak about the slow or fast switching limit, we must consider cases in which $\lambda/\alpha$ is much smaller or much greater than one.

For general systems of chemical reactions, the stationary probability distribution $P(X)$ can be obtained by solving the master equation. Here, we examine how the stationary probability distribution is modified in the limits of $\lambda \rightarrow 0, \infty$. For simplicity, we will first consider the case in which the copy number of the species undergoing conformation fluctuations is constant throughout the course of the reaction. We will later investigate network schemes for which this is not the case. We will denote by $P(X|K)$ the stationary probability of $X$ given a system with no dynamic disorder and a total rate given by $K$.

In the limit that $\lambda \rightarrow 0$, each individual rate $k_i$ remains at a certain value for increasingly
long amounts of time. In this limit, we assume that the total rate changes very slowly, and the chemical species $X$ can adiabatically follow the underlying rate parameter and equilibrate to the current value. At any given time, the distribution of $X$ should look like $P(X|K)$. Thus, the stationary distribution over $X$ measured from a long trajectory in this limit should look like marginalization:

$$\lim_{\lambda \to 0} P(X) = \int P(X|K)P(K)dK$$

(2.2)

where $P(K)$ is the probability that $\sum_i k_i = K$. In general $P(K)$ will not be analytically tractable, but can be approximated by a normal random variable in the limit of large copy number: $K \approx \mathcal{N}(\mathbb{E}[k], \mathbb{Var}[k])$. We have let $N$ represent the total number of the fluctuating chemical species, and $\mathbb{E}[k], \mathbb{Var}(k)$ denote statistics of the distribution that each $k_i$ draws from.

In the limit of $\lambda \to \infty$, we argue that

$$\lim_{\lambda \to \infty} P(X) = P(X|\mathbb{E}[K])$$

(2.3)

To see this, we must consider a generic time interval $[t_1, t_2]$ between successive reaction events. For a general time dependent parameter $K(t)$, the time-dependent propensity is proportional to $\int_{t_1}^{t_2} K(t)dt$ [14]. When each individual rate constant switches at discrete times, the total rate constant $K$ will take on a discrete set of values between these two time points. The total number of switching events in this period, $\Omega$, is Poisson distributed with parameter $N\lambda(t_2 - t_1)$. Further, the expected time interval $dt$ in between each switching event is of length $(N\lambda)^{-1}$. If we index the intervals between each switching event by $i$, we can rewrite the time dependent propensity as follows (dropping the combinatoric pre factor):

$$\int_{t_1}^{t_2} K(t)dt = \sum_{i=1}^{\Omega} K(t_i)dt_i \xrightarrow{\lambda \to \infty} (t_2 - t_1)\mathbb{E}[K]$$

(2.4)

To determine the limiting form, we have used the Renewal Reward theorem [28] (Section 2.9.1). This analysis demonstrates that as the switching rate becomes very large, the time-dependent propensity between any two reaction events will look like the average propensity.

2.3 Power consumption in Markov Chains

The entropy production for a non equilibrium steady state (NESS) can be computed for a general Markov chain with states indexed by $\sigma$ and transition rates between the $\sigma$ given by $W(\sigma, \sigma')$ [25],[26]:

$$\frac{dS}{dt} = \sum_{\sigma, \sigma'} P(\sigma)W(\sigma, \sigma') \log \left( \frac{W(\sigma, \sigma')}{W(\sigma', \sigma)} \right)$$

(2.5)
CHAPTER 2. DYNAMIC DISORDER

For certain birth death processes, a simple argument appealing to Jensen’s inequality motivates why the model for dynamic disorder we have considered should result in increased entropy production in the fast switch rate limit. Assume the rate of production is the fluctuating rate \( K (W(n, n+1) = K) \) and the rate of destruction is \( W(n, n-1) = n\alpha \). The entropy production for the process in which there is no dynamic disorder and the parameter \( K \) acts only at some average value \( \mathbb{E}[K] \) is given by

\[
\frac{dS}{dt} = \sum_n P(n|\mathbb{E}[K]) \mathbb{E}[K] \log \left( \frac{\mathbb{E}[K]}{\alpha(n+1)} \right)
\]

\[\text{or} \quad \frac{dS}{dt} = \sum_n P(n|\mathbb{E}[K]) \alpha n \log \left( \frac{\alpha n}{\mathbb{E}[K]} \right) \tag{2.6}\]

In the presence of dynamic disorder and a fast switch rate limit, the entropy production becomes

\[
\frac{dS}{dt} = \sum_n P(n|\mathbb{E}[K]) \int P(K) K \log \left( \frac{K}{\alpha(n+1)} \right) dK
\]

\[\text{or} \quad \frac{dS}{dt} = \sum_n P(n|\mathbb{E}[K]) \int P(K) \alpha n \log \left( \frac{\alpha n}{K} \right) dK \tag{2.7}\]

By the convexity of the functions \( x \log x \) and \( \log(1/x) \), and invoking Jensen’s inequality, the entropy production in equation (2.7) will be greater than in equation (2.6). What’s more, because the internal rate switching will equilibrate and the distributions reach a limiting form with increasing switch rate, the entropy production in equation (2.7) represents an asymptotic cost incurred by infinitely fast switching rate. This demonstrates that there is a finite price to pay even as the switching rate increases to infinitely large values.

In many cases, computing the entropy production is very hard, as it requires enumeration of all possible states and calculation of (or Monte Carlo sampling to determine) the steady state probability for the system to be in each state. However, one can numerically compute the irreversible entropy production for a NESS by looking at the average value of the fluctuating quantity \( Z(t) \) defined by Gaspard [27] as the log ratio of the probability for a trajectory to the probability of its time-reversed trajectory. On average, this quantity grows linearly with a slope equal to the irreversible entropy or power consumption required to maintain the NESS. For a sequence of reactions inducing the trajectory \( \mathbf{X}_0 \rightarrow \mathbf{X}_1 \rightarrow \cdots \rightarrow \mathbf{X}_n \), the quantity \( Z(t) \) is given by

\[
Z(t) = \log \left( \frac{W(\mathbf{X}_0|\mathbf{X}_1)W(\mathbf{X}_1|\mathbf{X}_2)\ldots W(\mathbf{X}_{n-1}|\mathbf{X}_n)}{W(\mathbf{X}_1|\mathbf{X}_0)W(\mathbf{X}_2|\mathbf{X}_1)\ldots W(\mathbf{X}_n|\mathbf{X}_{n-1})} \right) \tag{2.8}\]

For the model of dynamic disorder considered in this work, the transition for an individual rate \( k_i \rightarrow k'_i \) occurs with a rate given by the switch rate \( \lambda \) times the probability of drawing \( k'_i \).
CHAPTER 2. DYNAMIC DISORDER

Thus, an individual rate transition will contribute to $Z(t)$ a term of the form

$$\log \frac{p(k'_i)}{p(k_i)}$$

(2.9)

We note that switch rates in expectation should contribute nothing to irreversible entropy production as the above term in expectation becomes $H(k) - H(k) = 0$ where $H(k)$ is the entropy of the probability distribution that a single enzyme draws from.

2.4 Single enzyme, Two internal states

To illustrate the relationship between the switch rate and the probability distribution and entropy production, we will look now at a simple example which can be solved exactly for all values of the switch rate. This is the case of a simple birth death process with a rate of production that fluctuates between two internal states. This can be thought of as a single enzyme with two internal states catalyzing the production of an activated species:

$$\emptyset \xrightarrow{k_i} Y, \quad Y \xrightarrow{\alpha} \emptyset, \quad k_i \xrightarrow{\lambda} k_j$$

(2.10)

where $k_i, k_j$ denote the two rates, and $p_j$ is the steady state probability of the enzyme being in state $j$. The master equation for the joint PDF $p(n, i)$ where $n$ is the number of $Y$ and $i$ is the internal state is as follows:

$$\frac{\partial}{\partial t} p(n, i) = k_i p(n - 1, i) + \alpha(n + 1) p(n + 1, i) + \lambda p_i p(n, j)$$

$$- (\alpha n + k_i + \lambda p_j) p(n, i)$$

(2.11)

This equation is mathematically equivalent to that studied by Mehta for a model of receptor binding induced conformational change in a protein [26]. It is solved by introducing a probability generating function:

$$G_i(z) = \sum_{i=0}^{\infty} p(n, i) z^n$$

(2.12)

and the solution can be shown to be [26]:

$$G_i(z) = p_i e^{(k_i(z - 1)/\alpha)} _1 F_1 \left( \frac{\lambda p_i}{\alpha}, 1 + \frac{\lambda}{\alpha}, \frac{k_j - k_i}{\alpha}(z - 1) \right)$$

(2.13)

where $_1 F_1$ is the confluent hypergeometric function of the first kind.

We can see explicitly that the solution only depends on the ratio $\lambda/\alpha$. We plot the probability distribution with $\alpha = 10$ and the two internal rates to be $k = 10, 100$ with probability .25, .75. In the absence of dynamic disorder, the simple birth death process has a stationary distribution.
that is Poisson with parameter $k/\alpha$. In the fast switch rate limit, the distribution with dynamic disorder will approach Poisson with parameter $\mathbb{E}[k]/\alpha$, and in the slow switch rate limit, the distribution will approach a mixture of two Poissons, weighted by the stationary distributions. We show both limits (Figure 7.1B).

We also plot the entropy production associated with the system as a function of the switch rate (Figure 7.1A). In this case, it can be calculated exactly using

$$\frac{dS}{dt} = \sum_{n,i} P(n, i) k_i \log \left( \frac{k_i}{\alpha(n + 1)} \right)$$

$$+ \sum_{n,i} P(n, i) \alpha n \log \left( \frac{\alpha n}{k_i} \right)$$

The entropy production should have a sigmoidal shape. For low values of switching rate, it asymptotically approaches a weighted average of entropy productions for two birth death processes, each of which equal zero. For high switch rate limits, it asymptotically approaches a
Figure 2.2: A). For $n_x = 50$ we plot the probability distribution of species $Y$ in the birth death network. The curve labeled $E[K]$ indicates the Poisson distribution evaluated at the average $K$, for comparison with the fast switch rate limit. B). The capacity power consumption for the birth death network with multiple enzymes shows the characteristic sigmoidal shape. In this case, larger values of $E[k]$ (expected rate for a single enzyme) lead to lower power consumption in the large switch rate limit. C). The information in the birth death network increases with increasing switch rate, and reaches a limiting value predicted for the network in the absence of dynamic disorder and evaluated for the Poisson distribution at the average value of $K$, labeled here as $E[K]$. 

value predicted by equation (2.7), where $P(n|E[k])$ is Poisson with parameter $E[k]/\alpha$. Different values of $E[k]$ change the asymptotic value of the entropy production. In general, the specific topology of the signaling network will determine whether a greater $E[k]$ will increase or decrease the limiting value of entropy production. However, importantly, the asymptotic behavior of the curve is not influenced by the choice of $E[k]$.

2.5 Multiple Enzymes with three internal states

2.5.1 Information in a chemical network

To quantify the ability of a signaling network to transduce signals, we will study the mutual information between a signal input and a response, $I(S;R)$ [15]. This measures the Kullback Leibler (KL) divergence between the joint distribution $P(R,S)$ and a completely independent distribution $P(R)P(S)$. The KL divergence is an information theoretic measure of the distance between two distributions. For the purposes of this paper, we will work with simplified pictures in which the signal species copy number can only take on a finite number of values, and the
mutual information will quantify the ability to determine what the signal strength is based on our measurement of the response. We first look at the information in the birth death network:

\[
X \xrightarrow{k} X + Y, \quad Y \xrightarrow{0} 0
\] (2.15)

We allow the rate of production \(k\) to fluctuate, meaning the quantity \(k[X]\) becomes \(\sum_{i=1}^{n_x} k_i\) which we hereafter refer to as \(K\) (note that \(\mathbb{E}[K] = \mathbb{E}[k_i]n_x\)). We study the system using the Gillespie algorithm [19],[20],[21]. For these calculations, the internal states of the enzyme are chosen to be 1, 1, 10. First, for fixed \(n_x\), we verify that the probability distributions over \(Y\) tend to the limiting values predicted in Section II. The probability distribution in the absence of rate fluctuation is Poisson with parameter \(k[X]/\alpha\), and we compare in the fast switch rate limit to \(\mathbb{E}[k_i]n_X/\alpha\) for \(\alpha = 10\) and multiple values of \(\mathbb{E}[k_i]\) (Figure 2.2A).

Next, we study the information in this network by allowing \(X\) to take the values of 10 or 50. To analyze the information in the network, we must specify the prior probability that \(X = 50\), which we will denote by \(p\). We study the information \(I(X; Y)\) for all priors by computing separate steady states for each value of \(n_x\) (Figure 2.2C). The information for this network is maximized in the fast switch rate limit, and decreases as the switch rate decreases.

### 2.5.2 Capacity Power Consumption

One way to quantify the mutual information is to examine the channel capacity. For a generic channel, the channel capacity is defined as the maximal mutual information between a signal and a response, where the maximization is done with respect to the prior distribution over the signal. In this case, the channel capacity is \(I(p^*)\), where \(p^* = \arg \max_p I(X; Z)\).

To quantify the relationship between energy consumption and information content, we can define the capacity power consumption as below:

\[
p^* \left. \frac{dS}{dt} \right|_{n_x=50} + (1 - p^*) \left. \frac{dS}{dt} \right|_{n_x=10}
\] (2.16)

For a given \(p^*\) that optimizes the information content of a network, there is an associated energetic cost that corresponds to a weighted average of the entropy productions in each of the NESS of \(n_x\). In this sense, the capacity power consumption measures the energetic cost incurred for a network to transmit at its capacity. It is important to note that in general, the value of \(p^*\) depends on the switch rate.

For the parameters considered here, the value of \(p^*\) is .49. For the case of multiple enzymes with three internal states, we see that the capacity entropy production increases with the switch rate, but saturates at the high switch rate limit, as predicted before (Figure 2.2B).
2.6 Coherent Feedforward Type 1 network

2.6.1 Model and probability distributions

We now test these results in the context of a more complicated network scheme, and using various choices of continuous distribution for the fluctuating rate parameter. The network we will analyze here will be the Coherent Feedforward Type 1 (CFF1) network.

This particular network has been used as a model of gene transcription of arabinose in *E. coli* [16]. The network is chosen to make the analysis tractable. The reactions we consider are:

\[
\begin{align*}
X & \xrightarrow{k_1} X + Y \\
X & \xrightarrow{k_1} X + Z \\
Y & \xrightarrow{k_2} Y + Z \\
Y & \xrightarrow{\alpha_Y} \emptyset \\
Z & \xrightarrow{\alpha_Z} \emptyset
\end{align*}
\]  

(2.17)

An interesting property of the CFF1 network is its ability to filter noise [16]. The reactions above are chosen such that \(X\) is constant throughout the course of a trajectory. We first wish to demonstrate that the two limits of the switch rate are captured by marginalization and expectation over the random total rate. We will study the effects of allowing \(k_1\) to fluctuate. As such, the combination \(k_1[X]\) is replaced by \(\sum_{i=1}^{n_x} k_i\), hereafter denoted by \(K\). We study the system using the Gillespie algorithm [19],[20],[21]. We will do the analysis for various choices of distribution over the \(k_i\). To ensure that rates are always positive, we first study the system using a distribution that is proportional to the positive part of a normal PDF. In other words, \(k_i \sim \mathcal{N}(0, 1)[k_i > 0]\). Toward a better analytical understanding of the network, we first write down a system size expansion (section 2.9.2) to obtain an approximate stationary multivariate normal distribution \(P(Y, Z)\) [17]. We compare the probability distribution over \(Z\) in the fast and slow switch rate limits, and analytically compute the marginal distribution over \(Z\) by using the system size expansion to find \(P(Z|K)\) and integrating out \(K\) assuming that the copy number \(n_x\) is large enough that \(K\) is approximately normal (Figure 5.3A).

To show that the results hold for the case in which \(X\) fluctuates stochastically, we next analyze the network with the addition of the following two reactions:

\[
\begin{align*}
\emptyset & \xrightarrow{k_1} X \\
X & \xrightarrow{\alpha_Z} \emptyset
\end{align*}
\]  

(2.18)

In this case, we allow each \(X\) molecule to draw a new rate \(k_i\) when it is formed, and the rate then switches according to the above protocol. The slow switching limit is now set by the
Figure 2.3: A) We simulate the coherent feedforward network for $n_x = 100$, $k_2 = 5$, $a = 10$ and the distribution over the $k_i$ is proportional to the standard $\mathcal{N}(0,1)$ density when $k_i > 0$ and zero otherwise. We compute the distribution over downstream species $Z$ for fast switch rate ($50 s^{-1}$) and slow switch rate ($.1 s^{-1}$) limits. We analytically compute $P(z)$ by making a system size expansion to compute $P(Z|K)$ when $K$ does not fluctuate and integrating out $K$ by assuming $P(K)$ is approximately normal, with average $n_x \mathbb{E}[k_i]$ and variance $n_x \text{Var}[k_i]$. The slow switch rate limit approaches that computed in the SSE after marginalizing the stochastic switch rate parameter. B) The coherent feedforward network is simulated with additional reactions to degrade and produce $X$ with parameters $\alpha_x = 1, k_f = 500$. Gillespie simulations are performed for a switch rate of $.1, 30 s^{-1}$, and also for the network in the absence of dynamic disorder in which each $k_i$ is equal to the average value in the corresponding dynamic disordered simulations (No fluc). We compute the marginalization of $P(z)$ using the distributions for $P(K), P(Z|K)$ and performing the integration with the software package Mathematica. As the switch rate increases, it approaches the distribution predicted in the absence of rate fluctuation. As the switch rate slows, the distribution approaches that predicted by marginalizing the stochastic switch rate out of the distribution predicted by the SSE.
timescale of $1/\alpha_x$ since it shouldn’t matter if the rate $k_i$ persists for longer than the lifetime of an individual $X$. We can approximately calculate the marginal distribution in the slow limit for the case of fluctuating $X$ species by an adiabatic approximation, and by choosing the distribution of the $k_i$ to be closed to addition. As such, we let the $k_i$ draw from the chi-squared distribution with one degree of freedom (the distribution of the square of a normal random variable). The details are outlined in section 2.9.3. The results are shown in Figure 5.3B.

### 2.6.2 Channel capacity and rate variance

We would like to understand the relationship between the capacity for a biochemical network to convey information, and the noisiness of the underlying distribution for the $k_i$ (as measured by the variance).

We will analyze the Coherent Feedforward system as before treating $X$ as the signal/input, and $Z$ as the response/output. We will consider a two-state system where signal strength is high with probability $p$, and $X$ can be either 500 or 100. In other words, $P(X = 500) = p$, $P(X = 100) = 1 - p$. For these calculations, we let the $k_i$ draw from a lognormal distribution. To adjust the variance while holding the mean constant requires adjusting both the $\mu$ and $\sigma$ parameters of the lognormal distribution.

The graph of $I(X; Z)$ in Figure 5.4 as a function of $p$ shows that the information content of the network decreases as the variance of the underlying rate distribution increases. This is consistent with the intuition that the noisier an individual parameter becomes, the harder it is to convey information. Similar metrics related to binary decision making such as the area under the receiver operating characteristic (ROC) curve show similar trends.

### 2.6.3 Power consumption and channel capacity

From the approximate distribution $P(Y, Z)$, one can readily show that (section 2.9.2):

$$\text{Var}[Z|K] \propto K, \quad E[Z|K] \propto K$$  \hspace{1cm} (2.19)

From the law of total variance and the functional form of the variance for this example, we know that

$$\text{Var}[Z] \geq E[\text{Var}[Z|K]] = \text{Var}[Z|E[K]]$$  \hspace{1cm} (2.20)

This suggests that in the slow and fast switch rate limits, the average value of $Z$ is the same, and the distribution over $Z$ will be roughly centered at the same place (if we assume a symmetric distribution in $Z$). However, in the slow switch rate limit, the width of the distribution over $Z$ will be larger than in the fast switch rate limit. In our two state system, this means that peaks for low and high signal strength will be located in the same place regardless of the switch
Figure 2.4: Here we plot the mutual information between X and Z as a function of p, the probability of high signal strength. Different curves represent different values of the variance in distribution for an individual rate constant. Increasing variance leads to less information transduction in the network.
Figure 2.5: A). Plot of power consumption required for the NESS corresponding to the maximal information content of the network motif as a function of switch rate. Faster switch rates require more power consumption. B). A plot of mutual information between X and Z as a function of p. Different curves represent different switch rates, and the curve labeled ‘No fluc’ is calculated in the absence of dynamic disorder. High switch rates approach the limit of information predicted by the system size expansion (SSE) assuming the average value of the rate. The information evaluated at the expected value of the rate parameter and evaluated after marginalizing out the rate parameter assuming the central limit theorem (labeled SSE, Marg.) set bounds for the information.

rate being fast or slow, but will begin to overlap to a greater degree as the switch rate becomes slower. This makes it more difficult to discriminate a weak or strong signal if the switching rate is slow.

In the information theoretic framework, a slower switch rate corresponds to an increasing overlap between distributions \( P(Z|X = 500) \) and \( P(Z|X = 100) \), and thus it appears that slow switching should correspond to lower mutual information between a signal and response.

We can analytically compute the information using the distribution \( P(Y, Z) \) obtained from the system size expansion for the limits that \( \lambda \to 0, \infty \). Intermediate values are computed using the Gillespie algorithm. Indeed, we see that increasing switch rate increases the information content of the network (Figure 5.5B).

At this point, as it is clear that a faster switch rate should correspond to greater information content, we would like to comment on the energetic costs associated with a faster switch rate. Macroscopically, it can be shown that the CFF1 network is at equilibrium at steady state (section
2.9.2), but does dynamic disorder drive the network away from equilibrium?

Given the distribution $P(Y, Z)$ for the CFF1 network considered above, the entropy production in the absence of dynamic disorder can be calculated easily. It is not exactly zero (as would be expected macroscopically) due to finite size effects, but is very close to equilibrium. For cases of rate fluctuation, we must compute this quantity numerically. Nonetheless, we can see that dynamic disorder drives the system away further from equilibrium, with faster switching rates driving the system further away; the capacity power consumption increases with the switch rate (see Figure 5.5A). For these parameters, the value of $p^*$ that maximizes the mutual information is .5021 for all switch rates.

The enzyme transitions satisfy detailed balance, and indeed would even for a more complicated model in which the transition probabilities were more complex functions of the current and final state. This suggests that the energetic cost of this irreversibility which we document above cannot be explained by merely thermal energy required to allow the enzyme to transition between internal states.

### 2.7 Applications to Ras-SOS network

Here, we look at one example of a network from the earliest stages of T cell signaling in which dynamic disorder may be exploited to lower the false alarm probability of activation for the immune system. The specific network we consider involves activation of Ras proteins. Ras activation is an important step in the functions of T and B cells mounting an immunological response against an invading pathogen [32]. The reaction scheme is very simple. Ras proteins exist in both the inactive form, as RasGDP (bound to a GTP molecule) or in the active form as RasGTP. A molecule called SOS catalyzes the activation of RasGDP to RasGTP. SOS has an allosteric pocket that can bind either RasGDP or RasGTP, and its catalytic rate is larger when bound to RasGTP. This constitutes a positive feedback loop which leads to bimodality in the concentration of RasGTP [32].

Single molecule studies by Groves et. al. have shown that in fact the rate of SOS catalyzed activation of RasGDP to RasGTP is not fixed at one value, but instead fluctuates. Further, the distribution of catalytic rates that a single SOS molecule draws from depends on whether it has RasGDP or RasGTP bound to its allosteric site. The experiments indicate that the catalytic rate samples from a heavy tailed distribution, and when RasGTP is bound, the right tail becomes heavier, leading to the increase in average catalytic rate when RasGTP binds allosterically. Additionally, the switching rate is slower when RasGTP is bound[33].

To study the effects of fluctuating kinetics on the bimodal response of Ras activation in lymphocytes, we carried out stochastic simulations of a coarse-grained model of the network
using the Gillespie algorithm. In our simulations, we sought to understand and isolate the relative effects of both a heavier tailed distribution and a slower switching rate for SOS with RasGTP versus RasGDP allosterically bound (hereafter referred to as SOS-RasGTP and SOS-RasGDP respectively). By switching rate we refer here to the rate at which SOS stochastically draws a new catalytic rate. In our simulation, we consider a simple model in which the catalytic rate of SOS is allowed to fluctuate between a discrete set of states with probabilities chosen such that the average catalytic rate coincides with experimentally known values. We allow the SOS enzyme to draw from different catalytic distributions based on the allosteric occupancy. The amount of time that a SOS enzyme will remain in one catalytic state is itself a random variable governed by the aforementioned switching rate (details in section 2.9.4).

Our simulations suggest that there are two main results of allowing a fluctuating catalytic rate. First of all, the range of SOS concentrations that elicits a bimodal response is shifted to higher values when compared to the case of a non-fluctuating SOS catalytic rate (figure 2.6A,B). This implies that the Ras/SOS network with rate fluctuations becomes more conservative in the sense that greater signal strength (as measured by SOS concentration) is required to elicit a high RasGTP level. The second main result is that the range of SOS concentrations corresponding to a bimodal response is widened (figure 2.7). We were able to parse the relative contributions to these results from the slower switching rate and the heavier tailed distribution of SOS-RasGTP by performing simulations in which first the switch rate was identical but the distribution different, and then in which the distribution was identical but the switch rate different (figure 2.6C,D). What we have found is that if the SOS-RasGTP/GDP distributions are identical, bimodality will disappear even if there is a difference in switch rate. However, even if the switch rate is identical, bimodality is restored as long as the distributions are different.

We believe that these results are both due to the heavy-tailed nature of the catalytic distribution of SOS-RasGTP. Because the enzyme can sample catalytic rates below the average value with high probability, the effective concentration of SOS-RasGTP appears lower than the case in which there is no rate fluctuation. Having a slower switching rate for SOS-RasGTP versus SOS-RasGDP exaggerates this effect when compared to the case of equal switching rates, and pushes the bimodal response to even higher values of SOS concentration (figure 2.6C). This is because the enzyme now lingers on slow catalytic rates for a longer expected time due to the slow switch rate. Because of the heavy-tailed nature of the distribution, the states slower than the average have relatively high probability. The presence of a heavy tailed distribution for SOS-RasGTP is necessary however for a bimodal response to occur at all. When the SOS-RasGDP and SOS-RasGTP catalytic distributions are identical, there is no bimodality due to the absence of a positive feedback loop. When the distributions are different only because of the presence of one more highly active state for SOS-RasGTP, the bimodality is restored (figure 2.6D).
Figure 2.6: Many stochastic simulations are carried out of a coarse grained model of the Ras/SOS network (details in SI). Each result corresponds to an individual lymphocyte. The concentration of RasGTP is measured after ten minutes and the values for all such 8,000 'cells' are shown in the histograms above. The red highlighted boxes illustrate that bimodality has been shifted to higher SOS concentrations in various setups. A) Shows simulations for the Ras/SOS network in which there is no catalytic rate fluctuation. B) Shows simulations with catalytic rate fluctuations for which the switching rate of SOS with RasGTP or RasGDP allosterically bound is the same. C) Shows simulations in which SOS with RasGTP allosterically bound is slower than SOS with RasGDP allosterically bound. D) Shows simulations in which the catalytic distribution for SOS with RasGTP and RasGDP bound allosterically is identical. In this case, the only difference is that the switch rate when RasGTP is bound is slower.
Figure 2.7: Here, we plot the distribution of RasGTP as a function of increasing RasGRP concentration and increasing SOS concentration. Stochastic simulations in which the SOS catalytic rate is allowed to fluctuate are shown in blue, and those in which SOS acts only at the average value are shown in red. Blue highlighted boxes indicate parameter values for which the network displays bimodality only when the catalytic rate is allowed to fluctuate.

2.8 Discussion

In this chapter, we have analyzed a form of dynamic disorder in the context of signal transduction. We have analyzed the limits of the switching rate governing the time intervals in between transitions amongst rate values, and shown numerically and with analytical arguments that the infinitely fast limit leads to a system which is identical to that in the absence of dynamic disorder with all rate parameters at their average value. As the switch rate tends to zero, the system looks as if we have marginalized or integrated out the stochastic rate parameter. We analyzed a birth death network with multiple enzymes and three internal states, and the Coherent Feedforward Type 1 network, and demonstrated that the fast switch rate is most conducive to information transduction, but also leads to the most expensive energetic costs.

In certain networks, noise is exploited by a phenomenon known as stochastic resonance [29],[30],[31]. While it seems likely that in some contexts, dynamic disorder may reduce the signal to noise ratio that is characteristic of this behavior, in other cases where internal noise itself is used as the dynamical parameter that induces stochastic resonance, perhaps dynamic disorder
can help facilitate noise induced coherent resonance. While the metrics considered in this paper (entropy production, channel capacity) varied monotonically with the switch rate and variance of the underlying rate distribution, it is worth examining whether or not in certain regimes known to exhibit stochastic resonance some of these metrics are non-monotonic. However, for the purposes of signal transduction considered in this work, it appears that dynamic disorder can only hinder the ability of a signaling network to convey information, while at the same time incurring added energetic costs.

In the limit of small copy number, any cellular network will be subject to stochastic noise. The probabilistic nature of chemical kinetics implies that any such network will have a fundamental limit in terms of its ability to transduce a signal reliably (quantified in this work by the mutual information between a signal and a response). However, this work suggests that for at least some signaling network motifs, even relatively simple ones, dynamic disorder serves to further decrease the channel capacity. What’s more, for an enzyme to self average by making very rapid transitions between internal states seems to require greater energetic consumption. The asymptotic cost and channel capacity ultimately depend strongly on the parameters of the dynamic disorder, including the value of the average of the rate, the variance in the rate and the switching rate between internal states.

We analyzed a model of dynamic disorder that was experimentally observed in a simple network motif that demonstrates bimodality in the earliest stages of T cell signaling, and showed how stochasticity in the rate parameters can lead to a more robust signaling network which requires larger concentration of signaling molecule to become activated (thereby effectively reducing the false alarm probability, at the cost of a lower detection probability).

Ultimately, this work implies that the evolution of certain network motifs required for cellular computation must take into account dynamic disorder to optimally transmit information. In the next chapter, we will examine a more general model for a bistable network known as the genetic toggle switch model, and study how the kinetics of hopping from one stable basin to the other are influenced by stochasticity in the rate parameters.

2.9 Supplemental Information

2.9.1 The infinitely fast switching limit

Renewal theory involves processes for which inter arrival times are IID. An important result is the Renewal Reward Theorem. A renewal reward process is a process for which a reward $r_i$ is given in each inter arrival interval $dt_i$. If we define $\Omega(T)$ to be the counter function which equals the number of arrivals up to time $T$, and define $R(T) = \sum_{i=1}^{\Omega(T)} r_i$, the Renewal Reward theorem
CHAPTER 2. DYNAMIC DISORDER

states that, with probability one,

\[
\lim_{T \to \infty} \frac{R(T)}{T} = \begin{bmatrix} E[r_i] \\ E[dt_i] \end{bmatrix}
\] (2.21)

In the time-dependent propensity for the stochastic chemical network, the rewards are given by \( r_i = K_i dt_i \). To use the theorem, we will define a related process denoted with ' such that \( dt'_i = \lambda dt_i, T' = \lambda T \) and a counter function defined such that \( \Omega'(T') = \Omega(T) \), such that the limit of \( \lambda \to \infty \) implies \( T' \to \infty \) and vice versa for finite \( T \). Note that

\[
\frac{R(T')}{T'} = \frac{\sum_{i=1}^{\Omega(T')} K_i dt'_i}{T'} = \frac{\sum_{i=1}^{\Omega(T)} K_i dt_i}{T} = \frac{R(T)}{T}
\] (2.22)

The theorem says that

\[
\lim_{T' \to \infty} \frac{R(T')}{T'} = \lim_{\lambda \to \infty} \frac{R(T)}{T} = E[K]
\] (2.23)

which is the result stated in the text.

2.9.2 System size expansion of CFF1 network

The master equation for the reactions considered can be written in terms of step operators \( \mathcal{E} \) as

\[
\dot{P} = \begin{pmatrix}
(k_1 n_x [E_y^{-1} - 1]) + (k_1 n_x + k_2 n_y) [E_z^{-1} - 1] \\
+ \alpha [E_y^{-1} - 1] n_y + \alpha [E_z^{-1} - 1] n_z
\end{pmatrix}
\] (2.24)

Define macroscopic functions \( \phi \) and stochastic functions \( \xi, \eta \) such that

\[
n_y = \Omega \phi_y(t) + \Omega^{1/2} \xi \\
n_z = \Omega \phi_z(t) + \Omega^{1/2} \eta
\] (2.25)

where \( \Omega \) is the volume. Expanding the step operators \( (\mathcal{E}_y = \exp(\Omega^{-1/2} \partial_\xi)) \) to linear order in \( 1/\Omega \) yields the following macroscopic equations

\[
\dot{\phi}_y = k_1 \phi_x - \alpha \phi_y \\
\dot{\phi}_z = k_1 \phi_x + k_2 \phi_y - \alpha \phi_z
\] (2.26)

and the following Fokker Planck equation for probability distribution \( \Pi_t(\xi, \eta) \):

\[
\frac{\partial \Pi}{\partial t} = \alpha \frac{\partial}{\partial \xi} (\xi \Pi) + \frac{\partial}{\partial \eta} [(\alpha \eta - k_2 \xi) \Pi] \\
+ (k_1 \phi_x + \alpha \phi_y) \frac{1}{2} \frac{\partial^2 \Pi}{\partial \xi^2} + (k_1 \phi_x + k_2 \phi_y + \alpha \phi_z) \frac{1}{2} \frac{\partial^2 \Pi}{\partial \eta^2}
\] (2.27)
The steady state macroscopic concentrations can easily be shown to balance fluxes for all reactions, and thus the macroscopic steady state is at equilibrium. The above allows us to compute stationary means and stationary variances, leading to the stated results:

\[ \mathbb{E}[Z] = \frac{k_1 n_x}{\alpha} \left( 1 + \frac{k_2}{\alpha} \right) \]
\[ \text{Var}[Z] = \frac{k_1 n_x}{\alpha} \left( 1 + \frac{k_2}{\alpha} + \frac{k_2^2}{2\alpha^2} \right) \]  

(2.28)

### 2.9.3 Marginal distribution of \( P(Z) \)

Here, we seek to find the marginal distribution of \( P(Z) \) for the coherent feedforward type 1 network for comparison with the slow switch rate limit. We assume that \( \mathbb{E}[K] \) is sufficiently large such that downstream species \( Y, Z \) equilibrate at timescales much faster than the equilibration times of a single molecule of \( X \). This sort of adiabatic approximation in \( Y, Z \) will make calculations more tractable, and allow us to demonstrate the behavior of both limits in \( \lambda \). We can then make the claim that the probability distribution of a downstream species such as \( Z \) is given by

\[ P(z) = \int P(z|K)P(K)\,dK \]  

(2.29)

where \( P(z|K) \) is obtained from the system size expansion for the reaction scheme in which \( X \) is constant. Because the copy number \( n_x \) is now fluctuating, and we know that the value of \( K \) depends on the current copy number of \( X \), we can compute the stationary distribution over \( K \) by the following:

\[ P(K) = \sum_{n_x} P(K|n_x)P(n_x) \]  

(2.30)

where we know that \( P(n_x) \sim \text{Poisson}(\gamma) \) by properties of the birth death process, with \( \gamma \equiv k_f/\alpha_x \). For purposes of calculation, we can choose a distribution over \( k_i \) such that the \( n_x \)-fold convolution is easy to compute, and we can write down the distribution of the sum of \( n_x \) random variables \( k_i \). To ensure that the \( k_i \) are purely positive, we use the square of a standard \( \mathcal{N}(0,1) \) normal random variable, such that \( P(K|n_x) \sim \chi^2_{n_x} \), the chi-squared distribution.

The distribution \( P(K = x) \) is then

\[
P(K = x) = \sum_{n=0}^{\infty} \left( \frac{\gamma^n}{n!} e^{-\gamma} \right) \frac{1}{2^{n/2}\Gamma(n/2)} x^{n/2-1} e^{-x/2} \]
\[
= \frac{1}{x} e^{-(\gamma+x/2)} \sum_{n=0}^{\infty} \frac{\alpha^n}{n! \Gamma(n/2)}, \quad \alpha \equiv \gamma \sqrt{\frac{x}{2}}
\]  

(2.31)
The difficult sum required to compute the marginalization can be written in terms of Hypergeometric functions by first splitting the sum into even and odd powers:

\[
\sum_{n=0}^{\infty} \frac{\alpha^n}{n! \Gamma(n/2)} = \frac{\alpha^2}{2} \left( \sum_{n=0}^{\infty} \frac{\alpha^{2n}}{(2n + 2)! n!(1/2)} \right) + \frac{\alpha}{\Gamma(1/2)} \left( \sum_{n=0}^{\infty} \frac{\alpha^{2n}}{\Gamma(2n + 2)(1/2)_n} \right)
\]  

(2.32)

where we have used the Pochhammer symbol:

\[
(\gamma)_k = \frac{\Gamma(\gamma + k)}{\Gamma(k)}
\]  

(2.33)

and the fact that for integer argument, \(\Gamma(n) = (n-1)!\). Using the fact that \(\Gamma(3/2) = \sqrt{\pi}/2\), \(\Gamma(2) = 1\), \(\Gamma(1/2) = \sqrt{\pi}\), and the following property of gamma functions:

\[
\Gamma(z)\Gamma(z + 1/2)2^{2z-1} = \sqrt{\pi}\Gamma(2z)
\]

(2.34)

we can rewrite the above as

\[
\frac{\alpha^2}{2} \sum_{n=0}^{\infty} \frac{(\alpha^2/4)^n}{(3/2)_n (2n)_n n!} + \frac{\alpha}{\sqrt{\pi}} \sum_{n=0}^{\infty} \frac{(\alpha^2/4)^n}{n!(3/2)_n (1/2)_n}
\]

(2.35)

The above sums represent the generalized Hypergeometric functions \(pF_q\), and thus we get the identity:

\[
\sum_{n=0}^{\infty} \frac{\alpha^n}{n! \Gamma(n/2)} = \frac{\alpha^2}{2} {}_0F_2\left([3/2, 2], \alpha^2/4\right) + \frac{\alpha}{\sqrt{\pi}} {}_0F_2\left([3/2, 1/2], \alpha^2/4\right)
\]

(2.36)

Therefore:

\[
P(K = x) = \frac{1}{x} e^{-(\gamma + x/2)} \left[ \frac{\gamma^2}{4} {}_0F_2\left([3, 1/2], \frac{x}{8}\right) + \gamma \sqrt{\frac{x}{2\pi}} {}_0F_2\left([3, 1/2], \frac{\gamma^2 x}{8}\right) \right]
\]

(2.37)

2.9.4 Details of the Ras-SOS simulation

Stochastic simulations were carried out inside a simulation box with an area of \(4 \mu m^2\) total volume of \(V = 0.08 \mu m^3\). We have chosen the volume in such a way that diffusion within the box is much faster than the time scales of the chemical reactions. Thus, we can assume the simulation box is well mixed. We assume that cytosolic species such as ligand can interact with the membrane bound species only when it has come within a confinement length of \(d = 1.7\) nm.

We allow each SOS enzyme to independently switch its catalytic rate with a parameter \(K(\text{switch})\) which depends on the allosteric occupancy. At switching times which are stochastically determined in the simulation, the enzyme redraws a catalytic rate from a distribution outlined below, chosen such that the average value is the value recorded in the literature.
Table 1: Reactions and rate constants used

Rates used in the table below follow from [32].

<table>
<thead>
<tr>
<th>Reaction</th>
<th>$k_{on}, \mu M^{-1}s^{-1}$</th>
<th>$k_{off}, s^{-1}$</th>
<th>$k_{cat}, s^{-1}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Allosteric binding: SOS( allo) + RasGDP $\rightleftharpoons$ SOS( allo)-RasGDP</td>
<td>.12</td>
<td>3.0</td>
<td>N/A</td>
</tr>
<tr>
<td>Allosteric binding: SOS( allo)+RasGTP $\rightleftharpoons$ SOS( allo)-RasGTP</td>
<td>.11</td>
<td>.4</td>
<td>3.6</td>
</tr>
<tr>
<td>Deactivation by RasGAP: RasGAP+RasGTP $\rightleftharpoons$ RasGAP-RasGTP $\rightarrow$ RasGAP+RasGDP</td>
<td>1.74</td>
<td>.2</td>
<td>.1</td>
</tr>
<tr>
<td>DAG binding of Rasgrpl: DAG+Rasgrpl $\rightleftharpoons$ Dag-Rasgrpl</td>
<td>5</td>
<td>5</td>
<td>1</td>
</tr>
<tr>
<td>Activation by Rasgrpl: DAG-Rasgrpl+RasGDP $\rightarrow$ DAG-Rasgrpl+RasGTP</td>
<td>.33</td>
<td>1.0</td>
<td>.01</td>
</tr>
<tr>
<td>Catalytic pocket reaction: SOS+RasGDP $\rightarrow$ SOS-RasGDP $\rightarrow$ SOS+RasGTP</td>
<td>.27</td>
<td>4.0</td>
<td>.0005</td>
</tr>
<tr>
<td>Catalytic pocket run when allosteric site filled: SOS( allo)-RasGDP+RasGDP $\rightarrow$ SOS( allo)-RasGDP-RasGDP $\rightarrow$ SOS( allo)-RasGDP+RasGTP</td>
<td>.07</td>
<td>1.0</td>
<td>See Table 2</td>
</tr>
<tr>
<td>Catalytic pocket run when allosteric site filled: SOS( allo)-RasGTP+RasGDP $\rightarrow$ SOS( allo)-RasGTP-RasGDP $\rightarrow$ SOS( allo)-RasGTP+RasGTP</td>
<td>.05</td>
<td>.1</td>
<td>See Table 2</td>
</tr>
</tbody>
</table>

Table 2a: $k_{cat}$ distribution, SOS( allo)-RasGDP

<table>
<thead>
<tr>
<th>Value ($s^{-1}$)</th>
<th>$k_{cat}^2$</th>
<th>$k_{cat}^3$</th>
</tr>
</thead>
<tbody>
<tr>
<td>.000545</td>
<td>.00545</td>
<td></td>
</tr>
</tbody>
</table>

Table 2b: $k_{cat}$ distribution, SOS( allo)-RasGTP

| Probability | .5 | .5 |
Kcat values are chosen such that the average kcat matches experimentally determined results from [32]. The two distributions differ in that when RasGTP is bound allosterically, the kcat distribution becomes heavier tailed, thus leading to a larger average catalytic rate.

### Table 3: Rate switching reactions included

In the table below, i and j denote an arbitrary indexing over the various catalytic states of either SOS(allo)-RasGDP or SOS(allo)-RasGTP. Switching reactions are treated as first order reactions. A transition into state SOS(i) occurs with a rate $K_{\text{switch}} \cdot P_i$ where $P_i$ is the stationary probability of finding SOS in state i (drawn from the distributions in Table 2a, Table 2b.). This is mathematically equivalent to each SOS enzyme switching its catalytic rate with $K_{\text{switch}}$ and then drawing a catalytic rate from kcat distribution listed above. $K_{\text{on}}$ and $K_{\text{off}}$ values referenced below are listed in Table 1 above. The value of $K_{\text{switch}}$ used has been varied between 1s$^{-1}$ and .01s$^{-1}$. In simulations for which the switching rate of SOS(allo)-RasGTP is slower than that of SOS(allo)-RasGDP, we have used a ten fold difference in switch times.

### Table 4: Concentrations used

These concentrations have been shown to demonstrate bistability when kcat fluctuation is not allowed [32].
Chapter Two References


CHAPTER TWO REFERENCES

Chapter 3

Transitions in genetic toggle switches driven by dynamic disorder

3.1 Introduction

In this chapter, we investigate the effects of dynamic disorder in a general toggle switch model with bistability [9, 10, 11, 12, 13]. As in the previous chapter, we will allow some of the parameters of the transition rates to fluctuate stochastically, drawing from a heavy tailed distribution $p(k)$. We will consider two types of stochasticity, one in which the parameters remain at a fixed value for an exponentially distributed time interval, governed by a switching rate $A[s^{-1}]$, and another in which the stochastic parameters follow a continuous diffusion process governed by an underlying stochastic differential equation. We see that by tuning the shape of the distribution $p(k)$ from which a particular rate constant samples, or by tuning the switching rate $A$, the equilibrium probability mass function (PMF) is significantly changed and therefore one stable basin can be greatly stabilized relative to the other. We have already considered the implications of experimental evidence showing that biological systems can in fact tune or manipulate both $A$ and $p(k)$ by enzymatic allosteric occupancy (section 2.7).

While Chapter 1 focused primarily on steady state behavior, this chapter demonstrates that dynamic disorder (and specifically allosteric control of the parameters of the model) can dramatically influence the mean first passage time for transition from one stable basin to the other. We find that the results are qualitatively unchanged when the stochastic rate parameter follows a Markov jump process as opposed to stochastic Langevin dynamics.
CHAPTER 3. TOGGLE SWITCH

3.2 Discrete space, discrete jumps

In genetic toggle switches, a pair of genes mutually repress each other[9, 10, 11]. A pair of genes encodes proteins $A$ and $B$ respectively, called transcription factors. Protein $A$ and $B$ in turn form homodimers $A_2$ and $B_2$ which bind to regulatory regions of DNA, called operators ($O_a$ and $O_b$), of the respective other gene. The binding of $A_2$ to the operator $O_b$ represses the production of protein $B$ and vice versa. We count the total copy numbers of the transcription factors $A$ ($N_A$) and $B$ ($N_B$) which include those in homodimers and those bound to the operators. Within a region in parameter space, the genetic toggle switches have two possible stable states: a state with a large number of $N_A$ and a small number of $N_B$, or the other state with small $N_A$ and large $N_B$. [12]

To incorporate dynamic disorder into the model, we investigate a model in which, due to fluctuating conformational changes, the unbinding rate of $A_2$ from the operator $O_b$ is not fixed but instead fluctuates. We study an asymmetric model in which only polymer $A_2$ has a fluctuating unbinding rate.

To illustrate the model reactions in the context of a very simple example, we begin with a relatively simple model where the unbinding rate of $O_bA_2$ samples from a discrete probability distribution, $P_1 = 0.1$, $P_2 = 0.8$ and $P_3 = 0.1$ depending on the conformation of $A_2$. In other words, there are three conformations of polymer $A_2$, $A_2^1$, $A_2^2$ and $A_2^3$ with different unbinding rates, and these homodimers switch among each other governed by a rule of transition probability matrix:

$$R = \begin{bmatrix}
A_2^1 & A_2^2 & A_2^3 \\
A_2^1 & R_1 & R_2 & R_3 \\
A_2^2 & R_1 & R_2 & R_3 \\
A_2^3 & R_1 & R_2 & R_3
\end{bmatrix}$$

where, $R_1 = 0.1$, $R_2 = 0.8$, $R_3 = 0.1$ (chosen with corresponding off rates such that the average value equals the unbinding of $B_2$).

We model the genetic toggle switches as a discrete Markov jump process with the reaction scheme in Table 3.1. The values of the rate constants are chosen based on Warren’s paper. [12] We assume that there is only one copy of the genome in the genetic toggle switch.

This three state model has been studied in [25] to illustrate optimal transition paths calculated using large deviations theory [14, 15, 16, 17, 18].
CHAPTER 3. TOGGLE SWITCH

<table>
<thead>
<tr>
<th>Reactions</th>
<th>$k_f$</th>
<th>$k_b$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Production of proteins</td>
<td>$O_a \rightarrow O_a + A$</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>$O_b \rightarrow O_b + B$</td>
<td>1</td>
</tr>
<tr>
<td>Degradation of proteins</td>
<td>$A \rightarrow \emptyset$</td>
<td>0.8</td>
</tr>
<tr>
<td></td>
<td>$B \rightarrow \emptyset$</td>
<td>0.8</td>
</tr>
<tr>
<td>Formation of dimers</td>
<td>$A + A \rightleftharpoons A^1_2$</td>
<td>$10P_1$</td>
</tr>
<tr>
<td></td>
<td>$A + A \rightleftharpoons A^2_2$</td>
<td>$10P_2$</td>
</tr>
<tr>
<td></td>
<td>$A + A \rightleftharpoons A^3_2$</td>
<td>$10P_3$</td>
</tr>
<tr>
<td></td>
<td>$B + B \rightarrow B_2$</td>
<td>10</td>
</tr>
<tr>
<td>Binding/unbinding to operators</td>
<td>$O_b + A^1_2 \rightleftharpoons O_bA^1_2$</td>
<td>10</td>
</tr>
<tr>
<td></td>
<td>$O_b + A^2_2 \rightleftharpoons O_bA^2_2$</td>
<td>4.625</td>
</tr>
<tr>
<td></td>
<td>$O_b + A^3_2 \rightleftharpoons O_bA^3_2$</td>
<td>10</td>
</tr>
<tr>
<td></td>
<td>$O_b + B_2 \rightarrow O_bB_2$</td>
<td>10</td>
</tr>
<tr>
<td>Switching among dimers</td>
<td>$A^1_2 \rightleftharpoons A^2_2$</td>
<td>$\lambda R_2$</td>
</tr>
<tr>
<td></td>
<td>$A^1_2 \rightleftharpoons A^3_2$</td>
<td>$\lambda R_3$</td>
</tr>
<tr>
<td></td>
<td>$A^2_2 \rightleftharpoons A^3_2$</td>
<td>$\lambda R_3$</td>
</tr>
<tr>
<td></td>
<td>$O_bA^1_2 \rightleftharpoons O_bA^2_2$</td>
<td>$\lambda R_2$</td>
</tr>
<tr>
<td></td>
<td>$O_bA^1_2 \rightleftharpoons O_bA^3_2$</td>
<td>$\lambda R_3$</td>
</tr>
<tr>
<td></td>
<td>$O_bA^2_2 \rightleftharpoons O_bA^3_2$</td>
<td>$\lambda R_3$</td>
</tr>
</tbody>
</table>

Table 3.1: Reaction Scheme of Genetic Toggle Switches with Three Isomers of $A_2$

i $k_f$ and $k_b$ are the forward and backward rate constants of the corresponding reactions.

ii $P_{1,2,3}$ are the distribution probabilities of three polymers $A_2$

iii $R_{1,2,3}$ are the switching probabilities of three polymers $A_2$

iv $\lambda$ is the switching rate

3.3 Continuous Space Model

3.3.1 Discrete Jumps

We now extend the simple genetic toggle switch model to a more complex one where the unbinding rate samples from a continuous heavy tailed distribution instead of a discrete distribution. We begin by investigating the behavior of this toggle switch network in the small copy number limit using the Gillespie algorithm [19, 20, 21]. We first carry out the simulations for a model of dynamic disorder in which individual rate parameters remain at one value for a random, exponentially distributed amount of time (parameterized by a switching rate $\lambda [s^{-1}]$), before
redrawing a value from a prescribed distribution. Following experimental evidence which shows fluctuating rate constants draw from heavy tailed distribution [3, 5, 6], we allow the rate constants to sample from a log normal distribution. We carry out these simulations with only one copy of genome in the simulation box like the discrete distribution case in the last section. For this model, the state space is continuous as an individual rate constant samples from a continuous probability distribution, but the jumps are discrete.

Because of the fluctuating conformational changes of polymer $A_2$, the unbinding rate of $A_2$ to the operator $O_b$ samples from a lognormal distribution. We fix the mean value of the lognormal distribution at a value known to yield a bistable network, and look at the effect of changing the variance/skewness of the distribution. For the lognormal distribution, changing the variance while keeping the mean fixed requires us to change both the $\mu$ and $\sigma$ parameters of the exponentiated normal. We first investigate a model in which the fluctuation in rate parameter occurs only when the dimer $A_2$ is unbound from the operator $O_b$, but after dimer $A_2$ binds to the operator it is fixed in one conformational change.

Throughout, the average value that an individual rate constant draws from is chosen to be 5. We find that, upon increasing the standard deviation in the distribution that a rate constant $k$ chooses from over a range from less than one to ten, the mean first passage time to transition from a state of high $A$ to low $A$ (including those in homodimers and those bound to the operators) is significantly increased (Figure 3.1a).

The physical explanation behind the dramatic increase in mean first passage time is that, as we increase the standard deviation of the underlying lognormal distribution that an individual rate parameter draws from, we change the skew of the distribution. The consequence is that the most probable value (mode) of the probability distribution an off rate of $A_2$ is drawn from decreases to lower and lower values. This in turn means that a polymer $A_2$ will remain bound for a longer time on average, and thus does a better job at repressing the production protein $B$.

Mathematically, if we specify a mean $\mathbb{E}[k]$ and variance $\text{Var}(k)$ for an individual off rate $k$, we determine the parameters of the lognormal distribution by the inversion

$$
\sigma = \sqrt{\ln \left( \frac{\text{Var}(k)}{\mathbb{E}[k]^2} + 1 \right)}, \quad \mu = \ln(\mathbb{E}[k]) - \frac{\sigma^2}{2}
$$

The parameter $\sigma$ increases monotonically with $\text{Var}(k)$ and the skewness of the lognormal distribution (given by $(e^{\sigma^2} - 1) \sqrt{e^{\sigma^2} - 1}$) grows monotonically with $\sigma$. The parameter $\mu$ decreases monotonically with increasing $\sigma$, and the mode of the lognormal distribution, given by $e^{\mu - \sigma^2}$ decreases monotonically with $\text{Var}(k)$. Therefore, mathematically we shift the most probable values of the off rate to values that are much lower than the average $\mathbb{E}[k]$.

As expected, initializing the system in the low $A$ basin (using Monte Carlo sampling of the
Figure 3.1: a). Here we plot the mean first passage time to transition from a state of high A to a state of low A. The stable basins are found by Monte Carlo simulation, and we use \( A = 0, B \geq 7 \) for low A state and \( B = 0, A \geq 7 \) for high A state. The x axis is the standard deviation in the lognormal distribution for k, not the parameter \( \sigma \) of the lognormal. b). Here we plot the mean first passage time to transition from a state of low A to a state of high A as a function of increasing the standard deviation in the distribution of off rates for an individual polymer A from the operator. The trend is the reverse of that from Figure 3.1a.

Indeed, studying the probability mass function (PMF) for the toggle switch model with dynamic disorder shows that the basin of low A and high B is destabilized upon increasing the variance in the off rate of an individual polymer \( A_2 \). (Figure 5.7).

Of course, for a model in which the polymer does not switch when it is bound to the operator, we cannot see much interesting behavior with respect to the parameter \( \lambda \). As long as the average time for an individual polymer to rebind is larger than \( 1/\lambda \), the polymer will on average have resampled its kinetic off rate before rebinding. Upon increasing \( \lambda \) any further, the statistics of the network should be unchanged since it will not matter whether the polymer has resampled its kinetic off rate once or multiple times.

To examine a model that is more sensitive to \( \lambda \), we simulated the same network but instead let the polymer conformation fluctuate when it is bound to the operator. What we see in this case is also fairly intuitive. When the switching rate is very low and \( \sigma \) is large, a polymer that has just sampled a kinetic off rate that is far below the average value will get frozen at that
Figure 3.2: Joint PMF’s for the (number of A, number of B) molecules are plotted here (for $\lambda = 1.0$). These PMF’s are plotted for the model in which kinetic off rates vary continuously on the DNA (though the location of peaks is unchanged for the discrete model). By increasing the value of $\text{var}(k)$ of the underlying stochastic process, we see that the stable basin corresponding to high B and low A is destabilized. The colors vary between 0 and .08. These are calculated by running a single trajectory of simulation length $10^5$ seconds with a time increment of $dt = 10^{-3}$ and sampling every 1 second.

particular value for the duration of its time on the operator. It will then, on average, remain bound for a long time and do a better job at inhibiting the production of protein B. If we increase the switching rate $\lambda$, we expect that the system will begin to behave as if it was acting at its average value. This can be shown in more mathematically precise language using renewal theory [22]. Therefore, as we increase the switching rate $\lambda$, the mean first passage time for transition from high A to low A decreases. (Figure 4.8).

### 3.3.2 Continuous Jumps

To demonstrate that the results are robust to the type of model we consider, we also look at a continuous model in which the kinetic off rate evolves continuously following a Langevin dynamics of the following form:

$$k_t = \exp(\tilde{k}_t), \quad \text{d}\tilde{k}_t = \lambda(\mu - \tilde{k}_t)\text{d}t + \left(\sqrt{2\sigma^2}\right)\text{d}Z_t \quad (3.2)$$

That is, the rate parameter is an exponential of an Ornstein-Uhlenbeck (OU) process. We let $\tilde{k}_t$ denote the value of the OU process at time $t$, and $k_t$ the value of the actual kinetic parameter. This process is chosen since the steady state distribution of the OU process is a normal distribution, and thus the stationary distribution of the $k_t$ is lognormal, which will mimic the stationary distribution sampled in the previous model. The value $\lambda$ plays functionally
CHAPTER 3. TOGGLE SWITCH

Figure 3.3: Mean first passage times to transition from $A \geq 7, B = 0$ to $A = 0, B \geq 7$ are computed for various values of $\lambda$ and $\text{Var}(k) = 10, 25$. We do the calculation for both a discrete jump model and a continuous jump model, labeled DJCS,CJCS respectively. In these parameter regimes, the mean first passage times are comparable for both models. Importantly, increasing $\lambda$ allows faster transitions as the system behaves closer to the mean value. Increasing $\text{Var}(k)$ increases the skewness and therefore decreases the MFPT. For the $\lambda = 10^3$ value in the continuous jump model, a time discretization of $10^{-4}$ is used.
the same role as it did in the previous case. Though it does not appear in the overall stationary distribution of the OU process, it does modulate how rapidly the value \( k_t \) changes in an instant of time \( dt \).

Simulating the above process is much more computationally expensive. Now, we must discretize time by picking a \( dt \) such that the sum of all rates for various reaction events in the continuous time Markov process simulated by the Gillespie process above is small relative to \( dt \). Let the rate for reaction event \( i \) be \( \rho_i \). Then, in an instant of time \( dt \), we first ask whether a reaction occurred or not, where the probability for the reaction to occur is the cumulative distribution function (CDF) of the exponential distribution with parameter \( \sum_i \rho_i \). If a reaction did occur, we choose its value from the multinomial defined by the \( \rho_i \). Importantly, \( dt \) must be sufficiently small such that the probability for more than one reaction event to occur is essentially zero.

We find qualitatively the same results as before. Increasing the value of \( \text{Var}(k) \) at a fixed \( \lambda \) will increase the mean first passage time from high \( A \) to low \( A \), while increasing \( \lambda \) at a fixed value of \( \text{Var}(k) \) will decrease the mean first passage time from high \( A \) to low \( A \) (Figure 4.8). Quantitatively, the mean first passage time for both the continuous and discrete model is very similar, with the continuous model appearing to have a slightly larger MFPT for low \( \lambda \). We explore this in more detail in section 3.5.1.

### 3.4 Discussion

In this chapter, we have studied how kinetic switching between stable states in biological networks is influenced by dynamic disorder or conformational fluctuations in the rate coefficients. We carried out simulation and analysis based on a general genetic toggle switch model. In the model, the unbinding rate of polymers from the operators fluctuates with the switching rate, \( \lambda \).

We considered the genetic toggle switch model in which the switching rate draws from a continuous heavy tailed distribution. For this case, we have modeled the system first as a discrete Markov jump process and then under continuous Langevin dynamics. Both models produce qualitatively similar results. Under the condition of fixed switching rate \( \lambda \), the mean first passage time from high \( A \) to low \( A \) increases with the variance \( \text{Var}(k) \) in the distribution of off rates for an individual polymer \( A_2 \) from the operator. On the other hand, when we fix the variance \( \text{Var}(k) \), the mean first passage time from high \( A \) to low \( A \) decreases as the switching rate \( \lambda \) increases.

Overall, the regulation of the parameters modulating the dynamic disorder plays an important role in shaping the statistics of optimal transition paths, transition probabilities and the stationary probability distribution of the network.
Thus far, we have considered the effects that fluctuating parameters can have on the stationary distribution, entropy production, and mean first passage time transitions of continuous time Markov processes. In the next chapter, we will rephrase the question in the context of statistical inference. Namely, we will ask how one can make statistical inferences about the distribution of values that a kinetic rate parameter draws from based on observation of a time series of a stochastic process.

### 3.5 Supplemental Information

#### 3.5.1 Time correlation functions for discrete and continuous models

As was shown in the main text, both the discrete and continuous models have quantitatively very similar mean first passage times. Here, we compare the two models further to understand why they behave so similarly. To compare the discrete and continuous models further, we will look here at the time correlation function \( C(k_t, k_0) \) calculated as the correlation between the kinetic parameter \( k_t \) and the time 0 value \( k_0 \). The continuous model follows an exponential of an OU process. We denote the OU process by \( \tilde{k}_t \) such that \( k_t = \exp(\tilde{k}_t) \). The transition density for the OU process is given by

\[
\begin{align*}
    f(\tilde{k}_t, \tilde{k}_0) &= \frac{1}{\sqrt{2\pi \sigma^2 (1 - e^{-2\lambda t})}} \exp \left\{ -\frac{1}{2\sigma^2} \left( \frac{(x - \mu(1 - e^{-\lambda t}) - \tilde{k}_0 e^{-\lambda t})^2}{1 - e^{-2\lambda t}} \right) \right\} \\
    &= \mathcal{N} \left( \mu(1 - e^{-\lambda t}) + \tilde{k}_0 e^{-\lambda t}, \sigma^2(1 - e^{-2\lambda t}) \right) \\
    &\equiv \mathcal{N}(\mu(t), \sigma(t)^2) 
\end{align*}
\]

(we will make use of the time dependent mean and variance of the \( \tilde{k}_t \) later in calculating the correlation function). The stationary density of \( \tilde{k}_0 \) is of course \( \mathcal{N}(\mu, \sigma^2) \). Using the mean and variance of a lognormal distribution allows us to calculate the time correlation function as

\[
C(k_t, k_0) = \frac{\mathbb{E}[\exp(\tilde{k}_t) \exp(\tilde{k}_0)] - \mathbb{E}[\exp(\tilde{k}_0)]^2}{\text{Var}(\exp(\tilde{k}_0))} \\
= \frac{\mathbb{E}[\exp(\tilde{k}_t) \exp(\tilde{k}_0)] - e^{2\mu + \sigma^2}}{(e^{\sigma^2} - 1)e^{2\mu + \sigma^2}} \\
\]

(3.4)

Note that we can actually calculate the integral \( \mathbb{E}[e^{\tilde{k}_t} e^{\tilde{k}_0}] \) making use of a simple identity which can be proven easily by completing the square. For any random variable \( x \sim \mathcal{N}(\mu, \sigma^2) \) and any constant \( \lambda \), one can show that

\[
\mathbb{E}[e^{\lambda x}] = e^{\lambda \mu + \lambda^2 \sigma^2 / 2} \\
(3.5)
\]
Breaking the integral up and carrying out the \( \tilde{k}_t \) integrals first we get

\[
\mathbb{E}[e^{\tilde{k}_t \tilde{k}_0}] = \int \left( \int e^{\tilde{k}_t N(\tilde{k}_t; \mu(t), \sigma(t)^2)} d\tilde{k}_t \right) e^{\tilde{k}_0 N(\tilde{k}_0; \mu, \sigma^2)} d\tilde{k}_0 \\
= \exp \left\{ \mu(1 - e^{-\lambda t}) + \frac{\sigma^2 (1 - e^{-2\lambda t})}{2} \right\} \int e^{\tilde{k}_0 (\exp(-\lambda t) + 1) N(\tilde{k}_0; \mu, \sigma^2)} d\tilde{k}_0 \\
= \exp \left\{ \mu(1 - e^{-\lambda t}) + \frac{\sigma^2 (1 - e^{-2\lambda t})}{2} + \mu(e^{-\lambda t} + 1) + \frac{\sigma^2 (e^{-\lambda t} + 1)^2}{2} \right\} \\
= \exp \left\{ 2\mu + \sigma^2 + e^{-\lambda t} \sigma^2 \right\}
\]

(3.6)

This leads to the simple expression for the time correlation function given by

\[
C(k_t, k_0) = \frac{\exp \{ e^{-\lambda t} \sigma^2 \} - 1}{e^{\sigma^2} - 1}
\]

(3.7)

which we will denote by \( C_{OU}(t) \).

We now compare the above model with the discrete jump model in which each rate \( k_t \) remains at its value for a random exponentially distributed interval and then redraws from a prescribed distribution. In this case, the time correlation function is very simple to compute and does not depend on the parameters of the distribution, which we call below \( \mathbb{E}[k], \mathbb{E}[k^2] \):

\[
\mathbb{E}[k_t k_0] = \mathbb{E}_{k_0} [e^{\lambda t} \mathbb{E}[k|k_0] k_0] \\
= \mathbb{E}_{k_0} [k_0 e^{-\lambda t} + \mathbb{E}[k] (1 - e^{-\lambda t})] \\
= \mathbb{E}[k^2] e^{-\lambda t} + \mathbb{E}[k]^2 (1 - e^{-\lambda t})
\]

(3.8)

This demonstrates that the time correlation function of this behavior is \( C(k_t, k_0) = e^{-\lambda t} \). For small \( \sigma^2 \), the exponentiated OU correlation function approaches

\[
\frac{1 + e^{-\lambda t} \sigma^2 - 1}{1 + \sigma^2 - 1} = e^{-\lambda t}
\]

(3.9)

and the two correlation functions are approximately equal. We expect the most difference between the two processes for large \( \sigma^2 \), when the OU correlation function is

\[
\frac{\exp\{e^{-\lambda t} \sigma^2\}}{e^{\sigma^2}} = \exp\{\sigma^2 (e^{-\lambda t} - 1)\}
\]

(3.10)

and the correlations die off with a large magnitude negative exponent for the exponentiated OU process. The results are intuitive. Because the correlation function for the continuous process is a double exponential in \( \lambda \), it drops off more rapidly. For small values of \( \lambda \), we know that the discrete model will get frozen at a single parameter value for the duration of its dwell time, while the continuous model will still explore some of the surrounding values of its probability.
CHAPTER 3. TOGGLE SWITCH

distribution $p(k)$. This explains some of the differences seen in the mean first passage times from the continuous process and the discrete process (Figure 4.8).

To explain why the stochastic processes in the kinetic off rate do not lead to significantly big differences between the MFPT behavior for both networks (even at large values of $\sigma$ when the above analysis demonstrates the two models to be most different), we compute the average dwell time for a polymer following both above behaviors (by Monte Carlo sampling). For each sample, we initialize the system with a single polymer on the operator and draw the initial kinetic parameter from the lognormal with $\mu, \sigma$. For the exponentiated OU, we discretize time and in each instant $dt$ update the value of $k_t$, and also ask if unbinding occurred in the last increment, which does so with probability $1 - e^{-k_t dt}$.

For the jump Markov process, we simply simulate the continuous time Markov process. At any instant in time $t$ when the parameter is $k_t$, there are two competing exponentials: unbinding which occurs with rate $k_t$, and parameter switching which occurs with rate $\lambda$. We use the Gillespie method, and redraw the value of $k_t$ when the reaction event ‘rate switching’ occurs. We see that the expected dwell time is essentially the same for both models in the parameter regime that is shown to give bistability (Figure 4.10).

![Figure 3.4: We compute the expected dwell time of a polymer A on the operator for the continuous (labeled Exp. OU) and discrete model (labeled Standard) by Monte Carlo sampling. We find that the expected dwell times are comparable in both processes, which explains why the mean first passage times computed in the main text are similar for both models.](image-url)
Chapter Three References


58


Chapter 4

Detecting latent enzyme states from time series data

4.1 Introduction

The experimental evidence described earlier to characterize dynamic disorder was obtained through single molecule studies in which an individual enzyme was tethered to a surface and inundated with a high concentration of substrate. The macroscopic rate equations were studied and by observing what appeared to be discontinuous jumps in the underlying rate constant, a histogram of enzyme states was created. However, how can one do inference on the underlying enzyme state in the small (or mesoscopic) copy number limit? Here we describe a frequentist and bayesian approach to studying the latent enzyme state given a time series of copy number. Our toy example will be a simple birth death process $0 \rightarrow X$ in which the rate of X formation fluctuates. We will assume that one has a time series of $N_X(t)$, and can therefore isolate the arrival process of $X$. This resembles the mathematical framework of Markov Modulated Poisson Processes.

The strength in this inference approach is that we will be able to estimate transition probabilities between various enzyme states while simultaneously estimating the hidden enzyme state itself. It will also allow us to extend methods of model selection (such as the information criterion) to estimate how many hidden enzyme states there are. At the end of this chapter, we will speculate upon ways to extend this framework to more complex reaction networks.
CHAPTER 4. LATENT ENZYME STATES

4.2 EM algorithm for protein interaction networks

We will begin by making a simplifying assumption of discrete time periods (relaxed later) and a single enzyme. In particular, we will assume that the underlying Z(t) time series is discretized, and the enzyme occupies only one state in each time period (e.g. switching events happen at the very conclusion of a time period, henceforth called an 'epoch'). In this framework, we have a sequence of N latent or hidden Z enzyme states. In each epoch, the number of X formation events is Poisson with a parameter \( \lambda_{zi} \), the enzyme arrival rate in this current epoch. We assume 'K' hidden enzyme states. The parameters of the model are \( \theta = \{ q, \lambda \} \), where \( q \in \mathbb{R}^{K \times K} \) is a transition matrix between hidden enzyme states, and \( \lambda \in \mathbb{R}^{K} \) is a vector whose entries are the arrival rates in each of the K states. We will sometimes speak of \( P(Z_i = k) \), where little case \( k \) indexes one of the states in the vector \( \lambda \), and \( \lambda_k \) refers to the \( k^{th} \) entry of \( \lambda \) vector, while notation such as \( P(Z_{i|Z_{i-1}}) \) refers to transition probabilities between the enzyme state at time \( i-1 \) and \( i \), but for ease of notation drops the explicit reference to state \( k \).

First, the joint distribution of \( X, Z \) parametrized by \( \theta = \{ q, \lambda \} \) is

\[
P(X, Z|\theta) = P(Z_0)P(X_0|Z_0) \prod_{i} P(Z_i|Z_{i-1})P(X_i|Z_i)
\]

\[
= P(z_0) \frac{e^{-\lambda_{z_0}} \lambda_{z_0}^{x_0}}{x_0!} \prod_{i=1}^{N} q(z_{i-1} \rightarrow z_i) \frac{e^{-\lambda_{z_i}} \lambda_{z_i}^{x_i}}{x_i!}
\]

(4.1)

Of course, if the state \( Z \) was observable, maximum likelihood estimation of all the parameters would be straightforward. However, this is not possible, and we instead resort to the iterative EM algorithm for calculating maximum likelihood estimations when there is hidden data. In the EM framework, one seeks to solve:

\[
\partial_{\theta} \log \int P(X, Z|\theta) dZ = 0
\]

(4.2)

In other words, we seek to do maximum likelihood estimation of \( \theta \) on a marginal log likelihood (after integrating out unknown variables \( Z \)). In principle, one can resort to quasi Newton methods to solve the above problem, but the EM provides an iterative approach that avoids use of optimizers [1]. It follows from the following set of inequalities: (below, we introduce an arbitrary probability distribution \( Q(Z) \) over the hidden variables):

\[
\log \int P(X, Z|\theta) dZ = \log \int \frac{P(X, Z|\theta)}{Q(Z)} Q(Z) dZ
\]

\[
= \log \mathbb{E}_Q \left[ \frac{P(X, Z|\theta)}{Q(Z)} \right]
\]

\[
\geq \mathbb{E}_Q \left[ \log \frac{P(X, Z|\theta)}{Q(Z)} \right]
\]

(4.3)
(where the third line holds by Jensen’s inequality). One can see that we achieve the upper bound between second and third line by setting \( Q(Z) = P(Z|X, \theta) \). This immediately implies the following iterative scheme. Initialize some \( \theta^0 \). Let \( P^t(Z) \equiv P(Z|X; \theta^t) \). Calculate \( \mathbb{E}_{P^t}[\log P(X, Z|\theta)] \) and optimize with respect to \( \theta \) to find the new \( \theta^{t+1} \).

Typically, the quantity \( \mathbb{E}_{z|x,\theta^t}[\log P(X, Z|\theta)] \) is referred to as \( Q(\theta, \theta^t) \).

Let \( p^{(t)}(z_i = k) \equiv p(z_i = k|X, \theta^t) \) and \( p^{(t)}(z_i = k, z_{i-1} = k') \) defined the same way. In the E-step, we calculate the following:

\[
Q(\theta, \theta^t) = \mathbb{E}_{z|x,\theta^t}[\log P(X, Z|\theta)] = \sum_{i=0}^{N} \left\{ \sum_{k=1}^{K} (-\lambda_k + X_i \log \lambda_k) p^{(t)}(Z_i = k) \right\} \\
+ \sum_{i=1}^{N} \left\{ \sum_{k,k'} p^{(t)}(z_i = k, z_{i-1} = k') \log Q(k' \rightarrow k) \right\} \quad (4.4)
\]

In the M-step, we maximize the expectation above with respect to parameters. The updates are:

\[
\lambda_k^{t+1} = \frac{\sum_i X_i \cdot p^{(t)}(z_i = k)}{\sum_i p^{(t)}(z_i = k)} \\
q^{t+1}(k' \rightarrow k) = \frac{\sum_{i=1}^{N} p^{(t)}(z_{i-1} = k', z_i = k)}{\sum_{i=0}^{N-1} p^{(t)}(z_i = k')} \quad (4.5)
\]

We note that each iteration requires calculation of \( p(Z|X, \theta^{(t)}) \). In particular, we must compute pairwise and single node marginal probability tables over the latent enzyme state. We accomplish this using a message passing algorithm to calculate pairwise and single node marginal probabilities. In particular, if you have a probability distribution that factorizes in the following way:

\[
P(Z) \propto \prod_i \phi(z_i) \prod_{i,j} \psi(z_i, z_j) \quad (4.6)
\]

Define a message that node \( i \) sends to node \( j \) as:

\[
m_{i \rightarrow j}(z_j) = \sum_{z_i} \phi(z_i) \psi(z_i, z_j) \prod_{k \in N(i) \setminus j} m_{k \rightarrow j}(z_j) \quad (4.7)
\]

The messages we see are self-consistently defined by the above equations. Once one obtains them, the marginal probability of a single node variable \( z_i \) is given by

\[
p(z_i) \propto \phi(z_i) \prod_{k \in N(i)} m_{k \rightarrow i}(z_i) \quad (4.8)
\]

For tree-like graphs (such as the hidden markov model), one can solve the messages in a two pass procedure. For us, the single node functions are \( \phi(z_i) = p(x_i|z_i; \theta^{(t)}) \). The pairwise
CHAPTER 4. LATENT ENZYME STATES

potentials are \( \psi(z_i, z_{i+1}) = Q(z_i \rightarrow z_{i+1}) \). The two pass procedure is to first compute forward messages:

\[
m_{(i-1)\rightarrow i}(z_i) = \sum_{z_{i-1}} \phi(z_{i-1}) \psi(z_{i-1}, z_i) m_{(i-2)\rightarrow (i-1)}(z_{i-1})
\]

(4.9)

and then the reverse messages:

\[
m_{(i+1)\rightarrow i}(z_i) = \sum_{z_{i+1}} \phi(z_{i+1}) \psi(z_i, z_{i+1}) m_{(i+2)\rightarrow (i+1)}(z_{i+1})
\]

(4.10)

The messages are tuples in \( \mathbb{R}^K \) where \( K \) is the dimensionality of \( z_i \), the number of latent states of the enzyme. Marginal probabilities are then \( p(z_i) \propto m_{i-1\rightarrow i}(z_i) m_{i+1\rightarrow i}(z_i) \phi(z_i) \). Similarly, pairwise probabilities are

\[
p(z_i, z_{i+1}) \propto m_{i-1\rightarrow i}(z_i) m_{i+2\rightarrow i+1}(z_{i+1}) \phi(z_i) \phi(z_{i+1}) \psi(z_i, z_{i+1})
\]

(4.11)

The pairwise probabilities are tables in \( \mathbb{R}^{K \times K} \). For graphical networks not organized in tree-structures, one can solve for the fixed points of the message passing equations by iterative updates.

We test the algorithm and implementation on toy data generated from the following model: let \( k = 3 \), \( N = 1000 \) and \( \lambda = [1, 10, 20] \). We use the transition matrix

\[
q = \begin{bmatrix}
.1 & .2 & .7 \\
.4 & .2 & .2 \\
.2 & .2 & .6
\end{bmatrix}
\]

(4.12)

With our convention, the stationary distribution is a left eigenvector. We initialize the EM algorithm with \( \lambda^0 = [1, 2, 3] \) and a uniform transition matrix. The EM algorithm converges in fewer than 50 steps. In Figure 7.1 we show the estimates of the entries of \( \lambda \) as a function of EM iteration, while Figure 7.2 shows the estimates of the first row of the transition matrix as a function of EM iteration.
Figure 4.1: Here we plot current estimates of the three entries of $\lambda$ as a function of EM iteration. Dotted horizontal lines indicate the true parameters used in the data generation process.
Figure 4.2: Here we plot current estimates of the first row of the transition matrix $q$ as a function of EM iteration. Dotted horizontal lines indicate the true parameters used in the data generation process.

For the purposes of model selection, we know that when the algorithm has converged, the quantity

$$\mathbb{E}_{x|\theta^*} \left[ \log \frac{p(X,Z; \theta^*)}{p(Z|X, \theta^*)} \right] = Q(\theta^*, \theta^*) + H(Z) \approx \log P(X; \theta^{mle}) = \mathcal{L}(\theta^*)$$

(4.13)

will have converged to $\log p(x; \theta^*)$. This log likelihood can then be used to compute information criteria such as the BIC or AIC for purposes of model selection. The quantity $Q(\theta^*, \theta^*)$ was calculated in iterations of the EM algorithm. The notation $\mathcal{L}(\theta^*)$ refers to the log likelihood evaluated at maximum likelihood parameter estimate. To calculate $H(Z)$, we compute

$$H(Z) = - \sum_{z_0} p(z_0) \log p(z_0) - \sum_{z_i, z_{i+1}} p(z_i, z_{i+1}) \log p(z_{i+1}|z_i)$$

(4.14)
The Bayes and Aikaike information criterion (BIC, AIC) are methods of model selection [1]. Both represent a trade off between quality of fit and the tendency for high dimensional models to over fit. Unlike cross validation metrics which estimate the out of sample error (as defined by Hastie, chapter 7), information criteria estimate the in sample performance error, and are parsimonious in that they allow a fit on the entire data set. For our model, the number of parameters is $k + k(k - 1) = k^2$ (where each row of the transition matrix only has $k - 1$ free parameters). The AIC/BIC are calculated by

$$AIC = \frac{\mathcal{L}(\theta)}{N} - \frac{k^2}{N}$$

$$BIC = \frac{\mathcal{L}(\theta)}{N} - k^2 \log \frac{N}{N} \quad (4.15)$$

Figure 5.3 demonstrate that both information criteria are optimized by $K = 3$, the true dimensionality of hidden enzyme state.

Figure 4.3: Both the AIC and BIC correctly select 3 hidden states.
4.3 Gibbs sampler for protein interaction networks

The EM algorithm is conducive to calculating maximum likelihood parameter estimates in spite of hidden data. A Gibbs sampler approach can be used in the Bayesian framework to sample from the posterior distribution over parameters given hidden data. The Gibbs sampler samples from marginal distributions of single nodes in graphical probabilistic models. In particular, initialize all the nodes, then sample from one node $P(x_i|X_{-i})$, where $X_{-i}$ indicates all nodes except for $x_i$. Then, pick another node $j$ to update, and sample from $P(x_j|X_{-j})$, etc. For the hidden Markov model outlined above, we can imagine a graphical model with nodes consisting of $Z,X,\theta$. Given $\theta$ and $X$, we have outlined how we can use a message passing algorithm to calculate the distribution $P(Z|X,\theta)$, or in particular single node marginals $P(z_i|X,\theta)$ and pairwise tables $P(z_i, z_{i+1}|X,\theta)$. We can sample from this distribution by sampling $P(z_0|X,\theta)$ (multinomial), and then sequentially sampling from $z_{i+1} \sim P(z_i, z_{i+1}|X,\theta)/P(z_i)$.

Given a $Z, X$, we now determine a method for sampling from $\theta \sim P(\theta|Z, X)$. We will use the powerful approach of conjugate priors. For the Poisson parameters, we choose gamma priors, and for each row of the transition probability matrix we will use a Dirichlet prior (the prior to a multinomial distribution). The posteriors are easy to update and we can sample from them easily. In this way, we sample from the posterior by iteratively calculating $Z^t \sim P(Z|X, \theta^t)$, and $\theta^{t+1} \sim P(\theta|Z^t, X)$. Figures 4.4 and 5.5 show posterior distributions estimated by 100 samples from the Gibbs sampler (smoothed using a kernel density estimator).

Figure 4.4: A, B, C demonstrate the posterior distribution of $\lambda$ entries sampled by a Gibbs sampler, using conjugate priors. The true values used were 1, 10 and 20 respectively.
CHAPTER 4. LATENT ENZYME STATES

Figure 4.5: A,B,C demonstrate the posterior distribution of $q_{0 \rightarrow i}$, the first row of the transition probability matrix, sampled by Gibbs sampling using conjugate priors. The true values used were .1,.2 and .7 respectively.

4.4 Continuous switch times

Here, we develop a Gibbs sampler for a more realistic example in which the underlying latent enzyme state is following a continuous markov process. We treat as hidden variables $Z = (\{z_i\}_{i=0}^N, \{t_i\}_{i=1}^N, N)$ the number of switching events $N$ for the enzyme conformation, the arrival times for the switching events ($t_i$ distributed across the interval $[0, T]$) and the actual latent state of the enzyme $z_i$ in each inter-switch epoch (note the difference in index for the enzyme state and the switch-event times). Further, we treat as observed data $X = \{X_i\}_{i=1}^{N_x}$, where $X_i$ represents the time of the $i^{th}$ formation of X species, and $N_x$ represents the total number of X formations on the intervals $[0, T]$. Further, given a set of switch times $t_i$ and $\{X_i\}$, we can construct the statistic $\{N_i\}_{i=0}^N$ where $\sum_i N_i = N_x$ and each $N_i$ refers to the total number of X arrivals in a certain inter-switch epoch.

Each iteration of the Gibbs sampler will require sampling $Z|X, \theta$. To this end, we propose an MCMC Metropolis Hastings algorithm for sampling from $Z|X, \theta$. Note that

$$P(Z|X, \theta) \propto P(X|Z, \theta)P(Z|\theta)$$

$$= \left( \prod_{i=0}^N \lambda_{z_i}^{N_i} e^{-\lambda_{z_i} \delta_i} \right) \left( \prod_{i=1}^N q(z_{i-1} \rightarrow z_i) \right) \left( \lambda^N e^{-\lambda T} \right) \tag{4.16}$$

where we have used the fact that $\lambda^N e^{-\lambda T}$ is the joint density for $N$ arrivals and $\{t_i\}$ arrival times in the interval $[0, T]$ when the jump rate is $\lambda$. We use the notation that $\lambda_{z_i}$ is the arrival rate when latent enzyme state is $z_i$, and $\delta_i$ is the inter-switch epoch time length for latent state $z_i$.

For the Gibbs sampler, given the current state $Z$, we choose next state $Z'$ from a proposal
distribution \( Q(Z \rightarrow Z') \), and accept with probability

\[
\min \left\{ 1, \frac{P(Z'|X, \theta)Q(Z' \rightarrow Z)}{P(Z|X, \theta)Q(Z \rightarrow Z')} \right\}
\]

(4.17)

The ratio of \( P(Z|X, \theta) \) becomes simply the ratio of \( P(X|Z, \theta)P(Z|\theta) \). Below, we outline the proposal distribution we use, and also for each type of transition \( Z \rightarrow Z' \), the ratio \( Q(Z' \rightarrow Z)/Q(Z \rightarrow Z) \) and \( P(X|Z')P(Z')/P(X|Z)P(Z) \).

Given a current state \( Z \), we choose a next state \( Z' \) uniformly from the following set of simple rules. With a uniform probability of \( 1/4 \), we either add an arrival time, delete an arrival time, take one existing arrival time and place it uniformly over the interval \([0, T]\), or take an existing state of \( z_i \) and change it.

- When adding an arrival time, we will create a new inter-switch epoch \( z_i \). In other words, the old epoch becomes split into two. With equal odds, we choose the left or right epoch and uniformly choose a new state from the possible set of \( \lambda \) (the other epoch will be left in the same state as the original larger epoch was to begin with). Thus, the transition probability for \( Z \rightarrow Z' \) for any addition of a new arrival time is \( (1/4)(1/T)(1/2)(1/K) \).

Suppose we begin at state \( Z \) characterized by a time interval of \([0, 3]\), with two switch events at times 1, 2. Further, assume that the categorical state of the enzyme is \([0, 1, 2]\) to begin with (where the integers \((0, \ldots, K-1)\) index the different states). We uniformly draw a new arrival time on \([0, 3]\); let’s say we draw the time .5. Now, the current epoch from \([0, 1]\) in which the enzyme was in state 0 is now split into half. We randomly (with probability \( 1/2 \)) choose the left epoch \([0, .5]\) to retain the value of 0, and draw uniformly over \((0, \ldots, K-1)\) for the value of the enzyme in the rightmost half. Let’s say we draw value 1 with probability \( 1/K \). We are now at state \( Z' \) which has switch times at .5, 1, 2 and enzyme states 0, 1, 1, 2. Note that state \( Z \) had \( N = 2 \) switch events, whereas state \( Z' \) has \( N + 1 \) switch events. To transition from \( Z' \rightarrow Z \), we must delete a switch event.

We would have to choose the switch event that we just added, which would occur with probability \( 1/(N + 1) \). Then, we would have to choose the enzyme state from the previous left epoch \([0, .5]\) to represent the entire epoch now on \([0, 1]\). We choose the left side with probability \( 1/2 \). Thus, the ratio is

\[
\frac{Q(Z' \rightarrow Z)}{Q(Z \rightarrow Z')} = \frac{(1/2)(1/(N + 1))}{(1/T)(1/2)(1/K)} = \frac{KT}{N + 1}
\]

(4.18)

It is easy to see that the value of \( KT/(N + 1) \) still holds even if we had randomly picked the value of 0 instead of 1 for the new epoch on \([0, .5]\). In this case, there are two ways to transition from \( Z \rightarrow Z' \) and two ways to transition from \( Z' \rightarrow Z \).
As for the ratio of $P(X|'Z')P(Z')/P(X|Z)P(Z)$, imagine that the epoch we end up splitting is $Z_0$, and it is preceded by $Z_B$ (before) and followed by $Z_A$ (after). The ratio becomes.

Let the number of $X$ arrivals in this epoch originally be $N_0$, and after adding the new switch time becomes split into two epochs with $N_1, N_2$ such that $N_0 = N_1 + N_2$. The ratio is

$$
\frac{P(Z'|X, \theta)}{P(Z|X, \theta)} = \frac{q(z_B \rightarrow z_1)q(z_1 \rightarrow z_2)q(z_2 \rightarrow z_A)}{q(z_B \rightarrow z_0)q(z_0 \rightarrow z_A)} \times \lambda \times \\
\exp \{N_1 \ln \lambda_{z_1} + N_2 \ln \lambda_{z_2} - N_0 \ln \lambda_{z_0} - \lambda_{z_1} \delta_1 - \lambda_{z_2} \delta_2 + \lambda_{z_0}(\delta_1 + \delta_2)\}
$$

(4.19)

The factor of $\lambda$ comes from the ratio of the density of $N+1$ arrivals divided by the density of $N$ arrivals.

- When deleting an arrival time, we first uniformly choose which arrival time of the $N$ to delete. We now have to decide how to merge the two adjacent inter-switch epochs that are pushed into one. We randomly choose the left or right epoch and set the entire epoch to that value. The transition probability for deletion of an arrival event is thus $(1/4)(1/N)(1/2)$. To construct the ratio of reverse to forward choice, let's demonstrate with an example.

Working through a similar example as before, we can see that

$$
\frac{Q(Z' \rightarrow Z)}{Q(Z \rightarrow Z')} = \frac{(1/K)(1/2)(1/T)}{(1/N)(1/2)} = \frac{N}{K T}
$$

(4.20)

The value $N/KT$ is still true even if the arrival time we decide to delete separates two enzyme states which happen to be the same. We can see by analogy with the above case that

$$
\frac{P(Z'|X, \theta)}{P(Z|X, \theta)} = \frac{q(z_B \rightarrow z_0)q(z_0 \rightarrow z_A)}{q(z_B \rightarrow z_1)q(z_1 \rightarrow z_2)q(z_2 \rightarrow z_A)} \times \frac{1}{\lambda} \times \\
\exp \{-N_1 \ln \lambda_{z_1} - N_2 \ln \lambda_{z_2} + N_0 \ln \lambda_{z_0} + \lambda_{z_1} \delta_1 + \lambda_{z_2} \delta_2 - \lambda_{z_0}(\delta_1 + \delta_2)\}
$$

(4.21)

- To shuffle an existing switch-time, we randomly pick one of the $N$ switch-times, and place it uniformly over the interval $[0, T]$. This will require us to merge two epochs into one and split one epoch into two. For the merge, we randomly pick the previous left or right epoch's value, and for the split, we randomly pick left or right to retain the old value, and uniformly draw one of the states of $z$ for the other value. The probability is $(1/4)(1/N)(1/T)(1/2)(1/2)(1/K)$.
CHAPTER 4. LATENT ENZYME STATES

For this case:

$$\frac{Q(Z' \to Z)}{Q(Z \to Z')} = 1$$  \hspace{1cm} (4.22)

This holds even if the switch-time we shuffle is initially separating two enzyme states that are the same.

To calculate the ratio $P(Z'|X, \theta)/P(Z|X, \theta)$, we need only consider epochs that are changed by shuffling a switch-time. Before the shuffle, imagine that there are two sequences given by $[z_B, z_0, z_A]$ and somewhere else, $[z'_B, z'_1, z'_2, z'_A]$. Imagine that we take the switch time separating $z'_1$ from $z'_2$, combining them into a single epoch $z'_0$, and that we place that switch event in the middle of epoch $z_0$, splitting it into $z_1, z_2$. Define the numbers $N_0, N_1, N_2$ and $N'_0, N'_1, N'_2$ with the relationship $N_0 = N_1 + N_2$, $N'_0 = N'_1 + N'_2$. We therefore have that

$$\frac{P(Z'|X, \theta)}{P(Z|X, \theta)} = \frac{q(z_B \to z_1)q(z_1 \to z_2)q(z_2 \to z_A)}{q(z_B \to z_0)q(z_0 \to z_A)} \times \frac{q(z'_B \to z'_0)q(z'_0 \to z'_A)}{q(z'_B \to z'_1)q(z'_1 \to z'_2)q(z'_2 \to z'_A)} \times \exp \left\{ N_1 \ln \lambda_{z_1} + N_2 \ln \lambda_{z_2} - N_0 \ln \lambda_{z_0} - \lambda_{z_1}\delta_1 - \lambda_{z_2}\delta_2 + \lambda_{z_0} (\delta_1 + \delta_2) \right\} \times \exp \left\{ -N'_1 \ln \lambda_{z'_1} - N'_2 \ln \lambda_{z'_2} + N'_0 \ln \lambda_{z'_0} + \lambda_{z'_1}\delta'_1 + \lambda_{z'_2}\delta'_2 - \lambda_{z'_0}(\delta'_1 + \delta'_2) \right\}$$  \hspace{1cm} (4.23)

We make the simplification that if the randomly selected ‘new’ position for the old arrival time is WITHIN the same two arrival times $t_{left}, t_{right}$ as before, we will retain the same sequence of latent enzyme states $z_1, z_2$. In this case, the expression above simplifies greatly to

$$\frac{P(Z'|X, \theta)}{P(Z|X, \theta)} = \exp \left\{ (N_1 - N'_1) \ln \lambda_{z_1} + (N_2 - N'_2) \ln \lambda_{z_2} - \lambda_{z_1} (\delta_1 - \delta'_1) - \lambda_{z_2} (\delta_2 - \delta'_2) \right\}$$  \hspace{1cm} (4.24)

• For the case of changing an existing enzyme state $z_i$, it is easy to see that

$$\frac{Q(Z' \to Z)}{Q(Z \to Z')} = 1$$  \hspace{1cm} (4.25)

To calculate $P(Z'|X, \theta)/P(Z|X, \theta)$, imagine that we have a sequence of $z_B, z_0, z_A$, and we replace the enzyme state $z_0$ to $z'_0$ (time interval is denoted by $\delta_0$). The ratio is

$$\frac{P(Z'|X, \theta)}{P(Z|X, \theta)} = \frac{q(z_B \to z'_0)q(z'_0 \to z_A)}{q(z_B \to z_0)q(z_0 \to z_A)} \times \exp \left\{ N_1 \log(\lambda_{z'_0}/\lambda_{z_0}) - \delta_0 (\lambda_{z'_0} - \lambda_{z_0}) \right\}$$  \hspace{1cm} (4.26)

As each iteration of the Gibbs sampler requires sampling of $Z|X, \theta^t$, we must estimate the mixing time for the MCMC algorithm outlined above. We determine this numerically by running
a very long MCMC trajectory and calculating a time correlation function for $N$, the total number of latent enzyme state switching events (that is, the correlation function between $N(i)$ at iteration $i$ and $N(i + \Delta)$ as a function of $\Delta$). We pick a time such that the correlations have decayed to $1/e$ and use this as our mixing time.

Using our MCMC algorithm at each iteration of a Gibbs sampler (along with conjugate priors mentioned earlier) gives us a means to sample from the posterior distribution of $\theta | X$. In figures 4.8, 4.10, 4.11, we demonstrate posterior distributions over model parameters (and the first row of the transition probability matrix in underlying latent enzyme state) (for comparison, the priors used for $\lambda$ entries are shown in figure 4.9). From figure 4.8 we see that the algorithm correctly identifies as modes the true values of 1, 10, 20, but there is an identifiability problem. Due perhaps to the prior on $\lambda_0, \lambda_1$ overlapping, the algorithm estimated as posterior distribution for $\lambda_0$ a peak at around 10 and for $\lambda_1$ a peak at around 1. Of course, the choice of label is immaterial.

Figure 4.6: This is a sample MCMC trajectory. We show only one statistic, the total number of hidden enzyme switching events, as a function of MCMC iteration.
CHAPTER 4. LATENT ENZYME STATES

Figure 4.7: From a monte carlo trajectory of MCMC such as shown in Figure 5.6, we calculate an autocorrelation function, and use this to determine a characteristic time over which the markov chain reaches stationarity, a mixing time.

Figure 4.8: A,B,C demonstrate the posterior distribution of λ entries sampled by a Gibbs sampler, using conjugate priors. The true values used were 1,10 and 20 respectively. The underlying Z data is in the form of a continuous switching process.
CHAPTER 4. LATENT ENZYME STATES

Figure 4.9: A,B,C demonstrate the prior distribution of λ entries sampled by a Gibbs sampler, using conjugate priors. The underlying Z data is in the form of a continuous switching process.

Figure 4.10: A,B,C demonstrate the posterior distribution of q_{0 \rightarrow i} entries sampled by a Gibbs sampler, using conjugate priors. The true values used were .1,.2 and .7 respectively. The underlying Z data is in the form of a continuous switching process.
4.5 Chemical langevin limit: Kalman filter approach

We can extend the above analysis to the case in which the underlying latent enzyme is following a continuous diffusion process, and in which the copy number is large enough that the normal limit of the Poisson is appropriate (this is the chemical langevin limit). For this set up, we assume that there are conditional dynamics governing $f(z_{t+1}|z_t)$ where we let $z_{t+1} = z_{t+\delta t}$. The traditional Kalman filter applies to a hidden markov model in which states are conditionally gaussian. The latent state follows a gaussian linear dynamical system of the form

$$z_{t+1}|z \sim \mathcal{N}(Az_t + v, Q)$$

and the observed states are conditionally gaussian: $y_t|z_t \sim \mathcal{N}(Cz_t, R)$. Typically, one assumes a marginal on $z_0$ or $\mathcal{N}(0, \Lambda_0)$. The algorithm follows directly from properties of conditioning and marginalizing of multivariate gaussians, and again reduces the calculation of marginal parameters to a two pass algorithm preventing inversion of the large (sparse) covariance matrix of the entire joint density. (Details of the algorithm follow in section 4.6.1). The experimentalist now wishes to estimate the parameters of the dynamics governing the latent enzyme state, namely the $A, Q$ matrices. We assume that the dynamics of $y_t$ are known (these will generally be dictated by
CHAPTER 4. LATENT ENZYME STATES

the chemical langevin equation). We here outline a Gibbs sampler approach for the parameters $A, Q$.

Essentially, the Gibbs sampler exploits the fact that each row of $A$ is a parameter vector of an AR(1) auto-regressive model, estimated by least squares. A conjugate prior approach is to use Bayesian OLS (using an normal inverse gaussian distribution, outlined in Appendix II). We will again outline the approach for an enzyme catalyzed reaction of the form $\emptyset \to X$ in which the formation rate follows a continuous diffusion process.

If we denote by $\lambda$ the formation rate:

$$
\begin{align*}
\frac{dx_t}{dt} &= \lambda_t dt + \sigma_x dZ_t^x \\
\frac{d\lambda_t}{dt} &= -k(\lambda_t - \mu) dt + \sigma_\lambda dZ_t^\lambda
\end{align*}
$$

(4.28)

We’ll let $y_t = dx_t$. This implies that

$$
\begin{align*}
y_t|\lambda_t &\sim \mathcal{N}(\lambda_t \cdot dt, \sigma_x^2 \cdot dt) \\
\lambda_{t+1}|\lambda_t &\sim \mathcal{N}((1 - k \cdot dt)\lambda_t + k\mu dt, \sigma_\lambda^2 \cdot dt)
\end{align*}
$$

(4.29)

We can see that, in terms of model parameters above, this is equivalent to a Kalman filter with $A = (1 - k \cdot dt), v = k\mu \cdot dt, Q = \sigma_\lambda^2, C = dt, R = \sigma_x^2$.

Why are the dynamics in equation 4.28 a good model in the large copy number limit? In the case of multiple enzymes, the total rate of $X$ formation is $k = \sum_i k_i$, which is approximately normal: $k \sim \mathcal{N}(N\mu, N\sigma^2)$. If the copy number is large, the total rate $k$ evolves smoothly, and therefore we propose the dynamics in 4.28 with $k = O(N)$ and $\sigma_\lambda = O(N^{1/2})$.

We can implement a Gibbs sampler here as well, which will basically resemble Bayesian OLS. We propose initializing using the Empirical Bayes method. We know that (under the stationary distribution) $E[y] = \mu$ and

$$
\text{Var}(y) = dt^2 \frac{\sigma_\lambda^2}{2k - k^2 dt} + dt\sigma_x^2
$$

(4.30)

We assume that the parameter $\sigma_x$ is known (this would be related to the details of the chemical langevin equation). Let $\hat{\mu} = E[y]$, and for purposes of initialization, let’s assume that $\sigma_\lambda \approx \sigma_x$ to give

$$
\hat{k} = \frac{dt^2 \sigma_x^2 / 2}{\text{Var}(y) - dt \sigma_x^2}
$$

(4.31)

Let $\beta = [a, b]$ where $\lambda_{t+1} = a + b \cdot \lambda_t + \varepsilon_t$. Then the initialization is

$$
\beta_0 = \left[ \begin{array}{c} \hat{k}\mu dt \\ 1 - \hat{k} \cdot dt \end{array} \right]
$$

(4.32)

In the outer loop, we sample $\beta, \sigma_\lambda^2$ from the normal inverse gaussian distribution. In the inner loop, we run the Kalman filter to calculate $P(\lambda|Y)$, and sample $\lambda^t \sim P(\lambda|Y)$ (outlined in
section 4.6.2). The sampled $\lambda^t$ is then used to calculate a posterior $f(\beta, \sigma^2_\lambda | \lambda^t)$, from which we sample a new $\beta^{t+1}, \sigma^{t+1}_\lambda$ and iterate. Figure 4.12 shows the prior distribution used over $\sigma^2$. By storing the actual hidden sequence of $\lambda$, we can generate the true posterior distribution, which we overlay along with the Gibbs sampled posterior in figure 4.13.

We compute the posteriors over $a, b$ for the $\lambda_{t+1} = a + b \cdot \lambda_t + \epsilon_t$ model in figures 15 and 17 with corresponding priors shown in figures 14 and 16.

![Figure 4.12: The prior used over $\sigma^2$ for the Gibbs sampler to estimate the parameters of the hidden enzyme diffusion process.](image-url)
Figure 4.13: Here we plot the posterior distribution over $\sigma^2$ using a Gibbs sampler, conjugate priors seen in figure 4.12 (similar to Bayesian OLS) and a Kalman filter to calculate the distribution over hidden enzyme states at each time period.
Figure 4.14: The prior used over $a$ for the Gibbs sampler to estimate the parameters of the hidden enzyme diffusion process.
Figure 4.15: Here we plot the posterior distribution over $a$ using a Gibbs sampler, conjugate priors seen in figure 4.14 (similar to Bayesian OLS) and a Kalman filter to calculate the distribution over hidden enzyme states at each time period.
Figure 4.16: The prior used over $b$ for the Gibbs sampler to estimate the parameters of the hidden enzyme diffusion process.
CHAPTER 4. LATENT ENZYME STATES

Figure 4.17: Here we plot the posterior distribution over $b$ using a Gibbs sampler, conjugate priors seen in figure 4.16 (similar to Bayesian OLS) and a Kalman filter to calculate the distribution over hidden enzyme states at each time period.

4.6 Supplemental Information

4.6.1 Kalman Filter

Let’s formulate message passing (using information form multivariate normals) for a 2 node case. That is, $x_1, x_2 \sim \mathcal{N}^{-1}(h, J)$. We will make use of the fact that, for multivariate normals in information form, marginalization comes from $x \sim \mathcal{N}^{-1}(h', J')$ with $h' = h_1 - J_{12} J_{22}^{-1} h_2$ and $J' = J_{11} - J_{12} J_{22}^{-1} J_{21}$. From this, can we formulate the joint distribution as $\phi(x_1), \phi(x_2), \psi(x_1, x_2)$, and formulate a $m_{2\rightarrow1}(x_1)$? The joint clearly factorizes as

$$p(x_1, x_2) = \exp \left( -\frac{1}{2} x^T J x + h^T x \right)$$

$$= \exp \left( -\frac{1}{2} x_1^T J_{11} x_1 + h_1^T x_1 \right) \cdot \exp \left( -\frac{1}{2} x_2^T J_{22} x_2 + h_2^T x_2 \right) \cdot \exp (-x_2^T J_{21} x_1) \quad (4.33)$$

$$= \phi(x_1) \cdot \phi(x_2) \cdot \psi(x_1, x_2)$$
CHAPTER 4. LATENT ENZYME STATES

We know that

$$m_{2\rightarrow 1}(x_1) = \int \phi(x_2)\psi(x_1, x_2)dx_2$$

$$= \int \mathcal{N}^{-1} \left( \begin{bmatrix} 0 & J_{12} \\ h_2 & J_{22} \end{bmatrix} \right) dx_2$$

$$= \mathcal{N}^{-1}(x_1; -J_{12}J_{22}^{-1}h_2, -J_{12}J_{22}^{-1}J_{21})$$

$$= \mathcal{N}^{-1}(x_1; h_{2\rightarrow 1}, J_{2\rightarrow 1})$$

$$= \exp \left( -\frac{1}{2} x_1^T J_{2\rightarrow 1} x_1 + h_{2\rightarrow 1}^T x_1 \right) \quad (4.34)$$

It is not hard to verify that for a product of incoming nodes to $x_1$, if we calculated the messages by $m_{i\rightarrow 1}(x_1)$, the marginal of $x_1$ is

$$p(x_1) \propto \phi(x_1) \prod_{i \in \mathcal{N}(1)} m_{i\rightarrow 1}(x_1)$$

$$= \mathcal{N}^{-1}(x_1; h_1 + \sum_i h_{i\rightarrow 1}, J_{11} + \sum_i J_{i\rightarrow 1}) \quad (4.35)$$

Given messages coming into a node $i$ (in the form of $h_{k\rightarrow i}, J_{k\rightarrow i}$), how do we calculate the message sent from $i$ to $j$ $m_{i\rightarrow j}(x_j)$? The messages are:

$$m_{i\rightarrow j}(x_j) = \int \phi(x_i)\psi(x_i, x_j) \prod_{k \in \mathcal{N}(i)\setminus j} m_{k\rightarrow i}(x_i)dx_i$$

$$= \int \mathcal{N}^{-1} \left( \begin{bmatrix} h_i + \sum_k h_{k\rightarrow i} \\ 0 \end{bmatrix}, \begin{bmatrix} J_{ii} + \sum_k J_{k\rightarrow i} & J_{ij} \\ J_{ji} & 0 \end{bmatrix} \right)$$

$$= \mathcal{N}^{-1}(x_j; h_{i\rightarrow j}, J_{i\rightarrow j}) \quad (4.36)$$

with parameters given by

$$h_{i\rightarrow j} = -J_{ji} \left( J_{ii} + \sum_k J_{k\rightarrow i} \right)^{-1} \left( h_i + \sum_k h_{k\rightarrow i} \right)$$

$$J_{i\rightarrow j} = -J_{ji} \left( J_{ii} + \sum_k J_{k\rightarrow i} \right)^{-1} J_{ij} \quad (4.37)$$

In a loopy belief propagation update, the messages will be updated via

$$h_{i\rightarrow j}^{t+1} = -J_{ji} \left( J_{ii} + \sum_k J_{k\rightarrow i}^t \right)^{-1} \left( h_i + \sum_k h_{k\rightarrow i}^t \right)$$

$$J_{i\rightarrow j}^{t+1} = -J_{ji} \left( J_{ii} + \sum_k J_{k\rightarrow i}^t \right)^{-1} J_{ij} \quad (4.38)$$
And of course, at convergence, the marginals at each node are returned by

\[ x_i \sim N^{-1} \left( h_i + \sum_{k \in N(i)} h_{k \rightarrow i}, J_{ii} + \sum_{k \in N(i)} J_{k \rightarrow i} \right) \]  (4.39)

**Forward/backward for Gaussian HMM (Kalman filter)**

Let’s assume the model is of the form

\[ x_0 \sim N(0, \Lambda_0) \]
\[ x_{t+1} | x_t \sim N(Ax_t, Q) \]  (4.40)
\[ y_t | x_t \sim N(Cx_t, R) \]

What are the pairwise/single-node potential functions for the model \( X|Y \) where i.e. \( X = \{x_0, \ldots, x_T\} \)?

One can see that the single node potentials satisfy \( \phi(x_i) \sim N^{-1}(h_i, J_i) \) given by

\[ h_i = C^T R^{-1} y_i \]
\[ J_0 = \Lambda_0^{-1} + A^T Q^{-1} A + C^T R^{-1} C \]
\[ J_i = Q^{-1} + A^T Q^{-1} A + C^T R^{-1} C \]
\[ J_t = Q^{-1} + C^T R^{-1} C \]  (4.41)

The pairwise potentials are of the form

\[ \psi(x_i, x_{i+1}) = \exp \left( x_i^T A^T Q^{-1} x_{i+1} \right) \]  (4.42)

or, in general, if we let \( x_{i+1} = A_{i+1} x_i + \varepsilon \) in the data generation, we can let \( L_{i+1}^T = A_{i+1}^T Q_{i+1}^{-1} \)
and then the \( J_{i,i+1} = -L_{i+1}^T \). The forward pass can be defined in a single recursion if we let \( h_{-1 \rightarrow 0} = 0 \) and \( J_{-1 \rightarrow 0} = 0 \). For \( i \in \{0, \ldots, t-1\} \):

\[ h_{i \rightarrow i+1} = L_{i+1} (J_i + J_{i-1 \rightarrow i})^{-1} (h_i + h_{i-1 \rightarrow i}) \]
\[ J_{i \rightarrow i+1} = -L_{i+1} (J_i + J_{i-1 \rightarrow i})^{-1} L_{i+1}^T \]  (4.43)

Similarly, define \( h_{t+1 \rightarrow t} = 0, J_{t+1 \rightarrow t} = 0 \), the backward pass is given (for \( i \in \{t-1, t-2, \ldots, 0\} \)) by:

\[ h_{i+1 \rightarrow i} = -L_{i+1}^T (J_{i+1} + J_{i+2 \rightarrow i+1})^{-1} (h_{i+1} + h_{i+2 \rightarrow i+1}) \]
\[ J_{i+1 \rightarrow i} = -L_{i+1}^T (J_{i+1} + J_{i+2 \rightarrow i+1})^{-1} L_{i+1} \]  (4.44)

What is modified above if we assume a non-homogeneous linear dynamical system (LDS) of the form

\[ x_{t+1} | x_t \sim N(Ax_t + v, Q) \]  (4.45)
(where everything else is kept the same). Everything in the above analysis is the same except that the $h_i$ get modified as follows:

\begin{align*}
    h_0 &= C^T R^{-1} y_0 + A^T Q^{-1} v \\
    h_i &= C^T R^{-1} y_i + (A^T Q^{-1} - Q^{-1}) v \\
    h_t &= C^T R^{-1} y_t - Q^{-1} v
\end{align*}

(4.46)

How do we sample from the posterior $p(z|x)$? We sample $z_0$ from its marginal, and then sample repeatedly from $p(z_{i+1}|z_i)$. We know that this will be given by $N^{-1}(h_{i+1} + h_{i+2} - L_{i+1} z_i, J_{i+1,i+1} + J_{i+2,i+1})$.

### 4.6.2 Posterior for the continuous model

These results follow immediately from properties of Bayesian OLS [2]. For this set up, we have that

\[
f(Y|X, \beta, \sigma^2) \propto (\sigma^2)^{-N/2} \exp \left( -\frac{1}{2\sigma^2} (y - X\beta)^T (y - X\beta) \right)
\]

\[
f(\beta, \sigma^2) \sim NIG(\beta, \sigma^2|\beta_0, V_0, a_0, b_0)
\]

\[
= (\sigma^2)^{-(a_0+1)} \exp(-b_0/\sigma^2) \cdot \exp \left( -\frac{1}{2\sigma^2} (\beta - \beta_0)^T V_0^{-1} (\beta - \beta_0) \right)
\]

(4.47)

Where

\[
NIG(\beta, \sigma^2|\beta_0, V_0, a_0, b_0) = \mathcal{N}(\beta; \beta_0, \sigma^2 V_0) \cdot IG(\sigma^2|a_0, b_0)
\]

(4.48)

The $NIG$ is a conjugate prior [2]. We will now show what the updated parameters $\beta_N, V_N, a_N, b_N$ are.

To solve for the posterior, we will use a result which can be shown by using results of conditioning/marginalizing for multivariate normals in information form, that if $\beta \sim \mathcal{N}(\mu_\beta, \Sigma_\beta)$ and $Y|\beta \sim \mathcal{N}(X \beta, \Sigma_Y)$, the posterior $\beta|Y$ is normal with covariance matrix:

\[
\Sigma_{\beta|y} = (\Sigma_\beta^{-1} + X^T \Sigma_Y^{-1} X)^{-1} = \sigma^2 (V_0^{-1} + X^T X)^{-1}
\]

(4.49)

For which we get that $V_N = (V_0^{-1} + X^T X)^{-1}$. What is $\mu_{\beta|y}$?

\[
\mu_{\beta|y} = \Sigma_{\beta|y} (\Sigma_\beta^{-1} \mu_\beta + X^T \Sigma_Y^{-1} y) = V_N (V_0^{-1} \beta_0 + X^T y)
\]

(4.50)

This shows $\beta_0 \to V_N (V_0^{-1} \beta_0 + X^T y)$.

Of course, in rewriting the exponent as $-(1/2\sigma^2)(\beta - \beta_N)^T V_N^{-1} (\beta - \beta_N)$ we have to add $(1/2\sigma^2) \beta_N^T V_N^{-1} \beta_N$. Also, there is still a floating term of $-(1/2\sigma^2) \beta_0^T V_0^{-1} \beta_0$. 

The only terms not proportional to $\beta$ that still hit $1/\sigma^2$ in the exponent are $y^T y/2$, and the exponent on $(\sigma^2)^{-1}$ shows that $a_0 \to a_0 + N/2$. Thus:

$$
V_0 \to (V_0^{-1} + X^T X)^{-1} \equiv V_N
$$

$$
\beta_0 \to V_N (V_0^{-1} \beta_0 + X^T Y) \equiv \beta_N
$$

$$
b_0 \to b_0 + \frac{y^T y}{2} + \frac{\beta_0^T V_0^{-1} \beta_0}{2} - \frac{\beta_N^T V_N^{-1} \beta_N}{2}
$$

$$
a_0 \to a_0 + N/2
$$

(4.51)

The marginals of the NIG are given by a simple relationship. If $\beta, \sigma^2 \sim NIG(\beta_0, V_0, a_0, b_0)$ then $\sigma^2 \sim IG(a_0, b_0)$ and $\beta \sim T(\beta_0, (b_0/a_0)V_0, 2a_0)$.

By representing a multivariate $X \sim T(\mu, \Sigma, \nu)$ in stochastic form as

$$
W = \nu/\chi^2
$$

$$
X = \mu + \sqrt{W}AZ
$$

(4.52)

where $\Sigma = AA^T$ and $Z \sim N(0, \mathbb{1}_{N,N})$, we see that the marginals over entries of $X$ are simply

$$
X_i \sim T(\beta_0[i], (b_0/a_0)V_0[i, i], 2a_0)
$$

(4.53)
Chapter Four References


Chapter 5

Phosphorylation of a Tyrosine residue on Zap70 by Lck and its subsequent binding via a SH2 domain may be a key gatekeeper of T cell receptor signaling in vivo

5.1 Introduction

The initiation of signaling in T lymphocytes in response to the binding of the T cell receptor (TCR) to cognate ligands is a key step in the emergence of adaptive immune responses. Conventional models posit that TCR signaling is initiated by the phosphorylation of receptor-associated immune receptor activation motifs (ITAMs). The cytoplasmic tyrosine kinase, Zap70, binds to phosphorylated ITAMs, is subsequently activated, and then propagates downstream signaling. While evidence for such models is provided by experiments with cell lines, in vivo, Zap70 is bound to phosphorylated ITAMs in resting T cells. But, Zap70 is activated only upon TCR binding to cognate ligand. We report results of computational studies of a new model for the initiation of TCR signaling that incorporates these in vivo observations. Importantly, the new model is shown to allow for better and faster TCR discrimination between self and foreign
ligands at lower metabolic costs. The new model is consistent with many past experimental observations, and experiments that could further test the model are proposed.

5.2 Motivation for the model

T lymphocytes (T cells) play an important role in coordinating immune responses against infectious pathogens. They express T cell antigen receptor (TCR) molecules on their surface, which can recognize peptides bound to major histocompatibility (MHC) molecules (pMHC) that are displayed on the surface of infected, or antigen-presenting, cells. Sufficiently strong binding of the TCR to peptide-MHC molecules can initiate TCR signaling, which is a key step in T cell activation and the development of adaptive immune responses.

Peptides derived from both the host proteome and pathogenic proteins can bind to MHC molecules and be expressed on the surface of a cell. It is critical that productive TCR signaling resulting in T cell activation is initiated when pathogenic pMHC molecules (agonists) are encountered. T cells discriminate between self and pathogenic peptides with high specificity, and are extraordinarily sensitive to minute amounts of agonists[1]. Much effort has been devoted toward understanding the cellular machinery and topology of the membrane-proximal signaling network that enables T cells to exhibit these properties[2].

Because of processes that occur during development of T cells in the thymus, TCRs expressed on mature T cells bind weakly to some self peptide-MHC molecules present on peripheral tissues and antigen presenting cells. These weak interactions generate some signaling necessary for homeostasis and T cell survival but are insufficient to initiate a full activation response by the T cell. Full activation of the T cell requires a more complete and stronger signal to initiate a cellular response. Although the details of the molecular mechanisms differ, most postulated mechanisms for the ability of TCR signaling to discriminate between agonist and self ligands are variants of Hopfield’s kinetic proofreading idea, which was first adapted for T cells by McKeithan[3][4]. In brief, one posits that a set of biochemical transformations needs to be completed before productive downstream signaling can ensue. The ligand that binds more strongly to the TCR has a higher probability of remaining engaged for the time required for completing these biochemical reactions. The ability to discriminate between ligands that bind with only modestly dissimilar strength improves as the number of biochemical transformations to be completed increases. Importantly, this mechanism requires that the biochemical transformations are driven out of equilibrium, which is true as many of the transformations are phosphorylation reactions that consume ATP.

The conventional picture (Fig. 1a) for the earliest steps involved in initiation of TCR signal-
CHAPTER 5. ZAP70 MODEL

ing upon receptor engagement are recruitment of a CD4 or CD8 co-receptor-associated kinase Lck, and subsequent phosphorylation of the tyrosine residues in the cytoplasmic ζ and CD3 chain immunoreceptor tyrosine-based activation motifs (ITAMs). Some models also include a step where Lck is activated, while experimental work has established that some fraction of co-receptors are associated with already active Lck in the basal state[5, 6, 7]. Using its tandem SH2 domains, the kinase, Zap70, can bind to doubly phosphorylated ITAMs with high affinity and selectivity. Lck phosphorylates and activates Zap70 after which it can trans-autophosphorylate and activate other vicinal Zap70 molecules. Active Zap70 is required for further downstream signaling events leading to ERK activation and calcium increase[2].

Previous experimental evidence suggests that in vivo Zap70 is already bound to doubly phosphorylated ITAMs in thymocytes and unstimulated T cells[8]. Importantly, however, the Zap70 molecules are not phosphorylated. These in vivo observations suggest that the model described above, and its variants, needs modification. Specifically, an appropriate model must describe how continuous weak interactions with self ligands in vivo[9][10] allow for sufficient ITAM phosphorylation to result in binding of Zap70, but does not allow activation of Zap70 to initiate downstream signaling. It is also important to understand how removal of the kinetic proofreading steps associated with ITAM phosphorylation and binding of Zap70 impacts ligand discrimination.

In this paper, we carry out computer simulations to explore a variant of a model we proposed in a recent review article[2] to address these issues. Our results indicate that indeed this model is consistent with in vivo observations. Furthermore, the new model is better at discriminating between self and agonist pMHC ligands than the conventional model and also incurs a lower metabolic cost for the T cell. We describe how a specific experiment could test the veracity of this model.

5.3 Proposed signaling model and methods

Our model (Fig. 1b) is motivated by recent data showing that, after Zap70 binds to a doubly phosphorylated ITAM, Lck must first phosphorylate Tyrosines 315 and 319 on Zap70 in order to convert the latter molecule from its inactive to active conformation[11]. We further postulate that Lck can then bind to Tyrosine 319 with its SH2 domain, consistent with previous experimental data[12], thus stabilizing the active conformations of both Zap70 and Lck, and the entire complex. Active Lck and Zap70 can then further activate other Zap70 molecules enabling productive downstream signaling. If the rate at which Lck phosphorylates and binds to the Zap70 Tyrosine 319 is slower than the rate at which the co-receptor /Lck/TCR-pMHC complex
dissociates, then Lck will not activate Zap70 with high probability, and productive downstream signaling will not ensue. Even if Tyrosine 319 were to be phosphorylated by Lck, this would be transient as phosphatases would rapidly dephosphorylate this tyrosine if Lck does not bind to it with its SH2 domain. More stable TCR-pMHC bonds, such as with an agonist pMHC, will result in a longer lifetime for the co-receptor/Lck/TCR-pMHC complex, enabling activation of Zap70 following the mechanism described above. Note that the proposed model is consistent with experiments showing that the SH2 domain of Lck plays a role in T cell activation and coreceptor function[13, 14, 15, 16].

In our computational model, we lump the processes of Lck-mediated Tyrosine 319 phosphorylation and subsequent binding via the Lck SH2 domain into one composite step. It is the kinetic rate of these two sequential steps that is important. We have carried out calculations where, upon recruitment to the TCR-pMHC complex, Lck undergoes an activation step (as per some studies) as well as one where some fraction of the co-receptors have active Lck associated with it and there is no activation step. Qualitative results for these two cases are the same. Results shown in the main text are for the latter model in which some fraction of co-receptors have active Lck, whereas results for the model including Lck activation are shown in the Appendix (Figures S9-S12).

To simulate the protein interaction networks outlined above and sketched in Fig. 1b, we use the Gillespie algorithm for simulating Markov jump processes[17, 18, 19]. Details of the simulation including the simulation volume, the particular rate constants and initial copy number of all species used are included in the supplemental information. (Appendix IV). To compare the properties of the conventional and proposed signaling networks, we use the number of activated Zap70 molecules as a proxy for productive downstream signaling. We estimate steady state probability distributions of activated Zap70 when the network is stimulated by self pMHC alone, and then when a small percentage (2-4%) of agonist pMHC are also present. This is to mimic the fact that a very small amount of agonist pMHC molecules can trigger T-cell responses[20, 21, 22, 23, 24, 25, 26]. We carry out calculations over a wide range of values of the ratio of the dissociation rates of the TCR from self and agonist (non-self) pMHC ligands ($k_{off}^{self}/k_{off}^{non-self}$) to assess the ability of the networks to discriminate between self and non-self. Steady state probability distributions are estimated by simulating long trajectories and recording the number of activated Zap70 periodically (details about the simulation protocol follow in the Appendix IV).

To quantify signal discrimination, we calculate the relative increase in the average number of activated Zap70 molecules upon the introduction of a small quantity of agonist ligands. This is a reasonable metric because, following the intuition of Weber's Law, we expect biological systems to respond to the percent change in phosphorylated Zap70, as opposed to the absolute magnitude
CHAPTER 5. ZAP70 MODEL

of the increase. We also compute receiver-operator characteristic (ROC) curves, which measure the relative extent of true and false positives (see Appendix I for details) to further examine ligand discrimination by the conventional and new models.

Although cells have an abundant supply of ATP, we also compare the rates of ATP consumption for the two models. As T cells spend most of the time exposed to only self pMHC, we compare these metabolic costs only in the presence of self pMHC at steady state. Specifically, we compute the rate of entropy production to estimate the rate of energy consumption[27]. Details of this calculation are in supplemental information. (Appendix IV).

5.4 Results

5.4.1 The new model recapitulates in vivo observations

Upon scanning the values of unknown kinetic parameters, we could not find a set of values for which the conventional model could reproduce the in vivo observations that ITAMs are mostly phosphorylated, Zap70 is bound to them, and Zap70 is not phosphorylated. The conventional model can exhibit a high degree of ITAM phosphorylation with Zap70 bound upon stimulation with self pMHC alone, but we find that in these circumstances much of the bound Zap70 is also phosphorylated (Figure 2). The new model also exhibits a substantial amount of Zap70 bound to phosphorylated ITAMs upon stimulation by self pMHC alone, but only a negligible fraction of the bound Zap70 molecules are phosphorylated (Figure 2). Thus, the topology of the new model can recapitulate the in vivo observations, but the conventional model cannot.

An intuitive explanation for these results is that if the parameters are chosen such that stimulation with self pMHC molecules results in efficient ITAM phosphorylation and binding of Zap70, there is no mechanism to prevent Zap70 from becoming fully activated once it is recruited. The reason the new model is successful in recapitulating the in vivo observation is because we introduce a kinetic bottleneck, the phosphorylation of Tyrosine 319 on Zap70 followed by binding of Lck to this residue via its SH2 domain, which is necessary for downstream signal propagation. The kinetic proofreading steps of ITAM phosphorylation and recruitment of Zap70 are replaced by Lck mediated phosphorylation of Tyrosine 319 and binding. Note also that once this sequence of events occurs, it can act like a positive feedback loop since Zap70, now held in its active conformation, and localized Lck, whose occupied SH2 domain ensures its continued activity, can phosphorylate other Zap70 molecules, and unphosphorylated ITAMs, as well.

As noted in the previous section, we have carried out calculations for two variants of the models; one in which Lck is activated upon recruitment to the TCR-pMHC complex and the
other in which only a certain fraction of co-receptors are associated with active Lck but there is no Lck activation step upon recruitment to the receptor. Supplemental Figure S9 shows that qualitative results are the same for both models.

5.4.2 The new model is better at discriminating between self and agonist ligands

The new model results in a far larger percent increase in activated Zap70 upon introduction of a small fraction of agonists (Figure 3), indicating that it is better at ligand discrimination.

We find that the results shown in Figures 2 and 3 are robust to variations in the off rate of TCR from self ligands ($k_{off}^{self}$) and the rate at which Lck phosphorylates Tyrosine 319 and binds to it ($k_{open}$) as long as the latter process is slower than the first. When this condition holds, the new model is better at ligand discrimination (Figure 4a) and Zap70 molecules bound to ITAMs upon stimulation with self pMHC are not phosphorylated (Figure 4c). The results shown in Figure 4b demonstrate that, as expected, changing the rate $k_{open}$ does not change the fraction of ZapP70 molecules bound to phosphorylated ITAMs upon stimulation by self ligands alone. This robustness implies that it is possible to find kinetic parameters such that $k_{open} < k_{off}^{self}$ and use experimentally known Zap70 binding kinetics to robustly obtain the results we describe.

While these results show that at steady state the new model exhibits a larger percent increase in activated Zap70, we also wished to show that this model achieves this response quickly. This is an important metric to consider because if the new model outperforms the old one at discriminating between ligands, but achieves this goal in an unreasonable amount of time, it would not reflect reality (membrane proximal signaling occurs quickly[28]). We calculated the average relative change of activated Zap70 as a function of time after the introduction of a small percentage of agonist pMHC. We find that the new model responds faster to cognate ligands (Figure 5). This is because, upon stimulation by agonists the kinetic bottleneck is released, and since largely unphosphorylated Zap70 molecules were already bound to the ITAMs, they are rapidly phosphorylated. In the conventional model, in the parameter regime where Zap70 is already bound to ITAMs upon stimulation by self pMHC, a large fraction of these molecules are already phosphorylated. Therefore the percentage increase in phosphorylated Zap70 occurs slowly.
5.4.3 The new model is consistent with experiments showing that inhibiting Csk enhances the sensitivity of T cells to weak agonists, but not strong agonists

Csk is a cytoplasmic tyrosine kinase that negatively regulates Src family kinases (SFKs) such as Lck by phosphorylating an inhibitory site in the SFKs. Recent experiments demonstrate that increasing Lck activity by inhibiting the catalytic function of a PP1-analog sensitive Csk mutant makes T cells much more sensitive to stimulation by weak agonists, but does not significantly alter the sensitivity to strong agonists[29]. To explore whether the new model recapitulates this observation, we assume that a threshold amount of Zap70 molecules must be phosphorylated for T cell activation. This is because the number of phosphorylated Zap70 molecules is the proxy we are using for productive downstream signaling. Increasing Lck activity by modulating Csk corresponds to increasing the fraction of coreceptors that are bound to active Lck. We find that doubling the fraction of coreceptors with active Lck increases the numbers of phosphorylated Zap70 molecules for all ligands (Figure 6a). But, weak agonists can now exceed the threshold number required for T cell activation whereas the strong agonists already exceed this level of Zap70 phosphorylation with normal levels of Lck activity (Figure 6b). So, from the point of view of T cell activation downstream of Zap70, Csk inhibition makes a qualitative difference for stimulation by weak agonists, and not for strong agonists. Thus, the predictions of our model are consistent with the recently described experimental findings of Manz, et al[29].

In a model where Lck is activated upon recruitment to the TCR complex, we mimic the effects of inhibiting Csk by increasing the rate of Lck activation. A five-fold increase has the same qualitative effect as that shown in Figure 6b, and allows weaker agonists to exceed the threshold for activation.

5.4.4 The new model incurs lower metabolic costs for T cells

As long as the rate of the composite process of Lck-mediated phosphorylation of Tyrosine 319 on Zap70 and its subsequent binding via its SH2 domain is slower than the rate of dissociation of the TCR from self ligands, the qualitative results we have presented so far are valid. In this range of parameter space, the new model consumes less energy at steady state than the conventional model (Figure 7). This result can be understood intuitively by noting that in the conventional model, if Zap70 is bound to ITAMs upon stimulation by self ligands, a large fraction of bound Zap70 is phosphorylated. This means that, in the conventional model, there are many more phosphorylation and dephosphorylation events, which consume ATP. A
more technical explanation for the entropy production (measure of energy consumption in non-equilibrium systems) is that the new model behaves more like a birth death process in which most Zap70 molecules are bound and the state space counts the number of Zap70 molecules that are phosphorylated. Birth death processes are exactly reversible and thus have no entropy production at steady state.

5.4.5 Further experimental tests of the new model

The necessary condition for the new model to exhibit the properties described here is \( k_{\text{open}} < k_{\text{off}}^{\text{self}} \). Excellent estimates for the rates of dissociation of TCR from self pMHC molecules are available. What is required is a measurement of the rates at which Lck can phosphorylate Tyrosine 319 on Zap70, and subsequently bind to it with its SH2 domain\([30, 31, 32]\). The rate of production of Lck bound to Tyrosine 319 is encapsulated in \( k_{\text{open}} \).

Another consequence of the new model should be an enhanced stabilization of the TCR-CD8-Zap70 complex upon engagement of agonist ligands and productive signaling. Computational and experimental work has shown the kinetics of CD8 to play an important role in T-cell discrimination\([33, 34]\). Because the CD8 is now effectively bound to the complex not only through its interaction with the pMHC but also through the Lck-ZAP70 bond, we expect that an important feature of this model is that the off rate of CD8 from a TCR-pMHC will be effectively lowered. It is difficult to exactly determine the extent of stabilization by this mechanism because the binding affinity of Lck's SH2 domain to phosphorylated Tyrosine 319 on Zap70 is unknown. We make a conservative estimate of the dissociation rate of this complex using a discrete space Markov chain and finding the mean first passage time to dissociation (details in APPENDIX II). The inverse of the mean first passage time is the rate of dissociation. Our rough estimate suggests that the off-rate of CD8 from the complex should be reduced by roughly a factor of four upon the binding of Lck to Zap70 via its SH2 domain.

5.5 Discussion

According to conventional models, TCR signaling is initiated by the phosphorylation of ITAMs on ζ-chains. But in vivo, ITAMs are observed to be phosphorylated in resting T cells. In our proposed model, the steady state basal activity of Lck is sufficient to allow for significant ITAM phosphorylation in vivo as in this circumstance transient interactions between coreceptors, TCR, and endogeneous pMHC occur continuously. So, even though the probability that one
such interaction will lead to ITAM phosphorylation can be small, the multitude of events allows considerable amount of ITAM phosphorylation. Evidence that such basal signaling is ongoing is provided by the observation that stronger interactions with endogenous pMHC allow for more \( \gamma \)-chain phosphorylation and more basal signaling by the TCR\[9, 10\]. Although Zap70 is bound to these phosphorylated ITAMs in vivo, it is not in its active state. Unphosphorylated Zap70 is still autoinhibited as a consequence of the interaction between Tyr319 in interdomain B and the N-terminal catalytic domain. We propose that Lck-mediated phosphorylation of Zap70 at Tyr319 relieves the autoinhibited constraint imposed by Tyr319 on the N-lobe of the Zap70 catalytic domain, thereby leading to Zap70 activation. Lck may also bind to Tyr319 via its SH2 domain, further stabilizing the active state of Zap70. In our model, the phosphorylation of Tyr319 and the subsequent binding of Lck to it does not happen upon interactions of the TCR with endogenous peptides because the rate at which these processes occur is slower than the off-rate characterizing TCR-endogenous pMHC bonds. Thus, in the resting state, Zap70 is not activated. However, when longer-lived agonist pMHC-TCR bonds form, these processes can occur, leading to activation of Zap70. Thus, our model provides an explanation for in vivo observations.

Our computational results show that, compared to the conventional model, the model that we propose is better able to discriminate between endogenous and agonist pMHC ligands under in vivo conditions. This is because in the conventional model it is not possible to find a set of parameters wherein ITAMs are phosphorylated upon continuous interactions with endogenous pMHC without Zap70 also being activated. Thus, there is only a modest increase in active Zap70 molecules upon introduction of agonists. The new model also leads to faster Zap70 activation when agonist pMHC are expressed on antigen presenting cells because there are fewer steps to complete. Also, our results show that the new model is sensitive to small amounts of agonists present in a sea of endogenous ligands, as observed in experiments\[7, 22, 23, 25\]. Thus, our proposed model is consistent with in vivo observations and satisfies the key features of extraordinary selectivity and sensitivity that characterize the earliest events in TCR signaling.

The model we propose is also not in conflict with recent computational and experimental studies which suggest that the half-life of the TCR-agonist pMHC bond required for productive signaling is set by the time scale associated with a co-receptor bound active Lck molecule to find a TCR bound to agonist ligand\[5\]. Our new model would posit that once this happens, the next step is not ITAM phosphorylation, but rather, phosphorylation of Tyr319 on Zap70.

The binding of Lck to Tyr319 on Zap70 could also stabilize the active conformation of Lck, localize it to the TCR-agonist pMHC complex by hindering passive diffusion, and also stabilize the TCR-pMHC-co-receptor complex (as seen in our calculations). Stabilization of the active conformation of Lck promotes its ability to further phosphorylate other Lck molecules and Zap70.
molecules associated with vicinal TCRs that are bound to other agonist or endogenous ligands, thereby generating a positive feedback mechanism that could then overwhelm other negative feedback loops that maintain the resting state. By this means, our model is also in harmony with studies which suggest that signaling due to TCR-agonist pMHC ligands is amplified by endogenous pMHC molecules[6, 7, 24]. The importance of Lck's SH2 domain for its function has also been emphasized by studies wherein CD4 coreceptor function was reconstituted in a Lck-sufficient antigen-specific hybridoma with CD4-Lck fusion proteins[14].

Tyr493 in the Zap70 activation loop is 'preferentially' phosphorylated by trans-phosphorylation by Zap70, rather than by Lck[31]. The presence of multiple dimeric ITAMs may, thus, serve to position pairs of Zap70 molecules across from each other so that they can mediate trans-auto-phosphorylation and activation following release from autoinhibition by Lck phosphorylation of Tyr319. Triggering this positive feedback loop may represent an additional time scale that sets the threshold half-life of the TCR-pMHC bond required for productive downstream signaling.

The importance of Zap70 autoinhibition involving the Tyr319-catalytic domain interaction is highlighted by a recent report of a severe familial autoimmune syndrome that results from the inheritance of compound heterozygous mutations[35]. The two affected children inherited a weak hypermorphic mutation in Zap70, R360P, in the N-lobe of the catalytic domain which is combined with a loss of function allele. R360P is predicted to weaken the interaction of Tyr319 with the N-lobe. This weak hypermorphic mutation exhibits increased sensitivity to TCR stimulation and downstream signaling to Zap70 substrates in a reconstitution system. This mutation strongly supports the current model and emphasizes the importance of the autoinhibitory mechanism.

While our model is consistent with many old and recent observations, our computational results suggest key experiments that could test it further. A key assumption in the model is that the rate of phosphorylation of Tyr319 on Zap70 by Lck and the subsequent binding of Lck to this residue via Lck's SH2 domain is slower than the off-rate characterizing endogenous pMHC-TCR bonds. Thus, a critical test of our model requires measuring the rate at which Lck phosphorylates Tyr319 on Zap70, and subsequently binds to it. We hope that such experiments will be possible in the future.
Figure 5.1: Models for early steps of TCR signaling. (a) In the conventional model, coreceptor CD4/CD8 binds to MHC when agonist peptide is bound. In the absence of agonist pMHC, ITAM is unphosphorylated, and only in the presence of agonist peptide does ITAM get phosphorylated, leading to Zap70 binding and subsequent activation. (b) In contrast to the conventional model outlined above, it has been shown that even in the absence of agonist pMHC, Zap70 is bound to doubly phosphorylated ITAM but not activated. We suggest a mechanism by which Zap70 can bind to phosphorylated ITAM even in the absence of agonist pMHC, but due to slow subsequent phosphorylation of Tyr319 on Zap70 by Lck and binding of Lck to this residue, full activation is prevented until binding of agonist pMHC.
Figure 5.2: The old model has less Zap70 bound in the absence of agonist pMHC (a), and the percent of bound Zap70 that is phosphorylated is much higher than the new model (b).
**CHAPTER 5. ZAP70 MODEL**

Figure 5.3: The new model exhibits a much greater percent change in activated Zap70 after introducing a small fraction of agonist peptide into the system. The percent change is calculated by allowing the system to reach steady state before and after a small fraction of agonist peptide is added, and calculating the average amount of activated Zap70 in both cases. We calculate this measure of discrimination for various ratios of self/non-self pMHC-TCR off-rates, and show that the new model outcompetes the old model for all such ratios.

Figure 5.4: (a). If the TCR-endogenous pMHC off rate ($k^{self}_{off}$) is greater than the rate at which lck phosphorylates Tyr 319 on Zap70 and binds to it ($k_{open}$), there will be a significant increase in the amount of active Zap70 upon stimulation with a small amount of agonist peptide. (b). Changing the rate, $k_{open}$, does not change the fraction of Zap70 that is bound at steady state in the absence of agonist peptide. (c). Negligible amounts of bound Zap70 are active in the regime for which $k^{self}_{off} > k_{open}$.
Figure 5.5: The relative change in active Zap70 as a function of time after stimulation with a small amount (4%) of agonist peptide at time 0 shows the new model responds faster to stimulation. Calculations are based on the average of 5000 trials after a burn time of 500 seconds with data collected every .1 seconds.
Figure 5.6: The new model recapitulates experimental observations with cells with enhanced Lck activation. The dark blue bars represent average levels of activated Zap70 when the number of coreceptor molecules with active Lck is 133, and the light blue bars correspond to doubling the amount of coreceptor with active Lck. The images show dose curves of activated Zap70 as a function of the amount of agonist pMHC in the system. The left figure (a) is a dose curve for an agonist-TCR bond with an off rate equal to 0.44s⁻¹ (good agonist) and the right figure (b) is a dose curve for the case where this off rate equals 5.0s⁻¹ (weak agonist). If the threshold amount of active Zap70 required for T cell activation is set by the horizontal dark line, increasing the Lck activity (by increasing the concentration of active Lck) has no effect on functional outcome for a good agonist (a), since all conditions exceed the threshold. However, for a weak agonist (b), increasing Lck activity allows activation.
Figure 5.7: Energy consumption at steady state in absence of agonist peptide is minimized in the parameter regime for which $k_{off}^{self} > k_{open}$ (the parameter regime above the dotted line).

5.6 Supplemental Information

5.6.1 Non-monotonicity in optimal parameters: T cell as a binary asymmetric channel

An interesting prediction of the old model is that there is non-monotonicity in the signal discriminatory capabilities of the network as a function of the ZAP70 binding kinetics (Figure S3/S4). In other words, if we fix a binding rate for ZAP70 to phosphorylated ITAM and vary the unbinding rate, we will see the signal discrimination increase for intermediate values and then decrease when the off rate becomes too large. To calculate signal discriminatory capability of the null model over a wide range of ZAP70 binding kinetics, we use as a metric of discriminatory capability the area under the ROC curve[36] (Receiver Operator Characteristic) based on plots of (probability of detection, probability of false alarm) for various choices of a level of phosphorylated ZAP70 which sets the threshold for activation. We can think of this as a plot of (PD,PF) for every possible deterministic decision rule a T-cell can make, where a decision rule is uniquely defined by a threshold of activated ZAP70 that must be reached before
an immune response is mounted.

This result at first glance may seem strange since the McKeithan model of kinetic proofreading suggests that faster off rates should improve discriminatory abilities. However, the important point is that the McKeithan model actually predicts that a larger difference in off rate between self and non-self improves discrimination. By increasing the off rate of ZAP70, we are effectively making it harder for T cells engaged with self and non-self peptides alike to reach a state of activated ZAP70.

An intuitive explanation for this result is that when the ZAP70 off rate is too low, as soon as it binds it is likely to remain bound long enough to get phosphorylated regardless of whether or not the T-cell is exposed to self or non-self antigen. Thus, activated ZAP70 concentrations in the presence of self or non-self may be similar. If the ZAP70 off rate is too high, neither self nor non-self peptide can result in ZAP70 activation, so again the activated ZAP70 concentrations in both cases are the same (very low). Thus, the model predicts an intermediate regime of ZAP70 kinetics that maximizes signal discrimination (FIGURE S3). We note that this simple and intuitive result can be captured by a discrete-space Markov chain.

To explain the non-monotonicity in the signal discrimination capability of the old model as a function of ZAP70's unbinding rate, we built a very simple four state continuous time Markov chain to represent an individual TCR. The four states include a TCR unbound to pMHC (state 1) which transitions to TCR-pMHC complex (state 2). When the TCR-pMHC complex has formed, Zap70 can bind (state 3). It is possible for pMHC to unbind after ZAP70 has bound, leading to state 4, which has Zap70 bound to a TCR with no pMHC present; ZAP70 can also unbind from the TCR-pMHC complex before the pMHC does, leading back to state 2. We use state 3 as a proxy for activated T-cell in this simplified picture, since in the actual T-Cell, ZAP70 must be bound simultaneously with Lck associated coreceptor for activation.

We calculate two different rate matrices for self and non-self by using the same parameters for all transitions except for pMHC off rate, which is chosen to be larger in the case of non-self. We then calculate the steady state distribution by finding the kernel of the rate matrix. The steady state distributions under self and non-self allow us to compute \( P(activation|self) \) and \( P(activation|non\text{-}self) \), where \( P(activation) \) is equivalent to the steady state probability of the markov chain occupying state 3. A plot of the likelihood ratio as a function of ZAP70’s off-rate shows non-monotonicity for a fixed ratio of \( K_{off}^{non\text{-}self}/K_{off}^{self} \) and at a fixed \( K_{on}^{ZAP70} \) (Figure S1). To ensure that the non-monotonicity is seen when considering metrics besides just the likelihood ratio, we also calculated the mutual information between the random variables \( S \) the signal={self,non-self}, and \( R \) the response={activated or not activated}. Calculating the mutual information requires knowledge of the prior probabilities over \( S={self,nonself} \), but the channel capacity can be calculating by maximizing mutual information over the prior probability. The
channel capacity is zero when response is independent of signal (i.e. when both self and non-self lead to equal odds of activation), and is maximal when the signal is a deterministic function of the input, i.e. when \( p(activation|self) = 0 \), \( p(activation|non-self) = 1 \) (in which case the prior probability that maximizes the information is 50/50 over self and non-self).

We regard the set up as a binary asymmetric channel. To define the channel, we let the input variable be \( i \), where \( i \in \{ \text{self}, \text{non self} \} \), and the output variable be \( o \in \{0, 1\} \) to indicate not activated/activated. The information of the channel is

\[
I(i, o) = \sum_{i \in \{s,n\}, o \in \{0,1\}} p(i, o) \log \frac{p(i, o)}{p(i)p(o)}
\]

(5.1)

To find the channel capacity, we maximize the information over the prior distribution. In this case, the prior is parametrized by a single variable \( p = p(n) \). We will define the parameters of the channel as

\[
p(o = 0|n) = \epsilon_0, \quad p(o = 1|s) = \epsilon_1
\]

(5.2)

The marginals are

\[
p(0) = (1 - \epsilon_0) - (1 - \epsilon_0 - \epsilon_1)p
\]

\[
p(1) = \epsilon_0 + (1 - \epsilon_0 - \epsilon_1)p
\]

(5.3)

With this notation, the information of the channel is:

\[
I(p) = -(1 - \epsilon_1)p \log \left[ \frac{\epsilon_0 + (1 - \epsilon_0 - \epsilon_1)p}{1 - \epsilon_1} \right] - \epsilon_1p \log \left[ \frac{(1 - \epsilon_0) - (1 - \epsilon_0 - \epsilon_1)p}{\epsilon_1} \right]
\]

\[
- \epsilon_0(1 - p) \log \left[ \frac{\epsilon_0 + (1 - \epsilon_0 - \epsilon_1)p}{\epsilon_0} \right] - (1 - \epsilon_0)(1 - p) \log \left[ \frac{(1 - \epsilon_0) - (1 - \epsilon_0 - \epsilon_1)p}{1 - \epsilon_0} \right]
\]

(5.4)

At this point, we will introduce the notation \( h_i = h(\epsilon_i) \) to be the entropy of a binary random variable, where

\[
h(\epsilon_i) = -\epsilon_i \log \epsilon_i - (1 - \epsilon_i) \log(1 - \epsilon_i)
\]

(5.5)

We see that \( I'(p) \) is:

\[
I'(p) = -(1 - \epsilon_0 - \epsilon_1) \log[\epsilon_0 + (1 - \epsilon_0 - \epsilon_1)p]
\]

\[
+ (1 - \epsilon_0 - \epsilon_1) \log[(1 - \epsilon_0) - (1 - \epsilon_0 - \epsilon_1)p]
\]

\[
+ h_0 - h_1
\]

(5.6)

To find \( p^* \) that maximizes the information, set \( I'(p^*) = 0 \):

\[
\log \left[ \frac{\epsilon_0 + (1 - \epsilon_0 - \epsilon_1)p^*}{(1 - \epsilon_0) - (1 - \epsilon_0 - \epsilon_1)p^*} \right] = \frac{h_0 - h_1}{1 - \epsilon_0 - \epsilon_1}
\]

\[
\frac{\epsilon_0 + (1 - \epsilon_0 - \epsilon_1)p^*}{(1 - \epsilon_0) - (1 - \epsilon_0 - \epsilon_1)p^*} = 2^{(h_0 - h_1)/(1 - \epsilon_0 - \epsilon_1)} \equiv \lambda
\]

(5.7)
CHAPTER 5. ZAP70 MODEL

Solving gives

\[ p^* = \frac{(1 - \epsilon_o)\lambda - \epsilon_o}{(1 + \lambda)(1 - \epsilon_o - \epsilon_1)} \]  \hspace{1cm} (5.8)

At this point, what remains is to plug the \( p^* \) back into the original expression for \( I(p) \). Before we do this, note that the expressions for marginal \( p(0), p(1) \) given the optimal prior \( p^* \):

\[ p(0) = \epsilon_o + (1 - \epsilon_o - \epsilon_1)p^* = \frac{\lambda}{1 + \lambda} \]
\[ p(1) = (1 - \epsilon_o) - (1 - \epsilon_o - \epsilon_1)p^* = \frac{1}{1 + \lambda} \] \hspace{1cm} (5.9)

Plugging into \( I(p^*) \):

\[ I(p^*) = -p^*h_1 - (1 - p^*)h_0 + \log(1 + \lambda) - \left( \frac{\lambda}{1 + \lambda} \right) \log \lambda \] \hspace{1cm} (5.10)

Using the expressions for \( p^*, 1 - p^*, \lambda \), we can simplify the following:

\[
\begin{align*}
-p^* h_1 - (1 - p^*) h_0 &= \left( \frac{\lambda}{1 + \lambda} \right) \log \lambda \\
&= -(1 - \epsilon_o)\lambda h_1 + \epsilon_o h_1 - h_0 + \epsilon_1(1 + \lambda)h_0 - \left( \frac{\lambda}{1 + \lambda} \right) \left( \frac{h_0 - h_1}{1 - \epsilon_o - \epsilon_1} \right) \\
&= h_1 \left( \lambda - (1 - \epsilon_o)\lambda + \epsilon_o \right) + h_0 (-1 + \epsilon_1(1 + \lambda) - \lambda) \\
&= \frac{h_1\epsilon_o(1 + \lambda) - h_0(1 - \epsilon_1)(1 + \lambda)}{(1 + \lambda)(1 - \epsilon_o - \epsilon_1)} \\
&= \frac{h_1\epsilon_o - h_0(1 - \epsilon_1)}{(1 - \epsilon_o - \epsilon_1)}
\end{align*}
\] \hspace{1cm} (5.11)

All combined, we get

\[ I(p^*) = \frac{h_1\epsilon_o - h_0(1 - \epsilon_1)}{(1 - \epsilon_o - \epsilon_1)} + \log \left( 1 + 2^{(h_0 - h_1)/(1 - \epsilon_o - \epsilon_1)} \right) \] \hspace{1cm} (5.12)

The channel capacity is non-monotonic as a function of ZAP70’s kinetic off rate at a fixed on rate (Figure S2).

In the text, we calculated the AUROC for the null model based on plots of detection probability and false alarm probability for various thresholds. To quantify signal discrimination, we must choose a metric that is agnostic to the particular prior probabilities of encountering self and non-self, since such priors (though undoubtedly skewed towards encounters with self) vary by individual and are most likely not stationary. Almost every metric of binary signal discrimination used in decision theory takes into account both a probability of detection and a probability of false alarm. The probability of detection in our model is the probability for
a T-cell to mount a response in the presence of foreign antigen, while the probability of false alarm is the probability that a T-cell incorrectly activates in response to self pMHC. A decision rule could be deterministic, in which case it takes the form of a sharp threshold above which the T-cell decides to mount a response (in our case the threshold represents a sufficiently high concentration of phosphorylated ZAP70), or stochastic, meaning that the T-cell does not always respond in the same way to the same concentration of phosphorylated ZAP70, but responds in some instances and not in others.

Determining the optimal decision rule would require detailed knowledge of the prior probability distribution over self/non-self and a cost function that specifies the cost of various types of mistakes (namely, not detecting foreign antigen, and incorrectly responding to self). In this case, the optimal decision rule on the part of a T cell will be of the form of a likelihood ratio, and implies that the decision to mount a response occurs only if the amount of activated ZAP exceeds a threshold that is a function of the prior probabilities and cost function. This leads to the Bayes Least Cost decision rule[37]. (In certain cases in which there are outcomes that set the likelihood ratio equal to the optimal threshold, a randomized decision rule is optimal). If one does not know the prior probabilities but is instead willing to maximize the detection probability while insisting the false alarm probability remain below a certain threshold, the Neyman-Pearson lemma states that the optimal decision rule will still be of the form of a likelihood ratio with its own associated threshold[38].

In our case, it is not clear what the prior probabilities should be nor do we wish to specify a threshold for the probability of false alarm. Thus, we instead use as a metric of discriminatory capability the area under the ROC curve[36] (Receiver Operator Characteristic) based on plots of (PD,PF) for various choices of a level of phosphorylated ZAP70 which sets the threshold for activation. We can think of this as a plot of (PD,PF) for every possible deterministic decision rule a T-cell can make, where a decision rule is uniquely defined by a threshold of activated ZAP70 that must be reached before an immune response is mounted.

5.6.2 Stability of the signaling complex: mean first passage time for complex dissociation

One of the reasons the ZAP70-opening model (referred to in the text as the new model) has a discriminatory advantage over the old model is that the association of Lck with ZAP70 through its SH2 domain provides an extra degree of stabilization to the entire complex, and the effective rate at which any species leaves the complex is slower (Figure S4). Presumably a greater degree of complex-stabilization will lead to greater discriminatory advantages.
To calculate the effective rate at which various species leave the complex between co-receptor, TCR and pMHC, we built a discrete space continuous time Markov chain for a single complex, calculated the mean first passage time (mfpt) for various species to exit the complex, and calculated the effective rate by 1/mfpt. The complex has a total of three bonding interactions, namely that between the coreceptor and pMHC (interaction 1), between the TCR and the pMHC (interaction 2) and between Lck attached to the coreceptor and Zap70 on the ITAM (interaction 3).

The states of the Markov chain represent the various interactions that are still intact (Figure S5). In the figure S5, blue states are states for which 2 bonds are still in tact, and red states are those for which only one bond is in tact (i.e. one species has effectively left the complex). For instance, a transition from (1,2,3) to (1,2) indicates that bond 3 has broken but 1,2 are still in tact. This indicates that the pMHC-TCR bond is still in tact and CD8 is still engaged with the pMHC, but that Lck is no longer associated with ZAP70 through its SH2 domain. At this point, either the Lck-ZAP70 bond will reform (and return to state 1,2,3), or bond 1 or 2 can break. If for example bond 1 breaks next, the Markov chain exits to state 2, meaning that CD8 has effectively left the complex.

As an example of how we can use this Markov chain to calculate the effective rate at which any species will leave the complex, we can calculate the mean first passage time for the Markov chain beginning at state (1,2,3) to exit to any of the red states. Note that in principle this can happen an infinite number of ways, as the Markov process can bounce between any of the blue states an infinite number of times. To solve for the mean first passage times summing over all possible paths, we solve a standard set of recursive equations. Let $\tau_i$ indicate the mean first passage time for a Markov process starting in state $i$ to exit to a red state, and $\lambda_i$ be the total rate of leaving state $i$ (meaning that the expected time to leave state $i$ is $1/\lambda_i$). As an example:

$$\lambda_{(1,2,3)} = k^{CD8}_{off} + k^{pMHC}_{off} + k^{Lck}_{off}$$  \hspace{1cm} (5.13)

The recursive equations are obtained from

$$\tau_i = \frac{1}{\lambda_i} + \sum_j p_{i\rightarrow j} \tau_j$$  \hspace{1cm} (5.14)

The $p_{i\rightarrow j}$ is the probability to transition from state $i$ to $j$, and is obtained by dividing the rate of transition from $i \rightarrow j$ over the total rate of leaving state $i$.

We do the calculation assuming that on rates within the complex are the same as those in the well mixed state. For instance, the rate from state (2,3) to (1,2,3) is chosen to be the on rate between CD8 and a pMHC that would be recognized if CD8 and pMHC were not bound together in a complex. Undoubtedly the on rate within the complex will be much higher, and...
therefore the effective off rates from this calculation are likely to be conservative, in that the actual effective stabilization of the complex will be much greater than that predicted from this calculation.

To calculate the effective rate at which a species, for instance CD8, leaves the complex, we weight the rate calculated above by the probability that CD8 leaves first. This is calculated in a similar manner as before by a recursive set of equations. We call $p_i$ the probability that the Markov chain, beginning in state $i$, first reaches the red state corresponding to (2) before reaching any of the other red states. The recursive equations are

$$p_i = \sum_j p_{i\to j} p_j \quad (5.15)$$

The above equations are solved subject to appropriate boundary conditions: $p_2 = 1$, $p_1 = p_3 = 0$. Combining the results of the previous calculations, the effective rate at which CD8 leaves the complex is given by $\tau_{(1,2,3)} p_{(1,2,3)}$. What we find is that this calculation predicts the complex will be enhanced considerably due. The effective off rate of CD8 is reduced by a factor of roughly 4-10 for the range of peptide off rates considered here. We thus make an estimate that CD8 off rates become .25 the values considered in Appendix IV when the full complex is in tact.

### 5.6.3 A kinetic model for discrimination

To account for the fact that TCR is able to become sufficiently phosphorylated for a steady state proportion of ZAP70 binding, but ZAP70 is not able to become phosphorylated by Lck, it was suggested [2] that perhaps kinetics of ZAP70 are fast. Perhaps ZAP70 unbinds with a faster rate than Lck phosphorylation, and therefore Lck diffuses from the ITAM before it is able to phosphorylate ZAP70. (We note that the corresponding on-rate of ZAP70 would also have to be high enough for a steady state fraction of ZAP70 to be appreciably bound). An immediate implication of this model is that, since ZAP70 certainly does become phosphorylated and activated upon TCR engagement with foreign peptide-MHC, the kinetics of ZAP70 must be different in the presence of foreign antigen so that ZAP70 is then able to remain bound long enough for phosphorylation by active Lck.

One way to account for this is to postulate two sets of ZAP70 off rates, one off-rate for ZAP70 from a TCR that is not ligated with pMHC, and another slower off-rate for ZAP70 from TCR that is ligated with pMHC. This will result in ZAP70 unbinding faster on average from the TCR when only self peptide is around (since the TCR is more likely to be un-ligated due to the low binding affinity of self peptide) and ZAP70 unbinding slower on average from the TCR
in presence of foreign antigen. We note that there is not significant experimental evidence that
such a difference in kinetic off rates exists, nor is there a molecular mechanism by which this can
be explained. However, we aim here to explore whether or not such a model could recapitulate
the experimental results and also outperform the other two models, and if so, can it do so in a
parameter regime that is biologically realistic.

To simulate this model, we hold constant the ZAP70 kinetics when the TCR is ligated (and
choose these kinetics to coincide with values used for the main text calculations – see Appendix
D), but vary the second off rate for ZAP70 from an ITAM that is not ligated. What we find
is that the non-monotonicity described for the original model (Figure S3) disappears. That is,
increasing the second off rate for Zap70 from non-ligated ITAM only increases the discrimination
(Figure S6). The kinetic model outperforms the discriminatory capabilities of the original model.
However, we also see that the kinetic model predicts that very little ZAP70 will be bound in the
absence of agonist, and much of the ZAP70 bound is still phosphorylated (Figure S7). Therefore
the kinetic model cannot replicate experimental results. Note that Figure S7 shows results for
model of TCR signaling in which Lck must undergo an activation step.

We also find that, for the kinetic model to outperform the original model in terms of signal
discrimination requires a 10-20x difference in kinetic off rates for ZAP70 from ligated and non-
ligated TCR.

We also compared the energetic costs for both the kinetic and original model at steady state
in the presence of only self peptide; this is the relevant value to compare (as opposed to energetic
consumption in the presence of non-self, or some weighted combination of the two) since the
T-cell will spend the vast majority of its time exposed to endogenous peptide. What we find is
that increasing the second off rate monotonically increases the energetic consumption over the
range of values considered (Figure S8).

An intuitive explanation for this is that driving up the second off rate of ZAP70 leaves
the ITAM exposed and requires more frequent phosphorylation and dephosphorylation. The
technical explanation is that dephosphorylation of ITAM in the absence of coreceptor occurs at
a much faster rate than the reverse process, phosphorylation of ITAM by free Lck not attached
to coreceptor. Increasing the second off rate of ZAP70 and leaving ITAM open to more frequent
dephosphorylations means that a greater frequency of irreversible reactions must occur at steady
state, leading to a greater energetic cost. On the other hand, in the limit of very strong ZAP70
binding affinity, the network will look like a simple birth death process tracking the number of
phosphorylated ZAP70. Such networks are at equilibrium and satisfy detailed balance, meaning
they require no energy at steady state. This suggests that the kinetic model outperforms the
original model in discrimination, but does so at an energetic cost, with greater discrimination
requiring greater energetic expenditure.
5.6.4 Details of the stochastic simulation

To simulate the protein interaction networks outlined above, we use the Gillespie algorithm for simulating Markov jump processes\cite{17, 18, 19}. The Gillespie algorithm is based on the fact that time intervals between chemical reactions are exponentially distributed with a rate equal to the sum of all reaction events that can occur given the current state of the system (namely, the copy number of all molecules in the simulation volume). The system is updated by drawing from an exponential distribution to determine the time of the next reaction, and a multinomial distribution to determine the particular reaction that occurred.

Stochastic simulations of the membrane APC interface are carried out in a 1 micron by 1 micron simulation box. Simulations are carried out using both well-mixed and diffusive (spatially resolved) settings. For spatially resolved calculations, the box is divided into 10,000 boxes of .01 micron squared area. Parameter values listed in the table below carry ?bare? units of 1/s, but can easily be converted to conventional units of micromolar using a subvolume edge of .01 micron. Values of $k_{on}$ in the below tables are for well mixed simulations. For spatially resolved calculations, these must be multiplied by 10,000.

The reaction scheme involves TCR+pMHC+CD8 complex formation. Figures in the main text show results in which Lck only phosphorylates at a single rate, and in Figure 6 we show the effect of modulating the amount of coreceptor with active Lck. Calculations have also been done allowing Lck to be initially inactive, and requiring an additional step of Lck activation (Figures S7-S10). For calculations with an Lck activation step, we allow Lck activation when CD8 is in the complex, and deactivation occurs only off of the complex. Once TCR has been phosphorylated twice, ZAP70 can bind to the ITAM. After binding, ZAP70 undergoes an ‘opening’ step (with an opening rate that is varied in the main text). Once open, ZAP70 can become activated by Lck.

Note that, though not specified below, there is an implicit multiplication of 2 for phosphorylation events of TCR that are not phosphorylated at all, and for dephosphorylation of doubly phosphorylated ITAM.

<table>
<thead>
<tr>
<th>Reaction</th>
<th>$k_{on}$</th>
<th>$k_{off}$</th>
<th>$k_{cat}$</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>TCR+pMHC</td>
<td>.0052</td>
<td>Varied</td>
<td></td>
<td>24</td>
</tr>
<tr>
<td>TCR+CD8</td>
<td>.1</td>
<td>20</td>
<td></td>
<td>24</td>
</tr>
<tr>
<td>Lck activation</td>
<td></td>
<td></td>
<td>1</td>
<td>24</td>
</tr>
<tr>
<td>Phosphorylation by basal/active Lck</td>
<td>.05/6.26</td>
<td></td>
<td></td>
<td>13/24</td>
</tr>
<tr>
<td>Lck deactivation</td>
<td></td>
<td></td>
<td>.15</td>
<td>24</td>
</tr>
</tbody>
</table>
It is found that spatial resolution does not change the qualitative results of the calculations. All figures with the exception of Figure S9 are shown for well-mixed systems. Figure S9 (done with spatial resolution) demonstrates how the qualitative trend is unchanged when including diffusion.

Entropy production/energy consumption is calculated by keeping a running sum of the natural log of the ratio of forward reaction propensity over reverse reaction propensity for each reaction event that occurs. By reaction propensity, we mean the reaction rate constant times combinatorial factors related to protein number. Due to reference 10, it is shown that the slope of the time average of this quantity equals the rate of entropy production for non equilibrium steady states. To calculate entropy production, it is necessary to include reverse reactions for all involved steps. We choose rate constants to be sufficiently small for reactions not listed above that should not occur in the time scales of a single trajectory such that they are not observed (for instance, the phosphorylation rate by free Lck not associated with coreceptor is chosen such that we do not observe any of these reaction events in the course of a single trajectory—phosphorylation by free Lck serves as the reverse reaction to dephosphorylation of a TCR not associated with CD8-Lck/pMHC complex). Varying the reverse rate of these reactions between 10e-5 and 10e-20 shifts only the baseline entropy production but does not change the qualitative trends.
Figure 5.8: A very simple 4 state Markov chain was analyzed to explain non-monotonicity in signal discrimination of the null model (referred to in the text as 'old model') as a function of ZAP-70’s unbinding rate. We find that the likelihood ratio of non-self over self (calculated using the steady state probability of occupying state 3) is non-monotonic as a function of ZAP-70 off-rate.
Figure 5.9: Using the same 4 state markov chain as before, one can reframe the TCR signaling as a binary asymmetric channel, and calculate the channel capacity. We see that the non-monotonicity observed in the likelihood ratio is present in the channel capacity as well.
Figure 5.10: These are results from 1000 trajectories of length 500s averaged at every second over a burn time of 100s. The off rate for self is 10s$^{-1}$ and for non-self is 1s$^{-1}$, and 2% of the 100 total peptide is non-self. The AUROC is shown for various values of kinetic parameters of ZAP-70 binding/unbinding. We see that the null model demonstrates a non-monotonicity in the kinetic parameters of ZAP-70. For a fixed on rate, the signal discrimination as a function of off rate is non monotonic, and exhibits an optimal value.
To calculate the effective off rate of coreceptor from the TCR complex, we build a markov chain whose state space represents the interactions that are currently in tact in the complex. Here, we number the interactions as 1-3, between CD8, TCR and MHC.

Using the numbering convention from Figure S4, we build a markov chain whose state space represents the interactions that are currently in tact. The system begins in state (1,2,3) with all interactions in tact. A transition from (1,2,3) to (1,3) indicates that the pMHC-TCR bond has broken, but coreceptor/TCR interaction and Lck/ZAP-70 interactions are still in tact.
Figure 5.13: Here we use calculations similar to figure S3 but for a ‘kinetic model’ in which ZAP-70 has two sets of kinetic off rates. The off rate when TCR is ligated is fixed at .5s⁻¹, and the off-rate/on-rate for ZAP-70 from unligated TCR is varied. This demonstrates that for a fixed off rate when TCR is ligated, increasing the off rate when TCR is not ligated will only serve to increase the discriminatory capabilities.
Figure 5.14: Here, we compare various models of TCR signaling, including in the simulation an extra step of Lck activation. In panel 1, we see the percent of total ZAP-70 in the simulation that is bound to TCR in steady state in the absence of agonist peptide. The null model and ZAP-opening model have high percent of ZAP-70 bound, whereas the kinetic model has significantly lower bound ZAP-70. In panel 2, we see the percent of bound ZAP-70 that is activated. What we find is that the ZAP-opening model has a much lower fraction of ZAP-70 bound to be activated; this implies that the ZAP-opening model does the best job at recapitulating experimental results that allow for ZAP-70 to be both bound and not phosphorylated at steady state. In panel 3 we compare the percent increase in activated ZAP-70 upon introduction of 2% agonist peptide to the system. We find that both the kinetic model and the ZAP-opening model outperform the null model. However, a very large difference in the two kinetic parameters is required for the kinetic model to outperform the ZAP-opening model. Shown are results from simulations in which the kinetic off rate of ZAP-70 from a non-ligated TCR is 20x larger than the off rate from a ligated TCR.
Figure 5.15: For the kinetic model, we see that increasing the off rate of ZAP-70 from an unligated TCR increases the energetic costs (entropy production) of the system at steady state.

Figure 5.16: The average from 150 trajectories each (with self and self+non-self) for spatially resolved calculations to compare the null and Zap opening model. The results are qualitatively unchanged from well-mixed simulations. Spatial resolution serves only to enhance the trends seen before as there is the pronounced effect due to pMHC rebinding.
Figure 5.17: These figures are to be compared with Figure 4 a-c in the main text. We see that including an explicit step of Lck activation does not change the qualitative results.
Chapter Five References


121


CHAPTER FIVE REFERENCES


Chapter 6

Discrete and diffusion models for T cell decision making: Determining an optimal dwell time

6.1 Introduction

In this chapter, we develop both discrete time models and continuous diffusion models for a T cell searching for an infected antigen presenting cell (APC). In our very coarse grained model, we appeal to an important concept in the reinforcement learning community known as exploration versus exploitation. In our model, we assume that a T cell has a fixed amount of time $T$ (either a discrete number of decision making periods or some real valued time interval) to search antigen presenting cells and determine whether or not there is an infection. The T cell can allocate as much of this $T$ time at a certain APC before switching to another one. We assume a noisy stochastic signal transduction mechanism with which the T cell bound to a single APC gathers statistics to determine whether or not it is infected. In theory, the longer a T cell spends at one APC, the better it can gather statistics to determine beyond reasonable doubt whether the cell it is currently bound to is infected or not. Of course, by allocating too much of this time $T$ to a single APC, the T cell foregoes the opportunity to explore other cells, and risks missing a cell that it can more easily classify as infected. Also inherent in our framework is the notion that a T cell would like to correctly identify infected cells quickly.

Of particular interest to us is not only characterizing the existence of an optimal dwell time (corresponding in the discrete model to an optimal number of periods that a T cell remains bound to a single APC) and exploring how this varies with model parameters, but investigating whether or not a stochastic dwell time can improve the expected cost of the decision making...
process. Further, because of the biochemical machinery associated with taking measurements ('measurements' for us refers to an activated form of a signaling molecule), there is stochastic turnover as measurements are destroyed (corresponding to chemical reactions which de-activate aforementioned signaling molecules). How much memory of previous APC encounters should a T cell retain as it hops from one APC to the next? Of course, from the theoretical perspective of a decision-making agent operating based on noisy measurements, one would like to keep a detailed log or account of all measurements taken throughout the course of the decision making process. We investigate further a toy model to extend the discrete model here to one in which a detailed history of all statistics gathered throughout the course of the sequential decision making process are maintained. For this analysis (see chapter 7), we will use more traditional methods from reinforcement learning such as Markov Decision Problems with Bayesian updates.

However, in the biological setting, there is an energetic cost to storing previous measurements. Maintaining a growing reservoir of 'activated' signaling molecules would theoretically require a large amount of ATP. What's more, it seems that most signaling networks that we know of would have no way of marking which measurements came from which APC, meaning a T cell could not demarcate between measurements gathered at distinct APCs, a distinction which would be critical for any optimal sequential-decision rule.

Instead, here we assume that a T cell can spend several 'rest' or 're-set' periods in between hopping from one APC to another, which would serve to allow degradation mechanisms extra time to destroy previous measurements. If our decision rule is of the form of a simple thresholding (after activated signaling molecule number exceeds a threshold, classify the currently bound APC as infected and mount an immune response), this loss of memory in between APC encounters makes it more difficult to cross a threshold on any given encounter. In the limit of a very long re-set period, successive encounters with APC cells are IID. We note that there is an important distinction between increasing the number of reset periods and simply increasing the threshold of activated signaling molecule above which an immune response is mounted. This is due to the stochastic nature of measurement erasure (de-activation).

6.2 Discrete time model

6.2.1 Model details

Here, we outline the specific details of the discrete time model we consider. First we will give the biological intuition behind the various steps, and then provide pseudo code for the stochastic simulation. We assume that when a T cell binds to an APC that is infected, there is some spatial surface area corresponding to the (effectively 2D) interface between the two cells. This
interface contains some fraction $\varepsilon$ of pMHC presenting self peptide. The exact concentrations of TCR and pMHC will determine how many pMHC-TCR interactions form in an initial binding event between T cell and APC, but we coarse grain these specific details and assume a spatial poisson process with parameter $\lambda_1$.

In other words, each time a T cell binds to a new APC, the number of interactions formed between TCR and pMHC bearing self peptide is $Po(\lambda_1(1 - \varepsilon))$ whereas the number of interactions formed between TCR and pMHC bearing non-self peptide is $Po(\lambda_1\varepsilon)$. Of course, new interactions will form in subsequent periods as well, at a lower rate. Again, we coarse grain this into a second spatial poisson process with parameter $\lambda_2$, meaning that for each subsequent period a T cell remains at the same APC, $Po(\lambda_2(1 - \varepsilon))$ new interactions between pMHC with self peptide and TCR form, and $Po(\lambda_2\varepsilon)$ interactions between pMHC with non-self and TCR form.

We assume that pMHC-TCR interactions generate a downstream activator molecule (serving as the ‘measurement’ mentioned above) by a first order process:

$$pMHC - TCR \rightarrow pMHC - TCR + A \quad (6.1)$$

We assume the time interval in our simulation is such that each pMHC-TCR forms one activator molecule with probability $\gamma$. In other words, if there are $N_{tot}$ interactions between pMHC and TCR (which can be broken into $N_{tot} = N_{self} + N_{non-self}$), then each period, a stochastic number of activator molecules is formed, drawn from Binom($N_{tot}$, $\gamma$).

We assume that the number of periods before an activator molecule is destroyed is geometric, meaning that there is a probability $\alpha$ each period for any given activator to be destroyed (this is the discrete time analogue of the first order decay process). We therefore decrease the number of activator molecules $N_a$ by an amount equal to Binom($N_a$, $\alpha$) each period. We assume that there is some threshold $\Omega$ for activator number $N_a$ such that when $N_a > \Omega$ the T cell attacks the APC it is currently bound to.

Of course, TCR-pMHC interactions break with some rate which differs depending on whether the peptide is self or non-self. We again translate this into some probability $p_r$ (for probability of remaining bound) or equivalently a probability $1 - p_r$ that a particular TCR-pMHC bond is broken each period. To capture heterogeneity in the peptide-TCR affinity [1], we let each TCR-pMHC have its own $p_r$. The $p_r$ are drawn from two distributions based on whether or not the peptide is self or non-self. These are two beta distributions. At the start of each simulation for a particular T cell, we choose one value $p_r$ (non-self) from the beta distribution corresponding to non-self, which each pMHC-TCR bearing non-self peptide assumes. For each new interaction between pMHC-TCR bearing self peptide throughout the rest of the simulation, we choose $p_r$ from the beta distribution corresponding to self. Effectively, we have to probe each pMHC-
TCR interaction at each time period (with its own random number) to assess whether or not it breaks. By parametrizing this interaction strength by \( p_r \) instead of \( 1 - p_r \) we can interpret \( p_r \) as a measure of affinity, and therefore non-self peptides have a greater affinity for TCR (a larger \( p_r \)). A plot of the two beta densities used for the results shown in this work (with parameters (2, 4) and (4, 2) for self/non-self) are shown in figure 6.1.

![Density used for \( p_r \)](image)

Figure 6.1: Here we plot the pdf's used to generate \( p_r \) for both self and non-self. Note that \( p_r \) is the probability that a pMHC-TCR interaction remains bound for a given period.

The specific details of how many periods a T cell remains at one APC depend on whether or not the dwell time is stochastic or deterministic. If the dwell period is deterministic, a T cell spends \( D \) periods at each APC. If the dwell period is stochastic, the T cell spends \( \text{Geometric}(p) \) periods at each APC with \( p = 1/(D + 1) \).

The pseudo code for a time step of one interval for a T cell is given now, where \( N_s, N_{ns} \) represent pMHC-TCR interactions with pMHC bearing self/non-self peptides respectively:

- While \( t < T \) and \( N_a < \Omega \):
  - If hopping to a new cell:
    - Determine \( D \) dwell time
    - \( N_{s+} = P_0(\lambda_1(1 - \varepsilon)) \)
CHAPTER 6. OPTIMAL DWELL TIME

- \( N_{ns}^+ = P_0(\lambda_1 \varepsilon) \)
- \( N_a^- = \text{Binomial}(N_a, \alpha) \)

- else if resting:
  - \( N_a^- = \text{Binomial}(N_a, \alpha) \)

- else:
  - For \( p_r \in \{ p_r(i) \}_{i=1}^{N_s+N_{ns}} \), check if interaction has broken
  - \( N_a^- = \text{Binomial}(N_a, \alpha) \)
  - \( N_a^+ = \text{Binomial}(N_s + N_{ns}, \gamma) \)
  - \( N_s^+ = P_0(\lambda_1(1-\varepsilon)) \)
  - \( N_{ns}^+ = P_0(\lambda_1\varepsilon) \)

6.2.2 Cost function for a population of T cells

To say something about the optimality of a threshold/dwell time, we must be able to compare populations of T cells. Here, we discuss how to extend a cost function introduced in [2] which is adapted for sequential decision making problems. Denote \( f_0(t), f_1(t) \) to be the fraction of APCs bearing only self or also pathogenic pMHC to which T cells have activated (respectively) up to time \( t \):

\[
C = \sum_{t=1}^{T} \left( c_2 \left( e^{c_0 f_0(t)} - 1 \right) + \frac{1}{1 + c_1 f_1(t)} \right) \quad (6.2)
\]

We note that the cost \( C \) will be a function of the size of the T cell population and the number of APCs, as well as other model parameters. We evaluate \( E[C] \) on a grid of \((D, n)\) points (\( D \) being the dwell time, \( n \) being the threshold of \( N_a \) required for activation). A surface of \((D, n)\) values are compared for both stochastic and deterministic dwell times, and what we find is that the stochastic dwell time lowers every point on the surface (see figure 6.3).
Figure 6.2: We calculate the cost of a population of T cells searching for infected APCs. We calculate the expected cost by monte carlo simulation on a grid of $(T, n)$

Similarly, we can plot a particular point on the surface and calculate the cost at that point as a function of the number of reset/sleep periods. What we see is that this behavior decreases initially before increasing, suggesting that there is an optimal amount of memory to retain of previous APC encounters.
Figure 6.3: Here we plot the pdf’s used to generate $p_r$ for both self and non-self. Note that $p_r$ is the probability that a pMHC-TCR interaction remains bound for a given period.

6.3 Diffusion model for T cell decision makers

6.3.1 Model description

We would like to determine whether the result that stochasticity improves the cost of decision making is a general result, or specific to the details of the model above or the cost function specified above. To this point, we will define a very simple diffusion process that captures the essence of the model outlined above, and a very simple cost function to capture the intuition of the problem.

We make a simplistic analogy for the T-Cell process. Consider the following chemical langevin equation:

$$dx_t = -k(x_t - \mu_t)dt + \left(\sqrt{k|x_t| + k \mu_t}\right) dW_t$$  \hspace{1cm} (6.3)

where $\mu_t \in \{\mu_0, \mu_1, 0\}$ depending on if the T-cell is bound to a healthy cell, infected cell, or is ‘switching’ (respectively). Specifically, we will consider the following process. Suppose that $\mu_t$
remains constant for a stochastic time period (corresponding to the T-cell having a stochastic
dwell time at an individual APC) and then switches to 0 for a ‘sleep period’ (this corresponds to
a T-cell hunting for a new cell, or to some positive feedback loop which disables the formation
of x temporarily) before finding a new cell, at which point we stochastically choose \( \mu_t \) to be \( \mu_0 \)
or \( \mu_1 \). We choose \( \mu_1 > \mu_0 \) to capture the intuition that the overall rate of activator formation
is greater when a T-cell binds to an infected APC. The model is parametrized thus by \( \mu_0 \) and
\( \lambda = \mu_1/\mu_0 \). To compare to a deterministic dwell time, we will allow \( \mu_t \) to remain fixed for a
deterministic amount of time before switching.

We will assume further that there is a threshold \( x_u \) (for ‘upper’) above which the T-cell
mounts a response. Let there be two indicator functions \( I(t) \) and \( R(t) \) which respectively denote
whether or not the T-cell is currently at an infected cell and whether or not the T-cell has
responded. Assume that once the T-cell responds, it remains bound at the current cell for the
remainder of the time period \( T \).

We will define a simple cost function to be

\[
Cost = \int_0^T (1 - I(t)R(t)) \, dt \tag{6.4}
\]

This cost function simply measures the amount of time that the T-cell has made the correct
decision. The faster the T-cell responds \( (R(t) = 1) \) to an infected cell \( (I(t) = 1) \) the sooner the
cost drops to zero for the remainder of the time period. Of course, if the T-cell responds when
\( I(t) = 0 \), the total cost will equal the time period, as we integrate 1 over the entire time period.
The cost is limited by the time it takes for the T-cell to first encounter an infected cell, which
can easily be calculated based on the dwell time and geometric distribution of first encounter
time.

The above cost function assumes that there is always an infection. If this is not the case, we
can consider the following cost function. Let \( 1(\text{inf}) \) be the indicator for an infection. We can
modify the cost to be

\[
Cost = \int_0^T 1(\text{inf}) (1 - I(t)R(t)) + (1 - 1(\text{inf}))R(t) \tag{6.5}
\]

We will look to optimize over \( E[Cost] \), where in the first equation above the expectation is over
stochastic trajectories each of which assumes an infection, whereas the latter assumes expectation
over the probability of an infection.

Let’s imagine an extension of the stochastic process outlined above that does not terminate
once \( x_t \) exceeds \( x_u \). Instead, just allow \( x_t \) to evolve infinitely. We will let the random variable \( i \)
denote the first time for \( x_t \) to exceed \( x_u \) when the T-cell is currently bound to an infected cell.
Let \( h \) denote the first passage time for \( x_t \) to exceed \( x_u \) when the T-cell is currently bound to a
healthy cell. For the former equation above, we can see that the expected cost will be (letting the variables \( R, N \) correspond to Response and No response respectively, meaning the T cell did or did not exceed the threshold required for activation in time \( T \)):

\[
\mathbb{E}[\text{Cost}] = P(i < h|R) \cdot \mathbb{E}[i|i < h, R] + (P(i > h|R) + P(N)) \cdot T
\]

Roughly speaking, we separate the cost into three components.

- The first component, \( P(i < h|R) \cdot \mathbb{E}[i|i < h, R] \) states that, if we respond, we want to respond to the correct cell and we want to do it in a timely manner.

- The second component, \( P(i > h|R) \cdot T \), states that if we respond to the wrong cell, we will pay a penalty.

- The third component, \( P(N) \cdot T \) states that we run the risk of not responding at all if we set our boundary to be too conservative.

It is worth thinking about the limit in which the two stationary distributions are very far apart. In this case, let's assume that the stationary distribution over \( p(x|\mu_1) \) is centered at a location infinitely far from \( p(x|\mu_0) \). Conceptually, we would like a somewhat small dwell time since we will be limited by how long it takes to find the first infected cell. We also want a threshold that is sufficiently high that we make few mistakes, but sufficiently low that we can reach it in a somewhat small dwell time.

We see in fact that the relative cost of having a stochastic dwell time vs. a deterministic dwell time depends on the parameter regime. We pick \( \mu_1/\mu_0 = 1.1, \mu_0 = 10, k = 1 \) and choose \( \mu_1, \mu_0 \) with equal likelihood each hop. We plot the expected cost as a function of threshold in figure 6.4 (due to slow convergence of Monte Carlo sampling, the curve is noisy and we therefore show 95% confidence standard error bars). Figures 6.4, 6.5, 6.6, 6.7 show the case for \( D = \mathbb{E}[D] = .5 \), where \( D \) is the dwell time, and by abuse of notation we write \( D = \mathbb{E}[D] \) to mean that we choose \( D \) in the deterministic case to equal the mean value \( \mathbb{E}[D] \) for the stochastic dwell case. In the parameter regime considered, the second component of the cost function (outlined above), figure (6.5), is responsible for the trend observed in the total cost (compared to components 1 and 3 in figures 6.6, 6.7). While these figures are shown for a sleep period of 1.0, the results are quantitatively unchanged for a sleep period of .1 (see figure 6.8). In this parameter regime, we see that for most thresholds considered, the stochastic dwell time outperforms the deterministic dwell time, except at some very low thresholds.

However, when looking at \( D = \mathbb{E}[D] = 5.0 \), we see that the trend is reversed, and the cost function for deterministic dwell time outperforms the stochastic dwell time over almost all thresholds considered (figure 6.9).
Figure 6.4: Here we plot the expected cost for model eq. (6.3) as a function of threshold for activation.
Figure 6.5: This is a plot of the probability that $i$, the first time for a T cell to exceed the threshold while bound to an infected cell, is greater than $h$, the first time for a T cell to exceed the threshold while bound to a healthy cell (weighted by the length of the simulation time, $T$). This basically shows the probability for a T cell to mount a response to a healthy cell. This was referred to as component 2 of the total cost.
Figure 6.6: A plot of the probability that $i < h$ conditional on $R$ (a response), times the expected time to mount a response to an infected cell, conditional on $(i < h, R)$. This was referred to as component 1 of the total cost.
Figure 6.7: A plot of the probability for no response, weighted by the time of the simulation, $T$. This was referred to as component 3 of the total cost.
Figure 6.8: Here we plot the expected cost for model eq. (6.3) as a function of threshold for activation.
CHAPTER 6. OPTIMAL DWELL TIME

6.3.2 Density of $x$ after excursion $n$

To gain some insight into the results shown above, we examine the density of $f(x)$ after a given number of excursions. By excursion, we mean the following: upon each APC encounter, the value of $x$ increases per the dynamics in eqn (6.3). During the reset period, $x$ decreases since $\mu = 0$. If the dwell time is abundantly long, $x$ will approach a steady state distribution at the end of the T cell's time at its first APC. However, if the dwell time is not sufficiently long, then $x$ increases to some amount by the end of the encounter with a first APC (marking the end of its 'first excursion'), subsequently decays during a reset period, increases to some second amount by the end of the encounter with a second APC (marking the end of its 'second excursion'), and so on. We can estimate the density $f_n(x)$ of $x$ after $n$ excursions. To simplify matters, we can compare $f_n^h(x)$ and $f_n^i(x)$, indicating densities of $x$ assuming repeated encounters with only healthy OR infected cells (respectively). We note that this will not perfectly elucidate the mechanism for our observations since in the actual simulation, subsequent excursions may be with both healthy and then infected cells.

Figure 6.9: Here we plot the expected cost for model eq. (6.3) as a function of threshold for activation. In this figure, $D = \mathbb{E}[D] = 5$. 

$E[\text{cost}], \text{Dwell}=5.0; \text{Sleep}=0.1$

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{figure69.png}
\caption{Here we plot the expected cost for model eq. (6.3) as a function of threshold for activation. In this figure, $D = \mathbb{E}[D] = 5$.}
\end{figure}
CHAPTER 6. OPTIMAL DWELL TIME

Figure 6.10: Here we plot the expected cost for model eq. (6.3) as a function of threshold for activation. In this figure, \( D = \mathbb{E}[D] = 5 \).

In figure 6.10 we look at the density \( f_n^{nh}(x) \) for \( n = 1, 3, 4, 10 \) with \( D = \mathbb{E}[D] = .5 \). In these plots, red vs. blue denotes healthy vs. infected APCs, while dotted vs. solid densities indicate stochastic dwell vs. deterministic dwell. What we see is that the stochastic dwell distributions are heavy tailed to the right. While it appears that the healthy vs. infected stochastic dwell densities are less distinguishable than the case of deterministic dwell, they have also managed to place greater probability mass past the threshold in a fewer number of excursions. We can quantify both of these ideas by looking at both the KL divergence between healthy and infected \( KL(f_n^h || f_n^h) \) as a function of \( n \) (for both deterministic and stochastic dwell) and also comparing probability of detection and probability of false alarm plots for the thresholds considered previously. The KL divergence is estimated using the method outlined in [3]. We find that indeed the KL divergence between healthy and infected cases grows much more slowly for stochastic dwell as opposed to deterministic (figure 6.11). However, when we plot detection...
and false alarm probabilities as a function of threshold (at a fixed excursion number, shown is $n = 4$), we see that the stochastic case has a detection probability which outperforms the deterministic case, at the expense of a higher false alarm probability (figure 6.12).

Figure 6.11: We see that the KL divergence between healthy and infected grows much more slowly for stochastic dwell as opposed to deterministic dwell in the small $\mathbb{E}[D]$ limit.
Figure 6.12: For small excursion number, the stochastic dwell case has a probability of detection that outperforms the deterministic case, at the expense of a larger probability of false alarm.

We compare with the case in which $D = \mathbb{E}[D] = 5.0$, a relatively high dwell time. What we see is that after even 1 excursion (figure (6.13a)), the stochastic dwell case has a much heavier tail to the left, but the deterministic dwell limit appears to have already reach steady state, comparing the solid lines in figures (6.13a,b,c). In contrast to the low dwell limit, we can see that the KL divergence for the stochastic dwell case grows quickly and after four excursions has already matched the deterministic dwell case (figure 6.14). However, focusing on the range of thresholds considered above, if we plot the PD and PF vs. threshold, we see that the deterministic case greatly outperforms the stochastic case (in terms of PD) after even one excursion, but at the cost of a larger PF (figure 6.15).

What these results demonstrate is that, depending on the actual cost function of interest (whether a T cell would like to minimize PF at the expense of a lower PD, or maximize PD at the expense of a larger PF), by tuning the dwell time and adding stochasticity, the signaling network can achieve greater PD/PF or lower PD/PF than a deterministic case. Moreover, we have been able to achieve both results without changing the probability distribution that a dwell time chooses from (all results above are for a dwell time drawn from the exponential distribution).
Figure 6.13: Here we plot the expected cost for model eq. (6.3) as a function of threshold for activation. In this figure, $D = \mathbb{E}[D] = 5$.

Figure 6.14: The KL divergence between healthy and infected grows much more rapidly for stochastic dwell as compared to the small $\mathbb{E}[D]$ limit in figure (6.11). In this figure, $D = \mathbb{E}[D] = 5$. By excursion 4, stochastic and deterministic match.
CHAPTER 6. OPTIMAL DWELL TIME

6.3.3 Comparison with CIR model

Interestingly, if we integrate the dynamics using

\[ dx_t = -k(x_t - \mu_t)dt + \left(\sqrt{kx_t}\right) dW_t \]  

(6.7)

instead of the dynamics in eqn. (6.3), there is a closed form solution for the density of \( x \) at the end of its first excursion in the deterministic dwell case. Namely, for \( x_t \) following dynamics in (6.7), given an initial position of \( x_0 \) (which must be greater than zero for the case in which \( 2\mu_t > 1 \)), we have that at time \( t > 0 \), the density of \( x_t \) has a closed form solution. Define the following:

\[
\begin{align*}
c &= \frac{2}{1 - e^{-kt}} \\
q &= 2\mu - 1 \\
u &= cx_0 e^{-kt} \\
v &= cx_t
\end{align*}
\]

(6.8)

Then the density of \( x_t \), which we can denote by \( f(x|t) \) is given by

\[
f(x|t) = ce^{-u-v} \left(\frac{u}{v}\right)^{q/2} I_q(2\sqrt{uv})
\]

(6.9)
for $I_q(\cdot)$ the modified Bessel function of the first kind of order $q$. We can calculate numerically the density of $x$ at the end of the first excursion by integrating against the stochastic dwell time:

$$f(x) = \int_0^\infty f(x|t) f(t) dt = \int_0^\infty f(x|t) (1/D) e^{-t/D} dt$$  \hspace{1cm} (6.10)

We can compare the integration with estimates of the density from direct simulation. The CIR process admits a closed form solution for $x_t|x_0$ as a non central chi squared distribution (this interpretation follows immediately from the solution of the CIR by using Ito's lemma on a sum of squared OU processes) [4]. We simulate $Y \sim NC\chi^2(2cx_0e^{-kt}, 4\mu)$ and $x_t = Y/2c$ is a draw from $f(x|t, x_0)$. Simulation of the non central chi square is efficient given the Poisson weighted mixture representation. Our protocol to sample from the marginal (6.10) is

- Sample $t \sim (1/D) \exp(-t/D)$.
- Sample $n \sim Po(cx_0e^{-kt})$
- Sample $Y = \chi^2(2n + 4\mu) = \sum_{i=1}^{2n+4\mu} \varepsilon^2$ with $\varepsilon \sim \mathcal{N}(0,1)$.
- Set $x_t = Y/2c$.

While the exact simulation method outlined above and a simple Euler integration of the SDE confirm the numerical integration (figure 6.16), the computational ease of the numerical integration allows us to more efficiently sweep through expected dwell times to monitor how $PD$ curves for stochastic vs. deterministic dwell times compare. We compute the $PD$ curve for various thresholds in both the deterministic and stochastic dwell case, and by computing $PD(det) - PD(stoch)$ we can gauge when one system outperforms the other. What we see (figure 6.17) is that generally at higher expected dwell times, the deterministic system achieves a higher PD for all thresholds considered (of course this also comes at the cost of a higher PF as well). The trend does not appear to be monotonic though. If we look at a fixed threshold and plot the difference $PD(det) - PD(stoch)$ as a function of expected dwell time (figure 6.18), the curve appears to decrease before increasing (showing a distinct minimum at intermediate dwell times).

Next, we sought to understand to what extent we can tune the $PD(det) - PD(stoch)$ difference by tuning the distribution. To explore this, we fixed the mean of the dwell time distribution and systematically varied the variance. We accomplish this by letting $D$ draw from the two parameter gamma distribution, which allows fixing the mean and sweeping through the variance. By plotting the difference in detection probabilities vs. $1/var(D)$, we see converge of $PD(det) - PD(stoch)$ to zero as $1/var(D) \to \infty$ (figure 6.19). We also see that the T cell can tune the $PD(det) - PD(stoch)$ difference by tuning the distribution. This result is analogous
to earlier results shown in this thesis; we have seen that genetic toggle switch networks can tune mean first passage times between stable basins by tuning the distribution of repressor unbinding, and that allostERIC control of the distribution of catalytic rates of SOS in the Ras-SOS network for early T cell signaling can also modulate how likely the network is to be in the active state.

Figure 6.16: We compare numerical integration to find the marginal with an exact simulation and an euler integration using step size of \( dt = .001 \). For these calculations, we set the expected dwell time to be 5s.
CHAPTER 6. OPTIMAL DWELL TIME

Figure 6.17: For various dwell times (and expected dwell times) we compute the probability of detection (PD) curve for various thresholds. Taking the difference of the PD curves for deterministic vs. stochastic dwell times allows us to gauge when one system outperforms the other.

Figure 6.18: For various thresholds, we plot the difference $PD_{(det)} - PD_{(stoch)}$ as a function of dwell time or expected dwell time. The difference decreases before increasing, showing a distinct minimum for intermediate dwell times.
Figure 6.19: Letting $D$ the dwell time draw from the gamma distribution, we fix the mean at either 1.0s or 5.0s and sweep through different variances for the distribution. In both cases of course the $PD(det) - PD(stoch)$ converges to zero as we lower the variance, but we see by tuning the distribution we can achieve a wide range of detection probabilities.

### 6.4 Discussion

By considering both a discrete time model in which we could analyze a cost function that applies to a population of T cells searching for infected APCs, or a diffusion model in which we calculated the expected time a T cell mounts a response incorrectly, we have seen that stochasticity can be used to tune the responses of a T cell and make for a more robust network. Depending on the expected dwell time, stochasticity in the dwell time can be used either to increase the detection probability (at the expense of an increased false alarm probability) or decrease the false alarm probability (at the expense of a decreased detection probability). What’s more, by tuning the shape of the dwell time distribution we can also tune the detection probability thus achieving a continuum of points. This allows for a network that can be more robustly tuned to accomplish a desired PD,PF pair.
Chapter Six References


Chapter 7

T cell as an optimal learner

7.1 Introduction

In the previous chapter, we looked at a coarse grained model of T cells scanning multiple APCs (any of which may or may not be infected and thus displaying non-self pMHC). We saw that by remaining at each APC for a stochastic time interval (corresponding to a stochastic dwell time $t_i$ at the $i^{th}$ APC visited), and further by tuning the distribution of dwell times, the T cell could achieve a continuum of points along the PD,PF curve (of detection probability/false alarm probability pairs). The T cell can push to a higher PD than the deterministic dwell case (at the expense of a higher PF), or could push to a lower PF (at the expense of a lower PD) than the deterministic case.

As we alluded to in the introduction of the previous chapter, if one were to ignore the energetic costs of maintaining a detailed log of every measurement taken at each APC visited (this would correspond for instance to a time series of molecule copy number during each interaction), then the optimal dwell time would be a very complicated function of the entire trajectory. However, besides the energetic costs associated with storing such a log, actually implementing the optimal decision rule would require a very complex signaling network. From a theoretical perspective, however, it is worthwhile to explore what this optimal decision rule would look like. If we could characterize the optimal decision rule, we could understand ways in which the actual biological system has evolved to mimic such an optimal decision rule. As a very preliminary first step toward this end, we outline in this chapter methods to solve the optimization problem which mimic the discrete time model outlined in section 6.2.

We will begin in section 7.2 by computing the optimal decision rule for a T cell bound to a single APC for $T$ periods. At each period, the T cell makes a measurement, and decides whether or not to classify the APC as healthy or infected, or can defer the decision making for
one more period for an extra cost. In section 7.3 we look at a single T cell scanning multiple APCs for $T$ periods. At each period, the T cell decides whether to classify the current APC as infected, remain at the current APC for one more period and gather one more data point, or hop to a new APC. As we will see, this optimization suffers from the curse of dimensionality, and quickly becomes infeasible for large $T$. We move on to methods of approximate optimization, in particular Q-learning.

In section ??, we will use approximate optimization with Q-learning and function approximation. The Q-learning with function approximation is a useful paradigm we believe as it allows us to explore simple functions of a small number of variables, and thus look for simple variables (for instance those that satisfy the markov property and don't require access to the entire time series) that lead to networks with good performance. We can then compare with actual biological networks to see what topologies allow one to accurately track these variables.

### 7.2 Single APC Markov Decision Problem

Consider first a simplified model in which we have a two period decision process. In period one, we view a single draw from a Poisson distribution, parametrized by either $\lambda_0, \lambda_1$. We have to make a hard decision, that is either classify our draw as coming from distribution 0 (call this $\hat{H}_0$), classify it as coming from distribution 1 ($\hat{H}_1$), or we get a third choice: we can defer our decision making process for one more round, in which case we get to observe one more draw from the distribution.

How do we decide whether or not to defer the decision-making process? Clearly, if the expected gain in information will exceed the cost of waiting one more round, we should elect to defer the decision. To make this more rigorous, let's assume costs $C_{00}, C_{01}$ and $C$ to be the cost of claiming $\hat{H}_1$ when in fact $H_0$ is correct, vice versa, and $C$ to be the cost incurred in one period of waiting. We will assume priors $\pi_0, \pi_1$.

To simplify things, let's assume we have already deferred the decision-making process. That is, we have already now observed two draws from the Poisson distribution. We can either elect to classify the random variables as coming from $\lambda_0, \lambda_1$ or choose the third option again (make no decision, which in this case won't lead to an extra measurement since there are only two periods in this example), incurring a cost of $C$.

Let's calculate the expected loss $E[C(\hat{H}, H)|D_2]$, where $D_2 = \{N_1, N_2\}$ from both periods. To be clear, we are ignoring any costs that may have been incurred in the first period of decision making, solely calculating the expected cost (assuming we act optimally) in the second round. Bayesian decision making says that we should minimize the expected cost over the posterior $P(H_i|D_2)$. What is $P(H_0|D_N)$ (e.g. $D_N$ implies we have observed $N$ draws from the Poisson
distribution). One can show that
\[ \alpha_N = \mathbb{P}(H_0 | D_N) = (1 + \exp f(D_N))^{-1} \]
\[ f(D_N) = -N(\lambda_1 - \lambda_0) + \left( \sum_i N_i \right) \ln \left( \frac{\lambda_1}{\lambda_0} \right) + \ln \left( \frac{\pi_1}{\pi_0} \right) \] (7.1)

To calculate the \( C(\hat{H}) \equiv \mathbb{E}[C(\hat{H}, H) | D_2] \), we can calculate the cost under each decision separately:
\[ C(\hat{H}_1) = \alpha C_{10} \]
\[ C(\hat{H}_0) = (1 - \alpha) C_{01} \]
\[ C(\hat{H}_2) = C \] (7.2)

Acting optimally, we should choose the hypothesis to minimize the above expected cost. Thus, \( \hat{H}^* = \arg \min_{\hat{H}} C(\hat{H}) \), and the expected cost is \( f(D_2) = C(\hat{H}^*) \).

This naturally leads to a set of decision boundaries over \( \bar{N} = (N_1 + N_2)/2 \). By plotting the expected costs as a function of \( \alpha \) (itself a function of \( \bar{N} \)), we see the region of space in which we choose \( \hat{H}_0, \hat{H}_1, \hat{H}_2 \).

NOTE: How the decision boundaries change as we defer the decision-making process is not always clear a priori. For instance, consider the two-round case. In round 0, the cost of deferring the decision making process is \( C + \mathbb{E}[f(D_2)] > C \). Thus, in the second round, the cost of not answering has been lowered. However, we may expect that our estimate of \( \alpha \) will 'improve' (e.g. get further towards 1 or 0). The horizontal line in the graph of expected cost lowers, but we also move further to right or left. These are counteracting tendencies.

More generally, if we have data up to time t of the form \( D_t = \{ N_1, N_2, \ldots, N_t \} \), we have a set of possible actions \( \hat{A} = \{ \hat{H}_0, \hat{H}_1, \hat{H}_{NR} \} \) for hypothesis 0,1 and no response, and finally there is a true underlying state of the world \( A = \{ H_0, H_1 \} \). The basic principle of Bayesian decision theory is that we choose the action \( \hat{H} \) to minimize
\[ \hat{H}^* = \arg \min_{\hat{H} \in \hat{A}} \mathbb{E}[C(\hat{H}) | D] \] (7.3)
(where \( C(\hat{H}) \) is the cost of an action \( \hat{H} \) with respect to the posterior
\[ p(H_i | D) = \frac{p(D | H_i) \pi_i}{\sum_{i'} p(D | H_{i'}) \pi_{i'}} \] (7.4)

Call the expected cost at period t (if we act optimally) \( f_t(D_t) = \mathbb{E}[C(\hat{H}^*) | D_t] \). The expected costs given we choose \( \hat{H}_i, i \in \{ 0, 1 \} \) are easy to calculate. Let \( C_{i,j} \) be the cost of choosing hypothesis \( \hat{H}_i \) when the true state of the world is \( \hat{H}_j \):
\[ \mathbb{E}[C(\hat{H}_0) | D] = C_{01} \cdot p(H_1 | D) \]
\[ \mathbb{E}[C(\hat{H}_1) | D] = C_{10} \cdot p(H_0 | D) \] (7.5)
What is the expected cost of ‘no response’? We incur a fixed cost $C$ plus whatever the cost associated with the optimal decision is in the next state we end up in (where the next state is characterized by the expanded data $\mathcal{D}_t \cup N_{t+1}$). Of course, we don’t know what state we’ll end up in, so we have to take expectation over it:

$$\mathbb{E}[C(\hat{H}_{NR})|\mathcal{D}_t] = C + \sum_{N_{t+1}} P(N_{t+1}|\mathcal{D}_t) f_{t+1}(\mathcal{D}_t \cup N_{t+1})$$  \hspace{1cm} (7.6)

Note that above $P(N_{t+1}|\mathcal{D}_t) = \sum_{i \in \{0,1\}} P(N_{t+1}|H_i) P(H_i|\mathcal{D}_t)$.

As an example of how to solve this, assume a fixed $T$ periods. Solve by backward induction, also known as dynamic programming (this is an example of a markov decision problem). For the Poisson case, as shown above, the posterior $P(H_i|\mathcal{D}_T)$ only depends on the sample average $\bar{N} = (1/T) \sum_i N_i$. So for every possible $\bar{N}_T$, characterize $P(H_i|\bar{N}_T)$ and return the optimal decision as $f_T(\bar{N}_T) = \min\{P(H_0|\bar{N}_T)C_{0,0}, P(H_1|\bar{N}_T)C_{0,1}, C\}$. Working one step backwards, for every $\bar{N}_{T-1}$, calculate $P(H_i|\bar{N}_{T-1})$, and calculate

$$f_{T-1}(\bar{N}_{T-1}) = \min \left\{ \begin{array}{l} P(H_0|\bar{N}_{T-1})C_{1,0} \\
C + \mathbb{E}_{\bar{N}_T}[f_T(\bar{N}_T)|\bar{N}_{T-1}] \\
\end{array} \right.$$  \hspace{1cm} (7.7)

We solve the MDP numerically with values $\lambda_0 = 9, \lambda_1 = 10, \pi_1 = .5, c_{01} = c_{10} = 1., c = .01, N = 20$. The optimal decision bounds as a function of $n_{obs}$ are shown in figure 7.1. Note that by collapsing to the sample statistic $\bar{n}$, we can visualize the decision bounds in the same figure with the same y axis. We can also plot the expected cost as a function of $\bar{n}$ for various $n_{obs}$. 
Figure 7.1: After solving the MDP, we can calculate optimal decision bounds as a function of observation number. In other words, at observation \( n \), if one computes the statistic \( \sum_i x_i/n \), in what region of space should one classify as 0, 1 respectively, and in what region should one abstain from decision making and wait to observe another data point?
7.3 Multiple APC Markov Decision Problem

Here is the problem set up. There may or may not be an infection, with prior probability $\pi_1, \pi_0$. There is a fixed number of periods $T$. Let $H_1, H_0$ denote the presence or absence of a global infection, and $h_i$ denote whether or not an individual cell is infected given a global infection $\pi(h_i|H_1) \equiv \pi_{11}$. We assume that IF there is an overall infection, there will be a cost of $C$ each time period, although if you correctly identify an infected cell, the cost goes to 0 for every remaining period (including the one in which you respond). If you incorrectly respond to a cell that is not infected, there is a cost of $C_{10}$. If you incorrectly respond to a cell that is not infected in period $t$, AND there happens to be an overall infection, your cost is therefore
CHAPTER 7. T CELL AS AN OPTIMAL LEARNER

\[ C_{10} + (T - t + 1)C. \]

Decisions at each period are \( \mathcal{A} = \{ \hat{H}_{NR}, \hat{H}_1, \hat{H}_S \} \).

What is the data structure \( \mathcal{D}_t \) that stores our observations? It looks like

\[ \mathcal{D}_t = \{ [\#_0, \#_1, \ldots], \ldots, [\#_0, \ldots] \} \quad (7.8) \]

Each period, we collect a new observation, but the operation performed on our data structure depends on whether we ‘switch’ or ‘don’t respond’. These respectively look like \( \mathcal{D}_t.\text{append}([N_{t+1}]) \) and \( \mathcal{D}_t[-1].\text{append}(N_{t+1}) \).

So how do we solve the problem? Same as before. Use backward induction. We first enumerate every possible \( \mathcal{D}_T \) (note this requires us to enumerate every possible set of \( T' < T \) non zero integers that add up to \( T \), then search over the set of all possible \( \{ N_1, N_2, \ldots \} \) sample averages). For a given \( \mathcal{D}_T \), we can calculate the expected costs associated with each of the two final decisions:

\[
\begin{align*}
\mathbb{E}[C(\hat{H}_{NR})] &= C \times P(H_1|\mathcal{D}_T) \\
\mathbb{E}[C(\hat{H}_1)] &= C_{10} \times P(H_0|\mathcal{D}_T) + (C + C_{10}) \times P(h_i = 0, H_1|\mathcal{D}_T) \\
\mathbb{E}[C(\hat{H}_S)] &= C \times P(H_1|\mathcal{D}_T) + \sum_{N_{t+1}} f_{t+1} (\mathcal{D}_t.\text{append}([N_{t+1}])) p(N_{t+1}|\mathcal{D}_t) \\
\mathbb{E}[C(\hat{H}_{NR})] &= C \times P(H_1|\mathcal{D}_T) + \sum_{N_{t+1}} f_{t+1} (\mathcal{D}_t[-1].\text{append}(N_{t+1})) p(N_{t+1}|\mathcal{D}_t)
\end{align*}
\]

And of course the optimal decision is obtained by

\[
f_t(\mathcal{D}_t) = \min \left\{ \mathbb{E}[C(\hat{H}_{NR})], \mathbb{E}[C(\hat{H}_1)], \mathbb{E}[C(\hat{H}_S)] \right\} \quad (7.11)
\]

Some technical details in the above calculations. When we calculate \( p(N_{t+1}|\mathcal{D}_t) \):

\[
p(N_{t+1}|\mathcal{D}_t) = \sum_{h_i \in\{0,1\}, H_i \in\{H_0, H_1\}} p(N_{t+1}|h_i)p(h_i, H_i|\mathcal{D}_t) \quad (7.12)
\]

Of course the posteriors are

\[
p(H_i|\mathcal{D}_t) = \frac{p(\mathcal{D}_t|H_i)p_{i}}{\sum_{\nu} p(\mathcal{D}_t|H_{\nu})p_{\nu}} \quad (7.13)
\]
The likelihood function is the following. Assume we have seen some $T' < T$ number of cells, and let $N_i$ denote the data we observed in cell $i$:

$$p(D_t|H_1) = \sum_{\{h_{i'}\} \in [0,1]^{T'}} \left( \prod_{i=1}^{T'} p(N_i|h_i)p(h_i|H_1) \right)$$

$$= \prod_i \left( \sum_{h_i \in \{0,1\}} p(N_i|h_i)p(h_i|H_1) \right) \tag{7.14}$$

What about the joint?

$$p(h_i = 1, H_1|D_t) \propto p(D_t|h_i, H_1)\pi_1 \pi_1 \tag{7.15}$$

We can visualize the expected cost function and the decision boundaries for this problem by specifying two tuples. The first tuple indicates the total number of times we have remained at each cell we have visited so far (including the current one). It is of length $T' < T$, where $T'$ indicates the total number of APC cells we have seen thus far up to the current decision making interval. The second tuple is of length $T'$ or $T' - 1$, and indicates the total value of measurements taken at each cell (e.g. the sum of all measurements taken at each APC). For example, a state indexed by $(1, 1, 1), (10, 0)$ indicates that we stayed at the first cell for 1 period and saw $x = 10$, jumped to a second cell for 1 period and observed $x = 0$, and have just jumped to a third cell and are about to observe a measurement at this new cell. If we plot a cost function and decision boundaries for this next measurement, we could determine for what measurements we should jump to a new cell, for what measurements we classify as 1, and for what measurements we should remain at the current cell for one more measurement.

Figure (7.3) shows the expected cost and decision boundaries for two scenarios, $(1, 1, 1), (10, 0)$ and $(1, 1, 1), (0, 0)$ (out of a total of four decision periods). The x axis represents the possible first observation at a new cell in time period 3 (out of 4 total). Dashed lines indicate decision boundaries. A sufficiently low count at this new cell indicates that we should switch to a new cell for our final time period. A sufficiently high count indicates that we can classify the current cell as 1 and attack. An intermediate count indicates that we may be looking at an infected cell, but we don’t know for sure, so we should stay for one more measurement. The decision boundaries are intuitive. Both scenarios have identical lower bounds (for switching). The higher bound is much higher for the $(1, 1, 1), (0, 0)$ case. Because the first two cells we saw gave us measurements of 0, we believe there might not be a global infection. Therefore, it will take a much larger measurement at this current cell for us to suspect that it is infected. However if we saw $(10, 0)$ for the first two cells we stayed at, we may be much more certain of an overall
infection (since the first cell gave such a high measurement), so we have a much lower threshold to classify as '1'.

Figure (7.4) shows expected cost and decision boundaries for various scenarios, all of which have seen three cells thus far and remained at each one for a single period, and have now jumped to a new cell to take one more observation before time runs out (i.e. there is only one more chance after this final observation to make a decision). The x axis in this figure now represents the last observation that will be made at a new APC. Because there is only one observation left, there are only two possible decisions: 'no response' or 'classify as 1'. We again see intuitive trends. For a T-cell that has observed (10,10,10) at the three cells it has visited, the threshold for 'classify as 1' is very low. For the T-cell that has observed (5,0,0) at the three cells it has visited, the threshold is much higher.
Figure 7.3: Expected cost and decision boundaries for the switching problem. The x axis represents the possible first observation at a new cell in time period 3 (out of 4 total). Dashed lines indicate decision boundaries. A sufficiently low count at this new cell indicates that we should switch to a new cell for our final time period. A sufficiently high count indicates that we can classify the current cell as 1 and attack. An intermediate count indicates that we may be looking at an infected cell, but we don’t know for sure, so we should stay for one more measurement.
CHAPTER 7. T CELL AS AN OPTIMAL LEARNER

Figure 7.4: Expected cost and decision boundaries for the switching problem. For all of the above scenarios, we have seen three cells and remained at each one for one period each. We have now switched to a new cell, and we have one final observation to make before time runs out.
Chapter Seven References

Chapter 8

Conclusion

This thesis investigates the role of stochasticity in pivotal steps of decision making in the immune system. An overarching question is, with the health of the organism at stake, why have such critical decisions been left to chance? We can think about stochastic noise in two (not orthogonal) ways.

One idea is that noise is simply unavoidable. Consider the work presented in this thesis regarding dynamic disorder. Proteins are large molecules which rely on electrostatic and steric interactions between multiple domains to confer structure and ultimately function. It is perhaps impossible to assume that a protein can be constructed in such a rigid way as to preclude any chance of conformational fluctuations; perhaps such a rigid body might be unable to catalyze chemical reactions, and may hinder the regulation of copy number which necessitates degradation and turnover events.

It is almost certainly the case that some amount of noise is unavoidable, and with such a view in mind we can ask, how have biological systems evolved to mitigate the limitations that noise imposes? Is it possible however that some stochasticity is not necessarily a nuisance but can allow biological systems to 'improve' their functioning relative to a corresponding deterministic system?

We have presented computational experiments to investigate both questions above, and provide insight into ways in which the immune system is equipped to mitigate limitations imposed by stochasticity, and also exploit stochasticity for more robust immunological responses.

Stochasticity in protein interaction networks is a well studied problem. The typical approach is to model a well mixed system of reacting chemical species as a high dimensional Markov chain, in which the states are represented by an N-dimensional vector over non-negative integers \( \mathbb{N}^N \) whose entries index over the copy number (amount present) of different chemical species considered. Transition rates between adjacent states are proportional to a rate constant \( k_i \) for
the $i^{th}$ reaction (multiplied by a combinatorial factor accounting for all the ways this reaction can occur).

What is not as well understood is how stochasticity in the $k_i$ can affect the ability for a signaling network to transmit information about an extracellular signal. We have considered the case in which rate constants follow a jump Markov process and also a diffusion process. We have shown in chapter 2 that the network topology and distribution from which a $k_i$ samples from greatly influence the extent to which information is lost in the noisy network. We have also seen in chapters 2 and 3 that by tuning the parameters of the stochastic process in $k_i$ (which has been experimentally observed and is accomplished by allosteric regulation of a fluctuating protein) biological systems can modulate first passage transition times and minimize false positive error rates. In chapter 4, we have outlined how statistical inference algorithms can be used to detect and quantify the noise in $k_i$, with access only to a time series of copy number throughout the course of a signaling cascade.

A second important question we have explored involves the self vs. non-self problem in immunology. We have seen that infected cells present markers on their surface which may or may not indicate that the cell is infected. T cells must scan the surface of such so-called antigen presenting cells (APCs) and detect small traces of infection in a background sea of healthy, benign peptide. It is critical that T cells respond accurately, but we also know that the earliest stages of immune response include many steps which involve low copy number reactions, i.e. reactions in which stochastic noise is important. What are the properties of the underlying protein interaction networks that allow T cells to respond with great precision and accuracy to infections? We have studied this particular problem on multiple scales.

At the scale of an individual T cell at a single APC, we have explored the network topology of the underlying protein interaction network. In chapter 5 we proposed a new model that can account for recent experimental results which the conventional model cannot explain, and have also seen that the proposed model outperforms the previously held model in all metrics we considered. In chapter 6, we looked at the level of an individual T cell hopping across multiple APCs and investigated the optimal dwell time at each APC. By building discrete time and continuous diffusion models of the T cell, we showed that stochasticity in the dwell time can enhance the cost function of a population of T cells searching through a large number of APCs for an infection. By tuning the distribution that the dwell time samples from, we have seen evidence that T cells can achieve a continuum of points along the (probability of detection, probability of false alarm) curve, and depending on the particular cost function, a stochastic dwell time allows T cells to achieve either a higher PD than a deterministic dwell time (at the expense of a higher PF) or a lower PF than the deterministic dwell time (at the expense of a lower PD).
The results in this thesis can hopefully serve as the groundwork for future research aims. In particular, as synthetic biology makes inroads from gene expression networks to protein interaction networks, a comprehensive theoretical understanding of the ways nature has designed signal transduction pathways to learn about the extracellular environment will be crucial not just for the basic research but for engineering purposes. Knowing which sources of fluctuation can be ignored in theoretical analysis, which can be mitigated in network design, and which can be exploited to achieve a more robust or optimal response is not only an interesting problem to pursue as we come to know more about our immune system, but a very important one to consider in the age of synthetic network design.