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Mathematical Modelling of T Cell Activation: From Phenotypic Approaches to Mechanochemical Models

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Doctor of Natural Sciences

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*To my family and friends,
with love and gratitude.*

Declaration

I hereby declare that I wrote the submitted dissertation independently and without any unauthorized external assistance, and that I used only the sources and aids acknowledged in this work.

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Mainz, August 2026

Yogesh Bali

Abstract

T cell activation is a central process in adaptive immunity, initiated when T cell receptors recognise peptide–MHC complexes. A key question is how T cells achieve high sensitivity and specificity despite weak receptor–ligand binding and the presence of abundant self-peptides. This thesis studies this problem using mathematical models, moving from phenotypic descriptions of T cell signalling to mechanochemical models of force-dependent TCR–pMHC binding.

The first part of the thesis presents a comparative mathematical analysis of phenotypic models of T cell activation. Models based on receptor occupancy, kinetic proofreading, feedback, limited and sustained signalling, activation-chain stabilization, and incoherent feedforward motifs are analysed with respect to steady states, response functions, antigen discrimination, sensitivity, specificity, and parameter dependence. The KPR model with an incoherent feedforward loop and limited signalling captured the broadest range of experimentally observed features, because it balances activation by high-affinity ligands with restricted responses to low-affinity ligands. Sensitivity analysis further identifies phosphorylation-related parameters and phosphatase activity as major determinants of model behaviour.

The second part develops a mechanochemical energy-landscape model for TCR–pMHC binding under force. The model combines receptor extension, binding-site separation, hinge elasticity, ligand-dependent binding strength, and applied mechanical force. Mathematical analysis gives a cutoff force above which no bound state exists, while numerical calculations show how force reshapes energy barriers and bond lifetimes. Within this framework, agonist and weak agonist ligands can display catch–slip behaviour, whereas antagonist-like ligands exhibit slip-bond behaviour.

Together, the thesis shows how mathematical modelling can connect biochemical signalling architectures with force-dependent mechanisms of receptor–ligand recognition in T cell activation.

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Publications

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2. Bali, Y., Rendall, A. D. and Quapp, W. *Mechanochemical Energy Landscapes Under Force Support Catch-Slip Bonds in T-Cell Activation*. Accepted for publication in *Royal Society Open Science*.

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CHAPTER 1

Introduction

This thesis is a work in mathematical biology concerned with the mathematical modelling of biological processes involved in T-cell activation. The chapter begins by outlining the relevant biological background; the main biological terms used in this introductory discussion are defined more explicitly in Section 1.2.

1.1 Background and Biological Motivation

T-cell activation is a central event in adaptive immunity, linking antigen recognition to effector function and immunological memory [1, 2]. T cells migrate through lymphoid tissues and continuously scan antigen-presenting cells (APCs), especially dendritic cells, for cognate peptide antigens [3, 4]. These peptides are displayed by major histocompatibility complex molecules as peptide–MHC complexes, denoted by pMHC, and are recognized by T-cell receptors (TCRs) on the T-cell surface [5]. Productive TCR–pMHC engagement initiates intracellular signalling through the associated TCR–CD3 complex; early events include phosphorylation of immunoreceptor tyrosine-based activation motifs (ITAMs), recruitment and activation of ZAP-70, and propagation of downstream signalling pathways [6, 1]. Depending on the antigenic context and accompanying costimulatory and inflammatory signals, these pathways can lead to T-cell proliferation, cytokine secretion, cytotoxic effector activity, differentiation, and the formation of memory T cells [1, 7, 2].

These early molecular events provide the biological basis for the modelling questions considered in this thesis. A schematic overview of TCR–pMHC recognition and early TCR–CD3 signalling is shown in Figure 1.1.

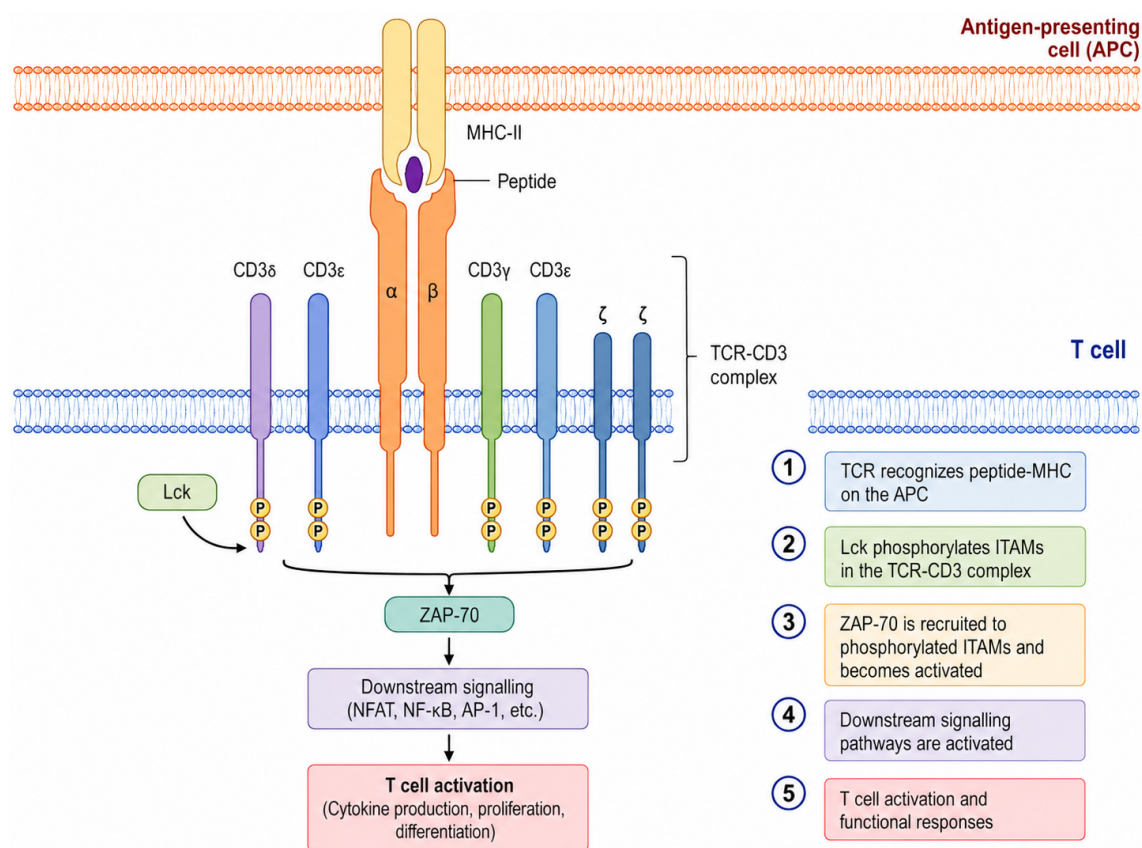


Figure 1.1. Early TCR signalling following peptide–MHC recognition. TCR–CD3 engagement leads to Lck-mediated ITAM phosphorylation, ZAP-70 recruitment, and downstream signalling.

The biological importance of this recognition process lies in its role as a cellular decision-making mechanism. A T cell must respond to foreign or abnormal peptides, such as those derived from pathogens or malignant cells, while avoiding inappropriate activation by self-peptides. This task is difficult because cognate foreign pMHC molecules may be rare, whereas self-pMHC molecules are much more abundant on the surface of antigen-presenting cells [8, 9]. Nevertheless, T cells can discriminate between closely related pMHC ligands, even when their TCR-binding properties are similar, and can make activation decisions on short time scales [10, 11]. They can also respond to extremely small numbers of agonist pMHC molecules; in some experimental systems, even a single peptide–MHC complex has been shown to trigger cytolytic activity or digital cytokine secretion [12, 13].

A central paradox is that this high sensitivity and specificity arise from molecular interactions that are relatively weak and transient. Many productive TCR–pMHC interactions measured in solution have dissociation constants in the micromolar range, often much weaker than classical antibody–antigen interactions [14, 15, 16, 17]. Nevertheless, T cells can convert weak and short-lived binding events into robust cellular responses. Activation cannot be explained by affinity alone, because dissociation kinetics, ligand density, and intracellular signalling dynamics also shape the outcome [18, 19, 20]. This

contrast between weak molecular binding and strong cellular discrimination motivates the mathematical study of T-cell activation developed in this thesis [21, 22].

Simple receptor-occupancy arguments provide a useful baseline but are not sufficient to explain antigen discrimination. In the simplest occupancy model, the cellular response is assumed to be proportional to the number of TCR–pMHC complexes formed at equilibrium. Under this assumption, ligand concentration and binding strength can compensate for one another, so that a weakly binding ligand could in principle produce a strong response if presented at sufficiently high concentration. This conflicts with the observation that weak or self-like ligands often fail to induce full activation even when abundant, whereas agonist ligands can activate T cells at much lower concentrations [20, 21].

Kinetic proofreading was introduced to address this limitation by making activation depend on binding duration rather than receptor occupancy alone. In this framework, a TCR–pMHC complex must remain bound long enough to complete a sequence of biochemical modification steps before reaching a signalling-competent state. Premature dissociation resets the receptor and reduces productive signalling [23]. This mechanism amplifies small differences in dissociation time into larger differences in response, thereby improving antigen discrimination. However, proofreading also creates a sensitivity–specificity trade-off: stronger proofreading can reject short-lived ligands more efficiently, while reducing the number of complexes that survive long enough to signal [24, 25, 21].

Additional experimental and theoretical observations further complicate this picture. Because antigenic pMHC molecules may be scarce, serial triggering has been proposed as a mechanism by which a small number of ligands sequentially engage many TCRs and amplify signalling [26, 27]. Efficient activation may also require an intermediate interaction lifetime rather than simply the longest possible binding duration, an idea captured by the optimal dwell-time concept [28, 18, 29]. Moreover, signalling may persist transiently after ligand dissociation, providing a way to reconcile rapid ligand turnover with sustained downstream activation [29, 20]. These mechanisms show that antigen discrimination is not controlled by affinity, occupancy, or dissociation time alone, but emerges from the combined effects of binding kinetics, ligand density, proofreading, serial engagement, signal persistence, feedback regulation, and downstream amplification [20, 30, 21].

The biochemical, spatial, and structural context of TCR triggering is also important. The antigen-binding TCR $\alpha\beta$ heterodimer signals through the associated CD3 complex, whose cytoplasmic ITAMs are phosphorylated by Src-family kinases such as Lck and subsequently recruit ZAP-70 [1, 6]. Spatial models such as kinetic segregation emphasize the geometry of the T-cell–APC contact, where close membrane apposition can exclude large phosphatases such as CD45 and shift the local kinase–phosphatase balance toward TCR phosphorylation [31, 32]. Structural and allosteric models add a complementary view by suggesting that pMHC engagement may alter the conformation or dynamics of

the TCR–CD3 complex, thereby linking extracellular ligand recognition to intracellular signalling [33, 34, 35, 36, 37].

Beyond biochemical and structural explanations, TCR triggering also raises the question of mechanotransduction. During contact with an APC, the TCR–pMHC complex is not only a biochemical binding pair but also part of a mechanically active interface. Forces can be generated or transmitted through actin dynamics, membrane deformation, microvillus motion, receptor movement, and traction at the immunological synapse. Experimental studies using micromanipulation, optical tweezers, biomembrane force probes, traction-force approaches, and molecular tension sensors show that piconewton-scale forces can act through the TCR or TCR–pMHC complex and influence early T-cell signalling [38, 39, 40, 41, 42, 43]. These findings motivate the view that TCR triggering is regulated not only by biochemical kinetics but also by mechanical loading.

In particular, force can alter TCR–pMHC bond lifetimes in a ligand-dependent manner. Several studies report that agonist TCR–pMHC interactions can exhibit catch–slip behaviour, in which moderate force prolongs the bond lifetime before higher force promotes dissociation. In contrast, non-stimulatory or antagonist-like ligands often show slip-bond behaviour, where increasing force shortens the bond lifetime [44, 45, 46]. This suggests that mechanical force may contribute to antigen discrimination by transiently stabilizing productive receptor–ligand interactions while destabilizing non-productive ones. However, this interpretation should be stated with care, because the role of force remains debated: some studies and models support force-amplified discrimination, whereas others suggest that force can reduce kinetic differences between ligands under certain experimental conditions [46, 47, 48].

The two modelling viewpoints developed in this thesis are summarized in Figure 1.2.

This thesis is motivated by the need to relate these different levels of explanation. Phenotypic models describe how T-cell responses depend on ligand concentration, dissociation time, proofreading, feedback regulation, and signalling architecture. Mechanochemical models ask how physical quantities such as receptor geometry, binding energy, and mechanical force shape the bond lifetimes that enter such signalling models. Accordingly, this thesis studies T-cell activation through two linked mathematical viewpoints: first, by comparing phenotypic reaction-network models of TCR signalling in terms of response properties such as specificity, sensitivity, antigen discrimination, optimal response, $E_{\max}$, and EC_{50} ; and second, by developing a mechanochemical energy-landscape model in which force modifies local minima, saddle points, energy barriers, and bond lifetimes. Together, these approaches connect cellular response patterns with possible physical mechanisms of TCR–pMHC bond stability [20, 21, 22].

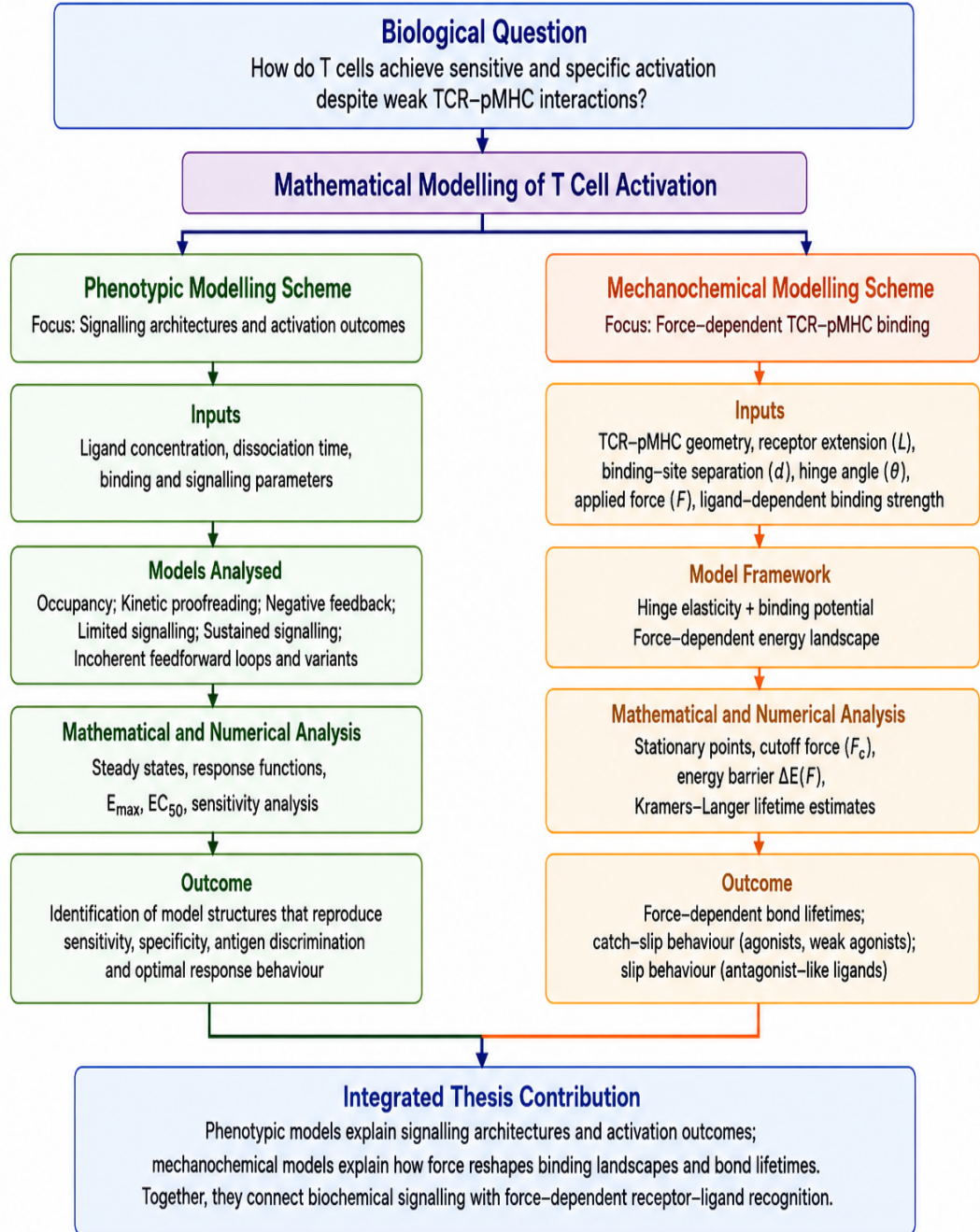


Figure 1.2. Modelling framework of the thesis. Phenotypic models describe signalling-level response properties, while mechanochemical models describe force-dependent TCR–pMHC bond stability.

1.2 Key Biological and Mathematical Terminology

This section introduces the core biological and mathematical concepts used throughout the thesis. The aim is not to provide a complete glossary, but to define the essential terminology needed to follow the phenotypic modelling, reaction-network analysis, and mechanochemical energy-landscape formulation developed in later chapters.

1.2.1 Biological and Immunological Terms

This subsection introduces the main biological objects involved in TCR–pMHC recognition and early T-cell signalling.

T cell. A T cell is a thymus-derived lymphocyte of the adaptive immune system that expresses a T-cell receptor (TCR) and participates in antigen-specific immune responses. Through TCR-mediated recognition of peptide–MHC complexes, T cells can initiate signalling programmes that lead, depending on cell type and context, to cytokine secretion, cytotoxic effector activity, proliferation, differentiation, and memory formation [49, 50, 1, 2].

Antigen-presenting cell (APC). An APC is a cell that displays peptide antigens to T cells through major histocompatibility complex molecules. Professional APCs, especially dendritic cells, are important for initiating T-cell responses because they capture antigens, migrate to lymphoid tissues, and interact dynamically with T cells during antigen scanning and priming [51, 3, 4].

Major histocompatibility complex (MHC). Major histocompatibility complex molecules are cell-surface proteins that bind peptide fragments and display them for recognition by T cells. A peptide–MHC complex, denoted by pMHC, is formed when a peptide is bound in the peptide-binding groove of an MHC molecule. T-cell antigen recognition is MHC-restricted, meaning that the T-cell receptor recognizes antigenic peptides in the context of MHC molecules rather than as free peptides. Structural and biophysical studies have shown that TCR recognition depends on contacts with both the peptide and the MHC molecule [52, 14, 5, 16].

T-cell receptor (TCR). The TCR is the antigen-recognition receptor on the T-cell surface. Early molecular studies identified the TCR as a heterodimeric receptor, and later structural and biophysical studies established how TCRs bind peptide–MHC ligands. In this thesis, the TCR is usually treated as the receptor and pMHC as the ligand [53, 54, 55, 5, 16].

TCR–pMHC complex. The TCR–pMHC complex is the bound receptor–ligand complex formed when a TCR binds a pMHC molecule. Its affinity, dissociation rate, dwell time, force-dependent lifetime, and geometry are central to the models studied in this thesis. Phenotypic models usually treat lifetime or dissociation rate as an input, whereas

the mechanochemical model studies how force and geometry can influence that lifetime [14, 16, 20, 22].

CD3 complex. The CD3 complex is the invariant signalling module associated with the antigen-binding TCR. It contains signalling chains whose cytoplasmic motifs transmit extracellular recognition into intracellular biochemical signalling [56, 57, 6, 34].

TCR–CD3 complex. The TCR–CD3 complex is the full receptor-signalling assembly composed of the antigen-binding TCR heterodimer and the associated CD3 signalling subunits. This complex links extracellular pMHC recognition to intracellular signalling, and structural studies have clarified the organization of the human TCR–CD3 assembly [56, 57, 34, 35, 37].

Immunoreceptor tyrosine-based activation motif (ITAM). An ITAM is a signalling motif located in the cytoplasmic tails of CD3 chains. Phosphorylation of ITAM tyrosines is one of the earliest biochemical events after productive TCR engagement and provides docking sites for downstream signalling proteins [6, 1].

Lck. Lck is a Src-family tyrosine kinase involved in early T-cell signalling. It phosphorylates tyrosine residues in CD3 ITAMs and thereby contributes to initiation of the TCR signalling cascade [1, 6].

ZAP-70. ZAP-70 is a tyrosine kinase recruited to phosphorylated ITAMs. Once recruited and activated, ZAP-70 helps propagate signalling downstream of the TCR–CD3 complex [1, 6].

1.2.2 Ligand Classes and Response Quantities

This subsection defines the ligand classes and response quantities used to compare T-cell activation models.

Agonist. An agonist is a pMHC ligand that binds to a T-cell receptor and induces productive T-cell activation. Agonist ligands are generally capable of triggering downstream responses such as cytokine production, proliferation, cytotoxic activity, or differentiation, depending on the T-cell type and biological context [20, 16, 21].

Weak agonist. A weak agonist is a pMHC ligand that can activate a T cell, but produces a weaker response than a full agonist. The reduced response may result from shorter TCR–pMHC dwell time, less efficient signalling, weaker ligand potency, altered receptor engagement, or less favourable structural or mechanical properties. Thus, weak agonism is defined by the functional response it produces, not only by binding affinity [20, 9, 21].

Antagonist. An antagonist is a pMHC ligand that can bind to a T-cell receptor but produces little or no productive activation on its own and can reduce the response induced by an agonist ligand [30, 20, 9].

Sensitivity. Sensitivity is the ability of a T cell to respond to very small numbers or low concentrations of agonist pMHC ligands. This property is important because antigen-

presenting cells may display only a few foreign or disease-associated pMHC molecules among a large background of self-pMHC molecules [12, 13, 20].

Specificity. Specificity is the ability of a T cell to respond selectively to appropriate antigenic ligands while avoiding activation by non-stimulatory or self-like ligands. In T-cell activation, specificity is closely related to the rejection of ligands that bind weakly, bind briefly, or fail to generate productive signalling [8, 20].

Antigen discrimination. Antigen discrimination is the ability of T cells to generate different cellular responses to different pMHC ligands, even when those ligands differ only slightly in binding kinetics, structure, or peptide sequence. This property allows T cells to respond to foreign antigens while avoiding inappropriate responses to self-like ligands [20, 11, 21].

Binding affinity and dissociation constant. Binding affinity describes the strength of a receptor–ligand interaction at equilibrium. For TCR–pMHC binding, affinity is commonly summarized by the dissociation constant K_D , where a smaller K_D indicates stronger equilibrium binding. For a simple one-step binding reaction,

$$K_D = \frac{k_{\text{off}}}{k_{\text{on}}}, \quad (1.1)$$

where k_{on} is the binding rate and k_{off} is the dissociation rate. Many TCR–pMHC interactions measured in solution have relatively weak affinities, often in the micromolar range [14, 16, 17].

Dissociation rate and dwell time. The dissociation rate k_{off} describes the rate at which a TCR–pMHC complex dissociates. In simple binding models, the reciprocal $1/k_{\text{off}}$ is often interpreted as an average dwell time or bond lifetime. This distinction is important because T-cell activation is influenced not only by equilibrium affinity, but also by how long the receptor–ligand complex remains bound.

Response function. A response function describes how the output of a model changes as a biological input, such as ligand concentration or dissociation time, is varied. In this thesis, response functions are used to compare the behaviour of different phenotypic models of T-cell activation.

Maximal response and half-maximal effective concentration. The maximal response E_{max} is the largest response that a biological system can produce in response to increasing ligand concentration or stimulus strength. The half-maximal effective concentration EC_{50} is the ligand concentration required to produce one half of E_{max} . Thus, E_{max} measures the maximal attainable effect, whereas EC_{50} measures potency: a lower EC_{50} means that a smaller ligand concentration is sufficient to produce a given level of response [20, 21].

Optimal response. Optimal response refers to a non-monotone response pattern in which the strongest activation occurs at an intermediate value of a biological variable,

rather than increasing continuously as that variable increases. In T-cell activation, this idea is often associated with the possibility that an intermediate TCR–pMHC interaction lifetime can be more effective than either very short or very long binding times. The exact location and existence of such an optimum may depend on ligand density, binding kinetics, and cellular context [28, 18, 21].

1.2.3 Phenotypic and Reaction-Network Terms

This subsection introduces the main modelling concepts used to describe T-cell activation at the level of observable response behaviour and biochemical reaction networks.

Phenotypic model. A phenotypic model is a mathematical model that describes observable input–output behaviour of a biological system without representing every underlying molecular detail. In T-cell activation, phenotypic models are used to describe how ligand binding, dissociation, proofreading, feedback, and signalling architecture shape response properties such as sensitivity, specificity, antigen discrimination, and dose–response behaviour [20, 21].

Biochemical reaction network. A biochemical reaction network is a representation of molecular processes as reactions between chemical or biochemical species. Such networks provide a structured way to describe binding, unbinding, modification, activation, inhibition, and signalling transitions. When reaction rates are specified, a reaction network can be translated into a system of differential equations [58, 59].

Species, complex, and reaction. In reaction-network terminology, a species is a basic chemical or biochemical substance whose concentration may change over time. A complex is a formal combination of species appearing as the source or product of a reaction, and a reaction is a directed transition from one complex to another. For example, a receptor and ligand may bind to form a receptor–ligand complex, and this complex may then progress through further signalling states [59].

Mass-action kinetics. Mass-action kinetics is the assumption that the rate of a reaction is proportional to the product of the concentrations of its reactant species. This principle gives a standard way to convert biochemical reaction networks into ordinary differential equations and is widely used in mathematical models of cellular signalling [58, 59].

Kinetic proofreading. Kinetic proofreading is a signalling mechanism in which a receptor–ligand complex must remain bound long enough to pass through a sequence of biochemical modification steps before productive signalling occurs. It can amplify small differences in ligand dissociation time into larger differences in cellular response [23, 20].

Feedback and feedforward regulation. Feedback regulation occurs when a downstream component of a signalling pathway modifies an earlier step in the same pathway. Negative feedback can reduce or terminate signalling, whereas positive feedback can amplify it. Feedforward regulation occurs when an input influences a response through more

than one pathway. In an incoherent feedforward loop, one pathway promotes the response while another opposes it. Such regulatory motifs can generate adaptation, antagonism, thresholds, and non-monotone response behaviour in cellular signalling systems [60, 30, 20].

1.2.4 Dynamical-Systems and Chemical-Reaction-Network Terms

This subsection introduces the main mathematical concepts used to analyze reaction-network models of biochemical signalling. The focus is on positivity, conservation laws, steady states, stability, and structural results from chemical reaction network theory.

Ordinary differential equation (ODE). An ordinary differential equation describes how an unknown quantity changes with respect to a continuous variable, usually time. In biochemical modelling, systems of ODEs are commonly used to describe how the concentrations of interacting molecular species evolve according to reaction rates [61, 62, 63].

State variables and solutions. A state variable is a quantity whose value changes over time, such as the concentration of a molecular species. A solution of an ODE system describes the time evolution of all state variables from a prescribed initial condition. In concentration-based models, biologically meaningful solutions are expected to remain non-negative.

Positive invariance. Positive invariance means that a set of states is preserved by the dynamics: if a solution starts inside the set, it remains inside the set for all future time. In biochemical reaction systems, the non-negative state space is usually positively invariant, reflecting the fact that concentrations cannot become negative [64, 59].

Conserved quantity. A conserved quantity is a combination of state variables that remains constant along solutions. Conservation laws commonly arise in receptor–ligand systems because the total amount of ligand or receptor may be fixed, even though these molecules can move between free and bound states. Conserved quantities restrict the possible dynamics to lower-dimensional regions of the state space [59].

Biologically relevant region. The biologically relevant region is the part of the state space where the variables satisfy biological constraints, such as non-negativity and conservation laws. Mathematical analysis is usually restricted to this region because only states inside it have biological meaning.

Steady state. A steady state, or equilibrium point, is a state at which all time derivatives vanish and the system no longer changes in time. In biochemical systems, steady states represent possible long-time outcomes of the reaction dynamics. A positive steady state is a steady state in which all relevant species have strictly positive concentration [62, 63].

Stability. Stability describes how solutions behave after small perturbations of a steady state. A steady state is stable if nearby solutions remain nearby, asymptotically stable if

nearby solutions converge to it over time, and globally asymptotically stable in a region if all solutions starting in that region converge to it [62, 63].

Stoichiometric subspace. The stoichiometric subspace is the linear space spanned by the reaction vectors of a reaction network. It describes the directions in which the state of the system can change as reactions occur [59].

Stoichiometric compatibility class. A stoichiometric compatibility class is the set of states that can be reached from a given initial condition while respecting the conservation laws of a reaction network. These classes are important because chemical reaction systems do not usually evolve through the whole state space, but only within the region allowed by their stoichiometry and conserved quantities [65, 59].

Deficiency. The deficiency of a chemical reaction network is a structural number defined by

$$\delta = n - \ell - s, \quad (1.2)$$

where n is the number of complexes, ℓ is the number of linkage classes, and s is the rank of the stoichiometric subspace. Deficiency relates the graph structure of the reaction network to the linear space generated by its reaction vectors [65, 59].

Weak reversibility. A reaction network is weakly reversible if every reaction pathway from one complex to another is accompanied by a pathway returning to the original complex. This is a structural property of the reaction graph and is one of the central hypotheses in several results of chemical reaction network theory [66, 65].

Deficiency Zero Theorem. The Deficiency Zero Theorem is a fundamental result in chemical reaction network theory. In one common form, it states that a weakly reversible mass-action network with deficiency zero admits a positive steady state in each stoichiometric compatibility class, and that this steady state is unique and locally asymptotically stable within that class [66, 65, 59].

1.2.5 Mechanochemical and Energy-Landscape Terms

This subsection introduces the main mechanochemical concepts needed to understand force-dependent molecular bond stability and energy-landscape modelling.

Mechanochemical model. A mechanochemical model describes how mechanical force influences molecular structure, reaction pathways, conformational change, or bond stability. Such models are used to study how physical quantities such as force, geometry, deformation, and binding energy affect molecular behaviour [38, 40, 22].

Mechanotransduction. Mechanotransduction is the conversion of mechanical input into biochemical or cellular response. In T-cell activation, mechanical forces acting on the TCR or TCR–pMHC complex may influence receptor triggering, early signalling, and ligand discrimination [38, 39, 40, 41].

Energy landscape. An energy landscape assigns an energy value to each possible configuration of a molecular system. Stable configurations correspond to local energy minima, while transitions between configurations usually require crossing energy barriers. Energy-landscape descriptions are useful for studying molecular stability, conformational change, and bond dissociation [67, 68, 69].

Force-loaded energy landscape. A force-loaded energy landscape is an energy landscape modified by an external or applied mechanical force. Force can tilt the landscape, shift the locations of stable and unstable stationary points, and change the height of energy barriers. This provides a mathematical way to describe how mechanical loading can stabilize or destabilize a molecular bond [70, 71, 69].

Reduced geometric coordinates. Reduced geometric coordinates are simplified variables used to describe the most relevant motions or deformations of a complex molecular system. Instead of representing every atom, a reduced model may describe bond opening, receptor extension, or angular deformation using a small number of collective variables. This approach improves mathematical tractability while retaining the main physical features of the problem [69, 22].

Stationary point. A stationary point is a point on an energy landscape where the gradient of the energy function is zero, that is,

$$\nabla E = 0. \tag{1.3}$$

Stationary points identify configurations where the system is locally balanced. Depending on the curvature of the landscape, a stationary point may be a local minimum, a saddle point, or another type of critical point [68, 69].

Local minimum. A local minimum is a stationary point whose energy is lower than that of nearby configurations. In molecular bond models, a local minimum usually represents a stable or metastable bound state. The stability of such a state depends on the surrounding energy barriers and on the effect of external force [67, 68].

Saddle point. A saddle point is a stationary point that is stable in some directions and unstable in at least one direction. In bond dissociation problems, an index-one saddle point often represents a transition state separating a bound state from an unbound or dissociated state [67, 68, 69].

Hessian matrix. The Hessian matrix is the matrix of second derivatives of an energy function. It describes the local curvature of the energy landscape near a stationary point. A positive-definite Hessian indicates a strict local minimum, whereas an index-one saddle has one unstable direction corresponding to a negative eigenvalue [68, 69].

Energy barrier. An energy barrier is the energy difference between a stable or metastable state and the transition state that must be crossed for a reaction or dissociation event to occur. For a force-dependent landscape, the barrier can change with applied force. A

larger barrier generally corresponds to greater bond stability, whereas a smaller barrier corresponds to easier escape or dissociation [67, 68].

Bond lifetime. Bond lifetime is the expected time for which a molecular bond remains intact before dissociation. In energy-landscape theory, bond lifetime is strongly influenced by the height of the energy barrier and by the local curvature near the bound state and transition state. Mechanical force can increase or decrease bond lifetime by changing these landscape features [67, 72, 68].

Arrhenius–Kramers–Langer approximation. The Arrhenius–Kramers–Langer approximation relates the mean escape time from a metastable state to the energy barrier and local curvature of the energy landscape. In simplified form, the lifetime contains an exponential dependence of the type

$$\exp\left(\frac{\Delta E}{k_B T}\right), \quad (1.4)$$

where ΔE is the energy barrier, k_B is the Boltzmann constant, and T is absolute temperature. The full approximation also includes prefactors depending on the local curvature near the bound state and transition state. This approximation provides a bridge between energy-landscape geometry and measurable bond lifetimes [73, 67, 72, 68].

Slip bond, catch bond, and catch–slip behaviour. A slip bond is a bond whose lifetime decreases as applied force increases. A catch bond is a bond whose lifetime initially increases under applied force. Catch–slip behaviour occurs when the bond lifetime first increases at moderate force and then decreases at higher force. These force-dependent lifetime patterns have been reported in several receptor–ligand systems, including TCR–pMHC interactions [70, 71, 44, 45, 46].

1.3 T-Cell Activation Remains a Modelling Problem

T-cell activation remains a modelling problem because the cellular decision to activate is not governed by a single biological variable. It depends on coupled molecular, biochemical, cellular, and mechanical processes, including TCR–pMHC binding and dissociation, ligand concentration, sequential signalling, feedback regulation, spatial organization at the T-cell–APC interface, receptor conformational changes, and mechanical force. None of these mechanisms alone fully explains the combination of sensitivity, specificity, antigen discrimination, and robustness observed in T-cell activation [20, 16, 1].

1.3.1 Multiple Mechanisms Acting at Different Scales

One difficulty is that T-cell activation involves mechanisms acting across different biological scales. At the molecular scale, the relevant processes include TCR–pMHC binding

affinity, binding rate, dissociation rate, and dwell time. At the biochemical scale, receptor engagement is followed by phosphorylation, recruitment of signalling proteins, proofreading-like progression through intermediate states, and feedback regulation. At the cellular scale, receptor triggering occurs in a spatially organized contact region between the T cell and the antigen-presenting cell, where membrane geometry, phosphatase exclusion, cytoskeletal motion, and mechanical loading can also influence signalling [23, 31, 60, 32, 44].

This multiscale structure makes T-cell activation difficult to understand by intuition alone. A ligand with a favourable binding lifetime may still fail to activate if the signalling architecture suppresses the response. Conversely, a small number of agonist ligands may trigger a strong response if serial engagement, signal persistence, or downstream amplification is effective. Therefore, the observed cellular response is not simply a direct measurement of affinity, occupancy, or dwell time, but the result of several interacting mechanisms [26, 29, 20]. Similar response patterns may arise from different mechanistic combinations, while the same mechanism may produce different outcomes depending on the surrounding signalling context.

1.3.2 Limitations of Single-Mechanism Explanations

Several mechanisms have been proposed to explain T-cell antigen discrimination, but each is limited when considered alone. Occupancy models provide a useful baseline by assuming that activation is proportional to the number of TCR–pMHC complexes, but they cannot explain why weak or self-like ligands often fail to activate T cells even when abundant. Kinetic proofreading improves discrimination by requiring sufficiently long binding, but it introduces a sensitivity–specificity trade-off: stronger proofreading can reject short-lived ligands more efficiently while reducing the number of complexes that survive long enough to signal [23, 24, 20].

Other mechanisms address different aspects of the problem. Serial engagement can explain how a small number of ligands may trigger many receptors, sustained signalling can explain how responses persist after ligand dissociation, and feedback or feedforward motifs can generate adaptation, antagonism, thresholds, or non-monotone responses. These mechanisms are not mutually exclusive; rather, they describe complementary aspects of the same biological decision process [27, 29, 30, 60, 21].

1.3.3 Need for Comparative Phenotypic Modelling

Because different mechanisms can produce similar qualitative behaviours, mathematical modelling is needed to compare them systematically. A model makes assumptions explicit and allows one to ask which mechanisms are sufficient to reproduce a given response property. For example, one can test whether a model can reproduce specificity, sensitivity,

antigen discrimination, optimal response, ligand antagonism, or non-monotone dose–response behaviour. Quantities such as the response function, $E_{\max}$, and EC_{50} provide a common language for comparing different models [20, 30, 21].

Mathematical analysis also helps distinguish between qualitative and quantitative success. A model may reproduce a response curve for one parameter choice but fail to do so robustly under parameter variation. Thus, comparison should ask not only whether a model can reproduce a response property, but also whether that property persists for biologically plausible parameter variation. Structural properties such as positivity, boundedness, uniqueness of steady states, and stability can also show whether a model gives well-defined long-time predictions. Therefore, mathematical modelling is not only a tool for simulation; it is also a way to understand what follows from the structure of the biological assumptions [59, 21].

1.3.4 Need for a Physical Description of Bond Lifetime

Phenotypic models usually treat dissociation time or bond lifetime as an input parameter. This is useful for studying how ligand lifetime affects signalling, but it leaves open the question of what determines the lifetime of the TCR–pMHC bond itself. Experimental studies suggest that mechanical force can alter TCR–pMHC bond lifetimes in a ligand-dependent way. In particular, some agonist interactions display catch–slip behaviour, whereas antagonist-like interactions more often show slip-bond behaviour [44, 45, 46].

This observation motivates a mechanochemical modelling perspective. Instead of prescribing bond lifetime directly, a mechanochemical model asks how force, receptor geometry, binding energy, and energy barriers generate the observed lifetime. In this framework, force can reshape an energy landscape by moving local minima and saddle points and by changing the barrier to dissociation. Such a model provides a possible physical explanation for the kinetic quantities that enter phenotypic signalling models [70, 71, 67, 68, 22].

Therefore, T-cell activation remains a modelling problem because it requires both response-level and physical-level explanations. Phenotypic models are needed to understand how signalling architectures generate cellular response patterns, while mechanochemical models are needed to understand how force and geometry may shape the bond lifetimes that feed into those response patterns. This motivates the research gap addressed in the next section.

1.4 Research Gap and Thesis Motivation

The previous sections show that T-cell activation requires both response-level and physical-level explanations. At the response level, phenotypic models describe how ligand concen-

tration, dissociation time, proofreading, feedback regulation, and signalling architecture shape cellular activation. At the physical level, mechanochemical models ask how force, receptor geometry, binding energy, and energy barriers determine the lifetime of the TCR–pMHC bond. The central motivation of this thesis is to study these two levels in a connected mathematical framework.

Existing models have explained important aspects of T-cell activation, including receptor occupancy, kinetic proofreading, feedback-based regulation, feedforward motifs, limited signalling, and sustained signalling [23, 60, 30, 20, 21]. Experimental and theoretical studies have also suggested that mechanical force can influence TCR–pMHC bond lifetimes and may contribute to ligand discrimination through catch–slip or slip–bond behaviour [44, 45, 46, 48]. However, a unified mathematical understanding is still lacking because these response-level and physical-level viewpoints are often studied separately.

1.4.1 Explanatory Power under Plausible Parameters

Phenotypic models of T-cell activation were developed to explain experimentally observed response properties such as specificity, sensitivity, antigen discrimination, ligand antagonism, optimal response, maximal response, and half-maximal effective concentration. These models are valuable because they provide simplified mathematical descriptions of how ligand concentration, dissociation time, proofreading steps, feedback regulation, and signalling architecture shape the cellular response [20, 30, 21]. However, an important gap remains: it is not enough to ask whether a model can reproduce a given property for one parameter choice. One must also ask whether the same property can be reproduced for biologically and experimentally plausible parameter ranges.

This issue is important because parameter values reported in the literature vary substantially across experimental sources and measurement contexts. Quantities such as receptor concentration, ligand–receptor binding rate, phosphorylation rate, phosphatase efficiency, dissociation rate, and feedback strength can differ between studies and between experimental techniques. As a result, the predicted behaviour of a phenotypic model may depend strongly on the parameter set being used. A response pattern that appears possible under one parameter choice may become weak, disappear, or change qualitatively under another [19, 20, 21].

The research gap in the phenotypic part of this thesis is therefore the need for a systematic comparison of model explanatory power under a common biologically motivated parameter framework. Such a comparison should determine which models can reproduce key response properties, how robust these predictions are across plausible parameter ranges, and which parameters are most responsible for variation in model output. This requires deriving response functions and summary quantities such as $E_{\max}$ and EC_{50} , but

also analyzing steady states, long-time behaviour, and parameter sensitivity across the family of models.

This thesis addresses this gap by studying nine phenotypic models within a unified mathematical framework and by comparing their ability to reproduce sensitivity, specificity, antigen discrimination, optimal response, and dose–response behaviour using parameter values derived from the literature. Where possible, shared parameters are evaluated through common mean, minimum, and maximum values, while model-specific parameters are treated according to their original modelling context. This allows the models to be compared not only as formal mathematical systems, but also as biologically interpretable explanations of T-cell activation [21].

1.4.2 Force-Dependent Bond Lifetime

A second research gap concerns the physical origin of the bond lifetimes used in phenotypic models. In many response-level models, the dissociation rate or dwell time is prescribed as a parameter. This is useful for studying how ligand lifetime affects signalling, but it does not explain how that lifetime arises from the physical behaviour of the TCR–pMHC bond. Thus, an important question remains: how can physical properties of the receptor–ligand interaction determine the kinetic quantities that control downstream signalling?

Mechanical force provides a possible route for addressing this question. Forces generated or transmitted at the T-cell–APC interface can act on the TCR or the TCR–pMHC complex and influence early signalling [38, 39, 40, 41]. Moreover, TCR–pMHC bond lifetimes can depend on both mechanical force and ligand class. Some agonist interactions display catch–slip behaviour, in which moderate force prolongs bond lifetime before higher force promotes dissociation. In contrast, antagonist-like or non-stimulatory ligands more often display slip-bond behaviour, in which force shortens the bond lifetime [44, 45, 46].

This raises an important mechanistic question: how can mechanical force acting on a molecular bond be translated into a cellular activation decision? If force stabilizes agonist interactions over a physiologically relevant range, it may increase the time available for proofreading and downstream signalling. If the same force destabilizes antagonist-like interactions, it may help suppress non-productive signalling. In this way, force-dependent bond lifetimes may contribute to the simultaneous achievement of sensitivity and specificity. However, this interpretation must be treated with care, because the role of force remains debated: some studies support force-amplified antigen discrimination, whereas others suggest that force can reduce kinetic differences between ligands under certain experimental conditions [47, 48].

The research gap is therefore to understand how mechanical force modifies TCR–pMHC bond stability and how this modification can be interpreted as a possible route toward T-cell activation. This requires a model in which bond lifetime is not simply imposed,

but emerges from physical quantities such as receptor geometry, binding energy, applied force, and the barrier to dissociation. Such a model can help explain how a mechanical perturbation at the receptor–ligand interface may influence the kinetic variables that enter phenotypic signalling models.

This thesis addresses this gap by developing a mechanochemical energy-landscape model for TCR–pMHC binding under force. In this framework, force reshapes the energy landscape, changes the positions and stability of local minima and saddle points, modifies the energy barrier to dissociation, and thereby determines the predicted bond lifetime. The model therefore provides a mathematical mechanism by which force-dependent molecular stability may be connected to the signalling timescales relevant for T-cell activation [70, 67, 72, 68, 22].

1.4.3 Linking Response-Level and Physical-Level Models

The central gap addressed in this thesis is the lack of a clear connection between phenotypic response models and mechanochemical models of TCR–pMHC bond stability. Phenotypic models explain how prescribed kinetic variables, such as ligand concentration and dissociation time, affect signalling. Mechanochemical models address the complementary question of how some of these kinetic variables may arise physically from receptor geometry, binding energy, and mechanical loading. These two viewpoints are closely related, but they are often studied separately.

The motivation for connecting them is that T-cell activation is both a signalling problem and a physical recognition problem. At the signalling level, the cell must convert ligand binding events into a robust activation decision. At the physical level, the TCR–pMHC bond must persist long enough, and in the appropriate mechanical context, to allow productive signalling. A mathematical description that considers only signalling architecture may miss how force controls the effective lifetime of the ligand–receptor interaction. Conversely, a description that considers only bond mechanics may miss how the resulting lifetime is interpreted by the intracellular signalling network.

This thesis therefore studies T-cell activation through two complementary mathematical frameworks. The phenotypic part analyzes a family of reaction-network models and compares their ability to reproduce key experimental response properties for biologically motivated parameter ranges. The mechanochemical part develops a force-dependent energy-landscape model in which bond lifetimes emerge from changes in local minima, saddle points, and activation barriers. Together, these approaches connect observable cellular response patterns with possible physical mechanisms of TCR–pMHC triggering.

The central research gap addressed in this thesis can therefore be summarized as follows: existing phenotypic models explain how cellular activation depends on kinetic variables, while mechanochemical models can explain how some of those kinetic variables

may arise from force-dependent bond stability. A combined mathematical study of both levels is needed to understand how T cells may achieve sensitivity, specificity, and antigen discrimination. This structure is reflected in the organization of the thesis: Chapters 2 and 3 develop the phenotypic modelling and analysis, whereas Chapters 4 and 5 develop the mechanochemical energy-landscape formulation and its application to force-dependent bond lifetimes.

1.5 Mathematical Framework of the Thesis

Mathematics plays a central role in explaining T-cell activation because the underlying biological process involves several interacting mechanisms that cannot be fully understood by qualitative reasoning alone. T-cell activation depends on receptor–ligand binding, ligand dissociation, proofreading steps, feedback regulation, downstream signalling, mechanical force, receptor geometry, and bond stability. Mathematical models allow these mechanisms to be formulated precisely, compared systematically, and analyzed in terms of their biological consequences.

In this thesis, mathematics is used in two complementary ways. First, it is used to study phenotypic models of T-cell activation, where the main question is how ligand concentration, dissociation time, proofreading, feedback, and signalling architecture determine cellular response. Second, it is used to study mechanochemical models of TCR–pMHC binding, where the main question is how mechanical force, receptor geometry, and energy barriers determine bond lifetime. These two mathematical viewpoints address different but connected levels of the same biological problem.

1.5.1 Mathematics in Phenotypic Models of T-Cell Activation

In the phenotypic part of the thesis, mathematics provides a framework for asking what different signalling architectures can explain about T-cell activation. The models are formulated as biochemical reaction networks and translated into systems of ordinary differential equations using mass-action kinetics. This makes it possible to represent ligand binding, ligand dissociation, kinetic proofreading, limited signalling, sustained signalling, negative feedback, and incoherent feedforward regulation in a common mathematical language [23, 60, 30, 20, 21].

This formulation is useful because it allows the models to be compared by their predicted response properties. Instead of asking only whether a model is biologically plausible in words, mathematics allows one to ask whether the model can reproduce experimentally motivated properties such as specificity, sensitivity, antigen discrimination, ligand antagonism, optimal response, and dose–response behaviour. Response functions, maxi-

mal response $E_{\max}$, and half-maximal effective concentration EC_{50} provide quantitative measures for comparing the explanatory power of different models [20, 21].

Mathematical analysis also helps determine whether a model is internally consistent and biologically meaningful. Since the variables represent concentrations, solutions should remain non-negative. Conserved quantities, such as total ligand and total receptor concentration, restrict the dynamics to biologically relevant regions. Steady-state analysis, stability analysis, and chemical reaction network theory can then be used to determine whether a model has a well-defined long-time response for fixed parameter values [66, 65, 59, 21].

Another important role of mathematics is to study parameter dependence. Phenotypic models often contain parameters whose values vary across experiments or are difficult to estimate precisely. Sensitivity analysis helps identify which parameters most strongly influence response functions, $E_{\max}$, EC_{50} , and other model outputs. In this way, mathematics helps distinguish model features that are robust from those that depend strongly on a particular parameter choice. This is essential for assessing whether a model can explain T-cell activation for biologically and experimentally plausible parameter ranges [74, 21].

Thus, in the phenotypic part of the thesis, mathematics informs us about the explanatory power of different model structures. It shows which models can reproduce key experimental properties, which mechanisms are needed for particular behaviours, whether the models have well-defined long-time dynamics, and which parameters control their predictions.

1.5.2 Mathematics in Mechanochemical Models of TCR–pMHC Binding

In the mechanochemical part of the thesis, mathematics is used to study how mechanical force can influence TCR–pMHC bond stability. Here the main object is not a signalling network, but a force-dependent energy landscape. The TCR–pMHC interaction is described using reduced geometric variables such as receptor extension, ligand-binding-site separation, and hinge angle. These variables allow receptor geometry, binding energy, and applied force to be combined in a single mathematical framework [38, 40, 22].

The energy-landscape formulation is useful because it converts the qualitative idea of force-dependent bond stability into a precise mathematical problem. Local minima represent mechanically stable bound states, while saddle points represent transition states for dissociation. The Hessian matrix is used to classify these stationary points and to determine whether a point is a stable minimum or an unstable saddle. The energy difference between the bound minimum and the saddle defines the force-dependent barrier to dissociation [67, 72, 68, 22].

Mathematics also explains how mechanical force can change bond lifetime. Applied force reshapes the energy landscape by moving minima and saddle points and by changing the height of the dissociation barrier. If force increases the barrier over an intermediate range, the bond lifetime can increase, producing catch-like behaviour. If force lowers the barrier, the lifetime decreases, producing slip-like behaviour. Thus, the model provides a mathematical way to understand how the same mechanical load may stabilize agonist-like interactions while destabilizing antagonist-like interactions [70, 71, 44, 45, 46].

The conversion from barrier height to lifetime is made through Arrhenius–Kramers–Langer type approximations. These approximations relate the expected escape time from a metastable bound state to the energy barrier and the local curvature of the energy landscape. In this thesis, they are used to compute force-dependent bond lifetimes from the geometry and energetics of the TCR–pMHC model [73, 67, 72, 68, 22].

Thus, in the mechanochemical part of the thesis, mathematics informs us about the physical origin of bond lifetime. It shows how force can reshape an energy landscape, how bound states and transition states change under mechanical load, how barriers determine lifetime, and how catch–slip or slip–bond behaviour can emerge from receptor geometry and binding energetics.

Together, these two mathematical viewpoints address complementary aspects of T-cell activation. Phenotypic models explain how kinetic variables and signalling architectures generate cellular response patterns, while mechanochemical models explain how some of those kinetic variables, especially force-dependent bond lifetime, may arise from physical bond stability. This combined mathematical approach provides the basis for the aims and contributions of the thesis.

1.6 Aim and Objectives of the Thesis

The overall aim of this thesis is to use mathematical modelling to explain key properties of T-cell activation arising from TCR–pMHC interactions, especially sensitivity, specificity, antigen discrimination, and ligand-dependent response. The thesis approaches this problem from two complementary viewpoints. First, it examines the explanatory power of phenotypic models of T-cell activation for biologically and experimentally plausible parameter ranges. Second, it develops a mechanochemical energy-landscape framework to investigate how mechanical force, receptor geometry, and binding energetics can shape TCR–pMHC bond lifetimes. The specific objectives of the thesis are as follows:

1. To examine which experimentally observed properties of T-cell activation can be explained by phenotypic models, including specificity, sensitivity, antigen discrimination, ligand antagonism, optimal response, $E_{\max}$, and EC_{50} .

2. To analyze positivity, boundedness, conserved quantities, steady states, long-time behaviour, and parameter sensitivity of these phenotypic models for experimentally plausible parameter ranges.
3. To investigate whether force-dependent TCR–pMHC bond lifetimes can contribute to the sensitivity–specificity problem in T-cell activation.
4. To formulate and analyze a mechanochemical energy-landscape model in which receptor geometry, binding energy, and applied force determine energy barriers and bond lifetimes.
5. To connect the phenotypic and mechanochemical viewpoints by interpreting force-dependent bond lifetime as a possible physical basis for the kinetic quantities used in response-level models of T-cell activation.

1.7 Main Contributions of the Thesis

This thesis contributes to the mathematical understanding of T-cell activation at two connected levels: phenotypic modelling of cellular response and mechanochemical modelling of TCR–pMHC bond stability. The main contributions are summarized as follows.

1. **Unified comparative framework for phenotypic models of T-cell activation.** This thesis presents a family of phenotypic models of T-cell activation within a common mathematical framework. The models include the occupancy model, kinetic proofreading, limited signalling, sustained signalling, negative feedback, stabilizing activation chains, incoherent feedforward motifs, and combined model variants. By expressing these models in a unified notation and reaction-network structure, the thesis makes it possible to compare different signalling architectures using the same biological benchmarks and mathematical response quantities.
2. **Evaluation for biologically plausible parameter ranges.** A central contribution of the thesis is the systematic evaluation of phenotypic models using biologically and experimentally plausible parameter ranges rather than isolated parameter choices. The models are compared in terms of their ability to reproduce key response properties such as specificity, sensitivity, antigen discrimination, ligand antagonism, optimal response, $E_{\max}$, and EC_{50} . This analysis shows that simple occupancy and standard kinetic proofreading models have limited explanatory power for several experimentally motivated behaviours, whereas extended models involving limited signalling, sustained signalling, negative feedback, and incoherent feedforward regulation can reproduce a broader range of response patterns. In particular, models with limited or sustained signalling and incoherent feedforward

structure generate richer non-monotone or optimal response behaviour, and the combined limited-signalling and incoherent-feedforward model produces a distinctive bimodal response with respect to dissociation time.

3. **Structural and sensitivity analysis of phenotypic models.** The thesis provides mathematical analysis of the phenotypic models, including positivity of solutions, boundedness, conserved quantities, steady states, and long-time behaviour. For several models, tools from chemical reaction network theory, including the Deficiency Zero Theorem, are used to establish the existence, uniqueness, and stability of positive steady states. The analysis shows that most models converge to a unique steady state, while the negative-feedback model requires separate treatment because it can exhibit more complex steady-state behaviour. In addition, sensitivity analysis is used to identify which parameters most strongly influence model outputs such as response functions, $E_{\max}$, and EC_{50} . These results highlight the importance of phosphorylation-related rates and phosphatase-related parameters in determining model behaviour.
4. **Development of a mechanochemical energy-landscape model.** The thesis develops a mechanochemical energy-landscape framework to investigate how mechanical force, receptor geometry, and binding energetics can shape TCR–pMHC bond stability. This contribution addresses a limitation of phenotypic models, where dissociation time or bond lifetime is usually treated as a prescribed kinetic input. In the mechanochemical model, bond lifetime is instead linked to physical quantities such as receptor extension, ligand-binding-site separation, hinge geometry, binding energy, mechanical work, local minima, saddle points, and energy barriers. This provides a mathematical route for studying how force may influence the kinetic quantities that enter T-cell activation models.
5. **Mechanochemical interpretation of catch–slip and slip-bond behaviour across ligand classes.** The thesis applies the mechanochemical model to agonist, weak agonist, and antagonist ligand classes. By analyzing force-dependent stationary points, Hessian-based stability, energy barriers, and Arrhenius–Kramers–Langer type bond lifetimes, the thesis shows how a reduced energy-landscape model can provide a possible mathematical mechanism for distinct ligand-class behaviours. In particular, the model offers an interpretation of how agonist-like interactions may exhibit catch–slip behaviour through force-induced barrier stabilization, whereas antagonist-like interactions may exhibit slip-bond behaviour through force-induced barrier reduction. The resulting force-dependent bond lifetimes provide a possible physical interpretation of how mechanical loading may contribute to ligand discrimination in T-cell activation.

1.8 Scope and Limitations

This thesis focuses on mathematical models of T-cell activation arising from TCR–pMHC interactions. Its scope is deliberately restricted to two connected levels of explanation. The first is the phenotypic level, where the aim is to compare how different signalling architectures reproduce experimentally motivated response properties of T-cell activation. The second is the mechanochemical level, where the aim is to investigate how force, receptor geometry, and binding energetics can influence TCR–pMHC bond stability and bond lifetime. The thesis does not attempt to construct a complete multiscale model of T-cell activation from molecular binding to cellular fate decisions.

In the phenotypic part of the thesis, the models are formulated mainly as deterministic systems of ordinary differential equations derived from biochemical reaction networks. These models are used to study response functions, $E_{\max}$, EC_{50} , specificity, sensitivity, antigen discrimination, ligand antagonism, optimal response, steady states, stability, and parameter sensitivity. The purpose is not to reproduce every molecular event in the T-cell signalling cascade, but to assess which simplified signalling architectures can explain key response properties for biologically plausible parameter ranges.

This modelling choice has important limitations. The deterministic ODE framework does not explicitly include stochastic fluctuations, spatial heterogeneity, receptor clustering, membrane diffusion, single-molecule noise, or cell-to-cell variability. These effects can be important in real T-cell activation, especially when ligand numbers are very small. However, this framework is appropriate for the aims of the thesis because it allows model structures to be compared systematically and their qualitative properties to be analyzed rigorously.

The mechanochemical part of the thesis is also intentionally coarse-grained. The TCR–pMHC interaction is represented using a reduced energy-landscape model involving receptor extension, ligand-binding-site separation, hinge geometry, binding energy, and applied force. This model does not attempt to provide an atomistic molecular-dynamics description of the full TCR–pMHC–CD3 complex, nor does it include the complete downstream signalling network. Instead, it focuses on how force can reshape local minima, saddle points, energy barriers, and bond lifetimes.

A further limitation is that the mechanochemical model treats force in an idealized way. In real T-cell–APC contacts, forces may vary in magnitude, direction, duration, and spatial location. They may arise from actin dynamics, membrane deformation, microvillus motion, receptor movement, or traction at the immunological synapse. The model abstracts these details into an effective applied force. This simplification is intentional because the objective is to obtain qualitative and mathematical insight into how force may stabilize or destabilize TCR–pMHC bonds.

The thesis also does not provide a fully coupled simulation linking molecular binding, force-dependent bond lifetime, intracellular signalling, gene expression, and final cellular fate. Instead, it studies two complementary mathematical viewpoints. Phenotypic models are used to analyze how kinetic variables and signalling architectures influence cellular response properties. Mechanochemical models are used to investigate how some of those kinetic variables, especially force-dependent bond lifetime, may arise from physical bond stability. The connection between these viewpoints is therefore conceptual and mathematical rather than a complete integrated multiscale simulation.

These restrictions are deliberate. By abstracting from some biological detail, the thesis gains mathematical clarity, analytical tractability, and interpretability. These simplifications make it possible to identify which mechanisms are sufficient to reproduce particular response properties, which predictions are robust for plausible parameter ranges, and how force-dependent energy barriers may contribute to TCR–pMHC bond lifetime and ligand discrimination.

1.9 Organization of the Thesis

The thesis is organized into six chapters. Each chapter develops one part of the overall argument, moving from the biological motivation and mathematical background to phenotypic modelling, mathematical analysis, mechanochemical modelling, and the final synthesis of the results.

Chapter 1 introduces the biological and mathematical motivation of the thesis. It presents T-cell activation as a problem of sensitive, specific, and ligand-discriminating recognition through TCR–pMHC interactions. The chapter discusses why T-cell activation remains a modelling problem, identifies the main research gaps, explains the role of mathematics in studying the problem, and states the aims, objectives, contributions, scope, and limitations of the thesis.

Chapter 2 presents the phenotypic modelling framework for T-cell activation. It introduces the main biological response properties used to evaluate models, including specificity, sensitivity, antigen discrimination, ligand antagonism, optimal response, $E_{\max}$, and EC_{50} . The chapter then formulates and compares a family of phenotypic models, including the occupancy model, kinetic proofreading, limited signalling, sustained signalling, negative feedback, stabilizing activation chains, incoherent feedforward motifs, and combined model variants.

Chapter 3 provides the mathematical analysis and systematic comparison of the phenotypic models. The chapter studies positivity, boundedness, conserved quantities, steady states, and long-time behaviour of the models. Where appropriate, tools from chemical reaction network theory, including the Deficiency Zero Theorem, are used to establish existence, uniqueness, and stability of biologically relevant steady states. The

chapter also evaluates model behaviour for biologically plausible parameter ranges and uses sensitivity analysis to identify parameters that strongly influence model predictions.

Chapter 4 develops the mechanochemical energy-landscape model for TCR–pMHC binding under force. The chapter introduces a reduced description based on receptor extension, ligand-binding-site separation, hinge geometry, binding energy, and mechanical work. It formulates the force-loaded energy landscape and analyzes its stationary points. Local minima, saddle points, Hessian classification, energy barriers, and bond-breaking conditions are studied to understand how force reshapes the stability landscape of the TCR–pMHC bond.

Chapter 5 applies the mechanochemical framework to force-dependent bond lifetimes and ligand-class discrimination. The chapter converts force-dependent energy barriers into bond lifetimes using Arrhenius–Kramers–Langer type approximations. It compares agonist, weak agonist, and antagonist ligand classes and investigates how catch–slip and slip–bond behaviours can arise from differences in geometry, binding energetics, and force-dependent barrier structure. The chapter concludes by relating the mechanochemical results to the kinetic quantities used in phenotypic models of T-cell activation.

Chapter 6 summarizes the main findings of the thesis and discusses their biological and mathematical implications. It brings together the phenotypic and mechanochemical parts of the work, emphasizing how response-level models and force-dependent energy-landscape models provide complementary perspectives on T-cell activation. The chapter also discusses limitations of the models, possible extensions, and future directions for mathematical modelling of TCR signalling and mechanochemical antigen discrimination.

Together, these chapters develop two complementary mathematical viewpoints. Chapters 2 and 3 examine what phenotypic models can explain about experimentally observed T-cell activation properties. Chapters 4 and 5 investigate how force-dependent molecular bond stability may provide a physical basis for some of the kinetic variables used in those response-level models. Chapter 6 then integrates these findings and outlines how the modelling approaches developed in the thesis may be extended in future work.

Phenotypic Models of T-Cell Activation

Phenotypic models of T-cell activation are simplified conceptual or mathematical descriptions designed to reproduce observable features of T-cell responses without committing to a fully specified molecular mechanism of receptor triggering [75, 76, 77]. In this context, the term *phenotypic* refers to experimentally visible input–output behavior, such as specificity, sensitivity, antigen discrimination, and optimal response, rather than to a complete biochemical account of all intracellular events. This viewpoint is especially useful in T-cell biology because the triggering process is highly complex, whereas several key response characteristics are already well established experimentally.

The biological motivation for studying these models comes from the remarkable ability of T cells to distinguish foreign ligands from self ligands despite often very small differences in binding properties. In particular, interactions between T-cell receptors (TCRs) and peptide–major histocompatibility complexes (pMHCs) have motivated a range of mathematical frameworks aimed at explaining how binding kinetics are translated into cellular activation. Experimental observations such as optimal dwell time, serial engagement, signal persistence after ligand dissociation, and strong discrimination between closely related ligands provide the main benchmarks that any successful model should capture [75, 76, 78].

The models considered in this chapter form a family of systems of ordinary differential equations derived from reaction-network assumptions and mass-action kinetics. They include the occupancy model, the kinetic proofreading model, and several extensions incorporating limited signaling, sustained signaling, negative feedback, stabilizing activation chains, and incoherent feedforward regulation. In addition, two further models are constructed by combining elements of existing formulations, bringing the total number of models considered to nine. Taken together, these models represent a progression from simpler descriptions of receptor occupancy toward richer formulations capable of reproducing a broader range of experimentally observed T-cell behaviors [78, 75].

The aim of this chapter is to present these phenotypic models in a unified mathematical framework and to establish the notation, assumptions, and structural features needed for their later analysis. In particular, we introduce the formulation of each model, the biologically relevant region arising from conservation laws, and the common response quantities that will be used to compare model behavior. For several models, the subsequent

mathematical analysis will rely on structural properties of the underlying reaction networks and on the Deficiency Zero Theorem [79, 80], while the negative-feedback model requires separate consideration because uniqueness of steady states does not always hold [78, 81]. The framework developed here provides the foundation for the mathematical and numerical investigations carried out in the following chapter.

The chapter is organized as follows. Section 2.2 describes the biological benchmarks that motivate the model family. Section 2.3 introduces the general modeling framework and the common assumptions underlying all models. Section 2.4 presents the nine models individually. Section 2.5 discusses the formal relations among the models. Section 2.6 introduces the response quantities used for later comparison, and Section 2.7 provides a brief summary.

2.1 Purpose of the Chapter

This chapter presents the family of phenotypic models of T-cell activation studied in this thesis within a common biological and mathematical framework. It also introduces the main response quantities—namely the response function, $E_{\max}$, and EC_{50} —that will be used in the subsequent comparative analysis.

2.2 Biological Benchmarks for Model Comparison

Any mathematical model of T-cell activation should be assessed by its ability to reproduce the main experimentally established features of TCR–pMHC recognition. In the phenotypic modeling literature, four criteria are especially central: specificity, sensitivity, antigen discrimination, and optimal response. In addition, serial engagement and signal persistence are important mechanistic ideas that help explain how T cells can generate strong responses despite low ligand numbers and rapid binding–unbinding dynamics [78, 75, 76, 82, 83].

The principal biological benchmarks considered in this thesis are the following:

1. **Specificity.** Specificity refers to the ability of T cells to remain largely unresponsive to self-ligands while still responding appropriately to foreign ligands. This means that self-peptides should fail to trigger activation even when presented in very large numbers, on the order of 10^5 or more. Thus, specificity expresses the requirement that the signaling system suppress activation by abundant nonagonist or self-pMHC ligands [76, 75].
2. **Sensitivity.** Sensitivity denotes the ability of T cells to respond to extremely small amounts of foreign antigen. It has been found that even approximately ten agonist ligands presented on an antigen-presenting cell can be sufficient to activate

lymphocytes. In this sense, sensitivity captures the capacity of the signaling system to detect rare foreign peptides despite the background presence of many self-ligands [76, 75].

3. **Antigen discrimination.** Antigen discrimination is the capacity to distinguish between ligands with only small differences in binding properties, especially differences in dissociation time, and to convert those small kinetic differences into large differences in downstream response. This requirement is central to proofreading-based explanations of T-cell activation and to the broader phenotypic modeling literature [76, 75].
4. **Optimal response.** Optimal response refers to the observation that T-cell activation need not increase monotonically with ligand dwell time. Instead, efficient activation may occur within an intermediate range of interaction times, so that neither very short nor very long binding durations are necessarily most effective. This idea is closely related to the concept of an optimal dwell time in TCR–pMHC interactions [82].
5. **Serial engagement and signal persistence.** Serial engagement refers to the idea that a small number of peptide–MHC complexes can sequentially trigger many T-cell receptors, thereby amplifying activation [83]. Signal persistence refers to the related possibility that signaling may continue, at least transiently, after ligand dissociation [84]. Together, these mechanisms help reconcile rapid ligand turnover with robust downstream signaling and motivate model extensions involving limited or sustained signaling [78, 75].

These benchmarks also help explain why a hierarchy of models is needed. A simple occupancy-based description is insufficient to account for the experimentally observed failure of weak ligands to trigger activation even at high concentration, whereas kinetic proofreading improves ligand discrimination by introducing sequential intermediate steps. At the same time, the biological tension between specificity, sensitivity, serial engagement, and dwell-time-dependent signaling motivates the development of more elaborate model variants with additional regulatory and signaling mechanisms [78, 76, 75].

In this thesis, these biological benchmarks are not treated only qualitatively but are also tracked through mathematical quantities derived from the models. The response function serves as the primary tool for studying how activation varies with ligand concentration and dissociation time, while $E_{\max}$ and EC_{50} provide complementary summary measures of maximal signaling output and ligand potency, respectively. Optimal response is identified through the presence of peaks or non-monotonic behavior in the response curves, antigen discrimination is evaluated through the extent to which small changes in dissociation time produce large changes in activation, sensitivity is reflected in the ability to respond at low

ligand concentrations, and specificity is assessed by whether weak or self-like ligands fail to trigger substantial activation even at high ligand concentrations.

Taken together, these benchmarks motivate the construction of a hierarchy of phenotypic models, ranging from simple occupancy-based descriptions to more elaborate proofreading models with additional regulatory and signaling motifs. The role of the models studied in this chapter is therefore not merely to reproduce receptor binding, but to explain how these benchmark properties can emerge from different signaling architectures [78, 75].

2.3 General Modelling Framework and Assumptions

The models considered in this chapter are formulated as systems of ordinary differential equations arising from biochemical reaction networks under mass-action kinetics [79]. This framework is common to the major phenotypic models of T-cell activation and makes it possible to compare different signaling mechanisms within a unified mathematical setting [75]. The state variables represent concentrations of ligands, receptors, and receptor–ligand complexes in different biochemical states, while the governing equations describe how these concentrations evolve through binding, dissociation, phosphorylation, and related transitions [85].

A central feature of this formulation is that the dynamics are studied in the non-negative orthant. More explicitly, if $x = (x_1, \dots, x_m)$ denotes the vector of concentrations in a given model, then the non-negative orthant is $\mathbb{R}_{\geq 0}^m = \{x \in \mathbb{R}^m : x_i \geq 0 \text{ for all } i\}$. Its interior, $\mathbb{R}_{> 0}^m$, is the positive orthant. This restriction is biologically natural because all state variables represent concentrations and must remain non-negative. In addition, these models possess conserved quantities, in particular the total ligand quantity L_T and the total receptor quantity R_T , which restrict the dynamics to an invariant region. In some model extensions, additional conserved total quantities also appear, such as the total phosphatase quantity S_T in the negative-feedback model. After using these expressions for the conserved quantities to reduce the systems, one obtains a compact state space whose interior will be referred to as the biologically relevant region. This common structure provides the basis for the later analysis of positivity, boundedness, steady states, and long-time behavior.

2.3.1 Common Variables and Parameters

Although the individual models differ in biological detail and mathematical complexity, they share a common notation. The principal variables used throughout this chapter are the following:

- L : concentration of free ligand,

- R : concentration of free receptor,
- C_0 : concentration of the initially bound TCR–pMHC complex,
- C_i : concentration of the complex in the i -th modified or phosphorylated state,
- C_N : concentration of the signaling-competent state,
- L_T : total ligand quantity,
- R_T : total receptor quantity.

Additional state variables appear in some model extensions. In sustained-signaling models, R^* denotes an unbound but still signaling receptor state. In negative-feedback models, S denotes the active phosphatase and S_T the corresponding conserved total quantity.

The most common kinetic parameters are:

- κ : ligand–receptor binding rate,
- ϕ : phosphorylation or forward transition rate between intermediate states,
- v : ligand dissociation rate.

Additional parameters appear in specific model extensions. The parameter ξ is used in limited-signaling models to describe the rate at which the signaling-competent state transitions to a non-signaling state. The parameter Ω appears in sustained-signaling models and governs the rate at which unbound but still active receptors return to their basal state. Parameters such as α , β , and γ arise in models involving feedback or regulatory mechanisms [78, 86]. In most proofreading-type formulations, the quantity C_N represents the signaling-competent state and therefore serves as the main upstream indicator of activation [85, 78].

2.3.2 Common Assumptions

The following assumptions underlie the formulation of the entire model family considered in this thesis:

1. Biochemical interactions are represented by reaction networks and translated into systems of ordinary differential equations using mass-action kinetics [78, 79].
2. All concentrations are assumed to be non-negative, and the dynamics preserve this non-negativity for all future times [78].

3. Ligand binding initiates the formation of receptor–ligand complexes, which may then pass through a sequence of intermediate biochemical states before reaching a signaling-competent state [85, 78].
4. In basic proofreading-type models, dissociation at an intermediate stage may terminate or reset progression along the activation chain, thereby reducing or eliminating signaling output [85]. In sustained-signaling variants, however, signaling may persist transiently even after ligand dissociation [84, 75].
5. The models possess conserved total quantities, most commonly the total ligand quantity L_T and the total receptor quantity R_T , which define stoichiometric constraints on the dynamics. Some extensions may involve additional conserved total quantities, such as the total phosphatase quantity S_T in the negative-feedback model [78, 86].
6. The models describe averaged, deterministic population-level behavior and therefore do not explicitly incorporate spatial heterogeneity or stochastic fluctuations in their basic formulation, although such effects may be considered in later refinements or extensions [76, 75].

2.4 Phenotypic Model Formulations

2.4.1 Occupancy Model

The occupancy model is one of the earliest conceptual descriptions of T-cell activation. In this framework, the response is assumed to depend directly on the number of TCR–pMHC complexes formed at equilibrium, so that receptor occupancy itself determines signaling output [87, 75]. In its simplest form, ligand binding is assumed to generate an immediately signaling-capable complex, without any intermediate proofreading steps or downstream regulatory motifs [75].

Let L denote the concentration of free ligand, R the concentration of free receptor, and C the concentration of the bound TCR–pMHC complex. The full reaction-style formulation is

$$\frac{dC}{dt} = \kappa LR - vC, \quad (2.1)$$

where κ is the ligand–receptor binding rate and v is the dissociation rate. In this model, T-cell activation is taken to be directly proportional to the concentration of the bound complex C .

At equilibrium, one obtains

$$LR = K_D C, \quad K_D = \frac{v}{\kappa}. \quad (2.2)$$

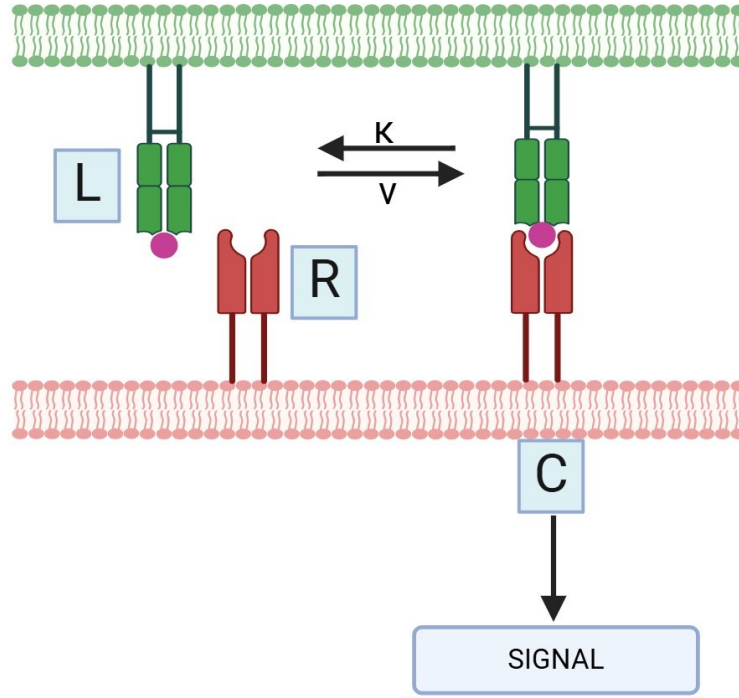


Figure 2.1. Ligand L binds receptor R to form the bound complex C , and the response is proportional to receptor occupancy.

The total amounts of ligand and receptor, which are conserved quantities, are given by

$$L_T = L + C, \quad R_T = R + C. \quad (2.3)$$

Substituting these expressions for the conserved quantities into the equilibrium relation yields the reduced form

$$C = \frac{L_T + R_T + K_D - \sqrt{(L_T + R_T + K_D)^2 - 4L_T R_T}}{2}. \quad (2.4)$$

In the occupancy model, the response is therefore identified with

$$\text{Response} = C. \quad (2.5)$$

The occupancy model therefore provides the simplest baseline description of T-cell activation in the model family considered here and serves as a reference point for the more detailed models introduced in the following sections. At the same time, its simplicity limits its biological scope, since it cannot explain the experimentally observed failure of weak ligands to trigger activation even at high concentration [78, 75].

2.4.2 Kinetic Proofreading Model

The kinetic proofreading (KPR) model was introduced to explain how T cells can distinguish between ligands with different binding lifetimes. In this framework, a pMHC ligand binds reversibly to a T-cell receptor to form an initial complex C_0 , which then passes through a sequence of intermediate biochemical modification steps before reaching a signaling-competent state C_N . If the ligand dissociates at any intermediate stage, these modifications are assumed to be rapidly undone, and the receptor returns to its basal state [85, 75].

These intermediate states may be interpreted as a coarse-grained representation of early signaling events in T-cell activation. In biological terms, they encompass processes such as receptor dimerization, the sequential phosphorylation of tyrosine residues within the TCR complex, and the subsequent recruitment and activation of ZAP-70. Experimental studies have reported differences in early ζ -chain phosphorylation patterns, including p21 ζ /p23 ζ , as well as differences in the recruitment and activity of ZAP-70 in response to ligands of varying potency [88, 89].

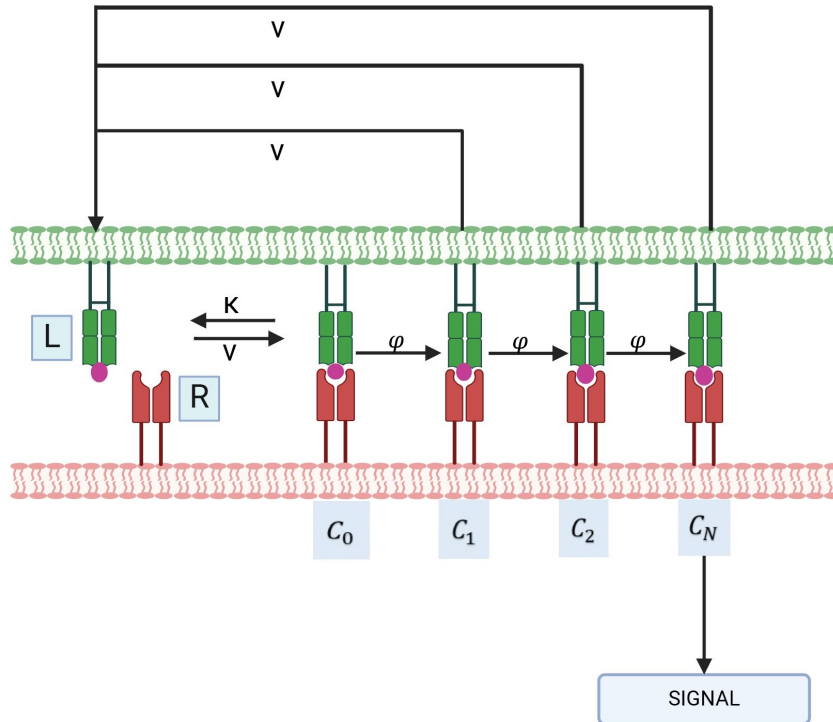


Figure 2.2. The bound complex progresses through sequential proofreading states before reaching the signalling-competent state C_N .

Let L denote the concentration of free ligand, R the concentration of free receptor, and $C_0, \dots, C_N$ the concentrations of the receptor–ligand complex at successive stages of

the proofreading cascade. The full reaction-style formulation is

$$\frac{dL}{dt} = -\kappa LR + v \sum_{i=0}^N C_i, \quad (2.6)$$

$$\frac{dR}{dt} = -\kappa LR + v \sum_{i=0}^N C_i, \quad (2.7)$$

$$\frac{dC_0}{dt} = \kappa LR - (\phi + v)C_0, \quad (2.8)$$

$$\frac{dC_i}{dt} = \phi C_{i-1} - (\phi + v)C_i, \quad 1 \leq i \leq N-1, \quad (2.9)$$

$$\frac{dC_N}{dt} = \phi C_{N-1} - vC_N. \quad (2.10)$$

Here, κ is the ligand–receptor binding rate, ϕ is the forward transition or phosphorylation rate, and v is the dissociation rate. In this model, T-cell activation is identified with the concentration of the signaling-competent state C_N .

Using the conserved quantities

$$L_T = L + \sum_{i=0}^N C_i, \quad R_T = R + \sum_{i=0}^N C_i, \quad (2.11)$$

the system can be rewritten in reduced form as

$$\frac{dC_0}{dt} = \kappa \left(L_T - \sum_{i=0}^N C_i \right) \left(R_T - \sum_{i=0}^N C_i \right) - (\phi + v)C_0, \quad (2.12)$$

$$\frac{dC_i}{dt} = \phi C_{i-1} - (\phi + v)C_i, \quad 1 \leq i \leq N-1, \quad (2.13)$$

$$\frac{dC_N}{dt} = \phi C_{N-1} - vC_N. \quad (2.14)$$

The KPR model provides the basic proofreading framework on which several of the later model extensions are built. At the same time, while kinetic proofreading improves ligand discrimination, it also introduces a trade-off between specificity and sensitivity that motivates the development of more elaborate variants [78, 75].

2.4.3 Kinetic Proofreading with Limited Signaling

The kinetic proofreading model with limited signaling extends the basic KPR framework by assuming that the signaling-competent state does not remain active indefinitely. Instead, once a receptor reaches the signaling state C_N , it undergoes a transition to a non-signaling state C_{N+1} at rate ξ . In this way, each receptor–ligand binding event is assumed to generate only a finite signaling packet [75, 78].

The biological motivation for this model comes from the observation that agonist pMHC molecules on the surface of antigen-presenting cells are typically sparse, often occurring only in the range of tens to hundreds of copies among roughly 10^5 MHC molecules on a single APC [90]. This limited availability constrains the number of T-cell receptors that can be engaged simultaneously. Experiments with T cells expressing two distinct TCRs have shown that activation is not transmitted to neighboring receptors, suggesting that each TCR must itself directly engage a pMHC in order to become activated [91]. To account for how strong signaling may nevertheless arise under such conditions, the serial triggering hypothesis proposes that a single antigenic pMHC can sequentially engage many TCRs over time [83]. In phenomenological terms, this suggests that each receptor contributes only a limited signaling event per productive binding episode, after which it no longer signals even if the complex remains bound. This interpretation is also consistent with observations that TCR signaling is sustained in peripheral microclusters but terminated as receptors migrate toward the central supramolecular activation cluster and are eventually targeted for degradation or internalization [92, 93].

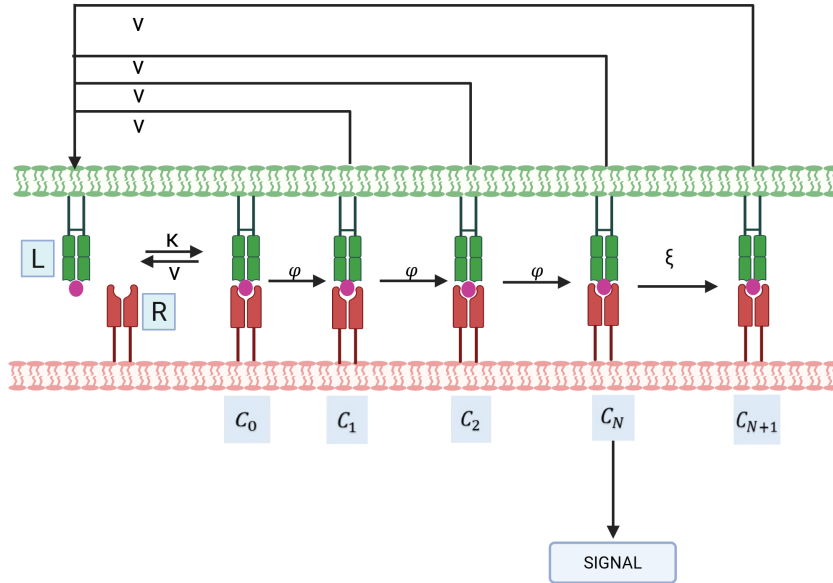


Figure 2.3. After reaching C_N , the complex enters a terminal non-signalling state C_{N+1} at rate ξ , limiting signal duration.

Let L denote the concentration of free ligand, R the concentration of free receptor, and $C_0, \dots, C_{N+1}$ the concentrations of the successive proofreading states. The full

reaction-style formulation is

$$\frac{dL}{dt} = -\kappa LR + v \sum_{i=0}^{N+1} C_i, \quad (2.15)$$

$$\frac{dR}{dt} = -\kappa LR + v \sum_{i=0}^{N+1} C_i, \quad (2.16)$$

$$\frac{dC_0}{dt} = \kappa LR - (\phi + v)C_0, \quad (2.17)$$

$$\frac{dC_i}{dt} = \phi C_{i-1} - (\phi + v)C_i, \quad 1 \leq i \leq N-1, \quad (2.18)$$

$$\frac{dC_N}{dt} = \phi C_{N-1} - (v + \xi)C_N, \quad (2.19)$$

$$\frac{dC_{N+1}}{dt} = \xi C_N - v C_{N+1}. \quad (2.20)$$

Here, κ is the ligand–receptor binding rate, ϕ is the forward transition or phosphorylation rate, v is the dissociation rate, and ξ is the rate at which the signaling-competent state becomes non-signaling. In this model, T-cell activation is identified with the concentration of the signaling-competent state C_N , while C_{N+1} represents receptors that remain bound but no longer contribute to signaling.

Using the conserved quantities

$$L_T = L + \sum_{i=0}^{N+1} C_i, \quad R_T = R + \sum_{i=0}^{N+1} C_i, \quad (2.21)$$

the system can be rewritten in reduced form as

$$\frac{dC_0}{dt} = \kappa \left(L_T - \sum_{i=0}^{N+1} C_i \right) \left(R_T - \sum_{i=0}^{N+1} C_i \right) - (\phi + v)C_0, \quad (2.22)$$

$$\frac{dC_i}{dt} = \phi C_{i-1} - (\phi + v)C_i, \quad 1 \leq i \leq N-1, \quad (2.23)$$

$$\frac{dC_N}{dt} = \phi C_{N-1} - (v + \xi)C_N, \quad (2.24)$$

$$\frac{dC_{N+1}}{dt} = \xi C_N - v C_{N+1}. \quad (2.25)$$

The limited-signaling model therefore refines the basic proofreading framework by incorporating the idea that signaling through an individual receptor is temporally restricted.

2.4.4 Kinetic Proofreading with Sustained Signaling

The kinetic proofreading model with sustained signaling extends the basic KPR framework by allowing signaling to persist for some time even after ligand unbinding. In this model, receptors that have reached the signaling-competent state C_N do not immediately lose

activity when the pMHC dissociates. Instead, they enter an unbound but still signaling state, denoted by R^* , and subsequently return to the basal receptor state at rate Ω [75, 78].

The biological motivation for this model comes from the need to reconcile two important ideas in T-cell activation. On the one hand, kinetic proofreading suggests that productive signaling requires sufficiently long TCR–pMHC engagement. On the other hand, serial triggering arguments suggest that pMHC molecules must dissociate rapidly enough to engage multiple receptors sequentially [83, 82]. The sustained-signaling framework addresses this tension by assuming that signaling can continue transiently even after pMHC has detached. From a mechanistic point of view, this can be interpreted as a coarse-grained description of signaling complexes or signalosomes that remain active for a limited period until they are dephosphorylated or internalized [75, 84, 78].

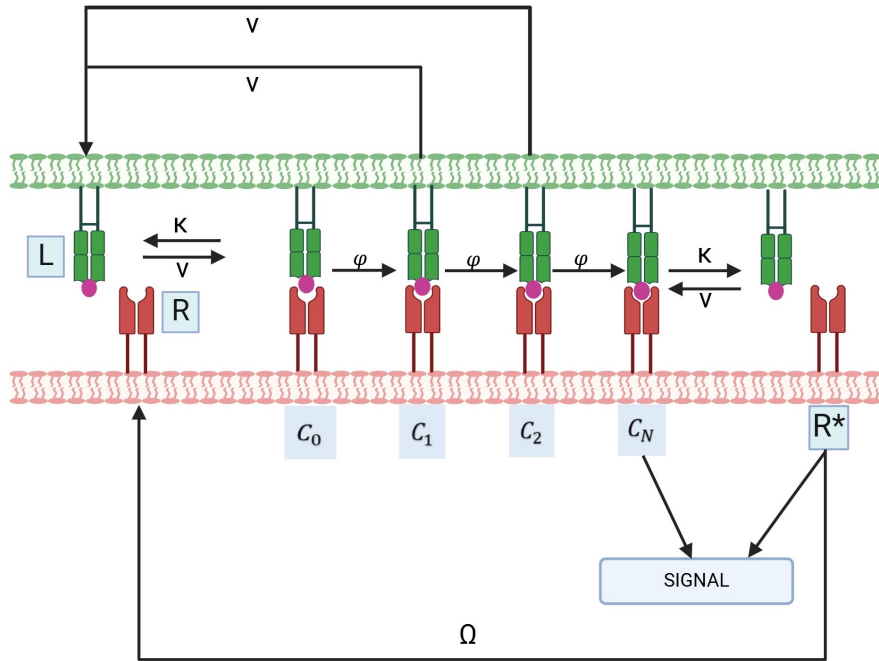


Figure 2.4. Ligand dissociation from C_N can generate a transient active receptor state R^* , which returns to the basal state at rate Ω .

Let L denote the concentration of free ligand, R the concentration of free receptor, $C_0, \dots, C_N$ the successive proofreading states, and R^* the unbound receptor state that remains transiently signaling after ligand dissociation. The full reaction-style formulation

is

$$\frac{dL}{dt} = -\kappa LR + v \sum_{i=0}^N C_i - \kappa LR^*, \quad (2.26)$$

$$\frac{dR}{dt} = -\kappa LR + v \sum_{i=0}^{N-1} C_i + \Omega R^*, \quad (2.27)$$

$$\frac{dR^*}{dt} = vC_N - \kappa LR^* - \Omega R^*, \quad (2.28)$$

$$\frac{dC_0}{dt} = \kappa LR - (\phi + v)C_0, \quad (2.29)$$

$$\frac{dC_i}{dt} = \phi C_{i-1} - (\phi + v)C_i, \quad 1 \leq i \leq N-1, \quad (2.30)$$

$$\frac{dC_N}{dt} = \phi C_{N-1} - vC_N + \kappa LR^*. \quad (2.31)$$

Here, κ is the ligand–receptor binding rate, ϕ is the forward transition or phosphorylation rate, v is the dissociation rate, and Ω is the rate at which the sustained-signaling state R^* returns to the basal receptor state. In this model, T-cell activation is associated with both the bound signaling-competent state C_N and the unbound but still active state R^* .

Using the conserved quantities

$$L_T = L + \sum_{i=0}^N C_i, \quad R_T = R + \sum_{i=0}^N C_i + R^*, \quad (2.32)$$

the system can be rewritten in reduced form as

$$\frac{dC_0}{dt} = \kappa \left(L_T - \sum_{i=0}^N C_i \right) \left(R_T - \sum_{i=0}^N C_i - R^* \right) - (\phi + v)C_0, \quad (2.33)$$

$$\frac{dC_i}{dt} = \phi C_{i-1} - (\phi + v)C_i, \quad 1 \leq i \leq N-1, \quad (2.34)$$

$$\frac{dC_N}{dt} = \phi C_{N-1} - vC_N + \kappa \left(L_T - \sum_{i=0}^N C_i \right) R^*, \quad (2.35)$$

$$\frac{dR^*}{dt} = vC_N - \kappa \left(L_T - \sum_{i=0}^N C_i \right) R^* - \Omega R^*. \quad (2.36)$$

The sustained-signaling model therefore refines the basic proofreading framework by incorporating the possibility that signaling may persist transiently after ligand dissociation.

2.4.5 Kinetic Proofreading with Negative Feedback

The kinetic proofreading model with negative feedback extends the basic KPR framework by allowing the proofreading cascade to be regulated by an inhibitory feedback mechanism.

In this model, the rates of progression through the activation chain are modulated by a phosphatase-mediated negative regulator, so that signaling is no longer determined solely by the forward proofreading steps [86, 75, 78].

The biological motivation for this model comes from the need to prevent excessive or inappropriate T-cell activation. In particular, negative regulation by phosphatases such as SHP-1 plays an important role in controlling both the strength and the duration of TCR signaling. In a coarse-grained formulation, this idea is represented by introducing an active inhibitory variable S , which feeds back on the proofreading chain and counteracts phosphorylation-driven progression toward the signaling state [86, 78]. Thus, unlike the basic KPR model, the present formulation includes an explicit regulatory mechanism that opposes activation.

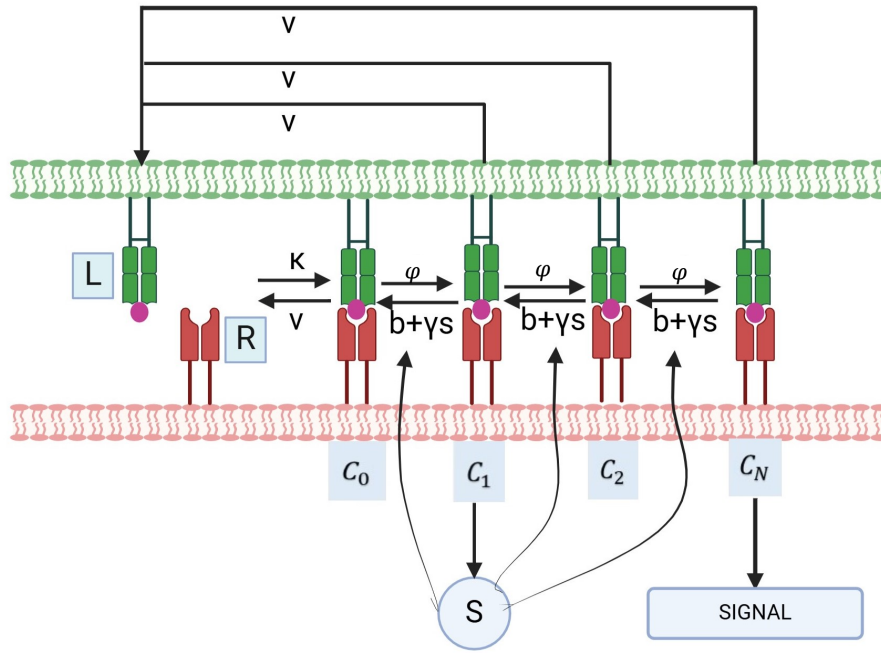


Figure 2.5. The inhibitory variable S feeds back on the proofreading cascade and reduces progression toward the signalling-competent state C_N .

Let L denote the concentration of free ligand, R the concentration of free receptor, $C_0, \dots, C_N$ the successive proofreading states, and S the active phosphatase. The full

reaction-style formulation is

$$\frac{dL}{dt} = -\kappa LR + v \sum_{i=0}^N C_i, \quad (2.37)$$

$$\frac{dR}{dt} = -\kappa LR + v \sum_{i=0}^N C_i, \quad (2.38)$$

$$\frac{dC_0}{dt} = \kappa LR + (b + \gamma S)C_1 - (\phi + v)C_0, \quad (2.39)$$

$$\frac{dC_i}{dt} = \phi C_{i-1} + (b + \gamma S)C_{i+1} - (\phi + v + b + \gamma S)C_i, \quad 1 \leq i \leq N-1, \quad (2.40)$$

$$\frac{dC_N}{dt} = \phi C_{N-1} - (b + \gamma S + v)C_N, \quad (2.41)$$

$$\frac{dS}{dt} = \alpha C_1(S_T - S) - \beta S. \quad (2.42)$$

Here, κ is the ligand–receptor binding rate, ϕ is the forward transition or phosphorylation rate, v is the dissociation rate, b is the spontaneous dephosphorylation rate, and γ measures the efficiency of the active phosphatase S . The parameters α and β denote, respectively, the activation and deactivation rates of the phosphatase, while S_T is the total phosphatase quantity [86]. In this model, T-cell activation is identified with the signaling-competent state C_N .

Using the conserved quantities

$$L_T = L + \sum_{i=0}^N C_i, \quad R_T = R + \sum_{i=0}^N C_i, \quad (2.43)$$

the system can be rewritten in reduced form as

$$\frac{dC_0}{dt} = \kappa \left(L_T - \sum_{i=0}^N C_i \right) \left(R_T - \sum_{i=0}^N C_i \right) + (b + \gamma S)C_1 - (\phi + v)C_0, \quad (2.44)$$

$$\frac{dC_i}{dt} = \phi C_{i-1} + (b + \gamma S)C_{i+1} - (\phi + v + b + \gamma S)C_i, \quad 1 \leq i \leq N-1, \quad (2.45)$$

$$\frac{dC_N}{dt} = \phi C_{N-1} - (b + \gamma S + v)C_N, \quad (2.46)$$

$$\frac{dS}{dt} = \alpha C_1(S_T - S) - \beta S. \quad (2.47)$$

The negative-feedback model therefore refines the basic proofreading framework by incorporating an explicit regulatory mechanism that modulates progression along the activation chain. Unlike several of the other models in this chapter, however, the negative-feedback model does not in general admit a unique steady state and therefore requires separate mathematical treatment in the later analysis.

2.4.6 Kinetic Proofreading with Stabilizing Activation Chain

The kinetic proofreading model with a stabilizing activation chain modifies the basic KPR framework by allowing the effective dissociation and progression rates to vary along the proofreading cascade. The model is intended to represent the possibility that receptor–ligand complexes are differentially stabilized or destabilized as signaling progresses, thereby allowing foreign and self ligands to be processed differently along the activation chain [77, 78].

The motivation for this model is to represent, in a coarse-grained way, the possibility that the proofreading process itself changes the quality of receptor–ligand interactions as signaling progresses. In this interpretation, the successive states $C_0, \dots, C_N$ do not merely record the passage of time, but also encode progressive strengthening or weakening of the underlying complexes. This effect is incorporated by allowing both the dissociation rates $v(i)$ and the forward transition rates $\phi(i)$ to depend on the position i in the activation chain [77, 78].

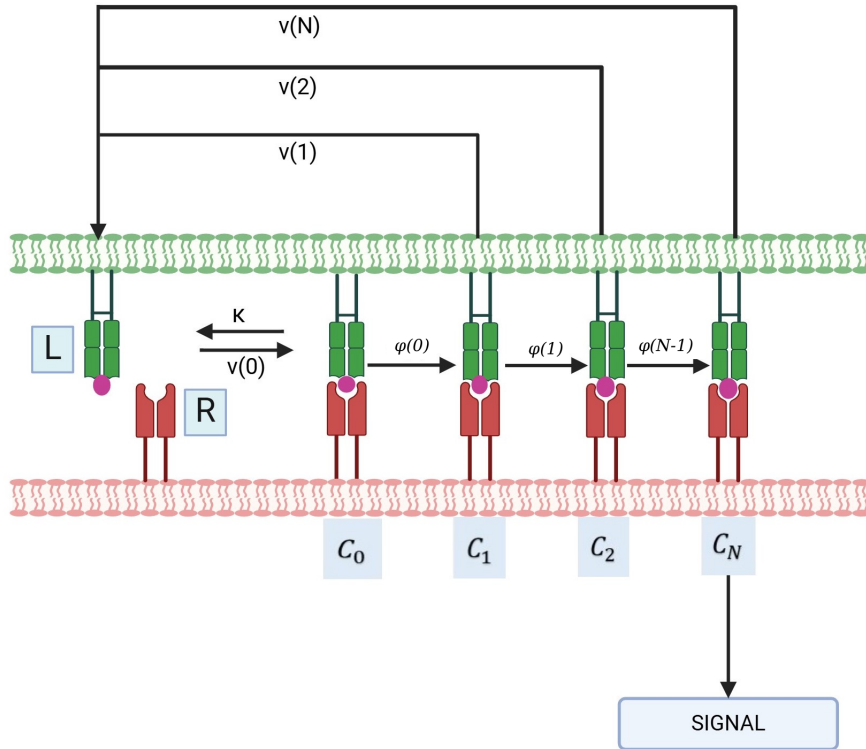


Figure 2.6. Forward progression and dissociation rates vary along the cascade, representing stage-dependent stabilization of receptor–ligand complexes.

Let L denote the concentration of free ligand, R the concentration of free receptor, and $C_0, \dots, C_N$ the successive proofreading states. The full reaction-style formulation is

$$\frac{dL}{dt} = -\kappa LR + \sum_{i=0}^N v(i)C_i, \quad (2.48)$$

$$\frac{dR}{dt} = -\kappa LR + \sum_{i=0}^N v(i)C_i, \quad (2.49)$$

$$\frac{dC_0}{dt} = \kappa LR - (\phi(0) + v(0))C_0, \quad (2.50)$$

$$\frac{dC_i}{dt} = \phi(i-1)C_{i-1} - (\phi(i) + v(i))C_i, \quad 1 \leq i \leq N-1, \quad (2.51)$$

$$\frac{dC_N}{dt} = \phi(N-1)C_{N-1} - v(N)C_N. \quad (2.52)$$

Here, κ is the ligand–receptor binding rate, while $v(i)$ and $\phi(i)$ are stage-dependent dissociation and forward transition rates, respectively. In this model, T-cell activation is identified with the signaling-competent state C_N [77, 78].

A common specification used in the literature is

$$v(i) = \frac{1+i}{1+r_{\text{off}}i} v, \quad \phi(i) = \phi r_p^i, \quad r_{\text{off}} > 1, \quad r_p > 1, \quad (2.53)$$

so that the effective rates vary systematically along the proofreading chain [77, 78].

Using the conserved quantities

$$L_T = L + \sum_{i=0}^N C_i, \quad R_T = R + \sum_{i=0}^N C_i, \quad (2.54)$$

the system can be rewritten in reduced form as

$$\frac{dC_0}{dt} = \kappa \left(L_T - \sum_{i=0}^N C_i \right) \left(R_T - \sum_{i=0}^N C_i \right) - (\phi(0) + v(0))C_0, \quad (2.55)$$

$$\frac{dC_i}{dt} = \phi(i-1)C_{i-1} - (\phi(i) + v(i))C_i, \quad 1 \leq i \leq N-1, \quad (2.56)$$

$$\frac{dC_N}{dt} = \phi(N-1)C_{N-1} - v(N)C_N. \quad (2.57)$$

The stabilizing-activation-chain model therefore refines the basic proofreading framework by allowing the effective kinetic rates to change as activation progresses.

2.4.7 Kinetic Proofreading with Incoherent Feedforward Loop

The kinetic proofreading model with incoherent feedforward loop (IFF) couples the basic KPR cascade to a regulatory motif in which the same upstream signal acts on a downstream

variable through both activating and inhibitory branches. Incoherent feedforward loops are common motifs in molecular regulatory networks and are known to generate biphasic responses with respect to time or dose [94, 78]. In the present framework, the signaling-competent state C_N directly inhibits the downstream variable X , but also activates an intermediate variable Y , which in turn promotes X . Thus, the proofreading state C_N has both a positive indirect effect and a negative direct effect on the final output.

The biological motivation for introducing this motif is to represent how T-cell activation may be fine-tuned by competing activating and inhibitory pathways. In this framework, the incoherent feedforward loop shapes the downstream response generated by the proofreading cascade, so that the final output depends not only on the presence of the signaling-competent state C_N but also on the balance between indirect activation through Y and direct inhibition of X [94, 78]. Although the original experimentally motivated architecture in the literature involved limited signaling together with an incoherent feedforward loop, the simpler KPR with IFF model is considered here as a useful standalone variant for comparison within the model family [94, 78].

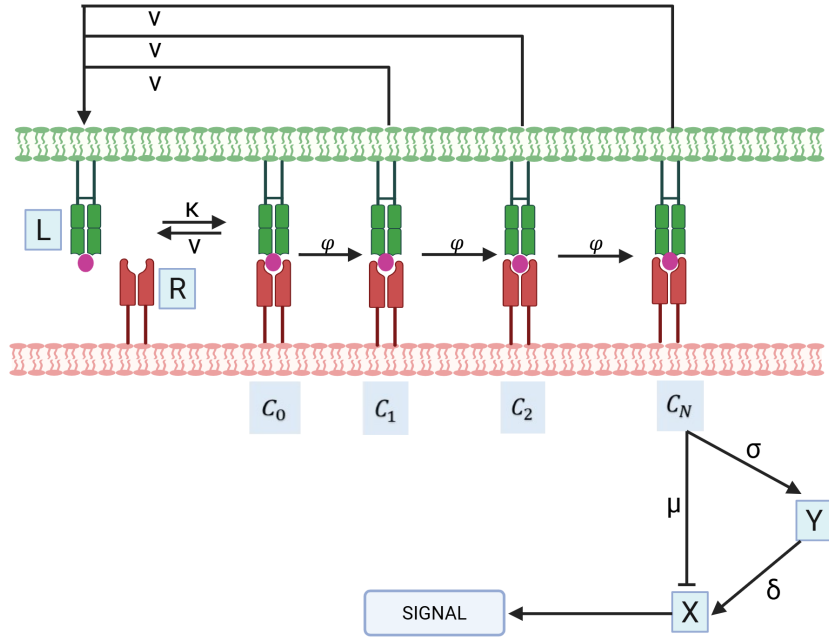


Figure 2.7. The state C_N regulates the downstream response through opposing activation and inhibition pathways.

Let L denote the concentration of free ligand, R the concentration of free receptor, and $C_0, \dots, C_N$ the successive proofreading states. The variables Y and X represent the activating and output branches of the incoherent feedforward loop, respectively. The form of the equations for Y and X follows directly from this regulatory assumption. The term $a(m - Y)$ represents basal production or activation of Y toward the upper level

m , while bY represents loss or deactivation of Y . The additional term $\sigma C_N(m - Y)$ expresses activation of Y by the proofreading output C_N , with saturation as Y approaches m . Similarly, $c(l - X)$ and dX describe basal production and loss of the output X , the term $\delta Y(l - X)$ represents indirect activation of X through Y , and the term $-\mu C_N X$ represents direct inhibition of X by C_N . Thus the same upstream variable C_N promotes X indirectly through Y while inhibiting X directly, which is the defining structure of an incoherent feedforward loop. The full reaction-style formulation is

$$\frac{dL}{dt} = -\kappa LR + v \sum_{i=0}^N C_i, \quad (2.58)$$

$$\frac{dR}{dt} = -\kappa LR + v \sum_{i=0}^N C_i, \quad (2.59)$$

$$\frac{dC_0}{dt} = \kappa LR - (\phi + v)C_0, \quad (2.60)$$

$$\frac{dC_i}{dt} = \phi C_{i-1} - (\phi + v)C_i, \quad 1 \leq i \leq N-1, \quad (2.61)$$

$$\frac{dC_N}{dt} = \phi C_{N-1} - vC_N, \quad (2.62)$$

$$\frac{dY}{dt} = a(m - Y) - bY + \sigma C_N(m - Y), \quad (2.63)$$

$$\frac{dX}{dt} = c(l - X) - dX + \delta Y(l - X) - \mu C_N X. \quad (2.64)$$

Here, κ is the ligand–receptor binding rate, ϕ is the forward transition or phosphorylation rate, and v is the dissociation rate. The parameters $a, b, c, d, \sigma, \delta, \mu, m$, and l govern the dynamics of the feedforward loop. In this model, T-cell activation is identified with the downstream output variable X , rather than directly with the signaling-competent proofreading state C_N . Using the conserved quantities

$$L_T = L + \sum_{i=0}^N C_i, \quad R_T = R + \sum_{i=0}^N C_i, \quad (2.65)$$

the system can be rewritten in reduced form as

$$\frac{dC_0}{dt} = \kappa \left(L_T - \sum_{i=0}^N C_i \right) \left(R_T - \sum_{i=0}^N C_i \right) - (\phi + v)C_0, \quad (2.66)$$

$$\frac{dC_i}{dt} = \phi C_{i-1} - (\phi + v)C_i, \quad 1 \leq i \leq N-1, \quad (2.67)$$

$$\frac{dC_N}{dt} = \phi C_{N-1} - vC_N, \quad (2.68)$$

$$\frac{dY}{dt} = a(m - Y) - bY + \sigma C_N(m - Y), \quad (2.69)$$

$$\frac{dX}{dt} = c(l - X) - dX + \delta Y(l - X) - \mu C_N X. \quad (2.70)$$

The KPR with IFF model therefore extends the basic proofreading framework by allowing the final response to be shaped by competing activating and inhibitory pathways.

2.4.8 Kinetic Proofreading with Limited Signaling and Incoherent Feedforward Loop

The kinetic proofreading model with limited signaling and incoherent feedforward loop combines two mechanisms introduced separately in earlier models: the assumption that signaling through an individual receptor is temporally limited, and the presence of a downstream regulatory motif with competing activating and inhibitory branches. In this framework, receptors reaching the signaling-competent state C_N signal only briefly before transitioning to a non-signaling state C_{N+1} at rate ξ , while the downstream output is regulated through an incoherent feedforward loop involving the variables Y and X [94, 78].

The biological motivation for this model is to capture two complementary aspects of T-cell activation. First, the limited-signaling assumption reflects the idea that each productive TCR–pMHC binding event generates only a finite signaling packet, which is consistent with serial triggering and the restricted signaling lifetime of individual engaged receptors. Second, the incoherent feedforward loop provides a mechanism by which activation can be fine-tuned through simultaneous enhancement and inhibition of the downstream response. The conversion from C_N to C_{N+1} can, in the model, penalize pMHC molecules with prolonged binding by reducing signaling efficacy and thereby adding an additional layer of quality control to antigen recognition, while the feedforward loop further shapes the final response.

Let L denote the concentration of free ligand, R the concentration of free receptor, $C_0, \dots, C_{N+1}$ the successive proofreading states, and Y and X the downstream variables of the incoherent feedforward loop. As in the previous model, the downstream equations are determined by the assumption that C_N has two competing effects on the final output.

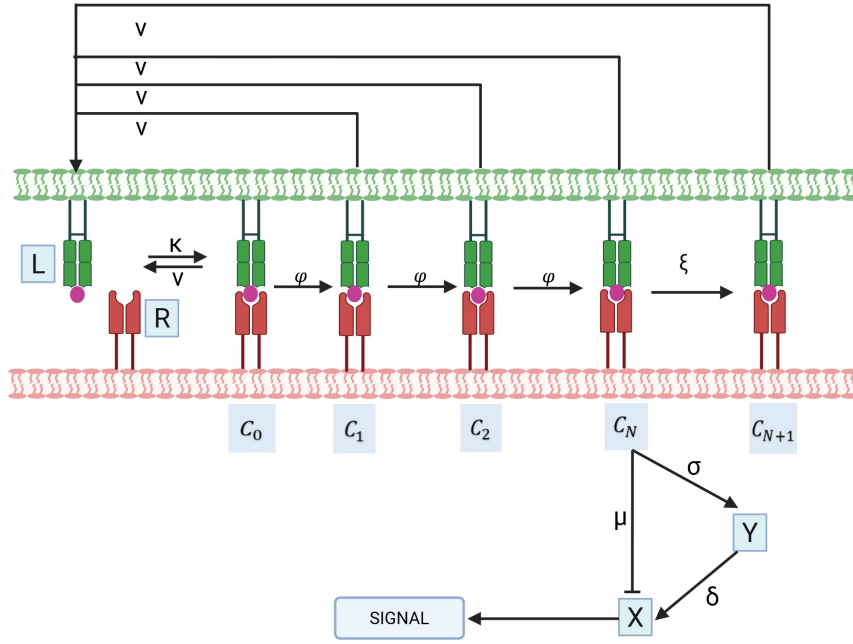


Figure 2.8. Kinetic proofreading with limited signalling and an incoherent feedforward loop. The model combines terminal signal limitation through C_{N+1} with feedforward regulation of the downstream output.

It activates Y through the saturating term $\sigma C_N(m - Y)$, and Y then activates X through $\delta Y(l - X)$. At the same time, C_N directly inhibits X through the loss term $-\mu C_N X$. The remaining terms, $a(m - Y) - bY$ and $c(l - X) - dX$, describe basal activation and loss of Y and X , respectively. The full reaction-style formulation is

$$\frac{dL}{dt} = -\kappa LR + v \sum_{i=0}^{N+1} C_i, \quad (2.71)$$

$$\frac{dR}{dt} = -\kappa LR + v \sum_{i=0}^{N+1} C_i, \quad (2.72)$$

$$\frac{dC_0}{dt} = \kappa LR - (\phi + v)C_0, \quad (2.73)$$

$$\frac{dC_i}{dt} = \phi C_{i-1} - (\phi + v)C_i, \quad 1 \leq i \leq N-1, \quad (2.74)$$

$$\frac{dC_N}{dt} = \phi C_{N-1} - (v + \xi)C_N, \quad (2.75)$$

$$\frac{dC_{N+1}}{dt} = \xi C_N - v C_{N+1}, \quad (2.76)$$

$$\frac{dY}{dt} = a(m - Y) - bY + \sigma C_N(m - Y), \quad (2.77)$$

$$\frac{dX}{dt} = c(l - X) - dX + \delta Y(l - X) - \mu C_N X. \quad (2.78)$$

Here, κ is the ligand–receptor binding rate, ϕ is the forward transition or phosphorylation

rate, v is the dissociation rate, and ξ is the rate at which the signaling-competent state becomes non-signaling. The parameters $a, b, c, d, \sigma, \delta, \mu, m$, and l govern the downstream feedforward motif. In this model, T-cell activation is identified with the output variable X , while C_N and C_{N+1} represent, respectively, the signaling and non-signaling terminal states of the proofreading cascade.

Using the conserved quantities

$$L_T = L + \sum_{i=0}^{N+1} C_i, \quad R_T = R + \sum_{i=0}^{N+1} C_i, \quad (2.79)$$

the system can be rewritten in reduced form as

$$\frac{dC_0}{dt} = \kappa \left(L_T - \sum_{i=0}^{N+1} C_i \right) \left(R_T - \sum_{i=0}^{N+1} C_i \right) - (\phi + v)C_0, \quad (2.80)$$

$$\frac{dC_i}{dt} = \phi C_{i-1} - (\phi + v)C_i, \quad 1 \leq i \leq N-1, \quad (2.81)$$

$$\frac{dC_N}{dt} = \phi C_{N-1} - (v + \xi)C_N, \quad (2.82)$$

$$\frac{dC_{N+1}}{dt} = \xi C_N - vC_{N+1}, \quad (2.83)$$

$$\frac{dY}{dt} = a(m - Y) - bY + \sigma C_N(m - Y), \quad (2.84)$$

$$\frac{dX}{dt} = c(l - X) - dX + \delta Y(l - X) - \mu C_N X. \quad (2.85)$$

The model therefore combines time-limited receptor signaling with downstream regulatory competition in a single phenotypic framework.

2.4.9 Kinetic Proofreading with Limited and Sustained Signaling

The kinetic proofreading model with limited and sustained signaling combines the two modifications introduced in the previous subsections. In this framework, each productive TCR-pMHC binding event generates only a finite signaling packet, represented by the transition from the signaling-competent state C_N to a non-signaling state C_{N+1} at rate ξ , while signaling may also persist transiently after ligand unbinding through an unbound but still active receptor state R^* , which returns to the basal state at rate Ω [78].

The biological motivation for this combined model is to capture two complementary features of T-cell activation within a single framework. On the one hand, the limited-signaling assumption reflects the idea that an individual receptor contributes only a restricted signaling event per productive engagement, consistent with serial triggering and the limited lifetime of signaling microclusters. On the other hand, the sustained-signaling assumption reflects the possibility that signaling can continue for a short period after

pMHC dissociation, thereby preserving activation even when ligand binding is transient [83, 82, 84, 78]. In this way, the model incorporates both temporal restriction and temporal persistence of signaling, providing a combined phenomenological description of receptor activation dynamics.

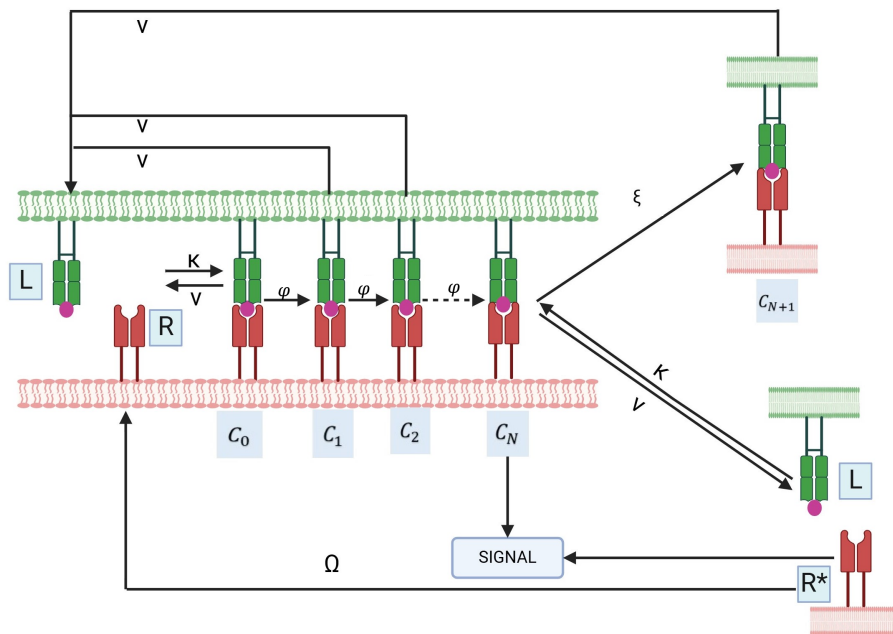


Figure 2.9. The signalling-competent state C_N can either enter the non-signalling state C_{N+1} or generate the transient active receptor state R^* .

Let L denote the concentration of free ligand, R the concentration of free receptor, $C_0, \dots, C_{N+1}$ the successive proofreading states, and R^* the unbound receptor state that remains transiently signaling after ligand dissociation. The full reaction-style formulation

is

$$\frac{dL}{dt} = -\kappa LR + v \sum_{i=0}^{N+1} C_i - \kappa LR^*, \quad (2.86)$$

$$\frac{dR}{dt} = -\kappa LR + v \sum_{i=0}^{N-1} C_i + v C_{N+1} + \Omega R^*, \quad (2.87)$$

$$\frac{dC_0}{dt} = \kappa LR - (\phi + v)C_0, \quad (2.88)$$

$$\frac{dC_i}{dt} = \phi C_{i-1} - (\phi + v)C_i, \quad 1 \leq i \leq N-1, \quad (2.89)$$

$$\frac{dC_N}{dt} = \phi C_{N-1} - (v + \xi)C_N + \kappa LR^*, \quad (2.90)$$

$$\frac{dC_{N+1}}{dt} = \xi C_N - v C_{N+1}, \quad (2.91)$$

$$\frac{dR^*}{dt} = v C_N - \kappa LR^* - \Omega R^*. \quad (2.92)$$

Here, κ is the ligand–receptor binding rate, ϕ is the forward transition or phosphorylation rate, v is the dissociation rate, ξ is the rate at which the signaling-competent state becomes non-signaling, and Ω is the rate at which the sustained-signaling state R^* returns to the basal receptor state. In this model, the activation output is determined by the combined contribution of the bound signaling-competent state C_N and the unbound but still active state R^* .

Using the conserved quantities

$$L_T = L + \sum_{i=0}^{N+1} C_i, \quad R_T = R + \sum_{i=0}^{N+1} C_i + R^*, \quad (2.93)$$

the system can be rewritten in reduced form as

$$\frac{dC_0}{dt} = \kappa \left(L_T - \sum_{i=0}^{N+1} C_i \right) \left(R_T - \sum_{i=0}^{N+1} C_i - R^* \right) - (\phi + v)C_0, \quad (2.94)$$

$$\frac{dC_i}{dt} = \phi C_{i-1} - (\phi + v)C_i, \quad 1 \leq i \leq N-1, \quad (2.95)$$

$$\frac{dC_N}{dt} = \phi C_{N-1} - (v + \xi)C_N + \kappa \left(L_T - \sum_{i=0}^{N+1} C_i \right) R^*, \quad (2.96)$$

$$\frac{dC_{N+1}}{dt} = \xi C_N - v C_{N+1}, \quad (2.97)$$

$$\frac{dR^*}{dt} = v C_N - \kappa \left(L_T - \sum_{i=0}^{N+1} C_i \right) R^* - \Omega R^*. \quad (2.98)$$

The model therefore combines limited receptor signaling and transient post-dissociation signaling within a single phenotypic framework.

2.5 Relationships Among the Model Structures

The phenotypic models introduced in this chapter are connected by several formal reductions, so that the model family forms a hierarchy rather than a disconnected collection of systems. In particular, some of the more elaborate models reduce to simpler ones under specific parameter choices [78].

Several of these relations are especially important:

- setting $\xi = 0$ in the model of Subsection 2.4.3 recovers the standard kinetic proofreading model of Subsection 2.4.2;
- in the modified form of the model in Subsection 2.4.3, the equation for C_{N+1} decouples and this variable tends to zero as $t \rightarrow \infty$, so that the dynamical properties of Subsection 2.4.2 may be recovered from those of Subsection 2.4.3;
- setting $\xi = 0$ and $\Omega = 0$ in the model of Subsection 2.4.9 reduces the system to the standard kinetic proofreading dynamics of Subsection 2.4.2;
- the model of Subsection 2.4.2 is a special case of the model in Subsection 2.4.6 when the stage-dependent parameters are chosen uniformly;
- the model of Subsection 2.4.7 is a limiting case of the model in Subsection 2.4.8, in the same way that the model of Subsection 2.4.2 is a limiting case of the model in Subsection 2.4.3.

These relations clarify how the different models are organized within a single framework of increasing biological and mathematical complexity. They will also be useful in the comparative analysis developed later in the thesis.

2.6 Response Quantities for Model Comparison

To compare the phenotypic models quantitatively, this thesis uses three main response measures: the response function, the maximum response $E_{\max}$, and the half-maximal effective concentration EC_{50} . These quantities are defined from the model-specific activation outputs and provide a common basis for comparing the different phenotypic models introduced in this chapter.

The response function describes how activation varies with ligand concentration and dissociation time, and therefore provides the primary basis for assessing specificity, sensitivity, antigen discrimination, and optimal response. The quantity $E_{\max}$ summarizes the maximal signaling output achievable under a given set of conditions, while EC_{50} measures the ligand concentration required to attain half-maximal activation and thus serves as an indicator of ligand potency.

In the next chapter, these quantities will be evaluated analytically and numerically using literature-based parameter values. Wherever possible, a common parameter set will be used across models, while additional model-specific parameters will be introduced for the relevant extensions.

2.7 Chapter Summary

In this chapter, the family of phenotypic models has been introduced within a common biological and mathematical framework. The biological benchmarks motivating the models have been described, the individual models have been presented in a unified form, and the formal relations among them have been outlined. The response quantities used for later comparison have also been introduced.

The next chapter is devoted to the mathematical and quantitative analysis of these models, including steady states, long-time behavior, response functions, and comparative evaluation using literature-based parameter values.

Mathematical Analysis and Comparative Model Results

The preceding chapter introduced the family of phenotypic models of T-cell activation and established the common mathematical framework, notation, and biological benchmarks that motivate our study. The present chapter is devoted to the mathematical and quantitative analysis of these models, and to a systematic comparison of their properties under literature-based parameter values.

The analysis proceeds in two complementary directions. The first is qualitative and concerns the long-time behavior of solutions. For each model, we investigate whether solutions converge to a unique steady state and, if so, whether that steady state is globally asymptotically stable within the biologically relevant region. These questions are addressed using tools from the theory of chemical reaction networks, including the Deficiency Zero Theorem, together with a general lemma on positivity of ω -limit points and a separate analysis for the negative-feedback model, which does not always possess a unique steady state [95, 65, 96, 66].

The second analytical direction is quantitative and concerns the response properties of the models. For each model, analytical expressions are derived for the steady-state response function, the maximal response $E_{\max}$, which quantifies efficacy, and the half-maximal effective concentration EC_{50} , which is commonly used as a measure of potency. These quantities are then evaluated numerically using literature-based parameter values drawn from multiple literature sources, allowing the models to be compared systematically with respect to specificity, sensitivity, antigen discrimination, and optimal response [20, 30, 97, 98]. The chapter concludes with a validation of previous findings for the negative-feedback model and a global sensitivity analysis based on LHS-PRCC in order to identify the parameters that most strongly influence model behavior [74].

3.1 Nature of Solutions

In this section we establish the basic well-posedness properties that apply across the model family: local existence and uniqueness of solutions, positivity and invariance of

the non-negative orthant, conserved quantities, boundedness of the biologically relevant region, and global existence. These properties provide the foundation for the steady-state and long-time analysis carried out in Section 3.2.

3.1.1 General ODE Framework

All models introduced in Chapter 2 are systems of ordinary differential equations arising from biochemical reaction networks under mass-action kinetics. In each case the governing equations take the form

$$\frac{dx_i}{dt} = f_i(x), \quad 1 \leq i \leq n, \quad (3.1)$$

where the state variables x_i represent concentrations of the chemical species involved, $x = (x_1, \dots, x_n)$, and the functions f_i are polynomials in the components of x arising from the mass-action kinetics assumption [65, 66]. Since polynomials are locally Lipschitz continuous, the standard theory of ordinary differential equations guarantees, for any initial time t_0 and any initial data $x(t_0) = x^0 = (x_1^0, \dots, x_n^0)$, a unique local solution $x(t)$ defined on some maximal interval $[t_0, t_1)$ [99]. Thus each model is locally well posed.

The question of whether solutions exist globally on $[t_0, \infty)$ will be resolved after positivity and boundedness have been established.

3.1.2 Positivity and Invariance of the Non-negative Orthant

Since all state variables represent concentrations of chemical species, only non-negative initial data are biologically meaningful. We therefore restrict attention to solutions satisfying $x_i(t_0) \geq 0$ for all i .

For systems arising from reaction networks under mass-action kinetics, the non-negative orthant

$$\mathbb{R}_{\geq 0}^n = \{x \in \mathbb{R}^n : x_i \geq 0 \text{ for all } i\} \quad (3.2)$$

is positively invariant. That is, if $x_i(t_0) \geq 0$ for all i , then $x_i(t) \geq 0$ for all $t \geq t_0$ whenever the solution exists [100]. The statement for strictly positive initial data follows from the structure of mass-action terms, while the more general statement allowing some initial concentrations to vanish follows by continuity of solutions with respect to initial data. Hence the non-negative orthant is invariant under the dynamics of every model in the family.

3.1.3 Conserved Quantities and Reduced Systems

Each core receptor–ligand model in the family possesses the conserved quantities given by the total ligand concentration L_T and the total receptor concentration R_T . In the simpler

proofreading models these conservation laws take the form

$$L_T = L + \sum_i C_i, \quad R_T = R + \sum_i C_i,$$

with the obvious modifications in models that also involve the sustained-signaling state R^* or the non-signaling transit state C_{N+1} .

Fixing positive values $\bar{L}_T$ and $\bar{R}_T$ for the conserved quantities allows the free ligand L and free receptor R to be eliminated from the system. This yields a reduced system for the remaining variables, defined on the invariant region

$$K = \{(C_0, \dots) \in \mathbb{R}_{\geq 0}^{n-2} : L \geq 0, R \geq 0\}, \quad (3.3)$$

where L and R are recovered from the conservation laws. The interior of this region will be referred to as the *biologically relevant region*.

For the proofreading-type core variables, each variable appears with a non-zero coefficient in at least one of the conserved quantities, so the corresponding reduced region K is compact. It follows that solutions of these reduced systems remain bounded and therefore exist globally on $[t_0, \infty)$ [99]. In the IFF-based extensions, the additional variables are handled separately in the model-specific analysis, using the triangular structure of the equations rather than relying solely on the conservation laws. Consequently, the original unreduced systems also admit global solutions.

3.1.4 A General Lemma for ω -Limit Points

A key step in establishing convergence to steady states in the interior of K is to show that ω -limit points cannot lie on the boundary of the state space. The following lemma provides a general criterion for this.

Lemma 3.1. *Let*

$$\frac{dx_i}{dt} = f_i(x), \quad 1 \leq i \leq n,$$

be a system of ordinary differential equations arising from a reaction network under mass-action kinetics. Then f_i can be written in the form

$$f_i(x) = -x_i g_i(x) + h_i(x), \quad (3.4)$$

where g_i and h_i are polynomials and $h_i(x) \geq 0$ on the non-negative orthant. Let $x(t)$ be a solution in the non-negative orthant, and suppose that for any ω -limit point x^ the condition $x_i^* = 0$ implies $h_i(x^*) > 0$. Then every ω -limit point of the solution lies in the positive orthant.*

Proof. Suppose for contradiction that some ω -limit point x^* has a zero component, say $x_j^* = 0$. Let $y(t)$ be the solution satisfying $y_i(t_0) = x_i^*$ for all i . Since ω -limit sets are invariant under the flow, the trajectory $y(t)$ through x^* lies in the ω -limit set of $x(t)$ for all times for which it is defined. In particular, $y(t)$ remains in the non-negative orthant, so $y_i(t) \geq 0$ for all t and all i . By hypothesis,

$$\frac{dy_j}{dt}(t_0) = h_j(x^*) > 0.$$

It follows that $y_j(t) < 0$ for $t < t_0$ sufficiently close to t_0 , contradicting the non-negativity of $y(t)$. Hence no ω -limit point can have a zero component, and every ω -limit point lies in the positive orthant. $\square$

Remark 3.2. There is a natural correspondence between points on the boundary of the reduced region K and points on the boundary of the non-negative orthant in the original variables. It follows that if Lemma 3.1 applies to the original unreduced system, then every ω -limit point of a solution of the reduced system starting in K lies in the interior of K . This observation will be used repeatedly in Section 3.2.

3.1.5 Analytical Observations on Monotonicity

Several basic monotonicity properties of the response functions can be established analytically from the equilibrium structure alone, before any numerical investigation. The arguments below follow Supplementary Section 1.8.

Occupancy model: monotonicity in dissociation time. The equilibrium complex concentration C satisfies the conservation and equilibrium conditions, which combine to give the quadratic

$$C^2 - (L_T + R_T + K_D)C + L_TR_T = 0, \tag{3.5}$$

whose biologically relevant root is the smaller one:

$$C = \frac{(L_T + R_T + K_D) - \sqrt{(L_T + R_T + K_D)^2 - 4L_TR_T}}{2}.$$

Set $a = L_T + R_T + K_D > 0$ and $b = L_TR_T > 0$, so that $C = \frac{1}{2}(a - \sqrt{a^2 - 4b})$. Differentiating with respect to a gives

$$\frac{\partial C}{\partial a} = \frac{1}{2} \left(1 - \frac{a}{\sqrt{a^2 - 4b}} \right).$$

Since $a^2 > a^2 - 4b > 0$, we have $\sqrt{a^2 - 4b} < a$, and hence $\partial C / \partial a < 0$. Combined with $da/dK_D = 1$, this gives

$$\frac{dC}{dK_D} < 0.$$

Since $K_D = v/\kappa$ and the dissociation time is $\tau = 1/v$, increasing τ decreases v and hence decreases K_D . Therefore C is a strictly increasing function of τ : longer receptor–ligand dwell times increase T-cell activation in the occupancy model.

Occupancy model: monotonicity in ligand concentration. Since a and b both depend on L_T , with $da/dL_T = 1$ and $db/dL_T = R_T$, the chain rule gives

$$\frac{dC}{dL_T} = \frac{\partial C}{\partial a} \frac{da}{dL_T} + \frac{\partial C}{\partial b} \frac{db}{dL_T}.$$

Differentiating with respect to b gives

$$\frac{\partial C}{\partial b} = \frac{1}{\sqrt{a^2 - 4b}} > 0.$$

Hence

$$\frac{dC}{dL_T} = \frac{1}{2} \left(1 - \frac{a}{\sqrt{a^2 - 4b}} \right) + \frac{R_T}{\sqrt{a^2 - 4b}} = \frac{\sqrt{a^2 - 4b} - a + 2R_T}{2\sqrt{a^2 - 4b}}.$$

To verify that the numerator is positive, note that $a - 2R_T = L_T + K_D > 0$, so it is enough to show $\sqrt{a^2 - 4b} > a - 2R_T$. Squaring both sides gives

$$a^2 - 4b > (a - 2R_T)^2,$$

which reduces to $4R_T K_D > 0$, which holds since $R_T > 0$ and $K_D > 0$. Therefore $dC/dL_T > 0$, so the occupancy response is a strictly increasing function of the ligand concentration L_T .

KPR model. In the kinetic proofreading model the response is $a^N C_T$, where

$$a = \frac{\phi}{\phi + v}.$$

Since a is independent of L_T , and C_T satisfies the same quadratic equation as in the occupancy model, the response is a strictly increasing function of L_T . With respect to dissociation time $\tau = 1/v$, both factors contribute positively: as τ increases, v decreases, so a increases, and C_T also increases by the same argument as in the occupancy model. Hence the KPR response $a^N C_T$ is an increasing function of τ .

Models with non-monotone responses. In contrast to the occupancy and standard KPR models, several extended models exhibit non-monotone behaviour. The KPR model with limited signaling possesses an optimal dissociation time arising from the competition between proofreading gain and the signaling-loss factor as τ increases. The KPR model with sustained signaling exhibits an optimal response with respect to ligand concentration.

The incoherent-feedforward-loop models can exhibit optimal responses with respect to both L_T and τ under certain parameter conditions, and the model combining limited signaling with an IFF can produce a bimodal response as a function of τ . These non-monotone features are identified and discussed in Section 3.5.

3.2 Steady States and Long-Time Behavior

This section investigates whether the models admit unique positive steady states and whether all solutions converge to them as $t \rightarrow \infty$. Different arguments are required for different models: a direct one-dimensional argument for the occupancy model, the Deficiency Zero Theorem for several proofreading variants, cascade arguments for models with downstream regulation, and a detailed polynomial analysis for the negative-feedback model, which does not always possess a unique steady state [101, 95]. The general framework and the key lemma used throughout are those established in Section 3.1.

Before proceeding to the individual models, we recall the principal terminology from chemical reaction network theory used in the analysis below [65, 96, 66]. A chemical reaction network consists of a finite set of species, a finite set of complexes, and a finite set of reactions between complexes. In the present setting, the species are the variables of the model, such as L , R , and the receptor–ligand complexes C_i . A complex is a formal linear combination of species with non-negative integer coefficients. Thus, if the species are denoted by $X_1, \dots, X_m$, a complex has the form

$$y = \sum_{i=1}^m y_i X_i, \quad y_i \in \mathbb{Z}_{\geq 0}. \quad (3.6)$$

For example, $L + R$, C_0 , and C_N are complexes in the receptor–ligand models considered here.

A reaction is an ordered pair of complexes, written

$$y \longrightarrow y'. \quad (3.7)$$

The reaction graph is the directed graph whose vertices are the complexes and whose directed edges are the reactions. The reaction vector associated with the reaction $y \rightarrow y'$ is

$$y' - y. \quad (3.8)$$

After choosing an ordering of the species and reactions, the stoichiometric matrix is the matrix whose columns are the reaction vectors:

$$\Gamma = \begin{bmatrix} y'_1 - y_1 & y'_2 - y_2 & \cdots & y'_r - y_r \end{bmatrix}. \quad (3.9)$$

Here r is the number of reactions. The stoichiometric subspace is the linear span of the reaction vectors,

$$\mathcal{S} = \text{span} \{y'_j - y_j : j = 1, \dots, r\}, \quad (3.10)$$

and its dimension is the rank s of the stoichiometric matrix.

For a given initial condition x_0 , the stoichiometric compatibility class is defined as

$$\mathcal{P}(x_0) = (x_0 + \mathcal{S}) \cap \mathbb{R}_{\geq 0}^m. \quad (3.11)$$

Thus, a stoichiometric compatibility class consists of all non-negative states that can be reached from x_0 without changing the conserved total quantities.

A linkage class is a connected component of the reaction graph when the directions of the reaction arrows are ignored. A reaction network is called weakly reversible if, whenever there is a reaction $y \rightarrow y'$, there exists a directed path in the reaction graph leading from y' back to y :

$$y' \longrightarrow y_1 \longrightarrow y_2 \longrightarrow \dots \longrightarrow y. \quad (3.12)$$

In other words, every reaction lies in a strongly connected component of the reaction graph.

The deficiency of a chemical reaction network is defined by

$$\delta = n_c - \ell - s, \quad (3.13)$$

where n_c is the number of complexes, ℓ is the number of linkage classes, and $s = \text{rank}(\Gamma)$ is the rank of the stoichiometric matrix. The deficiency always satisfies

$$\delta \geq 0. \quad (3.14)$$

Theorem 3.3 (Deficiency Zero Theorem [65, 96, 66]). *If a chemical reaction network has deficiency zero and is weakly reversible, then the corresponding system of ordinary differential equations with mass-action kinetics has a unique positive steady state in each stoichiometric compatibility class, and this steady state is asymptotically stable within its class. If in addition no solution has an ω -limit point on the boundary of the relevant region, then the steady state is globally asymptotically stable within its class.*

In all models considered here, the stoichiometric compatibility classes are the sets defined by fixed values of the conserved quantities L_T and R_T . The combination of Lemma 3.1 and Theorem 3.3 will be used repeatedly below to establish global asymptotic stability [101, 65].

3.2.1 Occupancy Model

The occupancy model reduces, after elimination of L and R via the conservation laws $L_T = L + C$ and $R_T = R + C$, to the single scalar equation

$$\frac{dC}{dt} = \kappa(L_T - C)(R_T - C) - vC \quad (3.15)$$

on the compact invariant interval $[0, \min(L_T, R_T)]$. The right-hand side is strictly positive at $C = 0$ and strictly negative at $C = \min(L_T, R_T)$, so by continuity there exists at least one steady state in the interior. The derivative of the right-hand side with respect to C is

$$-\kappa(R_T - C) - \kappa(L_T - C) - v < 0$$

throughout the relevant interval, so the steady state is unique. Since the system is one-dimensional and the vector field has a definite sign on each side of the unique steady state, every solution converges to this steady state as $t \rightarrow \infty$ [101].

3.2.2 Kinetic Proofreading Model

For the kinetic proofreading model, convergence to a unique globally asymptotically stable steady state is established by applying Lemma 3.1 to verify that all ω -limit points lie in the interior of the biologically relevant region, and then invoking the Deficiency Zero Theorem.

Let $\Sigma_1 = \sum_{i=0}^N C_i$. From the reduced system it follows that

$$\frac{d\Sigma_1}{dt} = \kappa(L_T - \Sigma_1)(R_T - \Sigma_1) - v\Sigma_1. \quad (3.16)$$

Since (3.16) is a closed scalar autonomous equation, any ω -limit point Σ_1^* of Σ_1 satisfies

$$\kappa(L_T - \Sigma_1^*)(R_T - \Sigma_1^*) = v\Sigma_1^*.$$

Since $v > 0$, this implies $\Sigma_1^* < L_T$ and $\Sigma_1^* < R_T$. If $C_0^* = 0$ at an ω -limit point of the full system, then the right-hand side of the C_0 -equation is strictly positive there, so Lemma 3.1 implies $C_0^* > 0$. The equations for $C_1, \dots, C_N$ then imply inductively that $C_i^* > 0$ for all $1 \leq i \leq N$. Thus all ω -limit points lie in the interior of the biologically relevant region.

Deficiency calculation. The network complexes are $\{L + R, C_0, C_1, \dots, C_N\}$, giving $n = N + 2$. There is a single linkage class, $l = 1$. The stoichiometric matrix is

$$\begin{bmatrix} a_1 & a_2 & \dots & a_{N+1} & b_1 & b_2 & \dots & b_N & c_1 \\ 1 & 1 & \dots & 1 & 0 & 0 & \dots & 0 & -1 \\ 1 & 1 & \dots & 1 & 0 & 0 & \dots & 0 & -1 \\ -1 & 0 & \dots & 0 & -1 & 0 & \dots & 0 & 1 \\ 0 & -1 & \dots & 0 & 1 & -1 & \dots & 0 & 0 \\ \vdots & \vdots & \ddots & \vdots & \vdots & \vdots & \ddots & \vdots & \vdots \\ 0 & 0 & \dots & -1 & 0 & 0 & \dots & 1 & 0 \end{bmatrix}.$$

The first $N + 1$ columns of this matrix are linearly independent, so $s \geq N + 1$. Hence

$$\delta \leq (N + 2) - 1 - (N + 1) = 0.$$

Since $\delta \geq 0$ always holds, we conclude $\delta = 0$. The network is weakly reversible, so Theorem 3.3 together with the interior ω -limit point property implies that the unique positive steady state in each stoichiometric compatibility class is globally asymptotically stable [65].

3.2.3 KPR with Limited Signaling

This model extends the KPR scheme by allowing the signaling-competent state C_N to transit to a non-signaling state C_{N+1} at rate ξ . Let $\Sigma_1 = \sum_{i=0}^{N+1} C_i$. From the reduced system it follows that

$$\frac{d\Sigma_1}{dt} = \kappa(L_T - \Sigma_1)(R_T - \Sigma_1) - v\Sigma_1. \quad (3.17)$$

Since (3.17) is a closed scalar autonomous equation, any ω -limit point Σ_1^* of Σ_1 satisfies

$$\kappa(L_T - \Sigma_1^*)(R_T - \Sigma_1^*) = v\Sigma_1^*.$$

Hence $\Sigma_1^* < L_T$ and $\Sigma_1^* < R_T$. If $C_0^* = 0$ at an ω -limit point of the full system, then the right-hand side of the C_0 -equation is strictly positive there, so Lemma 3.1 implies $C_0^* > 0$. The equations for $C_1, \dots, C_{N+1}$ then imply inductively that $C_i^* > 0$ for all $1 \leq i \leq N + 1$. Thus all ω -limit points lie in the interior of the biologically relevant region.

Deficiency calculation. The network complexes are $\{L + R, C_0, C_1, \dots, C_N, C_{N+1}\}$, giving $n = N + 3$. There is a single linkage class, $l = 1$. The stoichiometric matrix is

$$\begin{bmatrix} a_1 & a_2 & \dots & a_{N+2} & b_1 & b_2 & \dots & b_{N+1} & c_1 \\ 1 & 1 & \dots & 1 & 0 & 0 & \dots & 0 & -1 \\ 1 & 1 & \dots & 1 & 0 & 0 & \dots & 0 & -1 \\ -1 & 0 & \dots & 0 & -1 & 0 & \dots & 0 & 1 \\ 0 & -1 & \dots & 0 & 1 & -1 & \dots & 0 & 0 \\ \vdots & \vdots & \ddots & \vdots & \vdots & \vdots & \ddots & \vdots & \vdots \\ 0 & 0 & \dots & -1 & 0 & 0 & \dots & 1 & 0 \end{bmatrix}.$$

The first $N + 2$ columns of this matrix are linearly independent, so $s \geq N + 2$. Hence

$$\delta \leq (N + 3) - 1 - (N + 2) = 0.$$

Since $\delta \geq 0$ always holds, we conclude $\delta = 0$. Since the network is weakly reversible and has deficiency zero, and all ω -limit points lie in the interior of the biologically relevant region, Theorem 3.3 implies that the unique positive steady state in each stoichiometric compatibility class is globally asymptotically stable [65].

3.2.4 KPR with Sustained Signaling

In this model the conservation law for R_T includes the sustained-signaling state R^* :

$$R_T = R + \Sigma_1 + R^*, \quad \Sigma_1 = \sum_{i=0}^N C_i.$$

Summing the equations for $C_0, \dots, C_N$ and using the equation for R^* gives

$$\begin{aligned} \frac{d\Sigma_1}{dt} &= \kappa(L_T - \Sigma_1)(R_T - \Sigma_1 - R^*) - v\Sigma_1 + \kappa(L_T - \Sigma_1)R^* \\ &= \kappa(L_T - \Sigma_1)(R_T - \Sigma_1) - v\Sigma_1, \end{aligned} \tag{3.18}$$

which has the same form as (3.16). Since (3.18) is a closed scalar autonomous equation, any ω -limit point Σ_1^* of Σ_1 satisfies

$$\kappa(L_T - \Sigma_1^*)(R_T - \Sigma_1^*) = v\Sigma_1^*,$$

and hence $\Sigma_1^* < L_T$.

Suppose now that $\Sigma_1^* + R^{**} = R_T$. Then from the steady-state equation for C_0 it follows that $C_0^* = 0$. Using the equations for $C_1, \dots, C_{N-1}$ inductively gives $C_i^* = 0$ for

all $1 \leq i \leq N - 1$. Adding the steady-state equations for C_N and R^* then yields

$$0 = -\Omega R^{**}. \quad (3.19)$$

Since $\Omega > 0$, it follows that $R^{**} = 0$. Substituting back into the steady-state equation for C_N gives $C_N^* = 0$. Hence $\Sigma_1^* + R^{**} = 0$, contradicting $R_T > 0$. Therefore $\Sigma_1^* + R^{**} < R_T$. From this it follows that $C_0^* > 0$, inductively $C_i^* > 0$ for all $1 \leq i \leq N$, and $R^{**} > 0$.

Deficiency calculation. The network complexes are $\{L + R, C_0, C_1, \dots, C_N, L + R^*\}$, giving $n = N + 3$. There is a single linkage class, $l = 1$. The stoichiometric matrix is

$$\begin{bmatrix} a_1 & a_2 & \dots & a_N & a_{N+1} & b_1 & b_2 & \dots & b_N & c_1 & c_2 & c_3 \\ 1 & 1 & \dots & 1 & 1 & 0 & 0 & \dots & 0 & -1 & -1 & 0 \\ 1 & 1 & \dots & 1 & 0 & 0 & 0 & \dots & 0 & -1 & 0 & 1 \\ -1 & 0 & \dots & 0 & 0 & -1 & 0 & \dots & 0 & 1 & 0 & 0 \\ 0 & -1 & \dots & 0 & 0 & 1 & -1 & \dots & 0 & 0 & 0 & 0 \\ \vdots & \vdots & \ddots & \vdots & \vdots & \vdots & \vdots & \ddots & \vdots & \vdots & \vdots & \vdots \\ 0 & 0 & \dots & -1 & 0 & 0 & 0 & \dots & -1 & 0 & 0 & 0 \\ 0 & 0 & \dots & 0 & -1 & 0 & 0 & \dots & 1 & 0 & 1 & 0 \\ 0 & 0 & \dots & 0 & 1 & 0 & 0 & \dots & 0 & 0 & -1 & -1 \end{bmatrix}.$$

The first $N + 2$ columns of this matrix are linearly independent, so $s \geq N + 2$. Hence

$$\delta \leq (N + 3) - 1 - (N + 2) = 0.$$

Since $\delta \geq 0$ always holds, we conclude $\delta = 0$. Since the network is weakly reversible, has deficiency zero, and all ω -limit points lie in the interior of the biologically relevant region, Theorem 3.3 implies global asymptotic stability of the unique positive steady state [65].

3.2.5 KPR with Negative Feedback

The kinetic proofreading model with negative feedback occupies a special position within the model family because it does not always possess a unique steady state. A rigorous and complete analysis was carried out by Rendall and Sontag [95], whose main results are presented here. Their analysis considers the *agonist-only* case, meaning that only one activating ligand class is included explicitly. Thus, all receptor–ligand complexes $C_0, \dots, C_N$ arise from interactions between TCRs and agonist pMHC ligands, while competing ligand classes such as self ligands, nonagonists, weak agonists, or antagonists are not included in this reduced formulation. To remain consistent with the notation in [95], the dissociation rate is denoted by ν in this subsection.

Equations and positivity of ω -limit points. With this convention, the state variables are the concentration S of active SHP-1 and the concentrations C_j ($0 \leq j \leq N$) of the j -fold phosphorylated receptor–ligand complexes. Setting $\Sigma_1 = \sum_{j=0}^N C_j$, the governing equations are

$$\dot{S} = \alpha C_1(S_T - S) - \beta S, \quad (3.20)$$

$$\dot{C}_0 = \kappa(L_T - \Sigma_1)(R_T - \Sigma_1) + (b + \gamma S)C_1 - (\phi + \nu)C_0, \quad (3.21)$$

$$\dot{C}_j = \phi C_{j-1} + (b + \gamma S)C_{j+1} - (\phi + b + \gamma S + \nu)C_j, \quad 1 \leq j \leq N-1, \quad (3.22)$$

$$\dot{C}_N = \phi C_{N-1} - (b + \gamma S + \nu)C_N. \quad (3.23)$$

Summing equations (3.21)–(3.23) yields

$$\dot{\Sigma}_1 = \kappa(L_T - \Sigma_1)(R_T - \Sigma_1) - \nu \Sigma_1, \quad (3.24)$$

which has the same form as (3.16). Hence any ω -limit point satisfies $\Sigma_1^* < L_T$ and $\Sigma_1^* < R_T$. If $C_0^* = 0$ at an ω -limit point, then the right-hand side of (3.21) is strictly positive there, so Lemma 3.1 gives $C_0^* > 0$; then (3.22)–(3.23) imply inductively that $C_j^* > 0$ for all $1 \leq j \leq N$, and finally (3.20) with $C_1^* > 0$ gives $S^* > 0$. All ω -limit points lie in the interior of the biologically relevant region [95].

Why the Deficiency Zero Theorem is not used here. Unlike the previous proof-reading variants, the negative-feedback model is not analysed by the deficiency-zero argument. Instead, following [95], one reduces the steady-state problem to a polynomial equation for the active phosphatase variable S , which allows the multiplicity of positive steady states to be studied directly.

Reduction to a polynomial equation. At steady state, equation (3.20) gives the relation

$$S = S_T \frac{C_1}{C_1 + C^*}, \quad C^* = \frac{\beta}{\alpha}. \quad (3.25)$$

The steady-state form of equations (3.22) is a linear second-order recurrence in j . Its characteristic equation is

$$\phi + (b + \gamma S)r^2 - (\phi + b + \gamma S + \nu)r = 0, \quad (3.26)$$

whose two roots are

$$r_{\pm} = \frac{(\phi + b + \gamma S + \nu) \pm \sqrt{(\phi + b + \gamma S + \nu)^2 - 4\phi(b + \gamma S)}}{2(b + \gamma S)}. \quad (3.27)$$

These roots satisfy $r_- < 1 < r_+$, as can be verified by evaluating the left-hand side of (3.26) at $r = 1$ [95]. Steady-state solutions of (3.21)–(3.23) therefore admit the parametrisation

$$C_j = a_+ r_+^j + a_- r_-^j, \quad a_{\pm} > 0. \quad (3.28)$$

Summing (3.28) over $j = 0, \dots, N$ and using the boundary conditions from (3.21) and (3.23) one obtains

$$C_N = \frac{1 - r_-/r_+}{1 - (r_-/r_+)^{N+1}} r_-^N C_T, \quad (3.29)$$

which is the expression used in the response-function analysis of Section 3.3. Substituting (3.28) into (3.25) and using the conservation law then yields a polynomial equation of degree $N + 1$ in S [95].

Uniqueness of steady states for $N = 1$ and $N = 2$. For $N = 1$ the polynomial for S is the quadratic

$$\beta\gamma S^2 + [\beta(\phi + b + \nu) + \alpha\phi\Sigma^*]S - \alpha\phi\Sigma^*S_T = 0.$$

The leading coefficient is positive and the constant term is negative, so by Descartes' rule of signs the polynomial has exactly one positive root, and the steady state is unique [102, 95].

For $N = 2$ the polynomial for S is cubic with coefficients

$$\begin{aligned} a_0 &= -\alpha S_T(b + \nu)\phi\Sigma^* < 0, \\ a_1 &= \beta[b(\phi + b + \nu) + \nu(2\phi + b + \nu) + \phi^2] + \alpha(b + \nu)\phi\Sigma^* - \alpha\gamma S_T\phi\Sigma^*, \\ a_2 &= \beta\gamma(\phi + 2b + 2\nu) + \alpha\gamma\phi\Sigma^*, \\ a_3 &= \beta\gamma^2. \end{aligned}$$

The sequence of signs of the coefficients is either $(-, -, +, +)$ or $(-, +, +, +)$, giving precisely one sign change. By Descartes' rule the polynomial has exactly one positive root, so the steady state is again unique [95].

For $N = 1$ the vector field defining the dynamical system has negative divergence, so by Dulac's criterion and Poincaré–Bendixson theory every solution converges to the unique steady state as $t \rightarrow \infty$ [103, 95]. The system can exhibit damped oscillations: parameter values exist satisfying $\alpha C_1 + \beta = \phi + b + \gamma S + \nu$, at which the linearisation has eigenvalues with negative real part and non-zero imaginary part [95].

Multiple steady states for $N = 3$. For $N = 3$ the polynomial for S is of degree four with coefficients

$$\begin{aligned} a_0 &= -[(b + \nu)^2 + \phi\nu]\alpha\phi\Sigma^*S_T < 0, \\ a_3 &= \beta\{2\gamma(b + \nu) + \gamma^2(\phi + b + \nu)\} + \gamma^2\alpha\phi\Sigma^* > 0, \\ a_4 &= \beta\gamma^3 > 0. \end{aligned}$$

Unless $a_1 > 0$ and $a_2 < 0$, Descartes' rule implies only one positive root. To show that three positive roots can occur, let S_T and ν depend on a small parameter $\varepsilon > 0$ with

$$S_T = \bar{S}_T \varepsilon^{-1} + o(\varepsilon^{-1}), \quad \nu = \bar{\nu} \varepsilon^4 + o(\varepsilon^4),$$

while keeping all other parameters fixed and adjusting κ so that ν/κ (and hence Σ^*) remains constant. This gives asymptotic expansions

$$\begin{aligned} a_4 &= A_4, \quad a_3 = A_3 + o(1), \quad a_2 = A_2 \varepsilon^{-1} + o(\varepsilon^{-1}), \quad A_2 < 0, \\ a_1 &= A_1 + o(1), \quad A_1 > 0, \quad a_0 = A_0 \varepsilon^3 + o(\varepsilon^3), \quad A_0 < 0. \end{aligned}$$

Evaluating $p(1) \approx A_2 \varepsilon^{-1} < 0$ and $p(\varepsilon^2) \approx A_1 \varepsilon^2 > 0$ for small ε shows that the polynomial changes sign at least twice on $(0, \infty)$, confirming three positive roots [95]. These results are summarised as follows.

Theorem 3.4 (Rendall–Sontag [95]). *The agonist-only kinetic proofreading model with negative feedback has exactly one positive steady state for $N = 1$ and $N = 2$. For $N = 3$ there exist parameter values for which it has exactly three positive steady states, and the number of positive steady states cannot exceed three for $N = 3$.*

Concrete example. Setting $\alpha = \beta = \gamma = \phi = L_T = R_T = 1$, $S_T = 10$, $\kappa = 2 \times 10^{-4}$, $\nu = 10^{-4}$, and $N = 3$, the three positive steady states have approximate coordinates [95]:

$$\begin{aligned} (S^*, C_0^*, C_1^*, C_2^*, C_3^*) &\approx (1.1769, 0.1570, 0.1334, 0.1133, 0.0963), \\ &\approx (0.0005, 0.0001, 0.0001, 0.0003, 0.4996), \\ &\approx (0.2860, 0.0085, 0.0294, 0.1028, 0.3593). \end{aligned}$$

Numerical simulations for this parameter set reveal the following features. The first and second steady states appear to be asymptotically stable, while the third behaves as a saddle with a one-dimensional unstable manifold. A plot of the steady states as a function of L_T further indicates a fold bifurcation structure [95].

Global stability for small α . For $\alpha = 0$ and $S = 0$ the system reduces to the standard kinetic proofreading model, which has a unique globally asymptotically stable steady state (C_j^*) with associated deficiency-zero Lyapunov function h satisfying $\dot{h} \leq 0$ with equality only at the steady state. For small $\alpha > 0$ a perturbation argument shows that a unique positive hyperbolic steady state $(S^*(\alpha), C_j^*(\alpha))$ exists close to $(0, C_j^*)$. On the complement of a small ball about this steady state $\dot{h}$ remains strictly negative, while within the ball the only ω -limit point is the steady state itself. Hence for α sufficiently small the unique positive steady state is globally asymptotically stable [95].

3.2.6 KPR with Stabilizing Activation Chain

The argument parallels that for the standard KPR model, with the stage-dependent rates $v(i)$ and $\phi(i)$ replacing uniform rates. In this model the binding reaction follows $L + R = C_0$, where the bound receptor complex C_0 undergoes a sequence of phosphorylation steps to reach the signaling-competent state C_N . The stabilization and destabilization of the intermediate complexes C_i , as well as differences in activation timing, are encoded through the rate constants

$$v(i) = \frac{1+i}{1+r_{\text{off}}^i} v, \quad r_{\text{off}} > 1, \quad \phi(i) = \phi r_p^i, \quad r_p > 1,$$

for $i = 0, 1, \dots, N$. T-cell activation is determined by C_N . Each intermediate state C_i can decay, releasing L , R , and phosphate groups, making the network weakly reversible.

Let $\Sigma_1 = \sum_{i=0}^N C_i$. Summing the model equations gives

$$\frac{d\Sigma_1}{dt} = \kappa(L_T - \Sigma_1)(R_T - \Sigma_1) - \sum_{i=0}^N v(i) C_i. \quad (3.30)$$

If $\Sigma_1^* = L_T$ or $\Sigma_1^* = R_T$ at an ω -limit point, then the right-hand side of (3.30) would be strictly negative, since each $v(i) > 0$. Hence $\Sigma_1^* < L_T$ and $\Sigma_1^* < R_T$. If $C_0^* = 0$, then the right-hand side of the C_0 -equation is strictly positive at the ω -limit point, so Lemma 3.1 implies $C_0^* > 0$. The equations for $C_1, \dots, C_N$ then imply inductively that $C_i^* > 0$ for all $1 \leq i \leq N$. Hence all ω -limit points lie in the interior of the biologically relevant region.

Deficiency calculation. The network comprises $N+2$ complexes $\{L+R, C_0, C_1, C_2, \dots, C_N\}$, so $n = N + 2$. There is a single linkage class, $l = 1$. The stoichiometric matrix is

$$\begin{bmatrix} a_1 & a_2 & \cdots & a_{N+1} & b_1 & b_2 & \cdots & b_N & c_1 \\ 1 & 1 & \cdots & 1 & 0 & 0 & \cdots & 0 & -1 \\ 1 & 1 & \cdots & 1 & 0 & 0 & \cdots & 0 & -1 \\ -1 & 0 & \cdots & 0 & -1 & 0 & \cdots & 0 & 1 \\ 0 & -1 & \cdots & 0 & 1 & -1 & \cdots & 0 & 0 \\ \vdots & \vdots & \ddots & \vdots & \vdots & \vdots & \ddots & \vdots & \vdots \\ 0 & 0 & \cdots & -1 & 0 & 0 & \cdots & 1 & 0 \end{bmatrix}.$$

The first $N + 1$ columns of this matrix are linearly independent, so $s \geq N + 1$. Hence

$$\delta \leq (N + 2) - 1 - (N + 1) = 0.$$

Since $\delta \geq 0$ always holds, we conclude $\delta = 0$. The network is weakly reversible and has deficiency zero, and all ω -limit points lie in the interior of the biologically relevant region. Theorem 3.3 therefore implies that the unique positive steady state in each stoichiometric compatibility class is globally asymptotically stable [65].

3.2.7 KPR with Incoherent Feedforward Loop

Global asymptotic stability of the unique positive steady state is established using a cascade argument. The system splits naturally into two subsystems. The first, $Z_1 = (C_0, \dots, C_N)$, satisfies the same closed system as the standard KPR model:

$$\frac{dZ_1}{dt} = f(Z_1).$$

The second, $Z_2 = (Y, X)$, is governed by

$$\frac{dZ_2}{dt} = g(Z_1, Z_2).$$

By Section 3.2.2, the subsystem for Z_1 has a unique positive steady state $C_i^* > 0$ for each i , to which all solutions converge as $t \rightarrow \infty$.

Consider any ω -limit point of a solution (C_i, Y, X) of the full system. The solution starting at that point lies entirely in the ω -limit set, so C_i has the constant value C_i^* for all i . The equation for Y then reduces to the scalar linear equation

$$\frac{dY}{dt} = (a + \sigma C_N^*)(m - Y) - bY, \quad (3.31)$$

which has the unique positive steady state

$$Y^* = \frac{(a + \sigma C_N^*)m}{a + b + \sigma C_N^*}. \quad (3.32)$$

Every solution of (3.31) converges to Y^* as $t \rightarrow \infty$. Passing again to a solution starting at an ω -limit point, Y also takes the constant value Y^* , and the equation for X reduces to the scalar linear equation

$$\frac{dX}{dt} = (c + \delta Y^*)(l - X) - (d + \mu C_N^*)X, \quad (3.33)$$

which has the unique positive steady state

$$X^* = \frac{(c + \delta Y^*)l}{c + d + \delta Y^* + \mu C_N^*}. \quad (3.34)$$

Since these statements hold for all ω -limit points, every solution of the full system converges to (C_i^*, Y^*, X^*) as $t \rightarrow \infty$, and the unique positive steady state is globally asymptotically stable.

3.2.8 KPR with Limited Signaling and Incoherent Feedforward Loop

The cascade argument proceeds exactly as in Section 3.2.7, with the proofreading subsystem Z_1 now consisting of the variables $(C_0, \dots, C_{N+1})$. It has already been established in Section 3.2.3 that this extended proofreading subsystem has a unique positive steady state $C_i^* > 0$ for all i , to which all solutions converge as $t \rightarrow \infty$.

The downstream subsystem for Y and X is identical in structure to that in Section 3.2.7, with C_N^* now denoting the steady-state value of C_N from the limited-signaling proofreading subsystem. The equation for Y at an ω -limit point again reduces to (3.31), with the unique positive steady state Y^* given by (3.32). The equation for X at an ω -limit point similarly reduces to (3.33), with the unique positive steady state X^* given by (3.34). It follows that every solution of the full system converges to the unique positive steady state (C_i^*, Y^*, X^*) as $t \rightarrow \infty$, which is globally asymptotically stable.

3.2.9 KPR with Limited and Sustained Signaling

For the model combining limited and sustained signaling, we again apply Lemma 3.1 and the Deficiency Zero Theorem. Let $\Sigma_1 = \sum_{i=0}^{N+1} C_i$. From the reduced system one obtains

$$\frac{d\Sigma_1}{dt} = \kappa(L_T - \Sigma_1)(R_T - \Sigma_1) - v\Sigma_1. \quad (3.35)$$

Since (3.35) is a closed scalar autonomous equation, any ω -limit point Σ_1^* satisfies

$$\kappa(L_T - \Sigma_1^*)(R_T - \Sigma_1^*) = v\Sigma_1^*,$$

and hence $\Sigma_1^* < L_T$.

Suppose $\Sigma_1^* + R^{**} = R_T$. Then from the steady-state equation for C_0 it follows that $C_0^* = 0$. Using the equations for $C_1, \dots, C_{N-1}$ inductively gives $C_i^* = 0$ for all $1 \leq i \leq N-1$. Adding the steady-state equations for C_N, C_{N+1} , and R^* then yields

$$0 = -v C_{N+1}^* - \Omega R^{**}. \quad (3.36)$$

Since $v > 0$ and $\Omega > 0$, it follows that $C_{N+1}^* = 0$ and $R^{**} = 0$. Substituting $C_{N+1}^* = 0$ into the steady-state equation for C_N gives $C_N^* = 0$. Hence $\Sigma_1^* + R^{**} = 0$, contradicting $R_T > 0$. Therefore $\Sigma_1^* + R^{**} < R_T$, and from this it follows that $C_0^* > 0$, inductively $C_i^* > 0$ for all $1 \leq i \leq N+1$, and $R^{**} > 0$ [101].

Deficiency calculation. The network complexes are $\{L+R, C_0, C_1, \dots, C_{N+1}, L+R^*\}$, giving $n = N+4$. There is a single linkage class, $l = 1$. The stoichiometric matrix is

$$\begin{bmatrix} a_1 & a_2 & \dots & a_{N+2} & b_1 & b_2 & \dots & b_{N+1} & c_1 & c_2 & c_3 \\ 1 & 1 & \dots & 1 & 0 & 0 & \dots & 0 & -1 & -1 & 0 \\ 1 & 1 & \dots & 1 & 0 & 0 & \dots & 0 & -1 & -1 & 1 \\ 0 & 0 & \dots & 0 & 0 & 0 & \dots & 0 & 0 & 0 & -1 \\ -1 & 0 & \dots & 0 & -1 & 0 & \dots & 0 & 1 & 0 & 0 \\ 0 & -1 & \dots & 0 & 1 & -1 & \dots & 0 & 0 & 0 & 0 \\ \vdots & \vdots & \ddots & \vdots & \vdots & \vdots & \ddots & \vdots & \vdots & \vdots & \vdots \\ 0 & 0 & \dots & 0 & 0 & 0 & \dots & -1 & 0 & 1 & 0 \\ 0 & 0 & \dots & -1 & 0 & 0 & \dots & 1 & 0 & 0 & 0 \end{bmatrix}.$$

The first $N+3$ columns of this matrix are linearly independent, so $s \geq N+3$. Hence

$$\delta \leq (N+4) - 1 - (N+3) = 0.$$

Since $\delta \geq 0$ always holds, we conclude $\delta = 0$. Since the network is weakly reversible, has deficiency zero, and all ω -limit points lie in the interior of the biologically relevant region, Theorem 3.3 implies that the unique positive steady state in each stoichiometric compatibility class is globally asymptotically stable [65].

3.3 Response Functions, $E_{\max}$, and EC_{50}

In this section, we derive the analytical expressions for the response function, the maximum response $E_{\max}$, and the half-maximal effective concentration EC_{50} for the phenotypic models introduced in Chapter 2. These quantities will be used throughout the remainder of the chapter to compare the models quantitatively. In particular, they allow us to study how the steady-state response depends on ligand concentration and dissociation time, and thus to evaluate the extent to which each model reproduces the main biological benchmarks of T-cell activation.

We begin with the occupancy model, which provides the simplest setting in which these calculations can be carried out explicitly. In addition to deriving the response, $E_{\max}$, and EC_{50} , we also analyze the monotonicity of the response with respect to dissociation time and ligand concentration. These observations will serve as a useful baseline for the more elaborate proofreading-based models considered later.

3.3.1 Occupancy Model

In the occupancy model, a pMHC ligand L binds reversibly to a T-cell receptor R to form a receptor–ligand complex C . The governing equation is

$$\frac{dC}{dt} = \kappa LR - vC, \quad (3.37)$$

where κ is the and v is the dissociation rate.

At equilibrium, $\frac{dC}{dt} = 0$, and therefore

$$\kappa LR = vC. \quad (3.38)$$

Equivalently,

$$LR = K_D C, \quad (3.39)$$

where

$$K_D = \frac{v}{\kappa}. \quad (3.40)$$

The total ligand and receptor concentrations are conserved:

$$L_T = L + C, \quad R_T = R + C. \quad (3.41)$$

Substituting these expressions into (3.39) gives

$$(L_T - C)(R_T - C) = K_D C. \quad (3.42)$$

Expanding and rearranging, we obtain

$$C^2 - (L_T + R_T + K_D)C + L_TR_T = 0. \quad (3.43)$$

Thus C is given by

$$C = \frac{L_T + R_T + K_D - \sqrt{(L_T + R_T + K_D)^2 - 4L_TR_T}}{2}. \quad (3.44)$$

The smaller root is chosen because biologically meaningful solutions must satisfy $C < L_T$ and $C < R_T$.

In this model, the T-cell response is identified directly with the equilibrium concentration of the bound complex. Hence

$$\text{Response} = C. \quad (3.45)$$

Calculation of $E_{\max}$

The quantity $E_{\max}$ is defined as the limiting response as $L_T \rightarrow \infty$. From (3.44),

$$E_{\max} = \lim_{L_T \rightarrow \infty} \frac{L_T + R_T + K_D - \sqrt{(L_T + R_T + K_D)^2 - 4L_TR_T}}{2}. \quad (3.46)$$

Multiplying numerator and denominator by the conjugate gives

$$E_{\max} = \lim_{L_T \rightarrow \infty} \frac{2L_TR_T}{L_T + R_T + K_D + \sqrt{(L_T + R_T + K_D)^2 - 4L_TR_T}} \quad (3.47)$$

$$= \lim_{L_T \rightarrow \infty} \frac{2R_T}{1 + \frac{R_T}{L_T} + \frac{K_D}{L_T} + \sqrt{\left(1 + \frac{R_T}{L_T} + \frac{K_D}{L_T}\right)^2 - 4\frac{R_T}{L_T}}}. \quad (3.48)$$

Since

$$\frac{R_T}{L_T} \rightarrow 0, \quad \frac{K_D}{L_T} \rightarrow 0 \quad \text{as } L_T \rightarrow \infty,$$

it follows that

$$E_{\max} = R_T. \quad (3.49)$$

Calculation of EC_{50}

By definition, EC_{50} is the ligand concentration for which the response reaches one half of its maximal value. Since $E_{\max} = R_T$, we impose

$$C = \frac{R_T}{2}. \quad (3.50)$$

Substituting (3.50) into (3.39) gives

$$\left(L_T - \frac{R_T}{2}\right) \left(R_T - \frac{R_T}{2}\right) = K_D \frac{R_T}{2}. \quad (3.51)$$

This simplifies to

$$\left(L_T - \frac{R_T}{2}\right) \frac{R_T}{2} = K_D \frac{R_T}{2}, \quad (3.52)$$

and hence

$$L_T = K_D + \frac{R_T}{2}. \quad (3.53)$$

Therefore,

$$EC_{50} = K_D + \frac{R_T}{2}. \quad (3.54)$$

Collecting the results, the occupancy model satisfies

$$\text{Response} = C, \quad E_{\max} = R_T, \quad EC_{50} = K_D + \frac{R_T}{2}. \quad (3.55)$$

Thus, in the occupancy model, the response increases monotonically both with ligand concentration and with dissociation time. This model therefore provides the simplest baseline against which the more elaborate proofreading-based models can be compared.

3.3.2 Kinetic Proofreading Model

The kinetic proofreading model was introduced in Section 2.4.2. To avoid repeating the full system of differential equations, we recall here only the relations needed for the steady-state response analysis. Let

$$C_T = \sum_{i=0}^N C_i \quad (3.56)$$

denote the total concentration of bound receptor–ligand complexes. The conserved total quantities are

$$L_T = L + C_T, \quad R_T = R + C_T. \quad (3.57)$$

In this model, the T-cell response is identified with the signaling-competent state C_N :

$$\text{Response} = C_N. \quad (3.58)$$

Expression for C_T

At steady state, summing the equations for the bound receptor–ligand complexes gives

$$\frac{dC_T}{dt} = \kappa LR - vC_T. \quad (3.59)$$

At equilibrium this yields

$$LR = K_D C_T, \quad K_D = \frac{v}{\kappa}. \quad (3.60)$$

Substituting the conserved total quantities (3.57) gives

$$(L_T - C_T)(R_T - C_T) = K_D C_T. \quad (3.61)$$

This is identical to the equilibrium relation (3.42) derived for the occupancy model, so C_T is again given by equation (3.44) with C replaced by C_T .

Expression for the response

To express C_N in terms of C_T , define

$$a = \frac{\phi}{\phi + v}. \quad (3.62)$$

At equilibrium, the intermediate proofreading equations imply

$$C_i = a C_{i-1}, \quad 1 \leq i \leq N - 1. \quad (3.63)$$

Hence

$$C_i = a^i C_0, \quad 1 \leq i \leq N - 1. \quad (3.64)$$

From the steady-state equation for the terminal proofreading state, we obtain

$$C_N = \frac{\phi}{v} C_{N-1}. \quad (3.65)$$

Using (3.64), this gives

$$C_N = \frac{\phi}{v} a^{N-1} C_0. \quad (3.66)$$

Since

$$1 - a = \frac{v}{\phi + v}, \quad (3.67)$$

we have

$$\frac{\phi}{v} = \frac{a}{1 - a}. \quad (3.68)$$

Therefore

$$C_N = \frac{a^N}{1 - a} C_0. \quad (3.69)$$

Now

$$\begin{aligned}
 C_T &= \sum_{i=0}^{N-1} C_i + C_N \\
 &= \sum_{i=0}^{N-1} a^i C_0 + C_N \\
 &= \frac{1 - a^N}{1 - a} C_0 + \frac{a^N}{1 - a} C_0 \\
 &= \frac{1}{1 - a} C_0.
 \end{aligned} \tag{3.70}$$

Hence

$$C_0 = (1 - a)C_T. \tag{3.71}$$

Substituting (3.71) into (3.69), we obtain

$$C_N = a^N C_T. \tag{3.72}$$

Thus the response function for the kinetic proofreading model is

$$\text{Response} = a^N C_T. \tag{3.73}$$

Calculation of $E_{\max}$

Since a^N is independent of L_T and $C_T \rightarrow R_T$ as $L_T \rightarrow \infty$ by the same limiting argument as in Section 3.3.1, it follows immediately that

$$E_{\max} = a^N R_T. \tag{3.74}$$

Calculation of EC_{50}

Setting

$$a^N C_T = \frac{1}{2} a^N R_T \tag{3.75}$$

gives

$$C_T = \frac{R_T}{2}. \tag{3.76}$$

Since C_T satisfies the same quadratic equilibrium relation as in the occupancy model, substituting $C_T = R_T/2$ yields by exactly the same steps as in Section 3.3.1,

$$EC_{50} = K_D + \frac{R_T}{2}. \tag{3.77}$$

Thus, as in the occupancy model, the response of the kinetic proofreading model is monotone increasing both with ligand concentration and with dissociation time. The

essential difference is that the proofreading factor a^N suppresses the response relative to the occupancy model, thereby encoding the effect of the intermediate biochemical steps required for activation.

3.3.3 Kinetic Proofreading with Limited Signaling

The model with limited signaling was introduced in Chapter 2. To avoid repeating the full system of differential equations, we recall here only the relations needed for the steady-state response analysis. In this model, once the signaling-competent state C_N is reached, the bound receptor may transition to a non-signaling state C_{N+1} at rate ξ . Thus, the signaling produced by a single productive receptor–ligand interaction is temporally limited.

Let

$$C_T = \sum_{i=0}^{N+1} C_i \quad (3.78)$$

denote the total concentration of bound receptor–ligand complexes. The conserved total quantities are

$$L_T = L + C_T, \quad R_T = R + C_T. \quad (3.79)$$

In this model, the T-cell response is identified with the signaling-competent state C_N :

$$\text{Response} = C_N. \quad (3.80)$$

Expression for C_T

At steady state, summing the equations for the bound receptor–ligand complexes gives

$$\frac{dC_T}{dt} = \kappa LR - vC_T. \quad (3.81)$$

At equilibrium this yields

$$LR = K_D C_T, \quad K_D = \frac{v}{\kappa}. \quad (3.82)$$

Substituting the conserved total quantities (3.79) gives

$$(L_T - C_T)(R_T - C_T) = K_D C_T. \quad (3.83)$$

This is identical to the equilibrium relation (3.42) derived for the occupancy model. Hence C_T is again given by equation (3.44) with C replaced by C_T .

Expression for the response

Define

$$a = \frac{\phi}{\phi + v}. \quad (3.84)$$

At equilibrium, the intermediate proofreading equations imply

$$C_i = a C_{i-1}, \quad 1 \leq i \leq N - 1. \quad (3.85)$$

Hence

$$C_i = a^i C_0, \quad 1 \leq i \leq N - 1. \quad (3.86)$$

From the steady-state equation for C_N , we obtain

$$C_N = \frac{\phi}{v + \xi} C_{N-1}. \quad (3.87)$$

Using (3.86), this becomes

$$C_N = \frac{\phi}{v + \xi} a^{N-1} C_0. \quad (3.88)$$

Now

$$\phi = a(\phi + v), \quad 1 - a = \frac{v}{\phi + v}, \quad (3.89)$$

so that

$$\phi = \frac{av}{1 - a}. \quad (3.90)$$

Substituting this into (3.88), we find

$$C_N = \frac{v}{v + \xi} \frac{a^N}{1 - a} C_0. \quad (3.91)$$

The steady-state equation for C_{N+1} gives

$$C_{N+1} = \frac{\xi}{v} C_N. \quad (3.92)$$

We now express C_T in terms of C_0 . Using (3.86), (3.91), and (3.92), we have

$$\begin{aligned}
 C_T &= \sum_{i=0}^{N-1} C_i + C_N + C_{N+1} \\
 &= \sum_{i=0}^{N-1} a^i C_0 + C_N + \frac{\xi}{v} C_N \\
 &= \frac{1-a^N}{1-a} C_0 + \left(1 + \frac{\xi}{v}\right) C_N \\
 &= \frac{1-a^N}{1-a} C_0 + \left(\frac{v+\xi}{v}\right) \left(\frac{v}{v+\xi} \frac{a^N}{1-a} C_0\right) \\
 &= \frac{1-a^N}{1-a} C_0 + \frac{a^N}{1-a} C_0 \\
 &= \frac{1}{1-a} C_0.
 \end{aligned} \tag{3.93}$$

Hence

$$C_0 = (1-a)C_T. \tag{3.94}$$

Substituting (3.94) into (3.91), we obtain

$$C_N = \left(\frac{v}{v+\xi}\right) a^N C_T. \tag{3.95}$$

Thus the response function for the model with limited signaling is

$$\text{Response} = \left(\frac{v}{v+\xi}\right) a^N C_T. \tag{3.96}$$

Calculation of $E_{\max}$

By definition,

$$E_{\max} = \lim_{L_T \rightarrow \infty} \text{Response}. \tag{3.97}$$

Using (3.96), this gives

$$E_{\max} = \lim_{L_T \rightarrow \infty} \left(\frac{v}{v+\xi}\right) a^N C_T. \tag{3.98}$$

Since the factor $\left(\frac{v}{v+\xi}\right) a^N$ is independent of L_T , this becomes

$$E_{\max} = \left(\frac{v}{v+\xi}\right) a^N \lim_{L_T \rightarrow \infty} C_T. \tag{3.99}$$

Using (3.83), the same limiting calculation as in the occupancy model shows that

$$\lim_{L_T \rightarrow \infty} C_T = R_T. \tag{3.100}$$

Hence

$$E_{\max} = \left(\frac{v}{v + \xi} \right) a^N R_T. \quad (3.101)$$

Calculation of EC_{50}

The half-maximal condition is

$$\left(\frac{v}{v + \xi} \right) a^N C_T = \frac{1}{2} \left(\frac{v}{v + \xi} \right) a^N R_T. \quad (3.102)$$

Cancelling the common factor $\left(\frac{v}{v + \xi} \right) a^N$ gives

$$C_T = \frac{R_T}{2}. \quad (3.103)$$

Since C_T satisfies (3.83), the same substitution as in Section 3.3.1 yields

$$EC_{50} = K_D + \frac{R_T}{2}. \quad (3.104)$$

Compared with the standard kinetic proofreading model, the additional factor $\frac{v}{v + \xi}$ reduces both the response and the maximal attainable response, reflecting the fact that signaling is terminated once the complex reaches the non-signaling state C_{N+1} . The half-maximal effective concentration, however, remains unchanged.

3.3.4 Kinetic Proofreading with Sustained Signaling

The model with sustained signaling was introduced in Chapter 2. To avoid repeating the full system of differential equations, we recall here only the relations needed for the steady-state response analysis. In this model, receptors that have reached the signaling-competent state C_N may continue signaling for some time even after ligand unbinding. After dissociation, such receptors enter the transient signaling state R^* , which returns to the basal state at rate Ω .

Let

$$C_T = \sum_{i=0}^N C_i \quad (3.105)$$

denote the total concentration of bound receptor–ligand complexes. The conserved total quantities are

$$L_T = L + C_T, \quad R_T = R + C_T + R^*. \quad (3.106)$$

In this model, both the bound signaling state C_N and the sustained-signaling state R^* contribute to T-cell activation. Thus

$$\text{Response} = C_N + R^*. \quad (3.107)$$

Expression for R^*

At steady state, the equation for the sustained-signaling state gives

$$0 = vC_N - \kappa LR^* - \Omega R^*. \quad (3.108)$$

Solving for R^* , we obtain

$$R^* = \frac{vC_N}{\kappa L + \Omega}. \quad (3.109)$$

Expression for C_0

At steady state, the equation for the free receptor balance gives

$$0 = -\kappa LR + v \sum_{i=0}^{N-1} C_i + \Omega R^*. \quad (3.110)$$

The steady-state equation for C_0 gives

$$\kappa LR = (\phi + v)C_0. \quad (3.111)$$

Substituting (3.111) and (3.109) into (3.110) gives

$$-(\phi + v)C_0 + v \sum_{i=0}^{N-1} C_i + \Omega \frac{vC_N}{\kappa L + \Omega} = 0. \quad (3.112)$$

Define

$$a = \frac{\phi}{\phi + v}. \quad (3.113)$$

At equilibrium, the intermediate proofreading equations imply

$$C_i = a^i C_0, \quad 1 \leq i \leq N-1. \quad (3.114)$$

Therefore

$$\sum_{i=0}^{N-1} C_i = \sum_{i=0}^{N-1} a^i C_0 = \frac{1 - a^N}{1 - a} C_0. \quad (3.115)$$

Substituting (3.115) into (3.112) yields

$$-(\phi + v)C_0 + v \frac{1 - a^N}{1 - a} C_0 + \Omega \frac{vC_N}{\kappa L + \Omega} = 0. \quad (3.116)$$

Since

$$1 - a = \frac{v}{\phi + v}, \quad (3.117)$$

we have

$$\frac{v}{1 - a} = \phi + v. \quad (3.118)$$

Hence (3.116) becomes

$$-(\phi + v)C_0 + (\phi + v)(1 - a^N)C_0 + \Omega \frac{vC_N}{\kappa L + \Omega} = 0, \quad (3.119)$$

which simplifies to

$$-(\phi + v)a^N C_0 + \Omega \frac{vC_N}{\kappa L + \Omega} = 0. \quad (3.120)$$

Using again

$$\phi + v = \frac{v}{1 - a}, \quad (3.121)$$

we obtain

$$C_0 = \frac{\Omega(1 - a)}{a^N(\kappa L + \Omega)} C_N. \quad (3.122)$$

Expression for C_N

From the definition of C_T ,

$$\begin{aligned} C_T &= \sum_{i=0}^N C_i \\ &= \sum_{i=0}^{N-1} C_i + C_N \\ &= \sum_{i=0}^{N-1} a^i C_0 + C_N \\ &= \frac{1 - a^N}{1 - a} C_0 + C_N. \end{aligned} \quad (3.123)$$

Substituting (3.122) gives

$$\begin{aligned} C_T &= \frac{1 - a^N}{1 - a} \left(\frac{\Omega(1 - a)}{a^N(\kappa L + \Omega)} C_N \right) + C_N \\ &= \frac{\Omega(1 - a^N)}{a^N(\kappa L + \Omega)} C_N + C_N. \end{aligned} \quad (3.124)$$

Combining terms yields

$$\begin{aligned} C_T &= \left(\frac{\Omega(1 - a^N) + a^N(\kappa L + \Omega)}{a^N(\kappa L + \Omega)} \right) C_N \\ &= \left(\frac{\Omega + a^N \kappa L}{a^N(\kappa L + \Omega)} \right) C_N. \end{aligned} \quad (3.125)$$

Therefore

$$C_N = \frac{\kappa L + \Omega}{\Omega + a^N \kappa L} a^N C_T. \quad (3.126)$$

Expression for the response

Using (3.109) and (3.126), we obtain

$$\begin{aligned} \text{Response} &= C_N + R^* \\ &= C_N + \frac{v C_N}{\kappa L + \Omega} \\ &= \frac{\kappa L + \Omega + v}{\kappa L + \Omega} C_N \\ &= \frac{\kappa L + \Omega + v}{\kappa L + \Omega} \left(\frac{\kappa L + \Omega}{\Omega + a^N \kappa L} a^N C_T \right). \end{aligned} \quad (3.127)$$

Hence

$$\text{Response} = \left(\frac{\kappa L + \Omega + v}{\Omega + a^N \kappa L} \right) a^N C_T. \quad (3.128)$$

Calculation of $E_{\max}$

By definition,

$$E_{\max} = \lim_{L_T \rightarrow \infty} \text{Response}. \quad (3.129)$$

Using (3.128),

$$E_{\max} = \lim_{L_T \rightarrow \infty} \left(\frac{\kappa L + \Omega + v}{\Omega + a^N \kappa L} \right) a^N C_T. \quad (3.130)$$

Since the conservation law gives

$$L = L_T - C_T, \quad (3.131)$$

we obtain

$$E_{\max} = \lim_{L_T \rightarrow \infty} \left(\frac{\kappa(L_T - C_T) + \Omega + v}{\Omega + a^N \kappa(L_T - C_T)} \right) a^N C_T. \quad (3.132)$$

As in the previous models, $C_T \rightarrow R_T$ as $L_T \rightarrow \infty$. Dividing numerator and denominator by L_T , we obtain

$$E_{\max} = a^N R_T \lim_{L_T \rightarrow \infty} \left(\frac{\kappa \left(1 - \frac{C_T}{L_T}\right) + \frac{\Omega}{L_T} + \frac{v}{L_T}}{\frac{\Omega}{L_T} + a^N \kappa \left(1 - \frac{C_T}{L_T}\right)} \right). \quad (3.133)$$

Since

$$\frac{C_T}{L_T} \rightarrow 0, \quad \frac{\Omega}{L_T} \rightarrow 0, \quad \frac{v}{L_T} \rightarrow 0 \quad \text{as } L_T \rightarrow \infty, \quad (3.134)$$

it follows that

$$E_{\max} = R_T. \quad (3.135)$$

Calculation of EC_{50}

By definition, EC_{50} is the ligand concentration for which the response reaches one half of its maximal value. Since $E_{\max} = R_T$, we impose

$$\frac{R_T}{2} = \left(\frac{\kappa(L_T - C_T) + \Omega + v}{\Omega + a^N \kappa(L_T - C_T)} \right) a^N C_T. \quad (3.136)$$

Multiplying both sides by the denominator gives

$$\frac{R_T}{2} [\Omega + a^N \kappa(L_T - C_T)] = a^N C_T [\kappa(L_T - C_T) + \Omega + v]. \quad (3.137)$$

Expanding both sides yields

$$\frac{\Omega R_T}{2} + \frac{a^N \kappa R_T}{2} (L_T - C_T) = a^N \kappa C_T (L_T - C_T) + a^N C_T (\Omega + v). \quad (3.138)$$

Rearranging and solving for L_T , we obtain

$$EC_{50} = \frac{a^N C_T (\Omega + v + \kappa (\frac{R_T}{2} - C_T)) - \frac{\Omega R_T}{2}}{a^N \kappa (\frac{R_T}{2} - C_T)}. \quad (3.139)$$

Collecting the results, the kinetic proofreading model with sustained signaling satisfies

$$\text{Response} = \left(\frac{\kappa L + \Omega + v}{\Omega + a^N \kappa L} \right) a^N C_T, \quad E_{\max} = R_T, \quad (3.140)$$

and

$$EC_{50} = \frac{a^N C_T (\Omega + v + \kappa (\frac{R_T}{2} - C_T)) - \frac{\Omega R_T}{2}}{a^N \kappa (\frac{R_T}{2} - C_T)}. \quad (3.141)$$

Thus, unlike the standard kinetic proofreading model, the maximal response here is restored to R_T , reflecting the contribution of the sustained-signaling state R^* . The model

therefore allows signaling to persist even after ligand unbinding, which qualitatively changes the structure of the response while preserving a fully explicit steady-state description.

3.3.5 Kinetic Proofreading with Negative Feedback

The kinetic proofreading model with negative feedback was introduced in Chapter 2. To avoid repeating the full system of differential equations, we recall here only the relations needed for the steady-state response analysis. In this model, the progression through the activation chain is regulated by a phosphatase-mediated inhibitory feedback. The variable S represents the active phosphatase, and the dephosphorylation terms depend on S . The signaling output is again taken to be the concentration of the signaling-competent state C_N .

Let

$$C_T = \sum_{i=0}^N C_i \quad (3.142)$$

denote the total concentration of bound receptor–ligand complexes. The conserved total quantities are

$$L_T = L + C_T, \quad R_T = R + C_T. \quad (3.143)$$

In this model, the T-cell response is identified with the signaling-competent state C_N :

$$\text{Response} = C_N. \quad (3.144)$$

Expression for C_T

At steady state, summing the equations for the bound receptor–ligand complexes cancels the internal forward and backward transitions and gives

$$\frac{dC_T}{dt} = \kappa LR - vC_T. \quad (3.145)$$

At equilibrium this yields

$$LR = K_D C_T, \quad K_D = \frac{v}{\kappa}. \quad (3.146)$$

Substituting the conserved total quantities (3.143) gives

$$(L_T - C_T)(R_T - C_T) = K_D C_T. \quad (3.147)$$

This is identical to the equilibrium relation (3.42) derived for the occupancy model. Hence C_T is again given by equation (3.44) with C replaced by C_T .

Expression for the response

At steady state, solving the linear recurrence for the proofreading chain with feedback-dependent backward rate $b + \gamma S$ gives

$$C_N = \left(\frac{1 - r_-/r_+}{1 - (r_-/r_+)^{N+1}} \right) r_-^N C_T, \quad (3.148)$$

where

$$r_{\pm} = \frac{\phi + b + \gamma S + v \pm \sqrt{(\phi + b + \gamma S + v)^2 - 4\phi(b + \gamma S)}}{2(b + \gamma S)}. \quad (3.149)$$

Therefore the response function is

$$\text{Response} = \left(\frac{1 - r_-/r_+}{1 - (r_-/r_+)^{N+1}} \right) r_-^N C_T. \quad (3.150)$$

Calculation of $E_{\max}$

The feedback-dependent factor multiplying C_T in (3.150) is

$$A(S) = \left(\frac{1 - r_-/r_+}{1 - (r_-/r_+)^{N+1}} \right) r_-^N. \quad (3.151)$$

Thus

$$\text{Response} = A(S) C_T. \quad (3.152)$$

Since $C_T \rightarrow R_T$ as $L_T \rightarrow \infty$ by the same argument as in Section 3.3.1, the maximal response is

$$E_{\max} = A(S) R_T. \quad (3.153)$$

Equivalently,

$$E_{\max} = \left(\frac{1 - r_-/r_+}{1 - (r_-/r_+)^{N+1}} \right) r_-^N R_T. \quad (3.154)$$

Calculation of EC_{50}

The half-maximal condition is

$$\left(\frac{1 - r_-/r_+}{1 - (r_-/r_+)^{N+1}} \right) r_-^N C_T = \frac{1}{2} \left(\frac{1 - r_-/r_+}{1 - (r_-/r_+)^{N+1}} \right) r_-^N R_T. \quad (3.155)$$

Cancelling the common factor gives

$$C_T = \frac{R_T}{2}. \quad (3.156)$$

Since C_T satisfies (3.147), the same substitution as in Section 3.3.1 yields

$$EC_{50} = K_D + \frac{R_T}{2}. \quad (3.157)$$

Thus the negative-feedback mechanism modifies the response through the factor involving r_- and r_+ , which depends on the phosphatase activity S . In this way, the proofreading chain is no longer governed solely by forward transitions, but is also shaped by an inhibitory feedback that can substantially alter the magnitude and shape of the response.

3.3.6 Kinetic Proofreading with Stabilizing Activation Chain

The kinetic proofreading model with stabilizing activation chain was introduced in Chapter 2. To avoid repeating the full system of differential equations, we recall here only the relations needed for the steady-state response analysis. In this model, the forward transition rates and dissociation rates are allowed to vary along the proofreading chain. In this way, the model captures the possibility that the receptor–ligand complex becomes progressively stabilized or destabilized as proofreading proceeds.

The stage-dependent rates are taken to be

$$v(i) = \frac{1+i}{1+ri} v, \quad \phi(i) = \phi r^i, \quad r > 1. \quad (3.158)$$

Here $v(0) = v = 1/\tau$ represents the dissociation rate at the first step, while $\phi(0) = \phi$ denotes the forward transition rate from C_0 to C_1 .

Let

$$C_T = \sum_{i=0}^N C_i \quad (3.159)$$

denote the total concentration of bound receptor–ligand complexes. The conserved total quantities are

$$L_T = L + C_T, \quad R_T = R + C_T. \quad (3.160)$$

In this model, the T-cell response is identified with the signaling-competent state C_N :

$$\text{Response} = C_N. \quad (3.161)$$

Expression for the response

For this model, the steady-state concentrations can be written in the form

$$C_0 = \frac{C_T}{\mu}, \quad (3.162)$$

$$C_i = \gamma_i C_0, \quad 1 \leq i \leq N-1, \quad (3.163)$$

and

$$C_N = \delta C_T, \quad (3.164)$$

where

$$\mu = 1 + \frac{\phi(N-1)}{v(N)} \gamma_{N-1} + \sum_{i=0}^{N-1} \gamma_i, \quad (3.165)$$

$$\gamma_i = \prod_{j=1}^i \alpha_j, \quad \alpha_i = \frac{\phi(i-1)}{\phi(i) + v(i)}, \quad (3.166)$$

and

$$\delta = \frac{1}{\mu} \frac{\phi(N-1)}{v(N)} \gamma_{N-1}. \quad (3.167)$$

It follows immediately from (3.164) that the response function is

$$\text{Response} = C_N = \delta C_T. \quad (3.168)$$

Expression for C_T

The total concentration of bound receptor–ligand complexes is given by

$$C_T = \frac{2L_T R_T}{L_T + R_T + \epsilon + \sqrt{(L_T + R_T + \epsilon)^2 - 4L_T R_T}}, \quad (3.169)$$

where

$$\epsilon = \frac{1}{\mu} \frac{v(0) + \phi(0)}{\kappa}. \quad (3.170)$$

Thus the response can be written explicitly as

$$\text{Response} = \delta \frac{2L_T R_T}{L_T + R_T + \epsilon + \sqrt{(L_T + R_T + \epsilon)^2 - 4L_T R_T}}. \quad (3.171)$$

Calculation of $E_{\max}$

By definition,

$$E_{\max} = \lim_{L_T \rightarrow \infty} \text{Response}. \quad (3.172)$$

Using (3.168), we obtain

$$E_{\max} = \delta \lim_{L_T \rightarrow \infty} C_T. \quad (3.173)$$

From (3.169), the same asymptotic argument as in the previous models shows that

$$\lim_{L_T \rightarrow \infty} C_T = R_T. \quad (3.174)$$

Hence

$$E_{\max} = \delta R_T. \quad (3.175)$$

Calculation of EC_{50}

By definition, EC_{50} is the ligand concentration for which the response is equal to one half of its maximal value. Since $E_{\max} = \delta R_T$, we impose

$$\delta C_T = \frac{\delta R_T}{2}. \quad (3.176)$$

Therefore

$$C_T = \frac{R_T}{2}. \quad (3.177)$$

Substituting (3.177) into (3.169) gives

$$\frac{R_T}{2} = \frac{2L_T R_T}{L_T + R_T + \epsilon + \sqrt{(L_T + R_T + \epsilon)^2 - 4L_T R_T}}. \quad (3.178)$$

Equivalently,

$$L_T + R_T + \epsilon + \sqrt{(L_T + R_T + \epsilon)^2 - 4L_T R_T} = 4L_T. \quad (3.179)$$

Thus

$$\sqrt{(L_T + R_T + \epsilon)^2 - 4L_T R_T} = 3L_T - R_T - \epsilon. \quad (3.180)$$

Squaring both sides gives

$$(L_T + R_T + \epsilon)^2 - 4L_T R_T = (3L_T - R_T - \epsilon)^2. \quad (3.181)$$

Solving this equation for L_T , we obtain

$$L_T = \frac{R_T}{2} + \epsilon. \quad (3.182)$$

Therefore,

$$EC_{50} = \frac{R_T}{2} + \epsilon. \quad (3.183)$$

Collecting the results, the kinetic proofreading model with stabilizing activation chain satisfies

$$\text{Response} = \delta C_T, \quad E_{\max} = \delta R_T, \quad EC_{50} = \frac{R_T}{2} + \epsilon. \quad (3.184)$$

Thus, compared with the standard kinetic proofreading model, the stage-dependent rates modify the response through the factor δ and alter the effective half-maximal concentration through the parameter ϵ . In this way, the stabilizing activation chain changes both the magnitude of the response and the ligand concentration required to achieve half-maximal activation.

3.3.7 Kinetic Proofreading with Incoherent Feedforward Loop

The kinetic proofreading model with incoherent feedforward loop was introduced in Chapter 2. To avoid repeating the full system of differential equations, we recall here only the relations needed for the steady-state response analysis. This model couples the proofreading cascade to a downstream regulatory motif involving the variables Y and X . The signaling-competent state C_N activates the intermediate variable Y , while the final output variable X is regulated both by Y and directly by C_N . In this model, the T-cell response is identified with the downstream output X .

Let

$$C_T = \sum_{i=0}^N C_i \quad (3.185)$$

denote the total concentration of bound receptor–ligand complexes. The conserved total quantities are

$$L_T = L + C_T, \quad R_T = R + C_T. \quad (3.186)$$

In this model, the T-cell response is identified with the downstream variable X :

$$\text{Response} = X. \quad (3.187)$$

Expression for C_T

At steady state, summing the equations for the bound receptor–ligand complexes gives

$$\frac{dC_T}{dt} = \kappa LR - vC_T. \quad (3.188)$$

At equilibrium this yields

$$LR = K_D C_T, \quad K_D = \frac{v}{\kappa}. \quad (3.189)$$

Substituting the conserved total quantities (3.186) gives

$$(L_T - C_T)(R_T - C_T) = K_D C_T. \quad (3.190)$$

Expression for C_N

The proofreading subsystem is identical to that of the standard kinetic proofreading model. Therefore, if we define

$$q = \frac{\phi}{\phi + v}, \quad (3.191)$$

then

$$C_N = q^N C_T. \quad (3.192)$$

Expression for Y

At steady state, the equation for the intermediate regulatory variable Y gives

$$0 = a(m - Y) - bY + \sigma C_N(m - Y). \quad (3.193)$$

Expanding and collecting the terms involving Y , we obtain

$$(a + \sigma C_N)m - (a + b + \sigma C_N)Y = 0. \quad (3.194)$$

Hence

$$Y = \frac{(a + \sigma C_N)m}{a + b + \sigma C_N}. \quad (3.195)$$

Substituting (3.192) into (3.195), we get

$$Y = \frac{(a + \sigma q^N C_T)m}{a + b + \sigma q^N C_T}. \quad (3.196)$$

Expression for the response

At steady state, the equation for the downstream output variable X gives

$$0 = c(l - X) - dX + \delta Y(l - X) - \mu C_N X. \quad (3.197)$$

Expanding and collecting the terms involving X , we obtain

$$(c + \delta Y)l - (c + d + \delta Y + \mu C_N)X = 0. \quad (3.198)$$

Thus

$$X = \frac{(c + \delta Y)l}{c + d + \delta Y + \mu C_N}. \quad (3.199)$$

Using (3.192), this becomes

$$\text{Response} = X = \frac{(c + \delta Y)l}{c + d + \delta Y + \mu q^N C_T}, \quad (3.200)$$

where Y is given by (3.196).

Calculation of $E_{\max}$

By definition,

$$E_{\max} = \lim_{L_T \rightarrow \infty} X. \quad (3.201)$$

As in the previous models,

$$C_T \rightarrow R_T \quad \text{as } L_T \rightarrow \infty. \quad (3.202)$$

Hence

$$C_N \rightarrow q^N R_T. \quad (3.203)$$

From (3.195), it follows that

$$Y \rightarrow Y_\infty := \frac{(a + \sigma q^N R_T)m}{a + b + \sigma q^N R_T}. \quad (3.204)$$

Therefore,

$$E_{\max} = \frac{(c + \delta Y_\infty)l}{c + d + \delta Y_\infty + \mu q^N R_T}. \quad (3.205)$$

Calculation of EC_{50}

The quantity EC_{50} is defined as the ligand concentration for which the response reaches one half of its maximal value. Since the response depends on C_T both directly and indirectly through Y , the half-maximal condition is naturally written in implicit form:

$$\frac{(c + \delta Y(C_T))l}{c + d + \delta Y(C_T) + \mu q^N C_T} = \frac{1}{2} \frac{(c + \delta Y_\infty)l}{c + d + \delta Y_\infty + \mu q^N R_T}, \quad (3.206)$$

where

$$Y(C_T) = \frac{(a + \sigma q^N C_T)m}{a + b + \sigma q^N C_T}, \quad (3.207)$$

and Y_∞ is given by (3.204). Once the corresponding value of C_T has been determined from (3.206), the ligand concentration L_T can be obtained from the equilibrium relation (3.190).

Solving (3.190) for L_T gives

$$L_T = \frac{C_T(R_T + K_D - C_T)}{R_T - C_T}. \quad (3.208)$$

Therefore, EC_{50} is obtained by substituting into (3.208) the value of C_T which solves (3.206).

Collecting the results, the kinetic proofreading model with incoherent feedforward loop satisfies

$$\text{Response} = \frac{(c + \delta Y)l}{c + d + \delta Y + \mu q^N C_T}, \quad Y = \frac{(a + \sigma q^N C_T)m}{a + b + \sigma q^N C_T}, \quad (3.209)$$

and

$$E_{\max} = \frac{(c + \delta Y_\infty)l}{c + d + \delta Y_\infty + \mu q^N R_T}, \quad Y_\infty = \frac{(a + \sigma q^N R_T)m}{a + b + \sigma q^N R_T}. \quad (3.210)$$

Thus, in contrast to the previous models, the response here is determined by a downstream regulatory variable and not directly by the signaling-competent proofreading state. The incoherent feedforward structure therefore modifies both the magnitude and the qualitative dependence of the response on the biochemical parameters.

3.3.8 Kinetic Proofreading with Limited Signaling and Incoherent Feedforward Loop

The kinetic proofreading model with limited signaling and incoherent feedforward loop was introduced in Chapter 2. To avoid repeating the full system of differential equations, we recall here only the relations needed for the steady-state response analysis. This model combines the limited-signaling modification of the proofreading cascade with a downstream incoherent feedforward loop involving the variables Y and X . As in the previous feedforward model, the signaling-competent proofreading state C_N regulates the downstream response through the intermediate variable Y , while the final output X is taken as the measure of T-cell activation. In addition, once the state C_N is reached, the bound receptor may transition to the non-signaling state C_{N+1} at rate ξ .

Let

$$C_T = \sum_{i=0}^{N+1} C_i \quad (3.211)$$

denote the total concentration of bound receptor–ligand complexes. The conserved total quantities are

$$L_T = L + C_T, \quad R_T = R + C_T. \quad (3.212)$$

In this model, the T-cell response is identified with the downstream variable X :

$$\text{Response} = X. \quad (3.213)$$

Expression for C_T

At steady state, summing the equations for the bound receptor–ligand complexes gives

$$\frac{dC_T}{dt} = \kappa LR - vC_T. \quad (3.214)$$

At equilibrium this yields

$$LR = K_D C_T, \quad K_D = \frac{v}{\kappa}. \quad (3.215)$$

Substituting the conserved total quantities (3.212) gives

$$(L_T - C_T)(R_T - C_T) = K_D C_T. \quad (3.216)$$

Expression for C_N

The proofreading subsystem is the same as that of the model with limited signaling. Define

$$q = \frac{\phi}{\phi + v}. \quad (3.217)$$

Then, using the steady-state relation derived for the limited-signaling proofreading chain, we have

$$C_N = \left(\frac{v}{v + \xi} \right) q^N C_T. \quad (3.218)$$

Expression for Y

At steady state, the equation for the intermediate regulatory variable Y gives

$$0 = a(m - Y) - bY + \sigma C_N(m - Y). \quad (3.219)$$

Expanding and collecting the terms involving Y , we obtain

$$(a + \sigma C_N)m - (a + b + \sigma C_N)Y = 0. \quad (3.220)$$

Hence

$$Y = \frac{(a + \sigma C_N)m}{a + b + \sigma C_N}. \quad (3.221)$$

Substituting (3.218) into (3.221) yields

$$Y = \frac{\left(a + \sigma \left(\frac{v}{v + \xi} \right) q^N C_T \right) m}{a + b + \sigma \left(\frac{v}{v + \xi} \right) q^N C_T}. \quad (3.222)$$

Expression for the response

At steady state, the equation for the downstream output variable X gives

$$0 = c(l - X) - dX + \delta Y(l - X) - \mu C_N X. \quad (3.223)$$

Expanding and collecting the terms involving X , we obtain

$$(c + \delta Y)l - (c + d + \delta Y + \mu C_N)X = 0. \quad (3.224)$$

Therefore

$$X = \frac{(c + \delta Y)l}{c + d + \delta Y + \mu C_N}. \quad (3.225)$$

Using (3.218), the response function can be written as

$$\text{Response} = X = \frac{(c + \delta Y)l}{c + d + \delta Y + \mu \left(\frac{v}{v+\xi} \right) q^N C_T}, \quad (3.226)$$

where Y is given by (3.222).

Calculation of $E_{\max}$

By definition,

$$E_{\max} = \lim_{L_T \rightarrow \infty} X. \quad (3.227)$$

As in the previous models,

$$C_T \rightarrow R_T \quad \text{as } L_T \rightarrow \infty. \quad (3.228)$$

Hence

$$C_N \rightarrow \left(\frac{v}{v+\xi} \right) q^N R_T. \quad (3.229)$$

From (3.221), it follows that

$$Y \rightarrow Y_{\infty} := \frac{\left(a + \sigma \left(\frac{v}{v+\xi} \right) q^N R_T \right) m}{a + b + \sigma \left(\frac{v}{v+\xi} \right) q^N R_T}. \quad (3.230)$$

Therefore,

$$E_{\max} = \frac{(c + \delta Y_{\infty})l}{c + d + \delta Y_{\infty} + \mu \left(\frac{v}{v+\xi} \right) q^N R_T}. \quad (3.231)$$

Calculation of EC_{50}

The quantity EC_{50} is the ligand concentration for which the response reaches one half of its maximal value. Since the response depends on C_T both directly and indirectly through Y , the half-maximal condition is most naturally written in implicit form:

$$\frac{(c + \delta Y(C_T))l}{c + d + \delta Y(C_T) + \mu \left(\frac{v}{v+\xi} \right) q^N C_T} = \frac{1}{2} \frac{(c + \delta Y_{\infty})l}{c + d + \delta Y_{\infty} + \mu \left(\frac{v}{v+\xi} \right) q^N R_T}, \quad (3.232)$$

where

$$Y(C_T) = \frac{\left(a + \sigma \left(\frac{v}{v+\xi} \right) q^N C_T \right) m}{a + b + \sigma \left(\frac{v}{v+\xi} \right) q^N C_T}, \quad (3.233)$$

and Y_{∞} is given by (3.230). Once the corresponding value of C_T has been determined from (3.232), the ligand concentration L_T can be obtained from the equilibrium relation (3.216).

Solving (3.216) for L_T gives

$$L_T = \frac{C_T(R_T + K_D - C_T)}{R_T - C_T}. \quad (3.234)$$

Therefore, EC_{50} is obtained by substituting into (3.234) the value of C_T which solves (3.232).

Collecting the results, the kinetic proofreading model with limited signaling and incoherent feedforward loop satisfies

$$\text{Response} = \frac{(c + \delta Y)l}{c + d + \delta Y + \mu \left(\frac{v}{v+\xi} \right) q^N C_T}, \quad (3.235)$$

with

$$Y = \frac{\left(a + \sigma \left(\frac{v}{v+\xi} \right) q^N C_T \right) m}{a + b + \sigma \left(\frac{v}{v+\xi} \right) q^N C_T}, \quad (3.236)$$

and

$$E_{\max} = \frac{(c + \delta Y_{\infty})l}{c + d + \delta Y_{\infty} + \mu \left(\frac{v}{v+\xi} \right) q^N R_T}, \quad (3.237)$$

where

$$Y_{\infty} = \frac{\left(a + \sigma \left(\frac{v}{v+\xi} \right) q^N R_T \right) m}{a + b + \sigma \left(\frac{v}{v+\xi} \right) q^N R_T}. \quad (3.238)$$

Thus the limited-signaling modification enters this model through the additional factor $\frac{v}{v+\xi}$ in the proofreading contribution C_N . As a result, both the downstream response and its maximal value are reduced relative to the corresponding model without limited signaling, while the feedforward structure still governs the overall form of the output.

3.3.9 Kinetic Proofreading with Limited and Sustained Signaling

The kinetic proofreading model with limited and sustained signaling was introduced in Chapter 2. To avoid repeating the full system of differential equations, we recall here only the relations needed for the steady-state response analysis. This model combines two modifications of the proofreading cascade. On the one hand, once the signaling-competent state C_N is reached, the bound receptor may transition to a non-signaling state C_{N+1} at rate ξ , representing limited signaling. On the other hand, after ligand unbinding, signaling may persist transiently through the state R^* , which returns to the basal receptor state at rate Ω . In this model, both C_N and R^* contribute to the response.

Let

$$C_T = \sum_{i=0}^{N+1} C_i \quad (3.239)$$

denote the total concentration of bound receptor–ligand complexes. The conserved total quantities are

$$L_T = L + C_T, \quad R_T = R + C_T + R^*. \quad (3.240)$$

In this model, the T-cell response is identified with

$$\text{Response} = C_N + R^*. \quad (3.241)$$

Expression for C_T

At steady state, summing the equations for the bound receptor–ligand complexes gives

$$\frac{dC_T}{dt} = \kappa LR + \kappa LR^* - vC_T. \quad (3.242)$$

Using the conservation relation

$$R + R^* = R_T - C_T, \quad (3.243)$$

together with

$$L = L_T - C_T, \quad (3.244)$$

equation (3.242) becomes

$$\frac{dC_T}{dt} = \kappa(L_T - C_T)(R_T - C_T) - vC_T. \quad (3.245)$$

At steady state, this gives

$$(L_T - C_T)(R_T - C_T) = K_D C_T, \quad K_D = \frac{v}{\kappa}. \quad (3.246)$$

Thus C_T satisfies the same quadratic relation as in the occupancy model, and hence

$$C_T = \frac{L_T + R_T + K_D - \sqrt{(L_T + R_T + K_D)^2 - 4L_T R_T}}{2}. \quad (3.247)$$

Expression for R^*

At steady state, the balance equation for the sustained-signaling state gives

$$0 = vC_N - \kappa LR^* - \Omega R^*. \quad (3.248)$$

Solving for R^* , we obtain

$$R^* = \frac{vC_N}{\kappa L + \Omega}. \quad (3.249)$$

Expression for C_0

At steady state, the free receptor balance gives

$$0 = -\kappa LR + v \sum_{i=0}^{N-1} C_i + vC_{N+1} + \Omega R^*. \quad (3.250)$$

The steady-state relation for C_0 gives

$$\kappa LR = (\phi + v)C_0. \quad (3.251)$$

Moreover,

$$\sum_{i=0}^{N-1} C_i + C_{N+1} = C_T - C_N. \quad (3.252)$$

Substituting (3.252) and (3.249) into (3.250) gives

$$-(\phi + v)C_0 + v(C_T - C_N) + \Omega \frac{vC_N}{\kappa L + \Omega} = 0. \quad (3.253)$$

Define

$$a = \frac{\phi}{\phi + v}. \quad (3.254)$$

Then

$$1 - a = \frac{v}{\phi + v}. \quad (3.255)$$

Therefore, from (3.253), we obtain

$$C_0 = (1 - a)C_T - (1 - a)C_N + (1 - a) \frac{\Omega C_N}{\kappa L + \Omega}. \quad (3.256)$$

Expression for C_N

At steady state, the intermediate proofreading relations imply

$$C_i = a^i C_0, \quad 1 \leq i \leq N - 1. \quad (3.257)$$

The non-signaling terminal state satisfies

$$C_{N+1} = \frac{\xi}{v} C_N. \quad (3.258)$$

Using the definition of C_T , we have

$$\begin{aligned}
 C_T &= \sum_{i=0}^{N+1} C_i \\
 &= \sum_{i=0}^{N-1} C_i + C_N + C_{N+1} \\
 &= \sum_{i=0}^{N-1} a^i C_0 + C_N + \frac{\xi}{v} C_N \\
 &= \frac{1-a^N}{1-a} C_0 + C_N + \frac{\xi}{v} C_N.
 \end{aligned} \tag{3.259}$$

Substituting (3.256) into (3.259), we obtain

$$\begin{aligned}
 C_T &= \frac{1-a^N}{1-a} \left[(1-a)C_T - (1-a)C_N + (1-a)\frac{\Omega C_N}{\kappa L + \Omega} \right] + C_N + \frac{\xi}{v} C_N \\
 &= (1-a^N)C_T - (1-a^N)C_N + (1-a^N)\frac{\Omega C_N}{\kappa L + \Omega} + C_N + \frac{\xi}{v} C_N.
 \end{aligned} \tag{3.260}$$

Rearranging gives

$$a^N C_T = \left[a^N + \frac{\xi}{v} + (1-a^N)\frac{\Omega}{\kappa L + \Omega} \right] C_N. \tag{3.261}$$

Combining the terms in the bracket over the common denominator $v(\kappa L + \Omega)$, we obtain

$$a^N C_T = \frac{a^N v \kappa L + \Omega v + \xi \kappa L + \xi \Omega}{v(\kappa L + \Omega)} C_N. \tag{3.262}$$

Hence

$$C_N = \frac{v(\kappa L + \Omega)}{a^N v \kappa L + \Omega v + \xi \kappa L + \xi \Omega} a^N C_T. \tag{3.263}$$

Expression for the response

Using (3.249) and (3.263), we obtain

$$\begin{aligned}
 \text{Response} &= C_N + R^* \\
 &= C_N + \frac{v C_N}{\kappa L + \Omega} \\
 &= \frac{\kappa L + \Omega + v}{\kappa L + \Omega} C_N.
 \end{aligned} \tag{3.264}$$

Substituting (3.263), this becomes

$$\text{Response} = \frac{v(\kappa L + \Omega + v)}{a^N v \kappa L + \Omega v + \xi \kappa L + \xi \Omega} a^N C_T. \tag{3.265}$$

Calculation of $E_{\max}$

By definition,

$$E_{\max} = \lim_{L_T \rightarrow \infty} \text{Response}. \quad (3.266)$$

Using (3.265) and $L = L_T - C_T$, we get

$$E_{\max} = \lim_{L_T \rightarrow \infty} \frac{v(\kappa(L_T - C_T) + \Omega + v)}{a^N v \kappa(L_T - C_T) + \Omega v + \xi \kappa(L_T - C_T) + \xi \Omega} a^N C_T. \quad (3.267)$$

As in the previous models,

$$C_T \rightarrow R_T \quad \text{as } L_T \rightarrow \infty. \quad (3.268)$$

Dividing numerator and denominator by L_T , we obtain

$$E_{\max} = a^N R_T \lim_{L_T \rightarrow \infty} \frac{v \left(\kappa \left(1 - \frac{C_T}{L_T} \right) + \frac{\Omega}{L_T} + \frac{v}{L_T} \right)}{a^N v \kappa \left(1 - \frac{C_T}{L_T} \right) + \frac{\Omega v}{L_T} + \xi \kappa \left(1 - \frac{C_T}{L_T} \right) + \frac{\xi \Omega}{L_T}}. \quad (3.269)$$

Since

$$\frac{C_T}{L_T} \rightarrow 0, \quad \frac{\Omega}{L_T} \rightarrow 0, \quad \frac{v}{L_T} \rightarrow 0, \quad \frac{\xi \Omega}{L_T} \rightarrow 0 \quad \text{as } L_T \rightarrow \infty, \quad (3.270)$$

it follows that

$$E_{\max} = \frac{a^N v}{a^N v + \xi} R_T. \quad (3.271)$$

Calculation of EC_{50}

By definition, EC_{50} is the ligand concentration for which the response reaches one half of its maximal value. Since

$$E_{\max} = \frac{a^N v}{a^N v + \xi} R_T, \quad (3.272)$$

the half-maximal condition is

$$\frac{1}{2} \frac{a^N v}{a^N v + \xi} R_T = \frac{v(\kappa(L_T - C_T) + \Omega + v)}{a^N v \kappa(L_T - C_T) + \Omega v + \xi \kappa(L_T - C_T) + \xi \Omega} a^N C_T. \quad (3.273)$$

Cancelling the common factor $a^N v$ and rearranging, we obtain

$$\frac{R_T}{2(a^N v + \xi)} \left[a^N v \kappa(L_T - C_T) + \Omega v + \xi \kappa(L_T - C_T) + \xi \Omega \right] = C_T [\kappa(L_T - C_T) + \Omega + v]. \quad (3.274)$$

Solving for L_T yields

$$EC_{50} = \frac{R_T \left[\kappa C_T (a^N v + \xi) - \Omega(v + \xi) \right] + 2(a^N v + \xi) C_T (\Omega + v - \kappa C_T)}{\kappa(a^N v + \xi)(R_T - 2C_T)}. \quad (3.275)$$

Collecting the results, the kinetic proofreading model with limited and sustained signaling satisfies

$$\text{Response} = \frac{v(\kappa L + \Omega + v)}{a^N v \kappa L + \Omega v + \xi \kappa L + \xi \Omega} a^N C_T, \quad (3.276)$$

$$E_{\max} = \frac{a^N v}{a^N v + \xi} R_T, \quad (3.277)$$

and

$$EC_{50} = \frac{R_T \left[\kappa C_T (a^N v + \xi) - \Omega(v + \xi) \right] + 2(a^N v + \xi) C_T (\Omega + v - \kappa C_T)}{\kappa(a^N v + \xi)(R_T - 2C_T)}. \quad (3.278)$$

Thus this combined model incorporates both the finite duration of signaling through C_{N+1} and the transient persistence of signaling through R^* . As a consequence, both the response function and the maximal response differ from those of the models with only one of these two mechanisms.

3.4 Parameter Values

In this study, we investigated nine distinct models in order to assess how variation in parameter values influences the ability of the models to represent key phenotypic characteristics of TCR–pMHC interactions, such as specificity, sensitivity, and antigen discrimination. Considerable differences in parameter values are reported across the literature, which makes it challenging to select a consistent parameter framework for model comparison. To address this, we drew on four main literature sources [20, 30, 97, 98] and selected three representative values for each shared parameter—mean, maximum, and minimum—for use in the numerical simulations. This approach allows the comparative analysis to capture a biologically plausible range of outcomes while maintaining consistency across models. The resulting common parameter set is summarized in Table 3.1, while model-specific parameters are listed below.

The parameter values used here should be interpreted as a common phenomenological calibration for comparing model behaviour, rather than as a unique experimentally determined parameter set. In particular, many kinetic parameters for TCR–pMHC interactions have historically been obtained from three-dimensional experimental measurements, whereas TCR recognition *in vivo* takes place at a two-dimensional membrane interface. Since 2D and 3D measurements may produce different estimates of binding and dissocia-

tion kinetics, this issue is treated as an important limitation of the present comparison and is returned to in the Discussion [101].

3.4.1 Literature Sources for Parameter Values

The parameter values used in this comparative analysis were assembled from four published studies [20, 30, 97, 98]. These studies provided estimates for the principal quantities appearing across the different phenotypic models, including the receptor concentration R_T , ligand–receptor binding rate κ , phosphate concentration S_T , phosphorylation rate ϕ , spontaneous dephosphorylation rate b , and phosphatase efficiency γ . For the negative-feedback model, the SHP-1 activation and deactivation rates α and β were also taken from the literature. Since the reported values differ substantially across sources, we used them collectively to define a common and biologically plausible parameter framework for comparing all models.

This choice is useful for a controlled mathematical comparison, but it also has limitations. The sources used here do not necessarily estimate all parameters under identical experimental conditions, and kinetic parameters obtained from 3D solution-based assays are not directly interchangeable with parameters obtained from 2D membrane-based measurements. Thus, the present parameter set provides a consistent basis for comparing the models, while future refinements should incorporate improved 2D calibration whenever such data are available [101].

3.4.2 Common Parameter Set

To reduce dependence on any one source and to ensure comparability across models, we used a common parameter set for all shared parameters. In addition, parameters specific to particular model extensions were fixed according to the values used in the relevant source models and comparative framework.

In particular:

- in model (3), KPR with limited signaling [20], the value $\xi = 0.09$ is used; the same value is used for models (8) and (9);
- in model (4), KPR with sustained signaling [20], the value $\Omega = 0.001$ is used; the same value is used for model (9);
- in model (6), KPR with activation chain stabilization [97], the values

$$r_{\text{off}} = 1.5, \quad r_p = 1.03 \tag{3.279}$$

are used in the equations for $k_{\text{off}}(i)$ and $k_p(i)$, respectively;

- in model (8), KPR with limited signaling and incoherent feedforward loop [98],

$$d = 500 \text{ s}^{-1}, \quad c = 1 \text{ s}^{-1}, \quad b = 500 \text{ s}^{-1}, \quad a = 1 \text{ s}^{-1}, \quad (3.280)$$

$$m = 100, \quad l = 100, \quad \delta = 50 \text{ s}^{-1}, \quad \mu = 2.5 \text{ s}^{-1}, \quad \sigma = 0.5 \text{ s}^{-1}. \quad (3.281)$$

3.4.3 Mean, Minimum, and Maximum Values

To account for the variability in parameter values reported across the literature, we selected three representative values for each shared parameter: the mean, the maximum, and the minimum. These values were used in the numerical simulations to examine the robustness of the model predictions across a biologically plausible range of parameter choices. The resulting parameter values are summarized in Table 3.1.

Table 3.1. Literature-based parameter values used in the numerical simulations. The table reports mean, minimum, and maximum values for each parameter.

Parameter	Mean	Maximum	Minimum
Receptor per cell (R_T)	2.4×10^4	3×10^4	1.5708×10^4
Ligand–receptor binding rate (κ)	6.05×10^{-5}	10^{-4}	10^{-5}
Phosphate per cell (S_T)	5.25×10^5	6×10^5	3×10^5
Phosphorylation rate (ϕ)	0.535	1	0.05
Spontaneous dephosphorylation rate (b)	0.035	0.04	0.02
Phosphatase efficiency (γ)	2.93×10^{-4}	4.4×10^{-4}	1.2×10^{-6}
SHP-1 activation rate (α)	1.5×10^{-4}	2×10^{-4}	10^{-4}
SHP-1 deactivation rate (β)	0.503	1	6×10^{-3}

3.5 Numerical Comparison of Model Responses

In this section, we present the numerical simulations of the phenotypic models and compare their predictions with respect to the main biological benchmarks introduced earlier, namely specificity, sensitivity, antigen discrimination, and optimal response. Following the structure of the comparative analysis in the previous chapter, we examine for each model the response as a function of ligand concentration and dissociation time, together with the corresponding behaviour of $E_{\max}$ and EC_{50} . In the main text, we focus on the representative plots obtained using the mean parameter values, while the corresponding plots for the minimum and maximum parameter sets are presented in the appendix. This organization allows the behaviour of each model to be analysed individually before drawing an overall comparison across models.

3.5.1 Occupancy Model

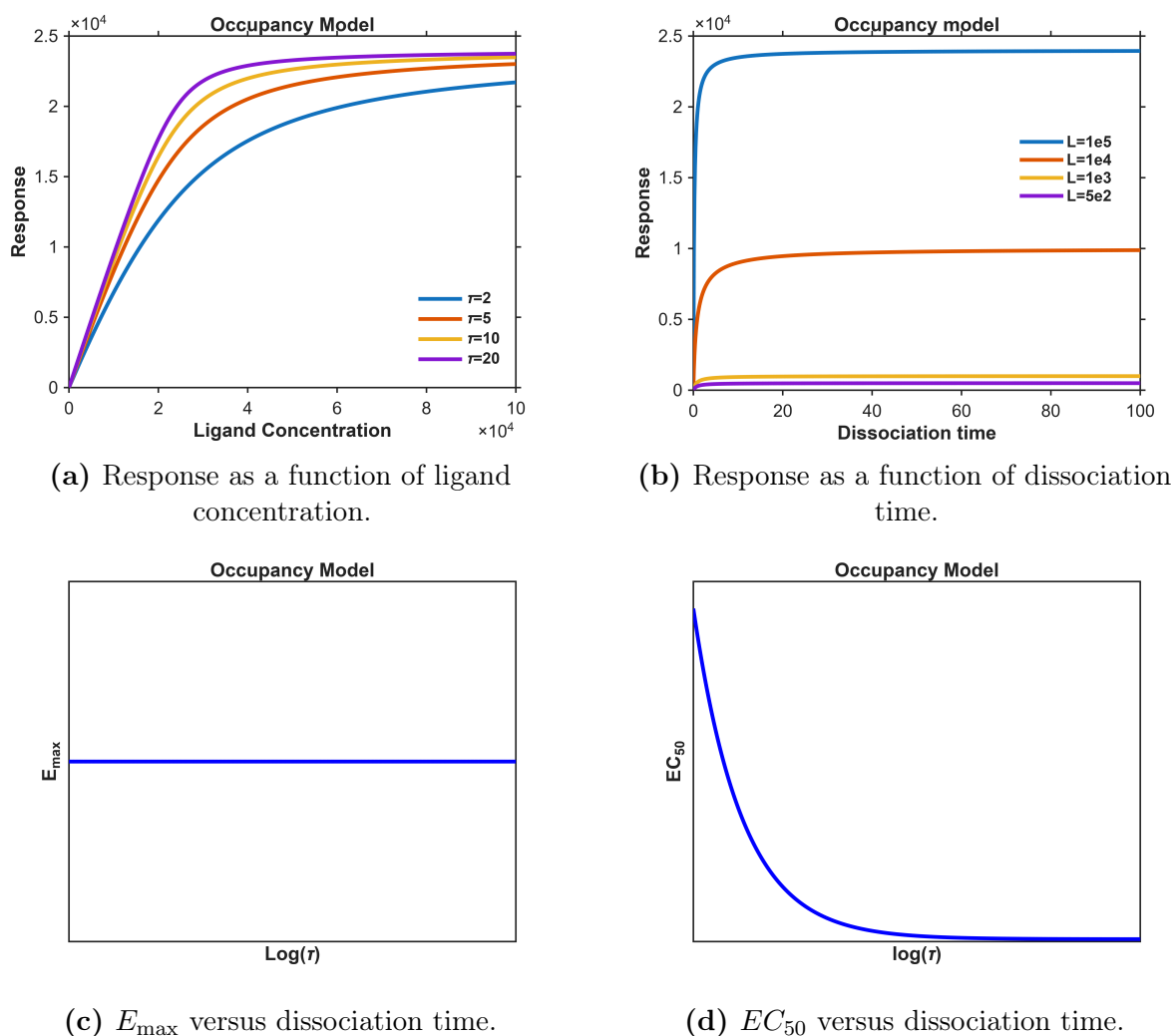


Figure 3.1. Numerical behaviour of the occupancy model for the mean parameter values. The four panels show the response as a function of ligand concentration and dissociation time, together with the corresponding behaviour of $E_{\max}$ and EC_{50} .

Figure 3.1 shows that the occupancy model produces purely monotone behaviour. In panel (a), the response increases with ligand concentration and approaches saturation, so no optimal response is observed with respect to ligand concentration. In panel (b), the response also increases with dissociation time, again without any interior optimum. Thus, increasing ligand availability or increasing binding persistence simply enhances activation in this model.

Panel (c) is the decisive feature of the model: $E_{\max}$ remains constant across dissociation times. This means that dissociation time affects how easily the response is reached, but not the highest response that can ultimately be attained. Panel (d) shows that EC_{50} decreases with dissociation time, indicating that ligands with longer binding times are more potent, since lower concentrations are required to reach half-maximal activation.

Taken together, these plots show that the occupancy model allows ligand concentration and binding quality to compensate for one another. In particular, even weakly binding ligands can in principle achieve the same maximal response as strongly binding ligands if their concentration is sufficiently high. This is the central limitation of the model. Although it captures the basic dependence of activation on receptor occupancy, it does not account for antigen discrimination and therefore fails to reproduce the experimentally observed distinction between weak and strong ligands. This result indicates that low-affinity pMHCs can still elicit a strong T-cell response when present at sufficiently high concentration. Since this does not agree with experimental observations, it reveals a central limitation of the occupancy model in describing antigen discrimination.

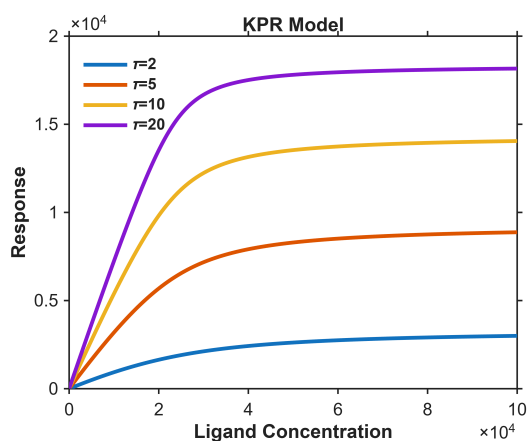
For this reason, the occupancy model is best viewed as a baseline for comparison with the subsequent models. It provides the simplest description of activation, but it cannot explain why strongly binding ligands can activate T cells at low concentrations whereas weakly binding ligands fail to do so even when their concentration is increased substantially.

3.5.2 Kinetic Proofreading

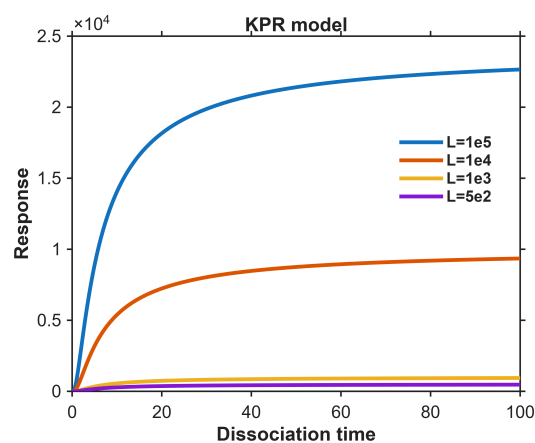
Figure 3.2 shows that the kinetic proofreading model differs qualitatively from the occupancy model by separating ligands according to dissociation time. In panel (a), the response increases with ligand concentration, but the dose-response curves remain clearly separated, with ligands of longer dissociation time producing stronger activation throughout. Thus, increasing ligand concentration enhances the response, but ligands with short binding times remain less effective at all doses.

Panel (b) shows that the response increases with dissociation time and does not exhibit an interior optimum. Ligands that remain bound for longer are more likely to complete the proofreading steps required for signaling, so activation increases monotonically with binding duration. Panel (c) shows that $E_{\max}$ also increases with dissociation time. Unlike the occupancy model, the maximum achievable response is therefore not fixed: ligands with short dissociation times are intrinsically limited in the level of activation they can produce. Panel (d) shows that EC_{50} decreases with dissociation time, indicating that ligands with longer binding times are more potent, since lower concentrations are required to reach half-maximal activation.

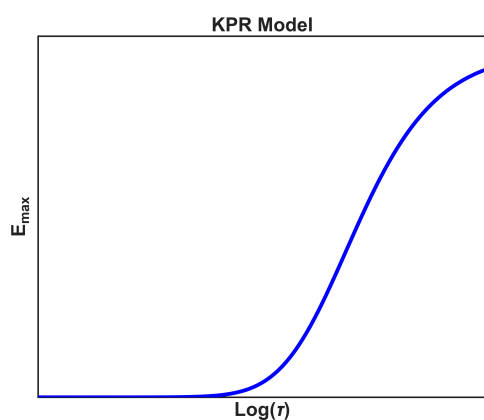
Taken together, these plots show that, in contrast to the occupancy model, high ligand concentration cannot compensate for short dissociation time. Merely increasing the concentration of pMHCs with short binding times does not produce the same level of response as pMHCs with longer binding times [104, 105]. In this sense, the kinetic proofreading model captures antigen discrimination through its dependence on dissociation time. However, since neither the response as a function of ligand concentration nor the



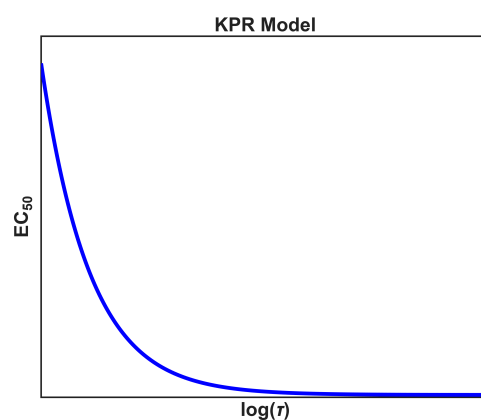
(a) Response as a function of ligand concentration.



(b) Response as a function of dissociation time.



(c) $E_{\max}$ versus dissociation time.



(d) EC_{50} versus dissociation time.

Figure 3.2. Numerical behaviour of the kinetic proofreading model for the mean parameter values. The four panels show the response as a function of ligand concentration and dissociation time, together with the corresponding behaviour of $E_{\max}$ and EC_{50} .

response as a function of dissociation time exhibits an optimum, the model still does not reproduce the full set of experimentally observed qualitative features of T-cell activation.

3.5.3 Kinetic Proofreading with Limited Signaling

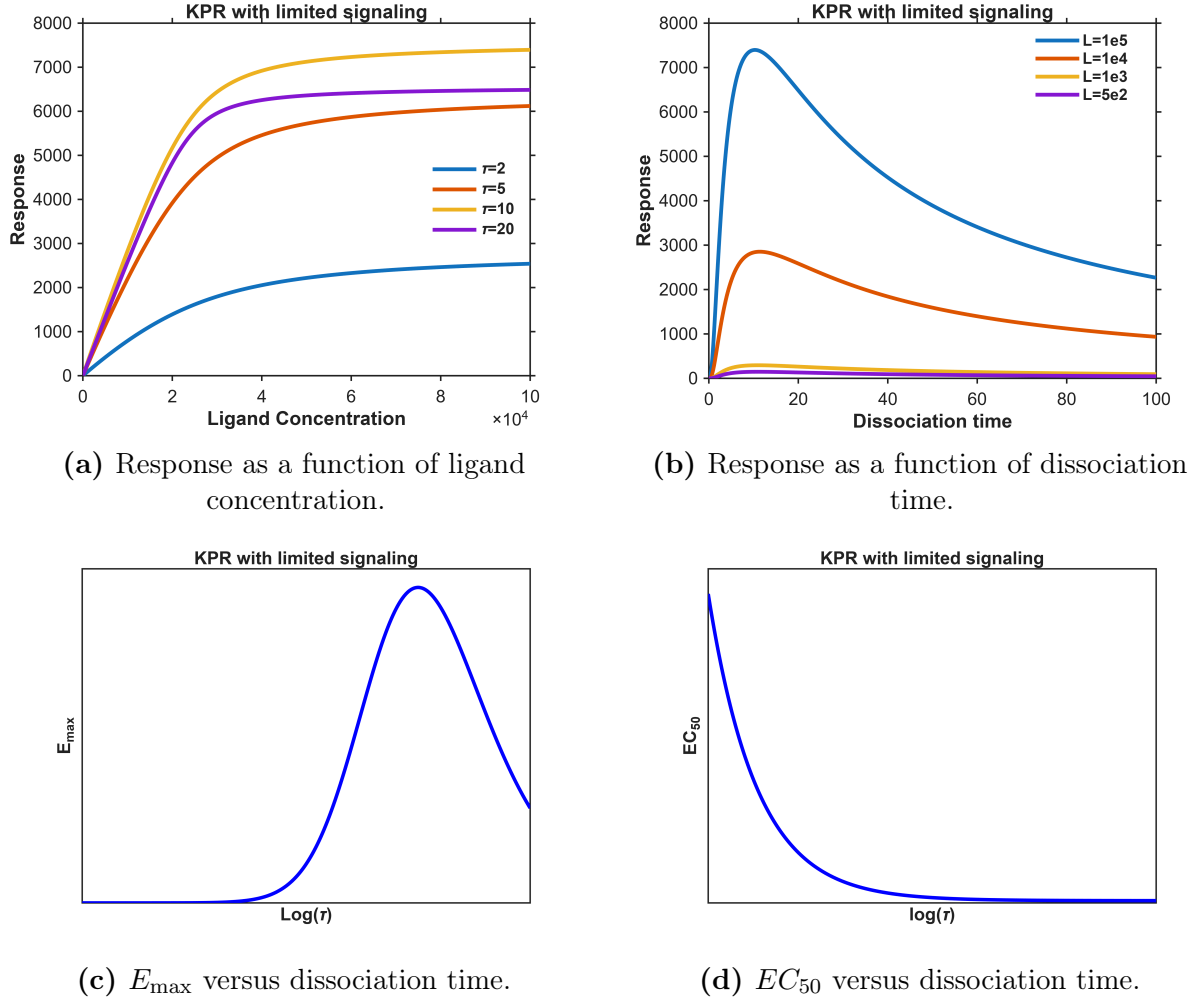


Figure 3.3. Numerical behaviour of the kinetic proofreading model with limited signaling for the mean parameter values. The four panels show the response as a function of ligand concentration and dissociation time, together with the corresponding behaviour of $E_{\max}$ and EC_{50} .

Figure 3.3 shows that the limited-signaling extension changes the qualitative behaviour of the basic KPR model in an important way. In panel (a), the response increases with ligand concentration and then approaches saturation, with longer-lived ligands producing stronger activation. However, the dose-response curves do not show a clear interior optimum with respect to ligand concentration.

Panel (b) shows the key feature of this model: the response as a function of dissociation time now exhibits an optimal dissociation time. For the mean parameter values, the response initially increases as ligands remain bound long enough to complete the proof-

reading steps, but then decreases once binding becomes too persistent. Thus, the most effective ligands are not those with the longest dwell times, but those with intermediate dissociation times.

This optimum can be understood as the result of a trade-off between proofreading and serial triggering. If the dissociation time is too short, ligands fail to remain bound long enough to complete the proofreading cascade efficiently. If it is too long, they remain trapped in stable but relatively unproductive complexes and are no longer able to engage multiple receptors sequentially. The response is therefore maximized at an intermediate dissociation time, where ligands remain bound long enough to trigger signaling but still dissociate quickly enough to permit repeated receptor engagement.

This pattern is also consistent with the broader simulation results, where an optimal dissociation time is clearly visible for the mean and maximum parameter values and becomes less pronounced for the minimum parameter set. It is likewise in line with experimental observations suggesting an optimal dissociation rate in T-cell activation [106, 107].

Panel (c) shows that $E_{\max}$ also exhibits optimality in this model. This indicates that the maximal achievable response is highest at an intermediate dissociation time rather than increasing indefinitely with binding persistence. In other words, very long-lived interactions are no longer always advantageous, because they reduce the ability of a ligand to sequentially trigger multiple receptors.

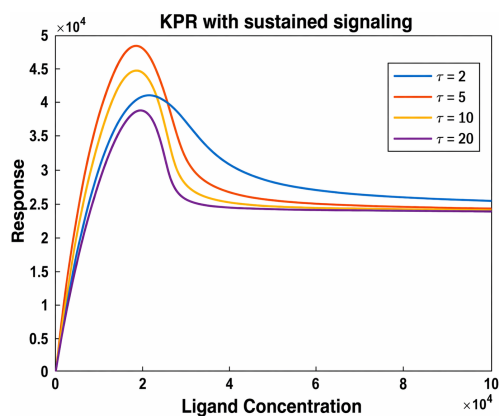
Panel (d) shows that EC_{50} continues to improve with dissociation time, so ligands with longer binding times remain more potent in the sense that they require lower concentrations to reach half-maximal activation. Nevertheless, the presence of an optimum in both the response and $E_{\max}$ means that potency alone is not sufficient to characterize overall effectiveness in this model.

Compared with the basic KPR model, this model provides a better qualitative account of experimentally observed T-cell behaviour. In particular, it captures the notion of an optimal dwell time and therefore improves the description of antigen discrimination and sensitivity, while still retaining the dependence of activation on dissociation kinetics.

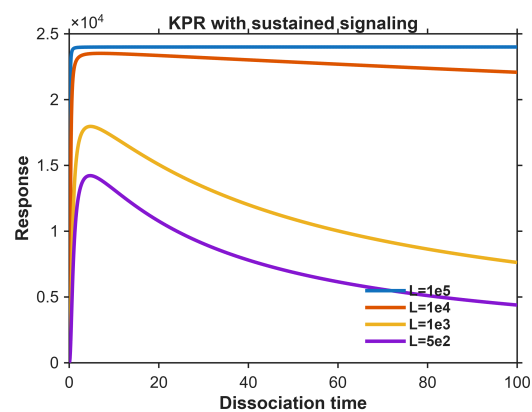
3.5.4 Kinetic Proofreading with Sustained Signaling

Figure 3.4 shows that the sustained-signaling extension produces a richer qualitative behaviour than the basic KPR model. In panel (a), the response as a function of ligand concentration is no longer purely monotone. Instead, the curves increase, reach a peak, and then level off. This indicates the presence of an optimum with respect to ligand concentration in this proofreading-based model.

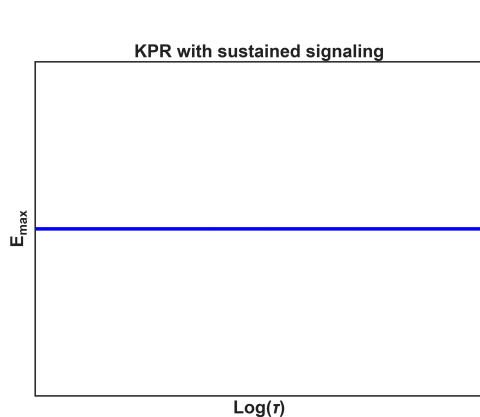
Panel (b) shows the response as a function of dissociation time. At lower ligand doses, the response displays a clear optimum at an intermediate dissociation time, whereas at



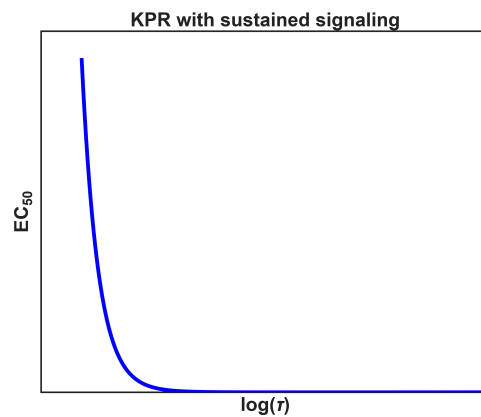
(a) Response as a function of ligand concentration.



(b) Response as a function of dissociation time.



(c) $E_{\max}$ versus dissociation time.



(d) EC_{50} versus dissociation time.

Figure 3.4. Numerical behaviour of the kinetic proofreading model with sustained signaling for the mean parameter values. The four panels show the response as a function of ligand concentration and dissociation time, together with the corresponding behaviour of $E_{\max}$ and EC_{50} .

higher doses this optimum becomes less visible or disappears. Thus, for small pMHC concentrations, neither very short-lived nor very long-lived interactions are most effective; rather, the strongest activation is achieved at an intermediate binding duration. At higher ligand doses, however, the requirement for an optimal dwell time is weakened.

This behaviour can be understood from the fact that signaling is allowed to persist after ligand unbinding. At low doses, activation is governed by a delicate balance between kinetic proofreading and serial engagement, so the strongest response is obtained at an intermediate dissociation time. If dissociation is too rapid, ligands fail to complete proofreading efficiently; if it is too slow, serial engagement is reduced. At higher pMHC doses, however, this balance changes. Even ligands with short dissociation times can maintain a substantial population of signaling-capable TCRs, because repeated encounters allow them to bind sustained-signaling receptors and prevent these receptors from returning to the basal state. As a result, the optimum with respect to dissociation time becomes less pronounced or may disappear at high doses.

Panel (c) shows that $E_{\max}$ is essentially independent of dissociation time in this model. This reflects one of the main consequences of sustained signaling: once a sufficient number of TCRs have reached a signaling-capable state, signaling can continue even after ligand unbinding, so the maximum achievable response is no longer strongly limited by the dissociation time itself. Panel (d) shows that EC_{50} remains correlated with dissociation time. Ligands with longer binding times achieve half-maximal activation at lower concentrations, indicating improved potency. However, as in the response plots, potency alone does not determine the overall effectiveness of the ligand, since the full response depends on both proofreading and the persistence of post-unbinding signaling.

Overall, this model captures several experimentally relevant features more successfully than the basic KPR model. In particular, it reproduces an optimal dissociation time under appropriate dose conditions and also shows an optimum with respect to ligand concentration. It therefore provides a more realistic description of T-cell activation, especially in situations where signaling is allowed to persist after ligand dissociation.

3.5.5 Kinetic Proofreading with Negative Feedback

Figure 3.5 shows that the introduction of negative feedback qualitatively changes the behaviour of the basic KPR model. In panel (a), the response as a function of ligand concentration rises initially, reaches a maximum, and then decreases sharply at higher concentrations. Thus, unlike the occupancy and basic KPR models, this model displays a clear optimum with respect to ligand concentration. The bell-shaped dose-response curves indicate that, at low ligand concentrations, the activation pathway dominates and the response increases with dose; beyond the peak, however, the negative regulator builds

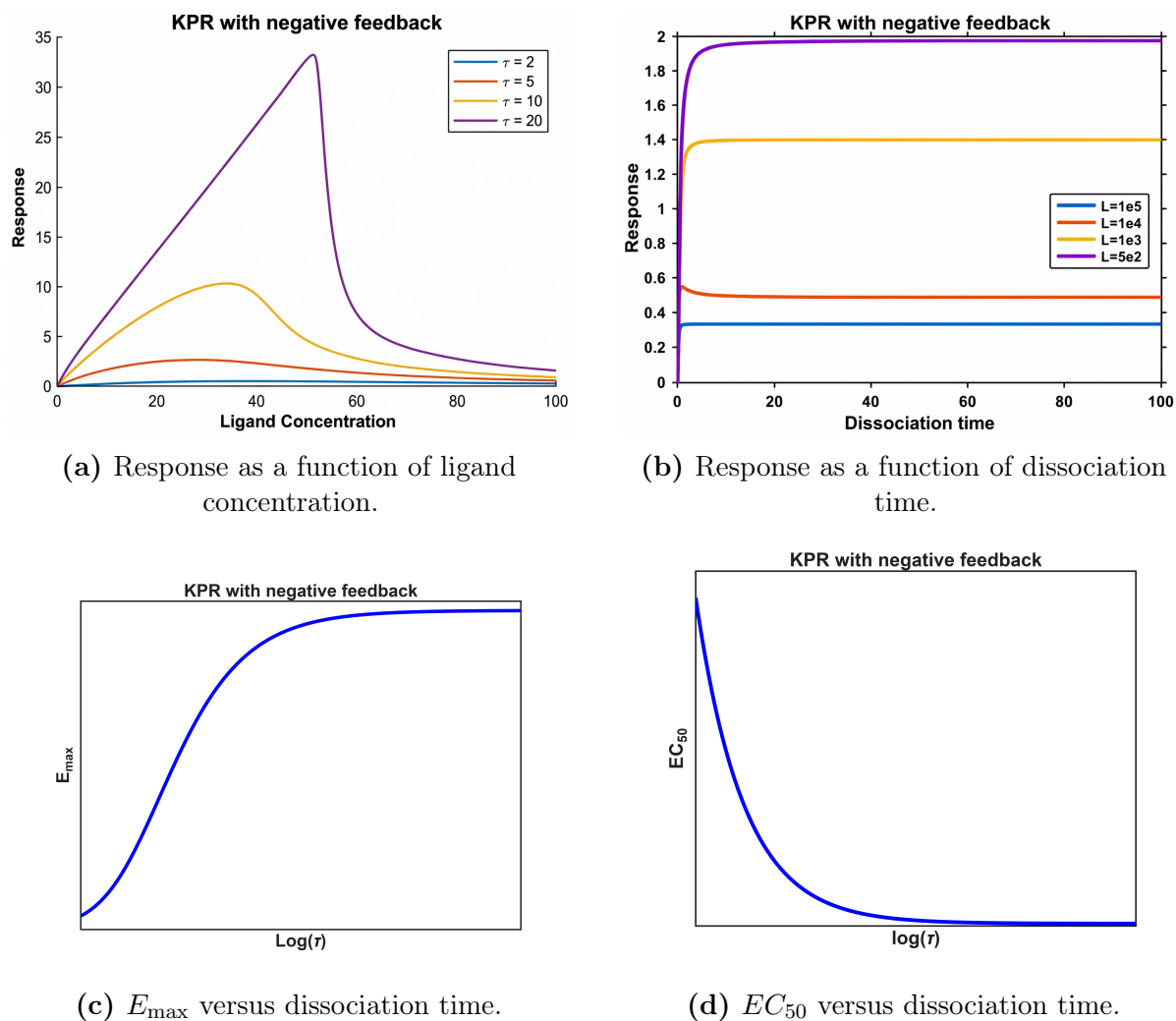


Figure 3.5. Numerical behaviour of the kinetic proofreading model with negative feedback for the mean parameter values. The four panels show the response as a function of ligand concentration and dissociation time, together with the corresponding behaviour of $E_{\max}$ and EC_{50} .

up sufficiently strongly to suppress the response, causing the curves to fall rapidly toward zero [60].

Panel (b) shows the response as a function of dissociation time. In the mean-parameter plots, the curves do not display a strong optimum across the full range shown. Nevertheless, the dependence on dissociation time is no longer purely monotone, and under suitable parameter conditions the model can produce a weak non-monotone response with respect to dissociation time [95]. Thus, the effect of dissociation time is more subtle here than in the models with limited or sustained signaling.

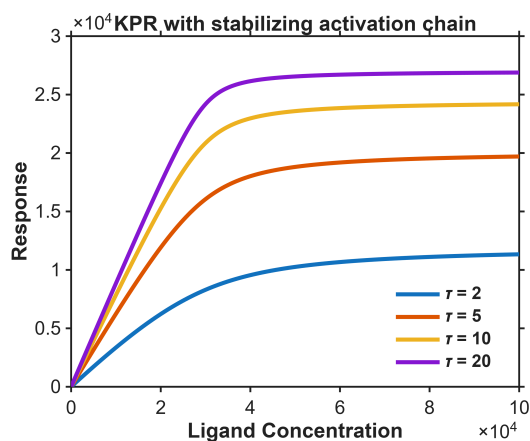
Panel (c) shows that $E_{\max}$ depends on dissociation time. Hence, the maximum achievable response is not fixed, but varies with ligand binding kinetics. In this respect, the model differs from the occupancy model and retains the proofreading feature that binding duration influences the level of achievable activation. Panel (d) shows that EC_{50} remains correlated with dissociation time. Ligands with longer dissociation times generally achieve half-maximal activation at lower concentrations, indicating improved potency. Nevertheless, the overall response is now shaped not only by proofreading but also by the buildup of the negative regulator, so potency alone is not sufficient to describe the full behaviour of the model.

Taken together, these plots show that negative feedback introduces an explicit regulatory mechanism that prevents unlimited growth of the response with increasing ligand concentration. This allows the model to reproduce an optimal dose response, which is absent in the simpler models. At the same time, the dependence of the response on dissociation time remains weaker and more parameter-sensitive than in models with a clear optimal dwell time. These results are consistent with earlier studies of negative-feedback models: the bell-shaped dose–response curve reflects inhibitory regulation at high ligand concentrations, while the weak optimum with respect to dissociation time agrees with earlier analyses showing that such non-monotone behaviour is possible only under suitable parameter conditions [60, 95].

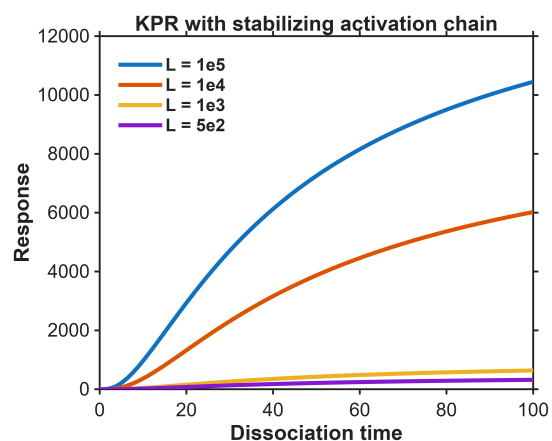
Overall, the KPR model with negative feedback provides a better qualitative description of regulated T-cell activation than the occupancy or basic KPR models. In particular, it captures the idea that activation should not increase indefinitely with ligand dose, but instead be limited by inhibitory control. However, since the optimum with respect to dissociation time is weak and parameter-dependent, the model does not fully reproduce all experimentally observed features of antigen discrimination.

3.5.6 Kinetic Proofreading with Stabilizing Activation Chain

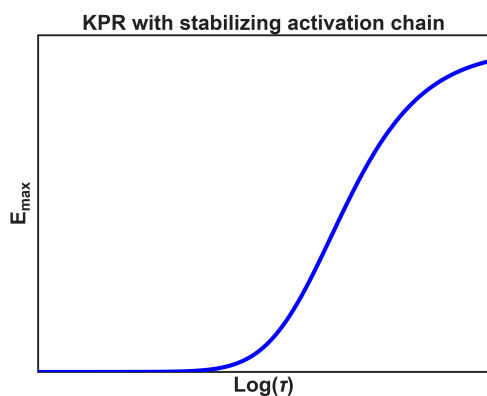
Figure 3.6 shows that the stabilizing-activation-chain model does not generate an optimal response with respect to either ligand concentration or dissociation time. In panel (a), the response increases with ligand concentration without exhibiting an interior maximum.



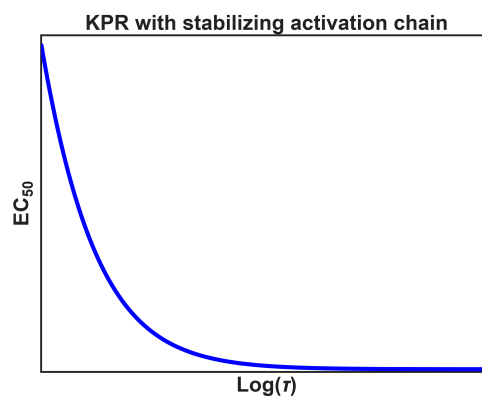
(a) Response as a function of ligand concentration.



(b) Response as a function of dissociation time.



(c) $E_{\max}$ versus dissociation time.



(d) EC_{50} versus dissociation time.

Figure 3.6. Numerical behaviour of the kinetic proofreading model with stabilizing activation chain for the mean parameter values. The four panels show the response as a function of ligand concentration and dissociation time, together with the corresponding behaviour of $E_{\max}$ and EC_{50} .

In panel (b), the response also varies monotonically with dissociation time, again without producing a clear optimum. Thus, despite the additional mechanism introduced in this model, the plots do not display the characteristic optimal behaviour observed in some of the other extensions of KPR.

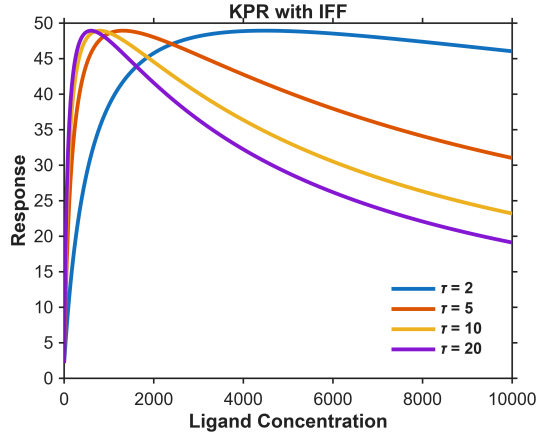
Panel (c), however, shows that $E_{\max}$ depends on dissociation time. This means that the maximum attainable response is still shaped by ligand binding kinetics, and in particular that ligands with shorter dissociation times are unable to generate the same level of activation as ligands with longer dissociation times. In this respect, the model retains the antigen-discrimination property associated with proofreading-based mechanisms. Panel (d) shows the corresponding behaviour of EC_{50} , which continues to vary with dissociation time. As in the previous proofreading-based models, ligands with longer binding times are more potent in the sense that lower concentrations are required to achieve half-maximal activation. However, this improvement in potency is not accompanied by an optimal response in either of the response plots.

These numerical results show that, although the model was designed to strengthen selectivity by progressively stabilizing some receptor–ligand complexes and weakening others, this mechanism does not produce an optimum in either ligand concentration or dissociation time [97]. Instead, its main effect is to preserve dissociation-time-dependent discrimination through the dependence of both $E_{\max}$ and EC_{50} on binding kinetics. In particular, ligands with short dissociation times remain unable to generate the same level of activation as ligands with longer dissociation times, even when their concentration is increased.

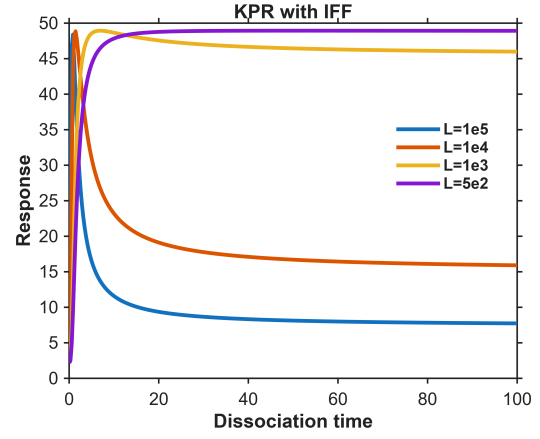
The main interpretation of these plots is that, although the model preserves antigen discrimination through its dependence on dissociation time, it does not reproduce the broader set of experimentally observed phenotypic characteristics. In particular, neither the response as a function of ligand concentration nor the response as a function of dissociation time shows an optimum. For this reason, the model improves on the occupancy model in terms of discrimination, but still fails to exhibit the key qualitative features expected of a more complete description of T-cell activation.

3.5.7 Kinetic Proofreading with Incoherent Feed Forward Loop

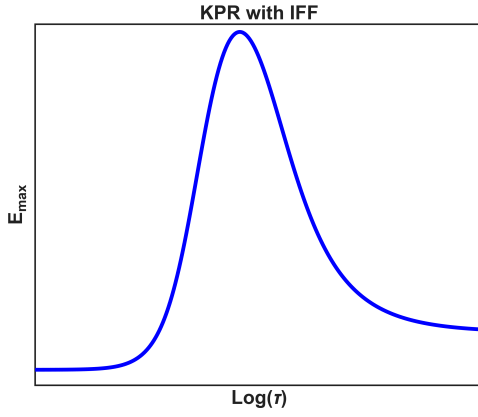
Figure 1.7 shows that the addition of an incoherent feed forward loop produces a markedly different behaviour from the previous proofreading-based models. In panel (a), the response as a function of ligand concentration exhibits an optimum for ligands with higher dissociation times, whereas this optimum is lost for lower dissociation times. Another important feature of this panel is that the relative ranking of ligands changes across the dose–response curves. At low ligand concentrations, the ligand with the longer dissociation time generates the strongest response on the left side of the peak, whereas this ordering



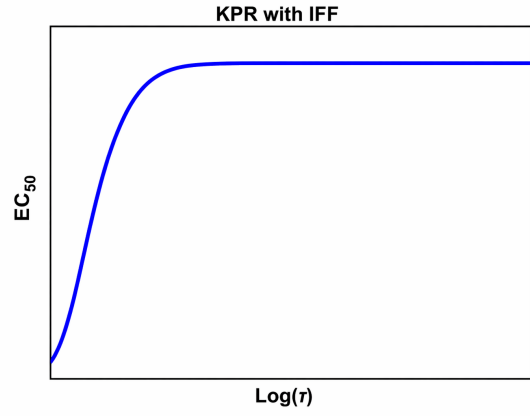
(a) Response as a function of ligand concentration.



(b) Response as a function of dissociation time.



(c) $E_{\max}$ versus dissociation time.



(d) EC_{50} versus dissociation time.

Figure 3.7. Numerical behaviour of the kinetic proofreading model with incoherent feed forward loop for the mean parameter values. The four panels show the response as a function of ligand concentration and dissociation time, together with the corresponding behaviour of $E_{\max}$ and EC_{50} .

can change as the ligand concentration increases. Thus, ligand effectiveness in this model depends not only on binding kinetics, but also on the dose regime in which the response curve is evaluated. This dose-dependent ranking differs from the behaviour reported in the original IFF-based study, where the model was not retained because it produced an ordering of ligand responses that was inconsistent with the expected biological hierarchy [30].

Panel (b) shows that the response as a function of dissociation time also displays an optimum, but only at higher ligand doses. At lower doses, the optimum disappears. Thus, in this model the presence or absence of an optimal dissociation time depends strongly on the dose regime. This can be explained by the fact that low-affinity ligands produce a lower peak level of the signaling component C_N . If this peak remains below the saturation threshold of the activating intermediate Y , then even at high concentrations the inhibitory branch is not sufficiently engaged to create an optimum. In this respect, the model differs from the sustained-signaling model: here the optimum with respect to dissociation time appears at higher doses and is lost at lower doses.

Panel (c) shows that $E_{\max}$ itself exhibits optimality. This is one of the distinguishing features of the IFF model and suggests that the dose-response curves may intersect for certain dissociation times. This intersection behaviour is visible in the plots and implies that ligand effectiveness is influenced not only by binding kinetics, but also by the dose at which the ligand is presented. In this sense, earlier studies also support the view that antigen effectiveness depends not only on receptor-binding properties, but also on the presentation context and dosage [108, 104].

Panel (d) shows that EC_{50} also varies with dissociation time. However, this variation must be interpreted together with the non-monotone response and the optimality in $E_{\max}$, since potency alone no longer determines the full activation profile.

Taken together, these plots show that the incoherent feed forward loop introduces a balance between activation and inhibition that can generate non-monotone responses in both ligand concentration and dissociation time. The model therefore captures a substantial part of the observed phenotypic characteristics. In particular, it can reproduce optimal response behaviour and dose-dependent ranking of ligands, features that are absent in simpler frameworks such as the occupancy model and the basic KPR model.

3.5.8 Kinetic Proofreading with Limited Signaling and Incoherent Feed Forward Loop

Figure 3.8 shows that combining limited signaling with an incoherent feed forward loop produces one of the richest response patterns among all the models considered. In panel (a), the response as a function of ligand concentration exhibits a clear optimum for high-affinity ligands, whereas this optimum becomes weaker or disappears for ligands with lower affinity.

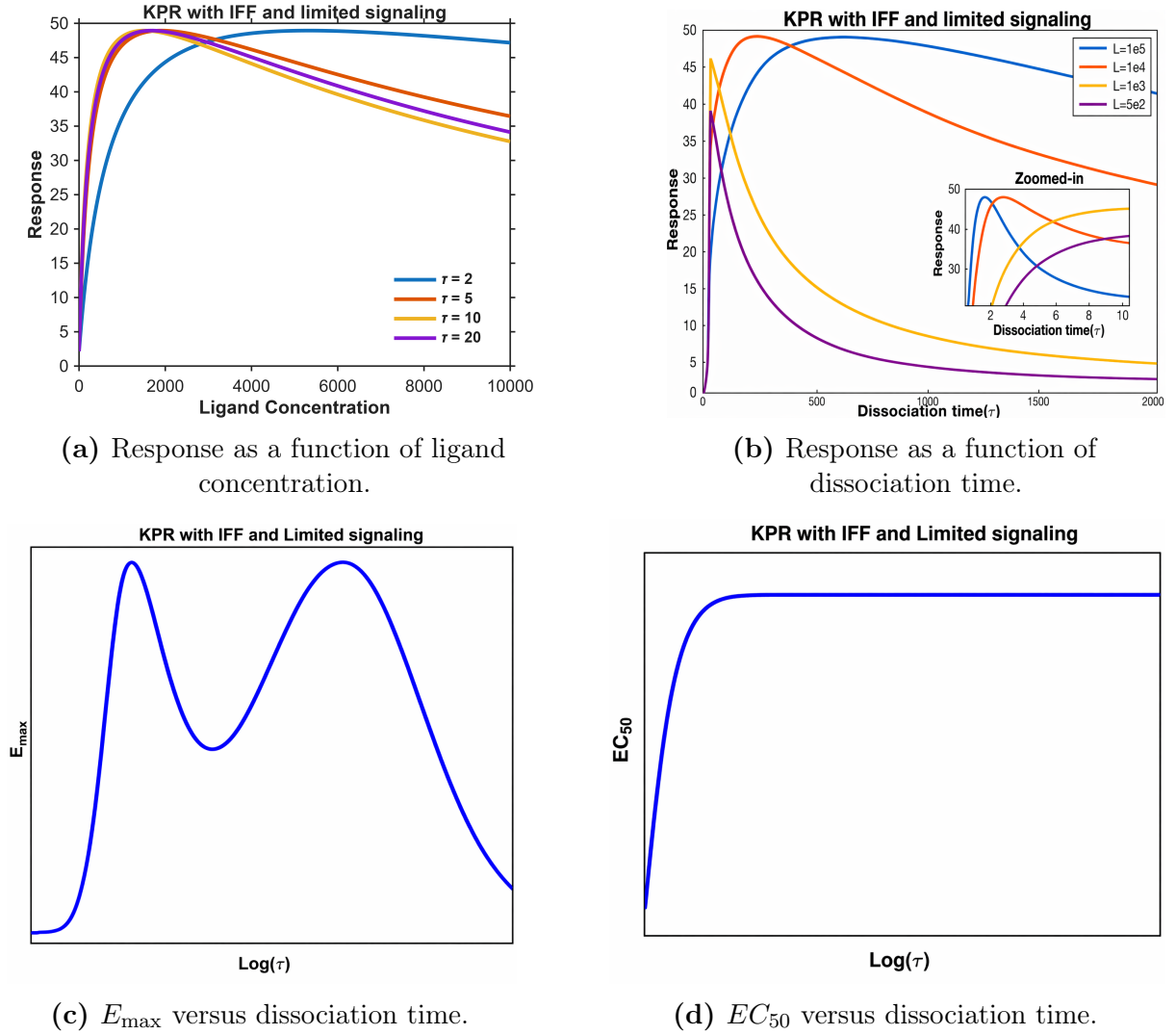


Figure 3.8. Numerical behaviour of the kinetic proofreading model with limited signaling and incoherent feed forward loop for the mean parameter values. The four panels show the response as a function of ligand concentration and dissociation time, together with the corresponding behaviour of $E_{\max}$ and EC_{50} .

Another important feature is that the high-affinity ligands produce the strongest response on the left side of the peak. This indicates that ligand ranking depends not only on dose, but also on where the response curve is sampled relative to the optimum. This qualitative behaviour is consistent with the findings reported for the original limited-signaling and IFF model [109, 101].

Panel (b) shows that the response as a function of dissociation time is no longer simply monotone or single-peaked. Instead, the curves display a bimodal pattern, with two distinct response maxima separated by a region of reduced activation. This bimodal behaviour is one of the most distinctive qualitative features of the model and is consistent with the observation in our published comparative study that the model combining limited signaling with an incoherent feedforward loop can generate a unique bimodal response with respect to dissociation time [101]. Mechanistically, the first peak can be interpreted as arising from the usual balance between kinetic proofreading and limited signaling: very short-lived ligands dissociate too rapidly to reach the signaling-competent state, whereas very long-lived ligands may reduce serial engagement. The second peak reflects the additional regulatory balance introduced by the incoherent feedforward loop, which creates another regime in which activation and inhibition interact differently.

Panel (c) shows that $E_{\max}$ also exhibits a bimodal dependence on dissociation time. Thus, the two-peak structure is not restricted to the response at fixed doses, but also appears in the maximum achievable activation. This indicates that the underlying mechanism generates two preferred kinetic regimes in which signaling can be especially effective. However, the biological interpretation of the second peak requires caution. While bimodal or biphasic responses have been reported in other immune and regulatory contexts, including biphasic regulation of IL-2 transcription, bimodal effects of endogenous IL-6, and two-phase metabolic adaptation in T cells [110, 111, 112], the relevant question in the present TCR-pMHC setting is whether both peaks occur within experimentally realistic ranges of dissociation time.

Panel (d) shows the behaviour of EC_{50} , which continues to vary with dissociation time. However, this variation must be interpreted together with the bimodal structure of the response, since the overall activation profile is no longer determined by a single monotone trend and potency alone no longer characterizes the full response. In particular, if the second response maximum occurs only at very long effective dissociation times, it should not be interpreted as an established biological feature of T-cell activation. Rather, it should be viewed as a model prediction whose physiological relevance depends on the kinetic parameter range used in the simulations.

This point is especially important because the kinetic parameters used in the present comparison are largely derived from three-dimensional measurements, whereas TCR-pMHC recognition occurs at the two-dimensional membrane interface between a T cell and an antigen-presenting cell. As discussed in [101], 2D and 3D measurements can differ

substantially in their kinetic values, units, and biological interpretation. Future simulations using 2D-calibrated values of k_{on} , k_{off} , and K_D , together with dense experimental sampling of ligand dissociation times, would therefore be needed to determine whether the second peak remains within a physiologically meaningful range. Experimental studies also show that T-cell responsiveness depends not only on receptor-binding kinetics, but also on antigen presentation format, ligand dose, and presentation context [104, 107, 106].

Taken together, these plots show that the simultaneous presence of limited signaling and incoherent feedforward regulation creates a more complex balance between activation, inhibition, and repeated receptor engagement. In particular, the model is able to produce both an optimal response with respect to ligand concentration and a bimodal response with respect to dissociation time. Overall, this model captures a broader range of phenotypic characteristics than the simpler proofreading-based frameworks. However, the bimodal kinetic signature should be treated as a hypothesis-generating prediction of the model, rather than as a confirmed experimental property, until it is tested using physiologically realistic kinetic measurements.

3.5.9 Kinetic Proofreading with Limited and Sustained Signaling

Figure 3.9 shows that the hybrid model combining limited and sustained signaling retains the most important qualitative features of both parent models. In panel (a), the response as a function of ligand concentration is non-monotone and exhibits a clear optimum. This behaviour is consistent with the sustained-signaling model, but here the peak is more pronounced and the non-monotone structure is easier to distinguish.

Panel (b) shows that the response as a function of dissociation time also has a clear optimum. This agrees with the behaviour of the limited-signaling model, but in the combined model the optimum is retained even at high pMHC doses, unlike the sustained-signaling model where the optimum may disappear at high doses. Thus, the present model preserves an intermediate preferred dissociation time over a broader range of ligand concentrations. This indicates that the combination of limited signaling and post-unbinding signal persistence creates a more robust balance between proofreading and serial engagement than either mechanism alone.

Panel (c) shows that E_{max} also exhibits an optimum. This is one of the strongest features of the model, since it implies that the maximum achievable response is highest at an intermediate dissociation time rather than increasing indefinitely. The presence of an optimum in E_{max} also suggests that the dose–response curves may overlap at particular dissociation times. In practical terms, this means that at low doses one antigen may induce a stronger T-cell response than another, while at higher doses their relative performance can be reversed [108, 104, 101].

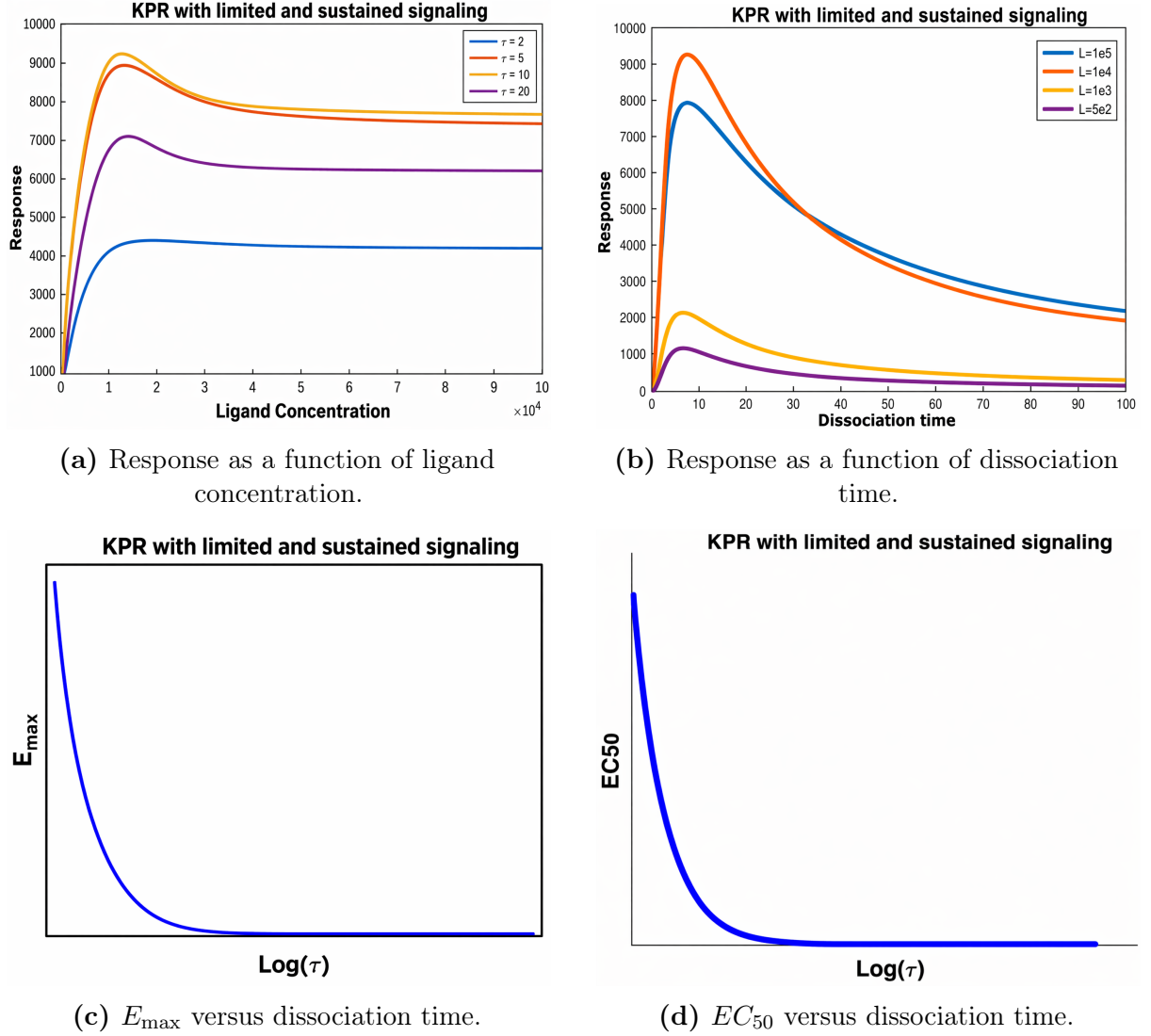


Figure 3.9. Numerical behaviour of the kinetic proofreading model with limited and sustained signaling for the mean parameter values. The four panels show the response as a function of ligand concentration and dissociation time, together with the corresponding behaviour of $E_{\max}$ and EC_{50} .

Panel (d) shows the corresponding behaviour of EC_{50} . As in the other models, potency remains correlated with dissociation time, so ligands with more favorable binding kinetics generally require lower concentrations to reach half-maximal activation. However, in this model the full response cannot be judged from potency alone, because both the response and $E_{\max}$ are themselves non-monotone.

Taken together, these plots show that the combination of limited signaling and sustained signaling produces a robust balance between proofreading, serial engagement, and signal persistence after ligand unbinding. This allows the model to retain an optimal dissociation time, display an optimum with respect to ligand concentration, and generate optimality in $E_{\max}$ at the same time. Among the models considered here, this hybrid architecture captures the broadest combination of sensitivity, discrimination, regulated specificity, and optimal response, making it one of the strongest candidates for reproducing the experimentally observed qualitative features of T-cell activation.

3.5.10 Comparative Assessment of the Models

Taken together, the numerical simulations show that the nine models differ substantially in their ability to reproduce the principal biological benchmarks of T-cell activation, namely specificity, sensitivity, antigen discrimination, and optimal response. The simpler models, especially the occupancy model and the standard kinetic proofreading model, provide useful baseline descriptions but do not account for the full range of experimentally observed behaviour. In the occupancy model, ligand concentration and affinity can compensate for one another, so weak ligands may still produce strong activation if supplied at sufficiently high concentration. The standard kinetic proofreading model improves on this by introducing discrimination through dissociation time, but it still does not generate strong optimality in the response.

The stabilizing-activation-chain model preserves dissociation-time-dependent discrimination, since both $E_{\max}$ and EC_{50} remain sensitive to binding kinetics. However, like the occupancy and standard KPR models, it does not produce an optimum with respect to either ligand concentration or dissociation time. Thus, although it sharpens kinetic discrimination, it does not recover the broader set of experimentally observed phenotypic features.

The models incorporating limited or sustained signaling provide a substantially better qualitative description of T-cell activation. Limited signaling introduces a natural trade-off between proofreading and serial engagement, which leads to an optimal dissociation time. Sustained signaling, on the other hand, allows signaling to persist after ligand unbinding and thereby reproduces additional experimentally relevant behaviour, including an optimum with respect to ligand concentration. These two model classes therefore capture important features associated with both sensitivity and discrimination.

The negative-feedback model contributes a different mechanism, namely regulatory control through inhibition. Its main strength is the appearance of a bell-shaped dose–response curve, showing that activation need not increase indefinitely with ligand concentration. In this way, it captures an important aspect of regulated activation by preventing uncontrolled responses at high ligand dose. However, its optimal response with respect to dissociation time is relatively weak and parameter-dependent, so its performance remains more limited than that of the most successful hybrid models.

The models containing incoherent feed forward loops add another layer of complexity and produce some of the most distinctive qualitative behaviours in the study. In particular, they reproduce non-monotone dose–response relationships, dose-dependent ranking of ligands, and intersection of response curves. These are important experimentally observed features, since they indicate that ligand effectiveness is determined not only by binding kinetics but also by the dose at which the ligand is presented. The model combining limited signaling with an incoherent feed forward loop is especially notable for its bimodal dependence on dissociation time, indicating that more than one preferred kinetic regime may exist for efficient activation.

Among all models considered here, the hybrid model combining limited and sustained signaling provides the broadest agreement with experimental expectations. It reproduces an optimal response with respect to both ligand concentration and dissociation time, exhibits optimality in $E_{\max}$, preserves strong antigen discrimination, and retains high sensitivity across a wide range of conditions. For this reason, it appears to offer the most complete phenotypic description among the models considered in this thesis.

Overall, the comparative analysis suggests that no single simple mechanism is sufficient to account for the full qualitative behaviour of T-cell activation. Instead, the most successful models are those that combine several interacting effects, including proofreading, limited signaling, sustained signaling, feedback regulation, and feed forward control. This supports the view that T-cell activation is not governed by a single isolated process, but rather by the interplay of multiple regulatory mechanisms acting across different kinetic and signaling regimes.

3.6 Validation of the Negative-Feedback Model

In this section, we revisit earlier results for the kinetic proofreading model with negative feedback, focusing on two qualitative properties established previously: the existence of multiple positive steady states and the possibility of a non-monotone response as a function of dissociation time [95]. This model is particularly important because, unlike most of the other phenotypic models studied in this thesis, it does not in general possess a unique positive steady state.

The earlier analysis of Rendall and Sontag showed that, for suitable parameter values, the negative-feedback model can admit three positive steady states and that this number cannot be exceeded [95]. The same study also demonstrated that the response function can become non-monotone with respect to dissociation time for appropriate parameter choices [95]. However, many of the parameter values used in that study were chosen in an arbitrary way, so it remained necessary to check whether the same conclusions persist under biologically motivated parameter choices.

To address this question, we repeated the analysis using the parameter framework employed throughout this thesis, namely values assembled from four literature sources and summarized through representative mean, minimum, and maximum values. In this way, the validation of the negative-feedback model was carried out under the same assumptions used in the comparative study of all nine models.

Our analysis confirmed the existence of exactly three positive steady states for the negative-feedback model, and all three were found to lie within the biologically feasible region. We further examined their stability and found that two of these steady states are stable. Thus, the multistationarity predicted in the earlier theoretical work is not merely an artifact of unrealistic parameter choices, but persists under experimentally motivated parameter values.

We next examined the response as a function of dissociation time. The earlier study predicted that the response can be non-monotone under suitable parameter conditions [95]. Our numerical results support this conclusion. In particular, we observed an optimal behaviour with respect to dissociation time, although for the mean parameter values this optimum is only weakly pronounced. A slight increase in the phosphorylation rate makes this peak much clearer, showing that the prominence of the non-monotone response depends sensitively on the parameter regime, especially on the phosphorylation rate.

Taken together, these results validate the main qualitative conclusions previously reported for the kinetic proofreading model with negative feedback. The model can indeed exhibit multiple positive steady states under biologically motivated parameter values, and it can also generate a non-monotone response with respect to dissociation time. At the same time, the present analysis clarifies that the strength of the optimal response is parameter-dependent and may remain modest for the mean values considered in this thesis.

3.7 Sensitivity Analysis

Sensitivity analysis is an essential tool for studying biological systems because it reveals how variations in parameter values influence system behavior and highlights the factors with the greatest impact on model outputs. It also supports model validation and refinement by distinguishing parameters that require precise estimation from those whose

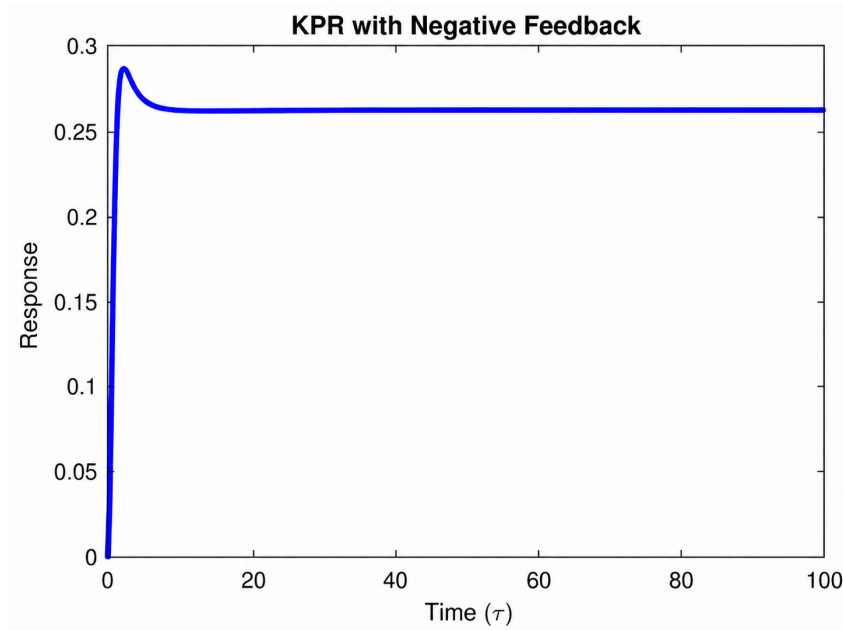


Figure 3.10. Validation of the negative-feedback model. The response is shown as a function of ligand dissociation time at fixed ligand concentration, illustrating how changes in phosphorylation rate affect the strength of the optimal response.

variation has a comparatively smaller effect on the dynamics. In this study, we used the Latin Hypercube Sampling–Partial Rank Correlation Coefficient (LHS–PRCC) approach to assess the robustness of the model family under parameter uncertainty [74, 113, 114, 115, 116].

3.7.1 Methodology: LHS–PRCC

To investigate parameter sensitivity across all model systems, we performed a global sensitivity analysis based on Latin Hypercube Sampling with Partial Rank Correlation Coefficients (LHS–PRCC). This approach is well suited to biological models with uncertain parameters because it allows efficient exploration of the multidimensional parameter space with a manageable number of simulations while quantifying the direction and strength of association between each parameter and the model output [74, 113, 114, 115, 116].

In the LHS step, model parameters were treated as uncertain quantities and sampled over biologically plausible ranges. The minimum and maximum values for each parameter were taken from the ranges reported in the literature, while the mean values were used as the baseline parameter set. Using these bounds, we carried out 10,000 simulations for each model in order to generate a broad ensemble of parameter combinations and thereby capture the uncertainty associated with parameter estimation.

The Latin Hypercube Sampling procedure ensures that the full range of each parameter is explored in a structured and space-filling way, making it more efficient than naive random sampling. For each sampled parameter set, the corresponding model response was

computed, and the PRCC was then used to evaluate the independent relationship between each parameter and the model output while controlling for the effects of the remaining parameters. In this way, PRCC provides a rank-based measure of the contribution of each parameter to variation in the response.

The sign of the PRCC indicates the direction of the parameter effect. A positive PRCC value means that increasing the parameter tends to increase the model output, whereas a negative PRCC value means that increasing the parameter tends to decrease the output. The magnitude of the PRCC reflects the strength of the association, so parameters with larger absolute PRCC values are interpreted as having a stronger influence on model behavior.

The sensitivity analysis results for all models are displayed in Fig. 3.11, where the PRCC profiles provide a direct visual comparison of the relative influence of parameters across the different signaling architectures.

Overall, this methodology provides a systematic framework for ranking sensitive parameters in each model and for comparing how parameter dependence changes across different signaling architectures.

The sensitivity analysis results for all models are summarized in Fig. 3.11, which enables direct comparison of the relative importance of parameters across the different signaling architectures. As shown by the PRCC profiles, the influence of parameters on model output is not uniform across the model family, but instead depends strongly on the underlying regulatory structure of each model.

A consistent result across several models is the strong positive influence of the phosphorylation rate ϕ . In the classical kinetic proofreading (KPR) model, the KPR model with negative feedback, the KPR model with limited signaling, and the KPR model with limited and sustained signaling, ϕ has a positive PRCC value and appears among the most influential parameters. This indicates that faster progression through the proofreading cascade promotes the accumulation of signaling-competent receptor states and thereby enhances the activation response.

By contrast, the ligand–receptor binding rate κ generally has only a small positive effect on the response. Although receptor engagement is necessary to initiate signaling, the PRCC results suggest that variation in κ is usually less important than variation in downstream parameters controlling proofreading, persistence of signaling, or feedback regulation.

The parameters associated with signal loss or deactivation tend to reduce activation. In the occupancy and standard KPR models, the off-rate v has a small negative effect, consistent with the fact that rapid dissociation decreases the probability that a receptor–ligand complex remains bound long enough to generate a productive signal. In the models with limited and sustained signaling, strongly negative PRCC values are associated with ξ and Ω , showing that faster transition to a non-signaling state or shorter persistence of

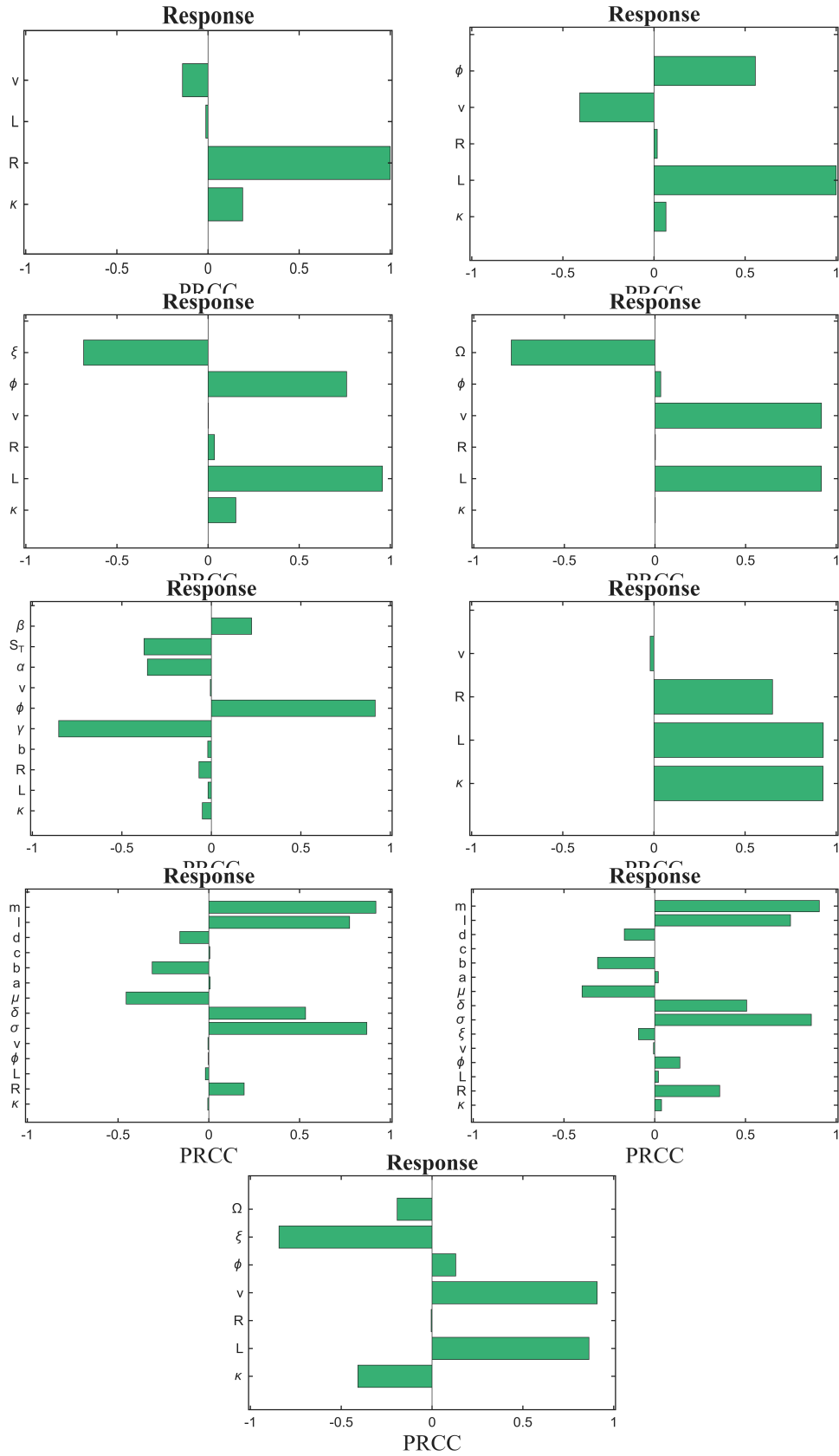


Figure 3.11. PRCC plots for the nine phenotypic models of T-cell activation, showing the comparative sensitivity of model responses to parameter variation.

post-unbinding signaling suppresses the overall response. In the more complex models, however, the off-rate v does not appear to influence activation as strongly.

A particularly clear antagonistic pattern appears in the KPR model with negative feedback. In this case, phosphorylation rate ϕ contributes positively to activation, whereas phosphatase efficiency γ contributes negatively. Thus, the sensitivity analysis reflects the competition between signal propagation and phosphatase-mediated suppression, which is central to the behavior of the feedback-regulated model.

For the models containing incoherent feedforward loops, the PRCC patterns reflect the balance between enhancing and inhibitory branches. The amplification parameters σ and δ have positive PRCC values, indicating that they strengthen activation, whereas the inhibitory parameter μ has a negative PRCC value, indicating suppression of the signal. These models therefore show particularly clearly how the final output depends on the interplay between activation and inhibition.

Finally, ligand concentration itself contributes positively to activation across the model family. Overall, the PRCC analysis shows that the dominant parameters are those controlling progression through the proofreading cascade, persistence of signaling, negative regulation, and amplification–inhibition balance. Among these, phosphorylation-related parameters emerge as the most consistently important determinants of the response.

3.7.2 Biological Interpretation of Sensitive Parameters

The sensitivity patterns shown in Fig. 3.11 provide a biological interpretation of how different molecular processes shape T-cell activation. The strong positive influence of the phosphorylation rate ϕ indicates that efficient receptor modification is a central requirement for productive signaling. Biologically, this means that faster phosphorylation increases the likelihood that receptor–ligand complexes progress through the proofreading cascade and reach signaling-competent states capable of triggering downstream immune responses.

The relatively small positive contribution of the ligand–receptor binding rate κ suggests that initial receptor engagement, although necessary, is not by itself sufficient to determine the strength of the response. In biological terms, ligand binding acts mainly as the initiating step, while the magnitude and persistence of T-cell activation depend more strongly on downstream signaling events than on binding alone.

The negative effects associated with v , ξ , and Ω reflect the importance of signaling duration. A larger off-rate v shortens receptor–ligand contact and thereby reduces the probability of successful proofreading. Similarly, larger values of ξ and Ω reduce the time for which signaling can persist, either by accelerating transition to non-signaling receptor states or by shortening post-unbinding signal maintenance. These results are biologically

consistent with the idea that T-cell activation depends not only on ligand recognition, but also on how long signaling is sustained once initiated.

In the negative-feedback model, the negative role of phosphatase efficiency γ highlights the suppressive function of phosphatase-mediated regulation. Biologically, increased phosphatase activity strengthens inhibitory feedback and limits excessive activation, whereas higher phosphorylation pushes the system in the opposite direction. The model therefore captures the regulatory balance between activation and suppression that controls the strength of T-cell signaling and helps prevent inappropriate activation.

For the incoherent feedforward loop models, the positive effects of σ and δ and the negative effect of μ reveal a biologically meaningful trade-off between amplification and inhibition. The enhancing branch promotes signal propagation, while the inhibitory branch restrains it. This architecture allows fine control of the activation response and helps explain how such models reproduce more complex phenotypic behaviors, including signal tuning and ligand antagonism.

Taken together, these findings show that the biologically most important parameters are those that govern receptor phosphorylation, signal persistence, inhibitory feedback, and the balance between amplification and suppression. The sensitivity analysis therefore supports the broader conclusion that T-cell activation is controlled not by a single mechanism, but by the interaction of multiple regulatory processes whose relative importance depends on the signaling architecture under consideration.

3.8 Summary and Interpretation

The analysis carried out in this chapter shows that phenotypic models of T-cell activation differ substantially in their ability to reproduce the central biological benchmarks of TCR–pMHC recognition, namely specificity, sensitivity, antigen discrimination, and optimal response. From the mathematical point of view, most of the models considered here possess a unique positive steady state and exhibit well-behaved long-time dynamics, whereas the kinetic proofreading model with negative feedback remains exceptional in allowing multiple positive steady states. From the biological point of view, the numerical results show that the simpler frameworks, especially the occupancy model and the standard kinetic proofreading model, are useful as baseline descriptions but do not reproduce the full range of experimentally observed behaviour.

The comparative analysis highlights that models incorporating additional regulatory mechanisms perform substantially better. Limited signaling introduces a trade-off between proofreading and serial engagement, which leads naturally to an optimal dissociation time. Sustained signaling allows receptor activation to persist after ligand unbinding and thereby reproduces additional dose-dependent effects, including an optimum with respect to ligand concentration. The negative-feedback model contributes a different mechanism

by introducing inhibitory regulation, which generates bell-shaped dose–response curves and, under suitable parameter regimes, a weak but genuine non-monotone dependence on dissociation time. The stabilizing-activation-chain model preserves dissociation-time-dependent discrimination, but does not recover optimality with respect to either ligand concentration or dissociation time. The models containing incoherent feedforward loops capture more complex behaviours, including non-monotone dose–response curves, dose-dependent ligand ranking, and intersections of response curves. In particular, the model combining limited signaling with incoherent feedforward regulation produces a distinctive bimodal dependence on dissociation time. Among all models considered here, the hybrid model combining limited and sustained signaling provides the broadest agreement with experimental expectations, since it reproduces optimality with respect to both ligand concentration and dissociation time while preserving strong discrimination and sensitivity.

The sensitivity analysis supports these conclusions by identifying the parameters that most strongly influence model behaviour. Across several models, phosphorylation-related parameters emerge as major determinants of the response, while parameters governing signal persistence, inhibitory regulation, and amplification–suppression balance shape the behaviour of the more specialized architectures. In biological terms, this means that T-cell activation cannot be understood as the result of a single isolated mechanism. Rather, it arises from the interaction of several regulatory processes acting across different kinetic and signaling regimes. The sensitivity patterns therefore reinforce the broader conclusion that the success of a model depends not only on its formal structure, but also on the balance of the kinetic parameters governing proofreading progression, feedback, and post-binding signaling.

At the same time, the present results point to an important limitation of the current modeling framework: model predictions depend strongly on parameter choice. This makes reliable parameter estimation a central issue for future work. Many parameter values used in phenotypic models of T-cell activation have historically been obtained from three-dimensional experimental techniques, such as surface plasmon resonance, single-molecule fluorescence microscopy, and fluorescence resonance energy transfer in solution. These techniques have been essential for estimating TCR–pMHC affinities, Binding rates, and dissociation rates, and they have strongly influenced the development of occupancy, kinetic proofreading, serial triggering, and optimal dwell-time models. However, they do not fully reproduce the physiological geometry of T-cell activation, where TCRs and pMHC molecules are both membrane-associated and interact at the two-dimensional interface between a T cell and an antigen-presenting cell [101].

This distinction between 3D and 2D parameter estimation has direct consequences for the interpretation of the models studied in this chapter. In two-dimensional experimental systems, such as supported lipid bilayers, micropipette adhesion-frequency assays, thermal fluctuation assays, and single-molecule imaging at the cell surface, TCR–pMHC interac-

tions are measured under membrane-constrained conditions that are closer to the natural cellular environment. Moreover, 2D and 3D measurements are not expressed in the same units: in 3D, affinity and Binding rates are typically measured using molar concentration units, whereas in 2D, affinity and Binding rates are expressed in surface-density units. For this reason, parameter values cannot be transferred directly from 3D experiments to 2D models without careful recalibration. Since the simulations and sensitivity analysis show that qualitative predictions can change substantially when kinetic parameters are varied, improved 2D calibration is essential for determining which model behaviours are robust biological predictions and which are artifacts of parameter choice [101].

Experimental implications and future calibration

The model comparison suggests several experimentally testable directions. First, activation should be measured over a two-dimensional panel of ligand dose and TCR–pMHC dissociation time. Such experiments would directly test whether the response is monotone, single-peaked, or bimodal as a function of binding duration. This is particularly important for distinguishing basic kinetic proofreading from models with limited signaling, sustained signaling, negative feedback, or incoherent feedforward regulation. A systematic comparison across both ligand concentration and dissociation time would also make it possible to test whether the predicted optima in response, $E_{\max}$, and EC_{50} persist when parameters are measured under membrane-proximal 2D conditions.

Second, the limited-signaling models predict that longer binding is not always better. If a bound receptor can signal only for a finite period, then very long-lived complexes may reduce serial engagement and thereby lower the overall response. This prediction could be tested using panels of altered peptide ligands or engineered TCR–pMHC pairs with controlled dissociation times, while measuring downstream activation across several ligand doses. A clear intermediate optimum in dissociation time would support the limited-signaling interpretation, whereas a purely monotone increase would favour simpler proofreading-based explanations.

Third, the sustained-signaling models predict that signaling can persist transiently after ligand unbinding. This suggests pulse–chase or ligand-washout experiments in which T cells are exposed to pMHC for a controlled time interval and downstream signaling is followed after ligand removal. If activation continues after ligand dissociation, this would support the inclusion of a sustained-signaling state such as R^* . If signaling instead stops rapidly after ligand removal, the sustained-signaling mechanism would be less plausible.

Fourth, models with incoherent feedforward regulation predict more complex dose-dependent effects, including intersections of dose–response curves and changes in ligand ranking across different dose regimes. These predictions should be tested by comparing weak, intermediate, and strong agonists across a wide range of ligand concentrations

rather than at a single fixed dose. If ligand effectiveness changes with dose, then EC_{50} alone is not sufficient to characterize antigen potency, and the full shape of the response curve must be considered.

Finally, the bimodal response predicted by the model combining limited signaling with incoherent feedforward regulation requires special experimental attention. A bimodal dependence on dissociation time would suggest that two distinct kinetic regimes can support activation, possibly reflecting competing phases of activation and inhibition. To test this, experiments would need sufficiently dense sampling of dissociation times, especially in the intermediate range where one response peak may decline and another may emerge. Such experiments would help determine whether the bimodal structure is a genuine feature of T-cell signaling or a model-dependent consequence of the chosen parameter values.

A further implication of this chapter is that, although the present phenotypic models are mathematically tractable and biologically informative, they still omit several mechanisms that are likely to be relevant for a more complete theory of T-cell activation. Promising directions for future development include kinetic segregation effects, conformational flexibility and mechanical coupling within the TCR–CD3 complex, and force-dependent catch-bond behaviour. Incorporating such mechanisms may help bridge the gap between phenomenological ODE-based descriptions and the structural and biophysical processes that underlie TCR triggering.

Overall, this chapter establishes both the usefulness and the limitations of current KPR-based phenotypic models of T-cell activation. It shows that these models remain effective tools for studying discrimination, sensitivity, and optimality, while also demonstrating that their predictive success depends crucially on parameter quality and on the biological mechanisms included in the model. These conclusions provide the basis for the broader interpretation developed in the next chapter, where the mathematical findings of this thesis are related to the central biological questions of T-cell activation and to possible directions for future model refinement.

Mechanochemical Energy-Landscape Model for TCR–pMHC Binding Under Force

4.1 Introduction

The previous chapter considered phenotypic models of T-cell activation. These models show how cellular response patterns can arise from receptor occupancy, ligand dissociation, kinetic proofreading, limited signalling, sustained signalling, feedback regulation, and related biochemical mechanisms. They are useful for comparing response properties such as specificity, sensitivity, antigen discrimination, and maximal activation. However, ligand dissociation rates, binding lifetimes, and signalling durations are treated as effective kinetic inputs. Thus, phenotypic models explain how these quantities influence activation, but not how they are generated by the underlying physical properties of the receptor–ligand interaction. The present chapter addresses this missing layer by developing a minimal two-dimensional mechanochemical energy-landscape model for the TCR–pMHC bond, in which receptor extension, ligand-binding-site separation, hinge geometry, binding energetics, and mechanical loading jointly determine the stability of the bound state.

The biological motivation for this approach comes from a central paradox in T-cell activation. TCR–pMHC interactions are relatively weak compared with many other specific protein–protein interactions, with equilibrium dissociation constants typically in the range 10^{-4} – 10^{-6} M [117, 118]. Nevertheless, T cells can detect rare foreign pMHC molecules among a large background of self-peptides and, in some settings, respond to very small numbers of agonist ligands [119, 120]. This contrast between weak molecular binding and highly sensitive cellular recognition has motivated several theoretical explanations, including kinetic proofreading, kinetic-segregation, allosteric triggering, and force-dependent models [121, 122, 123, 124, 125].

Mechanical force provides a natural additional mechanism for ligand discrimination. During contact between a T cell and an antigen-presenting cell, forces in the piconewton range can be transmitted through the TCR–pMHC complex by cytoskeletal motion,

microvillus dynamics, membrane deformation, and receptor pulling [126, 127, 128]. Force has been found to influence receptor–ligand binding in a ligand-dependent manner. For agonist TCR–pMHC interactions, moderate force can increase bond lifetime, producing catch-bond or catch–slip behaviour. In contrast, antagonist ligands tend to form slip bonds, whose lifetimes decrease under increasing force [127, 129, 130]. This suggests that force can act as a physical filter: productive ligands may be mechanically stabilised within a physiological force range, whereas non-productive ligands may dissociate more rapidly.

Force-dependent binding is also relevant because TCR triggering is not only a question of how long the ligand remains bound, but also of how binding is mechanically transmitted through the TCR–CD3 complex. A key early event in T-cell signalling is phosphorylation of immunoreceptor tyrosine-based activation motifs (ITAMs) located in the CD3 chains [131, 132]. In resting T cells, CD3 cytoplasmic tails, particularly CD3 ϵ , can associate with acidic lipids in the inner leaflet of the plasma membrane, which may limit access to their ITAMs [133]. Mechanical deformation of the TCR–CD3 complex, including changes in receptor orientation and hinge-like opening upon pMHC engagement, has therefore been proposed to contribute to signalling by altering the geometry of the complex and increasing accessibility of CD3 signalling motifs to kinases such as Lck [124, 134, 135]. In this sense, the hinge angle used in the present model is not merely a geometric variable; it represents a coarse-grained description of conformational changes that may couple extracellular ligand binding to intracellular ITAM phosphorylation.

To describe this coupling mathematically, the chapter uses an effective mechanochemical energy landscape. In this framework, receptor deformation, ligand-interface separation, angle-dependent binding strength, and applied force are represented by a small number of coarse-grained variables. The aim is not to resolve the full atomistic dynamics of the TCR–pMHC–CD3 complex, but to obtain a tractable model in which the existence, stability, and loss of a bound configuration can be analysed. In particular, ligand identity is represented through peptide-dependent parameters that define an allosterically modulated binding landscape, while applied force subsequently reshapes this landscape by moving stationary points and changing the activation barrier. The detailed construction of this force-dependent landscape, together with its stationary points, stability properties, and bond-breaking behaviour, is developed in the sections below [136, 137, 138, 139, 140].

Thus, this chapter provides the bridge between the phenotypic activation models of the previous chapter and the force-dependent barrier and lifetime analysis developed in the next chapter. The central idea is that cellular response models describe possible activation patterns, whereas the mechanochemical energy-landscape model explains how molecular geometry, binding energetics, and mechanical loading can generate the bound-state lifetimes underlying those patterns. The scope of the model is therefore deliberately restricted: it is a phenomenological description of force-dependent TCR–pMHC bond regulation, not a complete model of all biochemical events involved in T-cell activation.

The chapter is organised as follows. Section 4.2 provides the biological and structural background for TCR–pMHC recognition under force, including the sensitivity problem, catch–slip bond behaviour, and the structural basis for mechanochemical coupling. Section 4.3 introduces the reduced geometric coordinates used in the model, namely the receptor extension L , the ligand-binding-site separation d , and the hinge angle θ . Section 4.4 constructs the force-dependent energy-landscape formulation, including the harmonic hinge energy, Morse-type binding energy, angle-dependent binding depth, and mechanical work term. Section 4.5 derives the stationary-point equations and reduces the two-dimensional stationary system to a scalar angular equation $J(\theta) = 0$. Section 4.6 analyses local stability through the Hessian matrix, identifying the near-branch stability condition and the determinant criterion for a strict local minimum. Section 4.7 introduces Newton trajectories and bond-breaking points, explaining how force-dependent bond rupture is represented as the loss of a stable bound configuration. Section 4.8 interprets the mathematical results in biological terms. Finally, Section 4.9 summarises the main conclusions of the chapter and connects them to the force-dependent barrier and lifetime calculations developed in the next chapter.

4.2 Biological and Structural Background

4.2.1 The sensitivity problem in TCR–pMHC recognition

The interaction of T-cell receptors (TCRs) with peptides presented by major histocompatibility complex (MHC) proteins is central to T-cell activation. In this process, the TCR must recognize rare foreign pMHC molecules displayed by antigen-presenting cells (APCs), while avoiding activation by the much larger background of self-peptides. This recognition task is particularly striking because TCR–pMHC binding represents one of the weakest specific protein–protein interactions. Typical equilibrium dissociation constants lie in the range 10^{-4} – 10^{-6} M, approximately three orders of magnitude weaker than many antigen–antibody interactions [117, 118]. Despite this low affinity, TCRs exhibit extraordinary sensitivity and specificity, with T cells capable of detecting very small numbers of agonist pMHC ligands and, in some settings, responding to a single pMHC molecule [119, 120]. The mechanism by which such high functional sensitivity and specificity arises from weak molecular binding has therefore been a major focus of research.

Several non-exclusive frameworks have been proposed to explain this paradox of high sensitivity and weak binding. Kinetic proofreading models suggest that specificity is enhanced by a sequence of biochemical steps that must be completed before full activation occurs; short-lived receptor engagements are therefore more likely to dissociate before productive signalling is reached [121]. Kinetic-segregation and close-contact models emphasize the local geometry of the T-cell–APC interface and the exclusion of large

inhibitory phosphatases, such as CD45, from regions of close membrane contact [122, 123, 141]. Allosteric models instead focus on conformational rearrangements within the TCR–CD3 complex, through which extracellular ligand binding may be transmitted to intracellular signalling modules [124]. Accordingly, the molecular basis of TCR triggering remains an active and unresolved area of research.

These mechanisms are not mutually exclusive, and force-dependent mechanochemical models provide an additional layer of explanation. In this view, the relevant question is not only whether a ligand binds strongly or weakly under unloaded conditions, but how the stability of the bound state changes when mechanical load is applied. This distinction is important because the dissociation rate or bond lifetime used in phenotypic activation models is not necessarily an intrinsic, force-independent property of the ligand alone. Rather, it may depend on the mechanical state and conformational geometry of the receptor–ligand complex. Thus, force-dependent models ask how external load reshapes individual TCR–pMHC bonds and thereby alters the lifetimes that enter downstream activation processes [125].

4.2.2 Mechanical force and catch–slip bond behaviour

During contact between a T cell and an antigen-presenting cell, the TCR–pMHC complex is exposed to a mechanically active environment rather than acting as a passive receptor–ligand pair. Forces can be transmitted through the complex by actin retrograde flow, actin–myosin contractility, microvillus scanning, membrane deformation, and receptor movement. Experimental approaches including optical tweezers, biomembrane force probes, fluorescence-enabled force probes, and molecular tension sensors have shown that piconewton-scale loads can act on the TCR–pMHC–CD3 complex during antigen recognition [126, 127, 128].

A central observation from these studies is that force can change the lifetime of a TCR–pMHC bond in a ligand-dependent manner. In a classical slip bond, increasing force lowers the effective dissociation barrier and accelerates bond rupture, as described by Bell–Evans-type models [142, 143]. In contrast, agonist TCR–pMHC interactions can display catch–slip behaviour: the bond lifetime initially increases with force, reaches a maximum at an intermediate load, and then decreases at higher force. In TCR–pMHC recognition, such lifetime maxima are often reported around 10–15 pN [127, 129, 130]. Antagonist ligands, by contrast, more often show slip-bond behaviour, with lifetimes that shorten under increasing force [144, 130].

This ligand-dependent force response suggests that mechanical load may contribute directly to antigen discrimination. For agonist ligands, moderate force can prolong TCR–pMHC contact and increase the time available for productive downstream signalling [145, 146]. For antagonist ligands, the same applied force may instead promote dissociation

and limit signalling. Thus, force can have opposite effects on different ligand classes, depending on the geometry and energetics of the receptor–ligand complex.

The role of force in TCR ligand discrimination, however, remains an active area of debate. Some theoretical and experimental studies suggest that mechanical load can sharpen antigen discrimination by amplifying differences between agonist and non-agonist ligands [147, 125]. Other studies suggest that force may, under certain conditions, compress kinetic differences and thereby reduce discrimination [148]. This unresolved issue motivates a mathematical framework in which the force–lifetime relation is not imposed in advance. Instead, it should emerge from how mechanical load reshapes the geometry, stationary states, and energy barriers of the TCR–pMHC binding landscape.

4.2.3 Structural basis for mechanochemical coupling

The TCR is an $\alpha\beta$ heterodimeric glycoprotein. Each chain contains a variable region responsible for antigen recognition and a constant region providing structural support. The variable regions comprise complementarity determining regions (CDRs), with the CDR loops playing a central role in peptide antigen binding. Because the TCR itself lacks intrinsic signalling capacity, it depends on the associated CD3 complex for signal transduction [149, 150]. The CD3 complex is composed of six non-polymorphic chains, organized into CD3 γ –CD3 ϵ and CD3 δ –CD3 ϵ heterodimers, together with a CD3 $\zeta\zeta$ homodimer, all arranged in specific orientations relative to the TCR [151, 152]. A labelled view of the full TCR–pMHC–CD3 assembly is shown in Fig. 4.1.

Recent cryo-electron microscopy and molecular-dynamics studies have clarified the structural organization, membrane alignment, and conformational variability of the TCR–CD3 complex [153, 154, 155, 135]. These studies focus on interactions between TCR $\alpha\beta$ and CD3 subunits and on potential conformational changes in the extracellular domain of TCR $\alpha\beta$ relative to the membrane. Upon pMHC engagement, such rearrangements may couple to membrane disengagement of CD3 cytoplasmic tails, ITAM exposure, and Lck access, thereby linking receptor geometry to early biochemical signalling.

Single-particle cryo-EM resolved a near-complete human TCR–CD3 complex, including the extracellular domains and transmembrane helices, and mapped the chain–chain interfaces [153]. The TCR α chain interacts with CD3 δ at the DE loop and connecting-peptide region, with additional stabilization from CD3 ζ , whereas the TCR β chain interfaces with CD3 γ and CD3 ϵ mainly in extracellular and membrane-proximal regions [153]. Subsequent structural and NMR studies refined this picture: the TCR α DE loop engages the CD3 $\epsilon\delta$ module, while the TCR β CC' loop contacts the CD3 $\epsilon\gamma$ module. The TCR C β FG loop has also been implicated in TCR–CD3 coupling, although its contacts with CD3 $\epsilon\gamma$ appear more limited in some structural views, consistent with cooperative rather than uniformly strong binding across the assembly [156, 157, 158, 159].

Molecular-dynamics simulations extended these structural findings by revealing broader and more persistent contacts involving the TCR $C\beta$ FG loop and the TCR α variable domain than initially appreciated [154]. Notably, comparisons of TCR–CD3 alone with the TCR–CD3–pMHC complex suggest a marked reduction and redistribution of TCR–CD3 contacts upon pMHC engagement. This rearrangement has been proposed to constrain the spatial freedom of CD3 subunits and to expose or activate signalling motifs within CD3 cytoplasmic tails, thereby initiating downstream signalling and sharpening specificity [124].

These structural rearrangements are directly relevant for early signalling. A key early biochemical event is phosphorylation of immunoreceptor tyrosine-based activation motifs (ITAMs), which are located in the CD3 chains [131, 132]. In resting T cells, CD3 cytoplasmic tails, especially CD3 ϵ , can associate with acidic lipids in the inner leaflet of the plasma membrane [133]. Antigen engagement, together with local membrane deformation, may therefore promote an increased area of interaction for these tails from the membrane, making ITAMs more accessible to lymphocyte-specific protein tyrosine kinase Lck [134].

The TCR–CD3 complex also undergoes larger-scale conformational changes, structural studies suggest that the TCR extracellular domain can adopt a bent orientation in the resting state and a more extended orientation upon pMHC engagement [135, 160]. This change can be represented phenomenologically by an effective hinge angle θ , as illustrated in Fig. 4.2.

Thus, the structural picture suggests a minimal mechanochemical reduction: one coordinate can represent conformational extension of the receptor complex, and another can represent separation of the receptor–ligand interface. The model developed in the next section follows this logic. It does not attempt to resolve all atomistic details of the TCR–CD3 assembly, membrane curvature, or cytoskeletal dynamics. Instead, it uses the structural evidence for hinge-like deformation and binding-interface separation to construct a two-dimensional energy landscape in which mechanical force can shift bound minima and transition-state saddles.

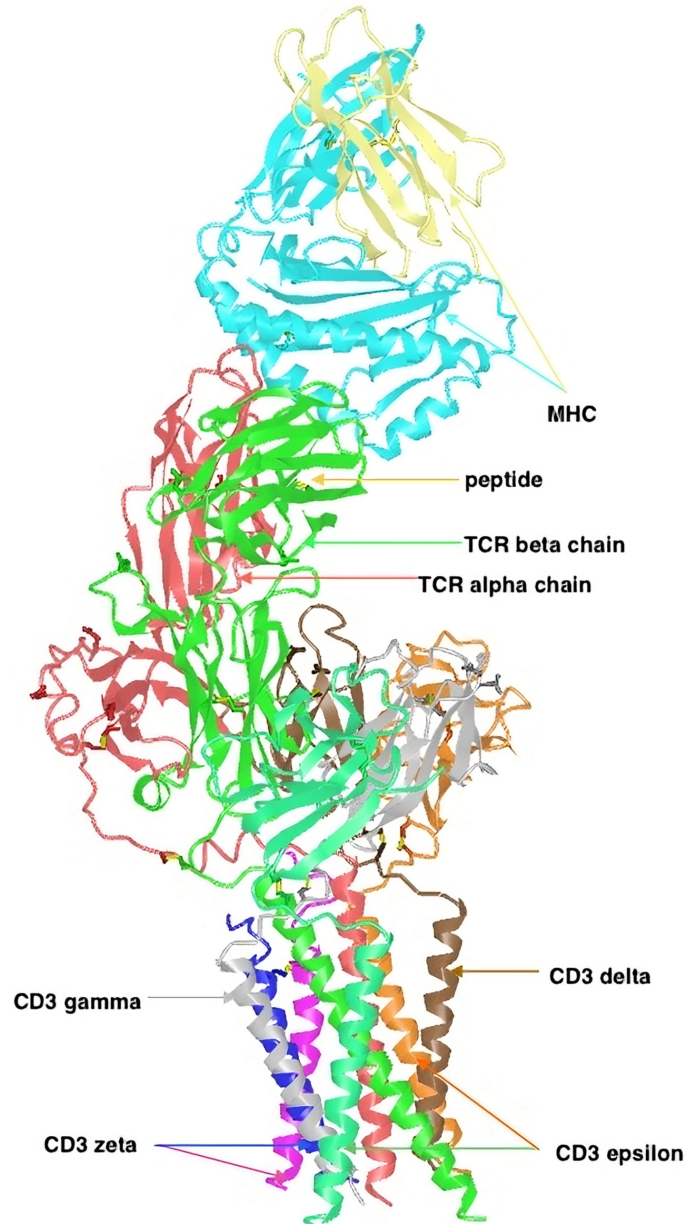


Figure 4.1. Labeled view of the TCR–pMHC–CD3 assembly. The peptide is shown in gold within the MHC groove, while the MHC is shown in cyan and pale yellow. The TCR β chain is shown in green and the TCR α chain in maroon. CD3 subunits are shown as CD3 ϵ (orange), CD3 δ (brown), CD3 γ (gray), and CD3 ζ (purple/blue). The structure was rendered in iCn3D from cryo-EM coordinates such as PDB 8ES8/8ES9; see Saotome *et al.* [161].

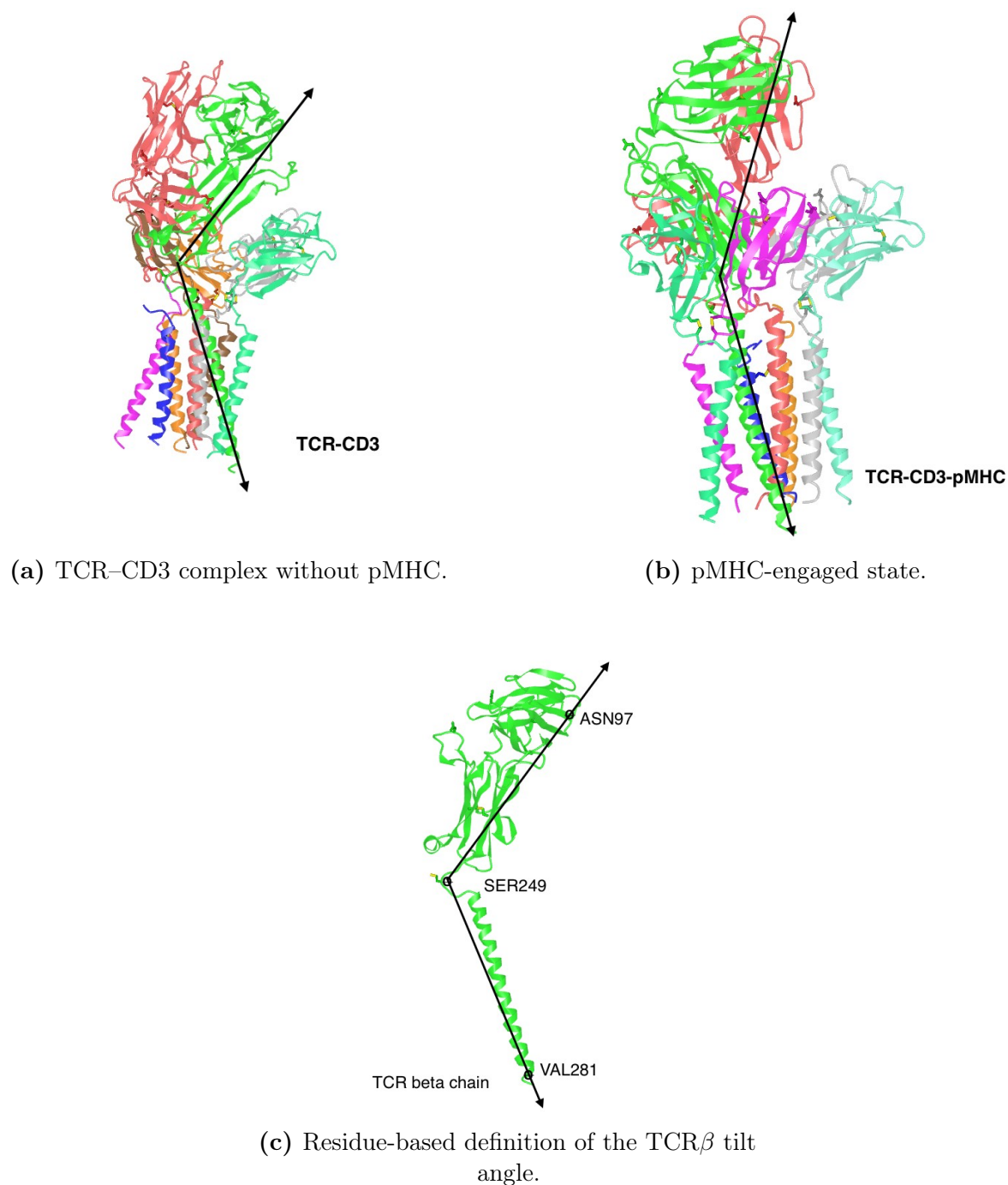


Figure 4.2. Tilt-angle comparison across the TCR-CD3 assembly. The upper-left panel shows the TCR-CD3 complex in the absence of pMHC. The upper-right panel shows the pMHC-engaged state, with increased TCR β tilt in the same viewing orientation. The lower panel illustrates the residue-based definition of the effective TCR β tilt angle θ , using ASN₉₇, SER₂₄₉, and VAL₂₈₁. This effective angle motivates the hinge coordinate used in the mechanochemical model.

4.3 From TCR Structure to Model Coordinates

The structural observations discussed above suggest that mechanical loading of the TCR–pMHC–CD3 complex involves two coupled processes: deformation of the receptor complex and separation of the ligand-binding interface. To represent these effects in an analytically tractable way, we introduce a two-dimensional mechanochemical description based on the receptor extension L , the ligand-binding-site separation d , and the associated hinge angle θ .

Choice of coarse-grained coordinates The structural interplay between receptor conformation and ligand binding is represented through two independent coarse-grained coordinates:

$$L \in [0, 2W], \quad d \geq 0.$$

Here, L denotes the effective extension of the TCR complex, while d denotes the separation of the TCR–pMHC binding interface along the pulling direction. The parameter W is an effective geometric length scale of the TCR complex.

The coordinate L captures conformational deformation of the receptor, especially changes associated with the orientation of the TCR β extracellular domain relative to the membrane. The coordinate d describes opening of the ligand-binding interface and therefore progression toward bond rupture. This phenomenological reduction projects the dominant mechanical effects of the full TCR–pMHC–CD3 assembly onto two collective coordinates relevant for force-dependent binding and dissociation.

Hinge angle and receptor extension The hinge angle θ provides a geometric description of the force-sensitive orientation of the TCR complex. Structural and molecular-dynamics studies suggest that pMHC engagement can induce conformational rearrangement of the TCR–CD3 complex, including a change in the orientation of the TCR β extracellular domain relative to the transmembrane and membrane-proximal regions [135, 162]. In the present model, this conformational change is represented by an effective hinge angle θ .

The receptor extension associated with this hinge geometry is denoted by $L(\theta)$. It represents the axial displacement produced by opening of the TCR complex and is related to the hinge angle by

$$L(\theta) = 2W \sin\left(\frac{\theta}{2}\right), \quad (4.1)$$

and hence

$$\theta(L) = 2 \arcsin\left(\frac{L}{2W}\right). \quad (4.2)$$

Here, W is the effective length scale of the TCR complex, estimated at approximately 7 nm [155]. This representation allows the free-energy landscape to be written in terms of L , while retaining the dependence of binding strength on the underlying receptor orientation θ .

Differentiating (4.2) gives

$$\frac{d\theta}{dL} = \frac{1}{W \sqrt{1 - \frac{L^2}{4W^2}}}. \quad (4.3)$$

Equivalently, using $L = 2W \sin(\theta/2)$, we obtain

$$\frac{d\theta}{dL} = \frac{1}{W \cos(\theta/2)}. \quad (4.4)$$

These identities will be used in the stationarity and Hessian calculations below.

Ligand-binding-site separation The second coordinate d denotes separation of the TCR–pMHC binding interface along the pulling direction. In the bound configuration, d is small and the receptor–ligand pair remains close to its preferred contact geometry. As d increases, stabilizing contacts weaken and the complex moves toward dissociation.

In the free-energy formulation below, this contribution is modeled by a Morse-type binding term, with d_0 setting the effective interaction range.

Physical interpretation of the two-dimensional reduction Together, (L, d) and $\theta(L)$ define the reduced geometry of the force-loaded TCR–pMHC complex. The extension coordinate describes receptor deformation, the separation coordinate describes ligand–interface opening, and the hinge angle links receptor geometry to binding strength. This geometry is illustrated in Fig. 4.3 and forms the basis for the force-dependent free-energy landscape constructed in the next section.

4.4 Mechanochemical Energy-Landscape Formulation

With the reduced geometry (L, d, θ) introduced above, we now construct the force-dependent energy landscape of the TCR–pMHC complex. The term “potential” is used here in an effective, coarse-grained sense. The model does not attempt to represent the full atomistic potential energy surface of the complete TCR–pMHC–CD3 complex, including solvent, membrane, and cytoskeletal degrees of freedom. Instead, it defines a reduced free-energy landscape in a small number of collective coordinates: the receptor extension L , the ligand-binding-site separation d , and the hinge angle $\theta = \theta(L)$. This reduced landscape is used to describe how receptor deformation, ligand-interface separation, angle-dependent binding strength, and applied force interact to determine whether a mechanically stable bound configuration exists.

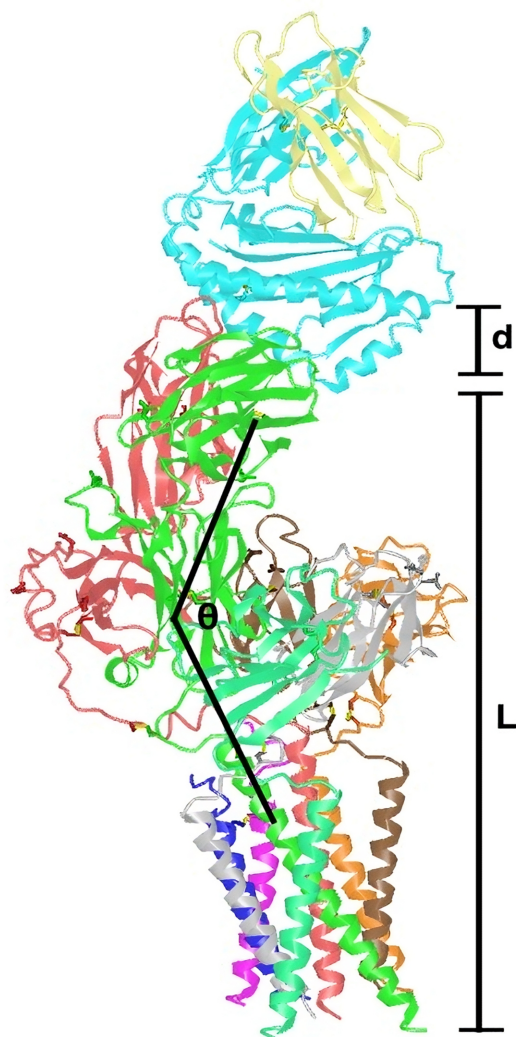


Figure 4.3. Geometric definitions used in the model. The hinge angle θ (black arc) is defined at the TCR hinge, L denotes the axial distance from the CD3 transmembrane region to the TCR-pMHC interface, and d represents the binding-site separation along the pulling axis. These coordinates, (L, d, θ) , enter the force-dependent free-energy formulation introduced below.

The purpose of this formulation is therefore not to provide an atomistic force field, but to give a mathematically tractable representation of force-dependent TCR–pMHC binding. In this setting, local minima represent stable bound configurations, saddle points represent transition configurations for dissociation, and differences in free energy between these points represent activation barriers. The two main coordinates, L and d , are treated as independent but energetically coupled variables: L describes receptor extension and hinge-associated conformational deformation, while d describes opening of the ligand-binding interface. This construction allows force to reshape both receptor geometry and interfacial separation, and provides the coordinate structure used below to track force-dependent minima, saddles, and bond-breaking points.

Force-tilted potential energy surfaces Mechanochemistry is concerned with how mechanical forces initiate or modulate chemical reactions [138, 139]. From a theoretical perspective, an external force perturbs the potential energy surface of a molecular system and changes the locations and stability of its stationary points [140]. In the general mechanochemical setting, the potential energy landscape of a molecular system under an external force, denoted $V_f(\mathbf{x})$, is commonly modeled as [136, 137]

$$V_f(\mathbf{x}) = V_0(\mathbf{x}) - F \mathbf{f}^\top \mathbf{x}, \quad (4.5)$$

where $V_0(\mathbf{x})$ is the unloaded potential energy surface, F is the magnitude of the applied force, and $\mathbf{f}$ is the direction of force application. Here, the superscript $^\top$ denotes transpose.

This representation expresses the fact that a constant external force acts as a linear tilt of the underlying energy landscape. In molecular systems, such a tilt can change the height of the barrier separating a local minimum from a neighbouring saddle point [163]. If $\mathbf{x}_m(F)$ denotes a local minimum and $\mathbf{x}_s(F)$ the adjacent saddle point on the loaded surface, then the corresponding force-dependent barrier is defined as

$$\Delta E(F) = V_f(\mathbf{x}_s(F)) - V_f(\mathbf{x}_m(F)). \quad (4.6)$$

Equivalently, substituting (4.5) gives

$$\Delta E(F) = V_0(\mathbf{x}_s(F)) - V_0(\mathbf{x}_m(F)) - F \mathbf{f}^\top (\mathbf{x}_s(F) - \mathbf{x}_m(F)). \quad (4.7)$$

Thus, the force-dependent barrier consists of the intrinsic energy difference between the saddle and the minimum, corrected by the mechanical work associated with their displacement along the pulling direction.

Total free energy of the TCR–pMHC complex For the TCR–pMHC system, we take the reduced configuration vector to be

$$\mathbf{x}^\top = (L, d), \quad (4.8)$$

where L is the receptor extension coordinate and d is the ligand-binding-site separation. The total force-dependent free energy is defined as

$$V_f(L, d) = V(\theta(L)) + B(d, \theta(L)) - F(L + d). \quad (4.9)$$

The first term describes the intrinsic energetic cost of changing the receptor hinge angle, the second term describes ligand-binding interactions, and the third term represents the mechanical work of the applied load. This form gives a minimal force-coupled landscape in which receptor deformation and ligand-interface separation are treated as coupled contributors to force-dependent binding.

Harmonic hinge energy The hinge energy $V(\theta(L))$ describes the energetic cost of deforming the TCR complex away from a preferred unloaded orientation. It is modeled by the harmonic potential

$$V(\theta) = \frac{1}{2}k_\theta(\theta - \theta_0)^2. \quad (4.10)$$

Here, $k_\theta > 0$ is the effective angular stiffness of the bound TCR–pMHC complex and θ_0 is the preferred unloaded hinge angle. In the parameterization used below, θ_0 is taken to be approximately 0.60π radians, consistent with structural estimates of the resting TCR orientation [135]. The quadratic term penalizes deviations from θ_0 , influencing the conformational stability of the bound receptor–ligand complex [164].

Morse-type ligand-binding energy The ligand-binding energy $B(d, \theta(L))$ characterizes the interaction between the TCR and the pMHC ligand along the separation coordinate. It is modeled by a Morse-type potential,

$$B(d, \theta) = D(\theta) \left[(1 - e^{-d/d_0})^2 - 1 \right]. \quad (4.11)$$

Here, $D(\theta)$ is an angle-dependent binding depth modulating the potential well, while $d_0 > 0$ sets the characteristic interaction range of the Morse potential along the separation coordinate [165]. The Morse form is used because it gives a finite binding well near the bound configuration and weakens nonlinearly as the receptor–ligand interface is opened.

For later use, we define

$$\Phi(d) = (1 - e^{-d/d_0})^2 - 1, \quad (4.12)$$

so that

$$B(d, \theta) = D(\theta)\Phi(d). \quad (4.13)$$

Angle-dependent binding depth The function $D(\theta)$ describes how receptor orientation modulates the depth of the ligand-binding well. It is given by

$$D(\theta) = D_0 \exp \left[-\frac{(\theta - \theta_1)^2}{2\sigma^2} \right]. \quad (4.14)$$

In this formulation, $D_0 > 0$ is the maximum binding-well depth, θ_1 is the preferred hinge orientation for binding, and $\sigma > 0$ sets how sharply $D(\theta)$ decays away from θ_1 [166]. Thus, $D(\theta)$ provides a phenomenological description of how receptor geometry modulates binding strength. Angles close to θ_1 correspond to more favourable binding, whereas deviations from θ_1 reduce the effective well depth.

Together, $V(\theta)$ and $D(\theta)$ separate two energetic effects. The hinge term $V(\theta)$ describes the intrinsic cost of deforming the receptor about the resting angle θ_0 , whereas $D(\theta)$ modulates how strongly ligand binding is favoured at different receptor orientations.

Mechanical work term The final term in (4.9),

$$-F(L + d), \quad (4.15)$$

represents the mechanical work of an applied load F . In the general notation of (4.5), this corresponds to using

$$\mathbf{x}^\top = (L, d) \quad (4.16)$$

and choosing an effective pulling direction whose projection onto the two coarse-grained coordinates gives the linear tilt

$$-F \mathbf{f}^\top \mathbf{x} = -F(L + d). \quad (4.17)$$

4.5 Stationary States and Scalar Reduction

We now derive the stationary configurations of the force-loaded free-energy landscape. In this chapter, a stationary state means a critical point of $V_f(L, d)$, that is, a pair (L^*, d^*) satisfying

$$\frac{\partial V_f}{\partial L}(L^*, d^*) = 0, \quad \frac{\partial V_f}{\partial d}(L^*, d^*) = 0.$$

Such points may represent stable bound configurations, transition-state saddles, or unstable configurations, depending on the Hessian classification developed in the next section.

Recall that

$$V_f(L, d) = V(\theta(L)) + D(\theta(L))\Phi(d) - F(L + d), \quad (4.18)$$

where

$$V(\theta) = \frac{1}{2}k_\theta(\theta - \theta_0)^2, \quad \Phi(d) = (1 - e^{-d/d_0})^2 - 1,$$

and

$$D(\theta) = D_0 \exp \left[-\frac{(\theta - \theta_1)^2}{2\sigma^2} \right].$$

We use the transformed separation variable

$$u := e^{-d/d_0} \in (0, 1], \quad \Phi(d) = u^2 - 2u.$$

Stationarity in the separation coordinate. Differentiating Φ with respect to d , using $du/dd = -u/d_0$, gives

$$\frac{d\Phi}{dd} = \frac{d}{dd}(u^2 - 2u) = 2u \frac{du}{dd} - 2 \frac{du}{dd} = \frac{2}{d_0}u(1 - u).$$

Since $D(\theta(L))$ does not depend on d , we obtain

$$\frac{\partial V_f}{\partial d} = D(\theta(L)) \frac{d\Phi}{dd} - F.$$

Thus the d -stationarity condition is

$$\boxed{\frac{2}{d_0}D(\theta)u(1 - u) = F.} \quad (A)$$

Introduce

$$\alpha := \frac{Fd_0}{2}, \quad C(\theta) := \frac{\alpha}{D(\theta)}. \quad (4.19)$$

Then Equation (A) is equivalent to

$$\boxed{u^2 - u + C(\theta) = 0, \quad u \in (0, 1].} \quad (4.20)$$

The discriminant condition gives

$$1 - 4C(\theta) \geq 0,$$

and hence

$$\boxed{D(\theta) \geq 2Fd_0.} \quad (4.21)$$

When this condition holds, the two roots are

$$u_{\pm}(\theta) = \frac{1 \pm \sqrt{1 - 4C(\theta)}}{2}. \quad (4.22)$$

The corresponding separations are

$$d_{\pm}(\theta) = -d_0 \ln u_{\pm}(\theta).$$

For $F > 0$, the Gaussian form of $D(\theta)$ gives the admissible angular band

$$\Theta = \{\theta \in [0, \pi] : D(\theta) \geq 2Fd_0\}. \quad (4.23)$$

Equivalently, when $D_0 \geq 2Fd_0$,

$$\Theta = [0, \pi] \cap [\theta_-, \theta_+], \quad \theta_{\pm} = \theta_1 \pm \delta, \quad \delta = \sigma \sqrt{2 \log \left(\frac{D_0}{2Fd_0} \right)}. \quad (4.24)$$

If $D_0 < 2Fd_0$, the admissible set is empty and no bound stationary state can exist.

Using the quadratic equation (4.20), we also have

$$u^2 = u - C(\theta),$$

and therefore

$$\Phi(d) = u^2 - 2u = -C(\theta) - u = -\frac{\alpha}{D(\theta)} - u. \quad (\star)$$

Stationarity in the extension coordinate. We next differentiate V_f with respect to L . Since d is independent of L , the chain rule gives

$$\frac{\partial V_f}{\partial L} = [V'(\theta) + D'(\theta)\Phi(d)] \frac{d\theta}{dL} - F.$$

Here

$$V'(\theta) = k_{\theta}(\theta - \theta_0), \quad D'(\theta) = -\frac{\theta - \theta_1}{\sigma^2} D(\theta),$$

and

$$\frac{d\theta}{dL} = \frac{1}{W \cos(\theta/2)}.$$

Hence the L -stationarity condition becomes

$$\left[k_{\theta}(\theta - \theta_0) - \frac{\theta - \theta_1}{\sigma^2} D(\theta)\Phi(d) \right] \frac{1}{W \cos(\theta/2)} = F. \quad (\text{B})$$

Multiplying by $W \cos(\theta/2)$, this can be written as

$$k_\theta(\theta - \theta_0) - \frac{\theta - \theta_1}{\sigma^2} D(\theta) \Phi(d) = FW \cos\left(\frac{\theta}{2}\right). \quad (\text{B1})$$

This equation is the geometric force-balance condition in the extension coordinate.

Reduction to one scalar equation. Substituting $(\star)$ into (B1) makes the L -stationarity equation affine in u . Define

$$A(\theta) := k_\theta(\theta - \theta_0) + \frac{\alpha}{\sigma^2}(\theta - \theta_1), \quad (4.25)$$

$$\mathcal{B}(\theta) := \frac{\theta - \theta_1}{\sigma^2} D(\theta), \quad (4.26)$$

and

$$R(\theta) := FW \cos\left(\frac{\theta}{2}\right). \quad (4.27)$$

Here $\mathcal{B}(\theta)$ is an auxiliary angular function and should not be confused with the binding energy $B(d, \theta)$.

Then (B1) becomes

$$\boxed{A(\theta) + \mathcal{B}(\theta)u = R(\theta)}. \quad (\text{B-linear})$$

If $\mathcal{B}(\theta) \neq 0$, then

$$u = \frac{R(\theta) - A(\theta)}{\mathcal{B}(\theta)}.$$

Enforcing the quadratic condition $u(1 - u) = C(\theta)$ yields

$$\left(\frac{R - A}{\mathcal{B}}\right) \left(1 - \frac{R - A}{\mathcal{B}}\right) = C(\theta).$$

Equivalently,

$$(R - A)\mathcal{B} - (R - A)^2 - C(\theta)\mathcal{B}^2 = 0.$$

Therefore the coupled stationary system reduces to the scalar equation

$$\boxed{J(\theta) := (R - A)\mathcal{B} - (R - A)^2 - C(\theta)\mathcal{B}^2 = 0} \quad (4.28)$$

on the admissible set

$$\Theta = \{\theta \in [0, \pi] : D(\theta) \geq 2Fd_0\}.$$

For $\theta \in \Theta$, Equation (4.20) has the roots $u_\pm(\theta)$. If $\theta \neq \theta_1$, the linear equation (B-linear) selects a unique value

$$u^* = \frac{R(\theta^*) - A(\theta^*)}{\mathcal{B}(\theta^*)}.$$

Thus each root $\theta^* \in \Theta$ of $J(\theta) = 0$ determines a stationary configuration by

$$d^* = -d_0 \ln u^*, \quad L^* = 2W \sin \left(\frac{\theta^*}{2} \right).$$

At the center $\theta = \theta_1$, where $\mathcal{B}(\theta_1) = 0$, the linear equation reduces to

$$A(\theta_1) = R(\theta_1),$$

or equivalently

$$k_\theta(\theta_1 - \theta_0) = FW \cos \left(\frac{\theta_1}{2} \right).$$

If this center condition holds, the admissible values of u are obtained directly from the quadratic equation

$$u^2 - u + C(\theta_1) = 0.$$

Existence and multiplicity. The preceding reduction leads to the following theorem.

Theorem 4.1 (Existence and multiplicity of stationary states). *Assume*

$$W > 0, \quad d_0 > 0, \quad D_0 > 0, \quad \sigma > 0, \quad k_\theta > 0, \quad F \geq 0.$$

Let

$$\begin{aligned} \alpha &:= \frac{Fd_0}{2}, \quad C(\theta) := \frac{\alpha}{D(\theta)}, \\ A(\theta) &= k_\theta(\theta - \theta_0) + \frac{\alpha}{\sigma^2}(\theta - \theta_1), \quad \mathcal{B}(\theta) = \frac{\theta - \theta_1}{\sigma^2}D(\theta), \\ R(\theta) &= FW \cos \left(\frac{\theta}{2} \right), \end{aligned}$$

and

$$J(\theta) = (R - A)\mathcal{B} - (R - A)^2 - C(\theta)\mathcal{B}^2.$$

Define the admissible set

$$\Theta := \{\theta \in [0, \pi] : D(\theta) \geq 2Fd_0\}.$$

Then the following statements hold.

(i) *If $D_0 < 2Fd_0$, equivalently*

$$F > \frac{D_0}{2d_0},$$

then $\Theta = \emptyset$, and there are no stationary states.

(ii) If $D_0 \geq 2Fd_0$, a pair (L^*, d^*) is a stationary state if and only if there exists $\theta^* \in \Theta$ such that

$$J(\theta^*) = 0.$$

The corresponding separation variable is obtained from

$$u^* = \frac{R(\theta^*) - A(\theta^*)}{\mathcal{B}(\theta^*)}, \quad \text{if } \mathcal{B}(\theta^*) \neq 0,$$

or from

$$u^2 - u + C(\theta^*) = 0$$

in the center case $\theta^* = \theta_1$. Then

$$d^* = -d_0 \ln u^*, \quad L^* = 2W \sin\left(\frac{\theta^*}{2}\right).$$

Moreover, the total number of stationary states is at most two.

(iii) Generically, excluding parameter values where a zero of J also satisfies $J'(\theta) = 0$, the number of stationary states is either 0 or 2. A single stationary state occurs only in degenerate situations: either a tangency with

$$J(\theta) = J'(\theta) = 0$$

on one side of θ_1 , or at the center when

$$k_\theta(\theta_1 - \theta_0) = FW \cos\left(\frac{\theta_1}{2}\right), \quad (4.29)$$

together with

$$D(\theta_1) = 2Fd_0.$$

(iv) If the center condition (4.29) holds and $D(\theta_1) > 2Fd_0$, then $\theta^* = \theta_1$ solves $J = 0$, and both quadratic roots $u_\pm$ are admissible. Thus, there are two distinct stationary states with the same extension

$$L^* = 2W \sin\left(\frac{\theta_1}{2}\right),$$

but different separations.

Proof. The condition $\partial_d V_f = 0$ gives

$$u(1 - u) = C(\theta),$$

with $u = e^{-d/d_0} \in (0, 1]$. Thus a stationary point must satisfy the discriminant condition

$$D(\theta) \geq 2Fd_0,$$

so that $\theta \in \Theta$. If $D_0 < 2Fd_0$, then $D(\theta) \leq D_0 < 2Fd_0$ for all θ , and therefore $\Theta = \emptyset$. This proves part (i).

Assume now that $D_0 \geq 2Fd_0$. From the quadratic equation $u^2 - u + C(\theta) = 0$, we have

$$\Phi(d) = u^2 - 2u = -C(\theta) - u.$$

Substituting this expression into the L -stationarity condition gives

$$A(\theta) + \mathcal{B}(\theta)u = R(\theta).$$

If $\mathcal{B}(\theta) \neq 0$, then

$$u = \frac{R(\theta) - A(\theta)}{\mathcal{B}(\theta)}.$$

Enforcing $u(1 - u) = C(\theta)$ gives the scalar equation

$$J(\theta) = (R - A)\mathcal{B} - (R - A)^2 - C(\theta)\mathcal{B}^2 = 0.$$

Conversely, if $\theta^* \in \Theta$ satisfies $J(\theta^*) = 0$, then the corresponding u^* obtained from the linear relation satisfies both stationarity equations. The original variables are recovered by

$$d^* = -d_0 \ln u^*, \quad L^* = 2W \sin\left(\frac{\theta^*}{2}\right).$$

At $\theta^* = \theta_1$, where $\mathcal{B}(\theta_1) = 0$, the linear condition reduces to $A(\theta_1) = R(\theta_1)$, while u^* is determined directly by the quadratic equation. This proves the equivalence in part (ii), apart from the bound on the number of solutions.

It remains to justify the multiplicity statement. At the center $\theta = \theta_1$, one has $\mathcal{B}(\theta_1) = 0$, and therefore

$$J(\theta_1) = -(R(\theta_1) - A(\theta_1))^2 \leq 0.$$

Equality holds precisely when

$$A(\theta_1) = R(\theta_1),$$

which is equivalent to

$$k_\theta(\theta_1 - \theta_0) = FW \cos\left(\frac{\theta_1}{2}\right).$$

At the admissible endpoints $\theta_\pm$, one has

$$D(\theta_\pm) = 2Fd_0,$$

and the quadratic equation in u has the double root

$$u = \frac{1}{2}.$$

Substituting $u = 1/2$ into the affine condition gives

$$J(\theta_{\pm}) = - \left(R(\theta_{\pm}) - A(\theta_{\pm}) \mp \frac{1}{2} \mathcal{B}(\theta_{\pm}) \right)^2 \leq 0.$$

Thus J is non-positive at the center and at the admissible endpoints.

Writing

$$G(\theta) := R(\theta) - A(\theta),$$

we can express

$$J(\theta) = -G(\theta)^2 + G(\theta)\mathcal{B}(\theta) - C(\theta)\mathcal{B}(\theta)^2.$$

For fixed θ , this quadratic expression in G is bounded above by

$$J_{\max}(\theta) := \mathcal{B}(\theta)^2 \left(\frac{1}{4} - C(\theta) \right). \quad (4.30)$$

Moreover,

$$J_{\max}(\theta) > 0 \iff C(\theta) < \frac{1}{4} \iff D(\theta) > 2Fd_0.$$

Therefore possible positive crossings of J can occur only in the interior of the admissible band.

To see the structure of the upper envelope, write

$$\varepsilon := \theta - \theta_1.$$

Since

$$\mathcal{B}(\theta)^2 = \frac{\varepsilon^2}{\sigma^4} D(\theta)^2, \quad C(\theta) = \frac{\alpha}{D(\theta)},$$

we obtain

$$J_{\max}(\theta) = \frac{\varepsilon^2}{\sigma^4} D(\theta)^2 \left(\frac{1}{4} - \frac{\alpha}{D(\theta)} \right) = \frac{\varepsilon^2}{\sigma^4} D(\theta) \left(\frac{D(\theta)}{4} - \alpha \right).$$

Using

$$D(\theta) = D_0 e^{-\varepsilon^2/(2\sigma^2)},$$

this can be written as

$$J_{\max}(\theta) = \underbrace{\left(\frac{\varepsilon^2}{\sigma^4} e^{-\varepsilon^2/(2\sigma^2)} \right)}_{h_1(\varepsilon)} \underbrace{D_0 \left(\frac{D(\theta)}{4} - \alpha \right)}_{h_2(\varepsilon)}.$$

On each side of θ_1 , the factor h_1 is single-humped, while h_2 is strictly decreasing in $|\varepsilon|$ and vanishes at the admissible endpoint where $D(\theta) = 2Fd_0$. Hence $J_{\max}$ is unimodal on each side of θ_1 . Consequently, J can cross the axis at most once on each side of θ_1 , giving at most two stationary states.

In nondegenerate regimes, zeros of J are transverse, so the number of stationary states is either 0 or 2. A single stationary state can occur only when a zero is degenerate, namely when $J = J' = 0$ on one side of θ_1 , or when the center condition holds at the threshold $D(\theta_1) = 2Fd_0$. If the center condition holds and $D(\theta_1) > 2Fd_0$, then the quadratic equation at θ_1 has two admissible roots $u_{\pm}$, producing two distinct separations with the same extension. This proves parts (iii) and (iv). $\square$

Corollary 4.2 (Critical force). *Let*

$$F_c := \frac{D_0}{2d_0}. \quad (4.31)$$

If $F > F_c$, then $\Theta = \emptyset$, and no stationary state exists. If $0 \leq F \leq F_c$, the number of stationary states is at most two.

The critical force F_c has a simple interpretation. Since D_0 is the maximum binding-well depth and d_0 is the effective interaction range, the ratio $D_0/(2d_0)$ gives the maximum force scale for which the model can support a bound stationary configuration. Above this threshold, the force-loaded landscape no longer contains an admissible bound state.

4.6 Local Stability of Stationary States

The stationary-point analysis above determines the critical points of the force-loaded free-energy landscape. To decide which of these critical points correspond to mechanically stable bound configurations, we examine the Hessian matrix of V_f . Throughout this section, we restrict attention to interior stationary points

$$(L^*, d^*) \in (0, 2W) \times (0, \infty),$$

so that the usual Hessian criterion for local stability applies.

Let

$$u^* := e^{-d^*/d_0}, \quad \theta^* := \theta(L^*),$$

and evaluate all quantities at (L^*, d^*) :

$$\theta' = \theta'(L^*), \quad \theta'' = \theta''(L^*), \quad D = D(\theta^*), \quad D' = \frac{dD}{d\theta}(\theta^*), \quad D'' = \frac{d^2D}{d\theta^2}(\theta^*), \quad \Phi = \Phi(d^*).$$

With the variable ordering (L, d) , the Hessian matrix is

$$H^* = \begin{pmatrix} H_{LL}^* & H_{Ld}^* \\ H_{Ld}^* & H_{dd}^* \end{pmatrix}. \quad (4.32)$$

Derivation of the Hessian entries. Starting from

$$V_f(L, d) = V(\theta(L)) + D(\theta(L))\Phi(d) - F(L + d),$$

the first derivatives are

$$\frac{\partial V_f}{\partial d} = D(\theta(L))\Phi'(d) - F, \quad (4.33)$$

$$\frac{\partial V_f}{\partial L} = \theta'(L) [k_\theta(\theta(L) - \theta_0) + \Phi(d)D'(\theta(L))] - F. \quad (4.34)$$

Here

$$\Phi(d) = u^2 - 2u, \quad u = e^{-d/d_0},$$

and therefore

$$\Phi'(d) = \frac{2}{d_0}u(1 - u), \quad \Phi''(d) = \frac{2}{d_0^2}u(2u - 1). \quad (4.35)$$

Since $D(\theta(L))$ is independent of d , differentiating (4.33) with respect to d gives

$$H_{dd} = D(\theta(L))\Phi''(d) = \frac{2D(\theta(L))}{d_0^2}u(2u - 1). \quad (4.36)$$

For the mixed derivative, differentiating (4.33) with respect to L gives

$$H_{Ld} = \frac{\partial}{\partial L} [D(\theta(L))\Phi'(d)] = D'(\theta(L))\theta'(L)\Phi'(d).$$

Thus

$$H_{Ld} = \theta'(L)D'(\theta(L))\frac{2}{d_0}u(1 - u). \quad (4.37)$$

The same expression is obtained by differentiating (4.34) with respect to d , confirming the symmetry $H_{Ld} = H_{dL}$.

For the L -derivative, define

$$G(\theta(L); d) := k_\theta(\theta(L) - \theta_0) + \Phi(d)D'(\theta(L)).$$

Then

$$\frac{\partial V_f}{\partial L} = \theta'(L)G(\theta(L); d) - F.$$

Differentiating with respect to L , and noting that d is independent of L , gives

$$\begin{aligned} H_{LL} &= \theta''(L)G(\theta(L); d) + \theta'(L)\frac{\partial}{\partial L}G(\theta(L); d) \\ &= \theta''(L) [k_\theta(\theta(L) - \theta_0) + \Phi(d)D'(\theta(L))] + (\theta'(L))^2 [k_\theta + \Phi(d)D''(\theta(L))]. \end{aligned} \quad (4.38)$$

At a stationary point, the first-order conditions give

$$\frac{2}{d_0}u^*(1 - u^*) = \frac{F}{D(\theta^*)}, \quad (4.39)$$

and

$$k_\theta(\theta^* - \theta_0) + \Phi(d^*)D'(\theta^*) = \frac{F}{\theta'}. \quad (4.40)$$

Substituting (4.39) and (4.40) into (4.36)–(4.38) yields the stationary-point Hessian entries

$$H_{dd}^* = \frac{2D(\theta^*)}{d_0^2}u^*(2u^* - 1), \quad (4.41)$$

$$H_{Ld}^* = \theta'F\frac{D'}{D}, \quad (4.42)$$

$$H_{LL}^* = \theta''\frac{F}{\theta'} + (\theta')^2 [k_\theta + \Phi(d^*)D''(\theta^*)]. \quad (4.43)$$

For the Gaussian depth function

$$D(\theta) = D_0 \exp \left[-\frac{(\theta - \theta_1)^2}{2\sigma^2} \right],$$

we have

$$\frac{D'(\theta)}{D(\theta)} = -\frac{\theta - \theta_1}{\sigma^2}, \quad D''(\theta) = D(\theta) \left[\frac{(\theta - \theta_1)^2}{\sigma^4} - \frac{1}{\sigma^2} \right]. \quad (4.44)$$

Therefore the mixed term can also be written as

$$H_{Ld}^* = -\theta'F\frac{\theta^* - \theta_1}{\sigma^2}. \quad (4.45)$$

The determinant of the Hessian is

$$\det H^* = H_{LL}^*H_{dd}^* - (H_{Ld}^*)^2. \quad (4.46)$$

Using the entries above, this becomes

$$\det H^* = \left[\theta''\frac{F}{\theta'} + (\theta')^2 (k_\theta + \Phi D'') \right] \left[\frac{2D}{d_0^2}u(2u - 1) \right] - \left[\theta'F\frac{D'}{D} \right]^2, \quad (4.47)$$

where all quantities are evaluated at (L^*, d^*) .

Local stability theorem.

Theorem 4.3 (Local stability at stationary points). *Let $V_f(L, d)$ be the force-loaded free energy on $(L, d) \in (0, 2W) \times [0, \infty)$, and let*

$$\theta(L) = 2 \arcsin \left(\frac{L}{2W} \right), \quad 0 < L < 2W.$$

Assume that $D \in C^2$ on the relevant range of θ . Let (L^, d^*) be a stationary point of V_f , with $d^* > 0$ in the force-loaded interior case. Then the following statements hold.*

(i) Necessary curvature in the separation direction.

$$H_{dd}^* > 0 \iff u^* > \frac{1}{2} \iff d^* < d_0 \ln 2. \quad (4.48)$$

Hence only the near branch $u^ > 1/2$ can be stable along the ligand-separation coordinate d .*

(ii) Strict local minimum. *For the symmetric 2×2 Hessian, the stationary point is a strict local minimum if and only if*

$$\boxed{H_{dd}^* > 0 \quad \text{and} \quad \det H^* > 0.} \quad (4.49)$$

If $H_{dd}^ > 0$ but $\det H^* < 0$, the point is a saddle. If $H_{dd}^* < 0$, the point is unstable along the separation coordinate d .*

(iii) Zero-force case. *For $F = 0$, the stationary point has the form $(\bar{L}, 0)$, with $\bar{\theta} = \theta(\bar{L})$ satisfying*

$$k_\theta(\bar{\theta} - \theta_0) = D'(\bar{\theta}). \quad (4.50)$$

It is locally stable if and only if

$$k_\theta - D''(\bar{\theta}) > 0, \quad (4.51)$$

since $H_{dd} = 2D(\bar{\theta})/d_0^2 > 0$ and $H_{Ld} = 0$.

Fold point. *At*

$$u^* = \frac{1}{2}, \quad d^* = d_0 \ln 2,$$

one has

$$H_{dd}^* = 0, \quad \det H^* = -(H_{Ld}^*)^2 \leq 0.$$

Thus no strict local minimum can occur exactly at the fold.

Sign structure on the d -stationary branch. The determinant can be further simplified along the d -stationary branch. Using

$$\frac{2}{d_0} u^*(1 - u^*) = \frac{F}{D(\theta^*)},$$

we obtain

$$D(\theta^*) = \frac{F d_0}{2u^*(1 - u^*)}.$$

Therefore

$$\begin{aligned} \frac{2D}{d_0^2} u(2u - 1) &= \frac{2}{d_0^2} \frac{F d_0}{2u(1 - u)} u(2u - 1) \\ &= \frac{F}{d_0} \frac{2u - 1}{1 - u}. \end{aligned} \quad (4.52)$$

Define

$$s(u) := \frac{2u - 1}{1 - u}. \quad (4.53)$$

Also define

$$S(L, u) := \frac{\theta'' F}{\theta'} + (\theta')^2 [k_\theta + \Phi(d) D''(\theta)], \quad (4.54)$$

and the nonnegative penalty term

$$K(L) := \left(\theta' F \frac{D'}{D} \right)^2 \geq 0. \quad (4.55)$$

Then, along the d -stationary branch,

$$\boxed{\det H^* = \frac{F}{d_0} S(L^*, u^*) s(u^*) - K(L^*)}. \quad (4.56)$$

Corollary 4.4 (Sign structure on the d -stationary branch). *Along the d -stationary branch, if $S(L^*, u^*)$ and $s(u^*)$ have opposite signs, then*

$$\det H^* < 0,$$

and the stationary point is a saddle.

On the near branch $u^ > 1/2$, one has $s(u^*) > 0$. Therefore, a strict local minimum requires*

$$S(L^*, u^*) > \frac{K(L^*)}{(F/d_0)s(u^*)}. \quad (4.57)$$

At the fold $u^ = 1/2$, $s(u^*) = 0$, and the determinant reduces to*

$$\det H^* = -K(L^*) \leq 0,$$

so no strict local minimum can occur there.

Interpretation. The stability analysis separates stationary points into bound minima and transition-state saddles. The first necessary condition is local positive curvature in the ligand-separation coordinate, which selects the near branch $u^* > 1/2$, or equivalently $d^* < d_0 \ln 2$. This means that a mechanically stable TCR–pMHC bound state can occur only while the binding interface remains sufficiently close. The determinant condition then tests whether curvature in the full (L, d) landscape is positive definite. If the determinant is negative, the stationary point is a saddle and represents a transition configuration on the pathway toward dissociation.

4.7 Newton Trajectories and Bond-Breaking Points

The previous sections characterized the stationary points of the force-loaded free-energy landscape and classified their local stability through the Hessian. We now describe how these stationary points vary as the applied force changes. This geometric viewpoint is useful because force-induced rupture is represented not only by increasing ligand separation, but by the loss of a stable stationary configuration on the force-loaded landscape.

Newton trajectory theory provides a framework for analysing how critical points of a potential energy surface move under an applied force [136, 137]. For a molecular configuration vector $\mathbf{x}$, the force-loaded potential energy surface can be written as

$$V_f(\mathbf{x}) = V_0(\mathbf{x}) - F \mathbf{f}^\top \mathbf{x}, \quad (4.58)$$

where $V_0(\mathbf{x})$ is the intrinsic, unloaded energy surface, F is the force magnitude, and $\mathbf{f}$ specifies the direction of force application. At a stationary point $\mathbf{x}_c$ of the loaded surface,

$$\nabla V_f(\mathbf{x}_c) = 0. \quad (4.59)$$

Equivalently,

$$\mathbf{g}(\mathbf{x}_c) := \nabla V_0(\mathbf{x}_c) = F \mathbf{f}. \quad (4.60)$$

Thus, as F varies, the critical point $\mathbf{x}_c$ traces a curve in configuration space. Such curves are Newton trajectories. For any point on the trajectory, the corresponding force magnitude is

$$\|\mathbf{g}(\mathbf{x}_c)\| = (\mathbf{g}^\top(\mathbf{x}_c) \mathbf{g}(\mathbf{x}_c))^{1/2}. \quad (4.61)$$

The motion of a critical point along a Newton trajectory can be expressed by the differential equation

$$\frac{d\mathbf{x}_c}{dt} = \text{Det}(H) H^{-1}(\mathbf{x}_c) \mathbf{g}(\mathbf{x}_c), \quad (4.62)$$

where H is the Hessian matrix of the intrinsic energy surface and t is a parameter along the trajectory [167, 168]. Since the force term in (4.58) is linear, it does not change the Hessian. Thus, the Hessian of the loaded surface and the unloaded surface coincide for the purpose of curvature and singularity analysis. To avoid explicit singularities associated with H^{-1} , one introduces the regularized matrix

$$\mathbf{A} = \text{Det}(H) H^{-1}. \quad (4.63)$$

This regularized form is useful near points where the Hessian becomes singular.

In the present TCR–pMHC model, the configuration vector is

$$\mathbf{x}^\top = (L, d), \quad (4.64)$$

and the force contribution is represented by the linear tilt

$$-F(L + d). \quad (4.65)$$

Thus, the effective pulling direction is the $(1, 1)$ direction in the (L, d) -plane. The Newton trajectory therefore describes how a stationary configuration of the TCR–pMHC landscape is displaced as the applied load is varied. In particular, it provides the geometric setting in which a bound minimum can approach a neighbouring saddle point as the force increases.

The key event is the coalescence of a local minimum with a first-order saddle point. At such a point, the Hessian becomes singular:

$$\text{Det}(H) = 0. \quad (4.66)$$

This condition marks the bond-breaking point (BBP). At the BBP, the local minimum representing the mechanically stable bound state disappears, and beyond this point the force-loaded landscape no longer supports the same bound stationary configuration.

This interpretation is consistent with the stability analysis above. Along the d -stationary branch, the two separation roots coalesce at

$$u^* = \frac{1}{2}, \quad d^* = d_0 \ln 2. \quad (4.67)$$

At this value,

$$H_{dd}^* = 0, \quad \det H^* = -(H_{Ld}^*)^2 \leq 0. \quad (4.68)$$

Thus no strict local minimum can occur at the fold of the separation branch. The full bond-breaking point is the corresponding loss of the bound minimum on the two-dimensional

landscape, identified by the Hessian-singularity condition

$$\text{Det}(H) = 0. \quad (4.69)$$

In biological terms, the BBP represents the loss of a mechanically admissible TCR–pMHC bound state. Before this point, the receptor–ligand complex can be locally stable on the force-loaded landscape. At the BBP, this local stability is lost through the merger of the bound minimum with a saddle point. Thus, bond rupture is described as a geometric loss of stability rather than simply as crossing a prescribed separation threshold.

4.8 Biological Interpretation of the Mathematical Results

The mathematical analysis developed in this chapter gives a mechanochemical interpretation of force-dependent TCR–pMHC binding. The main biological message is that a bound receptor–ligand configuration is not determined by binding strength alone. It also depends on receptor orientation, ligand-interface separation, and the applied mechanical load. In the reduced model, these effects are coupled through the force-loaded energy landscape $V_f(L, d)$, whose stationary points represent possible mechanically admissible configurations.

The admissibility condition

$$D(\theta) \geq 2Fd_0 \quad (4.70)$$

shows that an applied force can be sustained only when the local angle-dependent binding depth is sufficiently large. Since $D(\theta) \leq D_0$, this leads to the critical force scale

$$F_c = \frac{D_0}{2d_0}. \quad (4.71)$$

Thus, within the model, ligands with deeper effective binding wells can remain mechanically admissible over a larger force range, whereas a larger interaction range d_0 lowers the force threshold for loss of a bound stationary state. Biologically, this suggests that force sensitivity is controlled not only by the overall binding strength, but also by how binding strength is distributed over receptor orientation and separation.

The scalar reduction to the angular equation

$$J(\theta) = 0 \quad (4.72)$$

shows that force-dependent binding is fundamentally geometric in this model. The admissible angular band Θ identifies the hinge orientations for which the local binding well remains deep enough to support a stationary configuration. A ligand class whose preferred

binding orientation is compatible with the mechanically favoured receptor geometry is therefore more likely to maintain a bound state under load. Conversely, if force shifts the system outside the admissible angular band, no mechanically stable bound configuration can persist.

The stability analysis distinguishes a mathematically admissible stationary point from a physically stable bound state. The near-branch condition

$$u^* > \frac{1}{2}, \quad \text{equivalently} \quad d^* < d_0 \ln 2, \quad (4.73)$$

means that stable binding requires the TCR–pMHC interface to remain close to its preferred contact geometry. Stationary points on the far branch may solve the force-balance equations, but they are unstable in the separation direction and therefore cannot represent stable bound configurations.

The determinant condition

$$\det H^* > 0 \quad (4.74)$$

adds the requirement that the full two-dimensional landscape be locally stable with respect to both receptor extension and ligand separation. This condition is biologically important because it captures the coupling between deformation of the receptor complex and opening of the binding interface. Thus, the model does not treat dissociation as a purely one-dimensional escape process. Instead, a stable bound state requires simultaneous resistance to ligand separation and stability of the receptor geometry.

These results show that applied force does not merely perturb an already existing TCR–pMHC bond. Rather, force regulates the existence, multiplicity, and mechanical stability of the receptor–ligand configurations available under load. In this way, the force-loaded landscape constrains which bound states can persist and which configurations are driven toward dissociation.

Newton trajectory theory then provides the geometric interpretation of force-induced rupture. As the applied force changes, the bound minimum and the neighbouring saddle move on the energy landscape. Bond breaking occurs when the stable minimum is lost through coalescence with a saddle, marked by the Hessian singularity condition

$$\det H = 0. \quad (4.75)$$

Thus, rupture is not simply defined by reaching a prescribed separation distance. It is a loss of local stability on the force-reshaped energy landscape.

Together, these results provide the mathematical basis for interpreting catch–slip behaviour in the next chapter. Moderate force may shift the bound minimum and saddle in a way that increases the dissociation barrier, producing catch-like stabilization. At larger forces, the landscape approaches a fold or bond-breaking point, the barrier decreases,

and rupture is promoted. The model should therefore be interpreted as a phenomenological description of force-dependent TCR–pMHC bond regulation, rather than as a complete model of all biochemical events involved in T-cell activation. The next chapter uses this minimum–saddle structure to compute force-dependent energy barriers and bond lifetimes for agonist, weak agonist, and antagonist parameter regimes.

4.9 Summary

This chapter developed the mathematical foundation of a reduced mechanochemical energy-landscape model for TCR–pMHC binding under force. The model uses two collective coordinates: the receptor extension L and the ligand-binding-site separation d , with the hinge angle $\theta = \theta(L)$ linking receptor geometry to binding strength. The force-loaded free energy was written as

$$V_f(L, d) = V(\theta(L)) + D(\theta(L))\Phi(d) - F(L + d). \quad (4.76)$$

This expression combines harmonic hinge deformation, Morse-type ligand binding, angle-dependent binding depth, and a linear mechanical work term.

The stationary-point analysis showed that force-dependent binding is governed by the admissibility condition

$$D(\theta) \geq 2Fd_0. \quad (4.77)$$

This condition defines the admissible angular band and gives the critical force scale

$$F_c = \frac{D_0}{2d_0}. \quad (4.78)$$

For forces above F_c , the model cannot support a bound stationary configuration. For admissible forces, the two-dimensional stationary system can be reduced to the scalar angular equation

$$J(\theta) = 0, \quad (4.79)$$

which encodes the compatibility between hinge geometry, ligand separation, and applied force.

The Hessian analysis identified which stationary points correspond to stable bound states. Stability in the ligand-separation direction requires

$$u^* > \frac{1}{2}, \quad \text{or equivalently} \quad d^* < d_0 \ln 2. \quad (4.80)$$

A strict local minimum additionally requires

$$\det H^* > 0. \quad (4.81)$$

Thus, stable TCR–pMHC binding requires both local resistance to separation and stability of the coupled receptor–ligand geometry.

Finally, Newton trajectories were introduced to describe how stationary points move under applied force. The bond-breaking point occurs when a stable bound minimum coalesces with a neighbouring saddle, so that

$$\det H = 0. \tag{4.82}$$

This gives a geometric interpretation of force-induced rupture as the loss of a stable bound minimum on the force-loaded energy landscape.

Overall, the chapter shows how force can regulate the existence and stability of TCR–pMHC bound configurations by reshaping a reduced mechanochemical landscape. It therefore provides the mathematical bridge from phenotypic activation models, where lifetimes enter as effective inputs, to the force-dependent barrier and lifetime calculations developed in the next chapter.

Force-Dependent Energy Barriers and Bond Lifetimes in TCR–pMHC Binding

5.1 Introduction

Chapter 4 developed the mathematical foundation of the mechanochemical TCR–pMHC model. In that chapter, the TCR–pMHC–CD3 complex was described by a coarse-grained, force-loaded free-energy landscape $V_f(L, d)$, where L denotes receptor extension and d denotes ligand-binding-site separation. The extension coordinate was linked to the hinge angle θ through the geometric relation

$$L(\theta) = 2W \sin\left(\frac{\theta}{2}\right), \quad (5.1)$$

allowing receptor deformation and ligand separation to be represented within a two-dimensional energy landscape [153, 135]. The previous chapter derived the stationary-point equations, reduced the two-dimensional problem to a scalar angular condition $J(\theta) = 0$, obtained the critical force threshold

$$F_c = \frac{D_0}{2d_0}, \quad (5.2)$$

and classified the resulting stationary points using the Hessian matrix. Within that framework, a stable bound TCR–pMHC complex corresponds to a local minimum of the loaded landscape, whereas the transition state for dissociation is represented by a neighbouring saddle point. Bond rupture occurs when the bound minimum and the saddle coalesce and the local minimum disappears [137, 140].

Thus, the previous chapter established the mathematical conditions under which force-supported bound states can exist, lose stability, and undergo bond-breaking. However, those analytical results do not yet show what happens for different ligand classes. In particular, they do not determine whether agonist, weak agonist, and antagonist TCR–pMHC complexes produce different force-dependent barriers and lifetimes. The purpose of this chapter is to make this connection between the mathematical model and ligand-specific mechanochemical behaviour. Here, ligand identity is represented through class-specific

parameters that define a peptide-dependent mechanochemical landscape, and applied force subsequently reshapes this landscape by moving stationary points and changing the activation barrier. In this sense, we ask a more upstream physical question: how can mechanical force reshape ligand-dependent TCR–pMHC energy landscapes and thereby generate different bond-lifetime profiles [125, 127, 147].

This chapter has three main aims. First, we describe how representative parameter sets are constructed for agonist, weak agonist, and antagonist TCR–pMHC interactions by combining experimental, structural, and phenomenological information [169, 170, 125, 135, 171]. Second, we use the mathematical framework of Chapter 4 to compute the force-dependent energy barrier and bond lifetime for each ligand class. Third, we test the robustness of the results using sensitivity analysis, identifying which parameters control the overall lifetime scale and which parameters control the force-dependent shape of the response [172, 173].

For each applied force F , the bound state is denoted by $\mathbf{x}_m(F)$, and the adjacent saddle point is denoted by $\mathbf{x}_s(F)$. The force-dependent energy barrier is defined as

$$\Delta E(F) = V_f(\mathbf{x}_s(F)) - V_f(\mathbf{x}_m(F)). \quad (5.3)$$

This barrier measures the energetic cost of escaping from the bound state. If $\Delta E(F)$ increases over an intermediate force range, the applied force stabilizes the bond and produces a catch-like regime. If $\Delta E(F)$ decreases monotonically with force, the applied load promotes dissociation and the bond behaves as a slip bond [163, 140].

The barrier is then converted into a mean bond lifetime using an Arrhenius–Kramers–Langer approximation [174, 175, 176, 177]. This step is essential because the bond lifetime is the quantity most directly connected to force-spectroscopy experiments and to biological interpretation [125, 127]. Thus, the analysis links three levels of description: the geometry of the force-loaded effective free-energy landscape, the barrier height for dissociation, and the predicted survival time of the TCR–pMHC bond.

The main result of this chapter is that the same mathematical framework, when parameterized by ligand class, produces distinct mechanochemical phenotypes. Under this peptide-dependent landscape formulation, the agonist parameter set displays a pronounced catch–slip profile: the energy barrier and bond lifetime increase at moderate force and decrease at higher force. The weak agonist shows a weaker and shallower version of the same response. In contrast, the antagonist shows a monotonic decrease in barrier height and lifetime with increasing force, corresponding to slip-bond behaviour [125, 127, 178]. Thus, force does not simply weaken all TCR–pMHC interactions. Instead, its effect depends on the ligand-specific coupling between binding strength, receptor geometry, and ligand separation.

The sensitivity analysis further separates the roles of the main parameters. The

binding-well depth D_0 mainly controls the magnitude of the barrier and lifetime response, whereas the preferred binding angle θ_1 mainly controls the force-dependent shape of the catch-slip curve. This provides a mechanistic distinction between an energetic contribution, governed primarily by binding strength, and a geometric contribution, governed primarily by the preferred force-sensitive receptor orientation.

The scope of the chapter is therefore deliberately restricted. The model describes force-dependent TCR-pMHC bond regulation through a reduced mechanochemical landscape; it does not attempt to model the full biochemical process of T-cell activation. Instead, it provides an upstream physical mechanism by which ligand-dependent binding lifetimes may arise from the combined effects of binding energetics, receptor geometry, and applied force.

This chapter is organized as follows. Section 5.2 presents the parameterization for the agonist, weak agonist, and antagonist ligand classes. Section 5.3 introduces the Kramers-Langer approximation used to convert energy barriers into bond lifetimes. Section 5.4 presents the force-dependent energy-landscape geometry using potential-energy surface plots and Hessian-singularity curves. Section 5.5 discusses the force-dependent barrier curves, and Section 5.6 presents the corresponding bond-lifetime curves. Section 5.7 summarizes the sensitivity analysis, while Section 5.8 discusses the biological interpretation and scope of the model. Finally, Section 5.9 summarizes the main conclusions of the chapter.

5.2 Parameterization for Ligand Classes

To compute force-dependent barriers and bond lifetimes, we require representative parameter sets for agonist, weak agonist, and antagonist TCR-pMHC interactions. These parameters encode the main physical features of the coarse-grained landscape: binding strength, hinge stiffness, angular tolerance, preferred binding orientation, and the effective range of ligand separation.

The energy-landscape model introduced in Chapter 4 contains seven parameters: the binding-well depth D_0 , the angular stiffness k_θ , the angular width σ , the preferred binding angle θ_1 , the Morse range d_0 , the geometric length scale W , and the unloaded hinge angle θ_0 . Of these, $W = 7 \text{ nm}$ and $\theta_0 = 0.60\pi$ are shared structural constants fixed for all ligand classes from cryo-EM and structural modelling data [153, 135], and are not varied in the analysis.

The remaining parameters are ligand-class specific. They should be interpreted as effective parameters of the reduced mechanochemical landscape rather than as direct atomistic observables. In particular, ligand identity is represented by a peptide-dependent, allosterically modulated landscape: different ligand classes are assigned different values of D_0 , k_θ , σ , θ_1 , and d_0 , and applied force then reshapes these landscapes by moving

stationary points and changing the activation barrier. Thus, the parameterization below is intended to give representative agonist, weak agonist, and antagonist landscapes for force-dependent barrier and lifetime calculations.

Binding-Well Depth D_0 from Affinity Data The parameter D_0 measures the binding strength of a TCR–pMHC complex in the energy-landscape model. We define D_0 as a positive well depth per molecule. Thus, a larger value of D_0 means a deeper potential well and therefore stronger binding.

For each peptide–TCR pair, D_0 is obtained from the reported three-dimensional dissociation constant K_D . We use the standard-state relation

$$\frac{D_0}{k_B T} = -\ln \left(\frac{K_D}{1 \text{ M}} \right), \quad (5.4)$$

where $\ln$ is the natural logarithm, 1 M is the standard-state concentration, and T is the assay temperature. In this work we use $T = 310$ K. If a molar binding free energy ΔG° is reported instead of K_D , we use the equivalent one-molecule expression

$$D_0 = -\frac{\Delta G^\circ}{N_A}, \quad (5.5)$$

where N_A is Avogadro’s number.

We report D_0 in two equivalent ways: as the dimensionless ratio $D_0/k_B T$, and in mechanical energy units of pN nm. The conversion is

$$1 \text{ pN nm} = 10^{-21} \text{ J}, \quad k_B T|_{T=310 \text{ K}} = 4.28 \text{ pN nm}. \quad (5.6)$$

This sign convention is consistent with the energy landscape: favourable binding has negative ΔG° , but it corresponds to a positive well depth D_0 .

In practice, K_D , and therefore D_0 , differs from peptide to peptide even within the same ligand class. We therefore compute D_0 from the available peptide-level affinity data and then choose representative class-level values for the three ligand classes used in the simulations. The values are chosen so that they preserve the expected energetic ordering: agonists have the deepest binding wells, weak agonists have intermediate wells, and antagonists have the shallowest wells [169, 179, 170].

The representative values used in this chapter are

$$D_0 = 12.0 k_B T \quad (\text{agonist}), \quad D_0 = 9.4 k_B T \quad (\text{weak agonist}), \quad D_0 = 7.35 k_B T \quad (\text{antagonist}). \quad (5.7)$$

In mechanical units, these are approximately

$$51.4 \text{ pN nm}, \quad 40.2 \text{ pN nm}, \quad 31.5 \text{ pN nm}, \quad (5.8)$$

respectively.

As representative examples, the values are motivated by ligands spanning the agonist–antagonist range, including gp33-wt as an agonist [169], Y4A as a weak agonist [170], and Y4SC9M as an antagonist [170]. In the reduced model, D_0 should therefore be understood as an effective binding-well depth along the ligand-separation coordinate d , chosen to remain consistent with the measured three-dimensional binding free energy.

Angular Stiffness k_θ from Force–Lifetime Data The parameter k_θ describes the energetic cost of changing the hinge orientation of the TCR–pMHC complex. It appears in the harmonic hinge energy

$$V(\theta) = \frac{1}{2}k_\theta(\theta - \theta_0)^2, \quad (5.9)$$

where θ_0 is the preferred hinge angle in the unloaded state. A smaller value of k_θ corresponds to a more flexible angular response, whereas a larger value corresponds to a stiffer receptor–ligand complex.

Since direct ligand-resolved measurements of k_θ are not available, we estimate this parameter phenomenologically from published force-dependent TCR–pMHC bond-lifetime data [125]. The fitted values are

$$k_\theta = 76.3 [72.8, 79.7] \text{ pN nm} \quad (\text{agonist}), \quad (5.10)$$

$$k_\theta = 80.9 [73.8, 88.0] \text{ pN nm} \quad (\text{weak agonist}), \quad (5.11)$$

and

$$k_\theta = 85.3 [61.6, 109.1] \text{ pN nm} \quad (\text{antagonist}). \quad (5.12)$$

Here, the brackets denote bootstrap confidence intervals.

These estimates suggest that the agonist complex is represented as the most angularly compliant, while the weak agonist and antagonist are represented by progressively stiffer angular potentials. This ordering is consistent with the idea that stimulatory ligands allow a more favourable force-induced angular response, whereas weaker and antagonistic ligands are less mechanically adaptable [180]. Because k_θ is inferred from force–lifetime data within a coarse-grained fitting procedure, it should be understood as an effective model parameter rather than as a directly measured molecular stiffness.

Angular Width σ from Structural and Energetic Scaling The parameter σ determines the angular tolerance of the binding interaction. It appears in the angle-

dependent binding depth

$$D(\theta) = D_0 \exp \left[-\frac{(\theta - \theta_1)^2}{2\sigma^2} \right]. \quad (5.13)$$

Thus, σ controls how rapidly the binding strength decreases when the hinge angle θ moves away from the preferred binding angle θ_1 . A smaller σ means that strong binding is restricted to a narrow range of receptor orientations, whereas a larger σ means that binding is tolerated over a broader angular range.

It is important to distinguish σ from a directly measured angular variance. In the model, σ is an internal parameter of the binding-depth function $D(\theta)$. The observed angular spread of a bound TCR–pMHC complex also depends on the stiffness of the hinge potential. Near the preferred orientation, this combined effect can be viewed through the effective curvature

$$\kappa_{\text{eff}} = k_\theta + \frac{D_0}{\sigma^2}. \quad (5.14)$$

Therefore, σ should be interpreted as an effective angular tolerance of the binding interaction, not as a direct experimental measurement of the full angular distribution.

The agonist reference value is obtained from a structural angular-width anchor. Coarse-grained molecular-dynamics simulations of the pMHC-engaged TCR–CD3 complex report an approximate full width at half maximum of 30° for the TCR extracellular-domain tilt angle in the bound state [135]. This width is converted to a Gaussian standard deviation by

$$\sigma_0 = \frac{\text{FWHM}}{2\sqrt{2\ln 2}} \approx \frac{\text{FWHM}}{2.3548}. \quad (5.15)$$

For $\text{FWHM} = 30^\circ$, this gives

$$\sigma_0 \simeq 0.222 \text{ rad}. \quad (5.16)$$

We use this value as the agonist angular-width anchor. This anchor should be understood as a generic structural estimate for an agonist-bound pMHC-engaged $\alpha\beta$ TCR, rather than as a peptide-specific measurement for one ligand.

To represent weaker ligands, σ is then increased using a monotone binding-free-energy-based scaling. The motivation is that weaker binding interactions are expected to allow broader orientational variability, whereas strong agonist binding is more tightly concentrated around its preferred orientation. This gives the representative values

$$\sigma = 0.222 \text{ rad} \quad (\text{agonist}), \quad 0.238 \text{ rad} \quad (\text{weak agonist}), \quad 0.482 \text{ rad} \quad (\text{antagonist}). \quad (5.17)$$

Thus, the agonist landscape has the narrowest angular tolerance, the weak agonist has a slightly broader tolerance, and the antagonist is represented by the broadest angular tolerance. In the energy landscape, this means that agonist binding is concentrated near

a more specific receptor orientation, whereas antagonist binding is modeled as less tightly restricted around its preferred angle.

Preferred Binding Angle θ_1 The parameter θ_1 denotes the hinge angle at which the binding-strength function $D(\theta)$ reaches its maximum. It therefore represents the most favourable angular orientation for TCR–pMHC binding. When the hinge angle moves away from θ_1 , the effective binding depth decreases according to Eq. (5.13). Thus, θ_1 controls where the binding well is centred in angular space.

The values of θ_1 are assigned using the extension–angle relation

$$L(\theta) = 2W \sin\left(\frac{\theta}{2}\right), \quad \theta_1 = 2 \arcsin\left(\frac{L_{\text{bound}}}{2W}\right), \quad (5.18)$$

with the shared geometric scale $W = 7 \text{ nm}$ [153]. This relation converts a preferred bound extension into the corresponding hinge orientation used in the reduced energy landscape.

The representative values used in the simulations are

$$\theta_1 = 0.83\pi \quad (\text{agonist}), \quad \theta_1 = 0.80\pi \quad (\text{weak agonist}), \quad \theta_1 = 0.72\pi \quad (\text{antagonist}). \quad (5.19)$$

In degrees, these correspond approximately to 149.4° , 144° , and 129.6° , respectively. Thus, the agonist is represented by the most extended preferred binding orientation, the weak agonist by an intermediate orientation, and the antagonist by a more closed orientation.

This choice is consistent with structural and simulation-based observations that pMHC engagement is associated with a more extended TCR–CD3 configuration, with agonist-bound states showing tilt angles close to 150° [135]. In the model, θ_1 is therefore the main geometric parameter controlling how the preferred binding orientation aligns with the direction of mechanical loading.

Morse Interaction Range d_0 The Morse range parameter d_0 sets the characteristic length scale of the binding interaction along the ligand-separation coordinate d . The binding energy is written as

$$B(d, \theta) = D(\theta) \left[(1 - e^{-d/d_0})^2 - 1 \right]. \quad (5.20)$$

Here, d measures separation from the bound-state contact geometry. Therefore, d_0 determines how rapidly the binding energy weakens as the TCR–pMHC interface is pulled apart.

In the present model, d_0 is not treated as a directly measured equilibrium bond length. Instead, it is an effective coarse-grained interaction-range parameter. Since ligand-resolved experimental values of d_0 are not available for the systems considered here, we estimate it

phenomenologically from representative interfacial contact distances in crystallographic TCR–pMHC structures [171]. Using this procedure, the values used in the simulations are

$$d_0 = 0.185 \text{ nm} \quad (\text{agonist}), \quad 0.151 \text{ nm} \quad (\text{weak agonist}), \quad 0.125 \text{ nm} \quad (\text{antagonist}). \quad (5.21)$$

These values represent decreasing effective interaction ranges from agonist to weak agonist to antagonist. In the energy landscape, a larger d_0 means that the binding interaction persists over a slightly longer separation distance, whereas a smaller d_0 means that the interaction weakens more rapidly as the interface opens. The same parameter also enters the critical-force relation

$$F_c = \frac{D_0}{2d_0}, \quad (5.22)$$

and therefore contributes directly to the force at which stable bound stationary states cease to exist.

5.2.1 Summary of Parameter Values

The representative parameter values used for the agonist, weak agonist, and antagonist landscapes are summarized in Table 5.1. The table also indicates the interpretation of each parameter and how it was obtained.

Together, these parameter sets specify three class-level, force-loaded energy landscapes. The agonist landscape has the deepest binding well and the most extended preferred binding orientation, the weak agonist represents an intermediate case, and the antagonist has the shallowest binding well together with a more closed preferred orientation. These peptide-dependent landscapes are used in the following sections to compute force-dependent barriers and bond lifetimes.

5.3 From Energy Barriers to Bond Lifetimes

After specifying the ligand-class parameter sets, we next connect the geometry of the force-loaded energy landscape to bond stability. In mechanochemical descriptions, an applied force reshapes the potential-energy surface by shifting minima and saddle points, thereby changing the barrier that separates the bound state from the dissociation pathway [137, 140]. In the present model, this connection is made in two steps. First, for each force value F , we compute the energy barrier between the bound minimum and the neighbouring saddle point. Second, we convert this barrier into a mean bond lifetime using an Arrhenius–Kramers–Langer approximation [174, 175, 176, 177].

Table 5.1. Model parameters used in the coarse-grained TCR–pMHC energy-landscape formulation. Representative class-level values used in the force-dependent simulations are shown.

Parameter	Value(s) used	Interpretation	Source and method
D_0	Ag: $12.0 k_B T$ Wk: $9.4 k_B T$ An: $7.35 k_B T$	Maximum binding-well depth	Computed from reported 3D solution affinities, using either K_D or ΔG° , through the standard-state thermodynamic relation [169, 179, 170].
k_θ	Ag: 76.3 pN nm Wk: 80.9 pN nm An: 85.3 pN nm	Effective angular stiffness	Estimated phenomenologically from force-dependent TCR–pMHC bond-lifetime data; interpreted as an effective coarse-grained stiffness parameter [125].
σ	Ag: 0.222 rad Wk: 0.238 rad An: 0.482 rad	Angular tolerance of $D(\theta)$	Assigned using an agonist-bound structural angular-width anchor and binding-free-energy-based scaling across ligand classes [135, 170].
θ_1	Ag: 0.83π Wk: 0.80π An: 0.72π	Preferred hinge orientation for strongest binding	Assigned from the extension–angle relation using $W = 7$ nm; consistent with structural observations of TCR–CD3 conformational opening [153, 135].
d_0	Ag: 0.185 nm Wk: 0.151 nm An: 0.125 nm	Effective Morse interaction-range parameter	Estimated phenomenologically from representative interfacial contact distances in crystallographic TCR–pMHC structures [171].
W	7 nm	Effective geometric length scale	Fixed shared parameter in the extension–angle relation $L(\theta) = 2W \sin(\theta/2)$ [153].
θ_0	0.60π	Preferred hinge angle of the unloaded state	Fixed shared structural parameter representing the preferred unloaded orientation of the receptor complex [135].

Ag, agonist; Wk, weak agonist; An, antagonist. The quantities D_0 , k_θ , σ , θ_1 , and d_0 vary by ligand class, whereas W and θ_0 are fixed shared structural parameters.

5.3.1 Force-Dependent Barrier Height

For a fixed applied force F , the loaded energy landscape is denoted by $V_f(L, d)$. The bound TCR–pMHC state corresponds to a local minimum of this landscape, which we denote by

$$x_m(F) = (L_m(F), d_m(F)).$$

The transition state for dissociation is represented by the neighbouring index-one saddle point

$$x_s(F) = (L_s(F), d_s(F)).$$

The force-dependent energy barrier is then defined as

$$\Delta E(F) = V_f(x_s(F)) - V_f(x_m(F)). \quad (5.23)$$

This quantity measures the energetic cost of escaping from the bound minimum toward dissociation.

The dependence of $\Delta E(F)$ on force determines whether the landscape shows catch-like or slip-like behaviour at the barrier level. If moderate force raises the barrier, the applied load stabilizes the bound state and produces a catch-like regime. If force lowers the barrier, the applied load promotes dissociation and the bond behaves as a slip bond. This interpretation is consistent with the general physical picture of catch bonds, where force can either stabilize or destabilize a receptor–ligand complex depending on how it reshapes the underlying energy landscape [163, 140].

Equivalently, if the loaded potential is written as

$$V_f(x) = V_0(x) - Ff^\top x,$$

then the barrier can be expressed as

$$\Delta E(F) = V_0(x_s(F)) - V_0(x_m(F)) - Ff^\top (x_s(F) - x_m(F)). \quad (5.24)$$

This expression shows that force changes the barrier in two ways: it shifts the positions of the minimum and saddle point on the original landscape, and it adds a force-dependent energetic tilt [137, 140]. In the TCR–pMHC model considered here, the configuration vector is $x = (L, d)$, and the effective force enters through the term $-F(L + d)$. Thus, force reshapes the barrier by acting on both receptor extension and ligand-binding-site separation.

The barrier disappears when the bound minimum and the saddle point coalesce. This point is the bond-breaking point. Numerically, it is detected through the Hessian singularity condition

$$\det H = 0.$$

Beyond this point, the loaded landscape no longer supports a stable bound minimum, and the receptor–ligand complex cannot remain mechanically stable.

5.3.2 Kramers–Langer Lifetime Approximation

The barrier height $\Delta E(F)$ gives an energetic measure of bond stability, but force-spectroscopy experiments usually report bond lifetime [125, 127]. To connect the computed

barrier to a lifetime, we use an Arrhenius–Kramers–Langer approximation [174, 175, 176, 177]. The mean bond lifetime is written as

$$\tau(F) = \tau_0(F) \exp\left(\frac{\Delta E(F)}{k_B T}\right), \quad (5.25)$$

where k_B is the Boltzmann constant, T is the absolute temperature, and $\tau_0(F)$ is a force-dependent prefactor.

The exponential term in Eq. (5.25) contains the barrier height in thermal units. A higher barrier therefore gives a longer lifetime, whereas a lower barrier gives faster dissociation. The prefactor $\tau_0(F)$ accounts for the local curvature of the energy landscape near the bound minimum and the saddle point. Thus, the lifetime is controlled not only by the barrier height but also by the shape of the landscape around the escape pathway.

Following Langer’s metastable decay theory, the prefactor is written as

$$\tau_0(F) = \frac{2\pi}{\lambda_+(F)} \sqrt{\frac{|\det H^\ddagger(F)|}{\det H_A(F)}}, \quad (5.26)$$

where $H_A(F)$ is the Hessian matrix at the bound minimum $x_m(F)$, and $H^\ddagger(F)$ is the Hessian matrix at the saddle point $x_s(F)$. The absolute value is used because an index-one saddle has one unstable direction and therefore $\det H^\ddagger(F) < 0$.

The quantity $\lambda_+(F)$ is the positive escape rate associated with the unstable direction at the saddle. In the overdamped setting, it is related to the negative curvature along the unstable direction by

$$\lambda_+(F) = -\beta \mathcal{D} h_{uu}(F), \quad \beta = \frac{1}{k_B T}, \quad (5.27)$$

where $\mathcal{D}$ is an effective diffusion coefficient and $h_{uu}(F) < 0$ is the curvature of the saddle Hessian along the unit unstable direction. Equivalently, if $\lambda_-(F) < 0$ denotes the negative eigenvalue of $H^\ddagger(F)$, then

$$\lambda_+(F) = \beta \mathcal{D} |\lambda_-(F)|.$$

Combining Eqs. (5.25) and (5.26), the force-dependent lifetime becomes

$$\tau(F) = \frac{2\pi}{\lambda_+(F)} \sqrt{\frac{|\det H^\ddagger(F)|}{\det H_A(F)}} \exp\left(\frac{\Delta E(F)}{k_B T}\right). \quad (5.28)$$

This formula links the mechanochemical energy landscape to an experimentally interpretable quantity. The barrier $\Delta E(F)$ gives the dominant exponential contribution, while the Hessian terms and the unstable curvature describe how the local geometry of the landscape affects escape from the bound state.

In the following sections, this procedure is applied to the agonist, weak agonist, and

antagonist parameter sets. For each force value, we locate the bound minimum and neighbouring saddle point, compute $\Delta E(F)$, evaluate the local Hessian quantities, and estimate the corresponding bond lifetime $\tau(F)$.

5.4 Force-Dependent Energy-Landscape Geometry

We now examine how external force reshapes the TCR–pMHC energy landscape for agonist, weak agonist, and antagonist ligands. In pharmacological terminology, an agonist binds to a receptor and activates it, a weak or partial agonist binds but produces a weaker response, and an antagonist binds without productive activation and can block the action of an agonist [181, 182, 183, 184, 185]. In the present mechanochemical setting, these ligand classes are represented by different parameter sets in the same force-loaded energy landscape.

The applied force is taken along the $(1, 1)$ direction in the reduced (L, d) -coordinates. Thus, force acts simultaneously on receptor extension L and ligand-binding-site separation d , corresponding to the linear tilt term $-F(L + d)$ in the loaded energy landscape. This choice represents a coarse-grained pulling direction in which mechanical load both extends the TCR–pMHC–CD3 complex and promotes opening of the ligand-binding interface.

The potential-energy surface (PES) is analysed in the (L, d) -plane. Here, L denotes the effective receptor extension, and d denotes the separation of the ligand-binding interface. In the contour plots, each curve is an equipotential line of the loaded energy surface. The point labelled R denotes the reactant or bound state, corresponding to a local minimum of the PES. The point labelled TS denotes the transition state, represented by an index-one saddle point (SP1). The energy difference between these two points is the barrier that must be crossed for dissociation.

The Newton trajectory (NT) gives the path followed by stationary points as the applied force is varied. Along this curve, the local minimum and the saddle move through the energy landscape. The green curves labelled $\det(\mathbf{H}) = 0$ mark points where the Hessian matrix becomes singular. These curves are important because bond rupture occurs when the bound minimum and the saddle coalesce on such a curve. This coalescence point is the bond-breaking point (BBP). At the BBP, the energy barrier disappears and the loaded landscape no longer supports a stable bound state.

Figure 5.1 shows a representative three-dimensional PES for the agonist case at $F = 10$ pN. The bound minimum and the neighbouring saddle point are visible on the surface, and the vertical energy difference between them corresponds to the barrier height ΔE . This figure gives a direct geometric view of how the barrier arises from the shape of the energy landscape.

Figure 5.2 shows two-dimensional PES contour plots for the agonist and weak agonist cases at different force levels. In the agonist landscape at $F = 0$, the bound state R is

present as a stable minimum, while the transition state lies farther along the dissociation pathway. When force is increased to $F = 10$ pN, the PES contains a clearly visible minimum–saddle pair. The Newton trajectory bends through the landscape, and its turning behaviour provides a geometric indication of catch-like stabilization: the applied force initially moves the stationary points in a way that raises the barrier rather than immediately lowering it.

At still higher force, near $F = 116$ pN for the agonist, the bound minimum and transition state merge at the bond-breaking point. In the contour plot this appears as a shoulder-like structure rather than a separated minimum–saddle pair. This indicates that the local bound state has disappeared. Thus, the agonist landscape supports a stable bound configuration over a larger force range and shows a geometric mechanism for catch–slip behaviour.

The weak agonist landscape shows a similar but weaker pattern. At $F = 10$ pN, a minimum and saddle are again present, and the Newton trajectory also indicates a catch-like regime. However, the geometric stabilization is less pronounced than in the agonist case. At high force, near $F = 105$ pN, the minimum and saddle coalesce, marking the bond-breaking point for the weak agonist. This lower bond-breaking force indicates that the weak agonist landscape is less mechanically stable than the agonist landscape.

The antagonist case differs qualitatively. Its force response does not show the same intermediate stabilization. Instead, force progressively lowers the barrier, producing a slip-like response. In geometric terms, the applied force moves the system toward dissociation without first creating a substantial increase in the barrier. The bond-breaking point occurs at a lower force, around 75 pN, consistent with the weaker mechanical stability of the antagonist landscape.

Overall, the PES geometry shows that catch–slip behaviour is not simply a direct consequence of applying force. It depends on how force moves the bound minimum and transition state relative to one another. For agonists, moderate force transiently stabilizes the bond by increasing the barrier to dissociation. Weak agonists show the same mechanism in attenuated form, whereas antagonists behave as slip bonds, with force directly promoting dissociation. The quantitative barrier curves corresponding to these geometric observations are analysed in the next section.

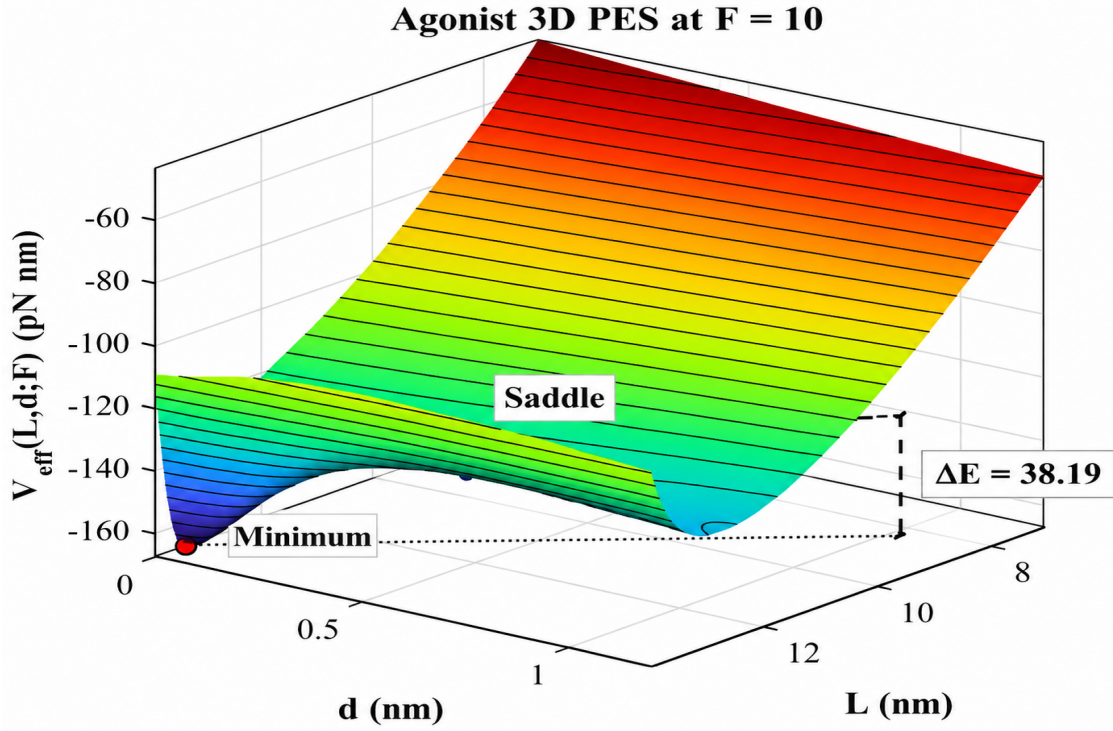


Figure 5.1. Representative three-dimensional effective potential-energy surface $V_{\text{eff}}(L, d; F)$ for the agonist at $F = 10$ pN. The bound minimum, saddle point, and barrier height ΔE are shown geometrically.

5.5 Force-Dependent Energy Barriers

The geometric changes described in the previous section can be quantified by the force-dependent energy barrier. For a given force F , the bound minimum is denoted by $\mathbf{x}_m(F)$, and the neighbouring saddle point by $\mathbf{x}_s(F)$. The barrier is defined as

$$\Delta E(F) = V_f(\mathbf{x}_s(F)) - V_f(\mathbf{x}_m(F)). \quad (5.29)$$

This quantity measures the energetic cost required for the TCR–pMHC complex to escape from the bound state and proceed toward dissociation.

The dependence of $\Delta E(F)$ on force gives a direct way to distinguish catch-like and slip-like behaviour at the level of the effective free-energy landscape. If the barrier increases over an intermediate force range, the applied force transiently stabilizes the bound state and produces a catch-like regime. If the barrier decreases as force increases, the applied load favours dissociation, corresponding to a slip-like regime.

Figure 5.3 shows the computed barrier curves for the agonist, weak agonist, and antagonist parameter sets. Under this peptide-dependent landscape formulation, the agonist case displays a clear non-monotone response. As force increases, the barrier rises

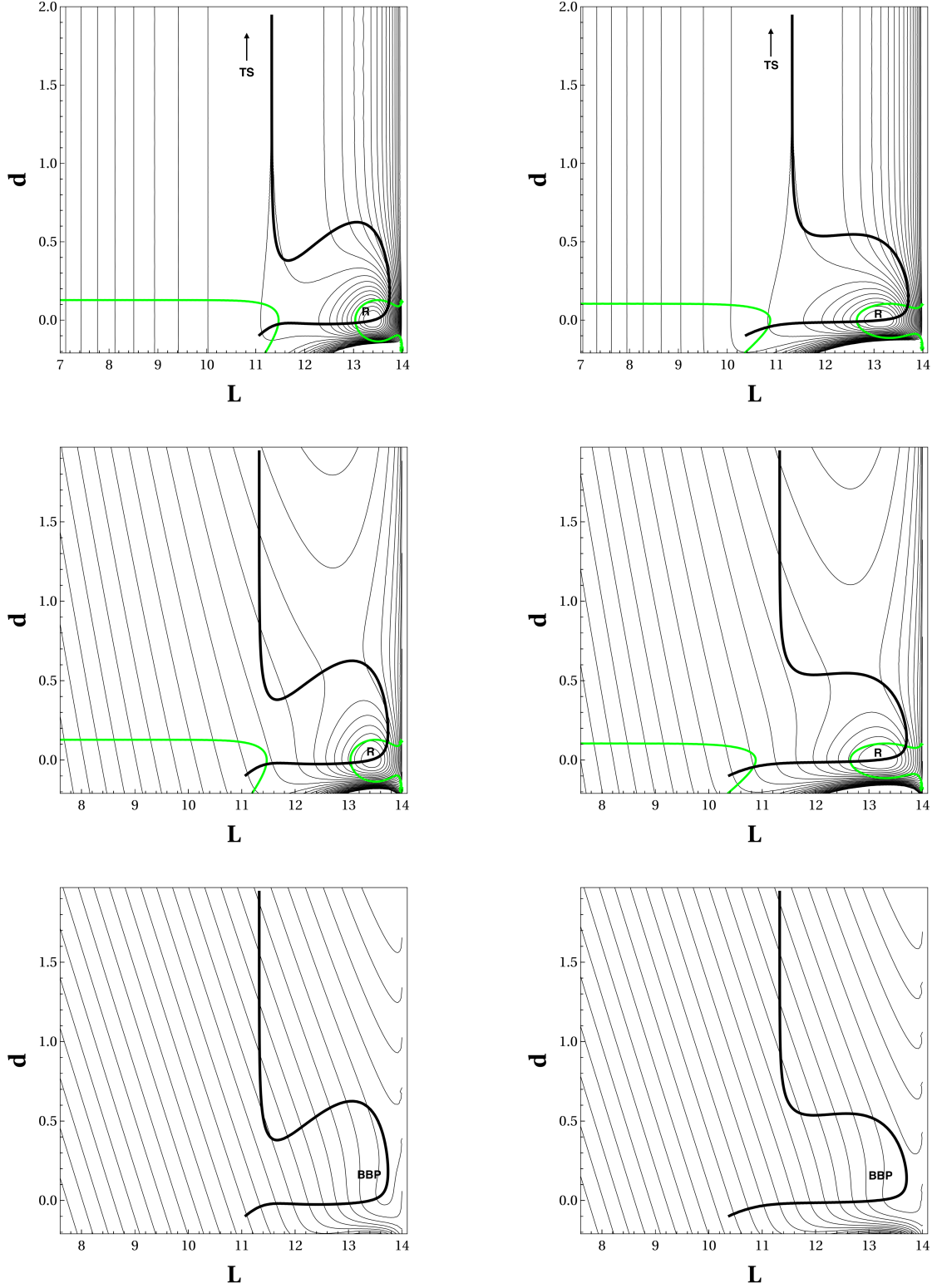


Figure 5.2. Two-dimensional potential-energy surfaces for TCR-pMHC interactions under different forces. The left column corresponds to the agonist and the right column to the weak agonist. From top to bottom: $F = 0$, $F = 10$ pN, and high-force conditions near bond rupture ($F = 116$ pN for the agonist and $F = 105$ pN for the weak agonist). Equipotential contours show the loaded energy landscape in the (L, d) -plane. R denotes the bound-state minimum, TS the adjacent index-one saddle point, and BBP the bond-breaking point where the minimum and saddle coalesce.

and reaches a maximum of approximately

$$\Delta E_{\max} \simeq 38.3 \text{ pN nm} \quad (5.30)$$

near

$$F \simeq 9 \text{ pN}. \quad (5.31)$$

Beyond this force, the barrier decreases. This biphasic profile is characteristic of catch–slip behaviour: moderate force stabilizes the bound state by raising the escape barrier, whereas larger forces reduce the barrier and promote dissociation.

The weak agonist shows the same qualitative pattern, but the effect is weaker. Its barrier increases only modestly and reaches a shallower maximum of approximately

$$\Delta E_{\max} \simeq 30.4 \text{ pN nm} \quad (5.32)$$

near

$$F \simeq 8\text{--}9 \text{ pN}. \quad (5.33)$$

Thus, the weak agonist still exhibits a catch-like phase, but the mechanical stabilization is smaller than in the agonist case.

The antagonist behaves differently. Its barrier decreases monotonically with force, beginning from a lower low-force value of approximately

$$\Delta E \simeq 27\text{--}28 \text{ pN nm}. \quad (5.34)$$

No intermediate barrier increase is observed. This indicates a slip-bond response, in which external force directly lowers the energetic cost of dissociation.

The same ordering is seen in the bond-breaking forces. The bound minimum and saddle coalesce at approximately

$$116 \text{ pN} \quad (\text{agonist}), \quad 105 \text{ pN} \quad (\text{weak agonist}), \quad 75 \text{ pN} \quad (\text{antagonist}). \quad (5.35)$$

Thus, the agonist landscape remains mechanically stable over the largest force range, the weak agonist is intermediate, and the antagonist loses stability at the lowest force.

These results show that the force response is ligand-class specific. Moderate force can increase the energy barrier for agonist and weak agonist landscapes, but not for the antagonist landscape. Therefore, force does not act simply as a universal destabilizing factor. Instead, its effect depends on how the applied load reshapes the ligand-specific combination of binding strength, receptor geometry, and ligand-interface separation.

The numerical values underlying the barrier curves are reported in Tables 5.2–5.4. For each force value, the tables list the coordinates of the bound minimum and adjacent

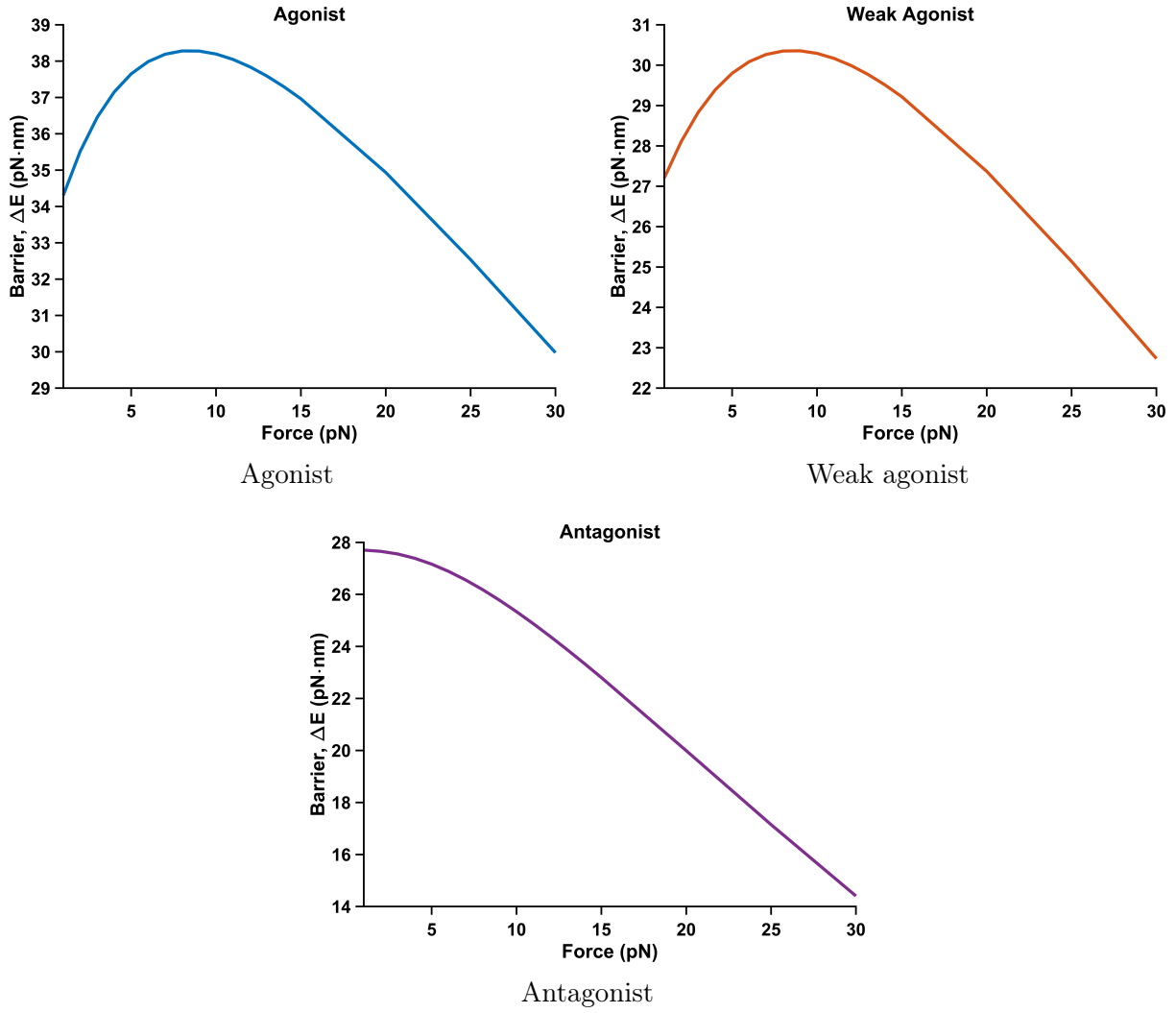


Figure 5.3. Force-dependent energy barriers for agonist, weak agonist, and antagonist TCR-pMHC interactions. The barrier is computed as $\Delta E(F) = V_f(\mathbf{x}_s(F)) - V_f(\mathbf{x}_m(F))$, where $\mathbf{x}_m(F)$ is the bound minimum and $\mathbf{x}_s(F)$ is the neighbouring saddle point. Under the peptide-dependent landscape formulation, the agonist shows a pronounced catch-slip profile, with a barrier maximum near 9 pN. The weak agonist shows a weaker catch-slip response, with a shallower maximum near 8–9 pN. The antagonist shows monotonic barrier decrease, corresponding to slip-bond behaviour.

saddle point, the corresponding free-energy values, and the resulting barrier height. These values make explicit how the barrier first increases and then decreases for agonist and weak agonist ligands, whereas it decreases monotonically for the antagonist.

Table 5.2. Stationary points of the effective free-energy landscape V_f for the agonist TCR–pMHC interaction, obtained along the Newton-trajectory direction $\frac{1}{\sqrt{2}}(1, 1)$. Energies are given in pN nm.

F [pN]	Min (L, d) [nm]	V_{Min} [pN nm]	SP (L, d) [nm]	V_{SP} [pN nm]	Barrier [pN nm]
1	(13.4101, 0.0003)	-46.2752	(11.6634, 0.3804)	-11.9705	34.3047
2	(13.4139, 0.0007)	-59.6878	(11.9297, 0.4167)	-24.1668	35.5210
3	(13.4177, 0.0010)	-73.1045	(12.1439, 0.4676)	-36.6497	36.4548
4	(13.4214, 0.0014)	-86.5253	(12.3204, 0.5114)	-49.3748	37.1505
5	(13.4251, 0.0017)	-99.9501	(12.4680, 0.5459)	-62.3005	37.6496
6	(13.4287, 0.0021)	-113.3789	(12.5930, 0.5721)	-75.3924	37.9865
7	(13.4323, 0.0024)	-126.8116	(12.6998, 0.5915)	-88.6225	38.1891
8	(13.4358, 0.0028)	-140.2483	(12.7919, 0.6054)	-101.9684	38.2799
9	(13.4393, 0.0031)	-153.6887	(12.8720, 0.6150)	-115.4082	38.2805
10	(13.4427, 0.0035)	-167.1330	(12.9421, 0.6210)	-128.9378	38.1952
11	(13.4461, 0.0038)	-180.5811	(13.0039, 0.6243)	-142.5343	38.0468
12	(13.4495, 0.0042)	-194.0328	(13.0586, 0.6255)	-156.1912	37.8416
13	(13.4528, 0.0045)	-207.4883	(13.1075, 0.6249)	-169.9000	37.5883
14	(13.4560, 0.0049)	-220.9474	(13.1512, 0.6229)	-183.6537	37.2937
15	(13.4593, 0.0052)	-234.4101	(13.1906, 0.6199)	-197.4464	36.9637
20	(13.4750, 0.0071)	-301.7768	(13.3396, 0.5947)	-266.8417	34.9351
25	(13.4899, 0.0090)	-369.2294	(13.4373, 0.5624)	-336.6935	32.5359
30	(13.5042, 0.0109)	-436.7647	(13.5056, 0.5291)	-406.7888	29.9759

5.6 Force-Dependent Bond Lifetimes

The force-dependent energy barrier $\Delta E(F)$ provides an energetic measure of bond stability. To express this stability in kinetic terms, we convert the barrier into a mean bond lifetime using the Arrhenius–Kramers–Langer form [174, 175, 176, 177]

$$\tau(F) = \tau_0(F) \exp\left(\frac{\Delta E(F)}{k_B T}\right), \quad (5.36)$$

where

$$\Delta E(F) = V_f(\mathbf{x}_s(F)) - V_f(\mathbf{x}_m(F)) \quad (5.37)$$

is the force-dependent barrier between the saddle point $\mathbf{x}_s(F)$ and the bound minimum $\mathbf{x}_m(F)$. Here, k_B is the Boltzmann constant, T is the absolute temperature, and $\tau_0(F)$ is the force-dependent prefactor. At human body temperature, $k_B T \simeq 4.28$ pN nm, which provides the thermal energy scale for comparing the computed barriers.

Table 5.3. Stationary points of the effective free-energy landscape V_f for the weak agonist TCR–pMHC interaction, obtained along the Newton-trajectory direction $\frac{1}{\sqrt{2}}(1, 1)$. Energies are given in pN nm.

F [pN]	Min (L, d) [nm]	V_{Min} [pN nm]	SP (L, d) [nm]	V_{SP} [pN nm]	Barrier [pN nm]
1	(13.1523, 0.0003)	-39.3487	(11.5877, 0.5744)	-12.1439	27.2048
2	(13.1613, 0.0006)	-52.5060	(11.8089, 0.5407)	-24.3981	28.1079
3	(13.1699, 0.0009)	-65.6723	(11.9974, 0.5368)	-36.8414	28.8309
4	(13.1784, 0.0012)	-78.8476	(12.1592, 0.5395)	-49.4596	29.3880
5	(13.1867, 0.0015)	-92.0315	(12.2991, 0.5431)	-62.2318	29.7997
6	(13.1948, 0.0018)	-105.2239	(12.4210, 0.5459)	-75.1378	30.0861
7	(13.2027, 0.0021)	-118.4245	(12.5278, 0.5473)	-88.1601	30.2644
8	(13.2105, 0.0024)	-131.6334	(12.6220, 0.5473)	-101.2834	30.3500
9	(13.2181, 0.0027)	-144.8502	(12.7056, 0.5460)	-114.4948	30.3554
10	(13.2255, 0.0029)	-158.0748	(12.7801, 0.5435)	-127.7832	30.2916
11	(13.2328, 0.0032)	-171.3071	(12.8468, 0.5402)	-141.1391	30.1680
12	(13.2400, 0.0035)	-184.5469	(12.9068, 0.5360)	-154.5546	29.9923
13	(13.2471, 0.0038)	-197.7941	(12.9610, 0.5312)	-168.0226	29.7715
14	(13.2540, 0.0042)	-211.0486	(13.0101, 0.5259)	-181.5371	29.5115
15	(13.2608, 0.0045)	-224.3104	(13.0549, 0.5201)	-195.0930	29.2174
20	(13.2933, 0.0060)	-290.7229	(13.2283, 0.4881)	-263.3533	27.3696
25	(13.3237, 0.0076)	-357.3004	(13.3458, 0.4543)	-332.1620	25.1384
30	(13.3523, 0.0093)	-424.0334	(13.4296, 0.4215)	-401.3006	22.7328

Table 5.4. Stationary points of the effective free-energy landscape V_f for the antagonist TCR–pMHC interaction, obtained along the Newton-trajectory direction $\frac{1}{\sqrt{2}}(1, 1)$. Energies are given in pN nm.

F [pN]	Min (L, d) [nm]	V_{Min} [pN nm]	SP (L, d) [nm]	V_{SP} [pN nm]	Barrier [pN nm]
1	(12.2427, 0.0003)	-39.9972	(11.5232, 0.7483)	-12.2956	27.7016
2	(12.2963, 0.0005)	-52.2673	(11.6993, 0.6688)	-24.6122	27.6551
3	(12.3468, 0.0008)	-64.5897	(11.8573, 0.6238)	-37.0367	27.5530
4	(12.3948, 0.0010)	-76.9616	(11.9993, 0.5922)	-49.5735	27.3881
5	(12.4403, 0.0013)	-89.3805	(12.1274, 0.5677)	-62.2174	27.1631
6	(12.4838, 0.0015)	-101.8441	(12.2431, 0.5474)	-74.9609	26.8832
7	(12.5253, 0.0018)	-114.3505	(12.3480, 0.5299)	-87.7958	26.5547
8	(12.5650, 0.0020)	-126.8976	(12.4434, 0.5143)	-100.7142	26.1834
9	(12.6031, 0.0023)	-139.4840	(12.5303, 0.5002)	-113.7088	25.7752
10	(12.6397, 0.0026)	-152.1079	(12.6097, 0.4872)	-126.7730	25.3349
11	(12.6749, 0.0028)	-164.7680	(12.6824, 0.4751)	-139.9007	24.8673
12	(12.7088, 0.0031)	-177.4629	(12.7491, 0.4638)	-153.0863	24.3766
13	(12.7415, 0.0034)	-190.1914	(12.8105, 0.4531)	-166.3249	23.8665
14	(12.7731, 0.0036)	-202.9523	(12.8671, 0.4428)	-179.6119	23.3404
15	(12.8037, 0.0039)	-215.7446	(12.9194, 0.4331)	-192.9434	22.8012
20	(12.9426, 0.0054)	-280.1424	(13.1295, 0.3897)	-260.1512	19.9912
25	(13.0623, 0.0071)	-345.1928	(13.2786, 0.3528)	-328.0457	17.1471
30	(13.1668, 0.0089)	-410.8113	(13.3883, 0.3205)	-396.4078	14.4035

The prefactor $\tau_0(F)$ accounts for the local geometry of the energy landscape near the minimum and saddle point. In Langer's metastable decay theory, it is written as

$$\tau_0(F) = \frac{2\pi}{\lambda_+(F)} \sqrt{\frac{|\det \mathbf{H}^\ddagger(F)|}{\det \mathbf{H}_A(F)}}, \quad (5.38)$$

where $\mathbf{H}_A(F)$ is the Hessian at the bound minimum and $\mathbf{H}^\ddagger(F)$ is the Hessian at the saddle point. The absolute value is used because an index-one saddle has one unstable direction and therefore a negative Hessian determinant. The quantity $\lambda_+(F)$ is the positive escape rate along the unstable direction at the saddle. In the overdamped setting,

$$\lambda_+(F) = -\beta \mathcal{D} h_{uu}(F), \quad \beta = \frac{1}{k_B T}, \quad (5.39)$$

where $\mathcal{D}$ is an effective diffusion coefficient and $h_{uu}(F) < 0$ is the unstable curvature of the saddle Hessian. Thus, the bond lifetime depends not only on the barrier height, but also on the local curvature of the energy landscape.

Figure 5.4 shows the predicted bond lifetime as a function of applied force for agonist, weak agonist, and antagonist TCR–pMHC interactions. The three ligand classes show distinct mechanochemical responses.

For the agonist, the lifetime increases from approximately 0.83 s at 1 pN to a maximum of about 1.29 s near 9 pN. At higher forces, the lifetime decreases, reaching approximately 0.24 s by 30 pN. This biphasic profile is characteristic of catch–slip behaviour. Moderate force stabilizes the complex by increasing the effective barrier to dissociation, whereas stronger force lowers the barrier and promotes bond rupture.

The weak agonist shows a much weaker form of the same behaviour. Its lifetime first decreases slightly from about 0.22 s at 1 pN to approximately 0.20 s at 2 pN. It then increases to a shallow maximum of approximately 0.24 s near 8 pN, before decreasing again to about 0.04 s at 30 pN. This small initial dip reflects competition between the force-dependent prefactor and the barrier term in Eq. (5.36). Thus, the weak agonist displays only a mild or transient catch-like response.

By contrast, the antagonist shows a monotonic decrease in lifetime with increasing force. No force-induced stabilization is observed. This behaviour is consistent with a slip bond, where external load directly facilitates dissociation rather than prolonging the bound state.

Thus, the lifetime curves provide the kinetic counterpart of the barrier analysis. Under this peptide-dependent landscape formulation, stimulatory ligand classes can show force-enhanced lifetimes, whereas the antagonist landscape is destabilized by force. This behaviour is consistent with force-spectroscopy studies reporting force-enhanced lifetimes for stimulatory TCR–pMHC interactions and slip-like behaviour for non-stimulatory

interactions [125, 127, 178].

The numerical values used to compute the lifetime curves are reported in Tables 5.5–5.7. These tables list the force-dependent barrier height, saddle curvature, Hessian determinants, Langer prefactor, and resulting lifetime.

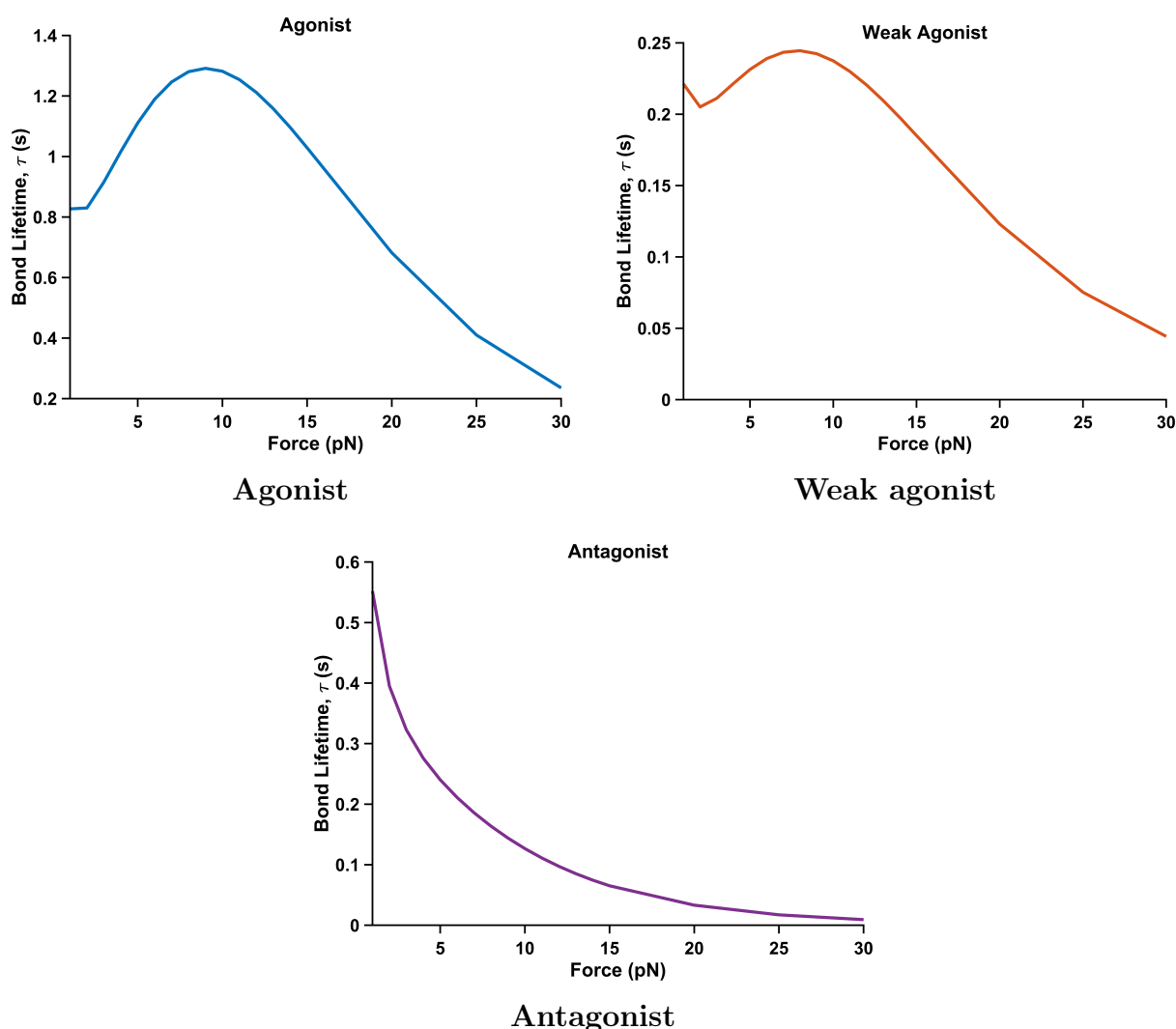


Figure 5.4. Force-dependent bond lifetimes for agonist, weak agonist, and antagonist TCR-pMHC interactions. Under the peptide-dependent landscape formulation, the agonist shows a pronounced catch-slip profile, with lifetime increasing at moderate force and decreasing at higher force. The weak agonist shows only a shallow and transient stabilization. The antagonist displays a monotonic decrease in lifetime, corresponding to slip-bond behaviour.

Table 5.5. Computed bond lifetime τ for the agonist case.

F [pN]	ΔE [pN nm]	λ_- [saddle]	$\det \mathbf{H}^\ddagger$	$\det \mathbf{H}_A$	τ_0 [ms]	τ [s]
1	34.3048	-5.8299	-26.5485	7.566×10^5	0.273	0.827
2	35.5210	-12.1652	-66.7134	7.651×10^5	0.206	0.830
3	36.4548	-18.4735	-122.2897	7.732×10^5	0.183	0.916
4	37.1505	-24.5660	-194.4368	7.811×10^5	0.173	1.016
5	37.6495	-30.4237	-284.9651	7.887×10^5	0.168	1.111
6	37.9865	-36.0693	-395.8185	7.960×10^5	0.166	1.189
7	38.1891	-41.5334	-528.9303	8.030×10^5	0.166	1.246
8	38.2799	-46.8453	-686.1825	8.098×10^5	0.167	1.280
9	38.2770	-52.0312	-869.3922	8.163×10^5	0.169	1.291
10	38.1952	-57.1133	-1080.3061	8.225×10^5	0.171	1.282
11	38.0468	-62.1108	-1320.5976	8.285×10^5	0.173	1.254
12	37.8417	-67.0397	-1591.8649	8.342×10^5	0.175	1.212
13	37.5883	-71.9133	-1895.6306	8.396×10^5	0.178	1.158
14	37.2937	-76.7431	-2233.3411	8.448×10^5	0.180	1.096
15	36.9637	-81.5383	-2606.3671	8.498×10^5	0.183	1.029
20	34.9351	-105.2252	-5043.9583	8.708×10^5	0.195	0.682
25	32.5359	-128.7637	-8527.7111	8.857×10^5	0.205	0.410
30	29.9758	-152.3138	-13149.1317	8.948×10^5	0.214	0.236

Table 5.6. Computed bond lifetime τ for the weak agonist case.

F [pN]	ΔE [pN nm]	λ_- [saddle]	$\det \mathbf{H}^\ddagger$	$\det \mathbf{H}_A$	τ_0 [ms]	τ [s]
1	27.2048	-6.8910	-36.1900	3.7371×10^5	0.384	0.221
2	28.1079	-13.7800	-83.5620	3.8296×10^5	0.288	0.205
3	28.8309	-20.5820	-144.2300	3.9189×10^5	0.251	0.211
4	29.3880	-27.2970	-219.8900	4.0050×10^5	0.231	0.221
5	29.7997	-33.9270	-312.1400	4.0880×10^5	0.219	0.231
6	30.0861	-40.4760	-422.6000	4.1679×10^5	0.212	0.239
7	30.2644	-46.9510	-552.8400	4.2447×10^5	0.207	0.243
8	30.3500	-53.3590	-704.4400	4.3186×10^5	0.204	0.245
9	30.3554	-59.7090	-878.9300	4.3896×10^5	0.202	0.242
10	30.2916	-66.0070	-1077.8000	4.4576×10^5	0.200	0.237
11	30.1679	-72.2590	-1302.6000	4.5228×10^5	0.200	0.230
12	29.9923	-78.4700	-1554.6000	4.5852×10^5	0.200	0.220
13	29.7716	-84.6470	-1835.4000	4.6447×10^5	0.200	0.210
14	29.5116	-90.7930	-2146.1000	4.7015×10^5	0.200	0.198
15	29.2174	-96.9110	-2488.1000	4.7555×10^5	0.201	0.185
20	27.3696	-127.1800	-4707.9000	4.9856×10^5	0.205	0.123
25	25.1383	-157.0000	-7868.6000	5.1508×10^5	0.212	0.075
30	22.7327	-186.3700	-12063.0000	5.2535×10^5	0.219	0.044

Table 5.7. Computed bond lifetime τ for the antagonist case.

F [pN]	ΔE [pN nm]	λ_- [saddle]	$\det \mathbf{H}^\ddagger$	$\det \mathbf{H}_A$	τ_0 [ms]	τ [s]
1	27.7016	-7.9994	-44.8780	6.9814×10^4	0.852	0.551
2	27.6551	-15.9415	-99.4022	7.4237×10^4	0.617	0.395
3	27.5530	-23.8688	-164.8882	7.8545×10^4	0.516	0.323
4	27.3881	-31.7354	-242.9386	8.2735×10^4	0.459	0.276
5	27.1631	-39.5920	-333.9471	8.6811×10^4	0.421	0.240
6	26.8832	-47.4255	-438.9301	9.0768×10^4	0.394	0.211
7	26.5547	-55.1657	-560.4789	9.4611×10^4	0.375	0.186
8	26.1834	-62.9197	-699.7389	9.8343×10^4	0.361	0.164
9	25.7752	-70.6318	-857.4608	1.0196×10^5	0.349	0.144
10	25.3349	-78.3595	-1035.6702	1.0547×10^5	0.340	0.127
11	24.8673	-86.0042	-1234.4197	1.0886×10^5	0.333	0.111
12	24.3766	-93.6234	-1453.0906	1.1214×10^5	0.327	0.097
13	23.8665	-101.1609	-1700.0355	1.1532×10^5	0.323	0.085
14	23.3404	-108.6744	-1972.5804	1.1839×10^5	0.319	0.075
15	22.8012	-116.2210	-2266.5316	1.2136×10^5	0.316	0.065
20	19.9912	-152.8850	-4176.5003	1.3465×10^5	0.310	0.033
25	17.1471	-187.8768	-6943.4706	1.4548×10^5	0.313	0.017
30	17.8206	-5346.1419	-144032.0222	1.5396×10^5	0.049	0.003

5.7 Sensitivity Analysis and Robustness

The barrier and lifetime results presented above were obtained from representative parameter sets for agonist, weak agonist, and antagonist TCR–pMHC interactions. Since several parameters are effective or phenomenologically assigned, it is important to check whether the predicted catch–slip and slip behaviours depend strongly on one particular choice of parameter values. We therefore performed a bounded sensitivity analysis around each ligand-class baseline.

For each ligand class, the parameters D_0 , k_θ , θ_1 , σ , and d_0 were perturbed independently within $\pm 5\%$ of their baseline values. The shared structural parameters W and θ_0 were kept fixed. For each sampled parameter set, the force-dependent energy barrier and bond lifetime were recomputed. Parameter influence was then quantified using Latin hypercube sampling together with partial rank correlation coefficients (LHS–PRCC) [172, 173].

To summarize the lifetime curves, we used four descriptors. The first was the peak lifetime, defined as the maximum lifetime attained along the force curve. The second was the force at peak lifetime. The third was the catch-phase amplitude, defined as the increase from the initial valid lifetime to the peak. The fourth was the slip-phase

drop, defined as the decrease from the peak to the final valid lifetime. Together, these quantities describe the magnitude, force location, and non-monotone structure of the lifetime response.

Figure 5.5 summarizes the main sensitivity results. Across the ligand classes, D_0 is the dominant positive determinant of peak lifetime. This means that increasing the binding-well depth mainly increases the overall lifetime scale. By contrast, θ_1 has the strongest influence on the force-dependent shape of the response, especially on the force at which the lifetime peak occurs and on the rise-and-fall structure of the catch-slip curve. Thus, the sensitivity analysis separates two roles: D_0 primarily controls how long the bond can persist, whereas θ_1 controls how the lifetime curve is reorganized under force.

The agonist case shows the clearest and most robust catch-slip structure. The dominant role of D_0 in setting the lifetime scale and the role of θ_1 in shaping the force response are both preserved under local parameter perturbations. The weak agonist retains the same broad sensitivity hierarchy but shows stronger secondary effects from d_0 , σ , and k_θ . This is consistent with its weaker and less sharply defined catch-like response.

For the antagonist, D_0 still controls the overall lifetime scale. However, because the antagonist lifetime curve does not show a stable interior maximum, catch-related descriptors such as the force at peak lifetime and catch-phase amplitude are less robustly defined. This agrees with the interpretation that the antagonist behaves as a slip bond rather than as a catch-slip bond.

Overall, the sensitivity analysis shows that the main conclusions are not artifacts of a single parameter choice. Agonists remain the most strongly force-stabilized, weak agonists remain intermediate, and antagonists remain force-destabilized under bounded perturbations around the baseline parameter sets. The analysis therefore supports the interpretation that catch-slip behaviour arises from a robust combination of binding strength and force-sensitive receptor geometry.

5.8 Biological Interpretation and Scope

The results of this chapter support a mechanochemical interpretation of TCR-pMHC ligand discrimination. In the model, force does not act only as a destabilizing perturbation. Instead, its effect depends on how the applied load reshapes a ligand-specific, peptide-dependent energy landscape. Ligand identity is represented through class-specific parameters that determine the underlying binding-well depth, angular stiffness, angular tolerance, preferred binding orientation, and interaction range. Applied force then reshapes this landscape by moving the bound minimum and neighbouring saddle point, thereby changing the barrier to dissociation and the corresponding bond lifetime.

For agonist ligands, moderate force increases the barrier to dissociation and prolongs the bond lifetime. For weak agonists, the same effect is present but much smaller. For

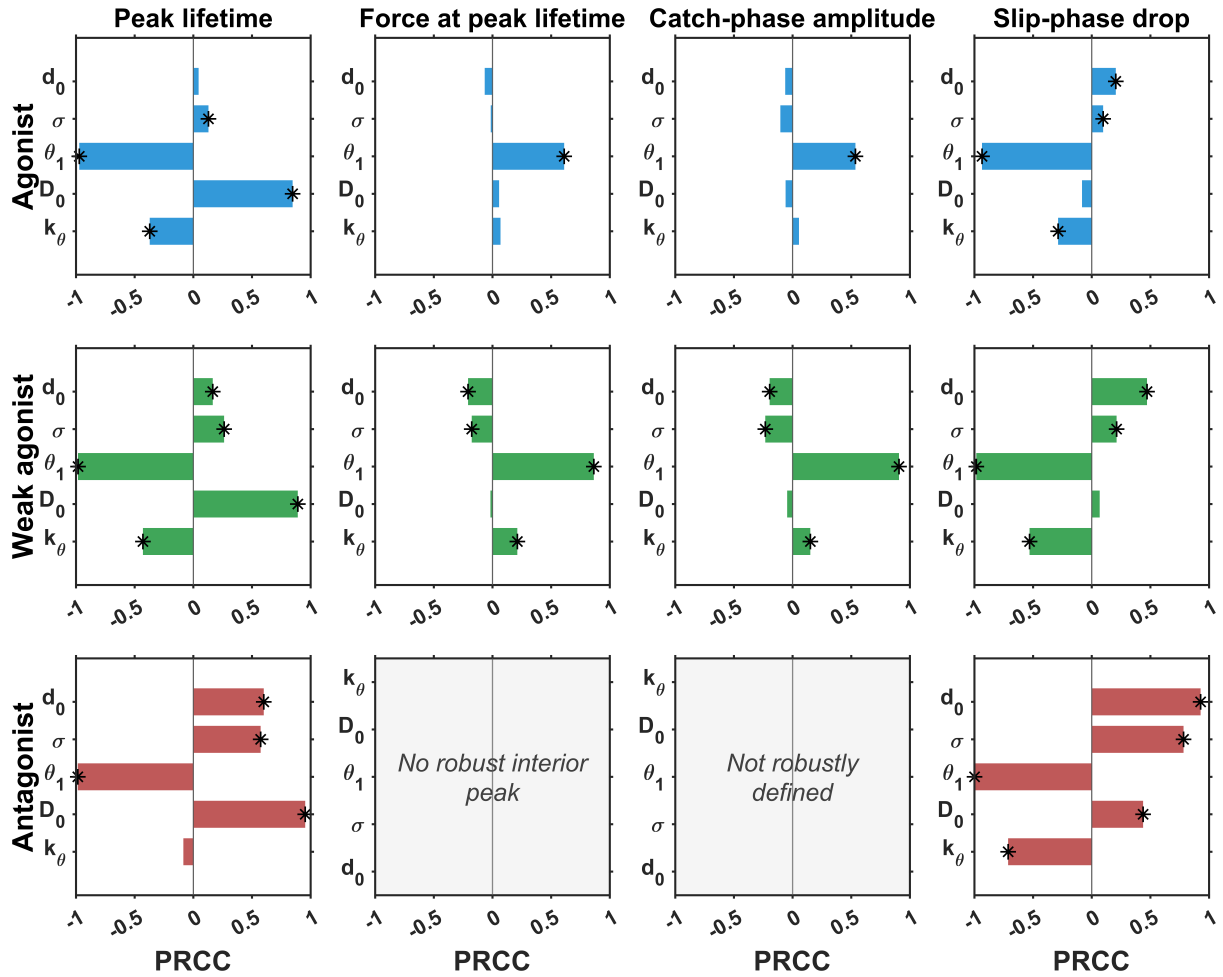


Figure 5.5. Sensitivity analysis of peak lifetime and catch-slip descriptors across ligand classes. Parameters D_0 , k_θ , θ_1 , σ , and d_0 were independently perturbed within $\pm 5\%$ of their class-specific baseline values. The shared structural parameters W and θ_0 were held fixed. The figure summarizes how each parameter influences peak lifetime, force at peak lifetime, catch-phase amplitude, and slip-phase drop.

antagonists, force lowers the barrier and shortens the lifetime, producing a slip-bond response. Thus, the model suggests that the effect of force is not universal across all TCR–pMHC interactions. Rather, it depends on how binding energetics, receptor geometry, and ligand-interface separation are combined within each ligand-class landscape.

This distinction is biologically meaningful because TCR–pMHC interactions are weak in equilibrium affinity, yet T cells can respond selectively to rare agonist peptides among many non-stimulatory ligands. The model suggests that mechanical force may contribute to this selectivity by amplifying differences in energy-landscape geometry. A ligand that aligns favourably with the force-sensitive receptor orientation can become transiently stabilized under moderate load, whereas a ligand with less favourable geometry is destabilized. In this sense, force may act as a physical filter that favours productive interactions while promoting dissociation of non-productive ones.

For agonists, the predicted catch-slip behaviour means that an intermediate range of

force increases the time available for productive receptor engagement. This is consistent with experimental observations that stimulatory TCR–pMHC interactions can display force-enhanced lifetimes and catch-bond behaviour [127, 125]. In biological terms, a longer bond lifetime may provide more time for early signalling processes, including conformational rearrangements of the TCR–CD3 complex, exposure of CD3 cytoplasmic tails, and phosphorylation of ITAM motifs by Lck. The present model does not simulate these downstream events directly, but it provides a possible upstream physical mechanism by which force could prolong the lifetime of a signalling-competent receptor–ligand interaction.

The weak agonist results indicate an intermediate mechanochemical phenotype. The barrier and lifetime curves show only weak stabilization, and the sensitivity analysis suggests a less robust catch-like response. This agrees with the idea that weak agonists can bind the receptor but do not support the same degree of mechanical reinforcement as full agonists. In this sense, weak agonists occupy a transition region between productive catch–slip behaviour and force-induced dissociation.

The antagonist results show the opposite behaviour. In the antagonist landscape, force decreases the barrier and lifetime monotonically. This means that load accelerates dissociation instead of stabilizing the interaction. Such a response is consistent with the biological role of antagonists as ligands that bind without producing productive activation and may interfere with agonist-mediated signalling. In the present framework, antagonism is therefore associated not only with weaker binding strength, but also with a mechanochemical landscape that fails to generate force-induced stabilization.

The sensitivity analysis gives a useful mechanistic interpretation of these differences. The binding-well depth D_0 mainly controls the overall magnitude of the lifetime response: deeper wells produce larger barriers and longer lifetimes. By contrast, the preferred binding angle θ_1 mainly controls the force-dependent shape of the response. It determines how the binding well is positioned relative to the direction of mechanical loading. Thus, the model separates two contributions to ligand discrimination: an energetic contribution through binding strength and a geometric contribution through receptor orientation.

The scope of the model should also be kept clear. The formulation is deliberately coarse-grained. It uses two reduced coordinates, L and d , to describe receptor extension and ligand separation, while the hinge angle $\theta = \theta(L)$ links receptor geometry to binding strength. The force is treated as a constant load acting through the term $-F(L + d)$. This captures how mechanical load tilts the effective free-energy landscape, but it does not explicitly represent the full cellular mechanics of the immunological synapse, including membrane deformation, microvillus motion, actin dynamics, or viscoelastic loading.

Similarly, the model does not explicitly simulate CD3 tail dynamics, Lck recruitment, ITAM phosphorylation, kinetic proofreading, or downstream biochemical signalling. These processes are essential for full T-cell activation, but they occur downstream of

the mechanochemical bond-stability step studied here. The model should therefore be interpreted as a phenomenological description of force-dependent TCR–pMHC bond regulation, not as a complete model of T-cell activation. Its purpose is to isolate one upstream physical layer: how applied force can reshape ligand-dependent energy barriers and bond lifetimes before those lifetimes feed into biochemical signalling networks.

Another limitation is that several parameters are effective quantities rather than directly measured molecular constants. For example, k_θ is inferred phenomenologically from force–lifetime data, σ is assigned using structural and energetic scaling, and d_0 is estimated from representative interfacial contact distances. These choices are useful for building a minimal and interpretable model, but more ligand-specific structural and force-spectroscopy data would allow future refinements. In particular, better measurements of force-dependent binding geometry and ligand-resolved mechanical response would help determine how robust the predicted catch–slip and slip behaviours are across different TCR–pMHC systems.

Despite these limitations, the model provides a compact mechanochemical framework linking force, energy barriers, and bond lifetimes. It shows how the same mathematical framework, when parameterized by ligand class, can generate three different mechanochemical behaviours: pronounced catch–slip behaviour for agonists, weak catch-like stabilization for weak agonists, and monotonic slip behaviour for antagonists. This supports the broader view that mechanical force may contribute to T-cell antigen discrimination by selectively stabilizing productive TCR–pMHC interactions while destabilizing non-productive ones.

5.9 Summary

This chapter applied the force-loaded energy-landscape framework to agonist, weak agonist, and antagonist TCR–pMHC interactions. Using the ligand-class parameter sets defined above, we tracked how the bound minimum and neighbouring saddle point move under applied force, computed the force-dependent energy barrier, and converted this barrier into a mean bond lifetime using the Arrhenius–Kramers–Langer approximation.

The results show that the same reduced mechanochemical framework can generate distinct ligand-class behaviours when the underlying landscape is parameterized by ligand identity. Under this peptide-dependent landscape formulation, agonists display a clear catch–slip response: the energy barrier and bond lifetime increase at intermediate forces and decrease at higher forces. Weak agonists show a weaker and more transient version of the same behaviour, whereas antagonists show monotonic barrier lowering and lifetime shortening, corresponding to slip-bond behaviour. Thus, force does not simply destabilize all TCR–pMHC bonds. Its effect depends on how the applied load reshapes the ligand-specific combination of binding strength, receptor geometry, and ligand-interface separation.

A key geometric feature of the analysis is the motion of minima and saddles on the effective free-energy landscape. Bond rupture occurs when the bound minimum and transition-state saddle coalesce at the bond-breaking point. This loss of local stability is associated with the Hessian singularity condition

$$\det H = 0. \quad (5.40)$$

The corresponding bond-breaking forces differ across ligand classes, with the agonist landscape remaining stable over a larger force range than the weak agonist and antagonist landscapes. This provides a geometric explanation for why agonist bonds can be transiently stabilized by moderate force, while antagonist bonds are driven more directly toward dissociation.

The lifetime analysis shows that barrier reshaping has direct kinetic consequences. Agonist lifetimes are prolonged under moderate load, weak agonist lifetimes are only mildly enhanced, and antagonist lifetimes decrease with force. The sensitivity analysis further indicates that the binding-well depth D_0 mainly controls the overall magnitude of the lifetime response, whereas the preferred binding angle θ_1 mainly controls the force-dependent shape of the catch-slip curve. This separates the mechanochemical response into an energetic contribution, governed primarily by binding strength, and a geometric contribution, governed primarily by force-sensitive receptor orientation.

The chapter also highlights the scope of the model. The formulation is deliberately minimal: it uses two reduced coordinates, assumes a Gaussian angular binding term, and treats force as a constant load. It does not explicitly simulate membrane mechanics, viscoelastic loading, CD3 tail motion, ITAM phosphorylation, or downstream signalling. Instead, it isolates one upstream physical mechanism: how force-dependent barrier reshaping can generate ligand-dependent TCR-pMHC bond lifetimes.

Overall, the results support a mechanochemical interpretation of TCR ligand discrimination. Mechanical force can reorganize the TCR-pMHC energy landscape in a ligand-dependent manner, stabilizing productive agonist interactions over an intermediate force range while destabilizing non-productive antagonist interactions. By linking force, effective free-energy geometry, barrier height, and bond lifetime, the model provides a compact framework for understanding how extracellular mechanical load may contribute to early T-cell recognition before the resulting bond lifetimes enter downstream activation pathways.

General Discussion, Conclusions, and Perspectives

6.1 Overview of the Thesis

This thesis has studied T-cell activation as both a signalling problem and a physical recognition problem. At the signalling level, the central question is how T cells convert receptor–ligand binding events into cellular response patterns such as specificity, sensitivity, antigen discrimination, ligand antagonism, optimal response, $E_{\max}$, and EC_{50} . At the physical recognition level, the central question is how the lifetime of the TCR–pMHC bond is shaped by receptor geometry, binding energetics, mechanical force, and the force-dependent structure of energy barriers. These two levels are complementary: signalling models describe how bond lifetimes are interpreted by the cell, whereas mechanochemical models describe how such lifetimes may arise from the physical behaviour of the receptor–ligand bond [20, 21, 22].

The motivation for combining these viewpoints comes from the central difficulty of T-cell activation. A T cell must respond to rare agonist peptides while avoiding inappropriate activation by abundant self or non-stimulatory ligands. This decision is not governed by a single biological variable. It depends on ligand concentration, receptor–ligand binding and dissociation, proofreading-like signalling steps, feedback regulation, spatial organization at the T-cell–APC interface, receptor conformational change, and mechanical loading [1, 6, 20]. Phenotypic models are well suited to study how kinetic variables and signalling architectures shape cellular response. However, in such models the ligand dissociation rate or bond lifetime is usually prescribed as an input parameter. Mechanochemical models address the complementary question of how such lifetimes may be generated by force-dependent bond stability [21, 22].

The central conclusion of the thesis is that these two levels should be viewed together. Phenotypic models explain how T-cell signalling can transform differences in ligand lifetime or dissociation rate into response properties such as discrimination, potency, and maximal activation. The mechanochemical model explains how ligand-dependent lifetimes may arise from force-induced changes in the energy landscape, including the motion of local

minima and saddle points and the resulting change in the dissociation barrier. In this way, the thesis connects response-level explanations of T-cell activation with a possible physical origin of the kinetic quantities that enter those response models [21, 22].

Chapters 2 and 3 developed and analysed the phenotypic part of the thesis. A family of T-cell activation models was formulated as biochemical reaction networks and translated into systems of ordinary differential equations using mass-action kinetics. These models were then compared using common response quantities, including response functions, $E_{\max}$, and EC_{50} , together with biologically motivated parameter values. The analysis identified which model structures can reproduce experimentally motivated properties such as specificity, sensitivity, antigen discrimination, optimal response, and ligand antagonism, and which parameters most strongly influence the resulting responses [21].

Chapters 4 and 5 developed the mechanochemical part of the thesis. There, the TCR–pMHC interaction was represented by a reduced force-loaded energy landscape involving receptor extension, ligand-binding-site separation, hinge geometry, binding strength, and mechanical work. The mathematical analysis characterized the existence and stability of force-supported bound states, the loss of stability at bond-breaking points, and the force-dependent energy barriers separating bound minima from saddle points. These barriers were then converted into predicted bond lifetimes using an Arrhenius–Kramers–Langer approximation, allowing agonist, weak agonist, and antagonist ligand classes to be compared within the same mechanochemical framework [67, 72, 68, 22].

The purpose of this final chapter is to synthesize the results of the thesis rather than to introduce another model. The chapter first summarizes the main conclusions from the phenotypic modelling framework and then the main conclusions from the mechanochemical energy-landscape framework. It then explains how the two viewpoints connect, discusses the biological and mathematical implications of the work, and outlines the main limitations and future directions. The final goal is to clarify the overall contribution of the thesis: a two-level mathematical description in which phenotypic models show how T-cell signalling interprets receptor–ligand lifetimes, while mechanochemical energy-landscape models show how force and receptor geometry may generate those lifetimes.

6.2 Synthesis of the Phenotypic Modelling Results

The phenotypic part of the thesis addressed the question of which simplified signalling architectures can reproduce key experimentally motivated features of T-cell activation. The models considered included receptor occupancy, kinetic proofreading, kinetic proofreading with limited signalling, kinetic proofreading with sustained signalling, kinetic proofreading with negative feedback, stabilizing activation chains, incoherent feedforward regulation, and combined variants. These models were formulated within a common reaction-network framework and translated into systems of ordinary differential equations using mass-action

kinetics. This common formulation made it possible to compare their assumptions, long-time dynamics, response functions, and parameter dependence in a systematic way [23, 20, 21].

The first important conclusion is that simple receptor occupancy provides a useful baseline but cannot explain the full range of experimentally observed T-cell response properties. In an occupancy model, activation is essentially proportional to the amount of TCR–pMHC complex formed at equilibrium. Such a model predicts that weak ligands can compensate for low affinity by being present at sufficiently high concentration. This is inconsistent with the biological observation that weak or self-like ligands often fail to activate T cells even when abundant, whereas agonist ligands can produce activation at much lower concentrations [20, 21]. Thus, receptor occupancy captures an important physical starting point, but it does not by itself explain specificity or antigen discrimination.

Kinetic proofreading improves on this limitation by introducing a sequence of intermediate biochemical steps. In this framework, a TCR–pMHC complex must remain bound long enough to progress through the proofreading chain before a signalling-competent state is reached [23]. This mechanism amplifies small differences in dissociation time and therefore provides a natural explanation for ligand discrimination. However, standard kinetic proofreading also has limitations. Strong proofreading increases specificity by rejecting short-lived ligands, but it can also reduce sensitivity because fewer complexes survive long enough to reach the signalling state [20, 21]. Thus, standard proofreading alone does not fully resolve the sensitivity–specificity trade-off.

The comparative analysis showed that extended models can reproduce a richer set of response behaviours. Models with limited signalling, sustained signalling, negative feedback, and incoherent feedforward motifs are able to generate response properties that are difficult or impossible for the simplest models. In particular, several extended models can produce non-monotone or optimal responses with respect to ligand concentration or dissociation time. This is biologically important because T-cell activation need not always increase monotonically with ligand dwell time. Instead, intermediate receptor–ligand lifetimes may be most effective in some signalling contexts [20, 21].

The model comparison also showed that different mechanisms generate non-monotone responses in different ways. Limited signalling can create an optimal response by introducing a loss of signalling at long dwell times. Sustained signalling can maintain response after ligand dissociation and can therefore change the dependence on ligand concentration. Incoherent feedforward motifs can generate adaptation-like or non-monotone response patterns because the same input influences activation through both promoting and opposing pathways. The combined models are especially informative because they show how several mechanisms can interact. For example, combining limited signalling with incoherent feedforward regulation can generate more complex response shapes, including bimodal dependence on dissociation time in some parameter regimes [21].

The analysis of $E_{\max}$ and EC_{50} further clarified the distinction between efficacy and potency. The maximal response $E_{\max}$ measures the largest response attainable by a model, whereas EC_{50} measures the ligand concentration required to reach half of that maximal response. By comparing these quantities across models and parameter sets, the thesis showed that different signalling architectures can have similar qualitative response curves but differ substantially in potency, maximal output, and robustness. This reinforces the importance of using more than one response quantity when assessing model performance. A model should not be judged only by whether it can produce activation, but also by how strongly it activates, how much ligand is required, and how these quantities change with dissociation time [20, 21].

The long-time analysis provided an additional mathematical contribution. For most models in the phenotypic family, solutions converge to a unique steady state in the biologically relevant region. This was established using positivity arguments, conservation laws, boundedness of the invariant region, and tools from chemical reaction network theory, including the Deficiency Zero Theorem [59, 21]. The kinetic proofreading model with negative feedback requires separate treatment because it can exhibit more complex steady-state behaviour and does not always fit into the same uniqueness framework. Thus, the mathematical analysis showed not only what response functions the models produce, but also whether those responses are well-defined as long-time outcomes of the underlying dynamical systems.

The sensitivity analysis showed that model predictions can depend strongly on particular kinetic parameters. In the phenotypic models, phosphorylation-related rates and phosphatase-related parameters play especially important roles in shaping response functions, $E_{\max}$, and EC_{50} . This finding has two important implications. First, experimental uncertainty in these parameters can significantly affect model predictions and may partly explain why different parameter sets lead to different response behaviours. Second, these parameters identify biological processes that should be measured more accurately in future experiments if the predictive power of phenotypic models is to be improved [21].

Overall, the phenotypic part of the thesis supports the view that no single simple mechanism is sufficient to explain all key properties of T-cell activation. Occupancy and standard kinetic proofreading provide important baseline models, but richer response properties require additional regulatory or signalling structures. Limited signalling, sustained signalling, negative feedback, and incoherent feedforward regulation provide mechanisms by which T-cell activation can display sensitivity, specificity, antigen discrimination, optimal response, and ligand antagonism in a more flexible and biologically realistic way [20, 21].

The main lesson from this part of the thesis is therefore that T-cell activation should be understood as a response-level decision process generated by signalling architecture, not as a direct readout of a single binding parameter. Ligand concentration and dissociation

time are important, but their effect depends on how the signalling network processes them. This conclusion sets up the mechanochemical part of the thesis: if bond lifetime is a key input to phenotypic signalling models, then it becomes important to ask how that lifetime is physically generated by receptor geometry, binding energetics, and mechanical force.

6.3 Synthesis of the Mechanochemical Energy-Landscape Results

The mechanochemical part of the thesis addressed a different but complementary question from the phenotypic modelling framework: how can the lifetime of a TCR–pMHC bond arise from physical properties of the receptor–ligand interaction? In the phenotypic models, the dissociation rate or dwell time is usually prescribed as a kinetic input. In the mechanochemical model, by contrast, bond lifetime is treated as an emergent quantity determined by the geometry and stability of a reduced force-loaded effective free-energy landscape. This landscape depends on receptor extension, ligand-binding-site separation, hinge geometry, angle-dependent binding strength, and applied mechanical force [70, 22].

A central point is that the model is deliberately coarse-grained. It is not an atomistic description of the full TCR–pMHC–CD3 complex and does not attempt to resolve all molecular degrees of freedom. Instead, it represents the receptor–ligand complex by a small number of collective variables that are chosen to capture the physical features most relevant for force-dependent bond stability. This reduction makes it possible to analyse the existence, multiplicity, and stability of bound configurations using explicit mathematical tools [22].

The model uses two main reduced coordinates. The first is the receptor extension L , which is related to the hinge angle θ through the geometric relation

$$L(\theta) = 2W \sin\left(\frac{\theta}{2}\right). \quad (6.1)$$

The second is the ligand-binding-site separation d , which represents opening of the TCR–pMHC binding interface. The force-loaded effective free-energy landscape is written as

$$V_f(L, d) = V(\theta(L)) + B(d, \theta(L)) - F(L + d). \quad (6.2)$$

Here $V(\theta)$ is the harmonic hinge energy, $B(d, \theta)$ is the Morse-type binding interaction with angle-dependent well depth, and $-F(L + d)$ is the mechanical work term. In the TCR-specific reduction used in the thesis, the force is treated as an effective constant load acting along a simplified pulling direction with equal projection onto the extension and separation coordinates [22].

The hinge contribution is modelled by

$$V(\theta) = \frac{1}{2}k_\theta(\theta - \theta_0)^2, \quad (6.3)$$

where k_θ is an effective angular stiffness and θ_0 is the unloaded preferred hinge angle. The binding interaction is represented by a Morse-type potential,

$$B(d, \theta) = D(\theta) \left[\left(1 - \exp \left(-\frac{d}{d_0} \right) \right)^2 - 1 \right], \quad (6.4)$$

where d_0 is the effective interaction-range parameter. The well depth is modulated by the hinge angle according to

$$D(\theta) = D_0 \exp \left[-\frac{(\theta - \theta_1)^2}{2\sigma^2} \right]. \quad (6.5)$$

Thus, ligand identity enters the model through an allosterically modulated, peptide-dependent landscape: different ligand classes are represented by different values of D_0 , k_θ , σ , θ_1 , and d_0 . Applied force then reshapes each of these landscapes by moving stationary points and changing the barrier to dissociation [22].

This formulation has several mathematical consequences. The stationary-point analysis showed that force-supported bound configurations can exist only if the binding landscape is sufficiently deep and short-ranged to balance the applied load. In the simplified model, this gives the critical force condition

$$F \leq F_c, \quad F_c = \frac{D_0}{2d_0}. \quad (6.6)$$

This threshold should be interpreted as a model-internal existence condition: for $F > F_c$, the reduced force-loaded landscape cannot support a bound stationary configuration. It is therefore not a universal biological constant, but a mathematical consequence of the chosen Morse-type binding term and the effective force-loading geometry [22].

The stationary-point equations also reduce to a scalar angular condition. This reduction is important because it converts the two-dimensional stationary problem into a tractable mathematical problem while preserving the coupling between receptor extension and ligand separation. Under the assumptions of the model, the force-loaded landscape admits at most two relevant stationary points: a bound minimum and a neighbouring saddle point. The bound minimum represents a mechanically stable TCR–pMHC configuration, whereas the saddle point represents the transition state that must be crossed for dissociation.

The Hessian analysis provided the criterion for distinguishing stable bound states from unstable stationary configurations. In particular, stable binding is restricted to the near-separation branch,

$$d < d_0 \ln 2. \quad (6.7)$$

Equivalently, if $u = \exp(-d/d_0)$, the stable branch satisfies $u > 1/2$. This condition has a clear mechanochemical interpretation: a strict local minimum can exist only when the ligand-binding interface remains sufficiently close to the bound contact geometry. Stationary points on the far-separation branch may solve the force-balance equations, but they are unstable in the separation direction and therefore cannot represent stable bound receptor–ligand configurations.

Bond rupture is represented geometrically as the loss of the local minimum. As the applied force changes, the bound minimum and the neighbouring saddle move on the force-loaded landscape. At a bond-breaking point, these two stationary points coalesce and the Hessian becomes singular:

$$\det H = 0. \quad (6.8)$$

Thus, rupture is not imposed by choosing an arbitrary separation distance. Rather, it is defined by the loss of local stability in the force-resaped effective free-energy landscape. This provides a precise mathematical description of force-induced bond breaking in the reduced model [22].

Chapter 5 then used this mathematical structure to compute force-dependent barriers and bond lifetimes for agonist, weak agonist, and antagonist parameter sets. For each applied force F , the bound state is denoted by $\mathbf{x}_m(F)$ and the adjacent saddle point by $\mathbf{x}_s(F)$. The force-dependent energy barrier is defined as

$$\Delta E(F) = V_f(\mathbf{x}_s(F)) - V_f(\mathbf{x}_m(F)). \quad (6.9)$$

This quantity measures the energetic cost of escaping from the force-supported bound state. If $\Delta E(F)$ increases over an intermediate force range, the applied load stabilizes the bond and produces a catch-like regime. If $\Delta E(F)$ decreases monotonically, force promotes dissociation and the bond behaves as a slip bond.

The barrier was then converted into a predicted mean bond lifetime using an Arrhenius–Kramers–Langer approximation,

$$\tau(F) = \tau_0(F) \exp\left(\frac{\Delta E(F)}{k_B T}\right). \quad (6.10)$$

Here $\tau_0(F)$ is a force-dependent prefactor determined by the local curvature of the landscape near the minimum and saddle. This step links the static geometry of the effective free-energy landscape to a kinetic observable: the mean lifetime of the TCR–pMHC bond [67, 72, 68, 22].

The numerical results showed distinct ligand-class behaviours. The agonist landscape displayed a pronounced catch–slip profile: the energy barrier and bond lifetime increased

at moderate force and decreased at higher force. The weak agonist displayed a weaker and shallower version of the same response. In contrast, the antagonist landscape showed monotonic barrier lowering and lifetime shortening, corresponding to slip-bond behaviour. Thus, force does not simply weaken all TCR–pMHC interactions. Its effect depends on the ligand-specific coupling between binding strength, receptor orientation, angular stiffness, angular tolerance, and ligand-interface separation [44, 45, 46, 22].

The sensitivity analysis of the mechanochemical model clarified the roles of the main parameters. The binding-well depth D_0 primarily controls the overall magnitude of the barrier and lifetime response: deeper wells generally produce larger barriers and longer lifetimes. The preferred binding angle θ_1 , by contrast, mainly controls the force-dependent shape of the catch-slip curve, because it determines how the strongest binding region is positioned relative to the force-sensitive hinge coordinate. This separates the mechanochemical response into an energetic contribution, governed mainly by binding strength, and a geometric contribution, governed mainly by receptor orientation under force [22].

Overall, the mechanochemical part of the thesis shows how force-dependent bond lifetimes can emerge from energy-landscape geometry. The model provides a minimal mathematical framework linking applied force, motion of minima and saddles, Hessian stability, activation barriers, bond-breaking points, and predicted lifetimes. It therefore supplies a possible physical origin for the kinetic variables that are used as inputs in phenotypic models of T-cell activation.

The main lesson from this part of the thesis is that ligand lifetime should not always be viewed as a fixed, force-independent parameter. In a mechanically active T-cell–APC contact, the effective lifetime of a TCR–pMHC bond may depend on how force reshapes the underlying receptor–ligand landscape. This provides the bridge to the phenotypic models: the mechanochemical framework explains how force and geometry may generate ligand-dependent lifetimes, while the phenotypic framework explains how signalling networks may interpret those lifetimes as cellular activation decisions.

6.4 Connecting Response-Level and Physical-Level Explanations

A central conceptual contribution of this thesis is the connection between response-level and physical-level explanations of T-cell activation. The phenotypic models and the mechanochemical energy-landscape model address different aspects of the same biological problem. Phenotypic models ask how T-cell signalling networks transform kinetic inputs into cellular response properties. The mechanochemical model asks how one of the most important kinetic inputs, the TCR–pMHC bond lifetime, may arise from force-dependent

bond stability. The two viewpoints are therefore linked by the role of bond lifetime [21, 22].

In the phenotypic models, ligand concentration, binding rate, dissociation rate, and dwell time enter as parameters controlling the progression of the signalling system. A longer or shorter TCR–pMHC lifetime affects whether a bound receptor complex can complete proofreading steps, whether signalling is terminated or persists after ligand dissociation, whether feedback or feedforward regulation is activated, and how much downstream response is ultimately produced. In this sense, the bond lifetime is interpreted by the signalling network. Its biological effect depends not only on the lifetime itself, but also on the architecture of the signalling model in which it is embedded [23, 20, 21].

The mechanochemical model addresses the complementary question of how such a lifetime may be generated physically. It does not attempt to describe the full downstream biochemical cascade of T-cell activation. Instead, it focuses on the upstream receptor–ligand bond and asks how applied force, receptor extension, hinge geometry, binding-well depth, and ligand-interface separation determine the stability of the bound state. In this setting, lifetime is not prescribed as an independent kinetic parameter. It is derived from the force-dependent energy barrier between a bound minimum and a neighbouring saddle point on the effective free-energy landscape [22].

The relationship between the two parts of the thesis can therefore be summarized as follows:

Phenotypic models describe how T-cell signalling interprets receptor–ligand lifetimes, whereas mechanochemical energy-landscape models describe how force and receptor geometry may generate those lifetimes.

This connection is important because it clarifies the meaning of dissociation time in T-cell activation models. In many phenotypic models, dissociation time is treated as a fixed ligand property. This is a useful abstraction, but it is not necessarily complete. The mechanochemical analysis shows that the effective lifetime of a TCR–pMHC bond may depend on the mechanical state of the receptor–ligand complex. The same ligand may have different lifetimes under different force conditions, and different ligand classes may respond differently to the same applied force. Thus, dissociation time should not always be viewed as a purely force-independent input; it may itself be an outcome of mechanochemical regulation.

This perspective also clarifies why T-cell activation is difficult to explain using a single mechanism. A signalling architecture may reproduce specificity, sensitivity, or antigen discrimination only for certain ranges of ligand lifetime. A mechanochemical landscape may generate such lifetimes only for certain ranges of force, geometry, and binding energy. Therefore, productive T-cell activation requires compatibility between two levels: the physical layer that generates receptor–ligand lifetimes and the biochemical layer that

interprets them. Neither layer alone gives a complete explanation of the full activation process.

The connection between the two levels also gives a more precise interpretation of catch-slip behaviour. In the mechanochemical model, catch-slip behaviour corresponds to a force range in which the dissociation barrier and bond lifetime increase before decreasing at higher force. In the phenotypic models, a longer lifetime can increase the probability that a receptor complex reaches a signalling-competent state or activates downstream regulatory motifs. Thus, force-dependent stabilization at the bond level may influence cellular activation only through the way the signalling network processes the resulting lifetime. The same physical lifetime can have different consequences in different signalling architectures [44, 45, 46].

The thesis does not yet build a fully coupled multiscale model in which the force-dependent lifetime computed from the mechanochemical landscape is inserted directly into the phenotypic signalling equations. Nevertheless, it establishes the mathematical pieces needed for such a connection. Chapters 2 and 3 identify how response properties depend on kinetic variables and signalling architecture. Chapters 4 and 5 show how force-dependent lifetimes can be computed from a reduced mechanochemical landscape. A natural next step is to couple these two descriptions explicitly, replacing prescribed dissociation times in the phenotypic models by lifetimes derived from force-dependent energy barriers.

The broader implication is that T-cell activation should be understood as a multilevel decision process. At one level, molecular mechanics determines how long receptor-ligand bonds persist under load. At another level, biochemical signalling networks determine how those binding events are converted into activation, non-activation, or partial response. The contribution of this thesis is to analyse both levels mathematically and to show how they can be connected through the central variable of bond lifetime.

6.5 Biological Implications

The biological implications of this thesis concern how T cells may combine sensitivity, specificity, and antigen discrimination despite relying on weak and transient TCR-pMHC interactions. The phenotypic analysis shows that these properties are difficult to obtain from receptor occupancy alone. More elaborate signalling mechanisms, such as proofreading, limited signalling, sustained signalling, feedback regulation, and incoherent feedforward regulation, can generate richer and more biologically plausible response patterns. The mechanochemical analysis adds a complementary point: the kinetic inputs to such signalling networks, especially dissociation time and bond lifetime, may themselves depend on mechanical force and receptor geometry [20, 21, 22].

One implication is that T-cell activation should not be interpreted as a direct readout of binding affinity alone. Affinity, dissociation rate, ligand concentration, receptor number, and signalling architecture all influence the cellular response. The phenotypic models show that the same kinetic input can lead to different response outcomes depending on how the signalling network is organized. For example, limited signalling, sustained signalling, feedback, and feedforward motifs can alter whether the response is monotone, optimal, or antagonistic. Thus, ligand potency is not determined only by how strongly a ligand binds, but also by how the resulting binding events are processed by the intracellular signalling system.

A second implication is that mechanical force may contribute to antigen discrimination, but not by acting as a universal stabilizing or destabilizing factor. In the mechanochemical model, force has different effects depending on the ligand-specific effective free-energy landscape. For agonist-like landscapes, moderate force can increase the barrier to dissociation and prolong the bond lifetime. For weak agonist-like landscapes, this stabilization is weaker and less pronounced. For antagonist-like landscapes, force lowers the barrier and shortens the lifetime. Thus, force may act as a physical filter that transiently stabilizes productive interactions while destabilizing non-productive ones [44, 45, 46, 22].

This interpretation is consistent with experimental observations that some stimulatory TCR–pMHC interactions display force-enhanced lifetimes or catch-bond behaviour, whereas non-stimulatory ligands more often display slip-bond behaviour [44, 45, 46]. However, this conclusion should remain cautious. The role of force in TCR ligand discrimination remains debated, and force may not sharpen discrimination under all experimental conditions [47, 48]. The mechanochemical model should therefore be understood as providing a possible physical mechanism for force-dependent ligand discrimination, not as a complete explanation of T-cell activation.

A third implication is that ligand potency may depend on both energetic and geometric factors. The binding-well depth D_0 captures an energetic contribution: deeper wells tend to produce higher barriers and longer lifetimes. The preferred binding angle θ_1 captures a geometric contribution: it determines how the strongest binding region is positioned relative to the force-sensitive receptor orientation. Thus, two ligands with similar unloaded binding strengths could, in principle, behave differently under force if their preferred geometries differ. Conversely, ligands with different affinities could produce similar cellular responses if their mechanochemical geometry and downstream signalling context compensate for those differences [22].

A fourth implication concerns weak agonists. In the mechanochemical model, weak agonists occupy an intermediate regime between agonists and antagonists. They can show a mild catch-like response, but the stabilization is weaker and less robust than for agonists. This is biologically meaningful because weak agonists often bind the TCR and produce partial or weaker signalling, but do not induce the same level of activation as

full agonists. The model suggests that this intermediate phenotype may reflect not only weaker binding strength, but also a less favourable mechanochemical coupling between receptor orientation, binding depth, and applied force.

A fifth implication concerns the relationship between early bond stability and downstream signalling. A longer TCR–pMHC bond lifetime may provide more time for early signalling events such as CD3 conformational rearrangement, ITAM exposure, Lck-mediated phosphorylation, and recruitment of ZAP-70 [1, 6]. The mechanochemical part of the thesis does not model these downstream biochemical events directly. However, the phenotypic models show how changes in ligand lifetime can be interpreted by signalling networks. Taken together, the two parts of the thesis suggest a route by which extracellular mechanical loading could influence intracellular signalling outcomes: force reshapes bond lifetime, and the signalling network interprets that lifetime.

A sixth implication is experimental. The thesis suggests that T-cell activation should be studied by combining response-level and physical-level measurements. At the response level, dose–response curves, $E_{\max}$, EC_{50} , ligand antagonism, and dependence on dissociation time provide information about the signalling architecture. At the physical level, force-dependent bond lifetimes, catch–slip or slip–bond profiles, and ligand-specific rupture behaviour provide information about the mechanochemical landscape. Measuring only one of these levels may miss important information. For example, a ligand may appear weak in an unloaded binding assay but behave differently under force, or a force-enhanced lifetime may lead to different cellular outcomes depending on the downstream signalling network.

Overall, the biological message of the thesis is that antigen discrimination may arise from the compatibility between two layers of regulation. The first is a physical layer, where force, receptor geometry, and binding energetics shape the lifetime of the TCR–pMHC bond. The second is a biochemical signalling layer, where proofreading, feedback, feedforward regulation, and signal persistence determine how that lifetime is translated into a cellular response. This multilevel view does not replace existing theories of T-cell activation; rather, it provides a mathematical framework for understanding how biochemical and mechanochemical mechanisms may cooperate in TCR triggering.

6.6 Mathematical Contributions

The thesis makes mathematical contributions at two connected levels. The first level is the response level, where T-cell activation is described through reaction-network models and ordinary differential equations. The second level is the physical bond level, where TCR–pMHC stability is described through a force-loaded effective free-energy landscape. The contribution of the thesis is not only to analyse these two levels separately, but also

to show how they can be related through the central variable of receptor–ligand bond lifetime.

At the response level, the thesis formulated a family of phenotypic models of T-cell activation within a common reaction-network framework. This allowed receptor occupancy, kinetic proofreading, limited signalling, sustained signalling, negative feedback, stabilizing activation chains, incoherent feedforward regulation, and combined model variants to be compared using the same mathematical language. Each model was expressed as a system of ordinary differential equations under mass-action kinetics, with biologically meaningful state spaces determined by non-negativity and conservation laws. This common framework made it possible to compare models that were originally proposed in different biological and mathematical contexts [23, 20, 21].

A first mathematical contribution of this framework was the qualitative analysis of the model dynamics. For the model family, positivity and invariance of the non-negative orthant were established, ensuring that biologically meaningful initial data lead to biologically meaningful solutions. Conservation laws for total ligand and receptor quantities were used to define reduced biologically relevant regions. Boundedness of these regions then provided a basis for global existence of solutions. These results are important because they show that the models are mathematically well posed before their biological response properties are interpreted.

A second contribution was the analysis of steady states and long-time behaviour. For several models, convergence to a unique positive steady state was obtained using ideas from chemical reaction network theory, including the Deficiency Zero Theorem, together with model-specific arguments excluding boundary ω -limit points [59, 21]. The kinetic proofreading model with negative feedback required separate consideration because it can exhibit more complex steady-state behaviour and does not always fall into the same uniqueness framework. This distinction is important because it shows that similar-looking signalling models can have different dynamical structures.

A third contribution was the systematic comparison of response functions. The thesis derived or computed steady-state response functions as functions of ligand concentration and dissociation time, and used these functions to compare specificity, sensitivity, antigen discrimination, optimal response, and ligand antagonism. The response quantities $E_{\max}$ and EC_{50} were used to separate efficacy from potency. This made it possible to distinguish between models that merely produce activation and models that reproduce experimentally motivated response patterns in a quantitatively meaningful way [21].

A fourth contribution at the response level was the use of parameter-based comparison and sensitivity analysis. By evaluating models under common literature-based parameter values, and by considering variation in those parameters, the thesis examined whether model predictions are robust or parameter-dependent. The sensitivity analysis identified which kinetic parameters have the strongest influence on response functions, $E_{\max}$, and

EC_{50} . This connects the mathematical analysis to experimental interpretation, because it indicates which biological rates need to be measured most accurately for predictive modelling.

At the physical level, the thesis developed a reduced force-loaded energy-landscape model for TCR–pMHC binding. The model introduced collective coordinates representing receptor extension and ligand-binding-site separation, with receptor orientation encoded through a hinge angle. This reduced description transformed the biological problem of force-dependent receptor–ligand binding into a tractable mathematical problem involving stationary points, Hessian stability, energy barriers, and bond-breaking points [70, 22].

A central mathematical contribution of the mechanochemical model was the derivation of the stationary-point conditions for the force-loaded landscape. The two-dimensional stationary system was reduced to a scalar angular condition, making it possible to analyse the number of stationary points and their dependence on force. This reduction showed how receptor geometry, binding strength, ligand separation, and mechanical loading are coupled in the model.

The stationary-point analysis also yielded a critical force condition,

$$F_c = \frac{D_0}{2d_0}. \quad (6.11)$$

This force cutoff is a model-internal existence threshold: above it, the reduced landscape cannot support a bound stationary configuration. The result links the maximum binding depth D_0 , the effective interaction range d_0 , and the maximum force compatible with a bound state in the model. It therefore provides a simple analytical relation between molecular binding parameters and force-supported stability.

Another contribution was the Hessian-based classification of stationary points. The analysis showed that stable bound configurations can occur only on the near-separation branch, where

$$d < d_0 \ln 2. \quad (6.12)$$

A stationary point is a strict local minimum only when the Hessian is positive definite, whereas a neighbouring saddle represents the transition state for dissociation. This analysis gives a precise mathematical criterion for distinguishing mechanically stable bound states from unstable configurations.

The thesis also represented bond rupture geometrically. As force varies, the bound minimum and the adjacent saddle point move on the force-loaded landscape. Bond breaking occurs when these stationary points coalesce and the Hessian becomes singular,

$$\det H = 0. \quad (6.13)$$

Thus, rupture is described as a loss of local stability rather than being imposed through

an arbitrary separation distance. This provides a mathematically defined bond-breaking mechanism within the reduced landscape framework [22].

The final physical-level contribution was the connection between energy barriers and bond lifetimes. For each applied force, the dissociation barrier was computed as the difference between the value of the force-loaded landscape at the saddle and at the bound minimum. This barrier was then converted into a mean lifetime using an Arrhenius–Kramers–Langer approximation. In this way, the thesis connected static energy-landscape geometry to a kinetic prediction for the TCR–pMHC bond lifetime. By applying the same calculation to agonist, weak agonist, and antagonist parameter sets, the model produced distinct catch–slip and slip–bond behaviours across ligand classes [67, 72, 68, 22].

The combined mathematical contribution of the thesis is therefore a two-level modelling framework. At one level, T-cell activation is studied as a dynamical systems problem involving reaction networks, steady states, response functions, and parameter sensitivity. At the other level, TCR–pMHC bond stability is studied as an energy-landscape problem involving force-dependent minima, saddles, barriers, and lifetimes. The bridge between these levels is bond lifetime: it is a physical output of the mechanochemical model and a kinetic input to the phenotypic signalling models.

This two-level structure provides a mathematical way to relate cellular response properties to physical features of receptor–ligand binding. It shows that sensitivity, specificity, and antigen discrimination can be studied not only through signalling architectures, but also through the force-dependent mechanisms that may generate the lifetimes interpreted by those architectures.

6.7 Limitations of the Present Work

The thesis has several limitations, many of which arise from the deliberate choice to use reduced mathematical models. These simplifications were necessary in order to obtain analytical insight, compare mechanisms systematically, and connect response-level modelling with mechanochemical bond-stability modelling. At the same time, they define the scope of the conclusions and indicate where future work is needed [21, 22].

A first limitation concerns the deterministic nature of the phenotypic models. The models studied in Chapters 2 and 3 are formulated as systems of ordinary differential equations under mass-action kinetics. They describe average concentrations of molecular species and are therefore most naturally interpreted as population-level or mean-field descriptions. This framework is well suited for analysing positivity, boundedness, steady states, response functions, $E_{\max}$, EC_{50} , and parameter sensitivity. However, it does not explicitly include stochastic fluctuations. This is an important restriction because T-cell activation can occur at very low ligand numbers, where random binding and unbinding events, stochastic phosphorylation, and single-cell variability may strongly influence the

response. Thus, the phenotypic models should not be interpreted as complete descriptions of single-cell activation decisions.

A second limitation is that the phenotypic models do not explicitly resolve spatial organization. The T-cell–APC interface is spatially structured, with receptor movement, receptor clustering, membrane apposition, cytoskeletal motion, kinase and phosphatase segregation, and microvillus-mediated scanning all potentially influencing signalling. In the present phenotypic framework, such effects are represented only indirectly through kinetic parameters or through simplified reaction-network motifs. This abstraction allows different signalling architectures to be compared in a common mathematical framework, but it does not capture spatial gradients, local membrane geometry, receptor nanoclusters, or spatially restricted signalling domains.

A third limitation concerns parameter uncertainty in the phenotypic models. The comparative study used parameter values derived from several experimental and modelling sources, and the simulations were repeated using mean, minimum, and maximum values in order to test the robustness of the conclusions. Nevertheless, parameters such as binding rate, dissociation rate, phosphorylation rate, phosphatase efficiency, receptor number, ligand density, feedback strength, and feedforward regulation can vary substantially between experimental systems and measurement techniques. In particular, differences between two-dimensional and three-dimensional experimental estimates of TCR–pMHC binding parameters can influence model calibration and interpretation. Therefore, the conclusions of the phenotypic comparison should be understood in relation to the available parameter ranges rather than as parameter-independent statements [21].

A related limitation is that sensitivity analysis identifies influential parameters but does not remove parameter uncertainty. The LHS–PRCC analysis showed that phosphorylation-related and phosphatase-related parameters can have strong effects on model outputs. This is useful because it indicates which biological rates are most important for model behaviour. However, it also means that predictions of response functions, $E_{\max}$, and EC_{50} may change when these parameters are estimated differently. Thus, improved experimental measurement of phosphorylation, dephosphorylation, phosphatase activity, and feedback regulation would be needed to increase the predictive power of the phenotypic models.

A fifth limitation is that the phenotypic models are intentionally phenomenological. They are designed to reproduce and compare response-level properties such as specificity, sensitivity, antigen discrimination, ligand antagonism, optimal response, $E_{\max}$, and EC_{50} . They do not attempt to reconstruct the complete intracellular signalling cascade. For example, detailed CD3 ITAM phosphorylation, Lck recruitment, ZAP-70 activation, LAT signalosome formation, calcium signalling, transcriptional regulation, and cell-fate decisions are not explicitly represented. The models therefore clarify which simplified signalling architectures are sufficient to generate particular response properties, but they do not provide a complete biochemical model of T-cell activation.

A sixth limitation concerns the scope of the mechanochemical model. The model developed in Chapters 4 and 5 is best viewed as a framework for TCR–pMHC barrier reshaping and bond lifetime, not as a complete model of T-cell activation or antigen specificity. It focuses on the upstream physical layer: how force, receptor geometry, binding energetics, and ligand-interface separation reshape an effective free-energy landscape. It does not explicitly include the downstream biochemical signalling network, CD3 tail dynamics, Lck recruitment, ITAM phosphorylation, ZAP-70 activation, or transcriptional responses. These downstream processes are essential for productive T-cell activation, but they lie outside the minimal mechanochemical formulation [22].

A seventh limitation is that the mechanochemical model is deliberately coarse-grained. It does not represent the full atomistic potential energy surface of the TCR–pMHC–CD3 complex. Instead, it uses two reduced coordinates, receptor extension L and ligand-binding-site separation d , together with an effective hinge angle θ . This reduction makes it possible to derive explicit stationary-point conditions, Hessian stability criteria, force thresholds, bond-breaking conditions, and force-dependent barriers. However, it cannot capture all molecular details of peptide-specific TCR–pMHC binding, CD3 conformational rearrangement, membrane coupling, lipid interactions, or coreceptor involvement.

An eighth limitation concerns the treatment of force. In the mechanochemical model, force is represented as an effective constant load and enters the landscape as a linear tilt. This force-clamp approximation is useful for analysing how mechanical load shifts minima, saddles, and barriers, and it is consistent with the simplified conditions often used in force-spectroscopy interpretations. In real T-cell–APC contacts, however, force is likely to be time-dependent, direction-dependent, spatially heterogeneous, and history dependent. It may arise from actin retrograde flow, actomyosin contractility, microvillus motion, membrane deformation, receptor movement, or traction at the immunological synapse. These cellular mechanics are abstracted into a single effective force in the present model.

A ninth limitation is that several mechanochemical parameters are effective or phenomenologically estimated. The binding-well depth D_0 is derived from affinity data, but parameters such as the angular stiffness k_θ , angular width σ , preferred binding angle θ_1 , and Morse interaction range d_0 are not all directly measured for each ligand class. They combine structural information, energetic scaling, literature estimates, and calibration to force-dependent lifetime data. This parameterization is useful for building a minimal and interpretable model, but more ligand-specific measurements would be needed for stronger quantitative predictions.

A tenth limitation is the use of simplified angular and geometric assumptions. The model uses a Gaussian angular dependence for the binding depth and a fixed geometric relation between receptor extension and hinge angle. These approximations keep the model analytically tractable and allow the role of θ_1 , k_θ , and σ to be interpreted clearly. However,

real TCR–pMHC–CD3 conformational dynamics may involve broader angular distributions, asymmetric motions, peptide-specific conformational pathways, and membrane-coupled rearrangements. Future versions of the model could include circular angle statistics, time-dependent loading, and membrane-coupled mechanical effects.

An eleventh limitation is that the model does not explicitly include CD8 or CD4 coreceptor engagement. Productive TCR triggering may require not only an appropriate TCR–pMHC bond lifetime, but also coreceptor binding, coreceptor-associated Lck recruitment, and downstream signalling context. A bond that is mechanically stable under force may not necessarily trigger a productive cellular response if the coreceptor and signalling environment are unfavourable. Thus, the mechanochemical model should be interpreted as describing one upstream contribution to T-cell activation, not the complete activation decision.

Finally, the thesis does not yet provide a fully coupled multiscale simulation from molecular force to cellular fate. The phenotypic and mechanochemical models are connected conceptually and mathematically through the role of bond lifetime, but the force-dependent lifetimes computed from the mechanochemical model are not yet inserted directly into the phenotypic signalling equations. Such a coupled model would allow one to study how force-dependent barrier reshaping influences response functions, $E_{\max}$, EC_{50} , antigen discrimination, and optimal response within a single integrated framework.

These limitations do not weaken the main conclusions of the thesis; rather, they clarify their intended scope. The phenotypic models identify which signalling architectures can reproduce response-level properties under biologically motivated parameter ranges. The mechanochemical model identifies a possible physical mechanism by which force and receptor geometry can generate ligand-dependent bond lifetimes. A more complete theory of T-cell activation will require coupling these levels with stochastic effects, spatial structure, membrane mechanics, coreceptor engagement, and downstream biochemical signalling.

6.8 Future Directions

Several future directions follow naturally from the results and limitations of this thesis. They concern both the phenotypic modelling framework and the mechanochemical energy-landscape framework, as well as the connection between them [21, 22].

The first and most direct direction is to couple the mechanochemical lifetime model to the phenotypic signalling models. In the phenotypic models studied in this thesis, the dissociation rate or dwell time is prescribed as an input parameter. In a fully coupled framework, this quantity would instead be computed from the force-dependent

energy barrier of the mechanochemical model. Thus, the dissociation rate would become force-dependent and ligand-class dependent:

$$v(F) \sim \frac{1}{\tau(F)}. \quad (6.14)$$

Such a model would connect mechanical force to signalling output in a single multiscale framework. Force would reshape the TCR–pMHC energy landscape, the energy landscape would determine the bond lifetime, and the resulting lifetime would enter the reaction-network models as a kinetic input. This would make it possible to ask how catch–slip or slip–bond behaviour affects specificity, sensitivity, antigen discrimination, $E_{\max}$, and EC_{50} .

A second direction is to improve parameter calibration for phenotypic models. The comparative analysis showed that model predictions depend strongly on parameter choice. Future work should therefore focus on obtaining more consistent and biologically realistic estimates of binding rates, dissociation rates, phosphorylation rates, phosphatase efficiencies, receptor numbers, and ligand densities. In particular, parameters measured in three-dimensional solution assays should not be transferred directly to two-dimensional membrane-based models without careful interpretation. Since TCRs and pMHC molecules interact at a membrane interface, future calibration should make greater use of membrane-proximal two-dimensional techniques such as supported lipid bilayers, micropipette adhesion-frequency assays, thermal fluctuation assays, and single-molecule imaging at the cell surface [21].

A third direction is to design experiments that test response functions over a two-dimensional panel of ligand concentration and dissociation time. Many models can produce similar behaviour if only one ligand dose or one ligand lifetime is examined. A systematic experimental design varying both ligand dose and dwell time would make it possible to distinguish monotone, single-peaked, and bimodal response patterns. This would be especially useful for separating basic kinetic proofreading from models with limited signalling, sustained signalling, negative feedback, or incoherent feedforward regulation.

A fourth direction is to test the specific predictions of limited-signalling models. These models imply that longer binding is not always better. If a bound receptor can signal only for a finite time, then very long-lived complexes may reduce serial engagement and lower the overall response. This prediction could be tested using altered peptide ligands or engineered TCR–pMHC pairs with controlled dissociation times, while measuring downstream activation across several ligand doses. Observation of an intermediate optimum in dissociation time would support limited-signalling mechanisms, whereas a purely monotone increase would favour simpler proofreading-based explanations.

A fifth direction is to test sustained-signalling mechanisms using pulse–chase or ligand–washout experiments. Sustained-signalling models predict that activation may persist transiently after ligand dissociation. This could be tested by exposing T cells to pMHC for

a controlled period, removing the ligand, and then measuring downstream signalling after removal. Persistence of signalling after ligand withdrawal would support the inclusion of an unbound but signalling-active state, whereas rapid loss of signalling would argue against sustained signalling as a dominant mechanism in that experimental setting.

A sixth direction is to test the dose-dependent predictions of incoherent feedforward models. These models can produce response-curve intersections, changes in ligand ranking across dose regimes, and in some cases bimodal dependence on dissociation time. Therefore, weak, intermediate, and strong agonists should be compared across a broad range of ligand concentrations rather than at a single fixed dose. If ligand potency changes with dose, then EC_{50} alone is not sufficient to characterize antigen potency; the full shape of the response curve must also be considered.

A seventh direction is to include stochastic effects. T cells can respond to very small numbers of agonist ligands, and therefore random binding, dissociation, and phosphorylation events may influence activation decisions. Stochastic simulations could test whether the deterministic conclusions of the ODE models persist in low-copy-number regimes. Such models would be especially relevant for single-cell variability, digital cytokine secretion, rare-ligand detection, and mixed self-foreign ligand environments.

An eighth direction is to include spatial organization and kinetic segregation more explicitly. The immunological synapse is spatially structured, with membrane apposition, CD45 exclusion, receptor diffusion, receptor clustering, kinase and phosphatase localization, microvillus scanning, and cytoskeletal motion all influencing signalling. Spatially resolved models could therefore connect the reaction-network approach more directly to the physical geometry of the T-cell-APC interface. Such models would also provide a natural bridge between phenotypic signalling models and mechanochemical descriptions of force generation [31, 32].

A ninth direction is to refine the mechanochemical parameterization using more ligand-specific experimental and structural data. The parameters D_0 , k_θ , σ , θ_1 , and d_0 determine the predicted force-dependent barriers and lifetimes. Future work should aim to estimate these quantities more directly using force-spectroscopy measurements, structural studies, molecular-dynamics simulations, and ligand-specific TCR-pMHC datasets. In particular, better estimates of angular distributions, angular stiffness, binding-interface separation, interaction range, and force-dependent rupture behaviour would allow stronger quantitative tests of the mechanochemical model [44, 45, 46, 22].

A tenth direction is to model time-dependent and direction-dependent force. The present mechanochemical model treats force as an effective constant load. In real T-cell-APC contacts, however, force may vary with time, direction, location, and mechanical history. Future models could include dynamic loading, oscillatory force, viscoelastic effects, cytoskeletal loading histories, or different pulling directions. This would help determine whether catch-slip behaviour is robust under more realistic mechanical conditions.

An eleventh direction is to include membrane mechanics and TCR–CD3 conformational coupling more explicitly. The present model uses receptor extension, hinge angle, and ligand-interface separation as reduced coordinates. Future refinements could include membrane deformation, lipid interactions, CD3 tail exposure, ITAM accessibility, and conformational changes in the TCR–CD3 complex. Such extensions would connect the energy-landscape model more directly to early biochemical signalling events such as Lck-mediated ITAM phosphorylation and ZAP-70 recruitment [1, 6].

A twelfth direction is to include CD4 and CD8 coreceptor effects. Coreceptors can influence TCR–pMHC binding, recruit Lck, alter the geometry of the receptor complex, and change the signalling threshold. They may also affect how mechanical force is transmitted through the receptor–ligand complex. Incorporating coreceptor engagement would therefore make the mechanochemical model more biologically complete and would help connect force-dependent bond stability to productive signalling initiation.

A thirteenth direction is to validate the mechanochemical predictions directly against force-spectroscopy experiments. The model predicts that agonist-like landscapes should display pronounced catch–slip behaviour, weak agonists a weaker intermediate response, and antagonist-like landscapes monotonic slip-bond behaviour. These predictions could be tested using biomembrane force probe, optical tweezer, fluorescence-enabled force probe, or molecular tension sensor experiments. A particularly useful experiment would measure force-dependent lifetimes for multiple ligands in the same TCR system and then compare the observed lifetime peaks, rupture forces, and slip regimes with the predicted landscape parameters [44, 45, 46].

Finally, future work could apply the combined phenotypic–mechanochemical framework beyond TCR–pMHC recognition. Many receptor–ligand systems in immunology and cell biology involve weak binding, force-dependent stability, and downstream signalling decisions. The mathematical ideas developed in this thesis, especially the connection between force-loaded energy landscapes and response-level signalling models, may therefore be useful for studying other mechanotransduction systems.

Taken together, these future directions point toward a more complete multiscale theory of T-cell activation. Such a theory would combine membrane-proximal parameter calibration, stochastic and spatial signalling, force-dependent bond lifetimes, TCR–CD3 conformational mechanics, coreceptor engagement, and downstream biochemical activation. The present thesis provides the mathematical foundation for this development by analysing the response-level and physical-level components separately and by identifying bond lifetime as the central bridge between them.

6.9 Concluding Remarks

This thesis has developed a mathematical study of T-cell activation at two linked levels. At the phenotypic level, it compared reaction-network models of TCR signalling and examined their ability to reproduce experimentally motivated response properties, including sensitivity, specificity, antigen discrimination, ligand antagonism, optimal response, $E_{\max}$, and EC_{50} . At the mechanochemical level, it developed a reduced effective energy-landscape model to study how mechanical force, receptor geometry, binding energetics, and energy-barrier structure can shape TCR–pMHC bond stability and lifetime [21, 22].

The main conclusion is that T-cell activation is best understood as both a signalling problem and a physical recognition problem. The signalling problem concerns how a cell interprets receptor–ligand binding events through proofreading, signal persistence, feedback, feedforward regulation, and other network mechanisms. The physical recognition problem concerns how long those binding events persist under force and how their lifetimes depend on the geometry and energetics of the receptor–ligand complex. These two problems are not separate: the bond lifetime generated at the physical level becomes a kinetic input to the signalling level.

The phenotypic part of the thesis shows that simple receptor occupancy and standard kinetic proofreading provide important baseline explanations, but they are not sufficient to reproduce the full range of T-cell response properties. More elaborate architectures, including limited signalling, sustained signalling, negative feedback, and incoherent feedforward regulation, can generate richer behaviours such as optimal responses, ligand antagonism, and more flexible discrimination between ligand classes. The analysis of response functions, $E_{\max}$, EC_{50} , steady states, and parameter sensitivity therefore shows that T-cell activation cannot be reduced to a single binding parameter. Instead, the cellular response depends on how the signalling network processes ligand concentration and dissociation time [21].

The mechanochemical part of the thesis shows that dissociation time itself need not be treated only as a fixed ligand property. In a mechanically active T-cell–APC interface, the effective lifetime of a TCR–pMHC bond may depend on how force reshapes the underlying energy landscape. Within the reduced model, force moves local minima and saddle points, modifies the activation barrier, and can ultimately remove the stable bound state at a bond-breaking point. When the model is parameterized by ligand class, agonist-like landscapes can display catch–slip behaviour, weak agonist-like landscapes can show a weaker intermediate response, and antagonist-like landscapes can show slip-bond behaviour. Thus, force does not simply strengthen or weaken all TCR–pMHC interactions in the same way; its effect depends on the ligand-specific coupling between binding strength, receptor geometry, angular stiffness, and ligand-interface separation [44, 45, 46, 22].

The connection between the two parts of the thesis is the bond lifetime. In the

mechanochemical framework, bond lifetime is a physical output generated from force-dependent energy barriers. In the phenotypic framework, bond lifetime is a kinetic input interpreted by the signalling network. This connection provides a bridge between two ways of thinking about T-cell activation. One begins with biochemical networks and asks how cellular responses are produced. The other begins with receptor–ligand mechanics and asks how the lifetimes of binding events are shaped by force. Together, they suggest that antigen discrimination may depend on the compatibility between physical bond mechanics and biochemical signalling architecture.

The thesis therefore supports a multilevel view of T-cell activation. At one level, receptor–ligand binding under force determines which molecular interactions persist long enough to be biologically relevant. At another level, intracellular signalling networks determine how those interactions are converted into activation, partial activation, antagonism, or non-activation. Neither level alone gives a complete explanation. Mathematical modelling is valuable precisely because it makes it possible to separate these levels, analyse their internal logic, and then identify how they may be connected.

By linking reaction-network dynamics, steady-state response functions, parameter sensitivity, force-loaded energy landscapes, Hessian stability, energy barriers, and bond lifetimes, this thesis provides a foundation for future multiscale models of T-cell activation. Such models could replace fixed dissociation times in phenotypic signalling networks by lifetimes computed from mechanochemical energy landscapes, and could thereby connect molecular force more directly to cellular response. In this way, the work contributes to a broader mathematical understanding of how T cells may combine sensitivity, specificity, and antigen discrimination in a mechanically and biochemically complex environment.

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