

Statistical Physics of Dynamic Self-Organization: From Field-Theoretic Self-Assembly to Allosteric Regulation

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ABSTRACT

Biological systems generate reliable, information-processing behavior from large numbers of interacting components operating far from equilibrium. At the molecular scale, this behavior emerges through self-assembly governed by local interaction rules, while at the cellular scale it manifests as gene regulatory networks that encode decision-making through nonlinear dynamics and multistability. Both are governed by the same fundamental questions: how do microscopic interaction rules give rise to robust, directly-tunable macroscopic states, and how can we capture the dynamics and fundamental physics of these processes?

In this thesis, I describe a rule-based operator algebra for representing multiparticle assemblies in stochastic chemical systems. The formalism enables rigorous analysis of complex formation and dissolution in systems where combinatorial complexity is otherwise intractable, and allows for both analytic and numerical explorations of scaling behavior and other statistical properties. The general language of the formalism renders it well-suited for studying self-assembly processes both in and out of equilibrium, and for examining not only the polymer systems discussed in this thesis but also broader applications in gene regulation and signaling protein-protein interactions.

I also discuss underlying dynamics of self-organization toward cell fate determination, and specifically the power of effector concentration as a tuning knob for examining the dynamics of gene regulatory motifs such as three-gene toggle switches and other biologically-relevant circuits in thermodynamic model descriptions. In particular, capturing shifts in the dynamical landscapes of these gene circuits through an explicit role for induction offers an actionable connection to experimental design.

PUBLISHED CONTENT AND CONTRIBUTIONS

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Chapter 1

INTRODUCTION

Living systems rely on vast numbers of interacting molecular components, with collective behavior giving rise to robust yet adaptable cellular functions. At the microscopic scale, proteins bind and unbind, assemble into complexes, undergo conformational changes, and regulate gene expression through intricate, highly-structured biochemical interaction rules. At the macroscopic scale, these interactions manifest as cell fate decisions, regulatory switches, oscillations, and multistable phenotypes. A central challenge in quantitative biology is to develop frameworks that connect such microscopic molecular rules with the resulting dynamical behaviors that emerge in genetic and biochemical networks. Establishing such integrative frameworks could even facilitate a new generation of experiments for probing regimes, perturbations, and design principles that might not be otherwise conceivable.

Over the past half-century, statistical physics has profoundly shaped insights into biological complexity. The language of partition functions, free energies, information theory, and response functions [1–4], for example, have become standard in the analysis of protein folding [5–7], ligand binding [8–12], and transcription regulation [10, 11, 13–16]. More recently, field-theoretic methods – originally developed for quantum many-body systems – have been adapted to describe stochastic chemical kinetics, reaction-diffusion systems, and population dynamics [17–21]. These analyses demonstrate the fundamental power of statistical physics in determining how noise and microscopic interactions coordinate to generate collective macroscopic dynamics and functions, and offer a natural language for elucidating physical principles across biological scales. This is true whether we are considering microscopic assembly processes, genotype to phenotype mapping toward cell fate determination and signaling response such as in immune cell differentiation, the dynamics of tissue development, or collective dynamics at the organismal community level. Fig. 1.1 highlights some of these advances. Regardless of application, however, these developments suggest that biological complexity, although historically treated as exceptional within the context of traditional physics, may be amenable to the same structural reasoning that underpins condensed matter and nonequilibrium physics.

BIOLOGICAL APPLICATIONS FOR STATISTICAL MECHANICS ACROSS SCALES

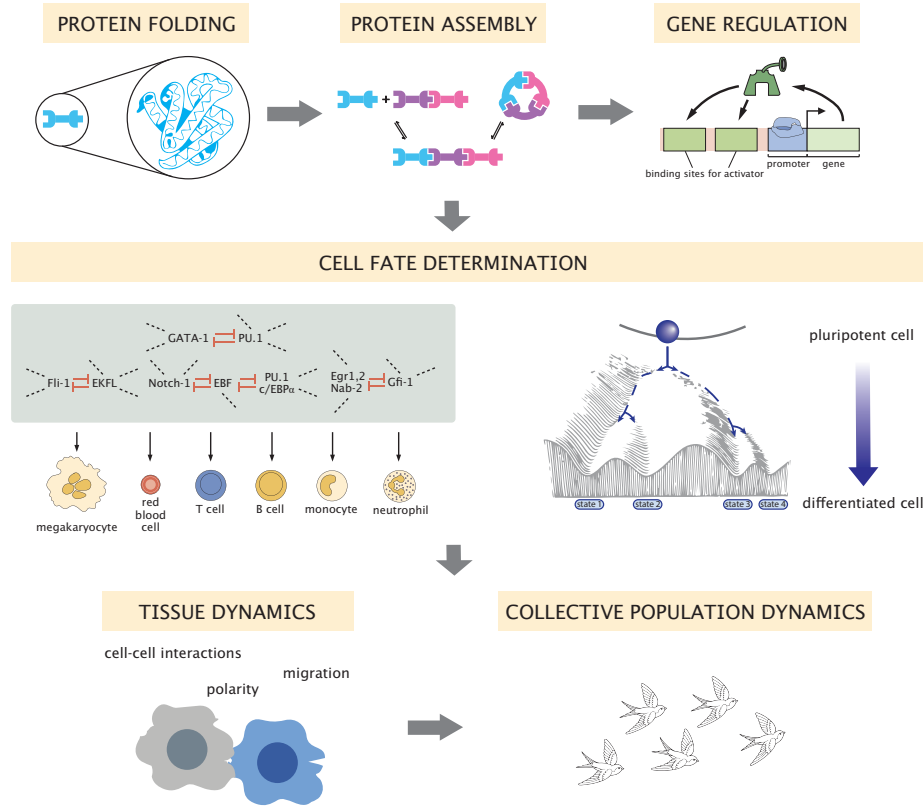


Figure 1.1: Principles of statistical physics determine how noise and microscopic interactions coordinate to generate collective macroscopic dynamics and function, providing a universal language across biological scales.

In this spirit, my thesis seeks to demonstrate the utility of fundamental physical principles in the modeling of self-assembly processes and gene regulatory network dynamics. The key unifying perspective across the work discussed in the following chapters is that biochemical networks are many-body systems with dynamics that can be described using operator algebras, statistical ensembles, and the study of parameter thresholds, or bifurcations, at which dynamical shifts occur. By embedding rule-based molecular interactions within algebraically-defined protein self-assembly, and by grounding gene circuit dynamics in thermodynamic models of transcription regulation, this research seeks to build mathematical, physics-driven frameworks to connect microscopic rules to emergent dynamical phenomena, and to form a predictive foundation that can directly translate to experimental inquiries. In this introduction, I briefly highlight relevant historical milestones in the use of statistical mechanics for studying biological systems, and elaborate on aspects therein

that require further understanding. I then outline my approach to these questions, which are the subjects of the following chapters.

1.1 Statistical mechanics as a lens for modeling biological systems

Applications of statistical mechanics to biological systems trace back to the early-to-mid twentieth century, notably in polymer theory [22] and in early treatments of cooperative ligand binding and allostery [9, 23, 24]. Classical models such as Monod-Wyman-Changeux (MWC) and related thermodynamic frameworks established that macroscopic observables, such as fractional state occupancy and gene expression levels, could be derived from partition functions over microscopic states. This approach reframed biological regulation as an equilibrium statistical mechanics problem, in which the probability of a given molecular configuration is determined by its free energy.

Equilibrium statistical mechanical descriptions are effective in biology due to the separation of timescales in dynamics at the microscopic and whole-cell levels, and because the nonequilibrium steady states that systems generally occupy often probabilistically resemble an equilibrium distribution of possible states when perturbed symmetrically. However, it is important to acknowledge that at a broader scale, biological systems are intrinsically out of equilibrium. Energy consumption through ATP hydrolysis, metabolic flux, and other processes drive systems away from detailed balance, which means that it is often insightful not to coarse grain descriptions to equilibrium mechanics alone. Nonequilibrium statistical mechanics provides a natural framework for such investigations, particularly in the presence of driven processes and stochastic fluctuations.

In parallel, the rise of nonequilibrium statistical mechanics has provided tools for treating systems driven by chemical fluxes. Reaction-diffusion equations, master equations, and stochastic processes have become central to theoretical biophysics. The chemical master equation, in particular, formalizes the time evolution of probability distributions over discrete molecular counts. Exact solutions are rare, however, and the combinatorial growth of possible molecular states quickly renders direct enumeration intractable.

Consider a system, for example, in which the expression of a given gene is regulated by a protein species that can bind non-exclusively at n binding sites along the DNA, as shown in Fig. 1.2(A). Assuming independent binding at all n sites, the total number of thermodynamic gene regulatory states is 2^n , and this number only

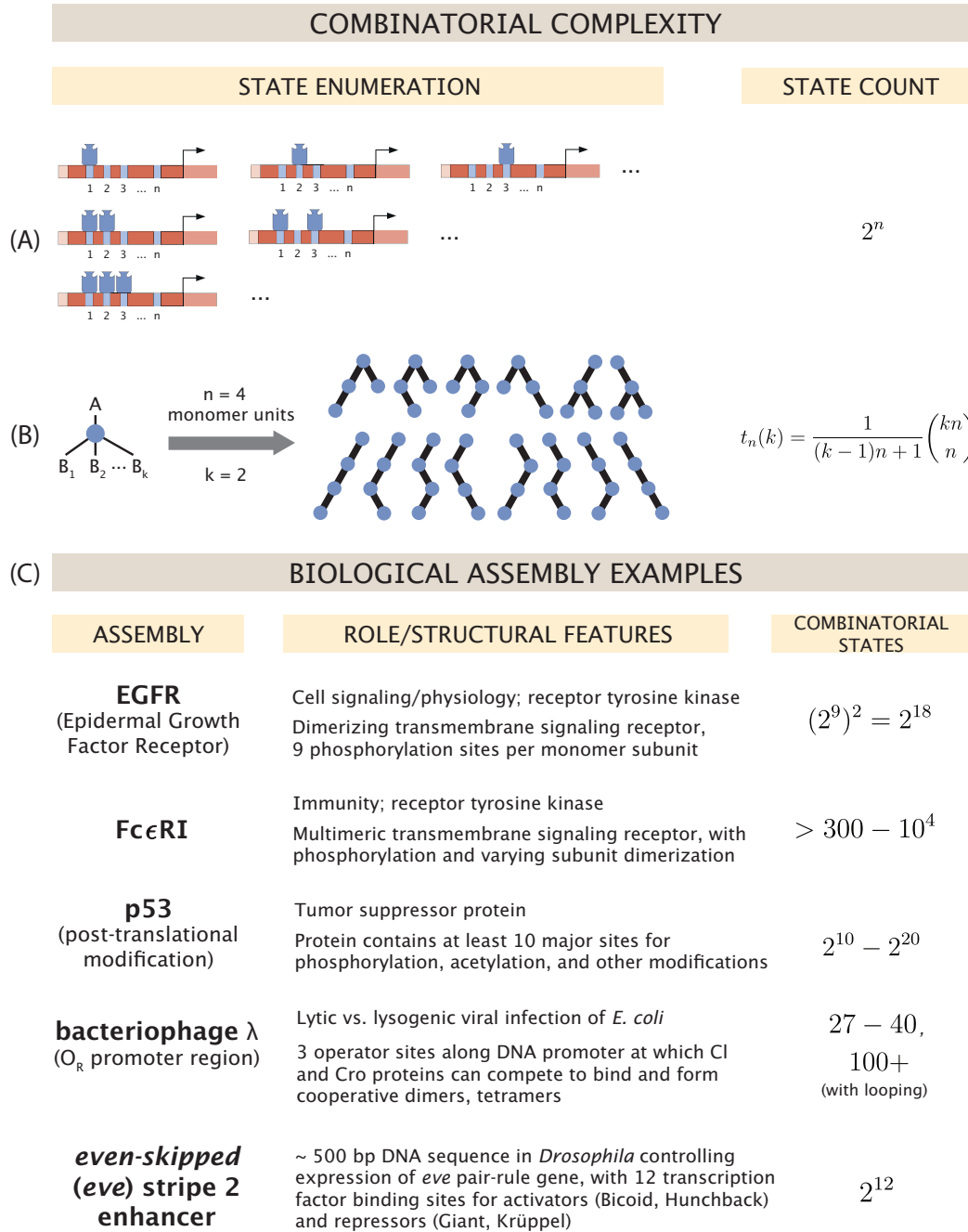


Figure 1.2: A combinatorial explosion of thermodynamic states arises when considering polymer growth and protein assembly in the contexts of signaling complexes and DNA-regulatory binding architectures. (A) State enumeration of transcription factor binding along the DNA with n independent binding sites. (B) State enumeration for branching homopolymers, modeled as trees, with $t_n(k)$ denoting the number of polymer states containing n monomers given k possible branches from a given parent node. (C) A table of biological examples, for which minimal models of structural binding generate a vast combinatorial number of thermodynamic assembly states [10, 13, 25–28].

increases upon the introduction of cooperative interactions among bound proteins and in the presence of additional regulatory protein species.

We also observe combinatorial complexity in polymer assembly settings, such as that shown in the branching homopolymer example of Fig. 1.2(B). In this model, every monomer can form a bond between its site A and one of k distinguishable interaction sites B on another monomer. Assuming a dilute, pre-gelling regime in which no cyclic structures form, each polymer containing n monomer subunits can be interpreted as a connected graph of n units, i.e., a tree, with $n - 1$ bonds. A given polymer tree of this form has a single canonical root, and a given parent node within the graph can form at most k branches.

Given this premise, we can determine the number of distinct assemblies containing n monomer units, t_n . From the canonical root, each of the k labeled B sites is either empty or occupied by a subtree of the same kind. The corresponding generating function for the system is then of the form

$$t(x) = \sum_{n=1}^{\infty} t_n x^n, \quad (1.1)$$

where x tracks monomer subunits. The canonical root contributes x , and each B site can be either empty or occupied by a subtree of the same kind, thus contributing $1 + t(x)$. Since the k interaction sites B are distinguishable and independent, the generating function satisfies the recursive implicit equation

$$t(x) = x[1 + t(x)]^k, \quad (1.2)$$

or more generally,

$$t = x\psi(t), \quad \psi(u) = (1 + u)^k. \quad (1.3)$$

This implicit solution leads to a closed form solution for t_n through the Lagrange inversion formula, which states that for $n \geq 1$

$$[x^n]t(x) = \frac{1}{n}[u^{n-1}](\psi(u))^n, \quad (1.4)$$

with the $[x^n]G(x)$ notation denoting the coefficient of x^n in the power series $G(x)$. From Eqn. 1.1, $t_n \equiv [x^n]t(x)$, and by substituting the power series of interest from Eqn. 1.3, we get

$$t_n = \frac{1}{n}[u^{n-1}](1 + u)^{kn}. \quad (1.5)$$

The number of enumerated states t_n with n monomers as a function of k sites thus derives from the coefficient of u^{n-1} in the power series

$$(1 + u)^{kn} = \sum_{m=0}^{kn} \binom{kn}{m} u^m, \quad (1.6)$$

which means that the number of distinct assemblies is

$$t_n = \frac{1}{n} \binom{kn}{n-1} = \frac{1}{(k-1)n+1} \binom{kn}{n}. \quad (1.7)$$

These are Fuss-Catalan numbers, a well-known result from combinatoric theory, which can quickly become intractably large with increasing n . As an example, binary branching $k = 2$ generates a total number of polymer states

$$t_n = \frac{1}{n+1} \binom{2n}{n} = C_n. \quad (1.8)$$

with C_n denoting the n th Catalan number. Fig. 1.2(B) plots all 14 states possible for $n = 4$ monomer subunits and $k = 2$ distinguishable B_i interaction sites. We can see that this state enumeration quickly expands as n increases, however, with $C_n > 100$ for $n = 6$ and $C_n > 1000$ for $n = 8$.

The problem of combinatorial complexity described above does not lie solely in theoretical systems. Fig. 1.2(C) highlights several biological examples of assemblies comprised of multiple distinct subunits that exhibit vast numbers of possible thermodynamic states. For protein signaling complexes such as EGFR and FcεRI ([25]), and p53 ([26]), these combinatorial states refer to different degrees of multimerization and modification through phosphorylation and other means. Modeling each monomer subunit of dimerizing EGFR as having nine available phosphorylation sites, we estimate $(2^9)^2 = 2^{18}$ possible states. A conservative model for FcεRI comprises a dimer of receptors where each monomer subunit can bind a ligand and two protein kinases (enzymes catalyzing phosphate group transfer). Accounting for the possible phosphostates of each component, the number of combinatorial states is $(\text{receptor phosphostates})^2 \times (\text{kinase occupancy})^2 \times (\text{kinase occupancy})^2 \approx 300$. If we consider additional phosphorylation sites or protein components, and the roles of scaffold and adaptor proteins binding to phosphorylated sites on the protein complex, then our estimate can expand to the order of 10^4 . Even if we were to ignore differentiation among subunits of a protein, it is clear that accounting for sites of post-translational modification leads to an explosion of possible combinatorial states. The tumor suppressor protein p53, for example, is known to have 10-20

major sites for phosphorylation, acetylation, and other modifications. Assuming independent binding across the sites, this translates to $2^{10} - 2^{20}$ possible states.

For DNA-binding transcription factor ensembles such as in bacteriophage λ ([13, 27, 28]) and in *Drosophila* pair-rule gene expression ([10]), the combinatorics refer to different possible regulatory states. While state counts vary in the literature cited above, depending on the proposed model’s molecular specificity (e.g., DNA looping effects at the λ O_R promoter region), they can easily reach the hundreds and thousands. Of course, limiting these counts to the number of *functional* states reduces enumeration complexity, but it remains fundamentally valuable to model systems by leveraging the complete set of combinatorial states because this approach shows *how* specific functional states can be reached. In a sense, such models indicate the energy landscape of dynamic assembly, revealing “hidden” intermediate states that are both kinetically accessible and influence overall binding dynamics.

A significant conceptual advance came with the recognition that stochastic reaction networks can be modeled with an operator formalism analogous to second quantization. In 1976, Masao Doi introduced a representation of classical multiparticle systems in Fock space, i.e., the space of all particle number states (vacuum, 1-particle states, 2-particle states, etc.) [17, 18]. This description maps the rise and fall of number counts for various protein complexes in a system with creation ($\hat{a}$) and annihilation ($\hat{a}^\dagger$) operators.

To illustrate, consider the most straightforward setting of a well-mixed system of particles, for which we would like to determine the probability $P(n, t)$ of having n particles present at time t . This probability evolves via a master equation which, for a simple death process in which particles are destroyed (i.e., decay) at a rate γ , is given by

$$\partial_t P(n, t) = \gamma(n + 1)P(n + 1, t) - \gamma n P(n, t). \quad (1.9)$$

Intuitively, the first term on the right hand side tracks probability flux into the n -particle state that can be attributed to the decay of one particle in an $n + 1$ -particle state. The second term corresponds to flux out of the n -particle state upon decay of one of the n particles.

Doi’s formalism encodes the probability distribution into a quantum-like state vector using an occupation number (Fock space) representation. The probability generating state then becomes

$$|\psi(t)\rangle = \sum_{n=0}^{\infty} P(n, t) (\hat{a})^n |0\rangle, \quad (1.10)$$

which is a weighted sum of basis states corresponding to particle number n with probability coefficients $P(n, t)$. One can then rewrite the master equation to track the evolution of a system vector, as in

$$\partial_t |\psi\rangle = \mathcal{L}(\hat{a}, \check{a}) |\psi\rangle \quad (1.11)$$

with a formal solution

$$|\psi(t)\rangle = e^{\mathcal{L}t} |\psi(0)\rangle, \quad (1.12)$$

where the Liouvillian $\mathcal{L}$ is an operation that encodes reaction rates.

Shortly thereafter, Luca Peliti converted this operator algebra into a coherent state path integral formulation. Rather than working with operators, Peliti's approach maps operators to complex numbers ($\hat{a} \rightarrow \phi, \check{a} \rightarrow \bar{\phi}$) such that $\mathcal{L}(\hat{a}, \check{a})$ transforms to an ordinary function $\mathcal{L}(\phi, \bar{\phi})$ governed by classical calculus. While this mapping is not *globally* allowable, since the creation and annihilation operators do not commute and order thus matters, the physics of coherent states makes this mapping possible *locally in time*. Since a coherent state $|\phi\rangle$ satisfies $a|\phi\rangle = \phi|\phi\rangle$, when we evaluate the inner product of $\mathcal{L}$ with coherent states that are an infinitesimal time step ϵ apart,

$$\langle \bar{\phi}_{t+\epsilon} | \mathcal{L}(\hat{a}, \check{a}) | \phi_t \rangle \rightarrow \mathcal{L}(\bar{\phi}_{t+\epsilon}, \phi_t). \quad (1.13)$$

Ultimately, Peliti [19] derives a mapping of the form

$$e^{\mathcal{L}t} \rightarrow \int \mathcal{D}\bar{\phi} \mathcal{D}\phi \exp \left(\int_0^T dt [\mathcal{L}(\bar{\phi}, \phi) - \bar{\phi} \partial_t \phi] \right), \quad (1.14)$$

where the $-\bar{\phi} \partial_t \phi$ term comes from the overlap between coherent states at neighboring time steps. Peliti and others demonstrated how this path integral and other field-theoretic methods (renormalization group and saddle-point approximation, among others) could be used to analyze reaction-diffusion systems [19, 20, 29–31]. Together, the Doi-Peliti technique established a fundamental correspondence between stochastic chemical kinetics and quantum many-body theory.

Single-species presentations of the Doi-Peliti formalism, however, are deceptively clean. Biological systems present additional challenges that these approaches do not entirely capture. In particular, Doi's formalism scales poorly with the number of species and the complexity of the interaction network. Since distinct operators are required for each species assembled, the exponential scaling of multistate species previously observed in standard sets of n master equations is instead transferred into the operator algebra complexity of $\mathcal{L}$. Interactions among multiple species during

reactions also entangle operators such that $\mathcal{L}$ no longer has sparse or factorizable structure that can be exploited to simplify the expression. This makes perturbation theory and renormalization group analysis methods impractical, rendering Doi *and* Peliti’s methods formal rather than particularly useful in modeling large biochemical networks.

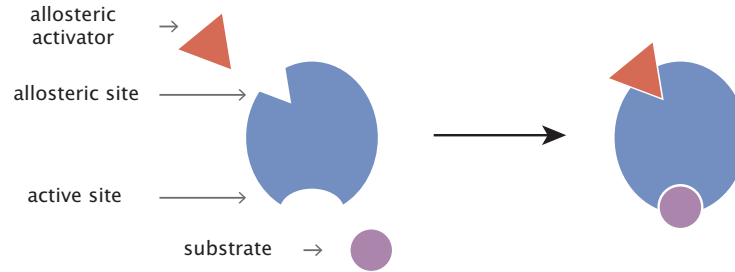
Beyond scaling, there is also a representational problem in that the Doi-Peliti formalism has no native concept of molecular structure. It can track that species C appears, for example, but not that C is a dimer of A and B with A’s binding site occupied. This makes it fundamentally unsuited to systems where the combinatorial variety of possible complexes and the structural conditions for their assembly are central to the system’s physical information.

Indeed, molecules are not indistinguishable particles with simple pairwise interactions. Rather, they possess internal states, multiple binding sites, and at times intricate rules for combinatorial assembly, where it is critical to preserve information about the internal characteristics and subunits making up larger assemblies. Thus, while the Doi-Peliti formalism provides a point of departure, it does not resolve the combinatorial explosion and assembly rule subtleties inherent to the formation of biological multiparticle complexes.

From the systems biology perspective, rule-based modeling has emerged as a practical alternative for approaching combinatorial complexity. Frameworks such as BioNetGen [32] and Kappa [33] encode interaction rules (e.g., binding of specific domains) without enumerating all possible complexes. While computationally efficient, however, these approaches generally lack a unified algebraic foundation facilitating statistical mechanical analysis of the physical properties of assembly.

Chapter 2 presents recently published work that addresses this gap in disciplines by developing a *rule-based* operator algebra formalism, drawing inspiration from the mathematical building blocks of Doi-Peliti and the rule-based emphasis of models from computational biology [34]. By constructing a Fock space in which particles possess internal states and binding sites, and by defining operators corresponding to rule-based assembly and disassembly events, we provide a mathematically transparent and physically grounded description of multiparticle complexes. This rule-based approach also allows for a diagrammatic representation echoing techniques from statistical field theory that enables compact visualization and manipulation of interaction rules for analytical study. In introducing the key innovation of rule-based modeling, this algebraic framework is compatible with both equilibrium and

(A) POSITIVE ALLOSTERIC MODULATION



(A) NEGATIVE ALLOSTERIC MODULATION

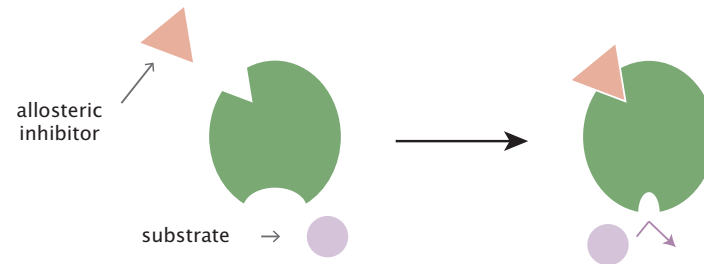


Figure 1.3: Allosteric proteins have an altered protein conformation upon the binding of an “effector” molecule, which can (A) activate or (B) inhibit the protein’s activity, i.e., its ability to bind a substrate at the reshaped active site.

nonequilibrium statistical mechanics concepts, and enables analytic and numeric analysis of assembly dynamics in either setting.

1.2 Allostery and its statistical mechanical origins

While multiparticle complex assembly highlights structural combinatorics, we are also interested at more macroscopic scales in the dynamics of how these assemblies coordinate to determine protein expression patterns and broader, nuanced cell functions. Allostery is an essential cellular tool for directing this mapping from microscopic interactions to macroscopic function, and is one of the central organizing principles of molecular biology, describing the regulation of a macromolecule’s activity through the binding of a ligand at a site distinct from its active or functional one (Fig. 1.3). First conceptualized in the mid-twentieth century, allostery has evolved from a biochemical description of enzyme regulation into a quantitatively tractable problem in statistical physics. Today, it provides a unifying framework for understanding cooperativity, signal integration, and decision-making in biological systems ranging from oxygen-binding hemoglobin in the bloodstream to multistability and phase transitions in gene regulatory networks.

The term “allosteric” was introduced by Jacques Monod and François Jacob in 1961 to describe regulatory proteins for which activity is modulated by ligands binding at topographically distinct sites [35], a phenomenon Monod himself considered “the second secret of life” [36]. Shortly thereafter, the quantitative foundations of allostery were established in two seminal models. The MWC model proposed that multimeric proteins exist in equilibrium between discrete conformational states—commonly denoted T (tense) and R (relaxed)—where ligand binding shifts the population distribution among the possible ligand-bound and unbound forms of these states. In contrast, the Koshland–Némethy–Filmer (KNF) model [37] introduced a sequential mechanism in which ligand binding induces conformational changes locally, propagating cooperativity without requiring global symmetry.

These early works framed allostery as a problem of conformational equilibria and cooperative binding. Importantly, they also implicitly invoked a statistical mechanical perspective: proteins were understood not as static structures but as ensembles of microstates, with ligand binding altering the relative statistical weights of these states. This shift from structural determinism to ensemble thermodynamics laid the groundwork for modern biophysical treatments of regulation.

From a physical standpoint, allostery can be described as the redistribution of probability mass over a discrete set of conformational states in response to ligand binding. In the MWC framework, consider a protein with two conformational states, relaxed (R) and tense (T), and n ligand-binding sites. The corresponding partition function can be written as

$$Z(c) = \left(1 + \frac{c}{K_R}\right)^n + e^{-\beta\Delta\epsilon} \left(1 + \frac{c}{K_T}\right)^n, \quad (1.15)$$

where c is the ligand concentration, $K_{R,T}$ are the dissociation constants of the relaxed and tense states, respectively, and $\Delta\epsilon = \epsilon_T - \epsilon_R$ is the energy difference between conformational states. In this notation, c/K is a dimensionless statistical weight for ligand binding to a single site, and corresponds directly in more traditional statistical mechanics notation to a Boltzmann factor for the statistical weight of a bound ligand relative to an empty site at a baseline chemical potential. Cooperative behavior emerges naturally when ligand affinity differs between conformations, so that binding effectively couples to a conformational phase variable. The sigmoidal binding curves characteristic of cooperative systems arise from this nonlinear reweighting of states.

The statistical mechanical interpretation of allostery was further complemented by Wyman’s theory [38] formalizing the thermodynamic coupling between ligand

binding and conformational changes in hemoglobin. Later work generalized these ideas, demonstrating that allostery need not require large conformational shifts but can arise from modulation of fluctuations within an ensemble [39]. In the ensemble view, regulation reflects shifts in occupancy across a rugged free-energy landscape, rather than transitions between two discrete structures alone.

Within this framework, allosteric proteins resemble finite-sized thermodynamic systems poised near transitions between competing states. The analogy to phase transitions becomes especially clear in the limit of high cooperativity. For example, in the MWC model with many subunits, the response function sharpens as the number of binding sites increases, approaching a discontinuous, switch-like transition in the thermodynamic limit. Although biological systems are finite, the mathematical parallels to mean-field models such as the Ising model are well established: conformational states correspond to spin variables, ligand concentration to an external field, and cooperative interactions to coupling constants.

This statistical mechanical perspective provides more than formal analogy – it offers predictive power. By writing down partition functions and enumerating microstates, one can derive response curves, Hill coefficients, and susceptibility-like quantities that measure sensitivity to ligand concentrations. Allosteric regulation thus becomes an experimentally testable mapping between microscopic free energies and macroscopic observables.

1.3 Allostery beyond enzymes: gene regulation as an allosteric process

The conceptual leap from enzymatic regulation to gene regulatory networks was catalyzed by the discovery of transcriptional repressors whose DNA-binding affinities are modulated by small molecules. The canonical example is the lac repressor characterized by François Jacob and Jacques Monod in their studies of the lac operon [35]. In this system, depicted in Fig. 1.4, effector molecules called inducers bind the repressor allosterically, decreasing its affinity for DNA and thereby activating transcription. Gene expression is then controlled by an effector molecule that tunes the occupancy of regulatory states, which is precisely the logic of allostery.

Modern quantitative models of transcriptional regulation adopt explicitly statistical mechanical formulations [40, 41]. Promoter occupancy is computed by enumerating binding configurations of RNA polymerase and transcription factors, weighted by Boltzmann factors determined by binding energies and ligand concentrations. In such models, effector molecules function as control parameters that alter the statis-

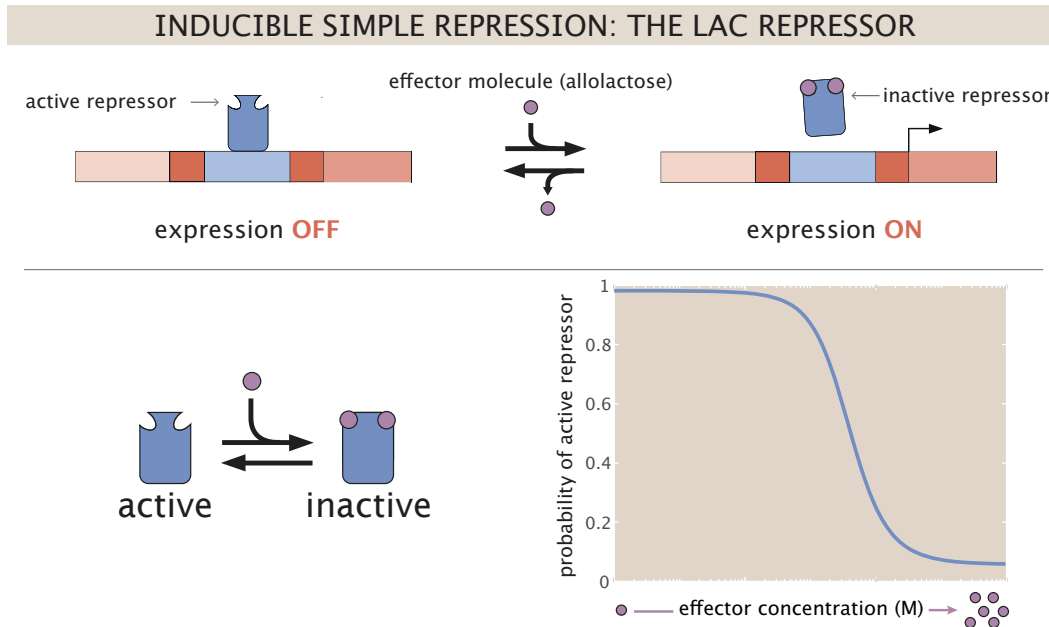


Figure 1.4: Allostery is a key cellular tool for controlling transcription. In a simple repression model for the lac repressor in *E. coli*, for example, if lactose is present it is converted to allolactose, which binds to the LacI repressor and renders it inactive, thus inducing gene expression. Within the broader context of the cell, an increase in the effector concentration alters the probability of encountering active repressors, thus shifting the equilibrium distribution to favor the inactive form and turn on expression. Effector molecules such as allolactose are called *inducers*.

tical weights of regulatory states. The resulting input–output functions, i.e., from ligand concentration to gene expression, often display sigmoidal or ultrasensitive behavior analogous to cooperative binding curves.

Crucially, when regulatory proteins oligomerize, bind cooperatively, or regulate their own expression, the system can exhibit multistability. Positive feedback loops, combined with nonlinear response functions derived from allosteric binding, generate bistable or multistable gene expression states. From a dynamical systems perspective, these states correspond to multiple stable fixed points in the space of expression levels. From a statistical mechanical perspective, they resemble coexisting phases separated by effective energy barriers.

1.4 Gene regulatory networks and multistability

The connection between allostery and phase transitions becomes particularly compelling in the context of gene regulatory networks that exhibit switch-like behavior. Consider a transcription factor with a DNA-binding affinity that depends alloster-

ically on an effector molecule. The effector concentration then acts as a thermodynamic field that shifts the balance between active and inactive conformations, as demonstrated by the change in the probability of active repressor plotted in Fig. 1.4. When the effector mechanism is embedded in a feedback loop, such as in the autoregulation of mutual repression, this nonlinear response can drive shifts in gene expression toward more diverse dynamic regimes, as will be clarified and explored in greater detail in Chapters 3 and 4. These bifurcations in dynamic phase space, such as a transition from monostable to bistable expression, are analogous to a second-order phase transition where, for example, a single stable state gives way to two symmetry-broken states. In stochastic descriptions, noise-induced switching between expression states parallels thermally-activated transitions between free-energy minima. Gene expression dynamics can then be derived from underlying microscopic binding equilibria, linking molecular allostery to macroscopic cell-fate decisions.

In this sense, effector molecule concentrations function as tuning knobs that control the position of a system relative to critical points. Near such points, susceptibility – defined as the derivative of expression with respect to effector concentration – can become large, indicating heightened sensitivity. This mirrors critical phenomena in physical systems, where response functions diverge at phase transitions. Although finite-size effects and intrinsic noise smooth these singularities in biological contexts, the conceptual and mathematical analogies remain powerful.

By framing regulation as a redistribution of ensemble probabilities, one can derive precise relationships between molecular parameters and system-level behavior. When extended to gene regulatory networks, this framework reveals deep connections between effector-mediated control, nonlinear feedback, multistability, and phase-transition-like phenomena. For example, the study of bistability and switches in genetic circuits has gained particular prominence with the synthetic genetic toggle switch [42], demonstrating experimentally that mutually repressing genes can produce two stable expression states. Theoretical models of such circuits typically use the Hill-function form to capture highly cooperative binding and sigmoidal behavior with coupled nonlinear differential equations. Given the high cooperativity regime, transcription factor binding in this approach is all-or-none, meaning that either all transcription factor binding sites are empty or all sites are occupied. If binding *activates* transcription, promoter activity is then proportional to the probability that

a transcription factor (concentration X) is bound, and the output fraction is

$$f_{\text{act}}(X) = \frac{X^n}{K^n + X^n}, \quad (1.16)$$

with Hill coefficient $n > 1$ denoting the strength of cooperativity and thus the steepness of the response curve to an increase in the transcription factor concentration. If binding *represses* transcription, promoter activity is instead proportional to the probability that the DNA remains unbound, with a corresponding output fraction

$$f_{\text{rep}}(X) = \frac{K^n}{K^n + X^n} = \frac{1}{1 + (\frac{X}{K})^n}. \quad (1.17)$$

These functions form the basis for models such as those in [42], where two genes expressing protein concentrations U and V that mutually repress each other can be written as

$$\frac{dU}{dt} = \frac{a_1}{1 + V^n} - bU, \quad \frac{dV}{dt} = \frac{a_2}{1 + U^n} - bV, \quad (1.18)$$

with $a_{1,2}$ are maximal production rates and b is the rate of protein decay. Such a system of equations generates bistable dynamic expression of protein concentrations U and V .

Yet such circuit designs only shed partial light on the coordinated emergence of multistable behavior that often naturally extends beyond two-state decision making. Further, these studies generally observe shifts in dynamics by tuning parameters intrinsic to the system, such as rates and dissociation constants, that are not explicitly tunable by the cell or by experimental design.

Chapter 3 addresses both of these points by systematically exploring how allosteric effector concentrations shape the multistable behavior of three-gene toggle switch motifs. This work [43] illustrates how effector molecules—through allosteric control of transcription factor activity—directly tune the locations of bifurcations and the qualitative structure of phase space in a three-gene network, expanding beyond simple bistable switches to tristability and even richer multistable regimes through the inclusion of autoactivation within the regulatory motif itself.

In this model, each gene encodes a protein that represses the others, and the effective concentration of active repressor is controlled by inducer molecules that bind allosterically to the transcription factors. As a function of inducer concentration, the system undergoes saddle-node bifurcations that create or annihilate a pair of fixed points, producing distinct dynamic phases distinguished by the number of stable steady states. At low inducer levels, the network supports multiple stable expression

patterns highlighting tristability, whereas sufficiently high inducer levels render dynamics partially or entirely irreversible, collapsing the system to bistable behavior in the presence of a single inducer and to monostable behavior in the presence of two. Importantly, the way in which effector binding alters transcription factor functionality (e.g., whether simply altering activity or causing a switch between repression and activation) reshapes both dynamic regimes and the corresponding bifurcation structure.

These analyses highlight a central conclusion: allosteric control is a useful, biologically relevant quantitative mechanism for driving multistability in gene networks, replacing abstract model parameters such as effective dissociation constants or rate parameters with quantities that cells actually tune endogenously. Instead of treating cooperativity and repression “parameters” as exogenous knobs, my work embeds them in mechanistic allosteric models that derive transcription factor activity from effector concentrations using statistical mechanics. Viewed from this perspective, the transitions between dynamical regimes – whether monostable, bistable, tristable, or more complex – are not mere mathematical artifacts but have direct interpretations as shifts in the underlying thermodynamic landscape induced by changes in effector binding equilibria.

This approach parallels classical phase transitions in physical systems. Effector concentration serves as an external field that biases the free-energy landscape of a regulatory network, inducing qualitative changes in global stability much like temperature or magnetic fields drive transitions in spin systems. The bifurcations observed in the three-gene toggle switch are thus reminiscent of critical points where symmetry breaks and new phases emerge. As in statistical mechanical phase transitions, the nature of these bifurcations reflects the interplay between nonlinearity (cooperativity) and feedback in shaping the landscape of stable states.

Most crucially, this discussion forms a foundation for expanding to higher-order gene networks and gaining insights into the mechanisms by which complex cellular decision-making can arise through control by effector molecules.

1.5 From Hill descriptions to thermodynamic models in simpler illustrative motifs

In modeling gene regulatory networks, Chapter 3 relies on Hill function models, which have been historically used to capture nonlinear input–output relationships. By defining state occupancies for transcription factors bound to DNA as either bound

or unbound, with bound states having cooperativity n , Hill models offer a tractable and conceptually transparent description of how regulator concentrations influence gene expression. In larger networks, and particularly those composed of multiple interacting feedback loops, this phenomenological representation facilitates investigations of bifurcations, multistability, oscillations, and other dynamical features without a combinatorial explosion of microscopic details. Indeed, for higher-order circuits, Hill functions offer the only computationally-viable means of surveying parameter spaces and identifying qualitative behaviors.

At the same time, Hill functions can be viewed as coarse-grained descriptions of underlying molecular processes. Through its coefficient n , the model encodes both cooperativity and oligomerization, and serves as a bridge between biochemical intuition and dynamical systems analysis. As such, it retains the essential nonlinearity required for multistability while remaining mathematically manageable.

When attention shifts from large composite networks to simpler regulatory motifs involving one or two genes and a smaller number of interaction components, it becomes feasible and pedagogically illuminating to resolve this coarse-graining. In these cases, one can replace phenomenological Hill expressions with full thermodynamic models that enumerate promoter microstates and assign each a statistical weight. Importantly, the thermodynamic framework does not contradict Hill descriptions, but rather clarifies their origins. Indeed, under appropriate conditions, such as strong cooperativity or dominant state competition, the resulting input–output relations reduce to Hill-like forms. Given that high cooperativity is a fundamental strategy for achieving precise and sensitive regulation, particularly in eukaryotes containing cis-regulatory elements such as enhancers and promoters, Hill models thus remain an effective description in many cases. More generally, however, thermodynamic models reveal how Hill coefficients and dissociation constants arise from combinations of fundamental microscopic parameters. This allows explorations of the impact of enumerating additional occupancy states in motifs where computational constraints do not dominate. In such settings, a detailed thermodynamic model can provide additional parametrizations that more precisely specify the biochemical underpinnings of binding interactions. By first characterizing the dynamical consequences of nonlinear regulation in complex networks, and then shifting to minimal motifs where microscopic detail is tractable, we obtain a layered understanding where global behaviors are identified at the network level, and their molecular origins can then be further clarified.

In Chapter 4, I explore thermodynamic modeling of inducible genetic circuits by focusing on simple illustrative motifs including auto-activation, mutual repression, and feed-forward loops [44]. While these motifs have already been studied extensively using Hill models [42, 45–50], features of these networks in thermodynamic models with tunable rate parameters, binding cooperativity, and in this case effector concentrations, remain less well understood. In continuing the theme of Chapter 3, I emphasize throughout the power of effector concentrations as natural tuning knobs for dynamic activity. Focusing on simpler circuit designs provides further freedom to explore the phase portraits that arise from thermodynamic model parametrization. Across all motifs we observe how effector concentration drives the diversity of and accessibility to different dynamic regimes of stability, driving these properties in a manner that cannot be fully realized by traditional abstract parametrization. These findings emphasize the ongoing importance of developing a comprehensive picture of the allosterome, i.e., a complete mapping of the effector molecules regulating transcription factors, to better understand large-scale gene regulatory network dynamics.

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Chapter 2

MODELING SELF-ASSEMBLY PROCESSES IN AND OUT OF EQUILIBRIUM FROM QUANTUM FIELD-THEORETIC PRINCIPLES¹

We begin our exploration across biological scales by focusing on those of fundamental building blocks. In traditional physics, this would mean dissecting the dynamics of particle interactions at the atomistic level, which is where quantum mechanics dominates. Under the appropriate conditions, electrons in this regime can then exhibit wave-like energy transfer, tunnel through a high potential energy barrier, superpose to exist in multiple states simultaneously, and entangle to share the same quantum state. These properties all rely on the quantum coherence of systems operating at this scale, which dictates that systems exist in a superposition of states with a well-defined phase relationship.

Quantum coherence is fragile, however, because it requires the system to be informationally isolated from its environment. The moment the environment “learns” a system’s state, the superposition collapses to a classical mixture. This irreversible process is referred to as *decoherence* [1], and is a fundamental feature of biological proteins in solution. Thermal “kicks” from solvent collisions and electromagnetic effects from polar solvent molecules act as “measurements” on the protein, entangling the system’s state with that of the thermal bath (solvent). The resulting decoherence is essentially instantaneous on biochemical timescales.

By building proteins, nature constructs systems made up of atomistic quantum components that, as collective units, behave *classically*. More concretely, a protein’s position and momentum is well-defined, thermal noise drives exploration and facilitates changes in protein conformation to find binding partners, and a protein complex’s 3D geometry (not its quantum state) determines its function. This classical collective behavior is not merely a physical curiosity — it represents the foundation upon which the logic of molecular assembly is built. Because proteins behave as classical objects with well-defined shapes and thermally driven dynamics, they can do something remarkably powerful: they can associate, and thus transcend

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the functional limitations of any individual component. When proteins oligomerize into complexes, entirely new capabilities emerge that no monomer could possess alone. The geometry of interfaces between subunits, for example, creates binding pockets that did not exist on any individual piece. Upon the binding of a ligand to a binding pocket, mechanical strain from the binding event can propagate across a complex, allowing the input signal to reshape a distant active site through a process known as allostery. Forming an enclosed chamber from collected subunits can sequester chemical properties, concentrate reactants, and prevent half-finished products from escaping into the crowded cell. Finally, the sheer size of an assembly can enforce kinetic delays that enable proofreading, thereby rejecting unsuitable substrates before committing to an irreversible step. In each case, the functional gain is inseparable from the act of assembly itself. Understanding why biology persistently builds large assemblies, then, requires us define how classical, thermally fluctuating objects can be arranged so that their interactions give rise to computational and catalytic capabilities of extraordinary sophistication.

If function emerges from the collective behavior of many interacting parts, however, this also implies that to understand or predict function one would need to track interactions explicitly. Across different biological contexts, systems of classically behaving protein complexes thus inherently exhibit a crucial theme of “combinatorial complexity” [2]. This means that an immense (and often infinite) variety of molecular complexes can form from a relatively small number of interaction rules governing the assembly of a relatively small number of molecular components. While it is this combinatorial complexity that makes the diverse, sophisticated collective functions of proteins possible, it is also this property that quickly renders self-assembly models intractable for practical analysis.

Consider the simplest possible case of a single protein with two modifiable sites (e.g., phosphorylation sites that can be either “on” or “off”). Assuming ligands bind independently at each site, such a protein can exist in $2^2 = 4$ possible distinct states. In a realistic signaling protein, it is more typical to have as many as 10-20 relevant sites across several subunits, leading to $2^{10} = 1024$ distinct states, or $2^{20} \approx 10^9$ states in an expanded model setting. These estimates, however, do not account for several key sources of interactions and functional transformations. Binding states, for example, can have multiple ligands. Proteins are also not in a single rigid state, but rather in an ensemble of conformations. Finally, post-translational modifications including phosphorylation, ubiquitination, acetylation, methylation, and more can

introduce combinatorial dependencies that raise the number of possible distinct states further. A few examples of this complexity from protein signaling and gene regulation were previously highlighted in Fig. 1.2. Predicting and managing the explosion of combinatorial states that can arise in biological assembly thus presents an outstanding challenge to statistical physicists and computational biologists alike.

In 1976, Masao Doi [3, 4] proposed a method for modeling many-body systems of classical particles, as outlined in Chapter 1 of this thesis. The approach roots assembly within a Fock space description capturing the space of all particle number states (vacuum, 1-particle states, 2-particle states, etc.). The formalism was further developed by others [5–7], and has proven useful in the study of diffusion-limited aggregation [8–10] and other problems in statistical physics [11–13]. As in quantum field theory, Doi’s formalism supports the creation and annihilation of particles, but does not support the assembly of preexisting particles into complexes. Consequently, analyzing systems that involve multi-particle complexes using this formalism requires specifying one distinct field for every distinct species of complex. This makes Doi’s formalism unwieldy for analyzing systems that exhibit substantial combinatorial complexity.

Consider, for example, the homopolymer system illustrated in Fig. 2.1. This system comprises one type of component particle having two sites capable of forming an interaction. In thermal equilibrium the system’s behavior is governed by two quantities: the chemical potential (μ) of the particles and the energy of interaction (ϵ) [Fig. 2.1(A)]. Out of equilibrium the system is governed by four rate parameters (r_{1-4}) describing the appearance and disappearance of particles, as well as their mutual binding and unbinding [Fig. 2.1(B)]. But despite how simple this system is to describe in words and pictures, modeling this system in Doi’s formalism is complicated because the above rules lead to an infinite number of possible polymeric complexes. To apply Doi’s formalism, one must define an infinite number of fields, one for every species of complex. For equilibrium systems, one must then manually specify the chemical potential of each species [Fig. 2.1(C)]. For systems out of equilibrium, one must manually specify the rate of reaction between all reacting sets of species [Fig. 2.1(D)]. And in doing so, one must take care that the chemical potentials and/or reaction rates written down are expressed correctly as functions of the underlying model parameters, as Doi’s formalism provides no means of computing these quantities.

Clearly something is missing. Ideally, the formalism one uses to describe systems

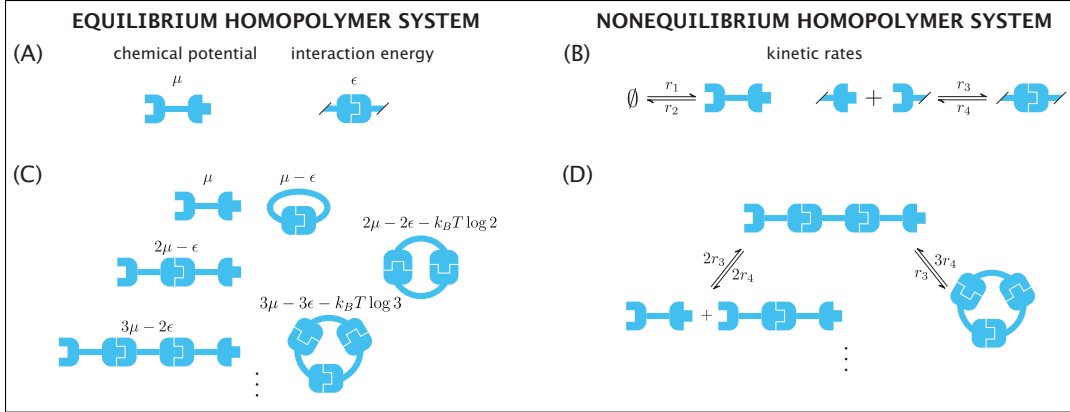


Figure 2.1: Homopolymer in zero dimensions. The system comprises a single species of component particle, with each particle having two domains capable of forming heterotypic interactions. (A) In thermal equilibrium, system behavior is governed by the chemical potential (μ) and interaction energy (ϵ). (B) Out of equilibrium, the system is governed by the kinetic rates for monomer creation (r_1), monomer annihilation (r_2), interaction formation (r_3), and interaction dissolution (r_4). (C) The rules in panel (A) generate an infinite number of possible complexes: x -chains and x -rings for all $x = 1, 2, \dots$, each complex with a distinct chemical potential. The log terms in the chemical potentials of the x -rings result from their rotational symmetry. (D) The rules in panel (B) result in an infinite web of reactions between different polymeric complexes, with reaction rates proportional to r_3 and r_4 in ways that depend nontrivially on the identities of the specific reactants and products.

of multi-particle complexes should allow one to mathematically derive the set of possible complexes, the chemical potentials of each complex, and the rates of reaction between complexes from the underlying rules and their associated parameters. This chapter develops a formalism that does this.

The problem of combinatorial complexity has long been recognized in the field of computational systems biology. Starting in the 2000s, researchers studying biological signaling pathways began developing “rule-based” approaches for simulating chemical systems of multi-particle complexes [2, 14–20]. Some of these efforts have produced sophisticated software ecosystems, such as BioNetGen [15, 17, 19, 21] and Kappa [18, 22]. These simulation approaches, however, are based on formal representations that do not lend themselves to analytical calculations using the mathematical methods of statistical physics. For example, BioNetGen is based on a process algebra describing the formation and dynamics of port graphs (i.e., graphs with edges attached through ports) [23] while Kappa is based on the κ -calculus process algebra language for linking protein “agents” [18, 22]. Neither approach

involves a native thermodynamic framework, and both describe interaction rules by specifying rates rather than energies. These limitations prevent such computational methods from providing a built-in notion of partition functions and free energy landscapes, which are fundamental foundations for statistical physics analyses. As a result, work in this area has been confined to computational rather than analytic calculations.

Here we bridge the divide between Doi's mathematical formalism and rule-based methods for computationally modeling biochemical systems. Using a Fock space for classical particles reminiscent of but distinct from that of Doi, we develop an operator algebra that allows not only for the creation and annihilation of particles, but also for the assembly of particles into complexes. We show that this operator algebra allows one to mathematically analyze equilibrium and nonequilibrium systems that are defined in a rule-based manner, and can also be used as a basis for computational analyses using stochastic simulations.

Section 2.1 establishes the formalism's foundation, introducing how microstates and macrostates are represented. We then apply the formalism to three systems in thermal equilibrium: a monomer system (Section 2.2), a homodimer system (Section 2.3), and a homopolymer system (Section 2.4). Next we show how our formalism can be used to define and analyze nonequilibrium systems, both through the analytic derivation of master equations in Section 2.5 and through computational analysis carried out using a stochastic simulation algorithm presented in Section 2.6. We end by illustrating the versatility of our formalism in Section 2.7, showcasing the variety and complexity of system behavior that can arise from positing different sets of rules.

2.1 Foundations

2.1.1 Microstates

Following Doi we define a set $\mathcal{S}$ of microstates where each microstate $s \in \mathcal{S}$ corresponds to a unit vector $|s\rangle$. The resulting set of pure states forms an orthonormal basis for the Fock space. The vector $|\psi\rangle$ describing the system is then given by a probabilistic mixture of pure states,

$$|\psi\rangle = \sum_{s \in \mathcal{S}} p_s |s\rangle, \quad (2.1)$$

where p_s represents the probability of the system being in state s . It is useful to define the sum of all possible states as the “sum vector”

$$|\text{sum}\rangle = \sum_{s \in \mathcal{S}} |s\rangle. \quad (2.2)$$

The expectation value of any operator $\mathbb{O}$ is then given by $\langle \text{sum} | \mathbb{O} | \psi \rangle$, and probability normalization requires that $\langle \text{sum} | \psi \rangle = 1$.

The Fock space supports systems both in and out of thermal equilibrium. In equilibrium, the system is taken to be in the grand canonical ensemble, such that the different possible states arising can vary in both total energy and total particle number (along with other thermodynamic variables for chemical potential, volume, and absolute temperature remaining constant). The state vector $|\psi\rangle$ can then be expressed in terms of a Hamiltonian operator $\mathbb{H}$ that assigns an energy to each microstate:

$$|\psi\rangle = \frac{e^{-\beta \mathbb{H}}}{Z} |\text{sum}\rangle, \quad \text{where } Z = \langle \text{sum} | e^{-\beta \mathbb{H}} | \text{sum} \rangle \quad (2.3)$$

is the partition function, $\beta = 1/k_B T$ where k_B is Boltzmann’s constant and T is temperature, and $\mathbb{H} |s\rangle = H_s |s\rangle$ where H_s denotes the free energy of state s . The dynamics of the system state $|\psi\rangle$ out of equilibrium is described by

$$\frac{d}{dt} |\psi\rangle = \mathbb{W} |\psi\rangle, \quad (2.4)$$

where $\mathbb{W}$ is a transition operator. We call this the “microstate master equation.” In terms of the scalar transition rates $W_{s \rightarrow t}$ from microstate s to microstate t , the transition operator is

$$\mathbb{W} = \sum_{s, t \in \mathcal{S}} W_{s \rightarrow t} (|t\rangle \langle s| - |s\rangle \langle s|). \quad (2.5)$$

Note that the first term in the summand reflects the flow of probability into p_t , while the second term reflects the flow of probability out of p_s . We call these the “reaction” and “depletion” terms, respectively.

2.1.2 Macrostates

Unlike in Doi’s formalism, the microstates in our formalism represent not only the externally observable properties of particles, but also their unobservable internal states. These internal states are, in fact, what make particles in our formalism identifiable and thus allow complexes to be constructed from preexisting particles. We therefore distinguish between the microstates of a system (represented by the $|s\rangle$ vectors) and the macrostates of the system.

In this work we focus on zero-dimensional (i.e., well-mixed) populations of particles and complexes. Assuming there are K possible observably distinct species of complex, each macrostate is characterized by a vector $\vec{n} = (n_1, \dots, n_K)$ where each n_k quantifies the number of complexes of species k . The corresponding macrostate vector is defined to be the sum of all microstate vectors consistent with the macrostate, i.e.,

$$|\vec{n}\rangle = \sum_{s|\vec{n}} |s\rangle. \quad (2.6)$$

Note that $|\text{sum}\rangle = \sum_{\vec{n}} |\vec{n}\rangle$, and that the probability of a the system being in a macrostate $\vec{n}$ given $|\psi\rangle$ is

$$P(\vec{n}) = \langle \vec{n} | \psi \rangle. \quad (2.7)$$

In equilibrium systems, each macrostate is an eigenstate of the Hamiltonian:

$$\mathbb{H} |\vec{n}\rangle = - \sum_k n_k \mu_k |\vec{n}\rangle, \quad (2.8)$$

where μ_k is the (bare) chemical potential of species k . Consequently, the probability of the system having macrostate $\vec{n}$ is a product of species-specific Poisson distributions, i.e.,

$$P(n_k) = \frac{1}{Z_k} \frac{e^{\beta \mu_k n_k}}{n_k!}, \quad \text{where } Z_k = \exp[e^{\beta \mu_k}]. \quad (2.9)$$

In nonequilibrium systems, this probability becomes a function of time t and evolves according to the “macrostate master equation”

$$\frac{d}{dt} P_t(\vec{n}) = \sum_{\vec{n}'} W_{\vec{n}\vec{n}'} P_t(\vec{n}'), \quad (2.10)$$

where $W_{\vec{n}\vec{n}'} = \langle \vec{n} | \mathbb{W} | \vec{n}' \rangle / \langle \vec{n}' | \vec{n}' \rangle$ are the macrostate-specific transition rates. In later sections we compute these $W_{\vec{n}\vec{n}'}$ from the transition operator $\mathbb{W}$, but instead of calculating each rate directly we find it simpler to calculate the vector

$$|J(\vec{n})\rangle = \mathbb{W}^\dagger |\vec{n}\rangle. \quad (2.11)$$

We call $|J(\vec{n})\rangle$ the “flux projector” since taking the inner product of it with $|\psi\rangle$ yields a vector of probability fluxes, i.e., $\dot{P}_t(\vec{n}) = \langle J(\vec{n}) | \psi(t) \rangle$.

2.2 Monomer in equilibrium

2.2.1 Microstates and macrostates

We now construct the Fock space on which our formalism is based, using a system of monomeric particles for concreteness. The particles are represented using a

hard-core boson field, A , which is assumed to have N excitation modes. Each mode A_i is indexed by a number $i \in \mathcal{N} = \{1, \dots, N\}$ that represents the internal state of a particle. This index allows the formalism to track individual particles that are outwardly identical. In what follows we keep N finite for concreteness, but all physically meaningful calculations are performed in the $N \rightarrow \infty$ limit.

Each mode A_i can be in one of two orthonormal states: $|1\rangle_i$ represents the presence of a particle with internal state i , while $|0\rangle_i$ represents its absence. Microstates are given by tensor products over all modes. Specifically, a microstate representing K particles having indices $\mathcal{I} \subseteq \mathcal{N}$ is represented by

$$|\mathcal{I}\rangle = \bigotimes_{i \in \mathcal{N}} \begin{cases} |1\rangle_i & \text{if } i \in \mathcal{I}, \\ |0\rangle_i & \text{otherwise.} \end{cases} \quad (2.12)$$

These states are orthonormal, i.e., $\langle \mathcal{I} | \mathcal{J} \rangle = \delta_{\mathcal{I}\mathcal{J}}$. The resulting macrostates of the system are

$$|n\rangle = \sum_{\mathcal{I}: |\mathcal{I}|=n} |\mathcal{I}\rangle, \quad \text{for } n = 0, \dots, N. \quad (2.13)$$

The vacuum state, $|0\rangle = |\emptyset\rangle$, is both a microstate and a macrostate.

These definitions readily extend to systems defined by multiple fields. Consider a mixture of monomer species A and B . The microstate comprising A monomers having indices $\mathcal{I}$ and B monomers having indices $\mathcal{J}$ is given by

$$|\mathcal{I}, \mathcal{J}\rangle_{A,B} = |\mathcal{I}\rangle_A \otimes |\mathcal{J}\rangle_B, \quad (2.14)$$

where the subscripts indicate the Fock space in which each state vector lives. The corresponding macrostates and sum states are given by analogous tensor products. Systems with three or more fields are defined similarly.

2.2.2 Mode and field operators

We now define four types of mode-specific operators: creation, annihilation, presence, and absence. The creation operator for mode i is defined to be $\hat{A}_i = |1\rangle_i \langle 0|_i$. When applied to a microstate $|\mathcal{I}\rangle$, this operator has the effect

$$\hat{A}_i |\mathcal{I}\rangle = \begin{cases} 0 & \text{if } i \in \mathcal{I}, \\ |\mathcal{I} \cup \{i\}\rangle & \text{otherwise.} \end{cases} \quad (2.15)$$

The corresponding annihilation operator is defined to be $\check{A}_i = \hat{A}_i^\dagger$, and has the effect

$$\check{A}_i |\mathcal{I}\rangle = \begin{cases} |\mathcal{I} \setminus \{i\}\rangle & \text{if } i \in \mathcal{I}, \\ 0 & \text{otherwise.} \end{cases} \quad (2.16)$$

The presence operator is defined as $\bar{A}_i = \hat{A}_i \check{A}_i$. $\bar{A}_i |\mathcal{I}\rangle$ is one if $i \in \mathcal{I}$ and zero otherwise. The absence operator is defined to be $\tilde{A}_i = \check{A}_i \hat{A}_i = 1 - \bar{A}_i$. Note that $\bar{A}_i$ and $\tilde{A}_i$ are self-adjoint.

We highlight several key algebraic properties of these operators. First, creation and annihilation operators are nilpotent, i.e., $\hat{A}_i^2 = \check{A}_i^2 = 0$. Second, the commutator

$$[\check{A}_i, \hat{A}_j] = \delta_{ij} (1 - 2\bar{A}_i) \quad (2.17)$$

is very different than one finds in the harmonic oscillator algebra, and thus in other algebras used to model classical particles [3, 7]. Third, the commutator

$$[\bar{A}_i, \hat{A}_j] = \delta_{ij} \hat{A}_j, \quad (2.18)$$

plays an important role later when constructing multi-particle complexes from component particles. Appendix 2.A lists some additional useful properties.

We further define field-specific creation, annihilation, presence, and absence operators as sums over all of the corresponding mode operators, i.e.,

$$\hat{A} = \sum_i \hat{A}_i, \quad (2.19)$$

and similarly for $\check{A}$, $\bar{A}$, and $\tilde{A}$. These field operators satisfy the useful commutation relations

$$[\check{A}, \hat{A}] = N - 2\bar{A}, \quad [\bar{A}, \hat{A}] = \hat{A}. \quad (2.20)$$

Macrostates are given by

$$|n\rangle = \frac{\hat{A}^n}{n!} |0\rangle. \quad (2.21)$$

Here the combinatorial factor corrects for each set of modes being summed over $n!$ times in the operator product $\hat{A}^n$. Note that $|n\rangle = 0$ if $n > N$, since this would cause every term in $\hat{A}^n$ to contain at least one factor of $\hat{A}_i^2$. Applied to a macrostate, one finds that

$$\hat{A} |n\rangle = (n+1) |n+1\rangle, \quad \bar{A} |n\rangle = n |n\rangle, \quad (2.22)$$

$$\check{A} |n\rangle = (N-n+1) |n-1\rangle, \quad \tilde{A} |n\rangle = (N-n) |n\rangle. \quad (2.23)$$

See Appendix 2.A for a derivation of these results.

In the large N limit, the number of modes that are excited in any macrostate $|n\rangle$ with substantial physical probability becomes negligible compared to N . In what follows we therefore approximate Eq. 2.23 as

$$\check{A} |n\rangle \approx N |n-1\rangle, \quad \tilde{A} |n\rangle \approx N |n\rangle. \quad (2.24)$$

By similar logic we can also approximate $[\check{A}, \hat{A}] \approx N$, etc.

Finally, it is useful to consider the coherent state,

$$|z\rangle = \sum_{n=0}^{\infty} z^n |n\rangle = e^{z\hat{A}} |0\rangle. \quad (2.25)$$

This allows one to express the generating function for the distribution over macrostates as $\langle z|\psi\rangle$. Note that setting $z = 1$ recovers the sum state, i.e., $|1\rangle = |\text{sum}\rangle$.

2.2.3 Hamiltonian operator

In what follows we assume that each system of interest is contained within a volume V . For a gas of monomers, the relevant Hamiltonian is $\mathbb{H} = -\mu\bar{A}$, where μ denotes a bare chemical potential. We use the term “bare” to emphasize that μ determines the excitation probability for each independent mode A_i , whereas the concentration of monomers depends on μ , N , and V . The generating function for the equilibrium probability distribution over macrostates is found by:

$$\langle z|\psi\rangle = \frac{1}{Z} \langle 0| e^{z\check{A}} e^{\beta\mu\bar{A}} e^{\hat{A}} |0\rangle \quad (2.26)$$

$$= \frac{1}{Z} \langle 0| e^{z\check{A}} e^{\lambda\hat{A}} |0\rangle \quad (\text{defining } \lambda = e^{\beta\mu}) \quad (2.27)$$

$$= \frac{1}{Z} \prod_i \langle 0|_i e^{z\check{A}_i} e^{\lambda\hat{A}_i} |0\rangle_i \quad (2.28)$$

$$= \frac{1}{Z} \prod_i \langle 0|_i (1 + z\check{A}_i)(1 + \lambda\hat{A}_i) |0\rangle_i \quad (2.29)$$

$$= \frac{1}{Z} (1 + z\lambda)^N \quad (2.30)$$

$$= \left(\frac{1 + z\lambda}{1 + \lambda} \right)^N. \quad (2.31)$$

In the first step we used the fact that $f(\bar{A})g(\hat{A})|0\rangle = g(f(1)\hat{A})|0\rangle$ for any functions f and g . The resulting quantity λ is the per-mode fugacity corresponding to chemical potential μ . In the second step we used the fact that operators for different modes commute. In the third step we used the nilpotency of $\hat{A}_i$ to truncate the expansions of each exponential. Finally, we used the normalization requirement $\langle 1|\psi\rangle = 1$ to determine the partition function $Z = (1 + \lambda)^N$. The result is the generating function for the binomial distribution corresponding to N modes with a per-mode excitation probability of $\lambda/(1 + \lambda)$. From this generating function we find that the expected concentration of monomers is

$$\frac{\langle \bar{A} \rangle}{V} = \frac{1}{V} \frac{d}{dz} \langle z|\psi\rangle \Big|_{z=1} = \frac{1}{V} \frac{N\lambda}{1 + \lambda} = \frac{\lambda'}{1 + V\lambda'/N}, \quad (2.32)$$

where $\lambda' = \frac{N}{V}\lambda$. Keeping $\langle \bar{A} \rangle / V$ constant while taking $N \rightarrow \infty$ requires holding λ' approximately constant and thus rescaling $\lambda \sim \frac{V}{N}$. In this limit we get

$$\langle z|\psi \rangle = e^{(z-1)V\lambda'} \Rightarrow |\psi \rangle = e^{-V\lambda'} |V\lambda' \rangle. \quad (2.33)$$

The corresponding partition function is $Z = e^{V\lambda'}$. Note that $\langle z|\psi \rangle$ is the generating function for a Poisson distribution with mean $V\lambda'$. We thus see that

$$\mu' = k_B T \log \lambda' = \mu + k_B T \log \frac{N}{V} \quad (2.34)$$

is the effective chemical potential, i.e., the chemical potential appropriately renormalized to account for the N modes available for excitation in volume V . It is therefore μ' , not μ , that reflects the physically measurable chemical potential.

Note that the appearance of the unitful quantity N/V within a logarithm may appear unusual, but it makes sense in the context of our formalism. At a fixed bare chemical potential μ , the concentration of particles $[A]$ will be proportional to the density of Fock space modes, N/V . The effective chemical potential μ' , which by definition is equal to $k_B T \log[A]$ up to an additive constant, must therefore receive an additive contribution of $k_B T \log N/V$ to its value. Looked at in a mathematically identical but conceptually different way, if we keep μ fixed and simply change the units in which V is measured, $[A]$ will scale with the quantity N/V (as it should), and so μ' should additively shift as $k_B T \log N/V$.

2.3 Homodimer in equilibrium

2.3.1 Composite operators

Multi-particle complexes are represented as products of mode operators for three kinds of fields: particle fields, interaction fields, and site fields. For example, we define the creation operator for a dimer of two A particles by the composite operator

$$\hat{D}_{ij} = \hat{I}_{ij} \hat{a}_i \hat{a}_j \hat{A}_i \hat{A}_j, \quad (2.35)$$

which is the product of mode operators for a particle field A , an interaction field I , and a site field a . More specifically, $\hat{A}_i$ and $\hat{A}_j$ create the two component particles, $\hat{I}_{ij}$ registers that these two particles interact with one another, and $\hat{a}_i$ and $\hat{a}_j$ respectively indicate that the A_i and A_j particles are each participating in an interaction and are therefore not free to interact with additional particles. Note that the index of the dimer creation operator is the pair of monomer indices, (i, j) . Since the monomer is symmetric, we assume that $\hat{I}_{ij} = \hat{I}_{ji}$, and so $\hat{D}_{ij} = \hat{D}_{ji}$. Note also that $\hat{D}_{ii} = 0$

because of the nilpotency of $\hat{A}_i$ and $\hat{a}_i$. The number of internal states for the dimer is therefore $N_D = \binom{N}{2} \approx N^2/2$. The dimer annihilation, presence, and absence operators are defined in terms of the creation operator in the same manner as for a single particle:

$$\check{D}_{ij} = \hat{D}_{ij}^\dagger = \check{I}_{ij} \check{a}_i \check{a}_j \check{A}_i \check{A}_j, \quad (2.36)$$

$$\bar{D}_{ij} = \hat{D}_{ij} \check{D}_{ij} = \bar{I}_{ij} \bar{a}_i \bar{a}_j \bar{A}_i \bar{A}_j, \quad (2.37)$$

$$\tilde{D}_{ij} = \check{D}_{ij} \hat{D}_{ij} = \tilde{I}_{ij} \tilde{a}_i \tilde{a}_j \tilde{A}_i \tilde{A}_j. \quad (2.38)$$

The field operator $\hat{D}$ is given by $\hat{D} = \frac{1}{2} \sum_{i,j} \hat{D}_{ij}$, where the factor $1/2$ compensates for double counting in the sum. The field operators $\check{D}$, $\bar{D}$, and $\tilde{D}$ are defined similarly.

The homodimer system also comprises free monomers. We represent these by a separate composite field M defined by the mode operator $\hat{M}_i = \hat{A}_i \tilde{a}_i$. The corresponding number of internal states is $N_M = N$, and the three related mode operators are $\check{M}_i = \check{A}_i \tilde{a}_i$, $\bar{M}_i = \bar{A}_i \tilde{a}_i$, and $\tilde{M}_i = \tilde{A}_i \tilde{a}_i$. The corresponding field operators are defined as sums over i . Note the inclusion of $\tilde{a}_i$ in $\hat{M}_i$ ensures that $\bar{M}$ does not count A particles that are components of dimers, $\check{M}$ does not annihilate such particles (which would leave dangling I and a modes), etc.

The macrostate comprising m monomers and d dimers is given by

$$|m, d\rangle = \frac{\hat{M}^m \hat{D}^d}{m! d!} |0\rangle. \quad (2.39)$$

Using $[\tilde{a}_i, \hat{a}_j] = -\delta_{ij} \hat{a}_i$ and $\hat{A}_i^2 = 0$, one can readily verify that $\hat{M}$ and $\hat{D}$ commute. The corresponding coherent state is therefore

$$|z_M, z_D\rangle = \sum_{m=0}^{\infty} \sum_{d=0}^{\infty} z_M^m z_D^d |m, d\rangle = e^{z_M \hat{M} + z_D \hat{D}} |0\rangle. \quad (2.40)$$

2.3.2 Sectoring by species

Now consider a Hamiltonian in which each A particle has chemical potential μ and each interaction has Gibbs free energy ϵ :

$$\mathbb{H} = -\mu \sum_i \bar{A}_i + \epsilon \frac{1}{2} \sum_{i,j} \bar{I}_{ij}. \quad (2.41)$$

To compute the equilibrium state of the system, we re-express the Hamiltonian as a sum of terms that operate separately on monomers and dimers. Using the identity

$1 = \tilde{a}_i + \bar{a}_i$, we split the Hamiltonian into two parts, $\mathbb{H} = \mathbb{H}_M + \mathbb{H}_D$, where

$$\mathbb{H}_M = -\mu \sum_i \bar{A}_i \tilde{a}_i, \quad \mathbb{H}_D = -\mu \sum_i \bar{A}_i \bar{a}_i + \frac{\epsilon}{2} \sum_{i,j} \bar{I}_{ij}. \quad (2.42)$$

These operators satisfy the commutation relations

$$\begin{aligned} [\mathbb{H}_M, \hat{M}] &= -\mu_M \hat{M}, & [\mathbb{H}_D, \hat{D}] &= -\mu_D \hat{D}, \\ [\mathbb{H}_M, \hat{D}] &= 0, & [\mathbb{H}_D, \hat{M}] &= 0, \end{aligned} \quad (2.43)$$

where $\mu_M = \mu$ and $\mu_D = 2\mu - \epsilon$ are the bare monomer and dimer chemical potentials. Next we compute the generating function:

$$\begin{aligned} \langle z_M, z_D | \psi \rangle &= Z^{-1} \langle 0 | e^{z_M \check{M} + z_D \check{D}} e^{-\beta(\mathbb{H}_M + \mathbb{H}_D)} e^{\hat{M} + \hat{D}} | 0 \rangle \\ &= Z^{-1} \langle 0 | e^{z_M \check{M} + z_D \check{D}} e^{\lambda_M \hat{M} + \lambda_D \hat{D}} | 0 \rangle \\ &\approx Z^{-1} \langle 0 | e^{z_M \check{M}} e^{\lambda_M \hat{M}} e^{z_D \check{D}} e^{\lambda_D \hat{D}} | 0 \rangle. \end{aligned} \quad (2.44)$$

In the first step we used Eqn. 2.43 and defined the fugacities $\lambda_M = e^{\beta\mu_M}$ and $\lambda_D = e^{\beta\mu_D}$. The second step follows from the approximation (see Appendix 2.B)

$$[\hat{M}, \check{D}] = \frac{1}{2} \sum_{i,j} (\hat{M}_i + \hat{M}_j) \check{D}_{ij} \approx 0. \quad (2.45)$$

This commutator is not exactly zero because annihilating a dimer frees up A modes that can be used to create two monomers. But by way of comparison,

$$[\hat{M}, \check{M}] = N_M - 2\bar{M} \quad \text{and} \quad [\hat{D}, \check{D}] = N_D - 2\bar{D} \quad (2.46)$$

have terms that scale as N and N^2 , respectively. The effect of the commutator $[\hat{M}, \check{D}]$ on a physical state is consequently negligible in the large N limit. This reflects the number of modes available to create a monomer not being limiting in the physically meaningful regime.

Next we insert a copy of the identity operator,

$$\mathbb{1} = \sum_{m,d} \frac{|m, d\rangle \langle m, d|}{m!d!}, \quad (2.47)$$

into the right-hand side of Eqn. 2.44 and observe that only the $|0\rangle \langle 0|$ term survives. Consequently,

$$\langle z_M, z_D | \psi \rangle \approx Z^{-1} \langle 0 | e^{z_M \check{M}} e^{\lambda_M \hat{M}} | 0 \rangle \langle 0 | e^{z_D \check{D}} e^{\lambda_D \hat{D}} | 0 \rangle$$

$$= Z^{-1} \langle z_M | \lambda_M \rangle \langle z_D | \lambda_D \rangle. \quad (2.48)$$

Setting $\langle 1, 1 | \psi \rangle = 1$ we find that $Z \approx Z_M Z_D$, where Z_M and Z_D are the respective partition functions for the monomer and dimer species. We thus obtain

$$|\psi\rangle \approx |\psi\rangle_M \otimes |\psi\rangle_D, \quad (2.49)$$

where $|\psi\rangle_M$ describes a monomer-only system, $|\psi\rangle_D$ describes a dimer-only system, and both have the same Poisson form as in Eqn. 2.33.

There are two important caveats to the result in Eqn. 2.49. First, the sectors for distinct species are only independent in the $N \rightarrow \infty$ limit. For example, the right-hand side of Eqn. 2.49 has nonzero $|m\rangle \otimes |d\rangle$ terms for all values of $m \leq N$ and $d \leq N/2$, whereas each $|m, d\rangle$ term on the left-hand side is nonzero only if $m + 2d \leq N$. Second, this sectoring result holds only in equilibrium systems; indeed, the populations of particles in different sectors will generally be coupled out of equilibrium.

Finally we discuss the scaling behavior of the system with N and V . As in the previous section, requiring the concentration of monomers $\langle \bar{M} \rangle / V$ to be constant as $N \rightarrow \infty$ reveals an effective monomer chemical potential of $\mu'_M = \mu_M + k_B T \log \frac{N_M}{V}$. Similarly, requiring the concentration of homodimers $\langle \bar{D} \rangle / V$ to be constant as $N \rightarrow \infty$ reveals an effective dimer chemical potential of $\mu'_D = \mu_D + k_B T \log \frac{N_D}{V}$. These relations are realized by renormalizing the parameters of the Hamiltonian so that

$$\mu' = \mu + k_B T \log \frac{N}{V} \quad \text{and} \quad \epsilon' = \epsilon - k_B T \log V \quad (2.50)$$

are held constant. In terms of these quantities, the effective dimer chemical potential is $\mu'_D = 2\mu' - \epsilon' - k_B T \log 2$, where the logarithmic term accounts for the symmetry of the molecule. This system is therefore exactly renormalizable. We thus see that, to maintain a fixed concentration of dimers, the bare interaction energy ϵ must become weaker as system volume increases. This makes sense: if V increases while monomer concentration stays fixed, the number of monomers available to bond to a given monomer will increase in proportion to V . To keep the probability of the given monomer forming a dimer constant, the bare interaction energy ϵ must weaken as V increases so that $e^{-\beta\epsilon} \propto V^{-1}$. This implies that $e^{-\beta\epsilon'} = V e^{-\beta\epsilon}$ will be fixed.

2.3.3 Gallery operators

Consider more generally a system that realizes K distinct species of complex. Let $\hat{G}_k$ denote the creation operator for complex k and assume that

$$[\bar{G}_k, \hat{G}_{k'}] = \delta_{kk'} \hat{G}_k. \quad (2.51)$$

We refer to the vector $\vec{\mathbb{G}} = (\hat{G}_1, \dots, \hat{G}_K)^\top$ as the “gallery,” as it exhibits creation operators for all possible complexes. The gallery allows us to define the coherent state

$$|\vec{z}\rangle = \exp \left\{ \vec{z}^\top \vec{\mathbb{G}} \right\} |0\rangle, \quad (2.52)$$

where $\vec{z} = (z_1, \dots, z_K)^\top$ is a vector of scalars. As in the monomer and homodimer systems, the generating function for a system $|\psi\rangle$ is $\langle \vec{z} | \psi \rangle$, and the sum state is

$$|\text{sum}\rangle = e^{\sum_k \hat{G}_k} |0\rangle = |\vec{1}\rangle. \quad (2.53)$$

The macrostates of the system are given by

$$|n_1, \dots, n_K\rangle = \left[\prod_{k=1}^K \frac{\hat{G}_k^{n_k}}{n_k!} \right] |0\rangle, \quad (2.54)$$

and the effects of the four field operators on macrostates are

$$\begin{aligned} \hat{G}_k |n_1, \dots, n_K\rangle &= (n_k + 1) |n_1, \dots, n_k + 1, \dots, n_K\rangle, \\ \bar{G}_k |n_1, \dots, n_K\rangle &= n_k |n_1, \dots, n_K\rangle, \\ \check{G}_k |n_1, \dots, n_K\rangle &\approx N_k |n_1, \dots, n_k - 1, \dots, n_K\rangle, \\ \tilde{G}_k |n_1, \dots, n_K\rangle &\approx N_k |n_1, \dots, n_K\rangle. \end{aligned} \quad (2.55)$$

where N_k is the number of internal states for species k .

Because different complexes can share internal components, the Hamiltonian can be defined in a rule-based manner as in Eqn. 2.41 instead of on a species-by-species basis. As we will see in later sections, such rule-based definitions can require far fewer than K terms. If there are no energetic interactions between separate complexes, the Hamiltonian can then be equivalently expressed as

$$\mathbb{H} \simeq - \sum_k \mu_k \bar{G}_k. \quad (2.56)$$

where μ_k is the bare chemical potential for species k . The generating function for the equilibrium state then factorizes, i.e.

$$|\psi\rangle = \bigotimes_k |\psi\rangle_k, \text{ where } |\psi\rangle_k = e^{-V\lambda'_k} |V\lambda'_k\rangle, \quad (2.57)$$

and where $\lambda'_k = e^{\beta\mu'_k}$ and $\mu'_k = \mu_k + k_B T \log \frac{N_k}{V}$ are the effective fugacity and chemical potential of species k . We note that it may or may not be possible to renormalize the parameters of the Hamiltonian so that all the effective chemical potentials are independent of V . For example, this is possible in the homodimer system, but not in the homopolymer system discussed in the next section.

2.3.4 Factory operators

Hamiltonians of the form in Eqn. 2.56 describe systems of non-interacting particles and might understandably be viewed as trivial. They become less trivial, however, in systems comprising large (or infinite) numbers of distinct complexes, each complex having a chemical potential that is a function of the parameters used to define the Hamiltonian. In such systems, merely enumerating different species of complex and determining their chemical potentials can be nontrivial. It is therefore natural to instead define the set of possible complexes implicitly by specifying the rules for their construction, and to use these rules to then compute the different species of complex and their associated chemical potentials. We now show how our formalism enables this.

In the case of the homodimer, the sum state can be expressed as

$$|\text{sum}\rangle = e^{\mathbb{F}_2} e^{\mathbb{F}_1} |0\rangle, \quad (2.58)$$

where

$$\mathbb{F}_1 = \sum_i \hat{A}_i \tilde{a}_i, \quad \mathbb{F}_2 = \frac{1}{2} \sum_{i,j} \hat{I}_{ij} \hat{a}_i \hat{a}_j \bar{A}_i \bar{A}_j. \quad (2.59)$$

Here, $\mathbb{F}_1 = \hat{M}$ creates free monomers, while $\mathbb{F}_2$ joins two monomers into a dimer. Specifically, $\mathbb{F}_2$ tests for the presence of two particles, A_i and A_j , and if these already exist it joins them into a dimer D_{ij} . Note that neither A particle can be part of an existing dimer due to the excitation of site fields a_i and a_j . For example,

$$\mathbb{F}_2 \frac{\mathbb{F}_1^2}{2} |0\rangle = \frac{1}{4} \sum_{i,j,k,l} \hat{I}_{ij} \hat{a}_i \hat{a}_j \bar{A}_i \bar{A}_j \hat{A}_k \tilde{a}_i \hat{A}_l \tilde{a}_l |0\rangle \quad (2.60)$$

$$= \frac{1}{4} \sum_{i,j,k,l} \hat{I}_{ij} \hat{a}_i \hat{a}_j \hat{A}_i \hat{A}_j (\delta_{ik} \delta_{jl} + \delta_{il} \delta_{jk}) |0\rangle \quad (2.61)$$

$$= |0, 1\rangle, \quad (2.62)$$

where the first step uses the identities $\hat{a}_i \tilde{a}_i = \hat{a}_i$ and $[\bar{A}_i, \hat{A}_j] = \delta_{ij} \hat{A}_i$. We will soon show more generally that

$$\frac{\mathbb{F}_2^p}{p!} \frac{\mathbb{F}_1^q}{q!} |0\rangle = \begin{cases} |q - 2p, p\rangle & \text{if } q \geq 2p, \\ 0 & \text{otherwise.} \end{cases} \quad (2.63)$$

Summing this over all p and q establishes the $|\text{sum}\rangle$ state in Eqn. 2.58.

The sum of states for complexes generated in any system can thus be specified by a vector of operators $\vec{\mathbb{F}} = (\mathbb{F}_1, \dots, \mathbb{F}_L)^\top$ via

$$|\text{sum}\rangle = e^{\mathbb{F}_L} \dots e^{\mathbb{F}_1} |0\rangle. \quad (2.64)$$

We call this vector the “factory.” We emphasize that the order of the operators within the factory is important, as these operators, unlike gallery operators, do not generally commute.

Using the factory instead of the gallery to define the set of possible complexes in a system can have an important advantage: the factory often comprises far fewer operators than the gallery. This is not the case for the homodimer system, but it is so for the homopolymer system presented in Section 2.4.

There is a disadvantage, however, to defining a system using the factory: one loses access to the generating function. One can, of course define a coherent state analogous to $|\vec{z}\rangle$ via

$$|\vec{x}\rangle = e^{x_L \mathbb{F}_L} \dots e^{x_1 \mathbb{F}_1} |0\rangle, \quad (2.65)$$

where $\vec{x} = (x_1, \dots, x_L)^\top$. It is questionable, however, how useful the corresponding generating function $\langle \vec{x} | \psi \rangle$ is for analysis. As we will see, each term in the expansion of Eqn. 2.65 can yield multiple distinct mixtures of complexes. One thus generally cannot read off the macrostate distribution $P(\vec{n})$ from the expansion of $\langle \vec{x} | \psi \rangle$.

2.3.5 Wick’s theorem

The algebraic manipulations needed to show Eqn. 2.63 become unwieldy as p and q become large. Wick’s theorem, a foundational result in quantum field theory, makes these calculations significantly more straightforward by providing a systematic procedure for reordering operators in a multi-operator product.

To see how Wick’s theorem can be applied to our formalism, define the compound operators $\bar{A}_i^a = \hat{a}_i \bar{A}_i$ and $\hat{A}_i^a = \hat{a}_i \hat{A}_i$. Ignoring the interaction field I for the moment, each term in the expansion of the left-hand side of Eqn. 2.63 has the form

$$\bar{A}_{i_1}^a \dots \bar{A}_{i_{2q}}^a \hat{M}_{j_1} \dots \hat{M}_{j_p} |0\rangle. \quad (2.66)$$

Note that all compound presence operators appear to the left of all creation operators. We refer to this as “productive ordering.” Given an operator product $X_1 X_2 \cdots X_n$, we denote its productive ordering by $\mathcal{P}(X_1 X_2 \cdots X_n)$. Each term in the Taylor expansion of the factory representation is productive ordered because the instructions for assembling each complex are applied after the instructions for creating its components. In contrast, each term in the expansion of the gallery representation in Eqn. 2.39 has the form

$$\hat{M}_{i_1} \cdots \hat{A}_{i_m} \hat{M}_{j_1}^a \cdots \hat{A}_{j_{2d}}^a |0\rangle. \quad (2.67)$$

The key difference from Eqn. 2.66 is that this term contains only creation operators, all of which commute.

Every term of the form in Eqn. 2.66 is in fact equal to a sum of terms having the form in Eqn. 2.67 with $m = q - 2p$ and $d = p$. To transform the former to the latter, we iteratively apply the exchange rule

$$\bar{A}_i^a \hat{M}_j = \hat{M}_j \bar{A}_i^a + \delta_{ij} \hat{A}_i^a \quad (2.68)$$

until no $\bar{A}_i^a$ operators appear to the left of any $\hat{M}_j$ operators. Each application of the exchange rule adds another term to the expansion. The result is a sum of operator products such that all $\bar{A}_i^a$ in each product appear to the right of all $\hat{M}_i$ and $\hat{A}_i^a$. Such products are said to be “normally ordered.” More generally, an operator product is normally ordered if all presence operators appear to the right of all creation operators. The normally ordered form of an operator product $X_1 X_2 \cdots X_n$ is denoted by $\mathcal{N}(X_1 X_2 \cdots X_n)$. Normally ordered products are useful because any such products containing presence operators vanish when applied to the vacuum state.

Wick’s theorem provides an equality between productive ordered and normally ordered operator products. To state Wick’s theorem, we define a “contraction” between two operators X_i and X_j to be

$$\overline{X_i X_j} = \mathcal{P}(X_i X_j) - \mathcal{N}(X_i X_j). \quad (2.69)$$

The contraction of two specific operators within a larger product $X_1 X_2 \cdots X_n$ removes these operators from the product and replaces them with their contraction, i.e.,

$$X_1 X_2 \cdots \overline{X_i \cdots X_j} \cdots X_n = X_1 X_2 \cdots X_n (\overline{X_i X_j}). \quad (2.70)$$

A key assumption of Wick’s theorem is that the contraction of any two operators in a product is “central,” i.e., it commutes with all other operators in the product.

For the homodimer algebra, the only contractions needed to transform Eqn. 2.66 to Eqn. 2.67 are of the form

$$\overline{\hat{A}_i^a} \hat{M}_j = \bar{A}_i^a \hat{M}_j - \hat{M}_j \bar{A}_i^a = [\bar{A}_i^a, \hat{M}_j] = \delta_{ij} \hat{A}_j^a. \quad (2.71)$$

These contractions are indeed central, i.e., $[\hat{A}_j^a, \hat{M}_j] = [\hat{A}_j^a, \bar{A}_i^a] = 0$.

Applied to our context, Wick's theorem states that any productive ordered operator product is equal to the sum of all possible normally ordered contractions:

$$\begin{aligned} \mathcal{P}(X_1 X_2 X_3 X_4 \cdots) &= \mathcal{N}(X_1 X_2 X_3 X_4 \cdots) + \\ &\quad \mathcal{N}(\overbrace{X_1 X_2} X_3 X_4 \cdots) + \mathcal{N}(X_1 \overbrace{X_2 X_3} X_4 \cdots) + \mathcal{N}(X_1 X_2 \overbrace{X_3 X_4} \cdots) + \cdots \\ &\quad \mathcal{N}(\overbrace{X_1 X_2} \overbrace{X_3 X_4} \cdots) + \mathcal{N}(\overbrace{X_1 X_2 X_3} \overbrace{X_4} \cdots) + \mathcal{N}(X_1 \overbrace{X_2 X_3 X_4} \cdots) + \cdots \\ &\quad \vdots \\ &= \sum_{\text{all contractions } C} \mathcal{N}(C(X_1 X_2 \dots X_n)) \end{aligned} \quad (2.72)$$

$$(2.73)$$

For example, applying Wick's theorem to the right-hand side of Eqn. 2.60 gives

$$\begin{aligned} \bar{A}_i^a \bar{A}_j^a \hat{M}_k \hat{M}_k &= \mathcal{N}(\bar{A}_i^a \bar{A}_j^a \hat{M}_k \hat{M}_k) + \\ &\quad \mathcal{N}(\overbrace{\bar{A}_i^a \bar{A}_j^a} \hat{M}_k \hat{M}_k) + \mathcal{N}(\bar{A}_i^a \overbrace{\bar{A}_j^a} \hat{M}_k \hat{M}_k) + \mathcal{N}(\bar{A}_i^a \bar{A}_j^a \overbrace{\hat{M}_k \hat{M}_k}) + \\ &\quad \mathcal{N}(\overbrace{\bar{A}_i^a \bar{A}_j^a} \overbrace{\hat{M}_k \hat{M}_k}) + \mathcal{N}(\overbrace{\bar{A}_i^a \bar{A}_j^a} \hat{M}_k \hat{M}_k) \\ &= \hat{M}_k \hat{M}_k \bar{A}_i^a \bar{A}_j^a + \\ &\quad \delta_{ik} \hat{A}_i^a \hat{M}_k \bar{A}_j^a + \delta_{il} \hat{A}_i^a \hat{M}_k \bar{A}_j^a + \delta_{jk} \hat{A}_j^a \hat{M}_k \bar{A}_i^a + \delta_{jl} \hat{A}_j^a \hat{M}_k \bar{A}_i^a + \\ &\quad \delta_{ik} \delta_{jl} \hat{A}_i^a \hat{A}_j^a + \delta_{il} \delta_{jk} \hat{A}_i^a \hat{A}_j^a. \end{aligned} \quad (2.74)$$

$$(2.75)$$

When applied to the vacuum state, only the last two terms in Eqn. 2.75 survive, thus yielding the expression in Eqn. 2.61,

$$\bar{A}_i^a \bar{A}_j^a \hat{M}_k \hat{M}_k |0\rangle = (\delta_{ik} \delta_{jl} + \delta_{il} \delta_{jk}) \hat{A}_k^a \hat{A}_l^a |0\rangle. \quad (2.76)$$

More generally, Wick's Theorem allows us to transform terms in the expansion of the factory expression for the sum vector (Eqn. 2.64) into a sum of terms in the expansion of the gallery expression for the sum vector (Eqn. 2.53).

2.3.6 Formal diagrams

We now introduce diagrammatic methods that aid in computations involving Fock space operators. Each diagram indicates an operator product or sums of such

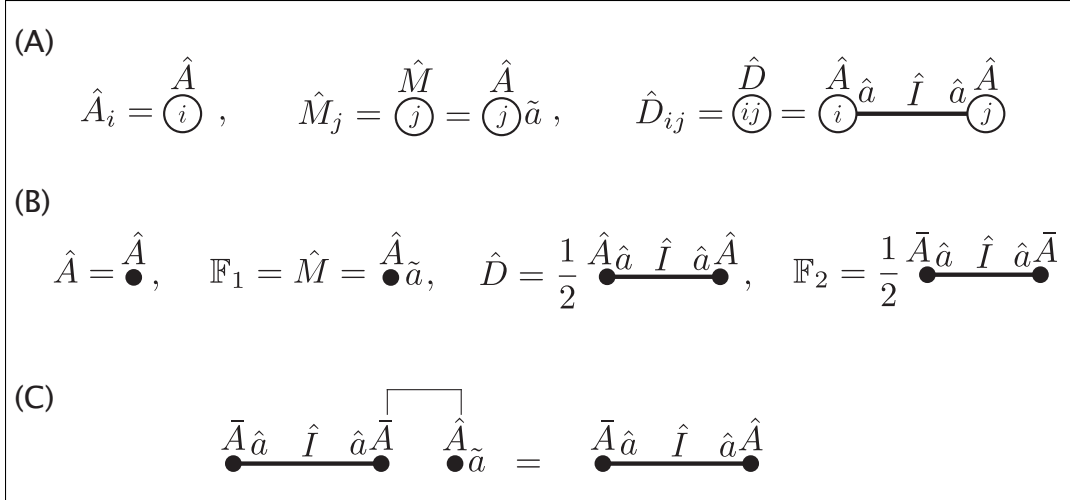


Figure 2.2: Diagrammatic notation for operator products and sums thereof over internal indices. (A) Examples of simple and compound mode operators. (B) Examples of simple and compound field operators. (C) Wick contractions relevant to the homodimer system.

products over internal states. Fig. 2.2 shows several examples. The indices of mode operators are written as index names inside open dots [Fig. 2.2(A)]. Mode operators are indicated by the decorated operator name written next to their respective dots. Multiple operator names written next to the same dot indicate that those operators share the same index. Modes that have two indices are written next to lines that connect the two dots representing these indices. A closed dot indicates summation over the corresponding index. Field operators are thus distinguished from mode operators through the use of closed rather than open dots. Symmetry factors are also kept explicit [Fig. 2.2(B)].

This diagrammatic notation is helpful in computations involving Wick contractions; we demonstrate this by deriving Eqn. 2.63. The effect of each Wick contraction is illustrated in Fig. 2.2(C): contracting an $\bar{A}^a$ operator (part of $\mathbb{F}_2$) with $\mathbb{F}_1 = \hat{M}$ eliminates the $\mathbb{F}_1$ and replaces the $\bar{A}^a$ in $\mathbb{F}_2$ with an $\hat{A}^a$. To avoid unnecessary notation going forward, we represent this operation using the same diagrams but showing only the decorations on the A operators. Now consider the left-hand side of Eqn. 2.63 with $p = 2$ and $q = 5$ [Fig. 2.3(A), line 1]. Because the two $\mathbb{F}_2$ operators are applied after the five $\mathbb{F}_1$ operators, this product is productive ordered [Fig. 2.3(A), line 2]. Next we use Wick's theorem to convert this to a sum of normally ordered products [Fig. 2.3(A), lines 3-8]. We also evaluate the combinatorial coefficients that arise due to distinct contractions producing topologically identical products.

(A)

$$\begin{aligned}
\frac{\mathbb{F}_2^2}{2!} \frac{\mathbb{F}_1^5}{5!} &= \frac{1}{2!} \left(\frac{1}{2} \bar{\bullet} \text{---} \bar{\bullet} \right)^2 \times \frac{1}{5!} (\hat{\bullet})^5 \\
&= \frac{1}{2!} \frac{1}{5!} \frac{1}{2^2} \mathcal{P}(\bar{\bullet} \text{---} \bar{\bullet} \bar{\bullet} \text{---} \bar{\bullet} \hat{\bullet} \hat{\bullet} \hat{\bullet} \hat{\bullet} \hat{\bullet}) \\
&= \frac{1}{2!} \frac{1}{5!} \frac{1}{2^2} \left[\begin{aligned}
&1 \times \mathcal{N}(\bar{\bullet} \text{---} \bar{\bullet} \bar{\bullet} \text{---} \bar{\bullet} \hat{\bullet} \hat{\bullet} \hat{\bullet} \hat{\bullet} \hat{\bullet}) \quad (i) \\
&+ 5 \cdot 4 \times \mathcal{N}(\bar{\bullet} \text{---} \bar{\bullet} \bar{\bullet} \text{---} \bar{\bullet} \hat{\bullet} \hat{\bullet} \hat{\bullet} \hat{\bullet} \hat{\bullet}) \quad (ii) \\
&+ \frac{(5 \cdot 4)(4 \cdot 1)}{2!} \times \mathcal{N}(\bar{\bullet} \text{---} \bar{\bullet} \bar{\bullet} \text{---} \bar{\bullet} \hat{\bullet} \hat{\bullet} \hat{\bullet} \hat{\bullet} \hat{\bullet}) \quad (iii) \\
&+ \frac{(5 \cdot 4)(4 \cdot 2)}{2!} \times \mathcal{N}(\bar{\bullet} \text{---} \bar{\bullet} \bar{\bullet} \text{---} \bar{\bullet} \hat{\bullet} \hat{\bullet} \hat{\bullet} \hat{\bullet} \hat{\bullet}) \quad (iv) \\
&+ \frac{(5 \cdot 4)(4 \cdot 3)(3 \cdot 2)}{3!} \times \mathcal{N}(\bar{\bullet} \text{---} \bar{\bullet} \bar{\bullet} \text{---} \bar{\bullet} \hat{\bullet} \hat{\bullet} \hat{\bullet} \hat{\bullet} \hat{\bullet}) \quad (v) \\
&+ \frac{(5 \cdot 4)(4 \cdot 3)(3 \cdot 2)(2 \cdot 1)}{4!} \times \mathcal{N}(\bar{\bullet} \text{---} \bar{\bullet} \bar{\bullet} \text{---} \bar{\bullet} \hat{\bullet} \hat{\bullet} \hat{\bullet} \hat{\bullet} \hat{\bullet}) \quad (vi)
\end{aligned} \right] \\
&= \stackrel{(i)}{\frac{1}{5!} (\hat{\bullet})^5} \times \frac{1}{2!} \left(\frac{1}{2} \bar{\bullet} \text{---} \bar{\bullet} \right)^2 + \stackrel{(ii)}{\frac{1}{4!} (\hat{\bullet})^4} \times \hat{\bullet} \text{---} \bar{\bullet} \times \frac{1}{2} \bar{\bullet} \text{---} \bar{\bullet} \\
&+ \stackrel{(iii)}{\frac{1}{3!} (\hat{\bullet})^3} \times \frac{1}{2} \hat{\bullet} \text{---} \hat{\bullet} \times \frac{1}{2} \bar{\bullet} \text{---} \bar{\bullet} + \stackrel{(iv)}{\frac{1}{3!} (\hat{\bullet})^3} \times \frac{1}{2!} (\hat{\bullet} \text{---} \bar{\bullet})^2 \\
&+ \stackrel{(v)}{\frac{1}{2!} (\hat{\bullet})^2} \times \frac{1}{2} \hat{\bullet} \text{---} \hat{\bullet} \times \hat{\bullet} \text{---} \bar{\bullet} + \stackrel{(vi)}{\hat{\bullet}} \times \frac{1}{2!} \left(\frac{1}{2} \hat{\bullet} \text{---} \hat{\bullet} \right)^2
\end{aligned}$$

(B)

$$\frac{\mathbb{F}_2^2}{2!} \frac{\mathbb{F}_1^5}{5!} |0\rangle = \hat{\bullet} \times \frac{1}{2!} \left(\frac{1}{2} \hat{\bullet} \text{---} \hat{\bullet} \right)^2 |0\rangle = \hat{M} \frac{\hat{D}^2}{2!} |0\rangle$$

Figure 2.3: Diagrams facilitate algebraic calculations. (A) Evaluation of the $p = 2$, $q = 5$ term of Eqn. 2.63 in terms of normally ordered operator products. (B) Result of the computation in panel (A) applied to the vacuum state.

Consider, for example, the coefficient for term (iii). For the first contraction, there are five choices of $\hat{M}$ and four choices of $\bar{A}^a$. For the second contraction, there are four remaining choices of $\hat{A}$ but only one possible choice for $\bar{A}^a$ – that which is linked with the first $\bar{A}^a$ through an I -field. Since interchanging the order in which the contractions are performed does not change the result, we divide the result by two. This yields a combinatorial coefficient of $(5 \cdot 4) \times (4 \cdot 1)/2!$. The combinatorial coefficients for the other terms follow similarly.

Each normally ordered term in lines 3-8 yields one of the resulting operator products shown in lines 9-11, as indicated. We leave it to the reader to check that the combinatorial coefficients computed in lines 3-8 do in fact match those shown in lines 9-11, which are as expected based on symmetry considerations. Note in particular that all terms except term (vi) contain $\bar{A}^a$ operators. Consequently, only term (vi) survives when applying this result to the vacuum state [Fig. 2.3(B)].

We are now in a position to evaluate the left-hand side of Eqn. 2.63 for general values of p and q (Fig. 2.4). If $q < 2p$, then $\mathbb{F}_1^q$ does not supply enough $\hat{M}$ operators to contract all the $\bar{A}^a$ operators supplied by $\mathbb{F}_2^p$. The expression therefore vanishes. If $q \geq 2p$, however, there are $q!/(q-2p)!$ ways to contract all the $\bar{A}^a$ with all the $\hat{M}$, thereby leaving p copies of $\hat{D}$ and q copies of $\hat{M}$. The resulting combinatorial factor replaces the $1/q!$ with $1/(q-2p)!$, thus providing the factors needed to correct the redundancies in $\hat{D}^p$ and $\hat{M}^q$. This completes the derivation of Eqn. 2.63, and thus proof of the factory/gallery equivalence for the homodimer system.

2.4 Homopolymer in equilibrium

We now turn here to a system that is far simpler to define in a rule-based manner than in a species-based manner. Consider a factory comprising two operators:

$$\mathbb{F}_1 = \sum_i \hat{A}_i \tilde{a}_i \tilde{b}_i, \quad \mathbb{F}_2 = \sum_{i,j} \bar{A}_i \bar{A}_j \hat{a}_i \hat{b}_j \hat{J}_{ij}. \quad (2.77)$$

This is similar to the homodimer factory, but $\mathbb{F}_2$ differs in that it forms an asymmetric (rather than symmetric) bond between two A particles. Specifically, the summand in $\mathbb{F}_2$ occupies a site a_i on the A_i monomer, a site b_j on the A_j monomer, and forms a bond J_{ij} between them. $\mathbb{F}_2$ is not multiplied by a symmetry factor because $J_{ij} \neq J_{ji}$. These factory operators are represented graphically in Fig. 2.5(A). We define a rule-based Hamiltonian for this system in the familiar way:

$$\mathbb{H} = -\mu \sum_i \bar{A}_i + \epsilon \sum_{i,j} \bar{J}_{ij}, \quad (2.78)$$

$$\begin{aligned}
\frac{\mathbb{F}_2^p \mathbb{F}_1^q}{p! q!} |0\rangle &= \frac{1}{p!} \left(\frac{1}{2} \overline{\bullet} \text{---} \overline{\bullet} \right)^p \times \frac{1}{q!} (\hat{\bullet})^q |0\rangle \\
&= \frac{1}{p!} \frac{1}{q!} \frac{1}{2^p} \times \mathcal{P} \left(\underbrace{\overline{\bullet} \cdots \overline{\bullet}}_p \underbrace{\hat{\bullet} \cdots \hat{\bullet}}_q \right) |0\rangle \\
\left(\text{assuming } q \geq 2p \right) &= \frac{1}{p!} \frac{1}{q!} \frac{1}{2^p} \times \frac{(q \cdot [2p])([q-1] \cdot [2p-1]) \cdots ([q-2p+1] \cdot 1)}{[2p]!} \\
&\quad \times \mathcal{N} \left(\underbrace{\overline{\bullet} \cdots \overline{\bullet}}_p \underbrace{\hat{\bullet} \cdots \hat{\bullet}}_{2p} \underbrace{\hat{\bullet} \cdots \hat{\bullet}}_{q-2p} \right) |0\rangle \\
&= \frac{1}{p!} \frac{1}{q!} \frac{1}{2^q} \frac{(q!/[q-2p]!) \cdot [2p]!}{[2p]!} \times (\hat{\bullet})^{q-2p} \times \left(\frac{1}{2} \overline{\bullet} \text{---} \overline{\bullet} \right)^p |0\rangle \\
&= \frac{1}{(q-2p)!} (\hat{\bullet})^{q-2p} \times \frac{1}{p!} \left(\frac{1}{2} \overline{\bullet} \text{---} \overline{\bullet} \right)^p |0\rangle \\
&= \frac{\hat{M}^{q-2p}}{(q-2p)!} \frac{\hat{D}^p}{p!} |0\rangle \\
&= |q-2p, p\rangle
\end{aligned}$$

Figure 2.4: Diagrammatic proof of the factory/gallery equivalence for the homodimer system.

as represented in Fig. 2.5(B). The resulting gallery [Fig. 2.5(C)] is far more complex than that of the homodimer: it comprises creation operators for polymer chains and polymer rings of all lengths. Here, x -chains and x -rings are created by the operators

$$\hat{C}_x = \sum_{i_1, \dots, i_x} \hat{A}_{i_1} \cdots \hat{A}_{i_x} \hat{a}_{i_1} \cdots \hat{a}_{i_{x-1}} \quad (2.79)$$

$$\begin{aligned}
&\quad \times \hat{b}_{i_2} \cdots \hat{b}_{i_x} \hat{J}_{i_1 i_2} \cdots \hat{J}_{i_{x-1} i_x}, \\
\hat{R}_x &= \frac{1}{x} \sum_{i_1, \dots, i_x} \hat{A}_{i_1} \cdots \hat{A}_{i_x} \hat{a}_{i_1} \cdots \hat{a}_{i_x} \\
&\quad \times \hat{b}_{i_1} \cdots \hat{b}_{i_x} \hat{J}_{i_1 i_2} \cdots \hat{J}_{i_{x-1} i_x} \hat{J}_{i_x i_1}.
\end{aligned} \quad (2.80)$$

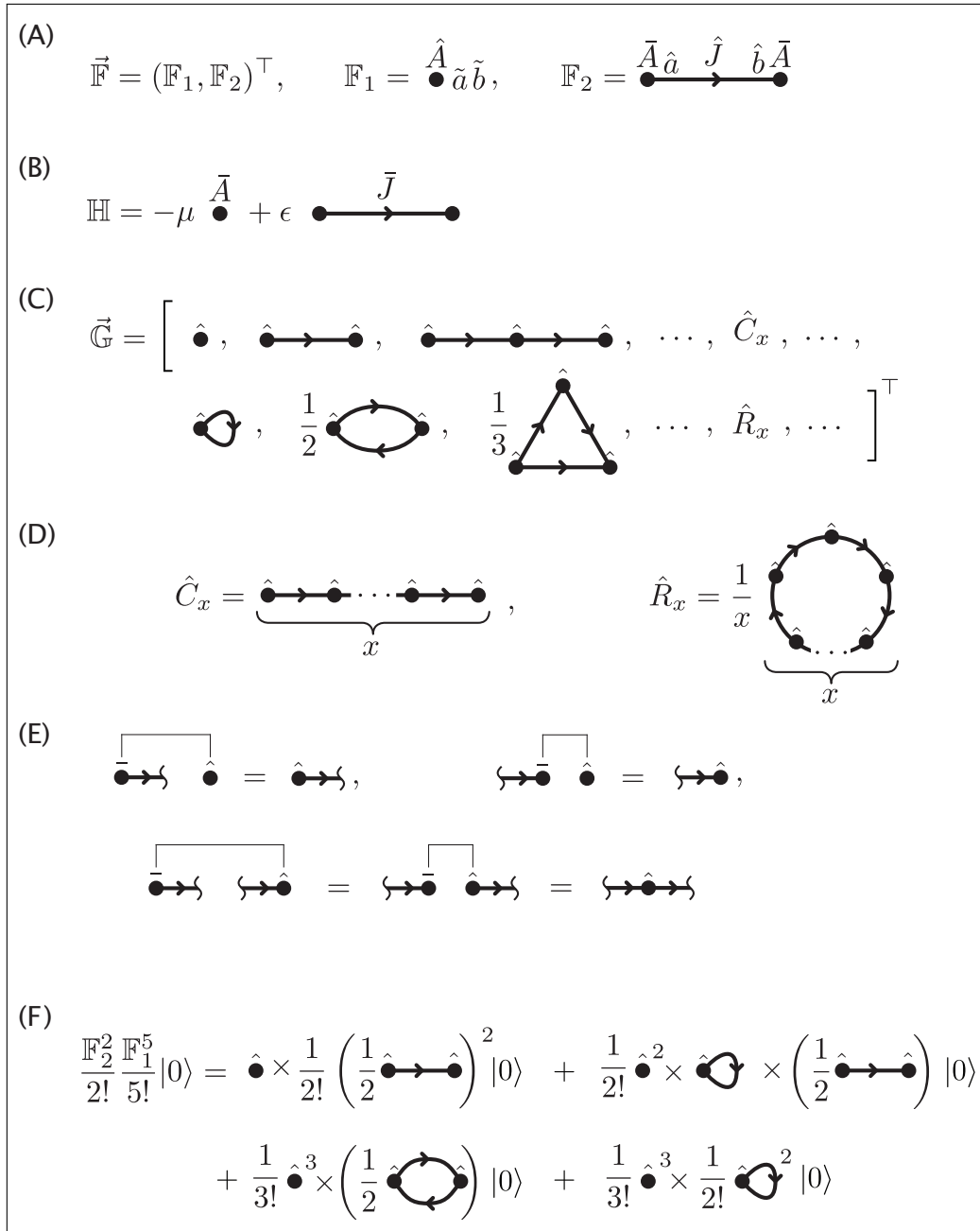


Figure 2.5: Diagrammatic specification of the homopolymer system. (A) The factory. (B) The Hamiltonian. (C) The resulting gallery. (D) The $\hat{C}_x$ and $\hat{R}_x$ comprising the gallery, and (E) the four contraction rules for the system. For conciseness, panels (C-E) hide all operator names except for the decorators on the A operators. (F) An example algebraic calculation carried out using the commutation rules in panel (E).

These operators are more clearly expressed in diagrammatic notation [Fig. 2.5(D)]. Note the factor of $1/x$ in Eqn. 2.80; this is needed to compensate for redundancy in the sum over internal indices that results from x -rings having rotational symmetry.

The equivalent species-based Hamiltonian has an infinite number of terms, each with its own bare chemical potential:

$$\mathbb{H} \simeq - \sum_{x=1}^{\infty} (\mu_{C_x} \bar{C}_x + \mu_{R_x} \bar{R}_x), \quad (2.81)$$

where $\mu_{C_x} = x\mu - (x-1)\epsilon$ and $\mu_{R_x} = x\mu - x\epsilon$ are the bare chemical potentials for x -chains and x -rings. The corresponding number of complex-specific microstates are $N_{C_x} = N^x$ and $N_{R_x} = \frac{1}{x}N^x$. Putting these together, we obtain the effective chemical potentials of each species:

$$\begin{aligned} \mu'_{C_x} &= x(\mu - \epsilon) + \epsilon + k_B T \log \frac{N^x}{V}, \\ \mu'_{R_x} &= x(\mu - \epsilon) + k_B T \log \frac{N^x}{xV}. \end{aligned} \quad (2.82)$$

Unlike in the homodimer system, it is not possible to renormalize μ and ϵ so that all μ'_{C_x} and μ'_{R_x} are independent of volume. Keeping μ'_{C_1} independent of N and V requires fixing the value of $\mu' = \mu + k_B T \log \frac{N}{V}$ as in the monomer and homodimer systems. Keeping all other μ'_{C_x} independent of N and V then requires fixing $\epsilon' = \epsilon - k_B T \log V$. The effective chemical potentials for all species thus become

$$\begin{aligned} \mu'_{C_x} &= x(\mu' - \epsilon') + \epsilon', \\ \mu'_{R_x} &= x(\mu' - \epsilon') - k_B T \log x - k_B T \log V. \end{aligned} \quad (2.83)$$

Constraining the concentrations of all C_x species independent of V therefore requires that the concentration of all R_x species scale as V^{-1} . We note, however, that the converse is not possible, i.e., one cannot choose a definition for μ' and ϵ' so that the concentrations of all ring species are independent of V .

We therefore conclude that, for the homopolymer system to behave sensibly in the $V \rightarrow \infty$ limit, the concentrations of all chain polymers must remain fixed whereas the concentrations of all ring polymers must vanish. This makes sense: as V increases, the number of free ends with which the free end of an x -chain can interact increases in proportion to V . To preserve the concentrations of all x -chains, $e^{-\beta\epsilon}$ must scale as V^{-1} . The probability of one free end of an x -chain interacting with the other free

end of the same polymer will thus scale as V^{-1} , and the concentration of all ring polymers will also scale as V^{-1} .

Given Eqn. 2.83, defining $\eta = e^{\beta(\mu' - \epsilon')}$ and computing

$$\log Z = \sum_{x=0}^{\infty} e^{\beta\mu C_x} + \sum_{x=0}^{\infty} e^{\beta\mu R_x}, \quad (2.84)$$

we find that the log partition function density of the system is, for $0 < \eta < 1$,

$$\frac{\log Z}{V} = \frac{e^{\beta\epsilon'}}{1 - \eta} - \frac{\log(1 - \eta)}{V}. \quad (2.85)$$

The left and right terms of the resulting expression are the respective contributions from chains and rings. The V^{-1} scaling of the second term reflects the vanishing of ring species as $V \rightarrow \infty$. The remaining chain contribution diverges as $\eta \rightarrow 1$ from below. In this limit, defining $\delta = 1 - \eta$, the concentration of A particles diverges as

$$\frac{\langle \bar{A} \rangle}{V} = \frac{1}{\beta} \frac{\partial}{\partial \mu'} \frac{\log Z}{V} = \frac{1}{\beta} \frac{\partial}{\partial \eta} \left(\frac{\log Z}{V} \right) \frac{\partial \eta}{\partial \mu'} = \eta \frac{\partial}{\partial \eta} \left(\frac{\log Z}{V} \right) \approx \frac{e^{\beta\epsilon'}}{\delta^2}. \quad (2.86)$$

Since the concentration of each chain species C_x is given by $e^{\beta\mu' C_x} = e^{\beta\epsilon'} \eta^x$, the distribution of chain lengths is distributed exponentially with decay rate $\log \eta$, and flattens out as $\eta \rightarrow 1$ from below. The mean and variance of these chain lengths diverge as

$$\langle x \rangle = \frac{\eta}{1 - \eta} \approx \frac{1}{\delta}, \quad \text{var}(x) = \frac{\eta}{(1 - \eta)^2} \approx \frac{1}{\delta^2}. \quad (2.87)$$

To show the equivalence of the factory and gallery representations of the homopolymer system, we again invoke Wick's theorem. In this case, however, the allowable contractions are more complex. Paralleling the analysis for the homodimer, we define the compound operators

$$\hat{M}_i = \hat{A}_i \tilde{a}_i \tilde{b}_i, \quad \hat{A}_i^a = \hat{A}_i \hat{a}_i \tilde{b}_i, \quad (2.88)$$

$$\hat{A}_i^b = \hat{A}_i \tilde{a}_i \hat{b}_i, \quad \hat{A}_i^{ab} = \hat{A}_i \hat{a}_i \hat{b}_i, \quad (2.89)$$

$$\bar{A}_i^a = \bar{A}_i \hat{a}_i \tilde{b}_i, \quad \bar{A}_i^b = \bar{A}_i \tilde{a}_i \hat{b}_i. \quad (2.90)$$

These six operators obey the contraction rules

$$\overbrace{\bar{A}_i^a \hat{M}_j} = \delta_{ij} \hat{A}_i^a, \quad \overbrace{\bar{A}_i^b \hat{M}_j} = \delta_{ij} \hat{A}_i^b \quad (2.91)$$

$$\overbrace{\bar{A}_i^a \hat{A}_j^b} = \overbrace{\bar{A}_i^b \hat{A}_j^a} = \delta_{ij} \hat{A}_i^{ab}. \quad (2.92)$$

These rules are shown diagrammatically in Fig. 2.5(E). The reader may notice that the first two contraction products are not central, and thus violate an assumption of Wick's theorem. We find, however, that Wick's theorem still holds if we allow contraction products to participate in additional contractions. Fully contracted operator products therefore have all $\bar{A}_i^a$ and $\bar{A}_i^b$ operators participating in one contraction each, whereas each $\hat{A}$ operator may participate in zero, one, or two contractions.

We can use the above contraction rules to derive the complexes generated from any given term in $|\text{sum}\rangle = e^{\mathbb{F}_2} e^{\mathbb{F}_1} |0\rangle$. Fig. 2.5(F) shows the result for one such term. A generalized version of this computation is used in Appendix 2.C to prove the factory/gallery equivalence for the homopolymer system.

2.5 Nonequilibrium systems

2.5.1 Species-based formalism

We now turn to the problem of determining the macrostate master equation (Eqn. 2.10) given a rule-based microstate master equation (Eqn. 2.4). We start, however, by investigating how to specify and analyze a more standard species-based microstate master equation.

Species-based master equations are built from individual reactions, each of which annihilates a specified set of complexes and creates a new set in their place. Suppose there are H distinct species-specific reactions. The transition operator in Eqn. 2.4 will then have the form

$$\mathbb{W} = \sum_{h=1}^H r_h \left(\mathbb{Q}_h - \dot{\mathbb{Q}}_h \right), \quad (2.93)$$

where r_h is the rate at which reaction h occurs, $\mathbb{Q}_h$ is a “reaction operator” that effects this reaction when applied to a macrostate $|\vec{n}\rangle$, and $\dot{\mathbb{Q}}$ is a corresponding “depletion operator.” Each reaction operator has the form

$$\mathbb{Q} = \prod_{k=1}^K \frac{\hat{G}_k^{p_k}}{p_k!} \frac{\check{G}_k^{q_k}}{q_k!}, \quad (2.94)$$

where $\vec{q} = (q_1, \dots, q_K)$ is an abundance vector that describes the reactants and $\vec{p} = (p_1, \dots, p_K)$ is a vector that describes the products. For the sake of simplicity we assume that $\vec{p}$ and $\vec{q}$ do not overlap (i.e., $p_k = 0$ and/or $q_k = 0$ for every k). Applying the conjugate of this operator to the macrostate, one finds that

$$\mathbb{Q}^\dagger |\vec{n}\rangle = N_{\vec{p}} \Omega(\vec{n}, \vec{q}, \vec{p}) |\vec{n} + \vec{q} - \vec{p}\rangle, \quad (2.95)$$

where

$$\Omega(\vec{n}, \vec{q}, \vec{p}) = \prod_k \binom{n_k + q_k - p_k}{q_k} \mathbb{1}(n_k \geq p_k), \quad (2.96)$$

with $\mathbb{1}$ denoting the indicator function. Ω is a coefficient that depends on $\vec{n}$, $\vec{p}$, and $\vec{q}$, but not otherwise on the details of the reaction, and

$$N_{\vec{p}} = \prod_k \binom{N_k}{p_k} \approx \prod_k \frac{N_k^{p_k}}{p_k!} \quad (2.97)$$

is the number of distinct product microstates created when $\mathbb{Q}$ is applied to a single reactant microstate.

In our formalism, the depletion operator $\mathring{\mathbb{O}}$ corresponding to any reaction operator $\mathbb{O}$ is given in terms of the microstate-specific components via

$$\mathbb{O} = \sum_{I, \mathcal{J}} o_{I\mathcal{J}} |\mathcal{J}\rangle \langle I| \Rightarrow \mathring{\mathbb{O}} = \sum_{I, \mathcal{J}} o_{I\mathcal{J}} |\mathcal{I}\rangle \langle I|, \quad (2.98)$$

where I and $\mathcal{J}$ index all microstates of the system and $o_{I\mathcal{J}}$ is the rate at which $|I\rangle$ is transformed into $|\mathcal{J}\rangle$. Note that, by this definition, all depletion operators are self-conjugate. In Appendix 2.D we show that Eqn. 2.98, together with the assumption of non-overlapping $\vec{p}$ and $\vec{q}$, leads to a depletion operator corresponding to $\mathbb{Q}$ of

$$\mathring{\mathbb{Q}} = \prod_k \binom{\tilde{G}_k}{p_k} \binom{\bar{G}_k}{q_k}. \quad (2.99)$$

Applying $\mathring{\mathbb{Q}}^\dagger = \mathring{\mathbb{Q}}$ to the macrostate then gives

$$\mathring{\mathbb{Q}} |\vec{n}\rangle = N_{\vec{p}} \Omega(\vec{n}, \vec{q}, \vec{q}) |\vec{n}\rangle. \quad (2.100)$$

Adding back the subscript h on $\vec{p}$ and $\vec{q}$ and using Eqn. 2.11, we obtain an expression for the flux projector:

$$\begin{aligned} |J(\vec{n})\rangle &= \sum_{h=1}^H r_h N_{\vec{p}_h} \{ \Omega(\vec{n}, \vec{q}_h, \vec{p}_h) |\vec{n} + \vec{q}_h - \vec{p}_h\rangle \\ &\quad - \Omega(\vec{n}, \vec{q}_h, \vec{q}_h) |\vec{n}\rangle \}. \end{aligned} \quad (2.101)$$

The difficulty with this species-based formulation is that, in systems that admit multi-particle complexes, the form of the transition operator in Eqn. 2.93 does not reflect the underlying simplicity of the system. Rather, the rates r_h and reaction

operators $\mathbb{Q}_h$ are derived quantities that follow from an (often much smaller) set of informally stated rules. Furthermore, even manually specifying the right-hand side of Eqn. 2.93 can be tricky: a small number of rules can lead to a very large (or even infinite) number of reactions H , and both r_h and $\mathbb{Q}_h$ can depend in nontrivial ways on the elemental parameters that govern those rules. We now show how our formalism addresses this problem by enabling the rule-based definition of $\mathbb{W}$.

2.5.2 Rule-based formalism

We specify the transition operator in a rule-based manner as follows. Suppose we have a system defined by L reaction rules. For each rule l , we specify a rate r_l and a “reaction rule operator” $\mathbb{R}_l$. The transition operator is then given by

$$\mathbb{W} = \sum_{l=1}^L r_l \left(\mathbb{R}_l - \hat{\mathbb{R}}_l \right), \quad (2.102)$$

where $\hat{\mathbb{R}}_l$ is the depletion operator corresponding to $\mathbb{R}_l$.

Suppose a rule operator $\mathbb{R}$ is able to drive M different species-specific reactions. For each reaction m , let $\vec{q}_m$ denote the number of reactant species and $\vec{p}_m$ the number of product species. We find that

$$\mathbb{R}^\dagger |\vec{n}\rangle = \sum_{m=1}^M \sigma_m \Omega(\vec{n}, \vec{q}_m, \vec{p}_m) |\vec{n} + \vec{q}_m - \vec{p}_m\rangle, \quad (2.103)$$

where σ_m is the number of distinct product microstates that can result from each reactant microstate in an m -type reaction. The depletion operator $\hat{\mathbb{R}}$ follows from the rule operator $\mathbb{R}$ using Eqn. 2.98. Applying $\hat{\mathbb{R}}^\dagger = \hat{\mathbb{R}}$ to $|\vec{n}\rangle$,

$$\hat{\mathbb{R}} |\vec{n}\rangle = \sum_{m=1}^M N_{\vec{p}_m} \Omega(\vec{n}, \vec{q}_m, \vec{q}_m) |\vec{n}\rangle. \quad (2.104)$$

Adding back the l indices, we obtain the flux projector

$$\begin{aligned} |J(\vec{n})\rangle = \sum_{l=1}^L \sum_{m=1}^{M_l} r_l \sigma_{lm} \{ & \Omega(\vec{n}, \vec{q}_{lm}, \vec{p}_{lm}) |\vec{n} + \vec{q}_{lm} - \vec{p}_{lm}\rangle \\ & - \Omega(\vec{n}, \vec{q}_{lm}, \vec{q}_{lm}) |\vec{n}\rangle \}. \end{aligned} \quad (2.105)$$

2.5.3 Macroscopic master equation for the homopolymer

We now use our rule-based formalism to derive the macrostate master equation for the homopolymer system. Out of equilibrium, the dynamics of this system can be

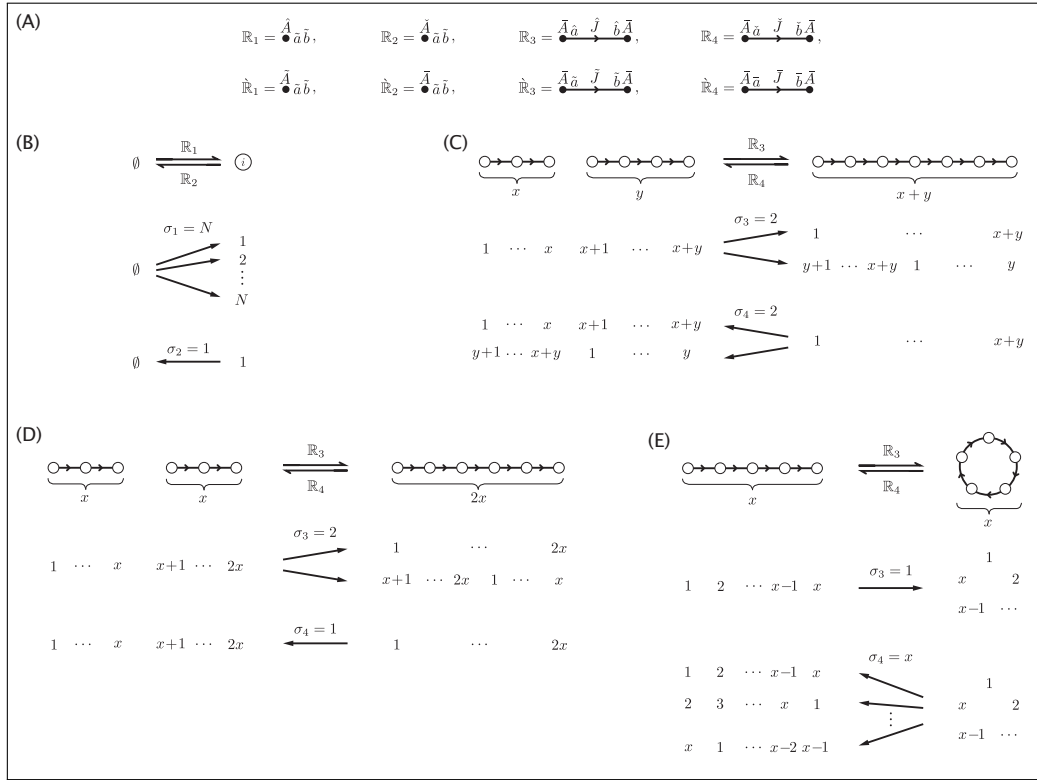


Figure 2.6: The eight types of species-specific reactions that occur in the nonequilibrium homopolymer system. (A) Diagrammatic representations of the four reaction rule operators and their corresponding depletion operators. (B) $\mathbb{R}_1$ maps the vacuum state to N different monomer microstates, while $\mathbb{R}_2$ maps each monomer microstate to a single vacuum state. (C-E) The three types of species-specific reactions effected by $\mathbb{R}_3$ and $\mathbb{R}_4$. (C) $\mathbb{R}_3$ can link an x -chain and y -chain together in two different ways, while $\mathbb{R}_4$ can split an $(x + y)$ -chain into an x -chain and y -chain in two different ways. (D) $\mathbb{R}_3$ can link two x -chains together in two different ways, while $\mathbb{R}_4$ can split a $2x$ -chain into two x -chains in only one way. (E) $\mathbb{R}_3$ can circularize an x -chain in only one way, while $\mathbb{R}_4$ can linearize an x -ring in x different ways. Nodes are shown as open dots in panels (B-E) to indicate specific internal states; the numbers below each node indicate example values for the internal index i of each component particle.

defined by $L = 4$ reaction rule operators:

$$\begin{aligned}\mathbb{R}_1 &= \sum_i \hat{A}_i \tilde{a}_i \tilde{b}_i, & \mathbb{R}_3 &= \sum_{i,j} \hat{J}_{ij} \hat{a}_i \hat{b}_j \bar{A}_i \bar{A}_j, \\ \mathbb{R}_2 &= \sum_i \check{A}_i \tilde{a}_i \tilde{b}_i, & \mathbb{R}_4 &= \sum_{i,j} \check{J}_{ij} \check{a}_i \check{b}_j \bar{A}_i \bar{A}_j.\end{aligned}\quad (2.106)$$

Here, $\mathbb{R}_1$ creates a monomeric particle, $\mathbb{R}_2 = \mathbb{R}_1^\dagger$ destroys a monomeric particle, $\mathbb{R}_3$ creates an interaction between two particles, and $\mathbb{R}_4 = \mathbb{R}_3^\dagger$ destroys an interaction. Note that these four operators are given by the factory operators of Section 2.4 and their conjugates. The corresponding depletion operators are

$$\begin{aligned}\hat{\mathbb{R}}_1 &= \sum_i \tilde{A}_i \tilde{a}_i \tilde{b}_i, & \hat{\mathbb{R}}_3 &= \sum_{i,j} \tilde{J}_{ij} \tilde{a}_i \tilde{b}_j \bar{A}_i \bar{A}_j, \\ \hat{\mathbb{R}}_2 &= \sum_i \bar{A}_i \tilde{a}_i \tilde{b}_i, & \hat{\mathbb{R}}_4 &= \sum_{i,j} \bar{J}_{ij} \bar{a}_i \bar{b}_j \bar{A}_i \bar{A}_j.\end{aligned}\quad (2.107)$$

Diagrammatic representations of these rule operators and depletion operators are shown in Fig. 2.6(A).

In Section 2.4 we showed that the macrostates of the homopolymer system are given by

$$|\vec{c}, \vec{r}\rangle = |c_1, r_1, c_2, r_2, \dots\rangle = \prod_{x=1}^{\infty} \frac{\hat{C}_x^{c_x}}{c_x!} \frac{\hat{R}_x^{r_x}}{r_x!} |0\rangle, \quad (2.108)$$

where c_x and r_x respectively indicate the number of chains and rings of length x . To compute the macroscopic master equation, we compute the flux projector

$$|J(\vec{c}, \vec{r})\rangle = \sum_{l=1}^4 r_l (\mathbb{R}_l^\dagger - \hat{\mathbb{R}}_l) |\vec{c}, \vec{r}\rangle. \quad (2.109)$$

We now evaluate Eqn. 2.109 term-by-term. To ease notation, we show in the macrostate only the elements of $\vec{c}$ and $\vec{r}$ that change upon application of each operator and denote the unchanged elements by "...". The $l = 1$ term corresponds to monomer creation. As illustrated in Fig. 2.6(B), $\mathbb{R}_1$ maps a single microstate (corresponding to no reactants) to $\sigma_1 \approx N$ microstates (corresponding to all possible monomer states). By Eqn. 2.103 and Eqn. 2.104,

$$(\mathbb{R}_1^\dagger - \hat{\mathbb{R}}_1) |\vec{c}, \vec{r}\rangle \approx N\{|c_1-1, \dots\rangle - |\dots\rangle\}. \quad (2.110)$$

$\mathbb{R}_2$ maps a single monomeric particle microstate to $\sigma_2 = 1$ microstate (i.e., no products), and so

$$(\mathbb{R}_2^\dagger - \hat{\mathbb{R}}_2) |\vec{c}, \vec{r}\rangle = (c_1+1) |c_1+1, \dots\rangle - c_1 |\dots\rangle. \quad (2.111)$$

The effects of $\mathbb{R}_3$ and $\mathbb{R}_4$ are more complex. $\mathbb{R}_3$ can effect three different types of reactions depending on the reactants. First, $\mathbb{R}_3$ can join together an x -chain and y -chain ($x < y$) to get an $(x + y)$ -chain; this can be done in $\sigma_3 = 2$ different ways [Fig. 2.6(C)]. Second, $\mathbb{R}_3$ can join together two x -chains to get a $2x$ -chain; this can be done in $\sigma_3 = 2$ ways [Fig. 2.6(D)]. Third, $\mathbb{R}_3$ can join together the ends of an x -chain to get an x -ring; this can be done in only $\sigma_3 = 1$ way [Fig. 2.6(E)]. We therefore find that

$$\begin{aligned} (\mathbb{R}_3^\dagger - \mathbb{R}_3) |\vec{c}, \vec{r}\rangle = & \sum_{x < y} 2\{(c_x + 1)(c_y + 1) |c_x + 1, c_y + 1, c_{x+y} - 1, \dots\rangle - c_x c_y |\dots\rangle\} \\ & + \sum_x 2\left\{\frac{(c_x + 2)(c_x + 1)}{2} |c_x + 2, c_{2x} - 1, \dots\rangle - \frac{c_x(c_x - 1)}{2} |\dots\rangle\right\} \\ & + \sum_x \{(c_x + 1) |c_x + 1, r_x - 1, \dots\rangle - c_x |\dots\rangle\}. \end{aligned} \quad (2.112)$$

Similarly, the inverse operator $\mathbb{R}_4$ can separate an $(x + y)$ -chain into an x -chain and y -chain ($x < y$) in $\sigma_4 = 2$ ways, can separate a $2x$ -chain into two x -chains in only $\sigma_4 = 1$ way, and can cut an x -ring to get an x -chain in $\sigma_4 = x$ different ways. Consequently,

$$\begin{aligned} (\mathbb{R}_4^\dagger - \mathbb{R}_4) |\vec{c}, \vec{r}\rangle = & \sum_{x < y} 2\{(c_{x+y} + 1) |c_x - 1, c_y - 1, c_{x+y} + 1, \dots\rangle - c_{x+y} |\dots\rangle\} \\ & + \sum_x \{(c_{2x} + 1) |c_x - 2, c_{2x} + 1, \dots\rangle - c_{2x} |\dots\rangle\} \\ & + \sum_x x\{(r_x + 1) |c_x - 1, r_x + 1, \dots\rangle - r_x |\dots\rangle\}. \end{aligned} \quad (2.113)$$

The depletion terms in these expressions can be simplified as follows

$$\sum_{x < y} 2c_x c_y + \sum_x c_x(c_x - 1) + \sum_x c_x = n_{\text{chain}}^2, \quad \sum_{x < y} 2c_{x+y} + \sum_x c_{2x} + \sum_x x r_x = n_{\text{link}}, \quad (2.114)$$

where $n_{\text{chain}} = \sum_x c_x$ is the total number of chains and $n_{\text{link}} = \sum_x [(x - 1)c_x + x r_x]$ is the number of links among all chains and rings.

Evaluating the inner product $\langle J(\vec{n}) | \psi(t) \rangle$, we thus obtain the macroscopic master equation for the homopolymer:

$$\begin{aligned} \frac{d}{dt} P(\dots) = & N r_1 P(c_1 - 1, \dots) + r_2 (c_1 + 1) P(c_1 + 1, \dots) \\ & + r_3 \left[\sum_{x < y} 2(c_x + 1)(c_y + 1) P(c_x + 1, c_y + 1, c_{x+y} - 1, \dots) \right. \end{aligned}$$

$$\begin{aligned}
& + \sum_x (c_x + 2)(c_x + 1)P(c_x + 2, c_{2x} - 1, \dots) + \sum_x (c_x + 1)P(c_x + 1, r_x - 1, \dots) \Big] \\
& + r_4 \left[\sum_{x < y} 2(c_{x+y} + 1)P(c_x - 1, c_y - 1, c_{x+y} + 1, \dots) + \sum_x (c_{2x} + 1)P(c_x - 2, c_{2x} + 1, \dots) \right. \\
& \quad \left. + \sum_x x(r_x + 1)P(c_x - 1, r_x + 1, \dots) \right] \\
& - [Nr_1 + r_2c_1 + r_3n_{\text{chain}}^2 + r_4n_{\text{link}}] P(\dots). \tag{2.115}
\end{aligned}$$

To verify this result we focus on the depletion term, i.e., the coefficient of $P(\vec{c}, \vec{r})$. The overall monomer creation rate is Nr_1 . This makes sense, as r_1 is the per-mode rate of excitation of the A field. r_1 must therefore scale as V/N . The r_2 term reflects our assumption that only A particles engaging in no interactions are able to be annihilated; it does not scale with V or N . The r_3 term reflects the fact that new interactions form between the a -end of one chain and the b -end of either another chain or the same chain (r_3 scales as V^{-1}). The total number of available reactants in the system is therefore n_{chain}^2 . The r_4 term reflects the assumption that any link can be annihilated regardless of the complex in which it occurs (r_4 does not scale with V or N).

2.6 Simulations

2.6.1 Numerical solutions

Our formalism enables the numerical solution of the microstate master equation – at least when N is sufficiently small. One first defines a set of modes $\mathcal{M}$, as well as a set of mode-specific rules $\mathcal{R} = \{(\mathbb{R}_l, r_l)\}$, where l indexes the qualitatively different rules as in Eqn. 2.102, and i indexes the internal states of the particles that each rule acts upon (so that $\mathbb{R}_l = \sum_i \mathbb{R}_{li}$). The transition operator is then computed using

$$\mathbb{W} = \sum_{l=1}^L r_l \sum_i (\mathbb{R}_{li} - \dot{\mathbb{R}}_{li}). \tag{2.116}$$

Given an initial state vector $|\psi(0)\rangle$, the state at time t is computed using

$$|\psi(t)\rangle = \exp\{t\mathbb{W}\} |\psi(0)\rangle. \tag{2.117}$$

Fig. 2.7(A)-(C) shows the results of this computation for three example systems: a monomer system ($N = 20$), a homodimer system ($N = 4$), and a homopolymer system ($N = 3$). All simulations evolve at infinitesimal time steps in arbitrary units over a time frame that is sufficient for observing the system in steady state.

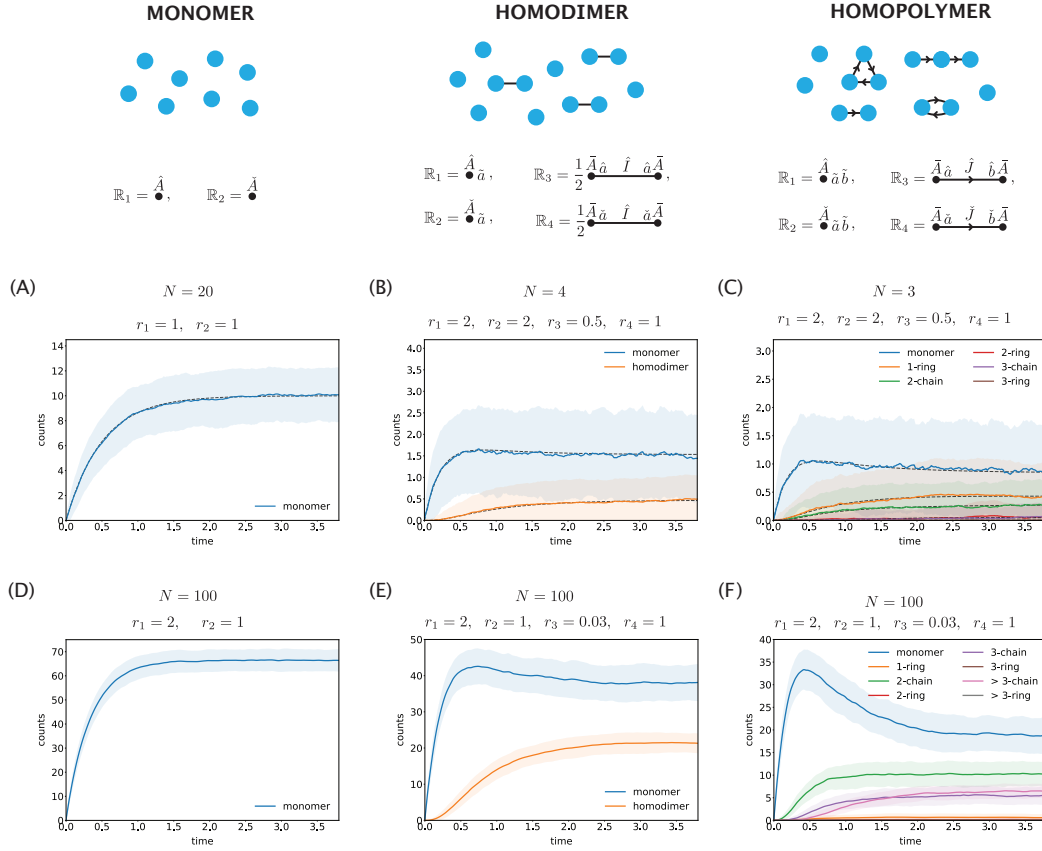


Figure 2.7: Simulations of nonequilibrium systems. (A-C) Deterministic and stochastic simulations for (A) a monomer system ($N = 20$), (B) a homodimer system ($N = 4$), and (C) a homopolymer system ($N = 3$). (D-F) Stochastic simulations for the same systems as in panels (A-C) using $N = 100$. Black dashed lines indicate mean abundances from deterministic simulations. Solid lines plot mean abundances from 500 stochastic simulations. Error bands indicate standard deviations in abundance across the stochastic simulations.

The primary limitation of this approach is that it requires computations involving very large vectors and matrices: $|\psi\rangle$ is a $2^{|\mathcal{M}|}$ -dimensional vector, $\mathbb{W}$ is a $2^{|\mathcal{M}|} \times 2^{|\mathcal{M}|}$ matrix, and $|\mathcal{M}|$ is polynomial in N , e.g., $|\mathcal{M}| = N$ for the monomer, $|\mathcal{M}| = 2N + \binom{N}{2}$ for the homodimer, and $|\mathcal{M}| = 3N + N^2$ for the homopolymer. Even using sparse matrix methods, we have found the direct evaluation of Eqn. 2.117 to be impractical for all but very small values of N . Nevertheless, these deterministic simulations provide a valuable way to check the accuracy of the stochastic simulations that we now turn to.

2.6.2 Stochastic simulations

Our formalism also enables stochastic simulations using the Gillespie algorithm [24, 25]. Importantly, these stochastic simulations can be carried out using much larger values of N . Algorithm 1 is one algorithm that does this. After explaining how the algorithm works, we illustrate its operation stepping through one iteration of the algorithm for a homodimer system. We then present computational results obtained using this algorithm and discuss the algorithm’s current limitations.

The input to Algorithm 1 consists of two strings, s_{trans} and s_{init} . The string s_{trans} specifies the set of rules and their corresponding rates, while s_{init} specifies the initial state of the system, $|s_0\rangle$. The output of the algorithm is a trajectory object $\mathcal{T}$, the downstream parsing of which provides time traces for the abundances of all single particles and complexes. Post-hoc processing of $\mathcal{T}$ then provides time traces for all species of complex. We emphasize that this algorithm only tracks the excitation states of modes. In particular, there is no need during the execution of the algorithm to enumerate the different possible species of complex or even to track which species occur. Rather, time traces for the abundances of different complexes are determined only during the post-processing of $\mathcal{T}$.

After processing its inputs, the algorithm sets time t to zero and the trajectory object $\mathcal{T}$ to be the empty set. Next, the function `Initialize` takes s_{trans} and s_{init} as inputs and outputs four sets of objects:

- $\mathcal{O}$ is the set of all mode-specific operators (i.e., creation, annihilation, presence, and absence operators) for all fields. Every $\mathbb{O} \in \mathcal{O}$ has the attributes $\mathbb{O}.mode$, $\mathbb{O}.rules$, and $\mathbb{O}.eligible$. $\mathbb{O}.mode$ is a reference to the operator’s mode $M \in \mathcal{M}$. $\mathbb{O}.rules$ is a set of references to the rules $\mathbb{R} \in \mathcal{R}$ that include $\mathbb{O}$ in their operator product. $\mathbb{O}.eligible$ is a Boolean flag indicating whether $\mathbb{O}$ can be applied to $|s_t\rangle$ without killing it.
- $\mathcal{M}$ is the set of all modes for all fields. Every $M \in \mathcal{M}$ has the attributes $M.operators$ and $M.excited$. $M.operators$ is a set of references to the four mode-specific operators (i.e., $\hat{M}$, $\check{M}$, $\bar{M}$, and $\tilde{M}$). $M.excited$ is a Boolean value indicating whether the mode is excited. If TRUE, $\hat{M}$ and $\tilde{M}$ are ineligible, while $\check{M}$ and $\bar{M}$ are eligible. If FALSE, $\hat{M}$ and $\tilde{M}$ are eligible, whereas $\check{M}$ and $\bar{M}$ are ineligible.
- $\mathcal{R}$ is the set of all mode-specific rules, each defined as a product of mode-specific operators. Every rule $\mathbb{R} \in \mathcal{R}$ has the attributes $\mathbb{R}.operators$ and

$\mathbb{R}.rate$. $\mathbb{R}.operators$ is a set of references to the mode-specific operators $\mathbb{O} \in \mathcal{O}$ that comprise $\mathbb{R}$. $\mathbb{R}.rate$ is the rate at which the rule is applied when eligible.

- C is the “state constructor,” i.e., the set of creation operators that, when applied to $|0\rangle$, yield $|s_t\rangle$, the system state at time t . The specific C returned by `Initialize` corresponds to $|s_0\rangle$, which is specified by the string s_{init} . In particular, if the user specifies that $|s_0\rangle = |0\rangle$, then $C = \{\}$.

$\mathcal{O}$, $\mathcal{M}$, and $\mathcal{R}$ are static, whereas C evolves in time. Also note that

$$M = \mathbb{O}.mode \Leftrightarrow \mathbb{O} \in M.operators, \quad (2.118)$$

$$\mathbb{R} \in \mathcal{R} \Leftrightarrow \mathbb{O} \in \mathbb{R}.operators \quad (2.119)$$

for all $\mathbb{O} \in \mathcal{O}$, $M \in \mathcal{M}$, and $\mathbb{R} \in \mathcal{R}$.

After initialization, Algorithm 1 enters a while loop. Each execution of the loop applies one rule $\mathbb{R}$ to the system state $|s_t\rangle$, then steps the system forward in time from t to $t + \Delta t$. The contents of this while loop are as follows:

- Line 5 identifies the set of eligible rules and saves them in set $\mathcal{R}^*$. This is the most computationally expensive part of Algorithm 1, since it must loop through every possible rule and test that rule for eligibility using the function `IsRuleEligible`. A rule $\mathbb{R}$ is eligible if and only if every operator in $\mathbb{R}.operators$ is eligible.
- Line 6 uses the rates of the rules in $\mathcal{R}^*$ to randomly sample a time step Δt and a corresponding rule $\mathbb{R}$ via the Gillespie algorithm, which is implemented by `GillespieStep`. Time t is then incremented by Δt .
- Lines 8-14 update the state constructor C and the excitation state of modes affected by the rule $\mathbb{R}$. To do this, a for loop is carried out over all operators $\mathbb{O} \in \mathbb{R}.operators$. If $\mathbb{O}$ is a creation operator, then $\mathbb{O}$ is added to the state constructor C . Alternatively, if $\mathbb{O}$ is an annihilation operator, then the corresponding creation operator $\mathbb{O}^\dagger$ is removed from the state constructor C . In either case, `FlipModeExcitation` is applied to the mode $M = \mathbb{O}.mode$. This flips the *excitation* attribute of M , as well as the *eligible* attribute of all operators in $M.operators$.

Algorithm 1: Gillespie simulation

Data: $s_{\text{trans}}, s_{\text{init}}, n_{\text{max}}$
Result: $\mathcal{T}$

```

/* Initialize time, trajectory, operators, modes, rules, and state
   constructor. */
1  $t \leftarrow 0$ 
2  $\mathcal{T} \leftarrow \{\}$ 
3  $O, M, \mathcal{R}, C \leftarrow \text{Initialize}(s_{\text{trans}}, s_{\text{init}})$ 
/* Carry out Gillespie algorithm. */
4 for  $n \in \{1, \dots, n_{\text{max}}\}$  do
5    $\mathcal{R}^* \leftarrow \{\mathbb{R} : \mathbb{R} \in \mathcal{R}, \text{IsRuleEligible}(\mathbb{R})\}$ 
6    $\mathbb{R}, \Delta t \leftarrow \text{GillespieStep}(\mathcal{R}^*)$ 
7    $t \leftarrow t + \Delta t$ 
8   for  $\mathbb{O} \in \mathbb{R}.operators$  do
9     if  $\mathbb{O}.type == \text{"creation"}$  then
10        $C \leftarrow C \cup \{\mathbb{O}\}$ 
11        $\text{FlipModeExcitation}(\mathbb{O}.mode)$ 
12     if  $\mathbb{O}.type == \text{"annihilation"}$  then
13        $C \leftarrow C \setminus \{\mathbb{O}^\dagger\}$ 
14        $\text{FlipModeExcitation}(\mathbb{O}.mode)$ 
15    $\mathcal{T} \leftarrow \mathcal{T} \cup (n, t, \mathbb{R}, C)$ 
/* Create operators, modes, rules, and initial state constructor. */
16 Function  $\text{Initialize}(s_{\text{trans}}, s_{\text{init}})$ :
17   ...
18   return  $O, M, \mathcal{R}, C$ 
/* Compute whether rule is eligible. */
19 Function  $\text{IsRuleEligible}(\mathbb{R})$ :
20   return  $\text{Prod}(\{\mathbb{O}.eligible : \mathbb{O} \in \mathbb{R}.operators\})$ 
/* Randomly choose rule and time step using the Gillespie algorithm. */
21 Function  $\text{GillespieStep}(\mathcal{R}^*)$ :
22    $\vec{r} \leftarrow \{\mathbb{R}.rate : \mathbb{R} \in \mathcal{R}^*\}$ 
23    $r_{\text{tot}} \leftarrow \text{Sum}(\vec{r})$ 
24    $\Delta t \leftarrow \text{SampleExponentialDist}(rate = r_{\text{tot}})$ 
25    $\mathbb{R} \leftarrow \text{Choose}(set = \mathcal{R}^*, weights = \vec{r})$ 
26   return  $\mathbb{R}, \Delta t$ 
/* Flip excitation state of mode and eligibility of corresponding
   operators. */
27 Function  $\text{FlipModeExcitation}(M)$ :
28    $M.excited \leftarrow \text{NOT } M.excited$ 
29   for  $\mathbb{O} \in M.operators$  do
30      $\mathbb{O}.eligible \leftarrow \text{NOT } \mathbb{O}.eligible$ 

```

- Finally, the loop stores a tuple reporting the updated time t , the rule $\mathbb{R}$, and the resulting state constructor C in the trajectory object $\mathcal{T}$.

We now illustrate how this algorithm works by following it through initialization and one execution of the while loop. Assume that the string s_{trans} specifies the following kinetic rules for a homodimer system:

$$\begin{aligned}\mathbb{R}_1 &= \sum_i \hat{A}_i \tilde{a}_i, & \mathbb{R}_3 &= \sum_{i < j} \bar{A}_i \bar{A}_j \hat{a}_i \hat{a}_j \hat{I}_{ij}, \\ \mathbb{R}_2 &= \sum_i \check{A}_i \tilde{a}_i, & \mathbb{R}_4 &= \sum_{i < j} \bar{A}_i \bar{A}_j \check{a}_i \check{a}_j \check{I}_{ij}.\end{aligned}\quad (2.120)$$

We further suppose that the string s_{init} specifies an initial state containing four monomers having indices 2, 3, 5, and 7, i.e.,

$$|s_0\rangle = \hat{A}_2 \hat{A}_3 \hat{A}_5 \hat{A}_7 |0\rangle. \quad (2.121)$$

Taking s_{trans} and s_{init} as input, the function `Initialize` returns the following sets of objects:

$$\begin{aligned}\mathcal{M} &= \{A_i\}_i \cup \{a_i\}_i \cup \{I_{ij}\}_{i < j}, \\ \mathcal{O} &= \{\hat{A}_i, \check{A}_i, \bar{A}_i, \tilde{A}_i\}_i \cup \{\hat{a}_i, \check{a}_i, \bar{a}_i, \tilde{a}_i\}_i \cup \\ &\quad \{\hat{I}_{ij}, \check{I}_{ij}, \bar{I}_{ij}, \tilde{I}_{ij}\}_{i < j}, \\ \mathcal{R} &= \{\hat{A}_i \tilde{a}_i\}_i \cup \{\check{A}_i \tilde{a}_i\}_i \cup \\ &\quad \{\bar{A}_i \bar{A}_j \hat{a}_i \hat{a}_j \hat{I}_{ij}\}_{i < j} \cup \{\bar{A}_i \bar{A}_j \check{a}_i \check{a}_j \check{I}_{ij}\}_{i < j}, \\ \mathcal{C} &= \{\hat{A}_2, \hat{A}_3, \hat{A}_5, \hat{A}_7\}.\end{aligned}\quad (2.122)$$

Here the indices i and j are understood to run over $1, \dots, N$, and the rates r_l corresponding to each $\mathbb{R}_l$ are kept implicit. `Initialize` also sets the values of $M.\text{excited}$ for all $M \in \mathcal{M}$, and of $\mathbb{O}.\text{eligible}$ for all $\mathbb{O} \in \mathcal{O}$. $M.\text{excited}$ is `TRUE` for modes A_2 , A_3 , A_5 , and A_7 , and is `FALSE` for all other modes (including all modes of the fields a and I). Consequently, the set $\mathcal{O}^*$ of eligible operators is

$$\begin{aligned}\mathcal{O}^* &= \{\hat{A}_i, \tilde{A}_i\}_{i \notin \{2,3,5,7\}} \cup \{\check{A}_i, \bar{A}_i\}_{i \in \{2,3,5,7\}} \cup \\ &\quad \{\hat{a}_i, \tilde{a}_i\}_i \cup \{\hat{I}_{ij}, \tilde{I}_{ij}\}_{i < j}.\end{aligned}\quad (2.123)$$

Now consider the first execution of the while loop. In line 5 of Algorithm 1, `IsRuleEligible` is evaluated on every rule $\mathbb{R} \in \mathcal{R}$. Based on the eligibility of each operator in $\mathbb{R}.\text{operators}$, the eligible rules are found to be

$$\mathcal{R}^* = \{\hat{A}_i \tilde{a}_i\}_{i \notin \{2,3,5,7\}} \cup \{\check{A}_i \tilde{a}_i\}_{i \in \{2,3,5,7\}} \cup$$

$$\{\bar{A}_i \bar{A}_j \hat{a}_i \hat{a}_j \hat{I}_{ij}\}_{i < j \in \{2,3,5,7\}}. \quad (2.124)$$

Next, `GillespieStep` chooses a random eligible rule $\mathbb{R} \in \mathcal{R}^*$ and a time increment Δt that is then added to t . Suppose

$$\mathbb{R} = \bar{A}_3 \bar{A}_7 \hat{a}_3 \hat{a}_7 \hat{I}_{37} \quad (2.125)$$

is chosen. Applying $\mathbb{R}$ to the initial state vector then yields the updated state vector,

$$|s_{\Delta t}\rangle = \mathbb{R} |s_0\rangle = \hat{A}_2 \hat{A}_3 \hat{A}_5 \hat{A}_7 \hat{a}_3 \hat{a}_7 \hat{I}_{37} |0\rangle. \quad (2.126)$$

To register this change, the state constructor is updated to

$$C = \{\hat{A}_2, \hat{A}_3, \hat{A}_5, \hat{A}_7, \hat{a}_3, \hat{a}_7, \hat{I}_{37}\}. \quad (2.127)$$

Calls to `FlipModeExcitation` then set the *excited* attributes of modes a_3 , a_7 , I_{37} to TRUE and flip the *eligible* attribute of the four operators corresponding to each of these modes. The resulting set of eligible operators is

$$\begin{aligned} \mathcal{O}^* = & \{\hat{A}_i, \tilde{A}_i\}_{i \notin \{2,3,5,7\}} \cup \{\check{A}_i, \bar{A}_i\}_{i \in \{2,3,5,7\}} \cup \\ & \{\hat{a}_i, \tilde{a}_i\}_{i \notin \{3,7\}} \cup \{\check{a}_i, \bar{a}_i\}_{i \in \{3,7\}} \cup \\ & \{\hat{I}_{25}, \tilde{I}_{25}, \check{I}_{37}, \bar{I}_{37}\}. \end{aligned} \quad (2.128)$$

Finally the updated time t , the chosen rule $\mathbb{R}$, and the updated state constructor C are added as a tuple to the trajectory $\mathcal{T}$.

In the next execution of the while loop, the set $\mathcal{R}^*$ of eligible rules is found by `IsRuleEligible` to be

$$\begin{aligned} \mathcal{R}^* = & \{\hat{A}_i \tilde{a}_i\}_{i \notin \{2,3,5,7\}} \cup \{\check{A}_i \tilde{a}_i\}_{i \in \{2,5\}} \cup \\ & \{\bar{A}_2 \bar{A}_5 \hat{a}_2 \hat{a}_5 \hat{I}_{25}, \bar{A}_3 \bar{A}_7 \check{a}_3 \check{a}_7 \check{I}_{37}\}. \end{aligned} \quad (2.129)$$

It is worth noting the changes to $\mathcal{R}^*$ vs. Eqn. 2.124. The monomer annihilation rules $\check{A}_3 \tilde{a}_3$ and $\check{A}_7 \tilde{a}_7$ have been removed because the modes a_3 and a_7 are now excited, making the operators $\tilde{a}_3$ and $\tilde{a}_7$ ineligible. This prevents monomers joined by interactions from being annihilated, thereby leaving “dangling” interactions. In addition, all interaction creation rules except $\bar{A}_2 \bar{A}_5 \hat{a}_2 \hat{a}_5 \hat{I}_{25}$ have become ineligible. This prevents the A_3 and A_7 monomers, which are already interacting with each other, from participating in multiple interactions. Finally, the interaction annihilation rule $\bar{A}_3 \bar{A}_7 \check{a}_3 \check{a}_7 \check{I}_{37}$ becomes eligible, allowing the newly-formed bond to dissociate.

Fig. 2.7 shows this stochastic algorithm applied to monomer, homodimer, and homopolymer systems. Panels (A-C) validate this algorithm by showing that the mean abundance of each species found across 500 simulations closely traces the mean abundance predicted by the deterministic algorithm. As noted above, however, these comparisons can only be carried out at small N due to limitations of the deterministic algorithm. This stochastic algorithm can be performed at much larger values of N , e.g., Fig. 2.7(D)-(F) shows such simulations performed using $N = 100$. Initialized from a vacuum, Fig. 2.7(E)-(F) in particular illustrates we observe how monomer production dominates until a critical threshold quantity is achieved, beyond which bond formation starts to take over until steady state.

The size of N is still a limitation for Algorithm 1. The bottleneck is line 5, which requires iterating through all mode-specific rules in $\mathcal{R}$ and testing each one for eligibility. This step takes $o(|\mathcal{R}|)$ time, and $|\mathcal{R}|$ is polynomial in N : $|\mathcal{R}| = 2N$ for the monomer, $|\mathcal{R}| = 2N + 2\binom{N}{2}$ for the homodimer, and $|\mathcal{R}| = 2N + 2N^2$ for the homopolymer. That said, Algorithm 1 was developed only as proof-of-principle and has not been optimized for efficiency. Indeed, we expect the bottleneck can be eliminated by using more sophisticated methods for tracking which operators and rules are eligible given the state constructor, thereby enabling simulations using arbitrarily large values of N .

2.7 Expressiveness

Our formalism provides a rule-based approach for defining and analyzing a diverse array of stochastic chemical systems in which multi-particle complexes can form. Here we illustrate the expressiveness of our formalism by briefly considering a variety of such systems, both in and out of equilibrium. Though we are not aware of existing literature reporting these specific results, our primary intention here is simply to demonstrate the richness of models that can be constructed from a small number of rules.

In equilibrium, systems are defined by a factory $\vec{\mathbb{F}}$ and a Hamiltonian $\mathbb{H}$. Putting the Hamiltonian aside for the moment, it is interesting to consider the qualitatively different sets of complexes that can arise from simple changes to the factory. This expressiveness is perhaps most apparent in polymer systems. Fig. 2.8 shows nine such polymeric systems derived from variations on the homodimer and homopolymer systems described in previous sections. Two such systems, the isotropic [Fig. 2.8(B)] and branched homopolymer [Fig. 2.8(C)], are further analyzed below.

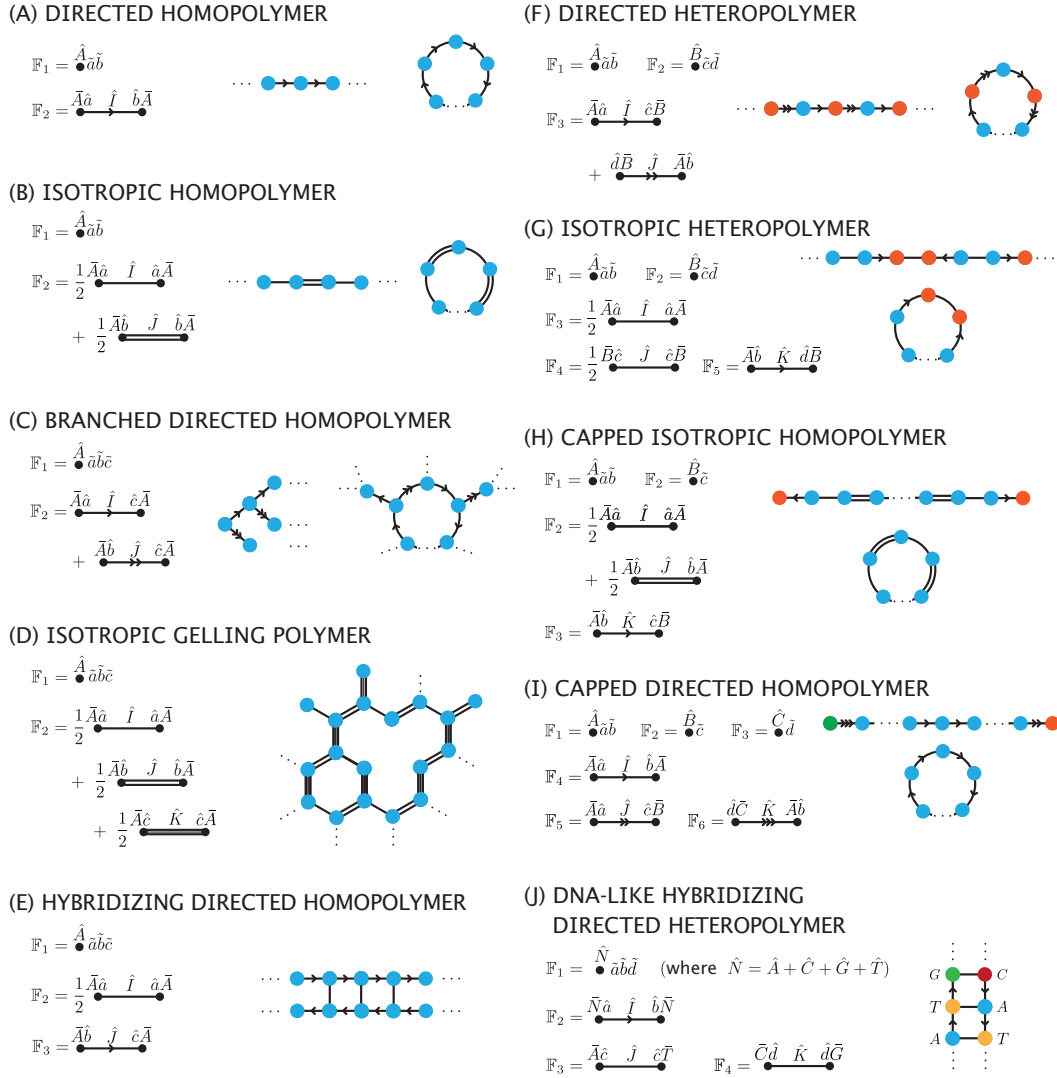


Figure 2.8: Factories defining various polymer systems. Each panel shows the factory operators used to define systems in equilibrium. The homopolymer system analyzed in Section 2.4 is shown in panel (A) for comparison.

Out of equilibrium, systems are defined by a set of rules, with each rule $\mathbb{R}_l$ assigned a corresponding rate r_l . Fig. 2.9 shows five such nonequilibrium systems. These rules again derive from variations on the homodimer and homopolymer systems. Fig. 2.9 also shows the results of stochastic simulations carried out using Algorithm 1 for specific choices of the rate parameters.

2.7.1 Isotropic homopolymer

We now analyze the isotropic homopolymer shown in Fig. 2.8(B). This system is defined by one species of monomeric subunit (A) with two sites (a and b) capable of participating in two classes of symmetric interaction (I and J). We aim to compute the partition function for the system assuming the Hamiltonian

$$\mathbb{H} = -\mu \sum_i \bar{A} + \frac{\epsilon}{2} \sum_{i,j} (\bar{I}_{ij} + \bar{J}_{ij}). \quad (2.130)$$

As with the homopolymer of Section 2.4, the two factory operators for this system generate chains and rings. However, the use of two distinct types of symmetric interaction complicates these species. First, there are no self-interacting monomers. Second, the I and J interactions alternate in all multimers. For x -chains this means that, when x is even, there are two different species related by the exchange of I and J interactions. When x is odd, there is instead only one-species of x -chain, as exchanging I and J is equivalent to flipping the order of the subunit indices. Moreover, x -rings occur only when x is even. These rings have 2-fold mirror symmetry and $x/2$ -fold rotational symmetry.

The log partition function density of the system is therefore given by

$$\begin{aligned} \frac{\log Z}{V} = & \sum_{x=1}^{\infty} \log Z_{(2x-1)\text{-chain}} + \sum_{x=1}^{\infty} \log Z_{2x\text{-chain}} \\ & + \sum_{x=1}^{\infty} \log Z_{2x\text{-ring}}. \end{aligned} \quad (2.131)$$

In terms of the effective chemical potential $\mu' = \mu + k_B T \log \frac{N}{V}$ and effective interaction energy $\epsilon' = \epsilon - k_B T \log V$, as well as the control parameter $\eta = e^{\beta(\mu' - \epsilon')}$ (which represents the Boltzmann weight of a single particle with a dangling bond), the single-complex partition functions in Eqn. 2.131 are

$$\begin{aligned} \log Z_{(2x-1)\text{-chain}} &= e^{\beta\epsilon'} \eta^{2x-1}, \\ \log Z_{2x\text{-chain}} &= 2e^{\beta\epsilon'} \eta^{2x}, \end{aligned}$$

$$\log Z_{2x\text{-ring}} = \frac{\eta^{2x}}{2xV}. \quad (2.132)$$

Summing the terms in Eqn. 2.131 we find that, for $0 < \eta < 1$,

$$\frac{\log Z}{V} = \frac{e^{\beta\epsilon'}\eta}{1-\eta} + \frac{e^{\beta\epsilon'}\eta^2}{1-\eta^2} - \frac{\log(1-\eta^2)}{2V}. \quad (2.133)$$

As with the homopolymer, the partition function diverges as $\eta \rightarrow 1$ from below. The concentration of A particles is then

$$\frac{\bar{A}}{V} = \frac{1}{\beta} \frac{\partial}{\partial \mu'} \frac{\log Z}{V} = \eta \frac{\partial}{\partial \eta} \frac{\log Z}{V} \quad (2.134)$$

$$= e^{\beta\epsilon'} \left[\frac{\eta}{1-\eta} + \frac{\eta^2}{(1-\eta)^2} + \frac{2\eta^2}{1-\eta^2} + \frac{2\eta^4}{(1-\eta^2)^2} \right] + \frac{2\eta^2}{1-\eta^2} \cdot \frac{1}{2V}. \quad (2.135)$$

Defining $\delta = 1 - \eta$, from which $1 - \eta^2 \approx 2\delta$ in the limit as $\eta \rightarrow 1$, we find that the concentration of A particles diverges in this limit, scaling as

$$\frac{\langle \bar{A} \rangle}{V} = e^{\beta\epsilon'} \left(\frac{1}{\delta} + \frac{1}{\delta^2} + \frac{2}{2\delta} + \frac{2}{4\delta^2} \right) + \frac{2}{2\delta} \cdot \frac{1}{2V} \quad (2.136)$$

$$= e^{\beta\epsilon'} \left(\frac{2}{\delta} + \frac{3}{2\delta^2} \right) + \frac{1}{\delta} \cdot \frac{1}{2V} \approx \frac{3e^{\beta\epsilon'}}{2\delta^2}. \quad (2.137)$$

As in the directed homopolymer case, this divergence is dominated by x -chain contributions even at finite V . The factor of $\frac{3}{2}$ difference between this result and the homopolymer result in Eqn. 2.86 reflects the fact that, while both even and odd chains contribute to Eqn. 2.137, there are twice as many species of even chains (but the same number of odd chains) as in the homopolymer system.

2.7.2 Branched directed homopolymer

Consider now the branched directed homopolymer system shown in Fig. 2.8(C). This system is defined by one species of monomeric subunit (A) with three sites (a , b , and c) capable of participating in two classes of directed interaction (I and J). We assume that the system is in thermal equilibrium and is governed by the Hamiltonian

$$\mathbb{H} = -\mu \sum_i \bar{A} + \epsilon \sum_{i,j} (\bar{I}_{ij} + \bar{J}_{ij}). \quad (2.138)$$

The two factory operators for this system generate two broad classes of complex: “trees,” which branch out from a single A in which site c is unoccupied, and “groves,”

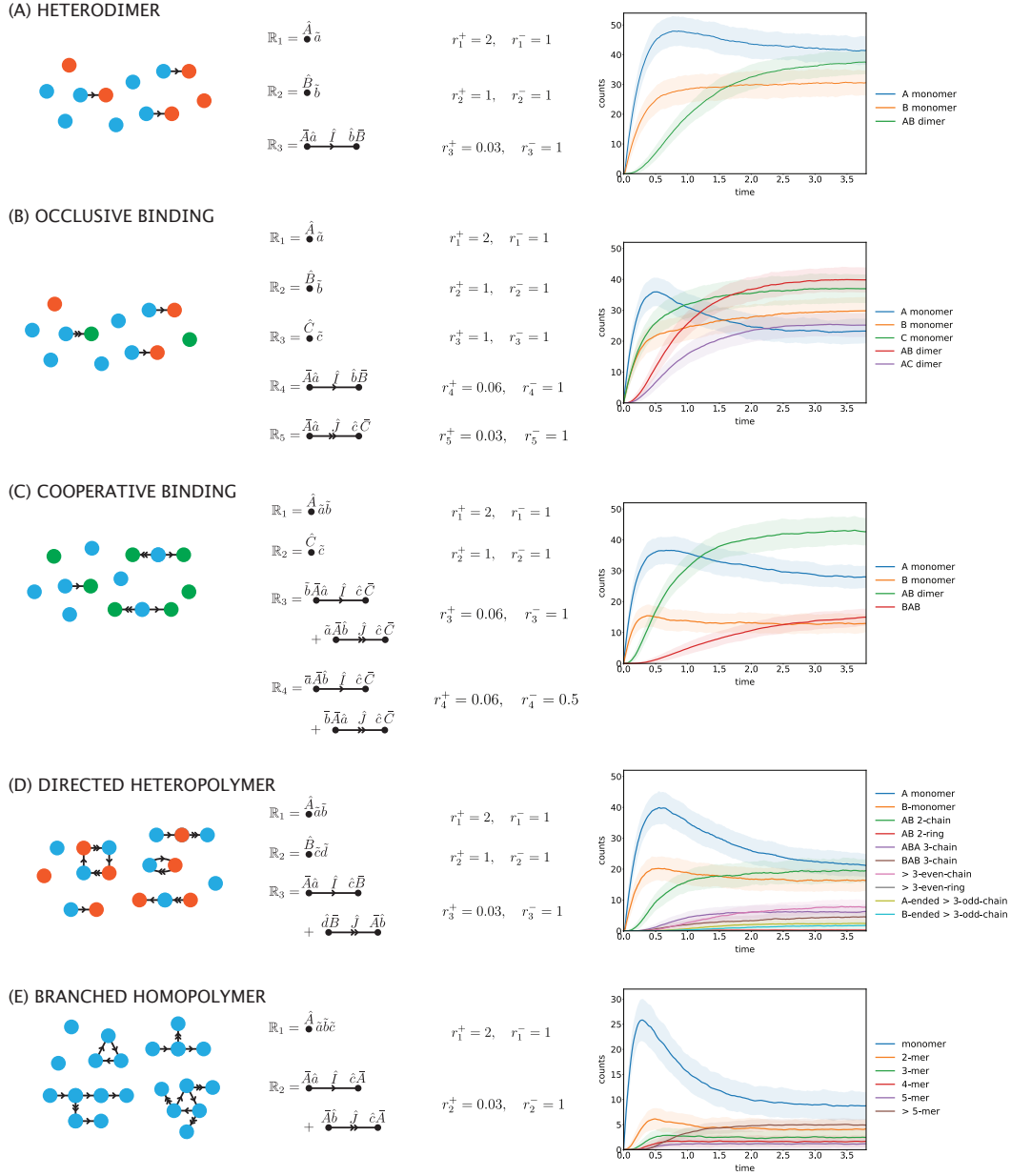


Figure 2.9: Stochastic simulations of various nonequilibrium models. Shown are results for (A) a heterodimer system, (B) an occlusive binding system, (C) a cooperative binding system, (D) a heteropolymer system, and (E) a branched homopolymer system. For each system, r_l^+ denotes the rate at which the forward rule, $\mathbb{R}_l$, is applied, while r_l^- denotes the rate at which the reverse rule, $\mathbb{R}_l^\dagger$, is applied. Solid lines indicate mean abundances from 500 stochastic simulations using $N = 100$. Error bands indicate abundance standard deviations across the simulations.

which consist of multiple trees branching out from a central closed ring. The log partition function density of this system is therefore given by

$$\frac{\log Z}{V} = Z_{\text{tree}} + \sum_{x=1}^{\infty} Z_{x\text{-grove}}, \quad (2.139)$$

where Z_{tree} is the partition function for all trees and $Z_{x\text{-grove}}$ is the partition function for all groves with trees extending off a central x -ring. To proceed, we let ξ represent the partition function for a tree with a dangling interaction extending off the root. In terms of this quantity,

$$Z_{\text{tree}} = e^{\beta\epsilon'} \xi, \quad Z_{x\text{-grove}} = \frac{2^x \eta^x (1 + \xi)^x}{V_x}, \quad (2.140)$$

where $\eta = e^{\beta(\mu' - \epsilon')}$ is again the Boltzmann weight of a single particle with a dangling bond. In Z_{tree} , the factor of $e^{\beta\epsilon'}$ removes the effect of the dangling bond. In $Z_{x\text{-grove}}$, the factor of η^x accounts for the particles and bonds in each x -ring, the factor of 2^x accounts for the fact that each bond in the x -ring can be of type I or J , and the $(1 + \xi)^x$ factor accounts for the fact that each A within the ring can either be bare or have a tree attached. As with the homopolymer, the factor of $\frac{1}{x}$ compensates for the rotational symmetry of the ring while $\frac{1}{V}$ reflects the entropic cost of self-circularization.

We solve for ξ by noting that the self-similar structure of each tree complex yields the recursion relation

$$\xi = \eta(1 + 2\xi + \xi^2). \quad (2.141)$$

Solving this quadratic equation and using the limiting behavior $\xi \approx \eta$ as $\eta \rightarrow 0$, we derive

$$\xi = \frac{1 - 2\eta - \sqrt{1 - 4\eta}}{2\eta}. \quad (2.142)$$

Expressing Z_{tree} and $Z_{x\text{-grove}}$ in terms of ξ and summing $Z_{x\text{-grove}}$ over all x , we find that for $0 < \eta < \frac{1}{4}$,

$$\frac{\log Z}{V} = e^{\beta\epsilon'} \left(\frac{1 - 2\eta - \sqrt{1 - 4\eta}}{2\eta} \right) - \frac{\log(1 - 4\eta)}{2V}. \quad (2.143)$$

As with the homopolymer system, the contribution from the circularized (i.e., x -grove) species vanishes in the $V \rightarrow \infty$ limit. We also find that the partition function

is not defined for $\eta > \frac{1}{4}$. In particular, the mean concentration of A particles is found to diverge as $\delta = \frac{1}{4} - \eta \rightarrow 0$ according to

$$\frac{\langle \bar{A} \rangle}{V} \approx \frac{e^{\beta\epsilon'}}{2\sqrt{\delta}} + \frac{1}{8V\delta}, \quad (2.144)$$

with the first and second terms respectively corresponding to trees and groves. This result is qualitatively different from the corresponding results for the directed homopolymer in Section 2.4 and for the isotropic homopolymer analyzed above. In particular, the circularized species (the groves) dominate over the linear species (the trees) in the $\delta \rightarrow 0$ limit when V is kept finite. The asymptotic behavior of the system therefore depends on which limit one takes first, $\eta \rightarrow \frac{1}{4}$ or $V \rightarrow \infty$. Moreover, the divergence is more mild than in the non-branched systems, scaling as either $\delta^{-1/2}$ or δ^{-1} (depending on how one handles V) rather than δ^{-2} .

2.8 Discussion

We have introduced an algebraic formalism for the rule-based modeling of multi-particle complexes in stochastic chemical systems. This algebra is based on a Fock space that allows not only the creation and annihilation of particles, but also the joining of particles into complexes based on specified rules. The Fock space comprises three types of hard-core boson fields; these represent particles, particle-particle interactions, and occupied binding sites. We have also described a formal diagrammatic approach that facilitates the use of this algebra.

For equilibrium systems, we showed that the set of all possible complexes can be rigorously specified by a “factory” and a Hamiltonian. The factory is an ordered set of operators that define how to construct complexes; the Hamiltonian is an operator that specifies rules for computing the Gibbs free energy of a complex based on its components. We showed for multiple systems how these rule-based definitions can be used to compute generating functions and partition functions, and to analyze scaling behavior near critical polymerization concentrations.

For nonequilibrium systems, we showed how to rigorously specify system dynamics in a rule-based manner. Specifically, we showed how a set of reaction rule operators and corresponding rates can be used to define the transition matrix of a “microstate” master equation. From this transition matrix one can then analytically compute the corresponding “macrostate” master equation, which governs the time evolution of observables. We also developed a Gillespie algorithm based on our

formalism for simulating stochastic chemical systems based on these rule operators and corresponding rates.

The essential feature of our formalism, one that distinguishes it from previous approaches for modeling many-body systems of classical particles, is that it explicitly represents internal particle states. These internal states endow each particle with its own identity, thus allowing preexisting particles to join together into multi-particle complexes. Notably, our approach to modeling these internal states is consistent with the behavior of quantum systems in the decoherence limit. In this limit, the reduced density matrix for each particle becomes diagonal with elements along the diagonal quantifying the probability of each energy eigenstate [1, 26]. The orthonormal microstates in our formalism correspond to these diagonal positions in the reduced density matrix (i.e., the energy eigenstates), and the probabilities that multiply these microstates correspond to the values of the reduced density matrix at these positions. Classical particles, such as proteins, have many distinct energy eigenstates corresponding to different internal excitation modes, and the internal states of particles in our formalism stand in for these modes. Our formalism assumes a specific number N of such modes, but this choice does not affect results when N is sufficiently large, and all physically meaningful calculations are performed in the $N \rightarrow \infty$ limit.

We envision a variety of potential analytic applications for our formalism. As in the work of Doi [3, 4], it may be possible to use the algebra we have introduced to carry out diagrammatic perturbation theory calculations. As in the work of Peliti [7] and Goldenfeld [6], it may also be possible to identify a path integral formulation of this algebra. While our analysis focused on zero-dimensional (i.e., well-mixed) systems, we expect that it should be straightforward to apply our formalism to spatially extended systems in which diffusion plays an important role (as in [3, 4, 6, 7]).

We also expect our results will enable improvements to existing rule-based modeling approaches. Our algebraic and diagrammatic representations are substantially more compact than those used by existing rule-based frameworks. In particular, the specification of reaction rules in BioNetGen and Kappa requires that the user identify relevant sub-complexes both before and after each transition, as well as identify which specific particles before correspond to which specific particles after. By contrast, our use of “hat” and “check” operators eliminates this need, allowing users to specify rules using a single operator product or diagram. Moreover, since the underlying objects in our formalism (hard-core bosons) are simpler than those

in existing algorithms (e.g., port graphs), the use of our formalism may allow existing Gillespie simulation approaches to be streamlined. This would first require reworking our proof-of-principle algorithm (Algorithm 1) so that its computational cost does not increase with N . We expect this should be possible using graph matching methods and more advanced bookkeeping when computing the set of eligible rules in line 5 of Algorithm 1.

Our formalism may further facilitate the development of new hybrid analytical and computational approaches for rule-based modeling. In particular, we envision the possibility of supplementing rule-based Gillespie simulations with computer algebra techniques, e.g., for identifying and coarse-graining fast reactions in nonequilibrium systems. There are other possibilities as well. Doi’s formalism has recently been used as the basis for analyzing reaction-diffusion systems using tensor networks and the time-dependent variation principle [27]. Our algebra may enable such approaches to be applied to chemical systems involving multi-particle complexes. Alternatively, our formalism may facilitate the use of finite state projection techniques [28].

In this work we have focused on polymer systems, which best illustrate how large complexes can arise from simple interaction rules. Our initial motivation in pursuing this project, however, was in biological systems. We specifically sought to develop methods for modeling the biophysical mechanisms of gene regulation. Gene regulation is controlled by large protein-nucleic acid complexes [29, 30], with individual DNA and RNA regulatory sequences able to nucleate the formation of large numbers of different macromolecular assemblies. A major goal in this field is to understand these complexes as well as their effects on gene expression using biophysical models, and substantial progress has been made using both equilibrium models [31–34] and nonequilibrium models [35–37]. In particular, biophysical modeling provides a principled approach to deciphering how gene regulatory programs are encoded in DNA and RNA sequences [38–42]. Such biophysical modeling efforts have been hindered by combinatorial complexity, however, and rule-based software has yet to be developed for modeling the sequence-dependent equilibrium and nonequilibrium processes that these models must describe. We anticipate that our work will help address this need by providing a formal rule-based language in which complex sequence-dependent biophysical models of gene regulation can be efficiently expressed and analyzed. Ultimately, we expect this work to facilitate the construction of large biophysical models able to describe genome-wide regulatory codes.

Appendices

2.A Algebra of mode and field operators

Since each mode represents a hard-core boson, mode-specific creation and annihilation operators are nilpotent, i.e.,

$$\hat{A}_i^2 = \check{A}_i^2 = 0. \quad (2.145)$$

When multiplied by presence and absence operators for the same mode, the creation and annihilation operators are readily seen to satisfy

$$\bar{A}_i \hat{A}_i = \hat{A}_i \tilde{A}_i = \hat{A}_i, \quad \tilde{A}_i \hat{A}_i = \hat{A}_i \bar{A}_i = 0, \quad (2.146)$$

$$\tilde{A}_i \check{A}_i = \check{A}_i \bar{A}_i = \check{A}_i, \quad \bar{A}_i \check{A}_i = \check{A}_i \tilde{A}_i = 0. \quad (2.147)$$

Presence and absence operators are both idempotent, i.e.,

$$\bar{A}_i^2 = \bar{A}_i, \quad \tilde{A}_i^2 = \tilde{A}_i, \quad (2.148)$$

and mixed products of presence and absence operators for the same mode vanish:

$$\bar{A}_i \tilde{A}_i = \tilde{A}_i \bar{A}_i = 0. \quad (2.149)$$

From these properties and the fact that operators for distinct modes commute, we get the following commutation relations for mode operators:

$$[\check{A}_i, \hat{A}_j] = \delta_{ij}(\tilde{A}_i - \bar{A}_i) = \delta_{ij}(1 - 2\bar{A}_i), \quad (2.150)$$

$$[\bar{A}_i, \hat{A}_j] = [\hat{A}_i, \tilde{A}_j] = \delta_{ij}\hat{A}_i, \quad (2.151)$$

$$[\tilde{A}_i, \check{A}_j] = [\check{A}_i, \bar{A}_j] = \delta_{ij}\check{A}_i. \quad (2.152)$$

Summing over indices gives the corresponding commutation relations for field operators:

$$[\check{A}, \hat{A}] = \tilde{A} - \bar{A} = N - 2\bar{A}, \quad (2.153)$$

$$[\bar{A}, \hat{A}] = [\hat{A}, \tilde{A}] = \hat{A}, \quad (2.154)$$

$$[\tilde{A}, \check{A}] = [\check{A}, \bar{A}] = \check{A}. \quad (2.155)$$

These commutation relations, together with $\bar{A}|0\rangle = 0$, allow us to compute the impact of each field operator on the macrostate $|n\rangle$,

$$\hat{A}|n\rangle = \hat{A} \frac{\hat{A}^n}{n!} |0\rangle = (n+1) \frac{\hat{A}^{n+1}}{(n+1)!} |0\rangle = (n+1) |n+1\rangle, \quad (2.156)$$

$$\begin{aligned}
\bar{A} |n\rangle &= \bar{A} \frac{\hat{A}^n}{n!} |0\rangle = \frac{1}{n!} [\bar{A}, \hat{A}^n] |0\rangle = \frac{1}{n!} \sum_{k=0}^{n-1} \hat{A}^{n-k-1} [\bar{A}, \hat{A}] \hat{A}^k |0\rangle \\
&= n \frac{\hat{A}^n}{n!} |0\rangle = n |n\rangle,
\end{aligned} \tag{2.157}$$

$$\check{A} |n\rangle = \check{A} \frac{\hat{A}^n}{n!} |0\rangle = [\check{A}, \frac{\hat{A}^n}{n!}] |0\rangle = \frac{1}{n!} \sum_{k=0}^{n-1} \hat{A}^{n-k-1} [\check{A}, \hat{A}] \hat{A}^k |0\rangle \tag{2.158}$$

$$\begin{aligned}
&= \frac{1}{n!} \sum_{k=0}^{n-1} \hat{A}^{n-k-1} (N - 2\bar{A}) \hat{A}^k |0\rangle = \frac{1}{n!} \sum_{k=0}^{n-1} (N - 2k) \hat{A}^{n-1} |0\rangle \\
&= \frac{1}{n!} \left(nN - 2 \frac{n(n-1)}{2} \right) \hat{A}^{n-1} |0\rangle = (N - n + 1) \frac{\hat{A}^{n-1}}{(n-1)!} |0\rangle \\
&= (N - n + 1) |n-1\rangle,
\end{aligned} \tag{2.159}$$

$$\begin{aligned}
\tilde{A} |n\rangle &= \tilde{A} \frac{\hat{A}^n}{n!} |0\rangle = (N - \bar{A}) \frac{\hat{A}^n}{n!} |0\rangle \\
&= (N - n) \frac{\hat{A}^n}{n!} |0\rangle = (N - n) |n\rangle.
\end{aligned} \tag{2.160}$$

2.B Non-commutation of monomer and dimer operators

Here we derive the expression for $[\hat{M}, \check{D}]$ in Eqn. 2.45. We begin by evaluating the commutator on the individual composite mode operators:

$$\begin{aligned}
[\hat{M}_k, \check{D}_{ij}] &= \check{I}_{ij} [\hat{A}_k \tilde{a}_k, \check{A}_i \check{A}_j \check{a}_i \check{a}_j] \\
&= \delta_{ki} \check{I}_{ij} \check{A}_j \check{a}_j [\hat{A}_k \tilde{a}_k, \check{A}_i \check{a}_i] \\
&\quad + \delta_{kj} \check{I}_{ij} \check{A}_i \check{a}_i [\hat{A}_k \tilde{a}_k, \check{A}_j \check{a}_j].
\end{aligned} \tag{2.161}$$

Considering the commutator in the $k = i$ term and dropping the subscripts for brevity, we find that

$$\begin{aligned}
[\hat{A} \tilde{a}, \check{A} \check{a}] &= \hat{A} [\tilde{a}, \check{A}] \check{a} + [\hat{A}, \check{A}] \tilde{a} \check{a} + \check{A} \hat{A} [\tilde{a}, \check{a}] + \check{A} [\hat{A}, \tilde{a}] \check{a} \\
&= \tilde{A} \check{a} \\
&= \hat{A} \tilde{a} \check{A} \check{a}.
\end{aligned} \tag{2.162}$$

The same holds for the $k = j$ term. Substituting these back into Eqn. 2.161 and summing over k gives

$$\begin{aligned}
\sum_k [\hat{M}_k, \check{D}_{ij}] &= \sum_k \delta_{ki} \hat{A}_i \tilde{a}_i \check{I}_{ij} \check{A}_i \check{a}_i \check{A}_j \check{a}_j \\
&\quad + \sum_k \delta_{kj} \hat{A}_j \tilde{a}_j \check{I}_{ij} \check{A}_i \check{a}_i \check{A}_j \check{a}_j \\
&= (\hat{M}_i + \hat{M}_j) \check{D}_{ij}.
\end{aligned} \tag{2.163}$$

From this we recover Eqn. 2.45.

2.C Factory/gallery equivalence for the homopolymer

We now prove the equivalence of the factory and gallery representations for the homopolymer system in Section 2.4. As with the homodimer, we do this by evaluating individual terms in the Taylor expansion of $e^{\mathbb{F}_2} e^{\mathbb{F}_1} |0\rangle$, with factory operators defined as in Fig. 2.5(a). By inspection we see that all full contractions of $\mathbb{F}_2^p \mathbb{F}_1^q$ must consist of a product of x -chain ($\hat{C}_x$) and x -ring ($\hat{R}_x$) operators. Wick's theorem therefore gives

$$\mathbb{F}_2^p \mathbb{F}_1^q |0\rangle = \sum_{\{c_x, r_x\} | p, q} \Omega_{\{c_x, r_x\}}^{(p, q)} \prod_{x=1}^{\infty} \hat{C}_x^{c_x} \hat{R}_x^{r_x} |0\rangle, \quad (2.164)$$

where c_x denotes the number of x -chains, r_x denotes the number of x -rings, $\{c_x, r_x\} | p, q$ denotes all sets of these numbers that are consistent with p bonds and q particles, i.e., which satisfy

$$q = \sum_{x=1}^{\infty} (x c_x + x r_x), \quad p = \sum_{x=1}^{\infty} ([x-1] c_x + x r_x), \quad (2.165)$$

and $\Omega_{\{c_x, r_x\}}^{(p, q)}$ is a combinatorial coefficient that quantifies the number of distinct contractions that yield $\{c_x, r_x\} | p, q$.

We now compute $\Omega_{\{c_x, r_x\}}^{(p, q)}$. The number of ways to partition q monomers among c_x distinct x -chains and r_x distinct x -rings is given by

$$\omega_q = \frac{q!}{\prod_{x=1}^{\infty} (x!)^{c_x + r_x}}. \quad (2.166)$$

Similarly, the number of ways to partition p bonds among the x -chains and x -rings is

$$\theta_p = \frac{p!}{\prod_{x=1}^{\infty} ([x-1]!)^{c_x} (x!)^{r_x}}. \quad (2.167)$$

Since one can rearrange the x -chains among themselves and the x -rings among themselves without changing the result, the number of unique partitions is the product of ω_q and θ_p divided by an exchange factor of $\pi_{p, q} = \prod_{x=1}^{\infty} c_x! r_x!$. Moreover, there are $\sigma_x = (x-1)!$ distinct ways of constructing each x -chain from a given set of x particles and $x-1$ bonds, and $\rho_x = x!$ ways to construct each x -ring from a set of x particles and x bonds. Note that the circular symmetry, which contributes a factor of $1/x$ to this second quantity, is already accounted for in the definition of the x -ring and should not be double-counted here. We therefore find that

$$\Omega_{\{c_x, r_x\}}^{(p, q)} = \frac{\omega_q \theta_p}{\pi_{p, q}} \prod_{x=1}^{\infty} \sigma_x^{c_x} \rho_x^{r_x} = \frac{p! q!}{\prod_{x=1}^{\infty} c_x! r_x!}. \quad (2.168)$$

Consequently,

$$\begin{aligned}
e^{\mathbb{F}_2} e^{\mathbb{F}_2} |0\rangle &= \sum_{p,q} \frac{1}{p!q!} \sum_{\{c_x, r_x\}|p,q} \frac{p!q!}{\prod_x c_x! r_x!} \prod_x \hat{C}_x^{c_x} \hat{R}_x^{r_x} |0\rangle \\
&= \sum_{c_1, c_2, \dots} \sum_{r_1, r_2, \dots} \prod_x \frac{\hat{C}_x^{c_x}}{c_x!} \frac{\hat{R}_x^{r_x}}{r_x!} |0\rangle \\
&= e^{\sum_x (\hat{C}_x + \hat{R}_x)} |0\rangle.
\end{aligned} \tag{2.169}$$

This establishes the factory/gallery equivalence for the homopolymer.

2.D Derivation for the species-specific depletion operator

Here we derive the species-specific depletion operator in Eqn. 2.99. First we express the species-specific reaction operator as

$$\mathbb{Q} = \prod_{k=1}^K P_k Q_k, \quad \text{where } P_k = \frac{\hat{G}_k^{p_k}}{p_k!}, \quad Q_k = \frac{\check{G}_k^{q_k}}{q_k!}. \tag{2.170}$$

The assumption of non-overlapping $\vec{p}$ and $\vec{q}$ implies that $P_k = 1$ and/or $Q_k = 1$ for all k . Because of this,

$$\mathring{\mathbb{Q}} = \prod_{k=1}^K \mathring{P}_k \mathring{Q}_k. \tag{2.171}$$

Note that this will generally not be true if any P_k and Q_k are both non-unity, since the depletion version of a product of operators for a given field is generally not the product of the depletion version of each operator.

Next we express P_k and Q_k in terms of mode-specific operators. Using the fact that the mode-specific creation and annihilation operators are nilpotent (and dropping k to ease notation),

$$P = \sum_{I:|I|=p} \prod_{i \in I} \hat{G}_i, \quad Q = \sum_{I:|I|=q} \prod_{i \in I} \check{G}_i. \tag{2.172}$$

Transforming $\hat{G}_i \rightarrow \tilde{G}_i$ and $\check{G}_i \rightarrow \bar{G}_i$, we obtain the corresponding depletion operators

$$\mathring{P} = \sum_{I:|I|=p} \prod_{i \in I} \tilde{G}_i, \quad \mathring{Q} = \sum_{I:|I|=q} \prod_{i \in I} \bar{G}_i. \tag{2.173}$$

Applying each of these operators to a macrostate $|n\rangle$, one finds that

$$\mathring{P} |n\rangle = \binom{N-n}{p} |n\rangle = \binom{\tilde{G}}{p} |n\rangle, \tag{2.174}$$

$$\hat{Q} |n\rangle = \binom{n}{q} |n\rangle = \binom{\bar{G}}{q} |n\rangle, \quad (2.175)$$

and therefore,

$$\hat{P} = \binom{\tilde{G}}{p}, \quad \hat{Q} = \binom{\bar{G}}{q}. \quad (2.176)$$

Reintroducing the k subscripts, we obtain Eqn. 2.99:

$$\hat{Q} = \prod_{k=1}^K \hat{P}_k \hat{Q}_k = \prod_{k=1}^K \binom{\tilde{G}_k}{p_k} \binom{\bar{G}_k}{q_k}. \quad (2.177)$$

2.E Code availability

Python code implementing Algorithm 1 as well as the Jupyter Notebooks used to perform the simulations in Fig. 2.7 and Fig. 2.9 are available at https://github.com/Rebecca-J-Rousseau/RousseauKinney2024_algebra.

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Chapter 3

BIFURCATIONS AND MULTISTABILITY IN INDUCIBLE THREE-GENE TOGGLE SWITCH NETWORKS¹

Having now established a mathematical framework for understanding how individual protein subunits collectively give rise to higher-order structural complexity through self-assembly, we turn to an analogous but distinct class of emergent biological organization, the gene regulatory network. While protein self-assembly is governed by the thermodynamic and geometric constraints encoded in molecular structure, gene regulation operates at a more macro scale that coordinates the activities of these protein assemblies to generate functional effects in the cell. Understanding such processes involves additional foundations such as information processing, feedback, and temporal coordination. In both cases, however, local interactions among discrete components produce system-level behaviors that cannot be predicted from the components alone. This shared principle of emergent behavior motivates our transition from structural to regulatory complexity, and positions the gene regulatory network as a natural next step in investigating how biological functions arise from network architecture.

Prior to the 1960s, the scientific view of the relationship between genes and proteins was as a passive, unidirectional process – sections of the DNA called genes encoded information that produced corresponding proteins. This notion was popularized in 1941 by the “one gene, one enzyme” hypothesis [1]. Gene expression was treated as broadly constitutive, with genes simply “on” by default and continuously producing protein products at a fixed rate. The idea that expression could be dynamically regulated in response to environmental conditions was not well established, even though the phenomenon of “enzymatic adaptation” (i.e., cells producing more of certain enzymes when supplied with specific molecules called substrates) had been observed empirically since the late 19th century. Louis Pasteur, for example, had noted that yeasts behave differently under different growth conditions [2]. Jacques Monod later made this and other experimental observations more concrete by plotting bacterial growth curves to visualize bacterial physiology [3]. The actual underlying

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mechanism for such phenomena, however, remained unclear.

The functional activities of genes and proteins were also considered fundamentally distinct. Following the experimental confirmation of DNA as the genetic material of life [4, 5], along with Watson and Crick's nearly concurrent discovery of DNA's structure in 1953 using Rosalind Franklin's X-ray diffraction images, the focus was overwhelmingly on DNA as an informational molecule. The thought that proteins could in turn act *back* on DNA to bind, read, and control it was not part of the mainstream framework. Likewise, there was no conceptual notion that an entire class of genes could exist solely to regulate the activity of other genes, and thus no basis for thinking about the logic of genetic circuits.

This all changed in the early 1960s, when the landmark work of François Jacob and Jacques Monod systematically investigated enzymatic adaptation in *Escherichia coli* and revealed a previously unrecognized layer of genetic organization. Working with the lactose (lac) operon, Jacob and Monod demonstrated that not all genes encode structural proteins or enzymatic machinery, but rather that some genes exist solely to control the expression of others [6–9]. Through a series of elegant genetic and biochemical experiments, they showed that the expression of the genes responsible for lactose metabolism is coordinated and regulated in response to environmental signals, switching “on” only when lactose is present and glucose is absent. This work culminated in their repressor-operator model, in which a regulatory protein (in this case a repressor) binds to a specific DNA sequence called the operator, physically blocking the transcription of downstream genes. When an inducer molecule is present, it binds the repressor and causes a conformational change that releases it from the DNA, permitting transcription to proceed. The conceptual significance of this model extended far beyond bacterial metabolism, and established that gene expression is not constitutive but conditional, governed by molecular logic that responds to context.

Jacob and Monod had, in effect, described the first genetic switch, i.e., a molecular device capable of converting a chemical signal into a discrete change in cellular state. This framework became the conceptual foundation upon which all subsequent study of gene regulatory networks would be built. Subsequent work, for example, discovered architectures that were mediated not only by repression, but also by activation [10], and even by combinations of both protein types [11]. The study of the lysis-lysogeny decision in bacteriophage lambda became a paradigm for the genetic switch [12, 13], and these ideas have since then been generalized, realized,

and exploited across the field of biology.

The repressor-operator model of Jacob and Monod provided not only a successful conceptual vision for gene expression, but also served as the basis for statistical mechanical models of transcription [14–16]. These models demonstrate that genetic circuits can be tuned directly and quantitatively as properties of the DNA’s spatial structure and genetic interactions change. Tuning could entail, for example, altering binding strengths, regulatory protein copy numbers, or DNA loop lengths [17–22]. These studies also encouraged the development of synthetic genetic circuits, where dynamic switches and oscillations can be observed in real-time for single cells using fluorescence [23–25].

These natural and synthetic examples from such earlier work, however, belie the true complexity of gene regulation required to generate a cell’s full repertoire of activities. Indeed, genes operate within sprawling, intricate networks of interactions which together encode a multitude of possible cellular functions. Often, these genes produce proteins, called transcription factors, that alter the expression of other genes. Whether considering resource consumption in *E. coli* [25] or stages of animal development such as digit formation [26–28], vulval development [29, 30], or stem cell differentiation [31–33], such biological systems can precisely tune relevant gene interactions toward many possible distinct phenotypes. This inherent multistability is crucial to cell function, allowing signaling from the environment, metabolic resource constraints, or other sources to induce systems toward what at times can be dramatically different end states.

Determining from experimental data alone the exact regulatory interactions that collectively drive multistable dynamics, however, is another matter. Indeed, the sensitivities of dynamics and steady state outcomes to both internal and external tuning are challenging to disentangle. To better understand these dynamics, we seek to explore motifs that establish the building blocks for multistability in higher-order networks.

One regulatory motif that has emerged as an integral component of natural and synthetic genetic circuit design is the bistable toggle switch, in which each gene produces a protein that represses expression of the other. Study of the lysis vs. lysogeny decision in bacteriophage lambda over the 1960-80s established the significance of this motif experimentally [12, 13, 35, 36], and subsequent research has modeled such motifs extensively using Hill models [23, 37, 38]. As a two-state model, however, the bistable switch sheds only partial light on multistability in

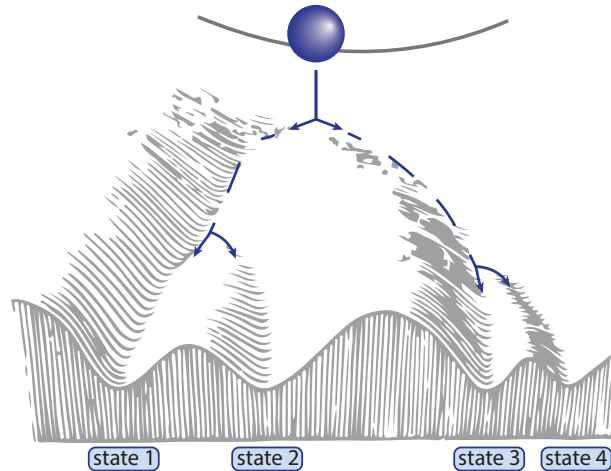


Figure 3.1: The Waddington (or epigenetic) landscape metaphor for cell differentiation, where the cell is represented as a ball rolling down a slope (adapted from [34]).

phenotypic expression.

Insights into the emergence of multistable dynamics in larger networks rely not only on the choice of model but also on the approach used. Existing literature on multistable cell fates during development, for example, has made significant strides by describing gene network dynamics with a Waddington landscape [34], a conceptual model that, while not strictly true, offers an intuitive analogy for visualizing how cells navigate developmental decisions. Originally proposed by the developmental biologist Conrad Waddington in 1957, the landscape metaphor, as depicted in Fig. 3.1, imagines a cell as a ball rolling across a contoured surface of hills and valleys, where the topography of the surface represents the collective influence of the underlying gene regulatory network on cell state. The valleys, or low-energy wells, correspond to stable cell fates, i.e., “attractors” in the language of dynamical systems, toward which cells are naturally drawn and in which they tend to remain once committed. The ridges and hills separating these valleys represent unstable states that cells must overcome to transition from one fate to another, with the height and steepness of these barriers encoding the relative stability of each fate and the difficulty of transitioning between them.

In this picture, a pluripotent stem cell then begins near the top of the landscape where the surface is relatively flat and multiple valleys are accessible, and rolls progressively downhill as development proceeds, passing through a series of bifurcation points, denoting critical junctures at which a single developmental trajectory splits into two or more distinct possibilities. The cell ultimately comes to rest in a

terminal differentiated fate. These bifurcations are not arbitrary, but rather emerge from the topology of the underlying gene regulatory network, and particularly from the presence of mutually antagonistic transcription factors that drive cells toward one of several discrete expression states through double-negative feedback or mutual repression.

The Waddington framework remains a powerful modern tool for mathematically grounding the theory of dynamical systems, where the landscape is reconstructed as a quasi-potential surface derived from the governing equations of the network, and where the valleys, ridges, and bifurcation points correspond to fixed points, saddle points, and bifurcations in the formal mathematical sense. This synthesis of Waddington’s developmental intuition with the quantitative tools of nonlinear dynamics has allowed researchers to further explore the question of how the architecture of a gene regulatory network determines the number, depth, and accessibility of stable cell fates. In turn, one can also ask how perturbations to that architecture, whether through mutation, noise, or external signals, can reshape the landscape and redirect developmental trajectories.

Recent studies in this spirit [39–43] have largely used geometric approaches that either coarse-grain representations to a set of network-free flow equations, or focus on systems that obey a gradient-like description. Given that many nontrivial networks contain feedback and cyclic interactions that break the monotonicity required for gradient-like behavior, however, other studies focus on the rotational effects that impact a system’s trajectory through the potential landscape [44–46]. These perspectives have been instrumental in characterizing a range of regulatory networks, often using abstract topological parametrizations and catastrophe theory. In this study, we build upon these rich traditions by directly linking bifurcation analysis of non-gradient multistable networks to experimentally accessible tuning knobs. In particular, we incorporate a concrete thermodynamic interpretation of induction to examine its explicit role in shaping multistable dynamics.

The activity of transcription factors often depends on the presence or absence of allosteric effector molecules [47–56]. When targeting a repressor, for example, an effector can act as an inducer by binding and stabilizing a protein in its inactive configuration, which suppresses its function and induces expression. Certain transcription factors are also known to have context-dependent behavior, acting as repressors at one promoter site along the DNA and as activators at another [57, 58]. Exploring different interpretations of the mechanistic role of effectors may then

reveal critical differences in tuned expression that allow for complementary experimental investigations.

Accordingly, our aim is to examine the role of the effector concentration as a system parameter, and by tuning this parameter alone, to directly observe corresponding dynamical shifts that occur. This framework offers a perspective with parametrizations that are accessible to the experimentalist while also inherent to cell activity itself. For example, researchers can directly input external non-metabolizable effectors to study the dynamic shifts observed in theory [59]. Cells also naturally leverage one or more effectors to tune dynamics, with two inducers driving cell fate determination in the lac operon and in cell differentiation during development, among other settings [38, 60]. It is important to recognize, of course, that tuning extracellular effector concentrations does not always exactly correspond to intracellular levels due to internal processes that can increase hypersensitivity, and that the internal concentrations of multiple distinct effectors can at times be codependent [21]. Nonetheless, effector concentration remains a valuable measure of a cell's ability to regulate its gene expression dynamics, especially in light of the extensive experimental evidence emphasizing their significance as natural tuning knobs.

To analyze the roles of effector molecules in the emergence of multistability, we specifically study extensions of the toggle switch to three genes. These motifs remain simple enough to visualize and physically interpret, yet also provide an additional layer of tunable gene interaction parameters that allows for more complex multistable dynamics – in this case, tristability and beyond. In exploring how a set of more than two genes couple to collectively decide among multiple possible cell fates, we also gain insights into how these interactions drive dynamics in larger and more complex networks.

The three-gene toggle switch, shown in Figure 3.2, provides a powerful basis for examining the rise of tristability and higher-order multistability in a range of biological contexts. The motif is most directly useful in a synthetic setting, where dynamic shifts can be tuned precisely through experiments. However, the model can also help uncover how various sets of three (or more) key genes interact to generate the multistable cell fates observed in nature. For example, experimental work has identified sets of three “master regulator” genes that interact to drive protein expression toward three or more distinct cell fate phenotypes, such as in T helper cell differentiation [61] and stem cell differentiation [62].

Recent work has also begun to build a comprehensive picture of stable expression in

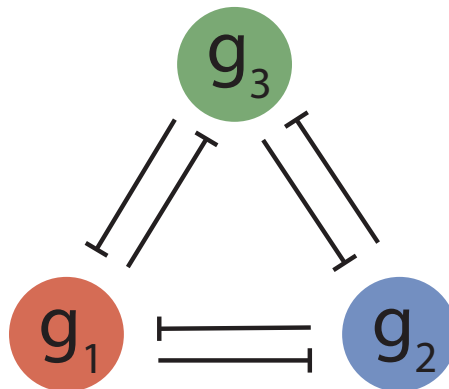


Figure 3.2: Network of three mutually-repressing genes. Transcribing each gene g_i produces a repressor with average concentration denoted by R_i .

such three-gene networks [39, 63, 64]. Our study focuses instead on alternative approaches to induction in a Hill model for the three-gene toggle. Within this reframed context for tuning dynamics, we then investigate not only how the mechanisms of induction shift transcription factor activities and stable state expression levels, but also how the dynamic topologies of the resulting fixed point landscapes evolve.

We begin in Section 3.1 by defining our model for the three-gene toggle switch, and showing how it mathematically incorporates the regulatory activity of transcription factors as allosterically-inducible proteins. From this initial framework, Section 3.2 demonstrates the different dynamics that become possible as inducer concentrations rise. In Section 3.3 we highlight how the thresholds, i.e., bifurcations, that separate different dynamic regimes shift when perturbed away from an initial assumed symmetry in gene interactions. Section 3.4 explores the impact of self-activation on the complexity of dynamics and the number of stable steady states possible for our three-gene switch. In particular, we find that the biological interpretation of dual repression and self-activation, whether arising from binding exclusivity or the effector's role, can significantly affect the types of dynamics that unfold. In so doing, our work sheds light on the complexity of dynamics possible from simple modeling choices, and on just how precisely inducer concentrations must be tuned under different conditions for a system to access various dynamic regimes. The diverse range of input-output responses demonstrated across the different three-gene networks presented here motivates further study of how our results generalize to higher-order gene regulatory architectures.

3.1 The three-gene toggle switch

We first consider the most direct extension of the two-gene bistable toggle switch to three genes, as depicted in Fig. 3.2. Transcribing a given gene g_i generates a protein R_i called a transcription factor, which can in turn bind to the promoter region of either remaining gene. In this particular form of the model, the transcription factor prevents RNA polymerase (RNAP) from binding and initiating transcription, thereby repressing expression. We begin by discussing how to model the dynamics of this system of repressors before demonstrating the role of induction in governing how dynamical behavior evolves as a function of inducer concentration(s).

3.1.1 The baseline model

The modeling of stability in basic switches and other regulatory motifs has long been explored using the tools of dynamical systems [37, 65–68]. To describe the expression patterns that arise from the three-gene toggle switch architecture shown in Fig. 3.2, we define a dynamic equation for each transcription factor repressor R_i of the form

$$\frac{dR_i}{dt} = a_i p_i^{\text{expr}}(R_i, \{R_{j \neq i}\}) - \frac{1}{\tau_i} R_i. \quad (3.1)$$

This general form assumes that the dynamics of gene transcription, i.e., the production of mRNA transcripts, evolves at a timescale such that production can be measured equivalently from the concentration of output repressor R_i . The first term of Eqn. 3.1 characterizes protein production at a maximal rate a_i , and depends on the probability p_i^{expr} that expression can occur. The second term accounts for protein degradation at a rate $1/\tau_i$.

Fig. 3.3(A) lists the possible regulatory states and corresponding statistical weights that contribute to the probability of expression p_i^{expr} . This approach to describing biological regulation draws from well-established statistical mechanical principles [14–16, 19–22, 69–72]. From this table, and equivalent definitions for expression of repressors 2 and 3, we define the dynamics of the corresponding three-gene circuit by the set of differential equations

$$\frac{dR_1}{dt} = \frac{a_1}{\left[1 + \left(\frac{R_2}{K_{12}}\right)^n\right] \left[1 + \left(\frac{R_3}{K_{13}}\right)^n\right]} - \frac{1}{\tau_1} R_1, \quad (3.2)$$

$$\frac{dR_2}{dt} = \frac{a_2}{\left[1 + \left(\frac{R_1}{K_{21}}\right)^n\right] \left[1 + \left(\frac{R_3}{K_{23}}\right)^n\right]} - \frac{1}{\tau_2} R_2, \quad (3.3)$$

$$\frac{dR_3}{dt} = \frac{a_3}{\left[1 + \left(\frac{R_1}{K_{31}}\right)^n\right] \left[1 + \left(\frac{R_2}{K_{32}}\right)^n\right]} - \frac{1}{\tau_3} R_3. \quad (3.4)$$

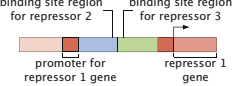
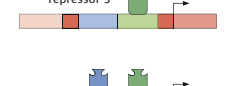
EXPRESSION OF REPRESSOR 1					
(A) WITHOUT INDUCTION			(B) WITH INDUCTION		
STATE	WEIGHT	RATE	STATE	WEIGHT	RATE
	1	a_1		1	a_1
	$\left(\frac{R_2}{K_{12}}\right)^n$	0		$\left(\frac{p_{\text{act}}(c_2)R_2}{K_{12}}\right)^n$	0
	$\left(\frac{R_3}{K_{13}}\right)^n$	0		$\left(\frac{p_{\text{act}}(c_3)R_3}{K_{13}}\right)^n$	0
	$\left(\frac{R_2}{K_{12}}\right)^n \left(\frac{R_3}{K_{13}}\right)^n$	0		$\left(\frac{p_{\text{act}}(c_2)R_2}{K_{12}}\right)^n \left(\frac{p_{\text{act}}(c_3)R_3}{K_{13}}\right)^n$	0

Figure 3.3: Expression of repressor 1 in the three-gene toggle switch, as described by a Hill function model. Equivalent definitions apply to expression of repressors 2 and 3. (A) Thermodynamic states, weights, and rates for expression of repressor 1 (R_1). The regulating repressors (R_2 and R_3) bind non-exclusively to the target promoter region to suppress gene transcription. Each repressor R_i binds at the promoter region for repressor R_1 with affinity K_{1i} and cooperativity n . (B) Thermodynamic states, weights, and rates for expression of repressor R_1 in the presence of inducers. Expression now depends on the *active* concentration of each repressor R_i , which is determined by a distinct inducer at concentration c_i , defining the probability of activity $p_{\text{act}}(c_i)$.

Each repressor R_j binds non-exclusively with affinity K_{ij} to the promoter region for the gene that expresses repressor R_i . This repression coefficient K_{ij} reflects the characteristic concentration of repressor R_j required to strongly regulate R_i 's expression. In other words, when $R_j = K_{ij}$, the produced concentration of repressor R_i is half its maximum possible value.

Note that these equations describe transcription factor binding phenomenologically through a Hill function model with coefficient n [73, 74]. This approach coarse-grains out the molecular detail of precise binding site occupancies found in a strictly thermodynamic model, instead representing its high cooperativity limit. The Hill coefficient n thus does not necessarily correspond to the number of bound repressor molecules, but rather measures the sensitivity of output response to binding.

While there are advantages to the specificity of thermodynamic models [75], using them to evaluate stability profiles quickly becomes intractable in higher-dimensional networks, including those analyzed here. We therefore take a Hill model approach for the remainder of the discussion. Even without full knowledge of precise binding site occupancies, the Hill function offers a strong fit to empirical data for gene regulatory dynamics [23, 76]. The model also reflects the Hopfield barrier for the

sharpness of input-output response [77], meaning that for a given coefficient n , the Hill model reflects the strongest, most sigmoidal input-output response possible for a system without energy expenditure. The approach thus allows us to investigate dynamics under the most input-sensitive model conditions, and to observe its maximum possible dynamic complexity.

We now have a model for the toggle switch that depends on various theoretically accessible parameters, specifically dissociation constants K_{ij} , production rates a_i , and degradation rates $1/\tau_i$. As pointed out previously, however, these parameters as written are not easily accessed experimentally, and are disconnected from the underlying biology that controls them within living cells. While the current model indicates that regulation is controlled by the total concentrations of transcription factors, these proteins are often themselves controlled by the allosteric binding of effector molecules. This implies that cells respond to changes in the number of *active* transcription factors.

In the following subsections we will use statistical mechanics to outline the role of effector molecules (specifically inducers) in defining transcription factor activity.

3.1.2 A statistical mechanical model for allosteric induction

Shifting to an allosteric description of gene regulation, we now argue formally that gene expression depends not on the total concentration of the regulating transcription factor, $[\text{TF}_{\text{tot}}]$, but rather on the *active* concentration thereof, i.e.,

$$[\text{TF}_{\text{act}}] = p_{\text{act}}(c)[\text{TF}_{\text{tot}}], \quad (3.5)$$

where $p_{\text{act}}(c)$ is the probability that the transcription factor is active as a function of effector concentration c . This probability can be defined by the Monod-Wyman-Changeux (MWC) model [47–50, 53–56], which states that when an effector at concentration c can bind allosterically at m sites on a transcription factor, the probability of that transcription factor being active is

$$p_{\text{act}}(c) = \frac{\left(1 + \frac{c}{K_A}\right)^m}{\left(1 + \frac{c}{K_A}\right)^m + e^{-\beta\Delta\epsilon} \left(1 + \frac{c}{K_I}\right)^m}, \quad (3.6)$$

where K_A and K_I are the dissociation constants for active and inactive repressor states, respectively, $\beta = 1/k_B T$, and $\Delta\epsilon = \epsilon_I - \epsilon_A$ is the energy difference between inactive (ϵ_I) and active (ϵ_A) states. Repressors are driven toward the inactive state upon effector binding when $K_I < K_A$. Effectors obeying this condition are referred

to as inducers. Conversely, when $K_I > K_A$, repressors are driven toward the active state, and effectors act as corepressors. Examples of both phenomena have been experimentally isolated in nature [78–80], but for simplicity we will assume the case of $K_I < K_A$ in what follows and refer exclusively to inducers.

Note that while we invoke the MWC model to describe allostery, we did not necessarily have to choose this definition for effector binding. We could also use the sequential model derived by Koshland, Némethy, and Filmer (KNF), which would describe effector binding as sequential and local [50, 56]. The transcription factor’s conformation, rather than changing through a single coordinated quaternary structure transformation as in the MWC interpretation, would instead change step-wise at the subunit level. The MWC model, however, is preferred, especially for its experimentally-supported accuracy in describing cooperative proteins and its simpler, elegant parametrization.

Fig. 3.4(A) plots one possible probability curve $p_{\text{act}}(c)$ in the induction setting with $K_I < K_A$ for a fixed set of parameters. This function follows standard sigmoidal behavior, with a sufficient increase in inducer concentration rendering repressor inactive. Depending on how we define parameters, we can alter several key properties of the input-output response from this probability function. The leakiness represents the amount of activity at saturating concentrations of inducer, i.e., $p_{\text{act}}(c \rightarrow \infty)$, and the saturation conversely denotes the amount of activity in the absence of inducer ($p_{\text{act}}(c = 0)$). The inducer concentration at which the probability is half maximal is denoted as the EC_{50} value, and the sharpness of the input-output response curve at this inflection point rises with an increasing Hill coefficient n . The MWC parameters chosen therefore determine how effectively inducers suppress the activity of repressors and the sensitivity of the output response to an increase in inducer concentration.

Fig. 3.4(B) highlights several viable probability curves, defined by MWC parameters bounded to biologically permissible ranges derived from Eqns. 3.50 and 3.51 in Appendix 3.A. We assume throughout that there are $m = 2$ binding sites for inducers on repressors. For fixed $\beta\Delta\epsilon = 4$ and $K_A = 150 \mu\text{M}$, Fig. 3.4(B) then maps the probability of activity as a function of molar inducer concentration for increasing dissociation constant $K_I < K_A$. As the ratio K_A/K_I decreases, approaching its lower bound (denoted in cyan as $K_I = 6.77 \mu\text{M}$ in Fig. 3.4), the curve’s inflection point shifts toward higher inducer concentrations. This occurs because as K_I rises and the inducer becomes less tightly bound to the inactive compared to the active

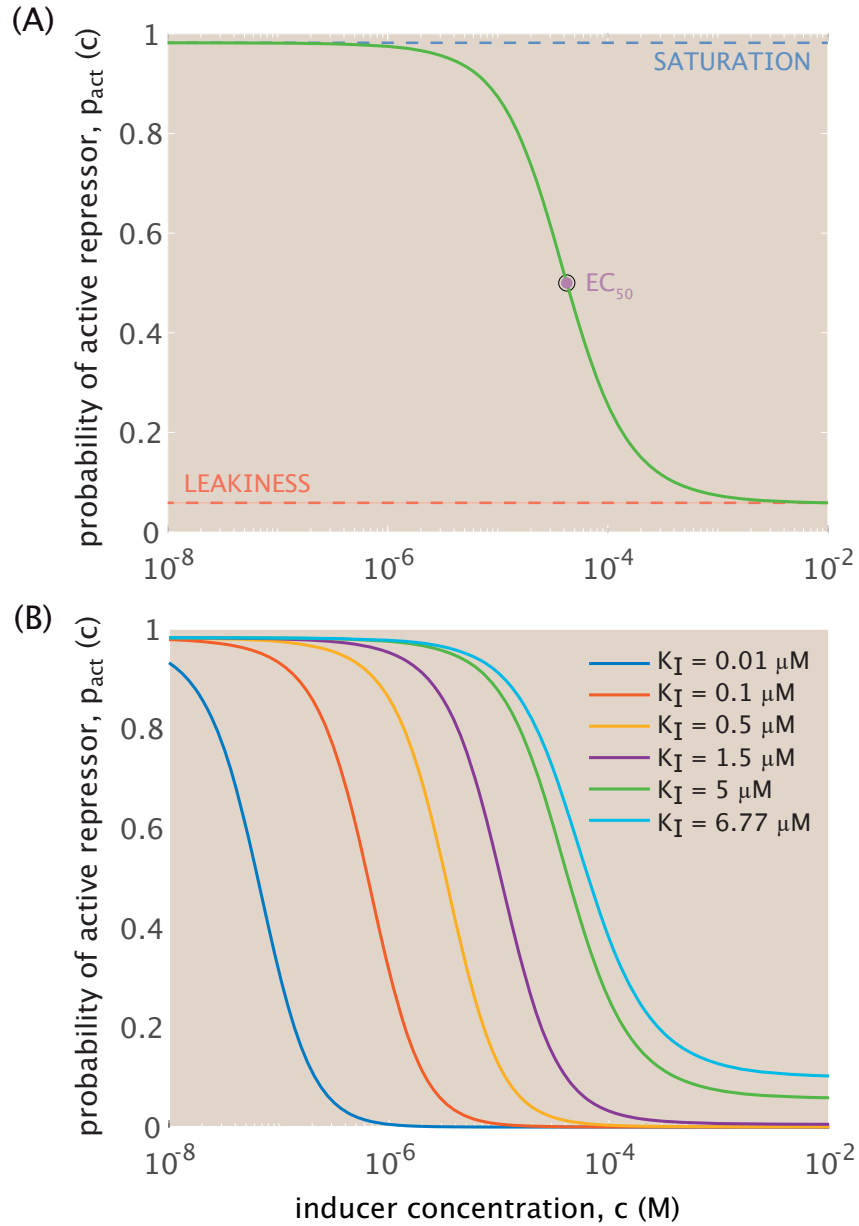


Figure 3.4: Repressor activity as a function of inducer concentration c , with $K_I < K_A$. (A) The probability of active repressor as a function of inducer concentration, with $m = 2$, $\Delta\epsilon = 4 k_B T$, $K_A = 150 \mu M$, and $K_I = 5 \mu M$. The saturation and leakiness limits are denoted in blue and orange, respectively, and the EC_{50} , i.e., inducer concentration at which the probability is half maximal, is marked in purple. (B) Evolution of the probability for different values of K_I , with the curve in panel (A) shown again in green. The shift in probability spans the range of allowable K_I values for the specified parameters up to the boundary at $K_I = 6.77 \mu M$, derived from Appendix 3.A.

repressor, a higher inducer concentration is necessary to sequester inactive repressors equivalently. Given that the choice of parameters in Fig. 3.4(A) is also within the biologically reasonable regime, we focus on these parameters for the remainder of our study, unless otherwise stated.

Note that increasing the number of inducer binding sites m for the target repressor protein would increase the cooperativity of inducer-repressor interactions, and thus increase the sensitivity of response to the presence of inducer. This would make the slope of the probability function steeper at the inflection point. Keeping all other properties fixed, an increase in m also decreases the inducer concentration required to suppress repressor activity.

While the role of induction has been widely discussed and used in experimental literature, theoretical studies leveraging this perspective for parametrization remain ongoing, with recent work demonstrating the utility of induction in modeling the dynamics of simple gene regulatory motifs [75, 81]. This approach, however, has not yet been expanded to the more intricate regulatory networks that are standard in nature. Toward this end, we now incorporate the MWC description for the probability of active repressor into our model for gene expression dynamics in the three-gene toggle switch.

3.1.3 The baseline model as a function of inducer concentrations

Suppose now that each repressor produced from the three-gene toggle circuit in Fig. 3.2 can be controlled by its own inducer, each at concentrations c_1 , c_2 , and c_3 . We will make the reasonable assumption that each repressor responds to induction with the same sensitivity and thus in accord with the same probability function. As shown in Fig. 3.3(B), this means that expression of repressor 1 depends on the concentrations of active repressors 2 and 3, namely, $p_{\text{act}}(c_2) \times R_2$ and $p_{\text{act}}(c_3) \times R_3$. We similarly scale the regulatory contribution of R_1 to expression of R_2 and R_3 by the probability $p_{\text{act}}(c_1)$. Eqns. 3.2 - 3.4 thus become

$$\frac{dR_1}{dt} = \frac{a_1}{\left[1 + \left(\frac{p_{\text{act}}(c_2)R_2}{K_{12}}\right)^n\right] \left[1 + \left(\frac{p_{\text{act}}(c_3)R_3}{K_{13}}\right)^n\right]} - \frac{R_1}{\tau_1}, \quad (3.7)$$

$$\frac{dR_2}{dt} = \frac{a_2}{\left[1 + \left(\frac{p_{\text{act}}(c_1)R_1}{K_{21}}\right)^n\right] \left[1 + \left(\frac{p_{\text{act}}(c_3)R_3}{K_{23}}\right)^n\right]} - \frac{R_2}{\tau_2}, \quad (3.8)$$

$$\frac{dR_3}{dt} = \frac{a_3}{\left[1 + \left(\frac{p_{\text{act}}(c_1)R_1}{K_{31}}\right)^n\right] \left[1 + \left(\frac{p_{\text{act}}(c_2)R_2}{K_{32}}\right)^n\right]} - \frac{R_3}{\tau_3}. \quad (3.9)$$

We can simplify the model to a dimensionless form through several assumptions. First, we assume for analytic convenience that all repressors are produced and degraded at the same maximal rates, independent of the underlying dynamics of transcription. This sets $a_1 = a_2 = a_3 \equiv a$ and $\tau_1 = \tau_2 = \tau_3 \equiv \tau$. We also assume that a given repressor binds to different promoter sites with the same affinity, such that $K_{21} = K_{31} \equiv K_1$, $K_{12} = K_{32} \equiv K_2$, and $K_{13} = K_{23} \equiv K_3$. This allows us to re-express Eqns. 3.7 - 3.9 in dimensionless form, transforming $\bar{t} = t/\tau$, $\bar{R}_i = R_i/K_i$, and $\bar{a}_i = \tau a/K_i$ to obtain

$$\frac{d\bar{R}_1}{d\bar{t}} = \frac{\bar{a}_1}{[1 + (p_{\text{act}}(c_2)\bar{R}_2)^n][1 + (p_{\text{act}}(c_3)\bar{R}_3)^n]} - \bar{R}_1, \quad (3.10)$$

$$\frac{d\bar{R}_2}{d\bar{t}} = \frac{\bar{a}_2}{[1 + (p_{\text{act}}(c_1)\bar{R}_1)^n][1 + (p_{\text{act}}(c_3)\bar{R}_3)^n]} - \bar{R}_2, \quad (3.11)$$

$$\frac{d\bar{R}_3}{d\bar{t}} = \frac{\bar{a}_3}{[1 + (p_{\text{act}}(c_1)\bar{R}_1)^n][1 + (p_{\text{act}}(c_2)\bar{R}_2)^n]} - \bar{R}_3. \quad (3.12)$$

Finally, for most of the discussion we consider a symmetric system in which all repressors bind at all sites with equal affinity such that $K_1 = K_2 = K_3 \equiv K$. (We will examine how breaking this symmetry affects shifts in dynamics in later sections.) This means that $\bar{a}_1 = \bar{a}_2 = \bar{a}_3 = \bar{a}$, and we arrive at the system of equations

$$\frac{d\bar{R}_1}{d\bar{t}} = \frac{\bar{a}}{[1 + (p_{\text{act}}(c_2)\bar{R}_2)^n][1 + (p_{\text{act}}(c_3)\bar{R}_3)^n]} - \bar{R}_1, \quad (3.13)$$

$$\frac{d\bar{R}_2}{d\bar{t}} = \frac{\bar{a}}{[1 + (p_{\text{act}}(c_1)\bar{R}_1)^n][1 + (p_{\text{act}}(c_3)\bar{R}_3)^n]} - \bar{R}_2, \quad (3.14)$$

$$\frac{d\bar{R}_3}{d\bar{t}} = \frac{\bar{a}}{[1 + (p_{\text{act}}(c_1)\bar{R}_1)^n][1 + (p_{\text{act}}(c_2)\bar{R}_2)^n]} - \bar{R}_3. \quad (3.15)$$

The original model in Eqns. 3.2 - 3.4 thus collapses from a high-dimensional parameter space to at most a three-dimensional space defined by inducer concentrations c_1 , c_2 , and c_3 , which can be directly controlled experimentally. Note that while the symmetry introduced here is not necessary, it makes the underlying dynamics more transparent.

3.2 Regimes of multistability

For a given Hill exponent n and maximal (dimensionless) production rate $\bar{a}$, we now explore the dynamic profiles possible for the three-gene toggle switch as a function of inducer concentrations. In particular, we quantify the thresholds, or bifurcations, as a function of inducer that bring about fundamental shifts in dynamics. Our graphical analysis in this section will consider the large cooperativity case where

$n = 4$, along with setting $\bar{a} = 2$. While we will also examine how much the dynamic thresholds shift as we alter parameters, our focus in this section is specifically on the effects of induction at various intensities. To this end, we begin by considering a system tuned by a single inducer.

3.2.1 Single inducer

Suppose a single inducer controls the dynamics of the three-gene toggle switch by regulating the activity of R_1 . This means that $p_{\text{act}}(c_1) \equiv p_{\text{act}}(c)$ and $p_{\text{act}}(c_2) = p_{\text{act}}(c_3) = 1$ such that $\bar{R}_2$ and $\bar{R}_3$ are always maximally active. Substituting these definitions into Eqns. 3.13 - 3.15, we obtain the single-inducer model

$$\frac{d\bar{R}_1}{d\bar{t}} = \frac{\bar{a}}{(1 + \bar{R}_2^n)(1 + \bar{R}_3^n)} - \bar{R}_1, \quad (3.16)$$

$$\frac{d\bar{R}_2}{d\bar{t}} = \frac{\bar{a}}{[1 + (p_{\text{act}}(c)\bar{R}_1)^n](1 + \bar{R}_3^n)} - \bar{R}_2, \quad (3.17)$$

$$\frac{d\bar{R}_3}{d\bar{t}} = \frac{\bar{a}}{[1 + (p_{\text{act}}(c)\bar{R}_1)^n](1 + \bar{R}_2^n)} - \bar{R}_3. \quad (3.18)$$

For a given inducer concentration c , one then sets the above equations to zero and solves to obtain the steady state expressions.

We can first gain some analytical insight from the model by considering certain limit cases, specifically those regarding the existence of single-repressor-dominant steady states, as a function of inducer concentration. Consider the existence of an $\bar{R}_2$ -dominant steady state whereby $\bar{R}_1 = \bar{R}_3 = \varepsilon$ such that $\bar{R}_1^n, \bar{R}_3^n \rightarrow 0$. It follows from setting Eqn. 3.17 to zero that such a steady-state requires $\bar{R}_2 = \bar{a}$. By similarly setting Eqns. 3.16 and 3.18 to zero, it also follows that $\bar{R}_{1,3} = \bar{a}/(1 + \bar{R}_2^n)$. We therefore conclude that the system always has the following steady state regardless of inducer concentration:

$$\bar{R}_{ss} = (\bar{R}_1, \bar{R}_2, \bar{R}_3) = \left(\frac{\bar{a}}{1 + \bar{a}^n}, \bar{a}, \frac{\bar{a}}{1 + \bar{a}^n} \right). \quad (3.19)$$

Similar logic for the existence of an $\bar{R}_3$ -dominant state leads to a corresponding predicted steady state

$$\bar{R}_{ss} = (\bar{R}_1, \bar{R}_2, \bar{R}_3) = \left(\frac{\bar{a}}{1 + \bar{a}^n}, \frac{\bar{a}}{1 + \bar{a}^n}, \bar{a} \right). \quad (3.20)$$

The conditions for an $\bar{R}_1$ -dominant steady state, where $\bar{R}_2^n, \bar{R}_3^n \rightarrow 0$, follow from the same logic in Eqns. 3.16 - 3.18 as $\bar{R}_1 = \bar{a}$ and

$$\bar{R}_2 = \bar{R}_3 = \frac{\bar{a}}{1 + (p_{\text{act}}(c)\bar{R}_1)^n}. \quad (3.21)$$

Unlike the $\bar{R}_2$ and $\bar{R}_3$ -dominant states in Eqns. 3.19 and 3.20, however, Eqn. 3.21 indicates that for the $\bar{R}_1$ -dominant steady state the concentrations of non-induced repressors $\bar{R}_2$ and $\bar{R}_3$ depend on inducer concentration c . As c increases and the probability of activity decreases, the corresponding repressor concentrations at steady state rise. This also means that at a sufficiently high inducer concentration, $\bar{R}_2^n, \bar{R}_3^n \rightarrow 0$ no longer holds and the steady state can no longer exist. Assuming $\bar{R}_2 = \bar{R}_3 \leq \epsilon$ satisfies the specified condition, we derive the inducer bifurcation threshold by substituting this inequality into Eqn. 3.21 and rearranging to obtain the result

$$p_{\text{act}}(c) \geq \frac{1}{\bar{a}} \sqrt[n]{\frac{\bar{a}}{\epsilon} - 1}. \quad (3.22)$$

At equality, the probability in Eqn. 3.22 represents a bifurcation threshold, where for lower probabilities (i.e, higher inducer concentrations) the system can no longer exist at an $\bar{R}_1$ -dominant steady state.

Eqns. 3.19 - 3.22 therefore indicate that at low inducer concentration the system can arrive at a steady state in which any gene among the three can dominate expression. When the probability of activity drops below the threshold in Eqn. 3.22, however, the presence of inducer is sufficient to suppress $\bar{R}_1$ activity, and the system can no longer maintain a steady state with $\bar{R}_1$ -dominant expression. The sensitivity of $\bar{R}_1$ activity to tuning then depends on cooperativity (as approximated by Hill coefficient n) and on the protein expression rate $\bar{a}$.

Fig. 3.5(A) plots the probability threshold in Eqn. 3.22 as a function of n and $\bar{a}$. Note that the beige region of this heatmap depicts a regime where an $\bar{R}_1$ -dominant steady state is not possible at any inducer concentration. When $\bar{a} \gtrsim 2$, increasing the expression rate has little effect on this cooperativity bound. Fig. 3.5(B) plots the same relationship between the parameters, now explicitly as a function of inducer concentration (from the probability definition of Eqn. 3.6 in Section 3.1.2).

Fig. 3.5 tracks cooperativities up to $n = 10$. While it is not common for known multimeric transcription factors to extend beyond tetramers, plotting higher n acknowledges the uncertainty that remains in the field regarding the cooperative mechanics of eukaryotic regulation and particularly of enhancers [82]. Indeed, there are emerging cases of eukaryotic transcription factors functioning as higher-order homomultimeric complexes, such as FOXP3 in regulatory T-cells [83]. It is therefore useful to observe whether such higher-order cooperativities would influence bifurcations in the system.

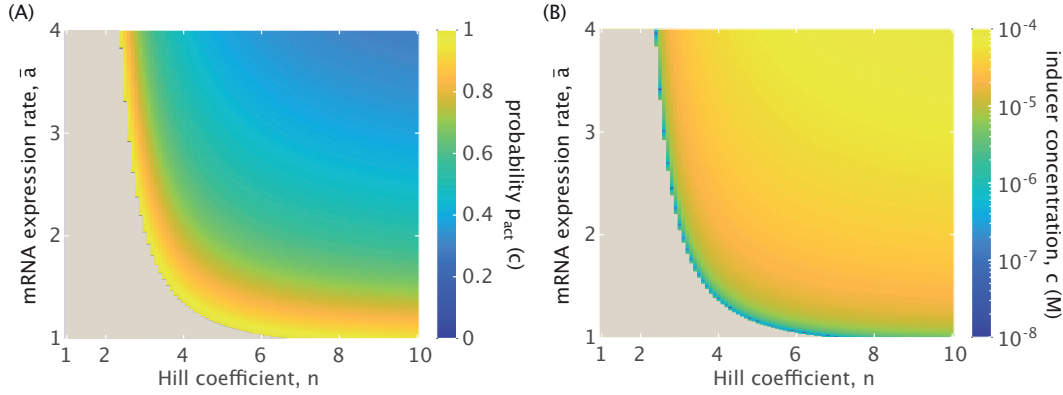


Figure 3.5: Heatmaps tracking the bifurcation threshold beyond which the three-gene toggle switch can no longer have an $\bar{R}_1$ -dominant steady state and be tristable. Note that we define the limit steady state case here as satisfying $\bar{R}_2^n, \bar{R}_3^n \rightarrow 0$, with $\bar{R}_2 = \bar{R}_3 \leq \varepsilon$. The results shown are specific to the choice $\varepsilon = \sqrt[4]{0.01}$. (A) The threshold probability of active repressor as a function of Hill coefficient n and mRNA expression rate $\bar{a}$. Note that the beige-shaded region for smaller axis values denotes a regime in which the steady state is never possible at any inducer concentration. (B) The threshold specified in Fig. 3.4(A), now in terms of inducer concentration for the MWC model.

We find that the bifurcation threshold for the shift between tristable and bistable dynamics is most sensitive to parameter changes at the onset of the expression domain (i.e., at minimum required n and $\bar{a}$). For $n \gtrsim 4$ and $\bar{a} \gtrsim 2$, both parameters are sufficiently large that increasing either has little effect on the threshold probability and corresponding inducer concentration. Given also the existence of homotetrameric transcription factors such as p53 that are crucial to cell fate decisions [84–86], it is therefore reasonable to choose $n = 4$ as representative of dynamics in the highly-cooperative regime, and we do this for the remainder of the chapter.

To show more completely how the dynamics evolve with increasing inducer concentration, we now apply the specific parameters $n = 4$ and $\bar{a} = 2$ to Eqns. 3.16 - 3.18. At each inducer concentration, we numerically solve for the fixed point repressor concentrations. We then perform a linear stability analysis on each fixed point. We do this by evaluating the Jacobian which, for our three-gene network modeled by differential equations of the form $d\bar{R}_i/dt = f_i(\bar{R}_1, \bar{R}_2, \bar{R}_3)$, is a 3×3 matrix with entries

$$J_{ij} = \frac{\partial f_i}{\partial \bar{R}_j}. \quad (3.23)$$

The Jacobian provides a first-order linear approximation of behavior evaluated near a fixed point, with its eigenvectors specifying the primary directions of dynamical

motion from the fixed point, and with its eigenvalues reflecting the rates at which perturbations to the fixed point grow or decay along the corresponding eigenvectors. If all eigenvalues have negative real parts, perturbation in any eigenvector direction of expression space will decay back to the fixed point, indicating it to be stable. If any of the eigenvalues have a positive real part, however, perturbation in the corresponding eigenvector direction(s) will grow exponentially and the system will move away from the fixed point, rendering the fixed point unstable.

For the three-gene models we examine, the unstable fixed points are characterized as saddle points. Mathematically, this distinction arises because at least one eigenvalue is negative. Dynamic trajectories local to the fixed point thus move away in some dimensional directions (positive eigenvalues) and approach in others (negative eigenvalues). The number of positive eigenvalues determines the number of dimensional directions of “escape” from the fixed point and thus the “index” of the saddle. If we consider the dynamics of gene expression within a potential landscape, expression levels local to the highest index saddle essentially follow a hierarchical dynamic flow through expression space toward stable states, guided by the presence of saddle points with decreasing index.

Fig. 3.6 plots the resulting changes in the number of fixed points and their expression levels through bifurcation diagrams, isolating the fixed point trajectories of the $\bar{R}_1$ coordinate in panel (A), and the (overlapping) $\bar{R}_2$ and $\bar{R}_3$ coordinates in panel (B). Three types of fixed points emerge from analysis of the Jacobian. Stable equilibria are denoted in blue, unstable index-1 saddle points in red, and unstable index-2 saddles in yellow.

As the inducer concentration increases, the system shifts across three regimes of decreasing dynamic complexity. Fig. 3.6(C)-(E) plot fixed points in expression space for a given inducer concentration within each of these regimes. At low inducer concentration, as highlighted in Fig. 3.6(C), the system is tristable, stabilizing to solely favor expression of one of the three repressors. A sufficiently stochastic system can be perturbed to transition between any pair of these stable points through one of the index-1 saddle points. The system also has an index-2 saddle point at which all repressors are half-maximally expressed. This means that if a system were to have a sufficient concentration of each repressor and initially exist near this index-2 saddle, it would be equally likely for the system to settle at any of the three possible stable states.

At an intermediate inducer concentration, a pair of fixed points annihilate each other

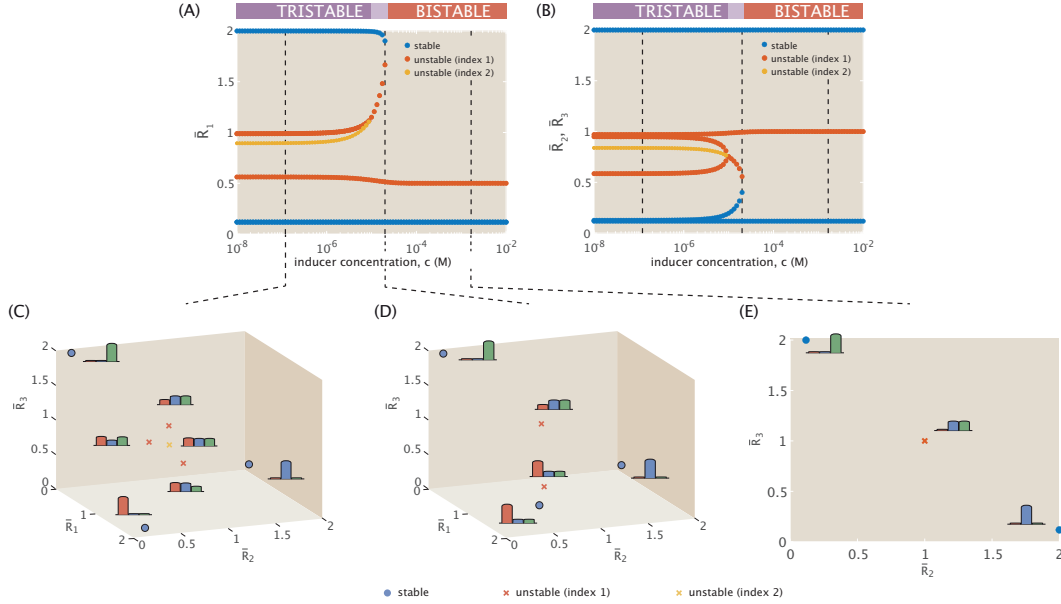


Figure 3.6: Dynamics of the three-gene toggle switch with $n = 4$ and $\bar{a} = 2$ for increasing inducer concentration c . Allosteric regulation is defined by the probability curve shown in Fig. 3.4(A). (A)-(B) Bifurcation diagrams for $\bar{R}_1$, $\bar{R}_2$, and $\bar{R}_3$ steady state expression as a function of inducer concentration. (C) Fixed points for the three-gene toggle switch at a low inducer concentration. (D) Fixed points for the three-gene toggle switch at an intermediate inducer concentration. (E) Fixed points for the three-gene toggle switch at a high inducer concentration. In (C)-(E) the stability of each fixed point is color-coded as in panels (A) and (B), and each fixed point is labeled with its corresponding expression levels, colored as in Fig. 3.2.

and a saddle bifurcation occurs. The resulting intermediate regime, represented in Fig. 3.6(D), is still tristable, but the three stable states are now only connected by two saddle points. From an initial state with sufficient concentrations of all three repressors, the system can now evolve dynamically in one of two ways: by either falling into a bistable potential well favoring $\bar{R}_2$ or $\bar{R}_3$ expression, or by following a trajectory directly stabilizing toward $\bar{R}_1$ -dominant expression. Dynamics are therefore more restricted in this regime than in the low inducer regime, and no longer equally likely to evolve toward any given stable state. Finally, Fig. 3.6(E) shows fixed point expression levels at a high inducer concentration, where the presence of inducer sufficiently suppresses the activity of $\bar{R}_1$ and the system undergoes a saddle bifurcation to collapse down to a bistable switch between $\bar{R}_2$ and $\bar{R}_3$ -dominant expression.

Fig. 3.6 thus allows us to determine the inducer concentration thresholds at which

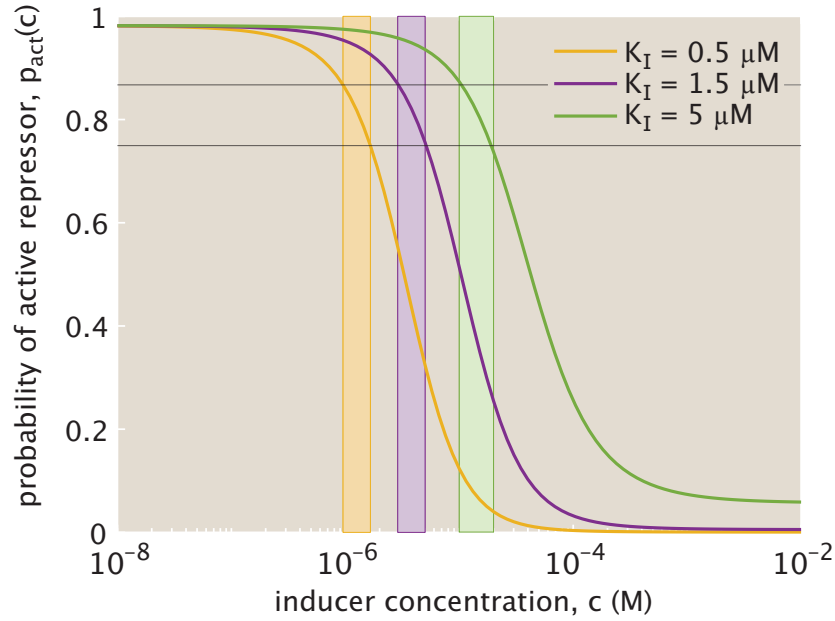


Figure 3.7: Change in inducer concentration range (shaded regions) of the intermediate dynamic regime for increasing dissociation constant K_I , overlaid with the corresponding activity probability curves from Fig. 3.4. Note that regardless of K_I parametrization, the intermediate regime is found within the same range of probabilities $0.749 \leq p_{\text{act}}(c) \leq 0.867$ (bounded in grey).

dynamic shifts occur, including the shift between tristable and bistable dynamics. Given a distribution of stable state expression levels from data, we could then distinguish between systems in the low inducer regime or in the more dynamically restricted intermediate regime. Fig. 3.6(B) also highlights that it is only at intermediate inducer concentrations that the system does not necessarily stabilize to a state exclusively favoring a single gene's expression, and that it is this characteristic that uniquely distinguishes the low vs. intermediate inducer concentration regimes of tristability.

Given the relatively narrow concentration range for the intermediate dynamic regime, it is natural to ask how sensitive this window is to changes in allosteric interaction and to changes in the cooperativity, or ultrasensitivity, of expression response to induction. Regarding sensitivity to allostery, altering the ratio of K_A and K_I for an inducer binding to a repressor shifts the inflection point of the probability curve for activity toward higher inducer concentrations, as previously seen in Fig. 3.4. We thus observe in Fig. 3.7 that the log scale range of inducer concentrations within the intermediate dynamic regime does not change but shifts toward increasing value

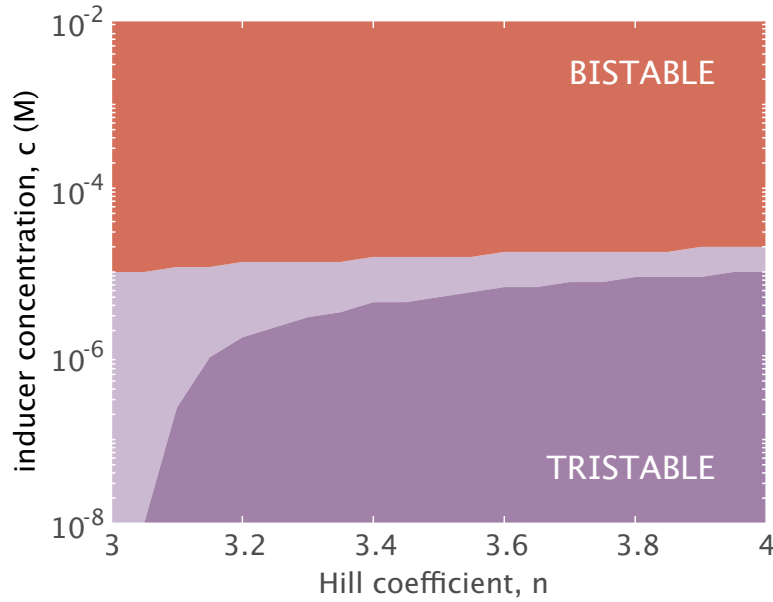


Figure 3.8: Change in the regions of multistability for $n \in [3, 4]$. Each dynamic region is color-coded as denoted in Fig. 3.6, with the seven-fixed point tristable regime (purple), five-fixed point tristable regime (light purple), and three-fixed point bistable regime (red). For $n \lesssim 3$, the light purple region illustrates the most complex dynamic regime that is accessible.

ranges with increasing K_I . Since altering the ratio of the dissociation constants does not affect the slope of the probability curve at the inflection point, the intermediate dynamic regime (for otherwise fixed values of $m = 2$ and $\beta\Delta\epsilon = 4$) exists across different K_A/K_I for the same probability range, $0.749 \leq p_{\text{act}}(c) \leq 0.867$.

The size of the intermediate regime differs, however, with changing cooperativity. Fig. 3.5(B) indicates that at $\bar{a} = 2$, bistability is possible for $n > 3$, but the threshold for shifting from tristability to bistability shifts toward lower inducer concentrations for Hill coefficients decreasing from $n < 4$. It is thus unclear from Fig. 3.5 alone whether the range of inducer concentrations allowing the intermediate dynamic regime would expand, contract, or remain the same for a smaller cooperative Hill coefficient. Indeed, Fig. 3.8 demonstrates how the regimes of multistability evolve in response to varying cooperativity. As the Hill coefficient increases from $n = 3$, the inducer concentration at which the system bifurcates from tristable to bistable dynamics rises slightly in the log scale. The threshold separating the two distinct regimes of tristability, however, does significantly change. The inducer concentration range corresponding to the intermediate regime narrows with increasing

cooperativity, since this implies a more sensitive response in gene expression to repressor binding, one that emphasizes the bias away from $\bar{R}_1$ expression as induction increases. The tristable threshold also begins to approach an apparent asymptote at $n = 4$, confirming our earlier finding that increasing cooperativity to $n = 4$ has little effect on the dynamics.

3.2.2 Two inducers

Activity in gene regulatory networks is not always restricted to control from a single effector. Our framework allows for induction by multiple effectors, whether synthetically through additional experimentally-inputted non-metabolizable inducers [59], or through naturally-occurring coordinated inducer activity. The direct involvement of small molecule effectors can be important, for example, in sugar-inducible genetic switches as they respond to metabolic nutrient availability [60, 87–90]. Much remains to be learned, however, about how natural allosteric effectors coordinate cell fate differentiation [38], and how existing regulators may play a role in inhibitory cross-talk, as evidenced in work on steroid hormone receptors [91]. These possibilities make our study of three-gene toggle switches in the presence of two inducers even more relevant.

We specifically extend to a system in which two repressors $\bar{R}_1$ and $\bar{R}_2$ are controlled by inducer concentrations c_1 and c_2 , such that dynamics evolve by the differential equations

$$\frac{d\bar{R}_1}{d\bar{t}} = \frac{\bar{a}}{(1 + p_{\text{act}}(c_2)\bar{R}_2^n)(1 + \bar{R}_3^n)} - \bar{R}_1, \quad (3.24)$$

$$\frac{d\bar{R}_2}{d\bar{t}} = \frac{\bar{a}}{[1 + (p_{\text{act}}(c_1)\bar{R}_1)^n](1 + \bar{R}_3^n)} - \bar{R}_2, \quad (3.25)$$

$$\frac{d\bar{R}_3}{d\bar{t}} = \frac{\bar{a}}{[1 + (p_{\text{act}}(c_1)\bar{R}_1)^n](1 + p_{\text{act}}(c_2)\bar{R}_2^n)} - \bar{R}_3. \quad (3.26)$$

Fig. 3.9 plots the shifts in dynamic regions of phase space (color-coded by the number of fixed points) for inducer concentrations c_1 and c_2 . Sufficiently high concentrations of both inducers render the target repressors largely inactive, such that the system enters a monostable regime (plotted in blue) that stabilizes to a single point reflecting expression of only the remaining non-induced repressor.

The bifurcation line seen earlier at $p_A(c) \approx 0.725$ in Fig. 3.6 now becomes a set of two linear thresholds in two dimensions, intersecting at approximate inducer concentration $2.3 \times 10^{-5} M$ (i.e., the approximate probability $p_A(c_1) = p_A(c_2) = 0.7$).

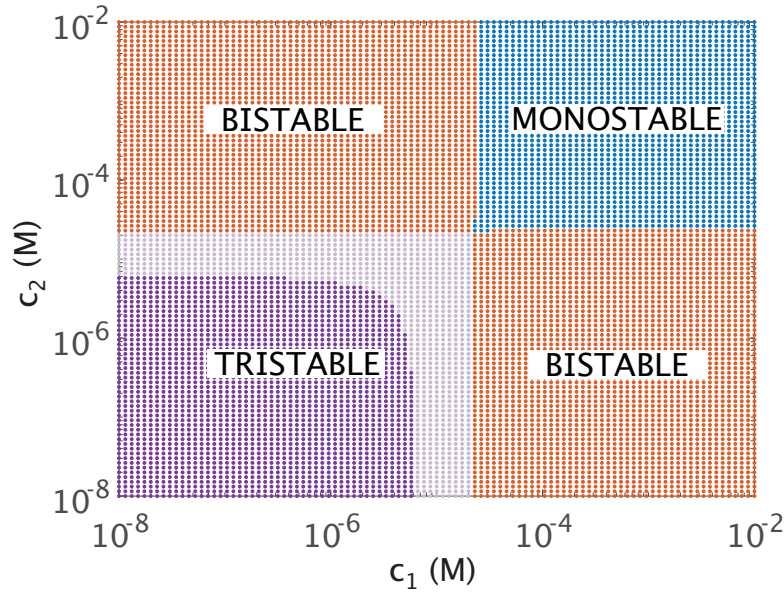


Figure 3.9: Phase diagram for evolving inducer concentrations c_1 and c_2 (regulating activity of $\bar{R}_1$ and $\bar{R}_2$, respectively). Each color-coded region corresponds to a phase defined by a different number of fixed points (seven in dark purple, five in light purple, three in red, and one in blue). Both inducers follow Eqn. 3.6 for the probability of activity with the same fixed parameters $m = 2$, $\Delta\epsilon = 4k_B T$, $K_A = 150 \mu M$, and $K_I = 5 \mu M$.

This intersection defines four regions with one (monostability in blue), two (bistability in red), or three stable points (tristability in purple). Note that analytically we can show that the thresholds separating tristable from bistable dynamics for c_1 and c_2 are equal to those in Eqn. 3.22 (see Appendix 3.B). In the purple region of Fig. 3.9, all three stable points become most accessible as both induction probabilities approach 1. Note that when the probability of active repressor reaches 1 for one of the repressors, the transition points along the axis of the other repressor reflect the bifurcations for the single-inducer system of Fig. 3.6, as expected. Increasing K_I for one of the inducers would shift the curve so that the intersection of its asymptote with the corresponding inducer axis occurs at a higher concentration. This trend also matches what we expect from the single inducer analysis.

The results observed for one and two inducers extend naturally in the three inducer regime, with tristability when all inducer concentrations are sufficiently small, monostability when all are sufficiently large, and bistability elsewhere. Similarly to how bifurcations transformed into a linear curve in two-dimensional phase space when moving from one to two regulating inducers, as in Fig. 3.9, the three inducer

case likewise extends these curves into corresponding bifurcation planes in three dimensions.

3.3 Effects of induction in systems perturbed away from symmetry

So far, we have assumed for simplicity that a given repressor binds different promoter sites with the same affinity (for example, $\bar{R}_1$ binds the promoters for $\bar{R}_2$ and $\bar{R}_3$ expression with the same affinity such that $K_{21} = K_{31} \equiv K_1$), and that protein expression is repressed with equal strengths $K_1 = K_2 = K_3 \equiv K$. While this symmetry allows us to observe the evolution of complex bifurcation diagrams, many biological systems are naturally perturbed away from symmetry. We now introduce biases into the relative repression strengths of a given protein, defined such that the model can still take a dimensionless form using the transformations that generated Eqns. 3.10 - 3.12 and Eqns. 3.13 - 3.15. This allows us to determine the sensitivity of the bifurcation thresholds to various perturbations.

Returning to the single-inducer case of Section 3.2.1, suppose that we keep all parameters fixed but introduce bias into regulation by the inducer-targeted $\bar{R}_1$. $\bar{R}_1$ regulates $\bar{R}_2$ and $\bar{R}_3$ expressions by binding to the respective promoter sites with affinities K_{21} and K_{31} , respectively. We now let $K_{31}/K_{21} = \alpha$, with K_{21} and all remaining dissociation constants equal to K as before. The dynamics are then defined by the differential equations

$$\frac{d\bar{R}_1}{d\bar{t}} = \frac{\bar{a}}{(1 + \bar{R}_2^n)(1 + \bar{R}_3^n)} - \bar{R}_1, \quad (3.27)$$

$$\frac{d\bar{R}_2}{d\bar{t}} = \frac{\bar{a}}{[1 + (p_{\text{act}}(c)\bar{R}_1)^n](1 + \bar{R}_3^n)} - \bar{R}_2, \quad (3.28)$$

$$\frac{d\bar{R}_3}{d\bar{t}} = \frac{\bar{a}}{\left[1 + \left(\frac{p_{\text{act}}(c)}{\alpha}\bar{R}_1\right)^n\right](1 + \bar{R}_2^n)} - \bar{R}_3. \quad (3.29)$$

In Section 3.2.1, we observed three distinct dynamic regimes, with decreasing complexity (i.e., number of fixed points) as the inducer concentration rose. Fig. 3.10(A) plots these shifts in dynamic regimes explicitly as changes in the number of fixed points, with the purple threshold denoting the bifurcation between distinct tristable regimes, and the green threshold denoting the bifurcation between tristable and bistable dynamics. The system sets equal interaction strengths among all repression-binding event types, i.e., $\alpha = 1$.

We now demonstrate how these bifurcations shift as we move away from symmetry. Fig. 3.10(B) reveals a narrow window of perturbation away from symmetry for

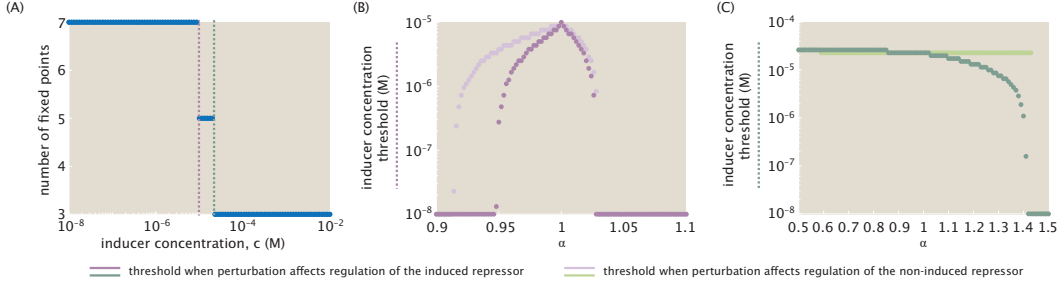


Figure 3.10: The change in bifurcation thresholds in a single inducer system as α rises. (A) The number of fixed points as a function of inducer concentration when $\alpha = 1$. The purple threshold denotes the transition between distinct tristable dynamic regimes (i.e., seven or five fixed points), and the green threshold denotes the transition between tristable and bistable dynamics (five or three fixed points, respectively). The system corresponds to that analyzed in Section 3.2.1. (B) The change in the inducer concentration threshold at which the tristable (purple) transition occurs as a function of α . The dark purple curve corresponds to a system where α targets regulation of the induced repressor, $\bar{R}_1$. The light purple curve is for a system where α affects regulation of a non-induced repressor, in this case $\bar{R}_2$. (C) The change in the inducer concentration defining the threshold separating tristable from bistable dynamics as a function of α . The specific colors again correspond to the different targets for perturbation bias, with dark green targeting the induced repressor and light green the non-induced repressor.

which a seven-fixed-point regime exists. Expressed differently, the dark purple curve indicates a window of only $0.946 \lesssim \alpha \lesssim 1.028$ within which a system with sufficiently high concentrations of all three repressors is equally likely to stabilize to any of the three possible stable states. The dark green curve in Fig. 3.10(C) shows that the threshold separating tristable from bistable dynamics is little affected by perturbations in $\alpha \leq 1$, where $\bar{R}_1$ has a weaker affinity for the $\bar{R}_2$ promoter than for the $\bar{R}_3$ promoter. Favoring affinity to $\bar{R}_2$ for $\alpha > 1$, however, begins to affect the threshold inducer concentration noticeably. At $\alpha \gtrsim 1.42$, the system becomes bistable regardless of inducer concentration.

Combining the information in dark purple and dark green in Fig. 3.10(B) and (C), we conclude that for $\alpha < 0.95$ and $1.028 \lesssim \alpha \lesssim 1.42$ only two dynamic regimes are accessible (with the seven-fixed point regime no longer viable at any inducer concentration). The perturbation threshold beyond which tristability of any kind is no longer possible occurs at $\alpha \approx 1.42$.

Alternatively, we can consider a perturbation bias that affects regulation by a repressor that is not targeted by an inducer, such as $\bar{R}_2$. In this case, setting $K_{32}/K_{12} = \alpha$

and all remaining coefficients (including K_{12}) to K , we obtain the equations

$$\frac{d\bar{R}_1}{d\bar{t}} = \frac{\bar{a}}{(1 + \bar{R}_2^n)(1 + \bar{R}_3^n)} - \bar{R}_1, \quad (3.30)$$

$$\frac{d\bar{R}_2}{d\bar{t}} = \frac{\bar{a}}{[1 + (p_A(c)\bar{R}_1)^n](1 + \bar{R}_3^n)} - \bar{R}_2, \quad (3.31)$$

$$\frac{d\bar{R}_3}{d\bar{t}} = \frac{\bar{a}}{[1 + (p_A(c)\bar{R}_1)^n] \left[1 + \left(\frac{\bar{R}_2}{\alpha}\right)^n\right]} - \bar{R}_3. \quad (3.32)$$

The light purple and green curves in Fig. 3.10(B) and (C) indicate the change in thresholds as α rises and alters the regulatory bias of $\bar{R}_2$. Fig. 3.10(B) shows that the change in this threshold as α increases from 1, such that $\bar{R}_2$ has a stronger binding affinity for the promoter of the induced $\bar{R}_1$ than for $\bar{R}_3$, is comparable to the change observed in dark purple as described by Eqns. 3.27 - 3.29. Decreasing α to favor stronger binding to the promoter for the non-induced $\bar{R}_3$, however, dampens the system's response to bias. The system denoted in light purple thus has a larger window of perturbation away from symmetry where the seven-fixed-point tristable regime is possible, with the window now favoring regulation of the non-induced over the induced repressor's expression.

The light green curve in Fig. 3.10(C), on the other hand, indicates that perturbation bias aimed to affect regulation by the non-induced repressor $\bar{R}_2$ in fact has no effect on the threshold between tristability and bistability until $\alpha \gtrsim 1.45$. We conclude that, unlike the first instance targeting the induced repressor, perturbations targeting a non-induced repressor generally influence only how complex the system's tristable dynamics can become.

3.4 The three-gene toggle switch with self-activation

The systems analyzed thus far focus exclusively on mutual repression. Toggle switches, however, can also be modeled with gene products not only repressing other genes but also stimulating their own expressions through self-activation, as shown in Fig. 3.11(A). Fig. 3.11(B) highlights protein production and regulatory binding at a given promoter site (in this case for gene g_1), where the protein produced can act either as a repressor targeting other genes or as an activator of its own expression. These dual repression and self-activation capabilities are observed across a range of natural gene network motifs with varying complexity, including applications in the bacteriophage lambda switch [12, 15, 35], stem cell and developmental

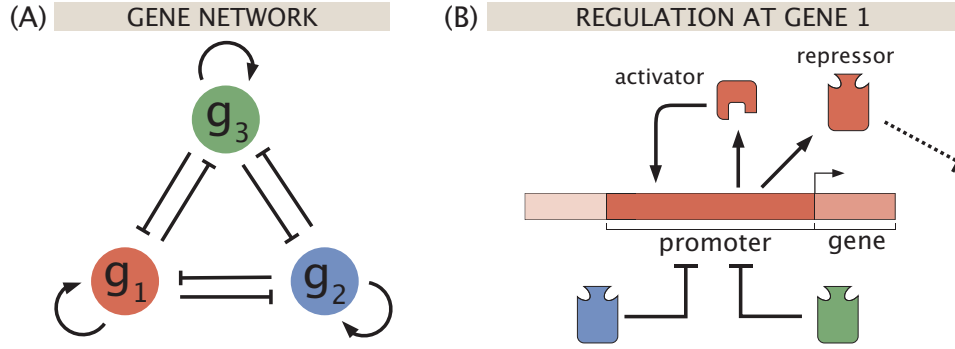


Figure 3.11: Gene expression in the three-gene toggle switch with self-activation. (A) Network of three mutually-repressing genes g_i , each producing a protein at average concentration R_i that can either repress expression of $R_{j \neq i}$ or activate its own expression. (B) Regulatory binding and expression at the promoter site for gene 1. Comparable illustrations would apply for genes 2 and 3.

differentiation [38], and mammalian cell cycle progression [92]. We now consider the effect of self-activation on stability in the three-gene context.

The following sections assume, as in our previous baseline model from Section 3.1.1, that different repressors can bind non-exclusively at a given gene promoter site to regulate expression. There are two possible ways, however, to incorporate activator binding. First, we analyze cases in which activators compete with repressors for the promoter such that an activator cannot bind if any repressor is bound, and vice versa. Section 3.4.1 then explores non-exclusive binding, where both an activator and a repressor can bind simultaneously at a given promoter site such that repressors moderate the effect of activation.

Given that all proteins in this self-activating network can act as either repressors or activators, we are interested not only in whether they bind competitively or non-exclusively to the DNA, but also in determining what defines a protein's activity as a repressor or an activator. To do this, we highlight two biological mechanisms, and how protein activities in each case are tuned by distinct quantitative roles for effector binding. In Fig. 3.12(A), it is the binding site identity alone that determines a protein's function. For example, if a protein binds to its own gene's promoter site then it functions as an activator, and otherwise as a repressor. Activator proteins are then defined by the same probabilistic effector binding that captures repressor activity, with both protein types rendered inactive by effector binding. We refer to this for the remainder of our study as Case I.

On the other hand, Fig. 3.12(B) depicts a setting in which effector binding directly

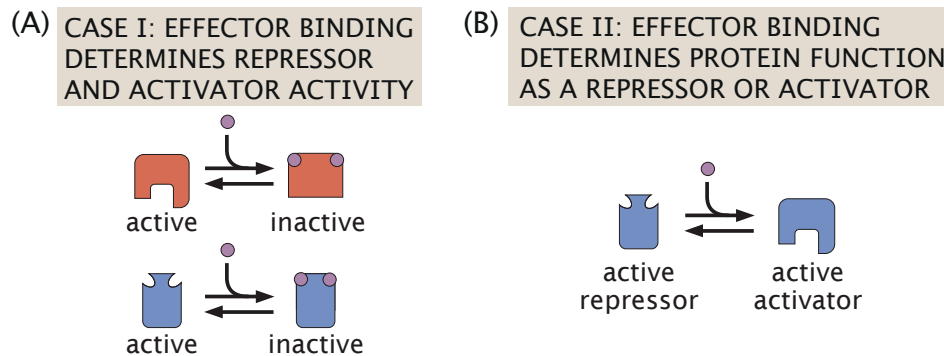


Figure 3.12: Two approaches to defining proteins as repressing or activating, depending on the role of effector binding. (A) Effector binding renders proteins inactive regardless of function because the protein's binding site to the DNA independently confers repressor vs. activator identity. (B) Effector binding directly determines whether a protein exists in a repressing or activating configuration, with binding transforming an active repressor to an active activator.

determines protein function. Since effector binding induces a protein conformational change (favoring expression in the inducer case), we propose in this case that effector binding alters a protein's configuration from that of an active repressor to that of an active activator. We refer to this for the remainder of our study as Case II. Past precedent exists for modeling such effector-driven dual function transcription factors. For the arabinose operon, for example, the presence of arabinose induces a conformational change in AraC and thus determines its interaction with the DNA as either a repressor or an activator [10, 93, 94]. Effector molecules can also indirectly control transcription factor function by determining whether transcription factors recruit corepressors or coactivators when bound to the DNA, such as for steroid hormone receptors [95, 96].

We demonstrate in the following discussion how these interpretations of effector-regulated activity lead to distinct dynamics for competitive repressor-activator binding and in particular for non-exclusive binding. In comparing the two effector mechanisms, we continue to define thermodynamic states and weights that do not explicitly involve long-range binding through DNA looping. Models incorporating such looping have been considered for simpler single operons through grand canonical ensemble interpretations [22, 97], and could be adapted to our structure. Our discussion here, however, will continue to focus on our three gene systems, but now with self-activation.

3.4.1 Competitive repressor-activator binding

We begin by considering competitive binding, with thermodynamic states, weights, and rates as depicted in Fig. 3.13. If we first assume that the binding site determines a protein's role as a repressor or an activator, then the activity of a given protein R_i tuned by inducer concentration c_i depends on the probability $p_{\text{act}}(c_i)$, as shown in Fig. 3.13(A). In line with the baseline model, a given protein is expressed at a maximal rate a in the absence of bound regulating proteins. A bound activator, however, now increases expression to $a + b$. From these states and weights, we then define the dynamics of protein expression by

$$\frac{dR_1}{dt} = \frac{a + (a + b)(\frac{R_1}{K_1})^n}{1 + (\frac{R_1}{K_1})^n + (\frac{R_2}{K_2})^n + (\frac{R_3}{K_3})^n + (\frac{R_2}{K_2})^n(\frac{R_3}{K_3})^n} - \frac{R_1}{\tau}, \quad (3.33)$$

$$\frac{dR_2}{dt} = \frac{a + (a + b)(\frac{R_2}{K_2})^n}{1 + (\frac{R_1}{K_1})^n + (\frac{R_2}{K_2})^n + (\frac{R_3}{K_3})^n + (\frac{R_2}{K_2})^n(\frac{R_3}{K_3})^n} - \frac{R_2}{\tau}, \quad (3.34)$$

$$\frac{dR_3}{dt} = \frac{a + (a + b)(\frac{R_3}{K_3})^n}{1 + (\frac{R_1}{K_1})^n + (\frac{R_2}{K_2})^n + (\frac{R_3}{K_3})^n + (\frac{R_2}{K_2})^n(\frac{R_3}{K_3})^n} - \frac{R_3}{\tau}. \quad (3.35)$$

By transforming $\bar{R}_i = R_i/K_1$, $\bar{t} = t/\tau$, $\bar{a} = \tau a/K_1$, and $\bar{b} = \tau b/K_1$, we obtain the dimensionless form

$$\frac{d\bar{R}_1}{d\bar{t}} = \frac{\bar{a} + (\bar{a} + \bar{b})\bar{R}_1^n}{1 + \bar{R}_1^n + (\frac{\bar{R}_2}{K^{(2)}})^n + (\frac{\bar{R}_3}{K^{(3)}})^n + (\frac{\bar{R}_2}{K^{(2)}})^n(\frac{\bar{R}_3}{K^{(3)}})^n} - \bar{R}_1, \quad (3.36)$$

$$\frac{d\bar{R}_2}{d\bar{t}} = \frac{\bar{a} + (\bar{a} + \bar{b})(\frac{\bar{R}_2}{K^{(2)}})^n}{1 + \bar{R}_1^n + (\frac{\bar{R}_2}{K^{(2)}})^n + (\frac{\bar{R}_3}{K^{(3)}})^n + (\frac{\bar{R}_2}{K^{(2)}})^n(\frac{\bar{R}_3}{K^{(3)}})^n} - \bar{R}_2, \quad (3.37)$$

$$\frac{d\bar{R}_3}{d\bar{t}} = \frac{\bar{a} + (\bar{a} + \bar{b})(\frac{\bar{R}_3}{K^{(3)}})^n}{1 + \bar{R}_1^n + (\frac{\bar{R}_2}{K^{(2)}})^n + (\frac{\bar{R}_3}{K^{(3)}})^n + (\frac{\bar{R}_2}{K^{(2)}})^n(\frac{\bar{R}_3}{K^{(3)}})^n} - \bar{R}_3, \quad (3.38)$$

where $K^{(2)} = K_2/K_1$ and $K^{(3)} = K_3/K_1$. To simplify, we impose equal binding affinities such that $K^{(2)} = K^{(3)} = 1$, and examine how the system's dynamic landscape evolves with increasing inducer concentration and rising activating bias in expression.

Induction in Case I

We introduce self-activation in the induced setting of Case I from Fig. 3.13(A) to observe how it alters the dynamics of the baseline model. Given the states and

COMPETITIVE REPRESSOR/ACTIVATOR BINDING

(A)	STATE	WEIGHT	RATE	(B)	STATE	WEIGHT	RATE
R ₁		1	a		1	a	
		$\left(\frac{p_{\text{act}}(c_1)R_1}{K_1}\right)^n$	a + b		$\left(\frac{[1-p_{\text{act}}(c_1)]R_1}{K_1}\right)^n$	a + b	
		$\left(\frac{p_{\text{act}}(c_2)R_2}{K_2}\right)^n$	0		$\left(\frac{p_{\text{act}}(c_2)R_2}{K_2}\right)^n$	0	
		$\left(\frac{p_{\text{act}}(c_3)R_3}{K_3}\right)^n$	0		$\left(\frac{p_{\text{act}}(c_3)R_3}{K_3}\right)^n$	0	
		$\left(\frac{p_{\text{act}}(c_2)R_2}{K_2}\right)^n \left(\frac{p_{\text{act}}(c_3)R_3}{K_3}\right)^n$	0		$\left(\frac{p_{\text{act}}(c_2)R_2}{K_2}\right)^n \left(\frac{p_{\text{act}}(c_3)R_3}{K_3}\right)^n$	0	
R ₂		1	a		1	a	
		$\left(\frac{p_{\text{act}}(c_2)R_2}{K_2}\right)^n$	a + b		$\left(\frac{[1-p_{\text{act}}(c_2)]R_2}{K_2}\right)^n$	a + b	
		$\left(\frac{p_{\text{act}}(c_1)R_1}{K_1}\right)^n$	0		$\left(\frac{p_{\text{act}}(c_1)R_1}{K_1}\right)^n$	0	
		$\left(\frac{p_{\text{act}}(c_3)R_3}{K_3}\right)^n$	0		$\left(\frac{p_{\text{act}}(c_3)R_3}{K_3}\right)^n$	0	
		$\left(\frac{p_{\text{act}}(c_1)R_1}{K_1}\right)^n \left(\frac{p_{\text{act}}(c_3)R_3}{K_3}\right)^n$	0		$\left(\frac{p_{\text{act}}(c_1)R_1}{K_1}\right)^n \left(\frac{p_{\text{act}}(c_3)R_3}{K_3}\right)^n$	0	
R ₃		1	a		1	a	
		$\left(\frac{p_{\text{act}}(c_3)R_3}{K_3}\right)^n$	a + b		$\left(\frac{[1-p_{\text{act}}(c_3)]R_3}{K_3}\right)^n$	a + b	
		$\left(\frac{p_{\text{act}}(c_1)R_1}{K_1}\right)^n$	0		$\left(\frac{p_{\text{act}}(c_1)R_1}{K_1}\right)^n$	0	
		$\left(\frac{p_{\text{act}}(c_2)R_2}{K_2}\right)^n$	0		$\left(\frac{p_{\text{act}}(c_2)R_2}{K_2}\right)^n$	0	
		$\left(\frac{p_{\text{act}}(c_1)R_1}{K_1}\right)^n \left(\frac{p_{\text{act}}(c_2)R_2}{K_2}\right)^n$	0		$\left(\frac{p_{\text{act}}(c_1)R_1}{K_1}\right)^n \left(\frac{p_{\text{act}}(c_2)R_2}{K_2}\right)^n$	0	

Figure 3.13: Expression of each protein R_i in the three-gene toggle switch with self-activation and competitive binding for repressors and activators. (A) Competitive binding with the role of effectors defined as in Fig. 3.12(A) (Case I). (B) Competitive binding with the role of effectors defined as in Fig. 3.12(B) (Case II).

weights listed and a single inducer c targeting $\bar{R}_1$, Eqns. 3.36 - 3.38 become

$$\frac{d\bar{R}_1}{d\bar{t}} = \frac{\bar{a} + (\bar{a} + \bar{b})[p_{\text{act}}(c)\bar{R}_1]^n}{1 + [p_{\text{act}}(c)\bar{R}_1]^n + \bar{R}_2^n + \bar{R}_3^n + \bar{R}_2^n\bar{R}_3^n} - \bar{R}_1, \quad (3.39)$$

$$\frac{d\bar{R}_2}{d\bar{t}} = \frac{\bar{a} + (\bar{a} + \bar{b})\bar{R}_2^n}{1 + [p_{\text{act}}(c)\bar{R}_1]^n + \bar{R}_2^n + \bar{R}_3^n + \bar{R}_2^n\bar{R}_3^n} - \bar{R}_2, \quad (3.40)$$

$$\frac{d\bar{R}_3}{d\bar{t}} = \frac{\bar{a} + (\bar{a} + \bar{b})\bar{R}_3^n}{1 + [p_{\text{act}}(c)\bar{R}_1]^n + \bar{R}_2^n + \bar{R}_3^n + \bar{R}_2^n\bar{R}_3^n} - \bar{R}_3. \quad (3.41)$$

To allow direct comparison with the original single-inducer model in Eqns. 3.16 - 3.18, we again set $n = 4$ and now choose maximal expression to satisfy $\bar{a} + \bar{b} = 2$.

Fig. 3.14 compares the dynamics that emerge. Panel (A) plots the bifurcations for the baseline setting shown earlier in Fig. 3.6, with panel (B) highlighting the fixed points at a low inducer concentration in three-dimensional expression space. Panel (C) plots the bifurcations for the comparable system with self-activation in Eqns. 3.39 - 3.41, with panel (D) again highlighting the low inducer regime. While any number of possible $(\bar{a}, \bar{b})$ pairs can satisfy $\bar{a} + \bar{b} = 2$, for closest comparison we choose the limit case of $\bar{a} = 2$ and $\bar{b} = 0$, where weak activator binding has a negligible impact on expression.

Fig. 3.14(C) shows that self-activation does not affect stable expression levels or the system's dynamics at high inducer concentrations. It does, however, influence the remaining saddle points, further accentuating expression of the dominant proteins. For example, the index-2 saddle becomes more centered in expression space at the half-maximal expression level. Also, for each index-1 saddle that favors $\bar{R}_1$, the dominant protein expression levels become more pronounced compared to those that are suppressed. This means that each saddle point allowing significant $\bar{R}_1$ expression is further from the stable points it dynamically links. If the system were to begin with half-maximal expression of each protein, for example, any trajectories leaning toward the $\bar{R}_1 - \bar{R}_2$ or $\bar{R}_1 - \bar{R}_3$ switches would take longer to stabilize because they must travel further to approach an index-1 saddle before veering off toward a stable point. We can think of these stable states as lying in deeper potential wells, making the related systems less likely to transition from one stable state to another.

As one might expect, the differences between the baseline model and our parametrization of the self-activation model are subtle, but the key advantage of self-activation lies in the additional tunability that becomes possible through the additive expression effect from $\bar{b}$. Increasing the strength of activation while maintaining a constant

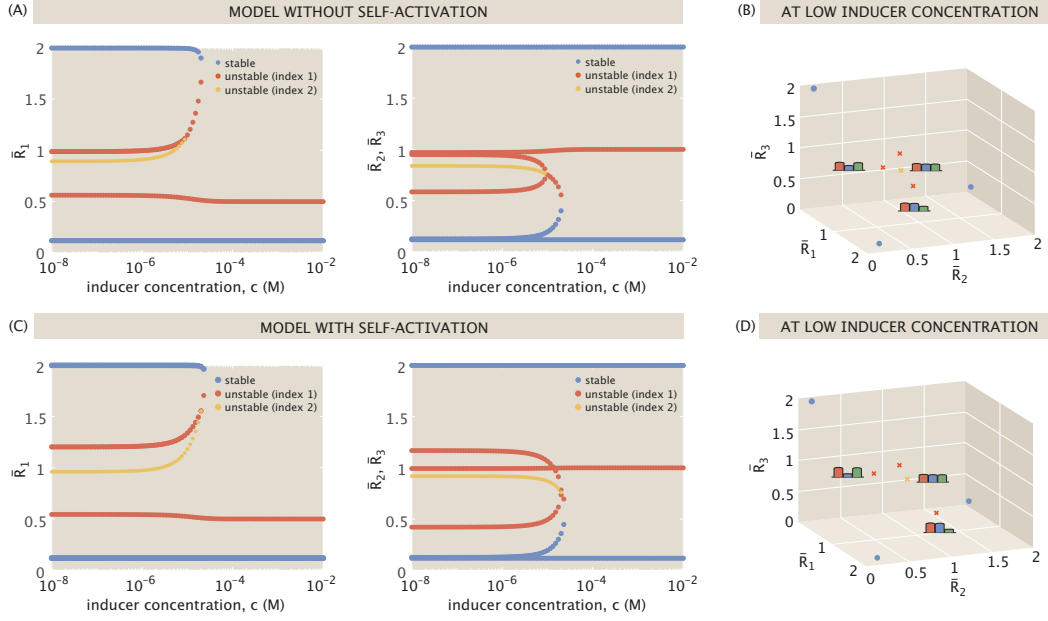


Figure 3.14: Comparison of the dynamics for the three-gene toggle switch without and with the presence of self-activation. In both settings, the Hill coefficient is $n = 4$ and we set the same maximal production level. (A) Bifurcation diagrams for $\bar{R}_1$, $\bar{R}_2$, and $\bar{R}_3$ steady-state expressions without self-activation as a function of inducer concentration, as previously shown in Fig. 3.6(A). Maximal production is set at $\bar{a} = 2$. (B) Fixed points at a low inducer concentration, with labels highlighting the index-2 saddle and the induced $\bar{R}_1$ -dominant index-1 saddles. (C) Bifurcation diagrams for steady-state expression with self-activation as defined by Eqns. 3.36 - 3.38. Rates are set at $\bar{a} = 2$ and $\bar{b} = 0$ for maximal production satisfying $\bar{a} + \bar{b} = 2$. (D) Fixed points at a low inducer concentration.

maximal expression level $\bar{a} + \bar{b}$ can lead to significant dynamic transformations. Fig. 3.15 fixes the system at a low inducer concentration for $\bar{a} + \bar{b} = 2$, and plots the bifurcation diagram with dynamics tuned as a function of activation $\bar{b}$. As $\bar{b}$ increases from zero, all fixed points shift toward lower expression while retaining the typical dynamic profiles observed in Fig. 3.14. Plotting how the fixed points shown for $(\bar{a}, \bar{b}) = (2, 0)$ would change with increasing inducer concentration, for example, would generate two dynamic phase shifts to arrive at a bistable switch between $\bar{R}_2$ and $\bar{R}_3$ expression, with results comparable to those shown in Fig. 3.6.

With sufficiently strong activation at $\bar{b} \approx 1.47$, however, the system no longer retains the index-2 saddle. This is evidenced by the fixed point plot highlighted at $\bar{b} = 1.5$ in Fig. 3.15. Moreover, $\bar{b} = 1.52$ marks a threshold from tristable to *quadristable* expression, where the index-2 fixed point transforms into a stable point. Rather than being comprised of a set of switches between $\bar{R}_i$ and $\bar{R}_j$ expression, the high $\bar{b}$

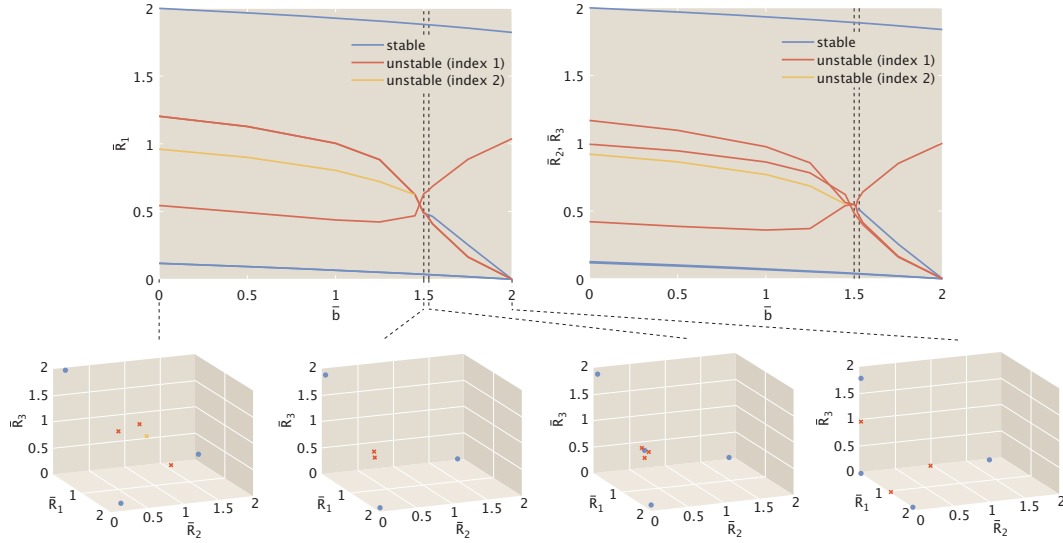


Figure 3.15: Bifurcation diagrams for $\bar{R}_1$, $\bar{R}_2$, and $\bar{R}_3$ expression at a low inducer concentration and at fixed $\bar{a} + \bar{b} = 2$ as a function of increasing activation strength ($\bar{b}$). The corresponding sets of fixed points are shown in three-dimensional expression space beneath, specifically highlighting each distinct dynamic phase for $(\bar{a}, \bar{b}) = (2, 0)$, $(\bar{a}, \bar{b}) = (0.5, 1.5)$, $(\bar{a}, \bar{b}) = (0.48, 1.52)$, and $(\bar{a}, \bar{b}) = (0, 2)$.

regime now brings together a set of switches between “on” and “off” expression for each protein. Note that, as the bifurcation point, $\bar{b} = 1.52$ shares properties between the two dynamic phases it straddles. As shown in Fig. 3.15, this point allows the quadristable dynamics observed for higher $\bar{b}$ at low inducer concentration, while still reducing to a single bistable switch at high inducer concentration (as is characteristic of the small $\bar{b}$ regime). Systems approaching the limit where expression can only occur in the presence of bound activator, on the other hand, as in the highlighted example $(\bar{a}, \bar{b}) = (0, 2)$ of Fig. 3.15, will reduce down to tristability at high inducer concentration, allowing either $\bar{R}_2$ -dominant expression, $\bar{R}_3$ -dominant expression, or none.

Induction in Case II

We now compare induction Case I above to the dynamics arising in an alternative setting, Case II, where the effector molecule binding determines whether a protein is active as a repressor or an activator. Fig. 3.13(B) outlines the thermodynamic states and weights in this setting, from which we model the system with the differential

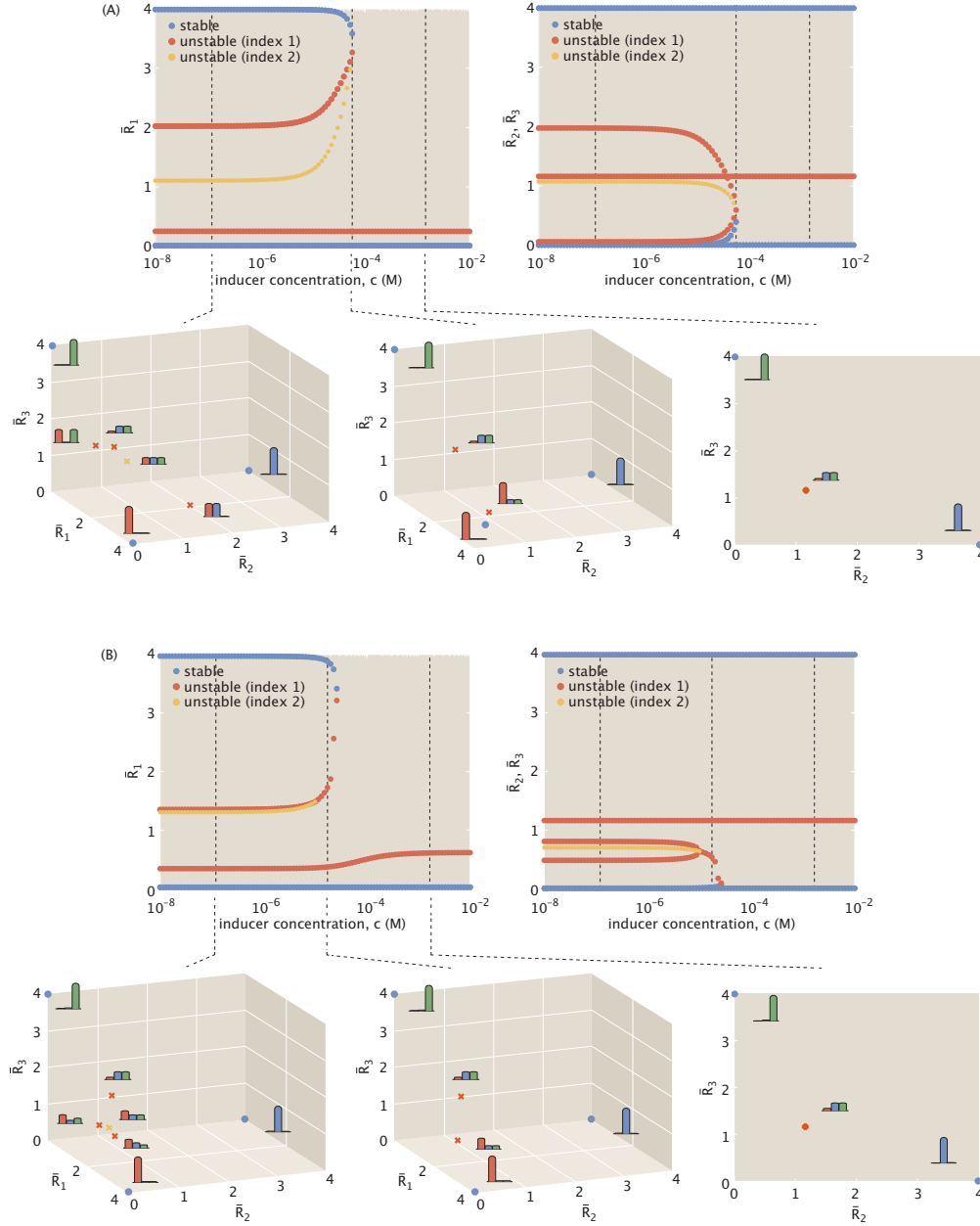


Figure 3.16: Induction Cases I and II for competitive repressor-activator binding, with $\bar{a} = 2$, $\bar{b} = 2$. Each panel plots the bifurcation diagrams for $\bar{R}_1$, $\bar{R}_2$, and $\bar{R}_3$ steady-state expressions as a function of inducer concentration. The fixed points are visualized in expression space beneath the corresponding bifurcations at low, intermediate, and high inducer concentrations. The low and high inducer concentrations chosen are the same in (A) and (B), and the intermediate concentrations selected highlight the same intermediate dynamic regime. (A) The competitive repressor-activator binding case in which inducer binding controls transcription factor activity regardless of its ultimate function as a repressor or activator (Case I). (B) The competitive repressor-activator binding case in which inducer binding transforms a protein from a repressing conformation to an activating form (Case II).

equations

$$\frac{d\bar{R}_1}{d\bar{t}} = \frac{\bar{a} + (\bar{a} + \bar{b})[(1 - p_{\text{act}}(c))\bar{R}_1]^n}{1 + [(1 - p_{\text{act}}(c))\bar{R}_1]^n + \bar{R}_2^n + \bar{R}_3^n + \bar{R}_2^n\bar{R}_3^n} - \bar{R}_1, \quad (3.42)$$

$$\frac{d\bar{R}_2}{d\bar{t}} = \frac{\bar{a} + (\bar{a} + \bar{b})\bar{R}_2^n}{1 + [p_{\text{act}}(c)\bar{R}_1]^n + \bar{R}_2^n + \bar{R}_3^n + \bar{R}_2^n\bar{R}_3^n} - \bar{R}_2, \quad (3.43)$$

$$\frac{d\bar{R}_3}{d\bar{t}} = \frac{\bar{a} + (\bar{a} + \bar{b})\bar{R}_3^n}{1 + [p_{\text{act}}(c)\bar{R}_1]^n + \bar{R}_2^n + \bar{R}_3^n + \bar{R}_2^n\bar{R}_3^n} - \bar{R}_3. \quad (3.44)$$

To aid in visual comparison with induction Case I, we consider systems allowing a larger maximal expression of $\bar{a} + \bar{b} = 4$. Specifically, we will set $\bar{a} = \bar{b} = 2$ for equally strong contributions from the basal and activated expression levels.

Fig. 3.16 compares the system defined by induction Case I, now for $\bar{a} = \bar{b} = 2$, with the corresponding model in panel (B) for the alternative induction approach highlighted in Eqns. 3.42 - 3.44. We observe that in the low inducer concentration regime, while the dynamical structure remains the same, the saddle points allowing $\bar{R}_1$ expression in panel (B) are now skewed toward favoring $\bar{R}_1$ expression. In this regime, a low inducer concentration implies a high probability $p_{\text{act}}(c)$ of $\bar{R}_1$ acting as a repressor, and a low probability $1 - p_{\text{act}}(c)$ of acting as an activator. With $[1 - p_{\text{act}}(c)]\bar{R}_1 \rightarrow 0$ in Eqn. 3.42, $\bar{R}_1$ expression is limited only by regulatory repression from $\bar{R}_2$ and $\bar{R}_3$, while the remaining two repressors are additionally regulated by $p_{\text{act}}(c)\bar{R}_1$. The resulting skewness of the central fixed point cluster to favor $\bar{R}_1$ places the $\bar{R}_2$ and $\bar{R}_3$ -dominant stable states in deeper potential wells. It thus requires a less significant perturbation to transition out of the $\bar{R}_1$ -dominant stable state into one of the other stable states.

Since all $\bar{R}_1$ -expressing saddle points are spatially closer together in the lower panel compared to the upper panel of Fig. 3.16, this indicates that Case II of induction does not require as high an inducer concentration to transition to an intermediate dynamic regime. Finally, at a sufficiently high inducer concentration, the saddle of the resulting bistable switch shifts the saddle point toward a slightly higher $\bar{R}_1$ concentration, which serves to deepen the potential well between the $\bar{R}_2$ and $\bar{R}_3$ -dominant stable states compared to Case I.

3.4.2 Non-exclusive repressor-activator binding

We now turn to systems in which repressors and activators bind non-exclusively to a gene's regulatory region. This means that at a given promoter site, a gene's expression can be regulated not only by the presence of one or both repressors

produced by the remaining two genes, but also by the possible additional presence of bound activator. As in the competitive binding case shown in Fig. 3.13, this system with non-exclusive binding retains the same possible states for a given promoter site, namely (i) the state with no bound transcription factors (with an expression rate a), (ii) states with one or both possible repressors bound (with an expression rate 0), and (iii) states with activator bound alone (with an expression rate $a + b$). Additional states account for activated expression in the presence of one or both available repressors, and in these states, activated expression is suppressed by the presence of bound repressor defined by a corresponding rate $a + b - d$.

Upon deriving a set of differential equations in dimensionless form for the expression of proteins $\bar{R}_1$, $\bar{R}_2$, and $\bar{R}_3$ from the corresponding thermodynamic states and weights (see Appendix 3.C for details), we have, as in the competitive binding setting, a system defined by basal expression $\bar{a}$ and additional activated expression $\bar{b}$, with the maximal expression level constrained once again to $\bar{a} + \bar{b} = 2$ to facilitate comparison across models. Given the non-exclusive binding conditions modeled, expression now also depends on the strength of repression from $\bar{d}$, where $\bar{d} \leq \bar{a} + \bar{b}$.

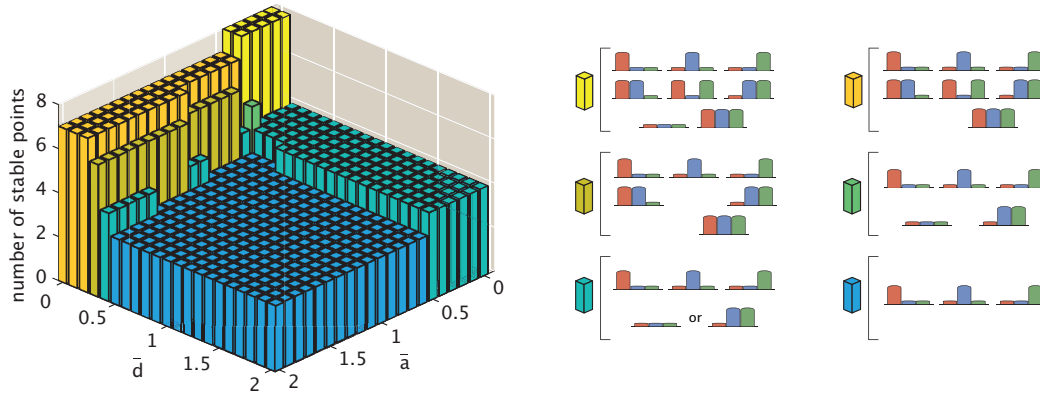
The following subsections focus on the impact of effector concentration and the relative strengths of activation and repression on dynamic stability. This allows us to determine the range of dynamic landscapes possible within the parameter space under different interpretations for effector activity, and the sensitivity of the resulting dynamics and bifurcations to each of the rate parameters.

Induction in Case I

We first consider Case I from Fig. 3.12(A), in which the binding of the effector molecule renders proteins inactive regardless of whether they are functioning as repressors or activators. Continuing our convention of choosing $\bar{R}_1$ as the target of effector binding, the concentration of $\bar{R}_1$ is scaled by the probability of activity $p_{\text{act}}(c)$, regardless of function, where we assume that all proteins interact with effectors through the same MWC model and binding affinities. Fig. 3.23 and Eqns. 3.59 - 3.61 of Appendix 3.C define the corresponding states and weights along with the resulting dimensionless differential equations incorporating the role of $p_{\text{act}}(c)$.

Evaluating the fixed points, we find that allowing non-exclusive binding expands the possible set of stable states to the maximum combinatorial number, i.e., no expression, one gene expressing, two genes expressing, or all three expressing.

(A) AT LOW EFFECTOR CONCENTRATION



(B) AT HIGH EFFECTOR CONCENTRATION

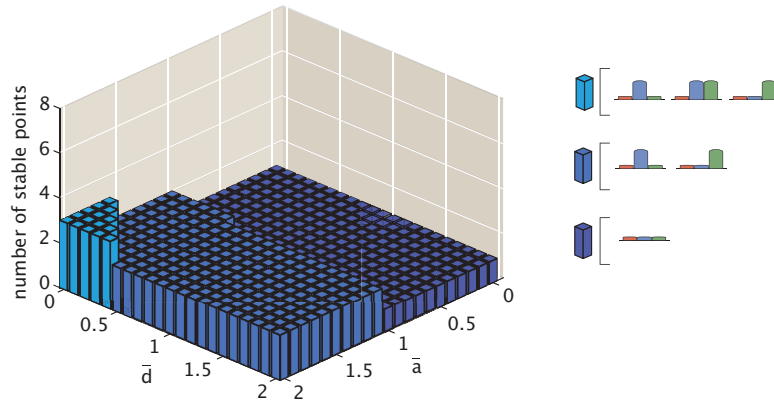


Figure 3.17: Number of stable fixed points in induction Case I of non-exclusive repressor-activator binding. The plots highlight stable points at low (10^{-8} M) and high (10^{-2} M) effector concentrations in panels (A) and (B), respectively, for varying rate parameters $\bar{a}$, $\bar{b}$, and $\bar{d}$, where $\bar{a} + \bar{b} = 2$ and $\bar{d} \leq \bar{a} + \bar{b}$. The diagrams on the right hand side of panel (A) represent the types of stable points observed in each regime at a low effector concentration, i.e., with eight (yellow), seven (orange), six (brown), five (green), four (teal), or three (blue) stable points. The diagrams on the right side of panel (B) represent the types of stable points observed in each regime at a high effector concentration, i.e., with three (blue), two (dark blue), or one (purple) stable point(s).

Determining the likelihood of each state being accessible within the allowable rate parameter space offers insights into how this more complex regulatory architecture prioritizes expression.

Fig. 3.17(A) tracks the number of possible stable states that exist at low effector concentration ($c = 10^{-8}$ M). The maximum number of stable states occurs in the yellow-shaded region where $\bar{d} \approx 0$ and $\bar{a} < 0.5$ ($\bar{b} > 1.5$). This represents systems in which repressor binding has negligible effects on expression, and where gene expression thus relies heavily on bound activators.

As $\bar{a}$ and $\bar{d}$ rise, the complexity of the dynamic landscape decreases, with the number of possible stable points falling until $\bar{a} \gtrsim 0.5$ and $\bar{d} \gtrsim 0.5$. This threshold combination is sufficient to render the system tristable at low effector concentrations, and is similar to what we observed in the previous competitive binding models of Sections 3.4.1 and 3.4.1.

Other than the three possible single-gene expression states, which exist at low effector concentrations for all possible rate parameter combinations, the stable state that survives across the broadest allowable parameter space is the one in which both $\bar{R}_2$ and $\bar{R}_3$ dominate. Such a state becomes no longer possible only if $\bar{a} \gtrsim 0.5$ and $\bar{d} \gtrsim 0.5$. The robustness of this state makes sense given that the model in question specifically tunes $\bar{R}_1$ expression via effector concentration. This targeted tuning more indirectly (and weakly) affects the existence of a stable $(0, \bar{R}_2, \bar{R}_3)$ state. Fig. 3.17(A) also indicates that the repression parameter $\bar{d}$ drives the loss of the $(\bar{R}_1, 0, \bar{R}_3)$ and $(\bar{R}_1, \bar{R}_2, 0)$ states. Essentially, once repressors play an active role in regulation (no longer $\bar{d} \rightarrow 0$), dual repressor and activator activity implies that the system can only express $\bar{R}_1$ significantly in a stable state when it is the only protein being expressed.

Generally, as effector concentrations increase, the number of available stable fixed points decreases until the system reaches monostable, bistable, or tristable dynamics, as shown in Fig. 3.17(B). When $\bar{a} \lesssim 1$, such that activation contributes more strongly than basal expression, a high effector concentration suppresses all gene expression. When the system approaches the limit case of largely unregulated expression (i.e., $\bar{a} \rightarrow 2$ and $\bar{d} \rightarrow 0$) it becomes tristable, expressing either $\bar{R}_2$, $\bar{R}_3$, or both. Otherwise, in the relatively weak activation regime that remains, the system collapses to the bistable toggle switch between $\bar{R}_2$ and $\bar{R}_3$.

Figs. 3.18 and 3.19 illustrate further how the system evolves toward these different

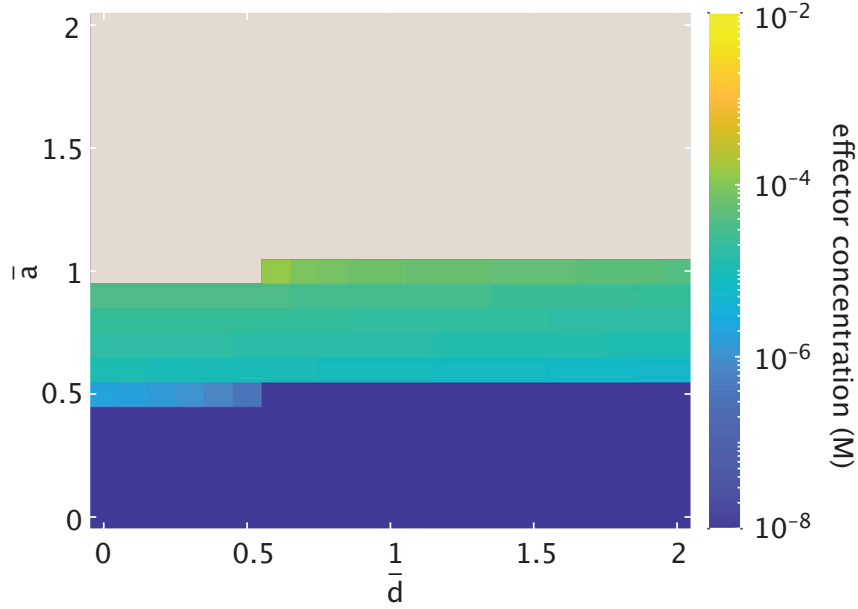


Figure 3.18: Heatmap tracking the minimum effector concentration (M) at which the stable state with no expression can exist with non-exclusive activator-repressor binding (induction Case I). Measurements span the range of rate parameters allowed by the constraints $\bar{a} + \bar{b} = 2$ and $\bar{d} \leq \bar{a} + \bar{b}$, with the x-axis denoting increasing strength of repression $\bar{d}$, and the y-axis denoting decreasing strength of activation with increasing $\bar{a}$. The beige region represents the parameter space in which a stable state without expression does not exist at any effector concentration.

regimes as the effector concentration rises, and how sensitive the different stable states are to these changes. The figures plot (respectively) the minimum and maximum effector concentrations that are required for different stable fixed points to exist, where Fig. 3.18 specifically highlights the minimum concentration for the stable state with no expression. At the maximum concentration threshold, a bifurcation occurs when a sufficiently high effector concentration prevents the system from stabilizing to the state with no expression. The beige-shaded region denotes the part of parameter space where a given fixed point does not exist at any effector concentration, and the yellow-shaded region denotes the space where the fixed point survives at high effector concentration.

Notably, the heatmaps of Fig. 3.18 and Fig. 3.19(A) directly overlap, indicating a close relationship between the existence of a stable state with no expression and the existence of a state that expresses one of the two proteins not impacted by the effector ($\bar{R}_2$ or $\bar{R}_3$). The existence of these three stable states is thus defined almost fully by the strength of activation as reflected by $\bar{a}$. As $\bar{a}$ rises, three regimes

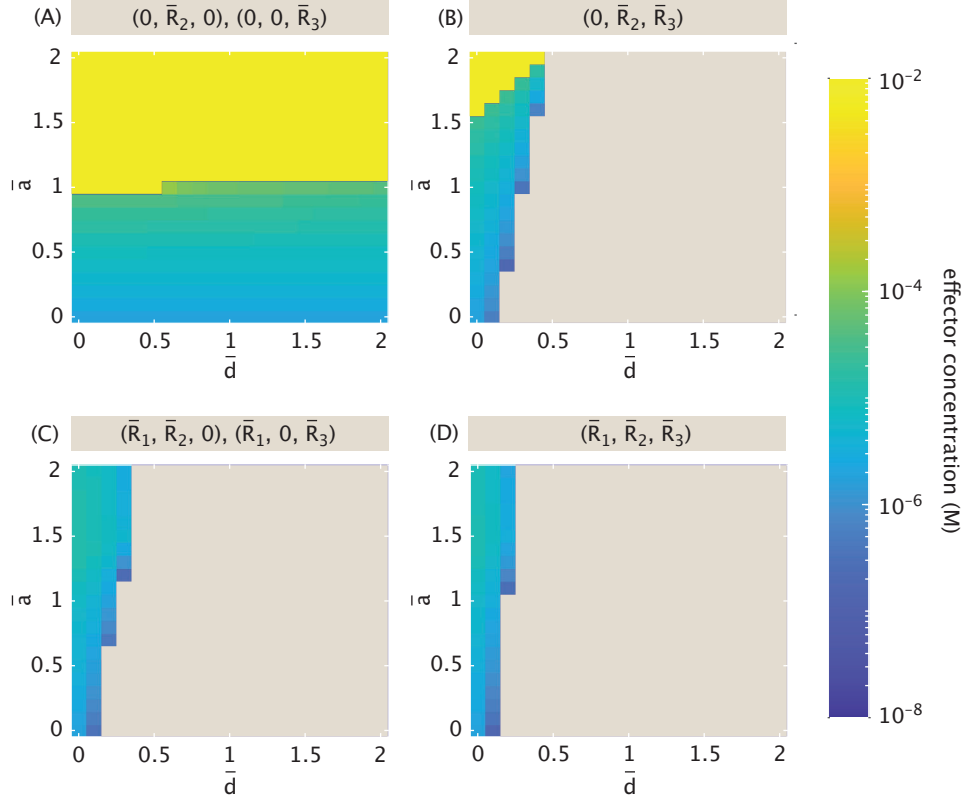


Figure 3.19: Heatmaps tracking the maximum effector concentrations (M) (bifurcation thresholds) at which stable states can exist for the non-exclusive activator-repressor binding model described in Fig. 3.12(A) (Case I). The heatmaps track (A) stable states expressing one of the genes that does not directly interact with an effector, i.e., $(0, \bar{R}_2, 0)$ and $(0, 0, \bar{R}_3)$, (B) the stable state in which the two genes not impacted by effector are expressed, i.e., $(0, \bar{R}_2, \bar{R}_3)$, (C) stable states expressing two genes including the effector-controlled $\bar{R}_1$, and (D) the stable state expressing all three genes. Measurements span the rate parameters allowed by the constraints $\bar{a} + \bar{b} = 2$ and $\bar{d} \leq \bar{a} + \bar{b}$, with the x-axis denoting increasing repression strength $\bar{d}$, and the y-axis denoting decreasing activation with increasing $\bar{a}$. Beige represents the region of parameter space in which the stable state cannot exist at any effector concentration, and the yellow-shaded region indicates where the stable state is possible at all effector concentrations.

with distinct dynamic trends emerge upon tuning the effector concentration. When $\bar{a} \lesssim 0.5$, all three states are possible until an intermediate effector concentration is reached, beyond which only the state suppressing all expression can exist among the three. When $0 \lesssim \bar{a} \lesssim 1$, we observe a tradeoff at an intermediate effector concentration, where the system's ability to stabilize to an $\bar{R}_2$ or $\bar{R}_3$ -dominant state at low concentrations is swapped for the system's ability to suppress all expression. This indicates a regime of strong activation compared to basal expression, where an increase in effector concentration causes a fixed point to emerge and replace states that existed at lower concentrations. Finally, in the weak activation regime of $\bar{a} \gtrsim 1$, the system can always stabilize to an $\bar{R}_2$ or $\bar{R}_3$ -dominant state at all effector concentrations, and never suppresses expression entirely. Note in all of these regimes that the strength of repression ($\bar{d}$) has negligible or no effect on the existence of the three states.

Fig. 3.19(B)-(D) highlights stable fixed points where more than one type of protein is expressed. The existence of such points relies on weak repression ($\bar{d} < 0.5$). As $\bar{a}$ rises and the strength of activation decreases accordingly, we observe levels of rising effector concentrations where the stable points vanish. The heatmaps indicate that as repression strength rises, a higher value of $\bar{a}$ and thus a weaker degree of activation is needed for the system to stabilize to these states, with the state in panel (B) responding most gradually and the state in panel (D) most sharply as repression strength increases. Finally, the span of effector concentrations indicates that the bifurcation thresholds for these stable points are more responsive to changes in activation strength than those of the single-protein expression states shown in Fig. 3.19(A).

We conclude from Fig. 3.19 that expressing more than one protein at a stable steady state is only possible in a system with weak repression, whereas repression has essentially no effect on expression that is not targeted by an effector ($\bar{R}_2$ and $\bar{R}_3$ here). Additionally, while weakening activation strength always expands the range of effector concentrations at which these stable fixed points can exist, only states that do not express the effector-targeted genes can exist at all effector concentrations (the yellow regions of Fig. 3.19). These yellow-shaded regions require weaker activation strengths compared to the strength of unregulated expression, with this requirement even stricter for the state expressing more than one protein. The results demonstrate that by tuning these parameters and testing varying effector concentrations, one can transform a system to express different numbers of genes at stable steady states

depending on which combinations are most relevant for a desired function.

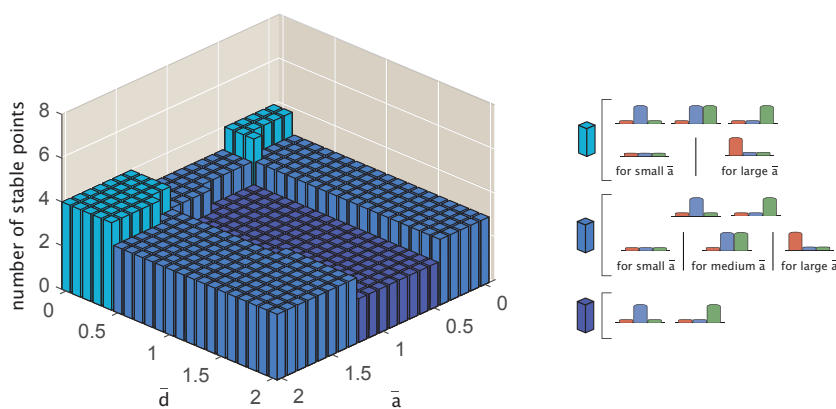
Induction in Case II

An alternative interpretation for effector activity, shown in Case II of Fig. 3.12(B), transforms the available states and weights from the non-exclusive case such that the concentration of transcription factor $\bar{R}_1$ is scaled by $p_{\text{act}}(c)$ when repressing expression of other proteins, and is scaled by $1 - p_{\text{act}}(c)$ when activating its own expression. (See Appendix 3.C and corresponding Fig. 3.24 for the thermodynamic states and weights and for the corresponding model form.)

By evaluating the resulting differential equations for fixed points, we obtain plots in Fig. 3.20 highlighting the types of stable points possible at low (10^{-8} M) and high (10^{-2} M) effector concentrations when tuning $\bar{a}$ and $\bar{d}$, with maximal production at a fixed $\bar{a} + \bar{b}$. Fig. 3.20(A) indicates that, regardless of activation strength ($\bar{a}$) and repression ($\bar{d}$), the system can always stabilize to a state in which either $\bar{R}_2$ or $\bar{R}_3$ dominates at low effector concentrations. When $0.5 \leq \bar{a} \lesssim 1.3$ and $\bar{d} \gtrsim 0.3$ (purple-shaded region), the system can only exist as a bistable switch between these two states. When repression is weak (small $\bar{d}$), the system can also stabilize to a state expressing both genes (regardless of $\bar{a}$). Meanwhile, the strength of activation solely determines whether it is possible for the system to suppress expression altogether or to stabilize to an $\bar{R}_1$ -dominant state. For sufficiently strong activation ($\bar{a} < 0.5$), it is possible for regulation to suppress all expression, and it is only under weak activation ($\bar{a} \gtrsim 1.3$) that it is possible for the system to express the effector-targeted $\bar{R}_1$.

At a high effector concentration, Fig. 3.20(B) indicates that the most complex dynamic landscape occurs when $\bar{d}$ is very small and $\bar{a}$ is very large, i.e., when there is effectively no positive or negative regulation in the network. Such a system can stabilize to either $\bar{R}_2$ or $\bar{R}_3$ -dominant states, or to a state in which any combination of two or more proteins dominates (including effector-targeted $\bar{R}_1$), as shown in the green-shaded region. Moving slightly away from this corner of parameter space, the system loses the ability to express all three genes, a change that is more sensitive to increased repression than increased activation. Increasing repression ($\bar{d}$) further while keeping $\bar{a}$ high suppresses the system's ability to express effector-targeted $\bar{R}_1$. In this case, the dynamics become comparable to a self-activated bistable switch, as in the dark blue region of Fig. 3.20(B), or the simple bistable switch between $\bar{R}_2$ and $\bar{R}_3$, as in the purple region. Increasing activation (decreasing $\bar{a}$) while keeping

(A) AT LOW EFFECTOR CONCENTRATION



(B) AT HIGH EFFECTOR CONCENTRATION

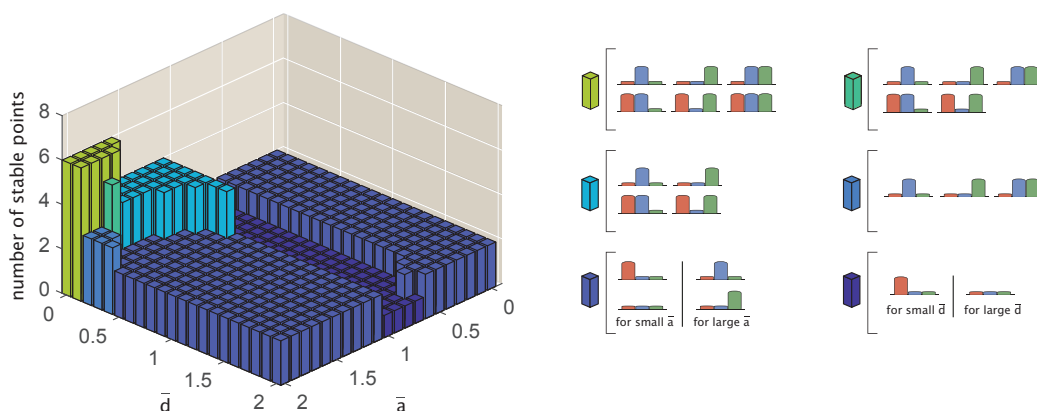


Figure 3.20: Number of stable fixed points in induction Case II of non-exclusive repressor-activator binding. The plots highlight stable points at low (10^{-8} M) and high (10^{-2} M) effector concentrations in panels (A) and (B), respectively, for varying rate parameters $\bar{a}$, $\bar{b}$, and $\bar{d}$, where $\bar{a} + \bar{b} = 2$ and $\bar{d} \leq \bar{a} + \bar{b}$. The diagrams on the right hand side of panel (A) represent the types of stable points observed in each regime at a low effector concentration, i.e., with four (blue), three (dark blue), or two (purple) stable points. The diagrams on the right side of panel (B) represent the types of stable points observed in each regime at a high effector concentration, i.e., with six (green), five (teal), four (blue), three (dark blue), two (purple), or one (dark purple) stable point(s).

repression relatively weak ($\bar{d} \lesssim 0.5$), on the other hand, implies that if the system expresses more than one gene in its stable state, one of them must be the effector-targeted $\bar{R}_1$, as seen in the blue-shaded region. In a strongly activating regime with small $\bar{a}$, dynamics center on expression of $\bar{R}_1$, either stabilizing to an $\bar{R}_1$ -dominant state or suppressing all expression (purple region). If $\bar{a} \rightarrow 1$ as in the dark purple region of Fig. 3.20(B), however, the system chooses one of these fates depending on $\bar{d}$, only expressing a gene if repression ($\bar{d}$) remains small.

As previously shown in Fig. 3.18 and Fig. 3.19 for induction Case I, we can now also investigate how different parametrizations for activation and repression in Case II influence the range of effector concentrations for which the different types of stable fixed points emerge. Fig. 3.21 tracks the minimum effector concentrations at which fixed points become possible within the parameter space, while Fig. 3.22 tracks the maximum effector concentrations. Given that Case II differs from Case I only in its treatment of $\bar{R}_1$, it follows that Fig. 3.19(A)-(B) and Fig. 3.22(C)-(D) show no difference in the expression patterns for those stable states that exclusively express one or both of the non-targeted genes, i.e., $(0, \bar{R}_2, 0)$, $(0, 0, \bar{R}_3)$, and $(0, \bar{R}_2, \bar{R}_3)$. A slight difference occurs for states expressing one of the two non-targeted genes when $\bar{a} = 1$ and $\bar{d} \leq 0.5$. Under these conditions, where Case I would allow these states to exist at all effector concentrations, there is instead still an upper bound shown in Fig. 3.22(C) for Case II beyond which the states cannot exist. This means that when effectors determine whether a protein acts a repressor or an activator, and the system neither favors nor disfavors activation, minimizing the effect of repression is not sufficient to allow expression at all effector concentrations, and that this can only be true if the system explicitly favors activation with $\bar{a} > 1$.

States expressing the effector-targeted $\bar{R}_1$ in Case II offer a stark contrast to those in Case I. In the latter setting, the $\bar{R}_1$ -dominant stable state is not included in Figs. 3.18 and 3.19 because it can exist for essentially the same range of low effector concentrations (from 10^{-8} M to approximately 5×10^{-5} M) regardless of $\bar{a}$ and $\bar{d}$. In Case II, however, this is only observed for $\bar{a} \gtrsim 1.4$, as seen in Fig. 3.21(B) and Fig. 3.22(B). Further, if $\bar{a} \leq 1$ (with some exceptions), the state can only exist at larger effector concentrations ranging from approximately 10^{-4} M to the highest parameter value, 10^{-2} M. Thus, when activation is particularly strong, the phase space for $\bar{R}_1$ -dominant expression spans the same range of concentrations observed for Case I. When activation is disfavored as the source of protein production compared to the basal level of expression, however, $\bar{R}_1$ -dominant expression emerges as a viable

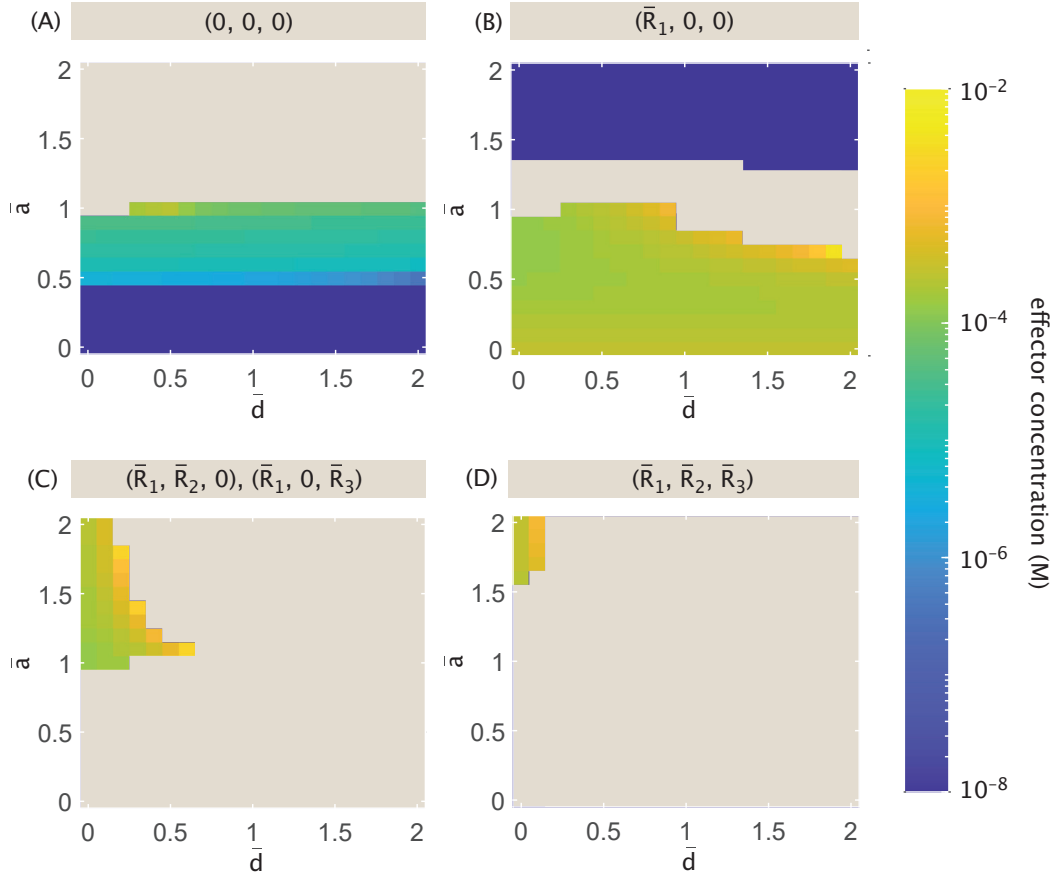


Figure 3.21: Heatmap tracking the minimum effector concentration (M) at which stable states can exist with the non-exclusive activator-repressor binding model described in Fig. 3.12(B) (Case II). Measurements span the rate parameters allowed by the constraints $\bar{a} + \bar{b} = 2$ and $\bar{d} \leq \bar{a} + \bar{b}$, with the x-axis denoting increasing repression strength $\bar{d}$, and the y-axis denoting decreasing activation with increasing $\bar{a}$. The heatmaps track (A) the stable state in which no genes are expressed, (B) the stable state in which only the effector-targeted $\bar{R}_1$ is expressed, (C) stable states expressing two genes including the effector-controlled $\bar{R}_1$, and (D) the stable state expressing all three genes. Beige represents the region of parameter space in which the stable state cannot exist at any effector concentration.

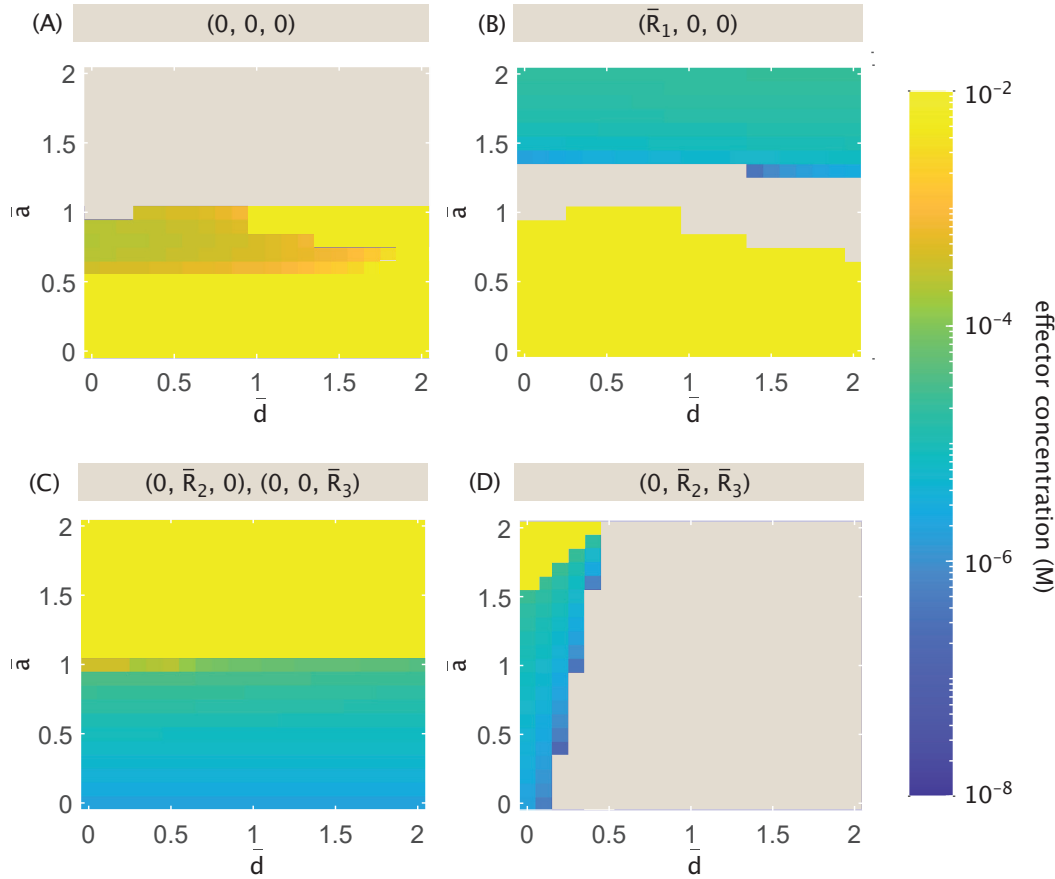


Figure 3.22: Heatmaps tracking the maximum effector concentrations (M) at which stable states can exist with non-exclusive activator-repressor binding model described in Fig. 3.12(B) (Case II). The measured values represent bifurcation thresholds for the existence of these states. The heatmaps track (A) the stable state in which no genes are expressed, (B) the stable state in which only the effector-targeted $\bar{R}_1$ is expressed, (C) stable states expressing one of the genes that does not directly interact with effector, i.e., $(0, \bar{R}_2, 0)$ and $(0, 0, \bar{R}_3)$, and (D) the stable state in which both genes not impacted by effector are expressed, i.e., $(0, \bar{R}_2, \bar{R}_3)$. Measurements span the rate parameters allowed by the constraints $\bar{a} + \bar{b} = 2$ and $\bar{d} \leq \bar{a} + \bar{b}$, with the x-axis denoting increasing repression strength $\bar{d}$, and the y-axis denoting decreasing activation as $\bar{a}$ rises. Beige represents the region where a stable state never exists at any effector concentration, and the yellow region indicates that the stable state is possible at all effector concentrations.

stable state only at high effector concentrations lying outside of the previously established range.

Considering the stable state in which all expression is suppressed, Fig. 3.18 and Fig. 3.21(A) demonstrate that regardless of the effector's role in regulating $\bar{R}_1$, this type of stable state can exist only when activation is favored over the basal rate ($\bar{a} \leq 1$). The strong resemblance between these plots also indicates that, in the viable region of parameter space, tuning $\bar{a}$ and $\bar{d}$ similarly affects the minimum concentration necessary for the state to exist. We observe small differences at the two visible thresholds: $\bar{a} = 0.5$ separates the high-activation regime in which the state always exists at low effector concentration from the regime characterized by a particular threshold effector concentration; and $\bar{a} = 1$ denotes the upper bound of $\bar{a}$ for which the $(0, 0, 0)$ state can exist at any effector concentration. Compared to Case I, when $\bar{a} = 1$ Case II requires an even weaker level of repression $\bar{d}$ to prevent the system from suppressing all expression.

Unlike Case I, for Case II the existence of the $(0, 0, 0)$ stable state does not immediately imply its viability for all effector concentrations above the minimum threshold. Fig. 3.22(A) shows that, in a regime of intermediate activation strength where $0.6 \leq \bar{a} \leq 1$, there is an upper bound on the effector concentration. These intermediate conditions mark a regime in which the effector concentration can be manipulated to tune stable expression off and on.

Finally, we consider stable states expressing more than one protein, including the effector-targeted $\bar{R}_1$. Whereas Fig. 3.19(C)-(D) indicates that these states can exist for Case I at low effector concentrations up to values between 10^{-6} M and 10^{-5} M for low repression $\bar{d}$, Fig. 3.21(C)-(D) indicates an overlapping regime of existence but with distinctly different dependencies on both rate parameters and on effector concentrations. In fact, these panels reveal that such states can only exist at higher effector concentrations above approximately 10^{-4} M, and that they further rely on there being sufficiently weak activation.

Our results for a range of possible stable states demonstrate that by probing the dynamics at various effector concentrations, we can clearly distinguish systems that utilize effectors in distinct ways. The tuning knob we present in this study can therefore be viewed as a foundation for building fitted-model descriptions of expression data, and for gaining insights into mechanisms that both regulate transcription factor activity and would otherwise not be observable.

3.5 Discussion

A large body of experimental and theoretical work has studied a number of commonly observed motifs including simple two-gene switches, oscillators, and feed-forward networks, among others [23–25, 38, 56, 98–103]. But even with these significant advances, there remains much more to explore. In fact, for *E. coli*, perhaps the most well-studied simple model organism, we still do not understand how more than $\sim 60\%$ of its genes are regulated [104]. We also do not fully understand the mechanism by which effector molecules control the transcription factors responsible for gene regulation. As the field progresses in obtaining and interpreting high-throughput data, we will likely uncover additional aspects of gene regulation that require more nuanced modeling. Our work expands upon significant and valuable existing studies to consider a range of other motifs, and draws inspiration from the prevalence of induction in the biophysical literature as an experimental tool for tuning expression.

At the core of our analysis is a baseline model of three genes in which each gene represses the other two. By harnessing inducers as tuning knobs controlling the concentration of active repressors, we observe how tuning through an MWC thermodynamic interpretation of allosteric induction limits or broadens the scope of dynamics, and how robust (or sensitive) bifurcations separating different dynamic regimes are to changes in cooperativity and relative gene interaction strengths.

In the first half of the chapter we observe that the simple three-gene toggle switch, in which only one of the three genes can dominate expression in a stable state, follows a trajectory of decreasing dynamic complexity as inducer concentration(s) rise. There are two notable takeaways from analysis of the three resulting dynamic regimes. First, we observe through bifurcation diagrams that the inducer concentration thresholds at which systems shift between tristable and bistable dynamics change little as the strength of cooperativity increases, and that the maximum level of gene expression holds steady as inducer concentrations rise. This facilitates the fitting of model parameters to experimental data, since altering the K_I of our $p_{\text{act}}(c)$ function becomes the only way to noticeably shift the bifurcation threshold. Meanwhile, when not at a low cooperativity level, the inducer concentration at which the systems shift between different forms of tristable dynamics depends most strongly on perturbation of the models' regulatory interaction structures away from symmetry. By determining the probabilities of stabilizing at each of the possible three stable points given different initial conditions at low inducer concentrations,

one can determine how close a system is to symmetry (i.e., equally strong repression among all genes). If a system tuned by one inducer is instead bistable at all inducer concentrations, it follows that this arises from a strongly skewed set of repression strengths explicitly favoring one gene, rather than from allosteric regulation.

While the baseline model demonstrates the utility of allosteric regulation as a tool to identify the physical parameters driving expression in experimental data, the second half of our study uses self-activation to highlight how the mechanism by which allostery controls transcription factor activity can significantly alter dynamics. Introducing activators that competitively bind to sites along the DNA does not affect the stable expression levels observed in the baseline model, but decreases the probability of transitioning from one stable state to another by placing stable states in deeper potential wells within the potential landscape. When self-activation is sufficiently strong, or when activators and repressors bind non-exclusively along the DNA, it becomes possible for the system to stabilize while expressing more than one gene. Understanding the parameter conditions, including the ranges of effector concentrations, that allow different stable states to emerge is a useful tool for determining how cells tune activity to coordinate expression of multiple genes.

We also find across several examples with competitive and non-exclusive repressor/activator binding that the biological interpretation of the effector's role in regulation matters, whether an effector's binding determines activity or whether it directly determines function as a repressor or an activator. Considering systems in which activators compete with repressors to bind along the DNA, changing the effector's role does not alter either the types of dynamic phases possible or the system's response to increasing effector concentrations. In the case where the effector determines protein function, however, the effector concentrations at which bifurcations occur shift to higher values, and it becomes easier to transition out of an $\bar{R}_1$ -dominant state and more difficult to transition out of an $\bar{R}_2$ or $\bar{R}_3$ -dominant state. In non-exclusive binding settings, we find that the two interpretations of effector activity reveal distinct differences in the types of phases observed across parameter space, as well as in the ranges of effector concentrations at which different types of stable points are observed.

Tuning effector concentration(s) thus allows us to distinguish among biologically distinct models for a single gene regulatory motif, even when the particular set of observed stable states is characteristic of multiple models. For instance, in both the competitive (with strong activation) and non-exclusive binding settings, it is

possible for the system to stabilize at low effector concentrations to any of the three single-gene-dominant states, or to a state suppressing all expression. As the effector concentration increases, however, the candidate models have divergent responses and are thus distinguishable. If the system becomes tristable to either suppress all expression or allow expression of a gene not targeted by the effector, the system identifies as competitive repressor-activator binding. If expression becomes suppressed entirely, the system instead allows non-exclusive binding, with the binding site determining transcription factor function and the effector only determining protein activity. Finally, if the system transforms to a bistable switch between no expression and expression of the effector-targeted gene alone, this implies non-exclusive binding where effector presence directly determines transcription factor function.

The mathematical interpretation of the biological mechanisms for induction embedded in the models we study have a significant impact on the types of dynamics observed. Our analysis therefore complements existing work on the direct incorporation of effector-driven transcription factor activity into models of gene regulation while further motivating the rigorous definition of *how* effectors function. In so doing, the work presents a theoretical approach through which it is possible to uncover such properties, along with the model parameters that allow them, in conjunction with experiments.

While our models ground the discussion of induction-driven bifurcations in the language of explicit gene circuits, it is worth noting the relevance of our framework to existing geometry-based descriptions of cell fate, which assume gradient or gradient-like behavior and abstract from mechanistic details. In geometric frameworks [39–43], cell fates arise from trajectories through a low-dimensional state space structured by attractor basins and their boundaries, where normal form polynomials tunable by abstract coefficients capture the relevant bifurcation structures near fate decisions. Several open questions remain, however, in relating this approach to single-cell RNA sequencing data. For example, which gene regulatory architectures produce these abstract landscape geometries, and how do external signals couple to those architectures to drive the bifurcation sequences that developmental biology requires? More concretely, how can one specify the relevant parameters as explicit functions of external inducer concentrations?

Our framework provides a direct answer by bridging phenomenological manifold descriptions with mechanistic transcriptional control. By defining the role of inducer concentration explicitly, we directly track corresponding shifts in repression thresh-

olds and can observe how nullclines translate and rotate in phase space. We thus establish induction as a mechanistically-defined geometric operator on the attractor landscape that reshapes the number and stability of fixed points.

In the context of normal form polynomials, the abstract parameters defining the phase space no longer implicitly depend on changes in external signals, but rather are explicit functions of inducer concentration. By directly mapping abstract parameters to experimentally accessible tuning knobs, we can determine exactly which regions of phase space are accessible to the cell and to the experimentalist, and what bifurcation sequences can be realistically traversed. Dynamical landscapes are thus no longer abstract objects for comparison to gene expression data, but rather settings that can be directly probed by inducer titration, facilitating reliable identification of topological models for single-cell experiments.

Finally, as evidenced by the diverse outputs we obtain from subjecting our three-noded networks to a range of effector inputs, there is considerable richness in the input-output responses possible for these systems. Our findings further underscore the importance and the challenges of understanding how such outcomes are realized in even more complex regulatory architectures, and invite dialogue with ongoing experimental efforts to reveal the full scope of the allosterome.

Appendices

3.A Biologically permissible ranges for MWC parameters in allosteric induction

To determine the biologically permissible ranges for the MWC parameters defined in Eqn. 3.6 (K_I , K_A , and $\Delta\epsilon$), we assume that the absence of inducer does not imply the complete absence of inactive repressor, given that there may be some natural fractional error q . We suppose, then, that in the absence of inducer,

$$p_{\text{act}}(c = 0) \geq 1 - q, \quad (3.45)$$

or alternatively,

$$1 - p_{\text{act}}(c = 0) = 1 - \frac{1}{1 + e^{-\beta\Delta\epsilon}} = \frac{1}{1 + e^{\beta\Delta\epsilon}} \leq q. \quad (3.46)$$

Rearranging Eqn. 3.46, we obtain a condition for the energy difference $\Delta\epsilon$:

$$e^{-\beta\Delta\epsilon} \leq \frac{q}{1 - q}. \quad (3.47)$$

It follows that for the probability of activity to saturate in the absence of inducer ($q = 0$), the energy of the inactive repressor state must far exceed that of the active state so that $\Delta\epsilon \rightarrow \infty$ and the repressor is always active.

Gene expression is generally “leaky,” meaning that there is typically a basal level of expression even in the presence of a large repressor concentration. We assume that this leakiness is also reflected by the fraction q such that a fraction of repressors less than q are active in the limit of infinite inducer concentration, with

$$\begin{aligned} p_A(c \rightarrow \infty) &\approx \frac{\left(\frac{c}{K_A}\right)^m}{\left(\frac{c}{K_A}\right)^m + e^{-\beta\Delta\epsilon}\left(\frac{c}{K_I}\right)^m} \\ &= \frac{(K_I)^m}{(K_I)^m + e^{-\beta\Delta\epsilon}(K_A)^m} \leq q. \end{aligned} \quad (3.48)$$

Rearranging Eqn. 3.48, we obtain a second condition

$$\left(\frac{K_A}{K_I}\right)^m \geq \frac{1-q}{q} e^{\beta\Delta\epsilon}. \quad (3.49)$$

Eqns. 3.47 and 3.49 establish bounds on the dissociation constants $K_{A,I}$ and the energy difference $\Delta\epsilon$ for a given “error” q and number of binding sites m .

Suppose, for example, that less than 10% ($q = 0.1$) of repressors are active in the presence of an infinite inducer concentration. MWC parameters are then bounded by

$$e^{-\beta\Delta\epsilon} \leq \frac{1}{9} \quad (3.50)$$

and

$$\left(\frac{K_A}{K_I}\right)^m \geq 9e^{\beta\Delta\epsilon}. \quad (3.51)$$

We use these bounds to determine a permissible range of K_I given fixed $K_A = 150 \mu M$ in Fig. 3.4(B).

3.B Analytic bifurcations for the two inducer model

In this appendix, we show analytically that the linear thresholds separating the (c_1, c_2) parameter space into tristable, bistable, and monostable regions in Section 3.2.2 are the same as those in Section 3.2.1.

The key bifurcation with respect to c_1 is the loss of the $\bar{R}_1$ -dominant stable state in transitioning from tristable to bistable dynamics. Assuming that $\bar{R}_{2,3}^n \rightarrow 0$ as in the single inducer setting, Eqns. 3.24 - 3.26 indicate a steady state solution

$$\bar{R}_{ss} = (\bar{R}_1, \bar{R}_2, \bar{R}_3)$$

$$= \left(\bar{a}, \frac{\bar{a}}{1 + [p_{\text{act}}(c_1)\bar{a}]^n}, \frac{\bar{a}}{1 + [p_{\text{act}}(c_1)\bar{a}]^n} \right). \quad (3.52)$$

Since our assumptions require $\bar{R}_2 = \bar{R}_3 \leq \varepsilon$,

$$\varepsilon \geq \frac{\bar{a}}{1 + [p_{\text{act}}(c_1)\bar{a}]^n} \quad (3.53)$$

and thus the $\bar{R}_1$ dominant steady state exists for

$$p_{\text{act}}(c_1) \geq \frac{1}{\bar{a}} \sqrt[n]{\frac{\bar{a}}{\varepsilon} - 1}, \quad (3.54)$$

the same as derived for a single inducer in Eqn. 3.22.

We derive the same bound for $p_{\text{act}}(c_2)$ by determining the bifurcation point with respect to c_2 at which the system no longer stabilizes to an $\bar{R}_2$ -dominant state.

3.C Non-exclusive repressor-activator binding in the three-gene toggle switch

In Section 3.4.2 we discuss the three-gene toggle switch with self-activation where repressors and activators can bind non-exclusively at a gene's regulatory region. This appendix outlines the analytic model and how it is modified under different interpretations of effector activity.

Fig. 3.23 shows the thermodynamic states, weights and rates related to non-exclusive binding. Note that these include the same states describing competitive binding in Fig. 3.13(A), but with three additional states in which an activator and either one or two repressors can be bound simultaneously at the relevant gene regulatory site. In these additional states, the presence of repressor(s) dampens expression to $a + b - d$. The states and weights translate into a corresponding system of equations for the evolution of expression. Omitting the role of effector, the change in R_1 expression, for instance, is

$$\begin{aligned} \frac{dR_1}{dt} &= \frac{a + (a + b)(\frac{R_1}{K_1})^n + (a + b - d)(\frac{R_1}{K_1})^n[(\frac{R_2}{K_2})^n + (\frac{R_3}{K_3})^n + (\frac{R_2}{K_2})^n(\frac{R_3}{K_3})^n]}{[1 + (\frac{R_1}{K_1})^n][1 + (\frac{R_2}{K_2})^n][1 + (\frac{R_3}{K_3})^n]} - \frac{R_1}{\tau} \\ &= \frac{a}{[1 + (\frac{R_2}{K_2})^n][1 + (\frac{R_3}{K_3})^n]} + \frac{b(\frac{R_1}{K_1})^n}{1 + (\frac{R_1}{K_1})^n} + \frac{(a - d)(\frac{R_1}{K_1})^n[(\frac{R_2}{K_2})^n + (\frac{R_3}{K_3})^n + (\frac{R_2}{K_2})^n(\frac{R_3}{K_3})^n]}{[1 + (\frac{R_1}{K_1})^n][1 + (\frac{R_2}{K_2})^n][1 + (\frac{R_3}{K_3})^n]} - \frac{R_1}{\tau}, \end{aligned} \quad (3.55)$$

where a is the rate of protein production when gene transcription is unregulated, $a + b$ is the rate with bound self-activator, and $a + b - d$ is the previous rate attenuated

NON-EXCLUSIVE REPRESSOR/ACTIVATOR BINDING: CASE I

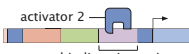
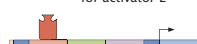
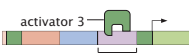
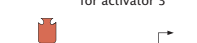
	STATE	WEIGHT	RATE	STATE	WEIGHT	RATE
R ₁	 binding site region for activator 1	1	a	 activator 1 binding site region for activator 1	$\left(\frac{p_{\text{act}}(c_1)R_1}{K_1}\right)^n$	a + b
	 binding site region for activator 1	$\left(\frac{p_{\text{act}}(c_2)R_2}{K_2}\right)^n$	0	 binding site region for activator 1	$\left(\frac{p_{\text{act}}(c_1)R_1}{K_1}\right)^n \left(\frac{p_{\text{act}}(c_2)R_2}{K_2}\right)^n$	a + b - d
	 binding site region for activator 1	$\left(\frac{p_{\text{act}}(c_3)R_3}{K_3}\right)^n$	0	 binding site region for activator 1	$\left(\frac{p_{\text{act}}(c_1)R_1}{K_1}\right)^n \left(\frac{p_{\text{act}}(c_3)R_3}{K_3}\right)^n$	a + b - d
	 binding site region for activator 1	$\left(\frac{p_{\text{act}}(c_2)R_2}{K_2}\right)^n \left(\frac{p_{\text{act}}(c_3)R_3}{K_3}\right)^n$	0	 binding site region for activator 1	$\left(\frac{p_{\text{act}}(c_1)R_1}{K_1}\right)^n \left(\frac{p_{\text{act}}(c_2)R_2}{K_2}\right)^n \left(\frac{p_{\text{act}}(c_3)R_3}{K_3}\right)^n$	a + b - d
R ₂	 binding site region for activator 2	1	a	 activator 2 binding site region for activator 2	$\left(\frac{p_{\text{act}}(c_2)R_2}{K_2}\right)^n$	a + b
	 binding site region for activator 2	$\left(\frac{p_{\text{act}}(c_1)R_1}{K_1}\right)^n$	0	 binding site region for activator 2	$\left(\frac{p_{\text{act}}(c_1)R_1}{K_1}\right)^n \left(\frac{p_{\text{act}}(c_2)R_2}{K_2}\right)^n$	a + b - d
	 binding site region for activator 2	$\left(\frac{p_{\text{act}}(c_3)R_3}{K_3}\right)^n$	0	 binding site region for activator 2	$\left(\frac{p_{\text{act}}(c_2)R_2}{K_2}\right)^n \left(\frac{p_{\text{act}}(c_3)R_3}{K_3}\right)^n$	a + b - d
	 binding site region for activator 2	$\left(\frac{p_{\text{act}}(c_1)R_1}{K_1}\right)^n \left(\frac{p_{\text{act}}(c_3)R_3}{K_3}\right)^n$	0	 binding site region for activator 2	$\left(\frac{p_{\text{act}}(c_1)R_1}{K_1}\right)^n \left(\frac{p_{\text{act}}(c_2)R_2}{K_2}\right)^n \left(\frac{p_{\text{act}}(c_3)R_3}{K_3}\right)^n$	a + b - d
R ₃	 binding site region for activator 3	1	a	 activator 3 binding site region for activator 3	$\left(\frac{p_{\text{act}}(c_3)R_3}{K_3}\right)^n$	a + b
	 binding site region for activator 3	$\left(\frac{p_{\text{act}}(c_1)R_1}{K_1}\right)^n$	0	 binding site region for activator 3	$\left(\frac{p_{\text{act}}(c_1)R_1}{K_1}\right)^n \left(\frac{p_{\text{act}}(c_3)R_3}{K_3}\right)^n$	a + b - d
	 binding site region for activator 3	$\left(\frac{p_{\text{act}}(c_2)R_2}{K_2}\right)^n$	0	 binding site region for activator 3	$\left(\frac{p_{\text{act}}(c_2)R_2}{K_2}\right)^n \left(\frac{p_{\text{act}}(c_3)R_3}{K_3}\right)^n$	a + b - d
	 binding site region for activator 3	$\left(\frac{p_{\text{act}}(c_1)R_1}{K_1}\right)^n \left(\frac{p_{\text{act}}(c_2)R_2}{K_2}\right)^n$	0	 binding site region for activator 3	$\left(\frac{p_{\text{act}}(c_1)R_1}{K_1}\right)^n \left(\frac{p_{\text{act}}(c_2)R_2}{K_2}\right)^n \left(\frac{p_{\text{act}}(c_3)R_3}{K_3}\right)^n$	a + b - d

Figure 3.23: Expression of each protein R_i in the three-gene toggle switch with self-activation and non-exclusive binding for repressors and activators. Effector binding is defined as in Fig. 3.12(A).

by the presence of bound repressor. Note that the second line of Eqn. 3.55 rearranges the first to separate protein production into three physically meaningful terms: (i) protein expression in the presence of repressors only, (ii) expression exclusively due to activation, and (iii) expression in the presence of both repressors and activators. Rescaling variables in Eq. 3.55 and in the equivalent equations for R_2 and R_3 expression such that $\bar{R}_i = R_i/K_1$, $\bar{t} = t/\tau$, $\bar{a} = \tau a/K_1$, $\bar{b} = \tau b/K_i$, and $\bar{d} = \tau d/K_1$, we define our non-exclusive binding model in dimensionless form by the set of differential equations

$$\frac{d\bar{R}_1}{d\bar{t}} = \frac{\bar{a}}{[1 + (\frac{\bar{R}_2}{K^{(2)}})^n][1 + (\frac{\bar{R}_3}{K^{(3)}})^n]} + \frac{\bar{b}\bar{R}_1^n}{1 + \bar{R}_1^n} + \frac{(\bar{a} - \bar{d})\bar{R}_1^n[(\frac{\bar{R}_2}{K^{(2)}})^n + (\frac{\bar{R}_3}{K^{(3)}})^n + (\frac{\bar{R}_2}{K^{(2)}})^n(\frac{\bar{R}_3}{K^{(3)}})^n]}{[1 + \bar{R}_1^n][1 + (\frac{\bar{R}_2}{K^{(2)}})^n][1 + (\frac{\bar{R}_3}{K^{(3)}})^n]} - \bar{R}_1, \quad (3.56)$$

$$\frac{d\bar{R}_2}{d\bar{t}} = \frac{\bar{a}}{[1 + \bar{R}_1^n][1 + (\frac{\bar{R}_3}{K^{(3)}})^n]} + \frac{\bar{b}(\frac{\bar{R}_2}{K^{(2)}})^n}{1 + (\frac{\bar{R}_2}{K^{(2)}})^n} + \frac{(\bar{a} - \bar{d})(\frac{\bar{R}_2}{K^{(2)}})^n(\bar{R}_1^n + (\frac{\bar{R}_3}{K^{(3)}})^n + \bar{R}_1^n(\frac{\bar{R}_3}{K^{(3)}})^n)}{[1 + \bar{R}_1^n][1 + (\frac{\bar{R}_2}{K^{(2)}})^n][1 + (\frac{\bar{R}_3}{K^{(3)}})^n]} - \bar{R}_2, \quad (3.57)$$

$$\frac{d\bar{R}_3}{d\bar{t}} = \frac{\bar{a}}{[1 + \bar{R}_1^n][1 + (\frac{\bar{R}_2}{K^{(2)}})^n]} + \frac{\bar{b}(\frac{\bar{R}_3}{K^{(3)}})^n}{1 + (\frac{\bar{R}_3}{K^{(3)}})^n} + \frac{(\bar{a} - \bar{d})(\frac{\bar{R}_3}{K^{(3)}})^n(\bar{R}_1^n + (\frac{\bar{R}_2}{K^{(2)}})^n + \bar{R}_1^n(\frac{\bar{R}_2}{K^{(2)}})^n)}{[1 + \bar{R}_1^n][1 + (\frac{\bar{R}_2}{K^{(2)}})^n][1 + (\frac{\bar{R}_3}{K^{(3)}})^n]} - \bar{R}_3, \quad (3.58)$$

with $\bar{d} \leq \bar{a} + \bar{b}$, $K^{(2)} = K_2/K_1$, and $K^{(3)} = K_3/K_1$. As in Section 3.4.1, our analysis in Section 3.4.2 assumes equal binding affinities such that $K^{(2)} = K^{(3)} = 1$, and a maximal production rate $\bar{a} + \bar{b} = 2$.

Unlike the competitive binding case, the model defined in Eqns. 3.56 - 3.58 depends on *two* independent parameters: (i) unregulated (basal) protein expression $\bar{a}$, with corresponding $\bar{b} = 2 - \bar{a}$ denoting additional expression from activations, and (ii) the strength of repression from $\bar{d}$. We now explicitly incorporate the role of effector molecules in two ways.

First, Fig. 3.23 defines effector activity as described by Case I of Fig. 3.12(A), where the active concentration of a protein indexed by i is $p_{\text{act}}(c)\bar{R}_i$ regardless of its function as a repressor or an activator. For the system containing one effector at concentration c that targets $\bar{R}_1$, the dimensionless Eqns. 3.56 - 3.58 in induction Case I become

$$\frac{d\bar{R}_1}{d\bar{t}} = \frac{\bar{a}}{[1 + \bar{R}_2^n][1 + \bar{R}_3^n]} + \frac{\bar{b}[p_{\text{act}}(c)\bar{R}_1]^n}{1 + [p_{\text{act}}(c)\bar{R}_1]^n} + \frac{(\bar{a} - \bar{d})[p_{\text{act}}(c)\bar{R}_1]^n[\bar{R}_2^n + \bar{R}_3^n + \bar{R}_2^n\bar{R}_3^n]}{[1 + [p_{\text{act}}(c)\bar{R}_1]^n][1 + \bar{R}_2^n][1 + \bar{R}_3^n]} - \bar{R}_1, \quad (3.59)$$

$$\frac{d\bar{R}_2}{d\bar{t}} = \frac{\bar{a}}{[1 + [p_{\text{act}}(c)\bar{R}_1]^n][1 + \bar{R}_3^n]} + \frac{\bar{b}\bar{R}_2^n}{1 + \bar{R}_2^n} + \frac{(\bar{a} - \bar{d})\bar{R}_2^n([p_{\text{act}}(c)\bar{R}_1]^n + \bar{R}_3^n + [p_{\text{act}}(c)\bar{R}_1]^n\bar{R}_3^n)}{[1 + [p_{\text{act}}(c)\bar{R}_1]^n][1 + \bar{R}_2^n][1 + \bar{R}_3^n]} - \bar{R}_2, \quad (3.60)$$

$$\frac{d\bar{R}_3}{d\bar{t}} = \frac{\bar{a}}{[1 + [p_{\text{act}}(c)\bar{R}_1]^n][1 + \bar{R}_2^n]} + \frac{\bar{b}\bar{R}_3^n}{1 + \bar{R}_3^n} + \frac{(\bar{a} - \bar{d})\bar{R}_3^n([p_{\text{act}}(c)\bar{R}_1]^n + \bar{R}_2^n + [p_{\text{act}}(c)\bar{R}_1]^n\bar{R}_2^n)}{[1 + [p_{\text{act}}(c)\bar{R}_1]^n][1 + \bar{R}_2^n][1 + \bar{R}_3^n]} - \bar{R}_3. \quad (3.61)$$

NON-EXCLUSIVE REPRESSOR/ACTIVATOR BINDING: CASE II

	STATE	WEIGHT	RATE	STATE	WEIGHT	RATE
R_1		1	a		$\left(\frac{[1-p_{\text{act}}(c_1)]R_1}{K_1}\right)^n$	a + b
		$\left(\frac{p_{\text{act}}(c_2)R_2}{K_2}\right)^n$	0		$\left(\frac{[1-p_{\text{act}}(c_1)]R_1}{K_1}\right)^n \left(\frac{p_{\text{act}}(c_2)R_2}{K_2}\right)^n$	a + b - d
		$\left(\frac{p_{\text{act}}(c_3)R_3}{K_3}\right)^n$	0		$\left(\frac{[1-p_{\text{act}}(c_1)]R_1}{K_1}\right)^n \left(\frac{p_{\text{act}}(c_3)R_3}{K_3}\right)^n$	a + b - d
		$\left(\frac{p_{\text{act}}(c_2)R_2}{K_2}\right)^n \left(\frac{p_{\text{act}}(c_3)R_3}{K_3}\right)^n$	0		$\left(\frac{[1-p_{\text{act}}(c_1)]R_1}{K_1}\right)^n \left(\frac{p_{\text{act}}(c_2)R_2}{K_2}\right)^n \left(\frac{p_{\text{act}}(c_3)R_3}{K_3}\right)^n$	a + b - d
R_2		1	a		$\left(\frac{[1-p_{\text{act}}(c_2)]R_2}{K_2}\right)^n$	a + b
		$\left(\frac{p_{\text{act}}(c_1)R_1}{K_1}\right)^n$	0		$\left(\frac{p_{\text{act}}(c_1)R_1}{K_1}\right)^n \left(\frac{[1-p_{\text{act}}(c_2)]R_2}{K_2}\right)^n$	a + b - d
		$\left(\frac{p_{\text{act}}(c_3)R_3}{K_3}\right)^n$	0		$\left(\frac{[1-p_{\text{act}}(c_2)]R_2}{K_2}\right)^n \left(\frac{p_{\text{act}}(c_3)R_3}{K_3}\right)^n$	a + b - d
		$\left(\frac{p_{\text{act}}(c_1)R_1}{K_1}\right)^n \left(\frac{p_{\text{act}}(c_3)R_3}{K_3}\right)^n$	0		$\left(\frac{p_{\text{act}}(c_1)R_1}{K_1}\right)^n \left(\frac{[1-p_{\text{act}}(c_2)]R_2}{K_2}\right)^n \left(\frac{p_{\text{act}}(c_3)R_3}{K_3}\right)^n$	a + b - d
R_3		1	a		$\left(\frac{[1-p_{\text{act}}(c_3)]R_3}{K_3}\right)^n$	a + b
		$\left(\frac{p_{\text{act}}(c_1)R_1}{K_1}\right)^n$	0		$\left(\frac{p_{\text{act}}(c_1)R_1}{K_1}\right)^n \left(\frac{[1-p_{\text{act}}(c_3)]R_3}{K_3}\right)^n$	a + b - d
		$\left(\frac{p_{\text{act}}(c_2)R_2}{K_2}\right)^n$	0		$\left(\frac{p_{\text{act}}(c_2)R_2}{K_2}\right)^n \left(\frac{[1-p_{\text{act}}(c_3)]R_3}{K_3}\right)^n$	a + b - d
		$\left(\frac{p_{\text{act}}(c_1)R_1}{K_1}\right)^n \left(\frac{p_{\text{act}}(c_2)R_2}{K_2}\right)^n$	0		$\left(\frac{p_{\text{act}}(c_1)R_1}{K_1}\right)^n \left(\frac{p_{\text{act}}(c_2)R_2}{K_2}\right)^n \left(\frac{[1-p_{\text{act}}(c_3)]R_3}{K_3}\right)^n$	a + b - d

Figure 3.24: Expression of each protein R_i in the three-gene toggle switch with self-activation and non-exclusive binding for repressors and activators. Effector binding is defined as in Fig. 3.12(B).

Evaluating the fixed points of Eqns. 3.59 - 3.61, we obtain the results discussed in Section 3.4.2.

An alternative interpretation for effector activity, described by Case II of Fig. 3.12(B), transforms the available states and weights to those in Fig. 3.24, where the concentration of $\bar{R}_i$ is scaled by $p_{\text{act}}(c)$ when repressing expression of other proteins, and scaled by $1 - p_{\text{act}}(c)$ when activating its own expression. Eqns. 3.56 - 3.58 then become

$$\frac{d\bar{R}_1}{d\bar{t}} = \frac{\bar{a}}{[1 + \bar{R}_2^n][1 + \bar{R}_3^n]} + \frac{\bar{b}[(1 - p_{\text{act}}(c))\bar{R}_1]^n}{1 + [(1 - p_{\text{act}}(c))\bar{R}_1]^n} + \frac{(\bar{a} - \bar{d})[(1 - p_{\text{act}}(c))\bar{R}_1]^n[\bar{R}_2^n + \bar{R}_3^n + \bar{R}_2^n\bar{R}_3^n]}{[1 + [(1 - p_{\text{act}}(c))\bar{R}_1]^n][1 + \bar{R}_2^n][1 + \bar{R}_3^n]} - \bar{R}_1, \quad (3.62)$$

$$\frac{d\bar{R}_2}{d\bar{t}} = \frac{\bar{a}}{[1 + [p_{\text{act}}(c)\bar{R}_1]^n][1 + \bar{R}_3^n]} + \frac{\bar{b}\bar{R}_2^n}{1 + \bar{R}_2^n} + \frac{(\bar{a} - \bar{d})\bar{R}_2^n([p_{\text{act}}(c)\bar{R}_1]^n + \bar{R}_3^n + [p_{\text{act}}(c)\bar{R}_1]^n\bar{R}_3^n)}{[1 + [p_{\text{act}}(c)\bar{R}_1]^n][1 + \bar{R}_2^n][1 + \bar{R}_3^n]} - \bar{R}_2, \quad (3.63)$$

$$\frac{d\bar{R}_3}{d\bar{t}} = \frac{\bar{a}}{[1 + [p_{\text{act}}(c)\bar{R}_1]^n][1 + \bar{R}_2^n]} + \frac{\bar{b}\bar{R}_3^n}{1 + \bar{R}_3^n} + \frac{(\bar{a} - \bar{d})\bar{R}_3^n([p_{\text{act}}(c)\bar{R}_1]^n + \bar{R}_2^n + [p_{\text{act}}(c)\bar{R}_1]^n\bar{R}_2^n)}{[1 + [p_{\text{act}}(c)\bar{R}_1]^n][1 + \bar{R}_2^n][1 + \bar{R}_3^n]} - \bar{R}_3, \quad (3.64)$$

describing a system containing only one effector at concentration c targeting $\bar{R}_1$ activity. Evaluating the fixed points of Eqns. 3.62 - 3.64, we obtain the results discussed in Section 3.4.2.

3.D Code availability

Matlab code used to generate figure graphs throughout this work are available ².

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²Supporting Matlab code is available on GitHub at github.com/Rebecca-J-Rousseau/inducible3genetoggle

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Chapter 4

THERMODYNAMIC MODELS OF SIMPLE INDUCIBLE GENETIC CIRCUITS¹

The previous chapter revealed the rich multistable dynamics that emerge in gene circuits as effector concentrations vary, and demonstrated how the specific mechanism of induction can fundamentally reshape the accessible landscape of cellular states. The three-gene network models discussed there are relevant to cell differentiation processes, such as those involving T-helper immune cells, and more broadly demonstrate methods that can be broadened to encompass other inducible circuit applications, such as those depicted in Fig. 4.1. Our explorations of transcription regulation in three-gene mutual repression networks made extensive use of Hill-function descriptions, which construct phenomenological expressions that efficiently capture cooperative binding and are well-suited to bifurcation analyses across large parameter spaces and in complex interaction networks. A long history in the literature supports the use of Hill functions as an approximation for describing the probability of promoter binding [5–13], but the approach assumes that gene regulation operates in a high cooperativity limit such that the set of possible regulatory states for a single type of transcription factor is binary – i.e., either bound or unbound. This facilitates the study of more complex network motifs and corresponding higher-order multistability [14, 15], and provides a foundation for expanding to larger networks.

In this chapter, we complement this powerful circuit-level perspective by considering ways to introduce additional molecular detail into our definitions for gene regulatory mechanics. Thermodynamic models, in particular, offer an alternate but related approach that explicitly enumerates the *complete* set of Boltzmann-weighted occupancy states for a regulatory region. Unlike the Hill function description, this means that more than one type of bound state could exist in a cooperative system, and that the number of regulatory protein copies that are bound, along with the strengths of interactions among those bound proteins, can be described more precisely. The framework also offers mechanistic transparency and interpretability, making it a nat-

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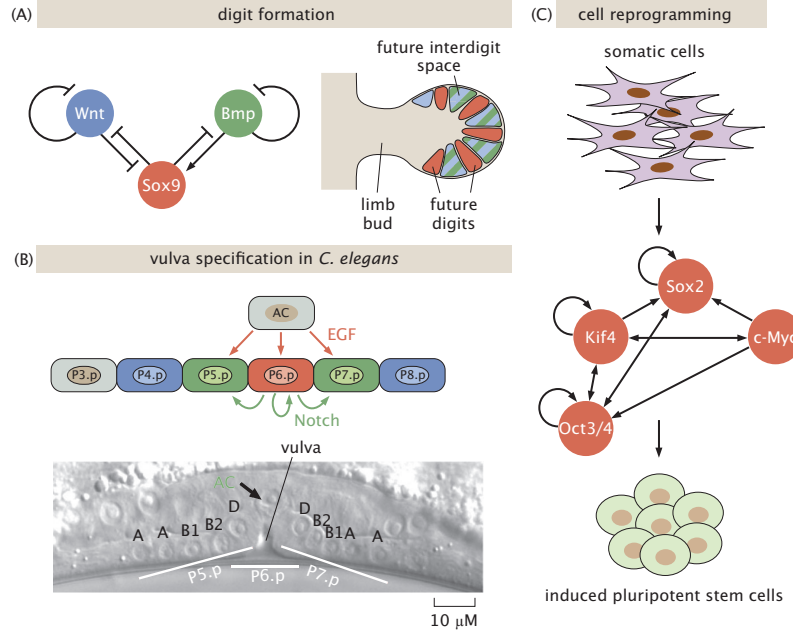


Figure 4.1: Gallery of examples of regulatory circuits participating in the genetic decisions of animal development. (A) A three-node network thought to be relevant to the control of digit formation, adapted from [1]. (B) An example involving vulval development in *C. elegans*, where epidermal growth factor (EGF) and Notch induce cells toward one of three possible fates, adapted from [2] and [3]. (C) Transcription factors compete and maintain cell pluripotency unless sufficiently induced to reprogram a cell to a differentiated fate, adapted from [4].

ural companion to Hill-based analyses, and one that grounds the emergent behaviors of complex networks in the microscopic interactions from which they arise. Accordingly, we shift our focus to simpler gene network motifs in this chapter, which allow us to employ thermodynamic models in a regime that is computationally feasible. While too detailed for application to higher-order networks, the insights gained, from a pedagogical perspective, render the additional rigor quite illuminating when applied to the definitions for molecular interactions during gene regulation.

To illustrate the thermodynamic models of interest, recall that the general way to describe a circuit involving n different transcription factor proteins is through a set of dynamical equations of the form

$$\frac{d[TF_i]}{dt} = f_i(\{[TF_j]\}), \quad (4.1)$$

where there is one such equation for each transcription factor (for which we will often use the shorthand notation). The function on the right side acknowledges that the dynamics of the i^{th} TF depend upon the concentrations of all the others,

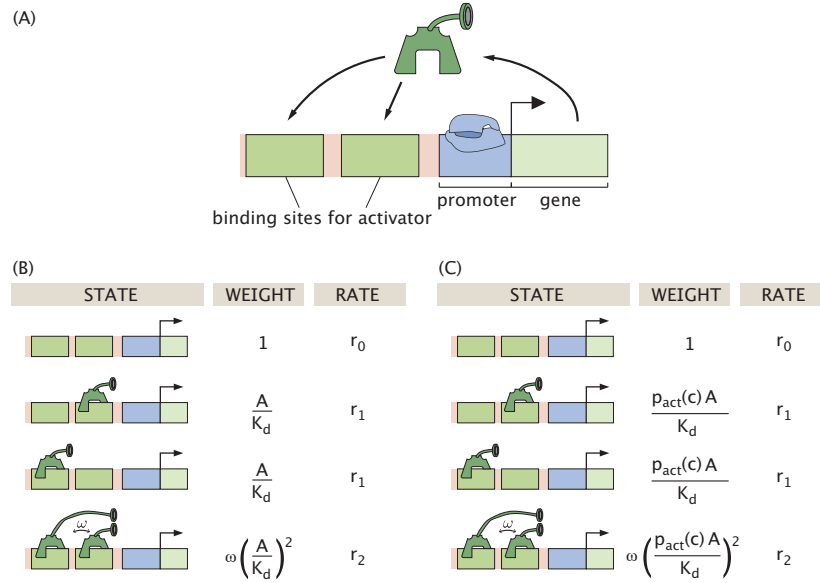


Figure 4.2: The auto-activation regulatory circuit. (A) Schematic of the operation of the circuit. Polymerase binding at the promoter (blue) transcribes the gene (encoded in the light green region), producing a protein that can activate its own expression at a sufficient concentration. In our model, an activator can bind at one of two possible sites to enhance gene transcription. (B) Thermodynamic states, weights, and rates for the circuit in the traditional model without induction. The parameter ω denotes the cooperative strength of two activators binding. (C) Thermodynamic states, weights and rates for the case in which the effector tunes the fraction of active activators. Note that in both of these cases the parameters K_d , ω , r_0 , r_1 and r_2 are effective parameters that have hidden dependence upon the number of polymerases and the strength with which polymerase binds the promoter. The explicit definitions of these effective parameters are worked out in Appendix 4.A.

represented here by the notation $\{TF_j\}$ signifying “the set of all n TFs.” Chapter 3 demonstrated one way to define the function f_i through a Hill-function description, and we now demonstrate what this function becomes in a thermodynamic model. For pedagogical simplicity, let us consider as a first case study the auto-activation switch, shown in Fig. 4.2(A). This regulatory motif allows two binding sites for an activating transcription factor, and two such proteins binding simultaneously are able to interact cooperatively. Since we do not necessarily assume a high-cooperativity limit for the biological activity of this network, however, it is also possible for the promoter of this gene to have single-bound activator. Fig. 4.2(B) lists the related states and thermodynamic weights, with each type of state allowing gene expression at a different rate. Expression of protein A is thus described by an equation of the

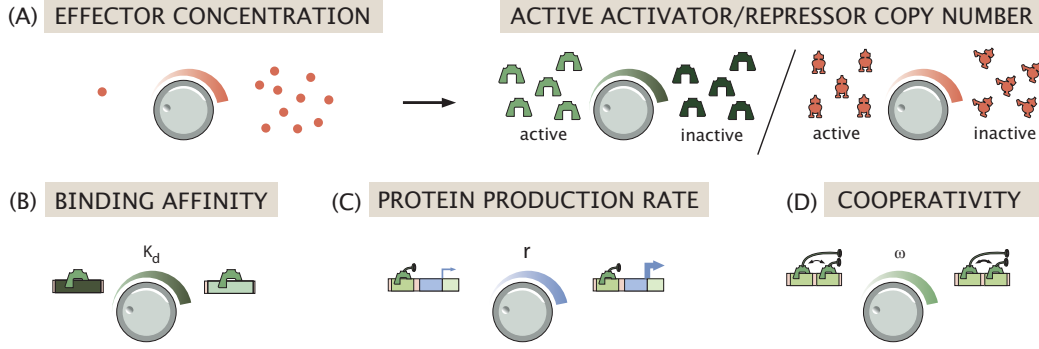


Figure 4.3: Tuning genetic circuits. The schematic shows different knobs that are available to the cell, the theorist and the experimentalist, namely (A) effector concentration (and by consequence the number of active activator or repressor molecules), (B) binding affinity K_d , (C) protein production rate r , and (D) cooperativity ω .

form

$$\frac{dA}{dt} = -\gamma A + \frac{r_0 + r_1 2 \frac{A}{K_d} + r_2 \omega \left(\frac{A}{K_d} \right)^2}{1 + 2 \frac{A}{K_d} + \omega \left(\frac{A}{K_d} \right)^2}, \quad (4.2)$$

where A is the number of activators, K_d is the dissociation constant for A in its interaction with DNA, and ω is the cooperativity between two activators bound to the gene promoter along the DNA. The first term on the right captures the degradation of activator at rate γ , and the second term characterizes protein production with a basal level of production r_0 and a saturating level r_2 . The production rate in Eqn. 4.2, and throughout this chapter, is modeled using a thermodynamic framework relating promoter occupancy to output [16–22]. Note that, as has been reported previously in the literature [22], this dynamical equation becomes a Hill function form in the high cooperativity limit.

Typically, analysis of dynamic stability in auto-activation and other simple genetic circuits proceeds by varying theoretically accessible parameters such as dissociation constants (K_d), transcription rates (r_i) and degradation rates (γ) as featured in Eqn. 4.2 and in Fig. 4.3(B-D), without reference to *how* such parameters are themselves controlled by living cells [23]. As emphasized in the previous chapter, however, the underlying protein expression response is in fact dictated by the presence and absence of experimentally accessible *effector molecules* that alter the balance between inactive and active forms of key regulatory molecules such as transcription factors, as shown in Fig. 4.3(A). Thus, while all of the tunable parameters in Fig. 4.3 make it possible to systematically tune the level of gene expression, some of these parameters are more conveniently accessible to the experimentalist and to

the cell itself as it rapidly tunes its behavior in response to stimuli. Our goal is to use explicit statistical mechanical models of the induction phenomenon to gain additional insights about the behavior of simpler genetic circuits as a function of the presence or absence of effectors. The reduced scale allows us another way to demonstrate the power of induction as a tuning knob in auto-activation and mutual repression, and even facilitates an extension to feed-forward loop circuits.

We will begin this expansion into inducible thermodynamic models by reviewing the key statistical mechanical principles for defining induction in Section 4.1. In particular, we highlight how tuning inducer concentration transforms a protein's dissociation constant to a restricted range of accessible values for an effective dissociation constant. Section 4.2 then demonstrates how tuning inducer concentrations drive auto-activation and mutual repression networks to shift between monostable and bistable dynamics. We also examine the range of effector concentrations for which systems can exhibit bistability, and how the thermodynamic rate and cooperativity parameters affect the accessibility of this dynamic phase. Finally, Section 4.3 examines the impact of effector-driven dynamics on expression in different types of feed-forward loops, where two proteins X and Y can either work together or against each other to regulate the expression of a third protein Z . This gene circuit setting provides useful insights into how the magnitude of time delays in expression onset and offset evolves within the parameter space of different logic gates, and into the emergence of pulsed expression in output Z .

Across these detailed discussions, we ultimately work through an array of increasingly sophisticated yet still structurally simple gene regulatory circuits, and leverage statistical mechanics to uncover how the presence of effectors dictates complex gene expression dynamics. We envision that the predictions and systematic analysis stemming from this work will make it possible to better understand how cells exploit these genetic circuits to regulate their decision making processes, as well as enable the predictive design of synthetic circuits with prescribed functions in response to input effector dynamics.

4.1 The Statistical Mechanics of Induction

We now review how we can explicitly incorporate an allosteric perspective into our definition for protein activity within dynamical equations of gene expression. This approach builds upon earlier work in which biological parameters were tuned by hand rather than by effectors [5, 6, 24–27]. Although some previous studies

STATE	THERMODYNAMIC WEIGHT	STATE	THERMODYNAMIC WEIGHT
active states		inactive states	
	1		$e^{-\beta \epsilon}$
	$\frac{c}{K_A}$		$e^{-\beta \epsilon} \frac{c}{K_I}$
	$\frac{c}{K_A}$		$e^{-\beta \epsilon} \frac{c}{K_I}$
	$\left(\frac{c}{K_A}\right)^2$		$e^{-\beta \epsilon} \left(\frac{c}{K_I}\right)^2$
$\underbrace{\left(1 + \frac{c}{K_A}\right)^2}$		$\underbrace{e^{-\beta \epsilon} \left(1 + \frac{c}{K_I}\right)^2}$	

Figure 4.4: States and weights for an allosteric transcription factor with an effector that can bind at two sites on the protein. Effectors can bind to both the active and inactive forms of the transcription factor with different dissociation constants K_A and K_I , which determine whether the effector stabilizes the protein more strongly in its active or inactive configuration. The sum of the thermodynamic weights for the active and inactive conformations are shown at the bottom.

also vary inducer concentration, they typically either address different regulatory contexts than ours, map inducer concentrations to protein activity without modeling the allosteric transition explicitly, or consider different types of dynamical systems analyses than we do [10, 13, 27–37].

In particular, we argue that often the number of active transcription factors TF_{act} is given by

$$TF_{\text{act}} = p_{\text{act}}(c)TF_{\text{tot}}, \quad (4.3)$$

where TF_{tot} is the total number of transcription factors and $p_{\text{act}}(c)$ is the probability that the transcription factor is active as a function of the effector concentration c . For example, effector binding can render a protein inactive, decreasing p_{act} as effector concentration c increases. As in Chapter 3, we again define the fraction of transcription factors that are active as given by the Monod-Wyman-Changeux (MWC) model, which can be used to compute $p_{\text{act}}(c)$ using statistical mechanics [23, 38–45].

In the MWC setting, an effector can bind to a transcription factor in both its active and inactive forms with different dissociation constants, with the two unbound states having an energy difference $\epsilon = \epsilon_i - \epsilon_a$. The values of the dissociation constants then determine whether increasing effector concentration stabilizes the active or inactive form of the transcription factor. The states and weights for such an

allosteric transcription factor with two binding sites is shown in Fig. 4.4. Appealing to these states and weights, the probability of a transcription factor being active is then of the form

$$p_{\text{act}}(c) = \frac{(1 + \frac{c}{K_A})^2}{(1 + \frac{c}{K_A})^2 + e^{-\beta\epsilon}(1 + \frac{c}{K_I})^2}, \quad (4.4)$$

where c is the concentration of effector molecules, $\beta = 1/k_B T$, and K_A and K_I are the dissociation constants for the transcription factor in its active and inactive states, respectively. We consider here a transcription factor with two effector binding sites.

Fig. 4.5(A) plots the probability of active transcription factor, as defined in Eqn. 4.4, generating an input-output function exhibiting characteristic sigmoidal behavior. As in Chapter 3, the transcription factors studied here are rendered inactive upon the binding of effector. Tuning the relative values of the active and inactive dissociation constants would generate situations in which activity increases with effector concentration. The corresponding expression behavior can be trivially extrapolated by inverting results for the former convention, so we need only choose one description to evaluate dynamics in this discussion.

The probability of activity $p_{\text{act}}(c)$ explicitly modulates the contribution of transcription factor concentrations to expression dynamics. However, we can also interpret the impact of this probability as rescaling the fixed physical dissociation constant K_d between transcription factors and DNA to an *effective* constant

$$K_d^{\text{eff}} = K_d / p_{\text{act}}(c), \quad (4.5)$$

a definition that proves to be informative in several ways. First, traditionally, theoretical models of the behavior of network motifs are studied by tuning K_d^{eff} , while experiments generally tune the effector concentration c . Incorporating $p_{\text{act}}(c)$ bridges theory and experiments by providing experimentally accessible “knobs” to control the behavior of the genetic circuit of interest. Second, since $p_{\text{act}}(c)$ is highly non-linear in c , other model variables might react differently to varying c than to varying K_d^{eff} . Third, $p_{\text{act}}(c)$ constrains the range of accessible K_d^{eff} values. When discussing input-output curves, there are a variety of summary parameters that help us understand their character qualitatively. For example, in the case where effector binding renders the protein inactive, the leakiness is the amount of activity at saturating concentrations of effector, namely, $p_{\text{act}}(\infty)$. Similarly, the maximal activity known as the saturation occurs in the zero-effector limit, namely, $p_{\text{act}}(0)$. These important

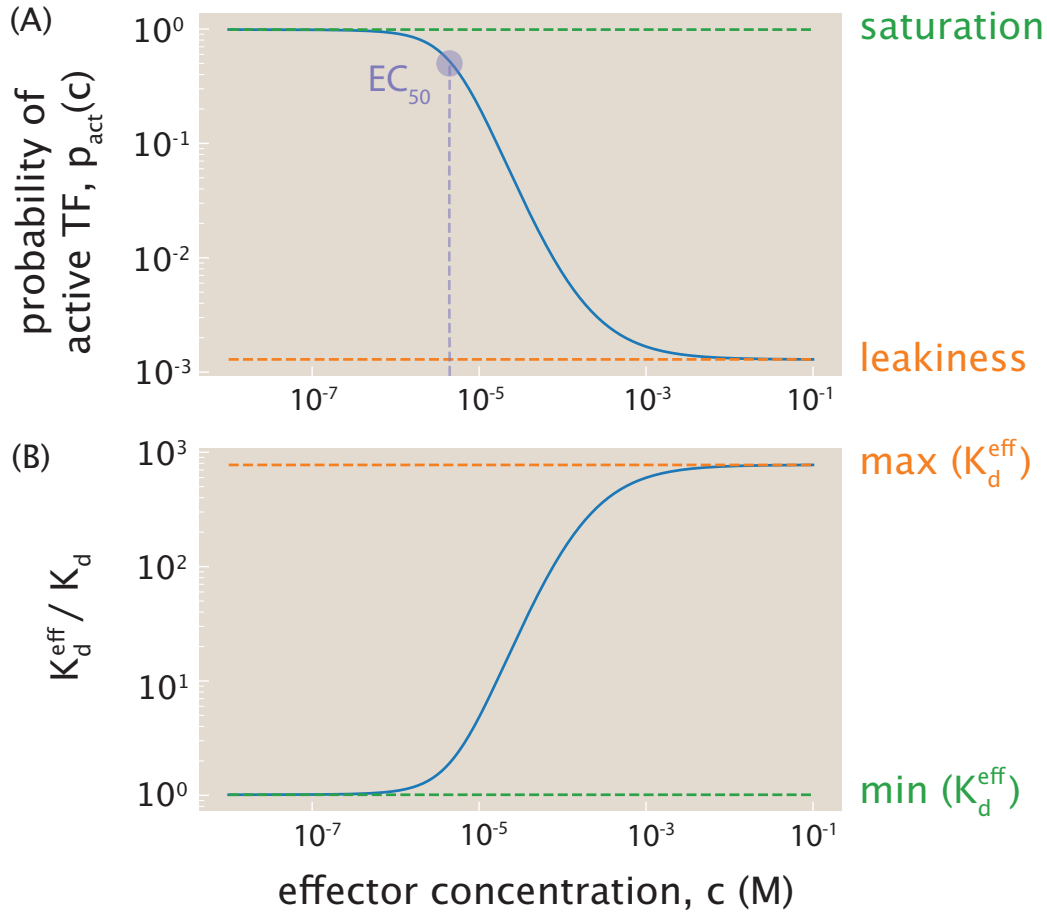


Figure 4.5: Activity of a transcription factor as a function of effector concentration c . Parameters used throughout the chapter, unless otherwise stated, are: $K_A = 140 \mu\text{M}$, $K_I = 530 \text{ nM}$, $\varepsilon = 4.5 k_B T$. (A) Probability of active transcription factor as a function of effector concentration, defined by Eqn. 4.4. The half maximal effective concentration EC_{50} , defined as the effector concentration c^* such that $p_{\text{act}}(c^*) = (p_{\text{act}}^{\text{max}} + p_{\text{act}}^{\text{min}})/2$, is plotted in purple. (B) The effective dissociation constant K_d^{eff} (dimensionless with respect to K_d) as a function of effector concentration. Saturation of $p_{\text{act}}(c)$ corresponds to minimal K_d^{eff} , and leakiness of $p_{\text{act}}(c)$ corresponds to maximal K_d^{eff} .

summary variables can be calculated as

$$p_{\text{act}}^{\text{max}} = \lim_{c \rightarrow 0} p_{\text{act}} = \frac{1}{1 + e^{-\beta\epsilon}} \quad (4.6)$$

and

$$p_{\text{act}}^{\text{min}} = \lim_{c \rightarrow \infty} p_{\text{act}} = \frac{1}{1 + e^{-\beta\epsilon} \bar{K}_c^2}, \quad (4.7)$$

where $\bar{K}_c = K_A/K_I$. With the parameters used in Fig. 4.5(A), $p_{\text{act}}^{\text{min}}$ and $p_{\text{act}}^{\text{max}}$ are separated by about three orders of magnitude, and thus K_d^{eff} is also only tunable across three orders of magnitude, as shown in Fig. 4.5(B). The restricted K_d^{eff} range can have important consequences. For example, consider a bistable system with a stability curve such as that shown in Fig. 4.6, where the x -axis is K_d^{eff} and the y -axis tracks the steady state concentration of some protein A . Tuning the parameter c imposes constraints on the range of achievable K_d^{eff} for a given K_d . The light blue range shown does not intersect the bistable regime, while the light green range does, illustrating that bistability may not be fully accessible due to the functional dependence of K_d^{eff} on $p_{\text{act}}(c)$. This restriction on K_d follows a tradition of relating thermodynamic binding interactions to experimentally measured biochemical parameters [46].

Our fundamental goal is to reconsider the classic stability analysis for a broad array of different regulatory architectures in light of the MWC model for transcription factor activity described above. For example, as seen in Fig. 4.6, tuning the K_d for the simple auto-activation circuit yields two stable fixed points. In the first section, we examine this circuit by modulating the concentration of effector molecules, which tunes the concentration of active transcription factors. From this simple genetic circuit, we then turn to the ubiquitous mutual repression switch, well known not only as a key part of the repertoire of natural genetic circuits as shown in Fig. 4.7, but also as one of the classic examples of synthetic biology [24]. Both auto-activation and mutual repression can exhibit bistable dynamics [5, 24–26]. We examine the conditions for bistability as well as the relaxation dynamics in each. Finally, we turn to three-gene feed-forward loops, in which an input gene regulates expression of another both directly and indirectly through regulation of an intermediary. Depending on the precise architecture, these circuits exhibit unique time-varying behavior in response to pulsing effector signals [47–49].

4.2 Bistability in Genetic Circuits

Different gene networks serve different biological functions. Among the most important classes of networks are those that yield bistability. Bistability refers to

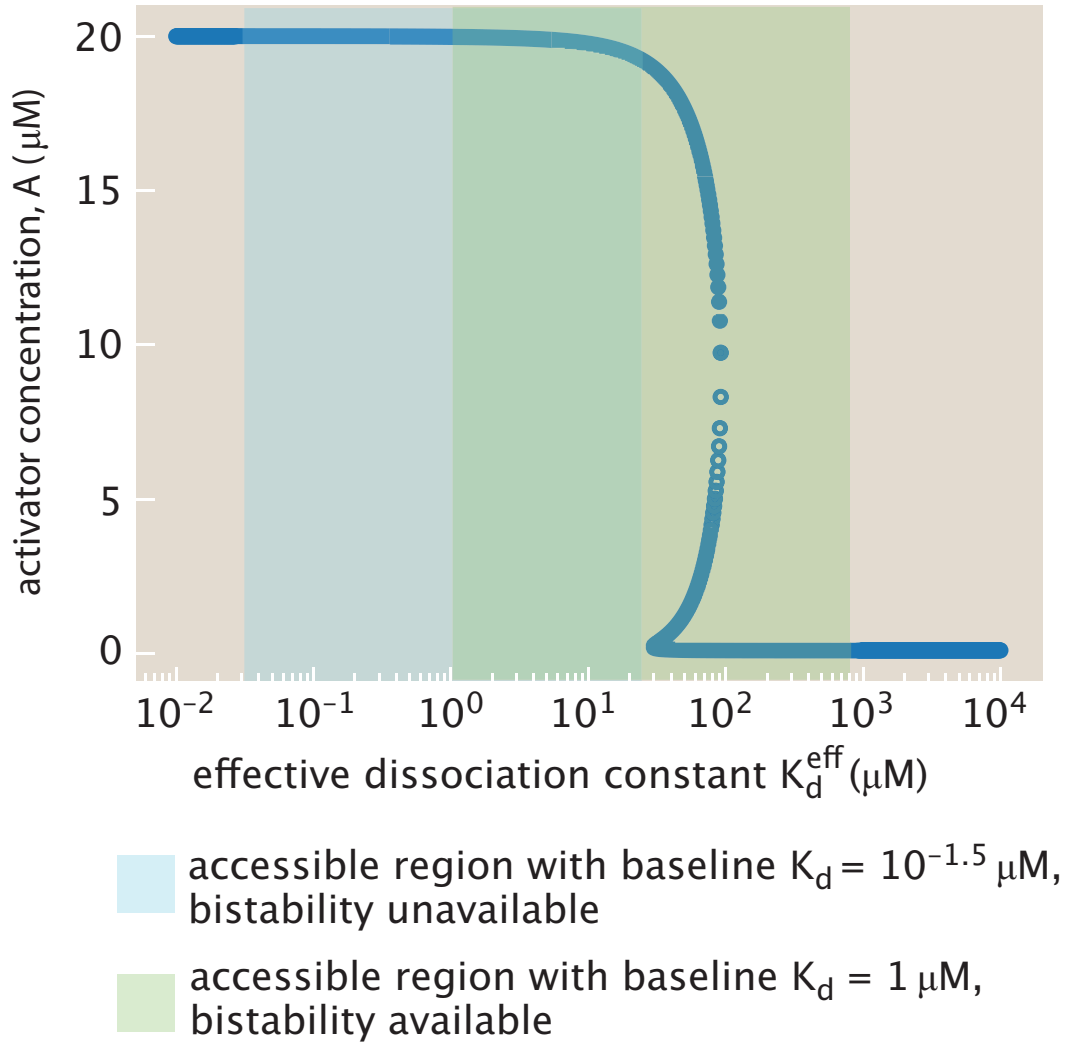


Figure 4.6: Allosteric tuning restricts the range of accessible K_d^{eff} . The dark blue curve shows the steady-state values of activator concentration A , with filled circles indicating stable fixed points and open circles indicating unstable ones. The light blue and light green shaded regions represent two example ranges of accessible $K_d^{\text{eff}} = K_d/p_{\text{act}}(c)$ values, obtained by varying the effector concentration c for two proteins with different DNA binding affinities (and thus different K_d values). For the blue region, $K_d = 3.2 \times 10^{-2} \mu\text{M} = 10^{-1.5} \mu\text{M}$; for the green region, $K_d = 1 \mu\text{M}$. The parameters for the auto-activation stability curve are: $\gamma = 1/\text{min}$, $r_0 = 0.1 \mu\text{M}/\text{min}$, $r_1 = 1 \mu\text{M}/\text{min}$, $r_2 = 20 \mu\text{M}/\text{min}$, $\omega = 100$.

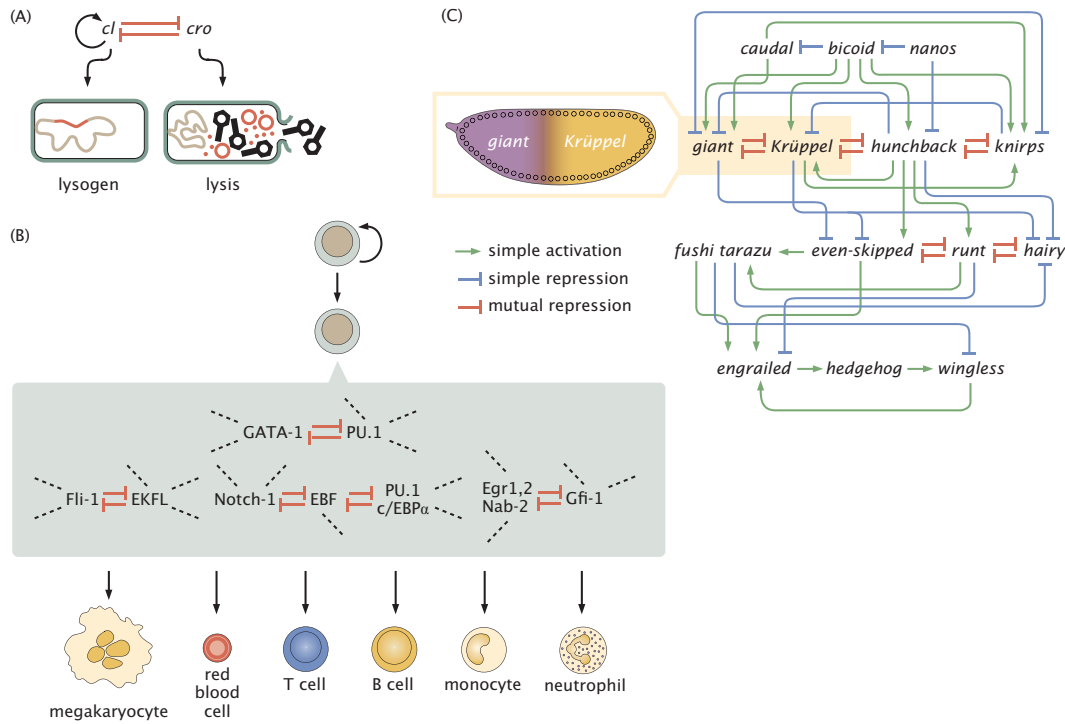


Figure 4.7: Mutual repression is ubiquitous in cellular decision making. (A) The bacteriophage lambda switch that mediates the phage decision to become a lysogen in the bacterium or engage in lysis through mutual repression of *cI* and *cro* (and some auto-activation of *cI* as well). (B) In hematopoietic development, mutual repression between different genes has been suggested to ensure the switch-like adoption of alternate cellular fates. Diagram adapted from [50] and [51]. (C) Mutual repression in the early fruit fly gene network has been associated with the emergence of discrete domains of gene expression. Diagram adapted from [52] and [53].

the situation in which, for a given set of parameters, a system can exist in one of two possible stable steady states. Such a feature is biologically significant. For example, often the expression level of one protein determines a cell's fate. To obtain cells with different functions, there might be some cells with high concentrations of the protein of interest and some cells with low concentrations, requiring a bistable system regulating the protein in question. Broad computational surveys have shown that switch-like, bistable behavior can emerge from a wide range of simple biochemical network architectures [54]. Here, we analyze the two simplest and most ubiquitous gene circuits that produce bistability [7], auto-activation and mutual repression, now through the new lens of how effector molecules modulate the dissociation constants of regulatory proteins binding to DNA. Although there are examples in the literature that also use this knob in other regulatory or modeling contexts (as noted in Section 4.1), the approach considered here offers a contrast to the conventional

setting often employed in studying auto-activation and mutual repression. In such cases, the tuning strategy is typically to modulate the binding parameters within thermodynamic models [5–7, 25], rather than those parameters being naturally tuned through the action of signaling molecules. For simplicity, the following sections do not consider transcription factor oligomerization, either in solution or when bound to DNA, which could give rise to more complex configurations such as DNA looping. We discuss modest generalizations to this effect in Appendix 4.B.

4.2.1 The auto-activation regulatory motif

Auto-activation circuits, in which a gene product enhances its own transcription, are among the simplest genetic regulatory motifs and are capable of generating bistable behavior [5]. Such motifs have been studied extensively in both synthetic and natural biological systems. In vitro synthetic networks have demonstrated robust switching between high and low expression states under controlled biochemical conditions [55], and positive feedback loops have been implicated in natural processes like cell differentiation, where they convert graded input signals into binary gene expression responses [56]. The inducible auto-activation circuit has also been explored with Hill function formulations and with p_{act} as an input parameter [57]. Motivated by this work, we now present our theoretical analysis of the auto-activation switch that incorporates a full thermodynamic model and explicit treatment of induction. The architecture we study is shown schematically in Fig. 4.2, where a transcription factor activates its own production, forming a feedback loop. Given the states, weights, and rates in Fig. 4.2(C), we can now write the kinetic equation governing the dynamics of the auto-activation system as

$$\frac{dA}{dt} = -\gamma A + \frac{r_0 + r_1(2p_{act}(c)\frac{A}{K_d}) + r_2\omega(p_{act}(c)\frac{A}{K_d})^2}{1 + 2p_{act}(c)\frac{A}{K_d} + \omega(p_{act}(c)\frac{A}{K_d})^2}, \quad (4.8)$$

where γ is the degradation rate of protein A , r_i is the production rate when i activators are bound, ω is the cooperativity of activator binding, and K_d is the biophysical dissociation constant specific to a gene and a transcription factor. The effect of allosteric regulation is included in $p_{act}(c)$, as we introduced earlier. This probability modifies the active transcription factor concentration from A to $p_{act}(c)A$.

Note that we could alternatively describe this auto-activation switch by explicitly considering all possible regulatory states with bound and unbound RNA polymerase (RNAP), and explicitly defining an energy for interaction between activators and RNAP. Appendix 4.A.1 demonstrates, however, that this representation is equivalent

to Eqn. 4.8, with the rates r_i , dissociation constant K_d , and cooperativity ω implicitly dependent on polymerase concentration, the strength of polymerase binding to the DNA, and the strength of interaction between polymerase and activator. The discussion throughout this work will thus remain in the equivalent coarse-grained realm, as depicted in Fig. 4.2, and effectively focus on polymerase-bound states.

It is helpful to write our dynamical equation in dimensionless form. To do so, we non-dimensionalize the system by using $1/\gamma$ as the unit of time and K_d as the unit of concentration. Within the p_{act} paradigm, we can write the dynamical equation in dimensionless form as

$$\frac{d\bar{A}}{d\bar{t}} = -\bar{A} + \frac{\bar{r}_0 + \bar{r}_1(2p_{\text{act}}\bar{A}) + \bar{r}_2\omega(p_{\text{act}}\bar{A})^2}{1 + 2p_{\text{act}}\bar{A} + \omega(p_{\text{act}}\bar{A})^2}, \quad (4.9)$$

where $\bar{t} = \gamma t$, $\bar{A} = A/K_d$ and $\bar{r}_i = r_i/\gamma K_d$.

At a given effector concentration, we can represent the gene expression dynamics that unfold through a phase portrait as in Fig. 4.8. The points of intersection of the production and degradation curves correspond to steady state activator concentrations. Fig. 4.8 highlights a system that can stabilize to one of two possible states with a high ($\bar{A}_{\text{high}}$) or low ($\bar{A}_{\text{low}}$) activator concentration. Depending on the initial concentration of activator protein, the system will converge to one of these stable points. At the unstable steady state $\bar{A}_{\text{unstable}}$, only a small perturbation is needed for the system to evolve toward one of the two stable steady states.

We can now qualitatively visualize how the dynamics of auto-activation transform at different effector concentrations. Specifically, as we will see explicitly in the next section, for each effector concentration c we generate a phase portrait analogous to that shown in Fig. 4.8. We then determine the number of stable and unstable fixed points and their corresponding activator concentrations. Performing this analysis as we tune effector concentration yields the bifurcation curve shown in Fig. 4.9. At a low effector concentration, activators are more likely to be found in their active configurations, enhancing gene expression such that the system always stabilizes to a state with a high concentration of activator. The magnitude of this concentration approaches a maximal value defined by the rate $\bar{r}_2$ for activated protein production. As the effector concentration increases, the system becomes bistable, allowing a bimodal distribution in protein concentrations for an ensemble of cells [26].

Ultimately, at sufficiently high effector concentration, activators are sequestered in their inactive configuration such that the system can only stabilize to a state with

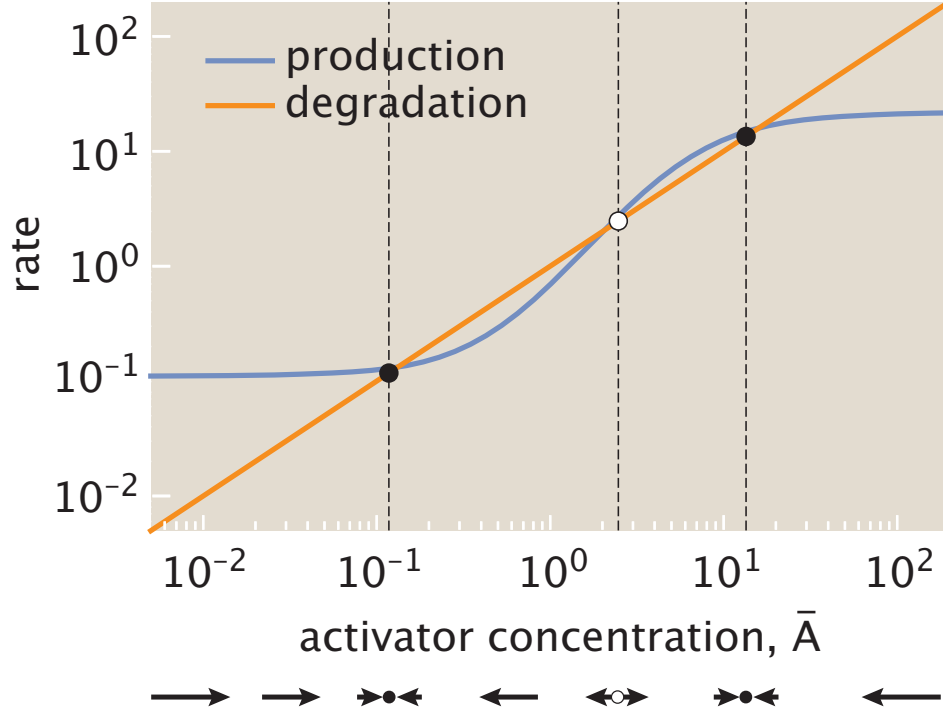


Figure 4.8: Plot of production and degradation rates for an auto-activation switch as a function of activator concentration. The figure illustrates the competition between the production and degradation terms for a system with rate constants $\bar{r}_0 = 0.1$, $\bar{r}_1 = 1$, $\bar{r}_2 = 20$, and cooperativity $\omega = 10$ at effector concentration $c = 25 \mu\text{M}$. Intersections of the curve denote stable (filled nodes at low concentration $\bar{A}_{\text{low}}$ and high concentration $\bar{A}_{\text{high}}$) and unstable (unfilled node $\bar{A}_{\text{unstable}}$) fixed points. The vectors denote a phase portrait representing the direction and magnitude of change in activator concentration as a function of activator concentration itself.

low activator concentration. The magnitude of activator expressed is then largely defined by the basal rate of production without bound activator, i.e., $\bar{r}_0$. Tracking the system's corresponding production and degradation curves through a series of snapshots in Fig. 4.9, we observe that these shifts between bistable and monostable dynamics emerge because the increasing effector concentration shifts the system production curve toward higher activator concentration. Expressed differently, as effector concentration increases, a higher activator concentration is necessary to achieve a given production rate.

Note that the threshold at which the system switches from one stable state to another differs when increasing and decreasing the effector concentration. If the system initially contains a high concentration of activator before tuning, a higher concentration of effector is necessary to switch to the low activator state than is required when

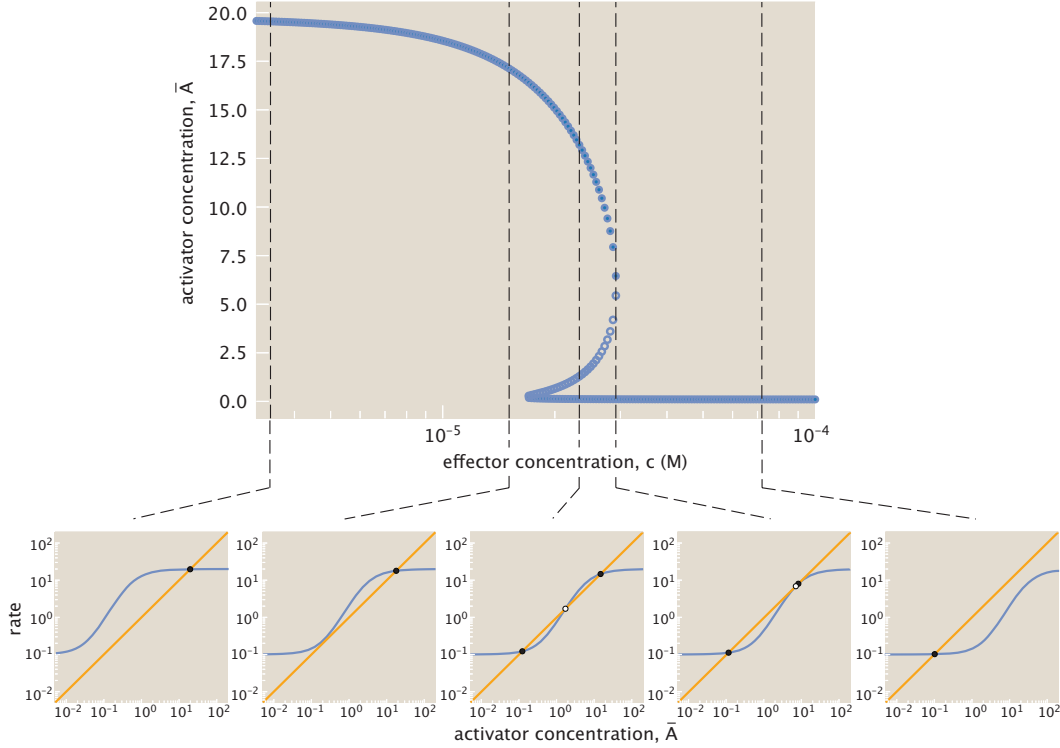


Figure 4.9: Bifurcation diagram for auto-activation system with rate constants $\bar{r}_0 = 0.1$, $\bar{r}_1 = 1$, $\bar{r}_2 = 20$, and cooperativity $\omega = 10$. This curve plots all stable (filled nodes) and unstable (unfilled nodes) fixed points, demonstrating a shift at intermediate effector concentrations from one to three fixed points. Each black dashed line denotes a specific effector concentration, pointing to a plot of the corresponding production (blue) and degradation (orange) rates as a function of activator concentration. Curve intersections mark the stable (filled nodes) and unstable (unfilled nodes) fixed points found at the given effector concentration.

decreasing effector concentration for a system with initially low activator concentration. Considering Fig. 4.9 again, the higher threshold corresponds to the maximum effector concentration at which the system is bistable, and the lower threshold to the minimum concentration at which the system is bistable. Fig. 4.10 illustrates this phenomenon of hysteresis more explicitly, overlaying the previously discussed bifurcation diagram, and showing how the threshold at which the blue stable equilibrium trajectory switches from high to low activator concentration differs from the threshold for the orange trajectory tracing the switch in reverse.

We are particularly interested in characterizing the conditions for bistability to occur at effector concentrations c , as well as how the bistable regime (if it exists) responds to changes in parameters such as production rates and cooperativity. While previous studies have analyzed bistability in auto-activation circuits using thermodynamic

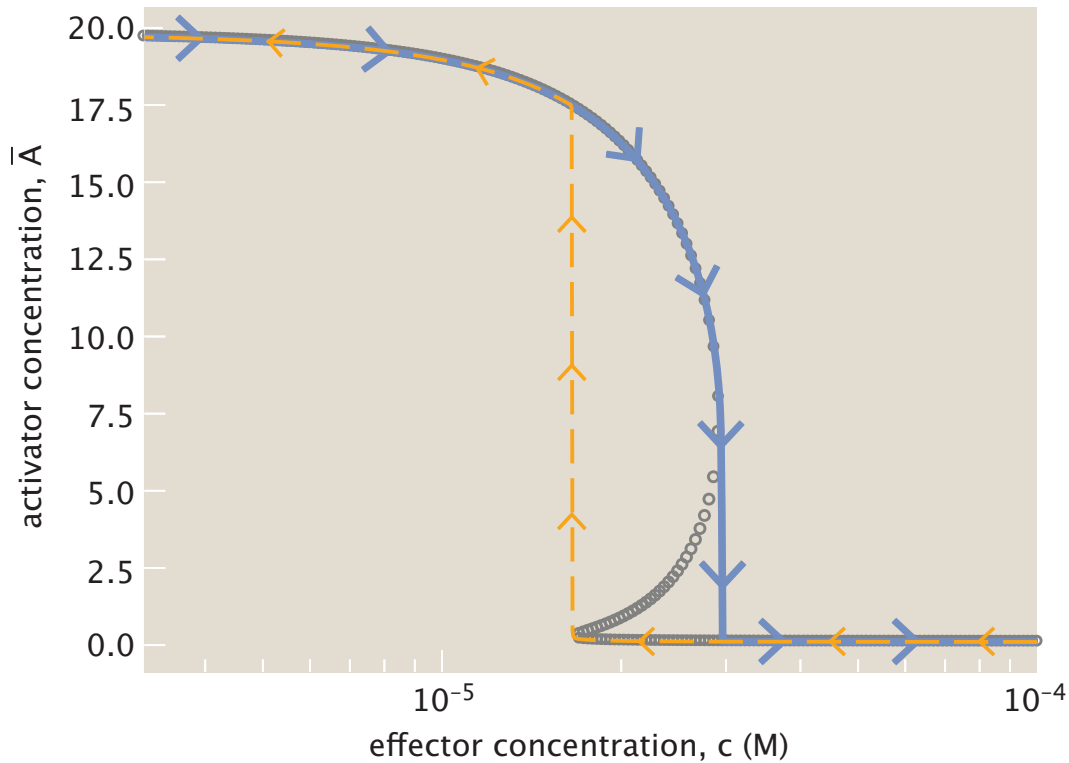


Figure 4.10: Plot of stable state evolution, exhibiting hysteresis under different trends for effector concentration. The blue curve plots how the stable state evolves with increasing effector concentration from an initial high concentration of activator. The orange dashed curve plots the change in stable activator concentration starting from an initially low level as effector concentration decreases. Comparing to the bifurcation diagram (gray) previously shown in Fig. 4.9, the history of steady-state evolution determines the threshold concentration of effector at which the system switches state, highlighting hysteretic behavior.

models without effectors [5, 58], our aim is to extend this to include effector dependence explicitly and systematically explore how the bistable region evolves across a broader parameter space.

Regimes of bistability

We now analyze the ways in which bistability emerges in the context of effector-mediated genes. One could begin with simply a fixed set of parameters and asking whether the system is bistable. In other words, can the concentration of gene product A settle at either a high or low steady state depending on the initial condition, thereby functioning as a binary switch?

The question becomes more nuanced, however, when taking the role of effector

molecules into account. Some parameters are more intrinsic and less readily tunable than others. For instance, molecular constants such as binding affinities or cooperativity are typically encoded in the system's molecular architecture. In contrast, cells can regulate the concentration of effector molecules relatively easily and rapidly, either through controlled expression, import/export mechanisms, or degradation pathways. Given this, the most relevant questions to ask are (i) under what constraints on the system's intrinsic parameters can bistability be achieved for at least some range of effector concentration c , and (ii) how wide is that range? Indeed, even if the effector concentration is a more accessible control knob, the cell is unlikely to operate at a single precise value, if only due to molecular noise and environmental fluctuations. It is therefore biologically relevant to assess not only whether bistability is possible, but also whether it is robust to such fluctuations in effector levels.

First, we can derive analytic bounds in the parameter space of rate and cooperativity constants required for bistability to be possible somewhere in the range of sampled effector concentrations. The details of the derivation are discussed in Appendix 4.C.1, but briefly, by setting $d\bar{A}/d\bar{t} = 0$ and rewriting the resulting expression in standard polynomial form, we can infer the number of positive real roots using classical results (i.e., Descartes' rule of signs) that relate sign changes in the coefficients of a polynomial to the number of positive roots. From this analysis, we then determine that for the bistability to be possible the system satisfy the inequalities

$$\frac{\omega\bar{r}_2}{2} > 1 + e^{-\beta\varepsilon}, \quad (4.10)$$

$$2\bar{r}_1 < 1 + e^{-\beta\varepsilon} \bar{K}_c^2, \quad (4.11)$$

$$\omega\bar{r}_2 > 4\bar{r}_1. \quad (4.12)$$

Notably, these conditions do not depend on the baseline expression level $\bar{r}_0$. However, as shown in Appendix 4.C.1, Eqns. 4.10 and 4.12 provide a necessary but not sufficient condition for bistability, so numerical analysis of parameter bounds is needed.

Fig. 4.11 numerically evaluates the range of effector concentrations at which a system exhibits bistability for different values of the rate and cooperativity constants (systematically modulating one parameter and leaving all other thermodynamic model parameters fixed). This figure demonstrates that even under the idealized assumption that the cell could freely choose its effector concentration, bistable behavior arises only within a restricted range of parameter values marked in red.

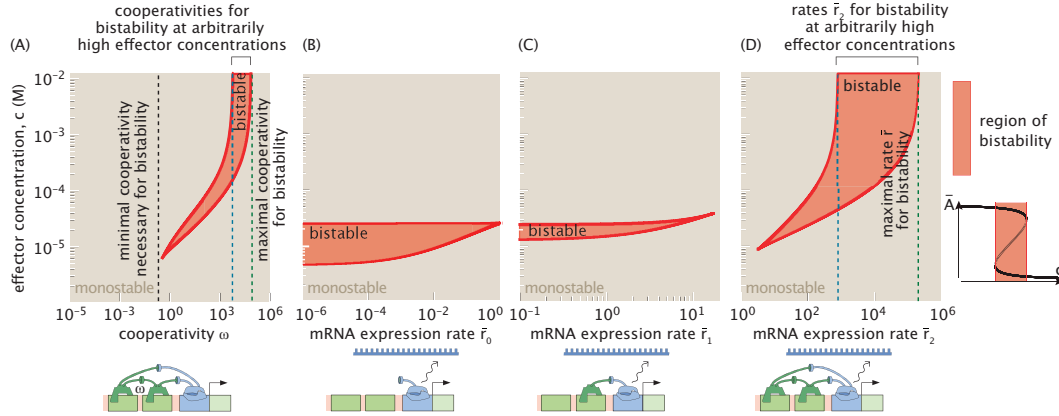


Figure 4.11: Range of effector concentrations for which the system exhibits bistability. The red shaded region indicates bistability. Outside this region, the system is monostable. Unless otherwise specified, parameters are fixed at: $\omega = 7.5$, $\bar{r}_0 = 0.1$, $\bar{r}_1 = 1$, and $\bar{r}_2 = 20$. (A) Bistable concentration range as a function of cooperativity ω , varied over the interval $\omega \in [10^{-6}, 10^6]$, corresponding to interaction energies between the two activators from approximately $+14, k_B T$ to $-14, k_B T$, since $\omega = e^{-\beta \varepsilon_{\text{int}}}$. The black dotted line shows the analytical lower bound on the minimal cooperativity required for bistability. (B) Bistable concentration range as a function of $\bar{r}_0$, sampled over the interval $\bar{r}_0 \in [10^{-6}, \bar{r}_1]$. (C) Bistable concentration range as a function of $\bar{r}_1$, sampled over the interval $\bar{r}_1 \in [\bar{r}_0, \bar{r}_2]$. (D) Bistable concentration range as a function of $\bar{r}_2$, sampled over the interval $\bar{r}_2 \in [\bar{r}_1, 10^6]$. The rate parameters are varied under the constraint of the auto-activation condition $\bar{r}_0 \leq \bar{r}_1 \leq \bar{r}_2$, which ensures that the production rate increases with the number of bound activators.

Fig. 4.11(A) tunes the strength of cooperative binding ω , evaluating a range of parameter values that correspond to a biologically relevant spectrum of bound-activator interaction strengths [20, 21, 25]. Fig. 4.11(B), (C), and (D) explore the effects of varying the protein production rate $\bar{r}_0$ in the absence of activator binding, production rate $\bar{r}_1$ when one activator is bound to the DNA, and production rate $\bar{r}_2$ when two activators are bound. To do so, we impose constraints on these parameters to ensure that the system remains within the auto-activation regime, as defined in Appendix 4.F. Specifically, we require that the production term in Eqn. 4.9 remains a monotonically increasing function of $\bar{A}$, such that $\bar{A}$ consistently acts as an activator across all concentrations. This condition imposes the inequality $\bar{r}_0 \leq \bar{r}_1 \leq \bar{r}_2$, which we enforce throughout our analysis when varying $\bar{r}_0$, $\bar{r}_1$ and $\bar{r}_2$.

First, we observe in Fig. 4.11(A) and (D) that there exists a critical threshold for both the cooperativity ω and the rate $\bar{r}_2$ below which the system is strictly monostable, indicating that a minimal level of nonlinearity is required for bistability. These

parameters thus play similar roles in shaping bistability, a conclusion that naturally follows from their presence in the same term of Eqn. 4.9. The analytical bounds derived in Eqns. 4.10 and 4.12 accurately capture this threshold.

Increasing $\bar{r}_2$ strengthens the contrast between states with high and low activator steady state concentrations in a positively cooperative system ($\omega > 1$), and as a result, the system becomes unstable for a broader range of effector concentrations. Therefore, as either ω or $\bar{r}_2$ increases, the region of effector concentrations that supports bistability broadens. This leads to a wider hysteresis zone and expands the range over which the system can toggle between high and low steady states under a fixed set of parameters. The region of bistability in effector concentration space is also displaced toward higher concentrations, where the activation probability $p_{\text{act}}(c)$ approaches the leakiness limit. This reflects the fact that the effector destabilizes the activator by decreasing its effective DNA binding affinity. In this sense, increasing effector concentration counteracts the effect of high ω and $\bar{r}_2$, which tend to promote high expression levels of A . These two opposing effects—activation-driven amplification and effector-driven destabilization—create a balance that enables bistability.

Due to the system's leakiness, complete inactivation of the activator is never achieved, even at high effector concentrations. As a result, for sufficiently large values of ω and $\bar{r}_2$, bistability can occur for all concentrations above a finite lower bound $c_{\min}(\omega, \bar{r}_0, \bar{r}_1, \bar{r}_2)$. This behavior corresponds to a limited region in parameter space where the system remains bistable at large c , as illustrated by the blue and green dotted lines in Fig. 4.11(A) and (C). In Appendices 4.C.2 and 4.E, we derive analytical bounds that predict the onset and breakdown of this upper-unbounded bistable regime.

Beyond a certain point and with fixed values of effector concentrations, further increases in either parameter have the opposite effect. When ω becomes too large, activators bind excessively tightly to the DNA, effectively locking the system into a high-expression state. Similarly, if $\bar{r}_2$ becomes too large, the system favors high levels of gene expression, and bistability is again lost (we analyze this transition using two-dimensional numerical sweeps and supporting analytical arguments in Appendix 4.E). This behavior is a direct consequence of how the effector enters the model. If, instead of varying the effector concentration, we varied an effective dissociation constant K_d^{eff} , the bistable region would always remain bounded within a finite range of values.

This behavior contrasts with the effect of increasing $\bar{r}_0$ or $\bar{r}_1$. As shown in

Fig. 4.11(B) and (C), higher values of either parameter reduce the width of the bistable region, and beyond a critical threshold, bistability disappears entirely. We can therefore infer that, in a positively cooperative system ($\omega > 1$), elevated values of $\bar{r}_0$ and $\bar{r}_1$ undermine the system's ability to function as a bistable switch.

Interestingly, we observe bistability for values of the cooperativity parameter ω that are less than one, as seen in Fig. 4.11(A). This can be reconciled in two complementary ways. First, we can define an effective cooperativity for the system, given by $\omega_{\text{eff}} = \omega \cdot \bar{r}_2/2$. From the necessary conditions for bistability derived in Eqns. 4.10 and 4.12 we find that $\omega_{\text{eff}} > 1$ is required for bistability. While this condition is necessary but not sufficient, it suggests that ω_{eff} captures the functional cooperativity of the system more accurately than ω alone, as it incorporates both the interaction between bound activators and the maximal rate of activator production. We can also reconcile our bistable results containing values of ω less than one by examining the effective Hill coefficient of the steady-state input–output function. As shown in Appendix 4.G, bistability is observed only when the effective Hill coefficient exceeds one, as anticipated from theoretical considerations [59]. This reinforces the idea that the system can exhibit bistability even when $\omega < 1$, provided the overall nonlinearity—quantified either by ω_{eff} or the effective Hill coefficient—is sufficiently strong.

Timescale for stabilization

In addition to the question of steady states, it is interesting to examine the timescale for an inducible genetic circuit, such as the auto-activation switch, to reach steady state. In doing this analysis we remind the reader that our treatment assumes separation of time scales between the dynamics of effector binding and allosteric transitions, and the dynamics of the relaxation to steady state. As a result, the binding of effector and the allosteric state of the activator rapidly equilibrate as the activator concentration changes dynamically.

As shown in Fig. 4.12(A), the relaxation timescale depends strongly on the initial activator concentration $\bar{A}_0$. In particular, as seen in the figure, the relaxation timescale to steady state increases as the initial value of activator concentration, $\bar{A}_0$, gets closer to the unstable fixed point. The increased time to reach steady state near the unstable point reflects the typical behavior of positive feedback systems near bifurcations, where the system lingers near the threshold before switching [60]. However, for large initial concentrations of A , the dimensionless relaxation timescale approaches

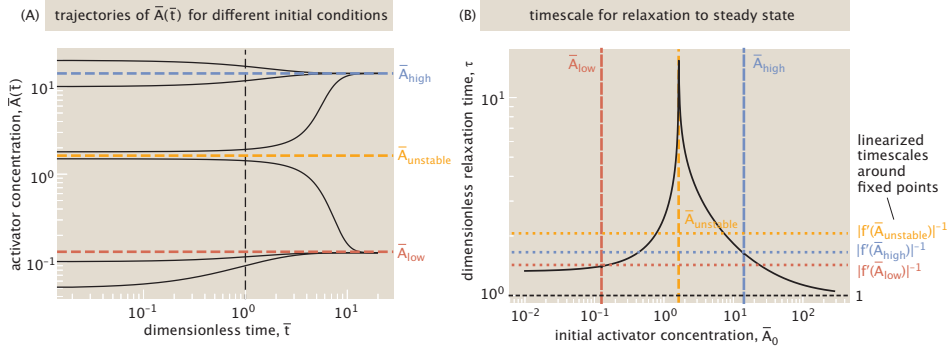


Figure 4.12: Dynamics of the auto-activation switch. The parameters of the system are fixed at: $\omega = 7.5$, $\bar{r}_0 = 0.1$, $\bar{r}_1 = 1$, $\bar{r}_2 = 20$, and $c = 2 \cdot 10^{-5}$ M. (A) Time evolution of the activator concentration for various initial conditions. Each black curve represents a trajectory $\bar{A}(\bar{t})$ starting from a different initial condition $\bar{A}_0$. Dashed horizontal lines indicate the stable ($\bar{A}_{\text{high}}$ and $\bar{A}_{\text{low}}$) and unstable ($\bar{A}_{\text{unstable}}$) fixed points. (B) Relaxation timescale obtained from exponential fits to the trajectory $\bar{A}(\bar{t})$ as a function of the initial concentration $\bar{A}_0$. Vertical lines indicate the positions of the steady states, while horizontal dashed lines mark the timescales associated with small perturbations around each fixed point, computed from the inverse slope $|f'(\bar{A})|^{-1}$ of the function f defined in Eqn. 4.14. The solid horizontal black line corresponds to the reference timescale $\bar{t} = 1$.

1, as shown by the line at $\bar{t} = 1$ in Fig. 4.12(A). This corresponds to the protein degradation timescale $1/\gamma$, which serves as the unit of time in our dimensionless form. This also corresponds to the relaxation timescale of a simply regulated gene, activated solely by an upstream transcription factor. We find that auto-activation consistently delays the response compared to simple regulation, although the two converge in the high- A limit. Within the Hill-function framework, positive autoregulation has likewise been shown to slow gene circuit response times [61], in contrast to the accelerating effect of negative feedback [62].

To further interpret these trends, we apply linear stability analysis to Eqn. 4.9, as is commonly done in the study of dynamical systems [63]. Linearizing around a point $\bar{A}_i$ with $\bar{A}(\bar{t}) = \bar{A}_i + \delta\bar{A}(\bar{t})$ and expanding the right-hand side, that we denote $f(\bar{A})$, to first order yields

$$f(\bar{A}(\bar{t})) \approx f(\bar{A}_i) + f'(\bar{A}_i) \delta\bar{A}(\bar{t}) \quad (4.13)$$

for sufficiently small $\delta\bar{A}(\bar{t})$, where $f'(\bar{A})$ is a derivative with respect to $\bar{A}$ rather than a time derivative. The function f is given by

$$f(\bar{A}) = -\bar{A} + \frac{\bar{r}_0 + \bar{r}_1(2p_{\text{act}}\bar{A}) + \bar{r}_2\omega(p_{\text{act}}\bar{A})^2}{1 + (2p_{\text{act}}\bar{A}) + \omega(p_{\text{act}}\bar{A})^2}. \quad (4.14)$$

Therefore, the linearized equation governing the evolution of a small perturbation $\delta\bar{A}(t)$ around the point $\bar{A}_i$ becomes

$$\frac{d\delta\bar{A}}{d\bar{t}} = f(\bar{A}_i) + f'(\bar{A}_i)\delta\bar{A}. \quad (4.15)$$

If $\bar{A}_i$ is a steady state, then $f(\bar{A}_i) = 0$, and the equation reduces to exponential relaxation with dimensionless timescale $|f'(\bar{A}_i)|^{-1}$. Stable fixed points, denoted $\bar{A}_{\text{low}}$ and $\bar{A}_{\text{high}}$, satisfy $f'(\bar{A}_{\text{low/high}}) < 0$, so perturbations decay over time. For unstable fixed points, where $f'(\bar{A}_{\text{unstable}}) > 0$, small deviations grow exponentially and drive the system away from the fixed point.

If $\bar{A}_i$ is not a fixed point, however, then $f(\bar{A}_i) \neq 0$ and the solution to Eqn. 4.15 does not represent convergence to a steady state. Instead, it predicts that $\delta\bar{A}$ asymptotes to a finite offset $f(\bar{A}_i)/|f'(\bar{A}_i)|$, breaking the assumption of linearity ($\delta\bar{A}(\bar{t}) \rightarrow 0$). In this case, the derivative $f'(\bar{A}_i)$ still encodes the local response to small perturbations, but only describes the dynamics while deviations remain small. Therefore, the timescale $|f'(\bar{A})|^{-1}$ is most meaningful in the vicinity of fixed points, even if it can still provide qualitative insights elsewhere.

These insights are reflected in Fig. 4.12(B), where we numerically compute the relaxation timescale as a function of the initial condition $\bar{A}_0$. For initial values near the stable fixed points, the timescale closely follows the linear prediction $|f'(\bar{A}_{\text{low/high}})|^{-1}$. In contrast, when the initial condition lies near the unstable fixed point, the system initially diverges exponentially and only later relaxes nonlinearly to a stable state. This leads to an overall increase in the time to reach steady state, consistent with the trajectories displayed in Fig. 4.12(A) (see Appendix 4.H for the precise numerical procedure used to compute these timescales). Together, these results reveal that the stabilization timescale in auto-activation circuits is not fixed but depends sensitively on initial conditions, particularly near unstable fixed points—highlighting the importance of considering nonlinear transient dynamics when predicting response times in bistable gene networks.

4.2.2 The mutual repression regulatory motif

Many regulatory circuits in prokaryotes and eukaryotes alike are mediated by the interaction between two genes that repress each other as shown in Fig. 4.13 [50, 51, 64]. Indeed, one of the signature achievements of the early days of synthetic biology was the consideration of a mutual repression switch in bacteria whose behavior was reported by the use of fluorescent proteins and controlled by a small

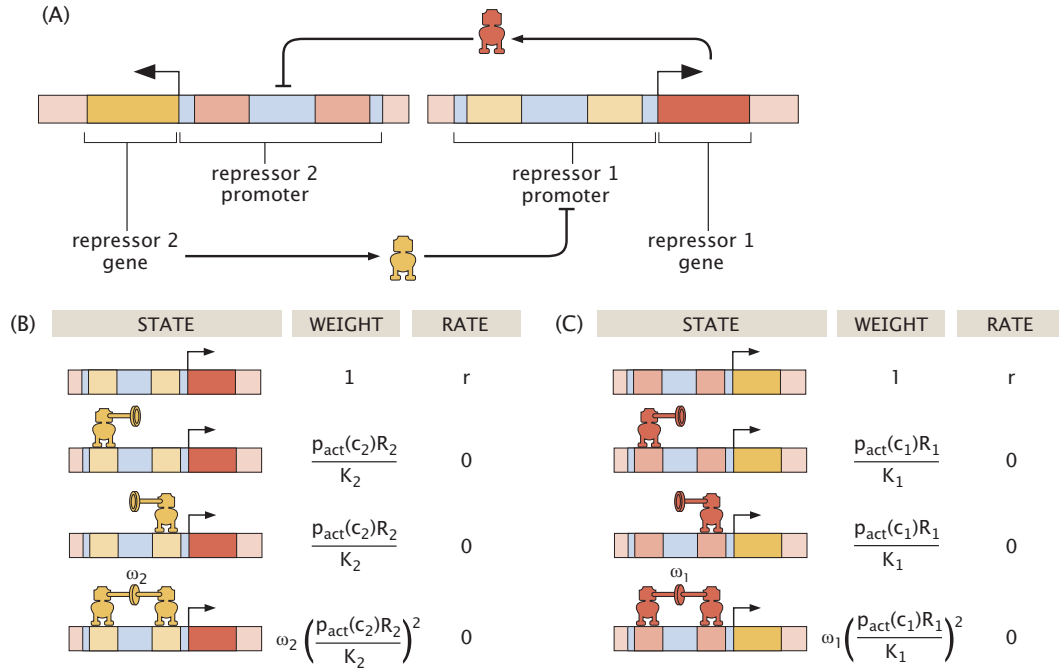


Figure 4.13: The mutual repression regulatory circuit. (A) Schematic of the operation of the circuit. When the gene for repressor 1 is expressed, the resulting protein downregulates the expression of the gene for repressor 2. Repressor 2, in turn, downregulates the expression of the gene for repressor 1. (B) Thermodynamic states, weights, and rates for expression of repressor 1. In our model, a repressor can bind non-exclusively at one of two possible sites within the target promoter region to suppress gene transcription. The parameter ω_2 denotes the cooperative strength between two bound repressors R_2 . (C) Thermodynamic states, weights, and rates for expression of repressor 2. The states and weights for the regulation of the promoter responsible for the production of repressor 2 are analogous to those shown for repressor 1. However, the dissociation constant of repressor 1 in this case is given by K_1 , and the cooperativity term for the interaction of two repressor 1 molecules bound to the DNA is ω_1 .

molecule inducer [24]. As the name suggests, two genes R_1 and R_2 mutually repress each other. To simplify our analysis we assume that the two genes share the same degradation rate γ and the same production rate r when no bound repressors are present.

By inspecting the thermodynamic states and weights listed in Fig. 4.13, we can describe the dynamics of repressor 1 expression as

$$\frac{dR_1}{dt} = -\gamma R_1 + \frac{r}{1 + 2\frac{p_{\text{act}}(c_2)R_2}{K_2} + \left(\frac{p_{\text{act}}(c_2)R_2}{K_2}\right)^2} \omega_2, \quad (4.16)$$

where we have defined an effector with concentration c_2 that regulates the activity of repressor 2. Similarly, the dynamics for R_2 expression are described by an equation analogous to Eqn. 4.16 but swapping R_1 and R_2 , given by

$$\frac{dR_2}{dt} = -\gamma R_2 + \frac{r}{1 + 2\frac{p_{\text{act}}(c_1)R_1}{K_1} + \left(\frac{p_{\text{act}}(c_1)R_1}{K_1}\right)^2} \omega_1, \quad (4.17)$$

where we have also defined an effector with concentration c_1 that regulates the activity of repressor 1. As in auto-activation, this analysis only accounts for the presence of RNA polymerase implicitly, which we discuss further in Appendix 4.A.2. With two effectors, our control knob now becomes a 2-dimensional vector (c_1, c_2) . The bistable region becomes an area, and (c_1, c_2) can be tuned following arbitrary trajectories in the inducer concentration space. We will explore these trends in this section.

To continue the analysis, we can write dimensionless forms of these kinetic equations by transforming $\bar{t} = \gamma t$, $\bar{R}_i = R_i/K_2$, and $\bar{r} = r/\gamma K_2$, and obtain

$$\frac{d\bar{R}_1}{d\bar{t}} = -\bar{R}_1 + \frac{\bar{r}}{1 + 2p_{\text{act}}(c_2)\bar{R}_2 + \omega_2 \left[p_{\text{act}}(c_2)\bar{R}_2 \right]^2}, \quad (4.18)$$

$$\frac{d\bar{R}_2}{d\bar{t}} = -\bar{R}_2 + \frac{\bar{r}}{1 + 2p_{\text{act}}(c_1)\frac{\bar{R}_1}{\bar{K}} + \omega_1 \left[p_{\text{act}}(c_1)\frac{\bar{R}_1}{\bar{K}} \right]^2}. \quad (4.19)$$

As a reminder, ω_1 and ω_2 are the cooperativity for $\bar{R}_1$ and $\bar{R}_2$, respectively, c_1 and c_2 are the inducer concentrations for each repressor, and $\bar{K} = K_1/K_2$ is the ratio of dissociation constants. Note that the equations above assume that each repressor responds to induction with the same inducer-protein binding chemistry, obeying the same activation probability function p_{act} . In the most general case, however, the two probability functions could differ.

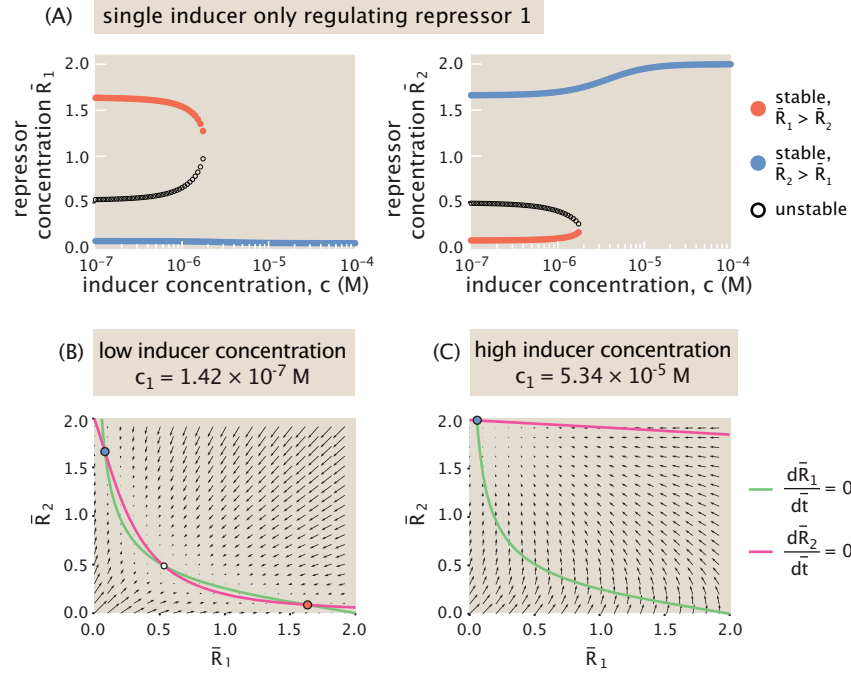


Figure 4.14: Bifurcation diagrams and phase portraits for mutual repression in the presence of a single inducer regulating the activity of repressor R_1 (with fixed parameters $\bar{K} = K_1/K_2 = 1$, $\bar{r} = 2$ and $\omega_1 = \omega_2 = 7.5$). (A) Bifurcation diagrams tracking steady-state $\bar{R}_1$ and $\bar{R}_2$ expression as inducer concentration increases. The red curves track expression in the steady state for which $\bar{R}_1 > \bar{R}_2$, the blue curves track expression in the steady state for which $\bar{R}_2 > \bar{R}_1$, and the unfilled nodes denote unstable saddle points. (B) Phase portrait at a low inducer concentration, demonstrating bistable dynamics between two possible stable states favoring either $\bar{R}_1$ (red) or $\bar{R}_2$ (blue). Steady states occur at the intersections of the nullclines as shown. (C) Phase portrait at a high inducer concentration, for which the system is monostable to favor $\bar{R}_2$.

This formulation now provides a two-dimensional knob for tuning the concentrations of the inducers corresponding to $\bar{R}_1$ and $\bar{R}_2$. The system may then be tuned to follow arbitrary trajectories in the two-dimensional parameter space spanned by c_1 and c_2 . Frequently, this tuning generates non-trivial bifurcation curves.

To build intuition about this system, we first consider a scenario in which only one of the repressors may be induced. Fig. 4.14(A) plots the resulting bifurcation diagrams for $\bar{R}_1$ and $\bar{R}_2$ steady-state expression. At low inducer concentrations, the system is bistable and can either evolve to favor $\bar{R}_1$ (red) or $\bar{R}_2$ (blue) expression. Fig. 4.14(B) shows an example phase portrait within this low induction regime that depicts vector field flows through expression space toward these fixed points. At a sufficiently high inducer concentration, $\bar{R}_1$ expression can no longer be maintained, and the system

transitions to a monostable regime in which only the state favoring $\bar{R}_2$ expression survives. A higher inducer concentration amplifies the expression of $\bar{R}_2$ up to its production limit. Fig. 4.14(C) depicts a phase portrait in this regime.

We now turn to a two-dimensional setting for allosteric regulation, with distinct inducers downregulating the activity of each repressor. Fig. 4.15(A) plots the phase diagram for the dynamics of mutual repression at different combinations of inducer concentrations. The dark green region denotes the region of parameter space with bistable dynamics, with monostable behavior elsewhere. Note the symmetry of this phase diagram with respect to the diagonal $c_1 = c_2$. This is unsurprising given the condition $\bar{K} = 1$, which amounts to saying that the two different repressors bind with the same dissociation constant. This condition will be discussed further in the following subsection.

Fig. 4.15(A) also considers different “protocols” for simultaneously varying the concentration of each inducer, demonstrating the varying ways that a system can evolve through phase space. For example, in Fig. 4.15(B), we examine a protocol in which the inducer concentration for each gene increases at the same constant rate (corresponding to the red trajectory in Fig. 4.15(A)). We then plot the corresponding bifurcation diagram. As inducer concentration increases, the scope of the bistable switch shrinks in expression space, with the stable states continuously approaching each other. At $c_1 = c_2 \approx 3.2 \cdot 10^{-6}$ M, the system then undergoes a pitchfork bifurcation to monostable expression, stabilizing at increasingly high concentrations of both $\bar{R}_1$ and $\bar{R}_2$.

We could follow an alternative trajectory (denoted by the purple arrow in Fig. 4.15(A)) through parameter space such that the inducer concentrations evolve at different rates, in this case with c_1 increasing more rapidly than c_2 . This purple trajectory then passes in and out of the green bistable region several times. Fig. 4.15(C) plots the corresponding bifurcation diagram tracking stable and unstable steady states as the inducer concentrations increase, and demonstrates the switches between bistability and monostability. Note that while the intermediate monostable regime favors $\bar{R}_1$ expression, the monostable regime at later times favors $\bar{R}_2$ instead, reflecting the swap in the dominant inducer concentration that occurs between these time periods. Thus, by modulating the induction dynamics of each repressor we can access a broad range of dynamical responses in repressor concentrations.

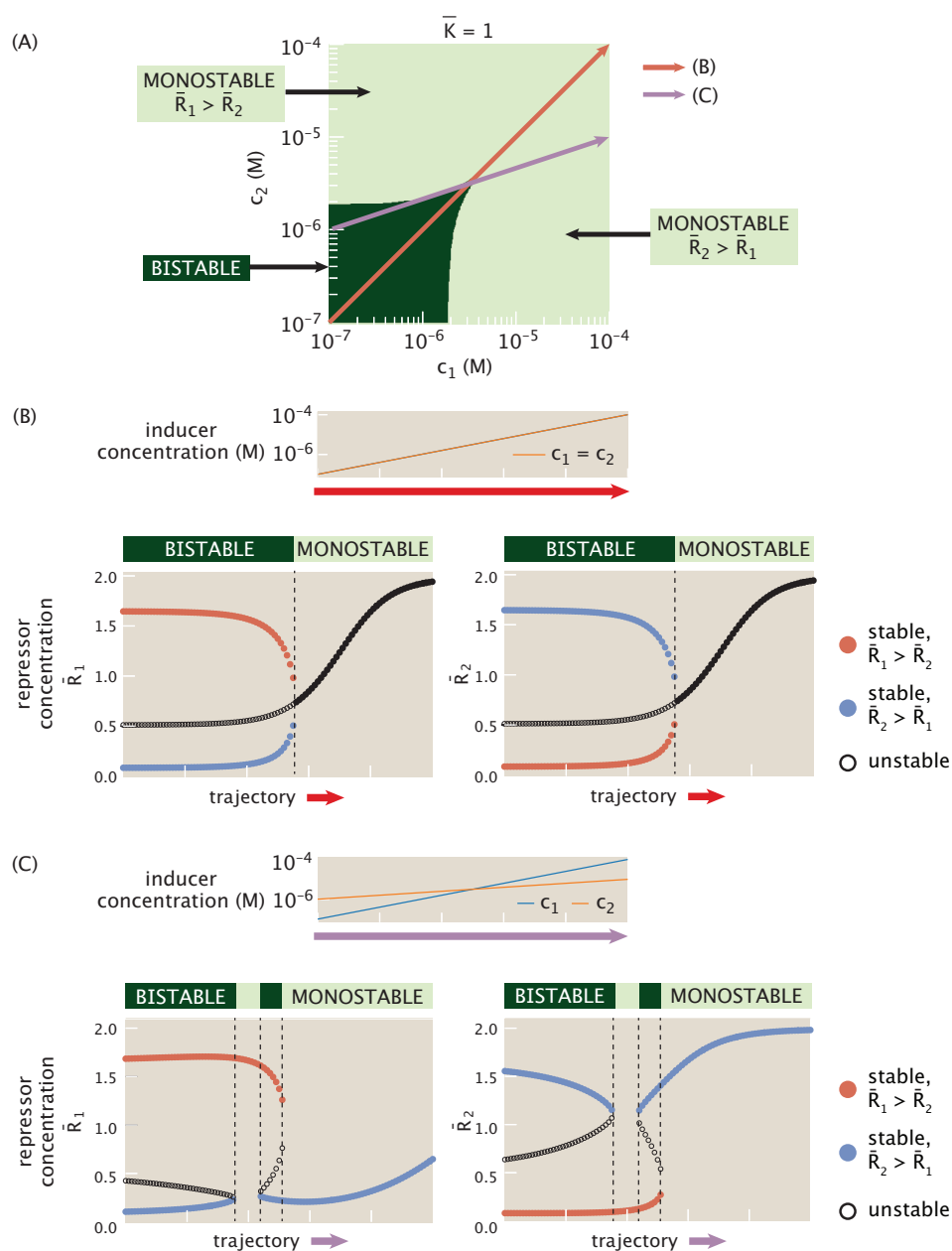


Figure 4.15: Phase diagram and bifurcation diagrams for mutual repression when each repressor's activity is regulated by an inducer (with fixed parameters $\bar{K} = K_1/K_2 = 1$, $\bar{r} = 2$ and $\omega_1 = \omega_2 = 7.5$.) (A) Phase diagram in which the dark green region denotes the inducer concentration regime for which the system has bistable dynamics. Trajectories (B) and (C) follow the shifts in dynamics moving through phase space along different paths, and are shown in the corresponding panels. (B) Bifurcation diagrams for $\bar{R}_1$ and $\bar{R}_2$ steady-state expression as the inducer concentrations controlling each repressor increase at the same rate. (C) Bifurcation diagrams as inducer concentrations increase at different rates.

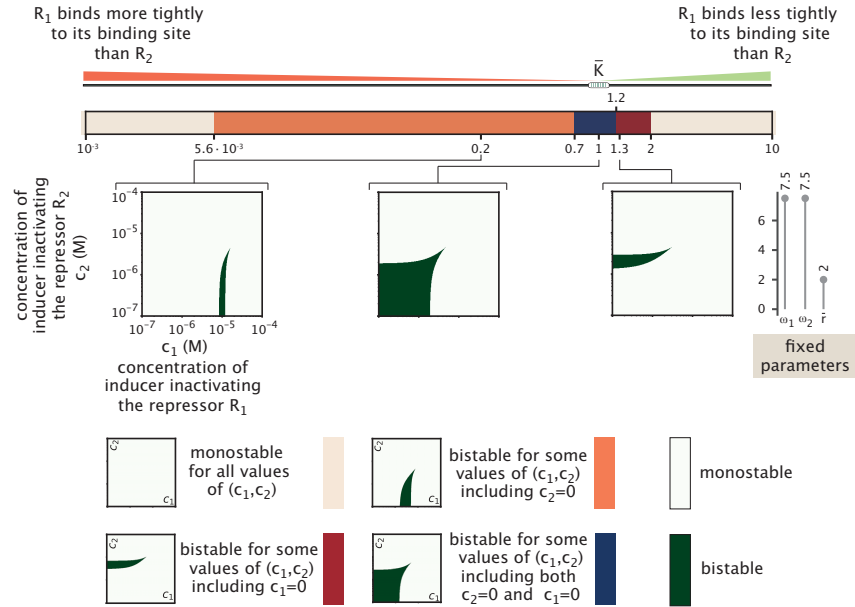


Figure 4.16: Bistability regimes in mutual repression as a function of the relative DNA-binding affinities of repressors R_1 and R_2 . Colored regions denote distinct bistable phase space geometries, defined by whether bistability occurs for a finite window of c_1 , a finite window of c_2 , or the presence of both at sufficiently small concentrations. Otherwise, the system is never bistable for any concentration (c_1, c_2) in the interval $[10^{-7}\text{M}, 10^{-4}\text{M}]$ considered. These geometries evolve as the binding affinity ratio $\bar{K} = K_1/K_2$ is varied, with $\omega_1 = \omega_2 = 7.5$ and $\bar{r} = 2$ held constant.

Conditions for bistability

We now study how the size, shape, and symmetry of the bistable region observed in the phase diagram of Fig. 4.15(A) varies with system parameters. Specifically, in Fig. 4.16 we first identify three distinct geometries for the bistable region in the (c_1, c_2) plane, each reflecting different limiting behaviors of the inducers.

The first geometry (marked orange in the legend of Fig. 4.16) corresponds to a situation where bistability is present only when c_1 lies within a finite interval $[c_1^{\min}, c_1^{\max}]$ for a given c_2 . In this case, both bounds of the interval are strictly positive and finite, and c_2 must be smaller than a certain threshold. The limiting factor is therefore c_1 , which must be finely tuned to enable bistability, while c_2 simply needs to remain below a critical level. Nevertheless, when decreasing c_2 the bistable interval in c_1 broadens, showing that lower c_2 expands the range of c_1 values supporting bistability.

A second geometry (marked red in the legend of Fig. 4.16) mirrors the first, but with the roles of c_1 and c_2 reversed. Here, bistability is present only when c_2 lies within

a finite interval, while c_1 must remain below a threshold. In this case, c_2 becomes the more constrained parameter to tune. In contrast, a third geometry (marked blue in the legend of Fig. 4.16) arises when bistability is supported broadly for small enough values of both c_1 and c_2 , with no lower bound required for either parameter. Although the bistable region remains upper-bounded, neither inducer is particularly limiting, with a broad range of concentrations allowing for bistability as long as neither becomes too large.

Fig. 4.16 illustrates how these phase space geometries for bistability depend on system parameters. We vary the relative DNA-binding affinity of the repressors by tuning $\bar{K} = K_1/K_2$, while keeping the basal production $\bar{r}$ fixed, and the cooperativities fixed and equal ($\omega_1 = \omega_2$). We observe that the most permissive bistable region—broad in both c_1 and c_2 , for the range of concentration studied—occurs when $\bar{K} \approx 1$, corresponding to a symmetric system where both repressors bind with comparable affinity. As $\bar{K}$ decreases (i.e., R_1 binds more tightly than R_2), the phase space becomes increasingly constrained in c_1 . If c_1 is too low, R_1 remains fully bound and strongly represses R_2 , suppressing bistability. Conversely, if c_1 is too high, R_1 becomes fully unbound, leaving R_2 unrepressed and again eliminating bistability. Only an intermediate range of c_1 supports bistability in this regime, while c_2 simply needs to be small enough. A similar scenario occurs when $\bar{K}$ becomes large (i.e., R_2 binds more tightly than R_1), but with c_1 and c_2 effectively reversed. Eventually, c_2 is no longer sufficient to counteract the tight binding of R_2 , and above a critical value of $\bar{K}$, bistability disappears entirely from the parameter space. Tuning the cooperativity parameter ω_2 produces similar qualitative changes in the bistability phase space as varying the relative binding affinity $\bar{K}$.

In both cases, we observe the same sequence of transitions in the structure of the bistable region, as shown in Appendix 4.I.3. High values of ω_2 reflect strong cooperative binding of repressor R_2 to the DNA, meaning that binding becomes more favorable when two repressors are present. This effect mirrors what happens when increasing $\bar{K}$: if $\bar{K} > 1$, then $K_1 > K_2$, implying that higher concentrations of R_1 are required for effective DNA binding compared to R_2 . As a result, increasing $\bar{K}$ effectively enhances the influence of R_2 , analogous to increasing ω_2 . Conversely, tuning ω_1 affects the system similarly, but with the roles of c_1 and c_2 reversed. The effects of cooperativity are examined in greater detail in Appendix 4.I.3.

From Fig. 4.16, we note that extreme (low or high) values of $\bar{K}$ tend to suppress bistability, as they strongly favor one repressor over the other across all concen-

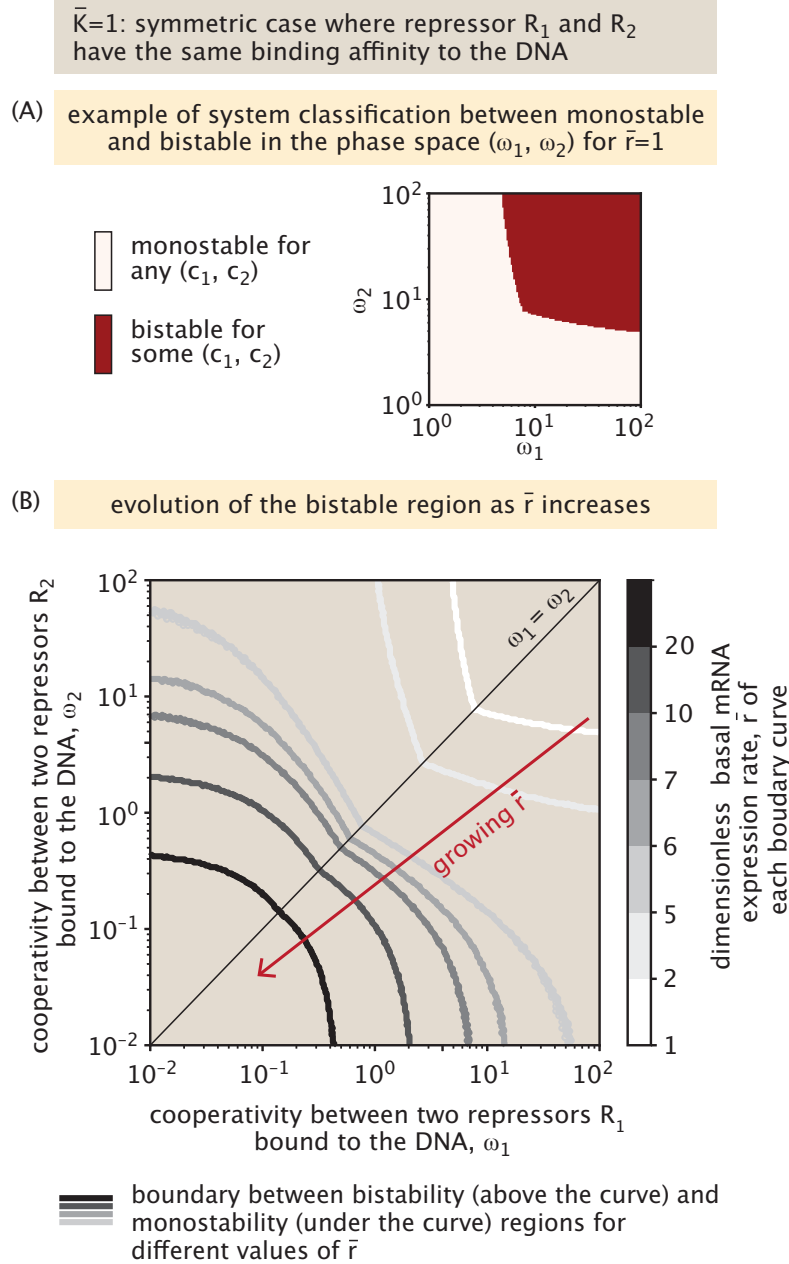


Figure 4.17: Bistability regions in mutual repression as a function of cooperativity and the production rate. In both panels, the two repressors have equal DNA-binding affinities, with $\bar{K} = 1$. We determine the boundary in (ω_1, ω_2) space that separates regions where bistability is possible for some (c_1, c_2) from those where it is not possible. (A) Example for $\bar{r} = 1$. (B) Boundary curves in the (ω_1, ω_2) plane showing how the region of possible bistability expands as $\bar{r}$ increases. Each curve corresponds to fixed $\bar{r}$ values and separates parameter combinations that are monostable for all (c_1, c_2) (below the curve) from combinations that are bistable for some (c_1, c_2) (above the curve). Colors indicate the value of $\bar{r}$ used to compute each boundary curve.

trations. In contrast, high values of ω_1 or ω_2 amplify repression mostly when the corresponding repressor is present at high concentration. As a result, the system requires finely tuned inducer concentrations to counteract this cooperative imbalance and sustain bistability, effectively constraining the range of inducer concentrations for which the system can be bistable—as shown in Appendix 4.I.3.

We next explore how the interplay between cooperativity and the production rate controls the presence and extent of bistability in mutual repression systems, focusing on the symmetric case $\bar{K} = 1$ shown in Fig. 4.17. We note that, in contrast to parameters like ω_1 , ω_2 , or K , tuning the rate parameter $\bar{r}$ does not break the symmetry between the two genes, as it controls the production rate of both repressors equally.

In Fig. 4.17(A), we classify parameter combinations in the (ω_1, ω_2) plane for $\bar{r} = 1$ according to whether the system exhibits bistability for any inducer concentrations (c_1, c_2) . Fig. 4.17(B) shows how the boundary between monostable and bistable regimes shifts with $\bar{r}$, separating regions where bistability is either inaccessible or achievable for at least some inducer pairs. We observe that the production rate, when coupled to cooperativity, plays a critical role in enabling bistability—much like in the auto-activation system, where the product $\omega\bar{r}_2$ must exceed a threshold to generate bistability. We quantify this cooperative relationship between ω_i and $\bar{r}$ further in Appendix 4.I.1 by deriving a necessary condition for bistability where

$$\bar{r} > \frac{1}{p_{\text{act}}^{\text{max}} - p_{\text{act}}^{\text{min}} + \min(\omega_2, \omega_1)p_{\text{act}}^{\text{max}}/2}, \quad (4.20)$$

with $p_{\text{act}}^{\text{max}}$ and $p_{\text{act}}^{\text{min}}$ defined in Eqns. 4.6 and 4.7.

Increasing $\bar{r}$ systematically expands the range of cooperativity values that can support bistability in the range of $\bar{r}$ evaluated in Fig. 4.17. We observe from the figure that at low production rates, bistability only arises when both repressors exhibit stabilizing cooperative binding to the DNA ($\omega_i > 1$). As $\bar{r}$ increases, this constraint relaxes: bistability becomes possible even without cooperativity ($\omega_1 = \omega_2 = 1$), and for sufficiently high $\bar{r}$, bistability can occur even in the presence of destabilizing interactions between the two repressors ($\omega_i < 1$). Intermediate production rates typically require at least one positively cooperative repressor.

Timescale for stabilization

Beyond identifying the final steady state of the mutual repression system, it is important to characterize the time required for the system to reach steady state

starting from different initial conditions. We define the relaxation time τ as the maximum of the times τ_1 and τ_2 taken by the two trajectories, $\bar{R}_1(\bar{t})$ and $\bar{R}_2(\bar{t})$, to reach 95% of their respective steady states. Fig. 4.18(A) illustrates the influence of the initial condition on the final steady state in a symmetric system, where $\bar{K} = 1$, $\omega_1 = \omega_2 = 7.5$, and $c_1 = c_2 = 10^{-6}$ M. The figure reveals that the phase space is divided into two basins of attraction, each leading to one of the two stable steady states. The separatrix, plotted in green, denotes the boundary between these basins, and is derived analytically in Appendix 4.J.

Fig. 4.18(B) and (C) quantify the relaxation time τ across the phase space $(\bar{R}_1^0, \bar{R}_2^0)$ for both symmetric and asymmetric parameter regimes. In both cases, the relaxation time is shorter when the initial condition lies far from the separatrix and near the final stable steady state. In contrast, initial conditions close to the separatrix result in significantly longer relaxation times, as the system evolves slowly near the unstable fixed point before diverging toward a stable state. Exactly on the separatrix, the relaxation to steady state is more difficult to quantify because the system may remain indefinitely near an unstable manifold without converging to either stable fixed point. In practice, however, even minimal noise in a real system will eventually drive the system away from this unstable region toward a stable state. For this reason, we disregard the white line—signifying artificially short relaxation times—observed exactly on the separatrix in Fig. 4.18(B). This feature is absent in Fig. 4.18(C), as the separatrix has a more complex shape and the numerical sweep over initial conditions does not sample it precisely.

In the asymmetric case (Fig. 4.18(C)), with $\omega_1 = 50$, $\omega_2 = 7.5$, and distinct inducer concentrations ($c_1 = 5 \cdot 10^{-6}$ M, $c_2 = 10^{-6}$ M), the phase space becomes skewed. The separatrix, corresponding to the ridge of maximal relaxation time in the grayscale colormap, delineates the boundary between the two basins of attraction. We do not overlay it explicitly, as doing so would interfere with the visualization of the relaxation times, which are particularly sensitive near this boundary. Compared to Fig. 4.18(B), where the separatrix coincides with the diagonal $\bar{R}_1^0 = \bar{R}_2^0$ due to the symmetry of the system, we observe that the separatrix is now deformed, and the relative sizes of the basins of attraction have shifted. Despite these geometric changes, the maximal relaxation times across both regimes remain comparable. This indicates that, although asymmetry reshapes the phase space and can affect which attractor is reached, it does not substantially alter the overall timescale required for the system to stabilize. The main contributor to long relaxation times remains the

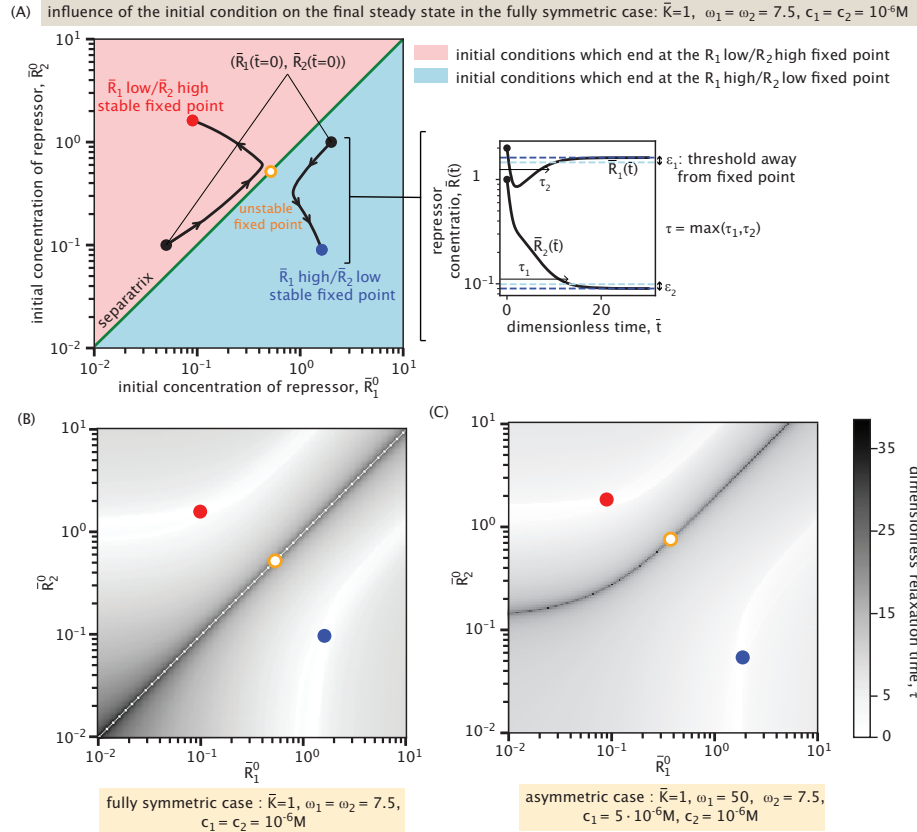


Figure 4.18: Dynamics and relaxation time in a mutual repression system. (A) Influence of the initial condition on the final steady state for a symmetric case ($\bar{K} = 1$, $\omega_1 = \omega_2 = 7.5$, $c_1 = c_2 = 10^{-6}\text{ M}$). Two stable steady states and one unstable steady state are shown. The colored regions correspond to sets of initial conditions that converge to each respective stable steady state. The green curve separating these regions is the separatrix. Dimensionless relaxation times τ_1 and τ_2 are defined as the time required for the system to approach within thresholds ϵ_1 and ϵ_2 of the stable fixed points, where these thresholds correspond to 95% of the respective steady-state values. The global relaxation time of the system, denoted τ , is then defined as $\tau = \max(\tau_1, \tau_2)$. (B-C) Dimensionless relaxation time as a function of initial concentrations of repressors R_1 and R_2 . Panel (B) corresponds to the symmetric case, whereas panel (C) plots an asymmetric case ($K = 1$, $\omega_1 = 50$, $\omega_2 = 7.5$, $c_1 = 5 \cdot 10^{-6}\text{ M}$, $c_2 = 10^{-6}\text{ M}$).

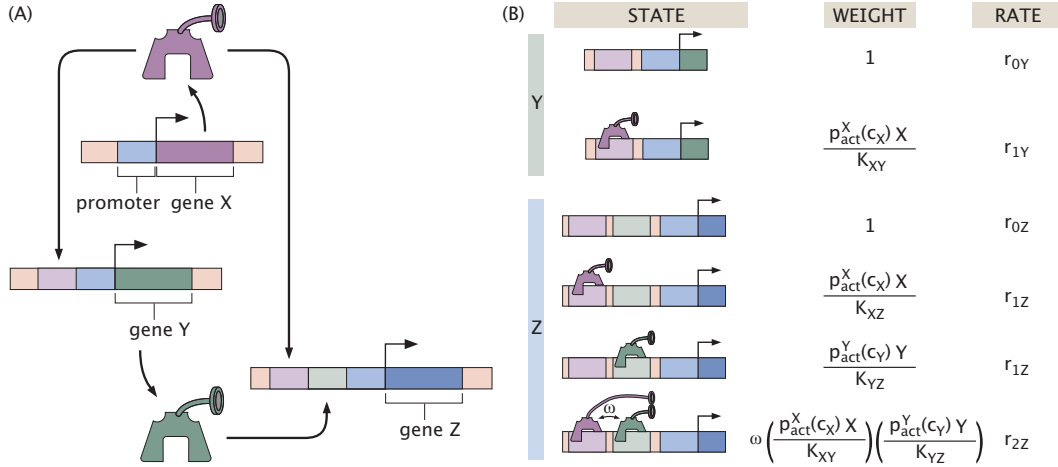


Figure 4.19: The coherent feed-forward loop. (A) Schematic representation. Expression of output protein Z is controlled by expression of protein X, either by direct activation or indirectly, first activating expression of Y, which in turn activates Z. The regulatory circuit is coherent because both pathways have the same activating effect on Z. (B) Thermodynamic states, weights, and rates for expression of activator Y and output protein Z. Note that the model assumes both X and Y can bind together to activate expression at their respective binding sites, interacting with cooperativity ω .

initial conditions—and how close they are to the separatrix—regardless of symmetry.

This section has had as its primary ambition to carefully consider the famed mutual repression genetic switch from a new perspective in which two repressors are controlled separately by different effector molecules. We have seen that the steady-states and the dynamics in this case are extremely rich, making it clear that there is much freedom in the biological context to exploit various behaviors.

4.3 Kinetics and Time Delays in Feed-Forward Loops

In this section, we consider gene circuits whose functionality appears in their dynamics rather than in their steady state responses. Specifically, we focus on a three-gene circuit called the feed-forward loop shown schematically in Fig. 4.19(A) [47]. Here, we denote the input genes as X and Y, and the output gene as Z. In a feed-forward loop, X regulates Y, and X and Y together regulate Z. X thus controls expression of output Z through both direct and indirect regulatory paths. This network typically features a sign-sensitive delayed or accelerated response (depending on architecture) to a step-wise change in the effector concentration for protein X [47]. That is, while the qualitative nature of the response (delay or acceleration) remains fixed, its magnitude depends on the sign (an increase or a decrease) of the input change. This

delayed or accelerated response has been hypothesized to have important biological consequences, particularly in information-processing systems that filter noisy inputs [47]. Beyond their role in shaping temporal responses, coherent feed-forward loops have been shown to attenuate input noise, thereby enhancing the reliability of gene expression [65–67].

Interestingly, there are various architectures of feed-forward loop depending on whether X and Y work together or at cross purposes. We will largely focus on the particular architecture where X activates Y and Z , and Y also activates Z , which is the so-called type I coherent feed-forward loop, with the word “coherent” attached to this architecture since X and Y alter the expression of Z in the same way. We consider this particular motif primarily because at the time of the most recent census of regulatory architectures in *E. coli*, this version of the feed-forward loop appeared most frequently [68, 69]. The logic of our analysis can be applied to any of the other feed-forward architectures as well.

Previous literature explores feed-forward loops from a dynamical systems perspective using Hill functions to model transcription factor-DNA interactions and considering the effector concentration for X as a Boolean variable that is either fully on or fully off [47]. We build upon that earlier analysis also by making a systematic search for network parameters that give rise to various functions. Our goal is to expand the theoretical understanding of the feed-forward loop architecture by incorporating the thermodynamic model to describe transcription factor binding to the DNA and to explicitly include effector function. Specifically, we explore what gives rise to the dynamical features of the feed-forward loop, the robustness of such features, and the effect of continuously tuning the effector concentration.

As usual when writing the dynamical equations, we begin with the states, weights and rates for the regulatory architecture of interest. Fig. 4.19(B) provides the states, weights and rates for the coherent feed-forward loop architecture, where we assume one binding site per transcription factor. In light of the states and weights, we can write the time-evolution equations for the coherent feed-forward loop as

$$\frac{dY}{dt} = -\gamma Y + \frac{r_{0Y} + r_{1Y} p_{\text{act}}^X(c_X) \frac{X}{K_{XY}}}{1 + p_{\text{act}}^X(c_X) \frac{X}{K_{XY}}} \quad (4.21)$$

for the regulation of Y by X and

$$\frac{dZ}{dt} = -\gamma Z + \frac{r_{0Z} + r_{1Z} \left(p_{\text{act}}^X(c_X) \frac{X}{K_{XZ}} + p_{\text{act}}^Y(c_Y) \frac{Y}{K_{YZ}} \right) + r_{2Z} \omega p_{\text{act}}^X(c_X) \frac{X}{K_{XZ}} p_{\text{act}}^Y(c_Y) \frac{Y}{K_{YZ}}}{1 + p_{\text{act}}^X(c_X) \frac{X}{K_{XZ}} + p_{\text{act}}^Y(c_Y) \frac{Y}{K_{YZ}} + \omega p_{\text{act}}^X(c_X) \frac{X}{K_{XZ}} p_{\text{act}}^Y(c_Y) \frac{Y}{K_{YZ}}} \quad (4.22)$$

for the regulation of Z by both X and Y . We assume here that the proteins Y and Z have the same degradation rate γ . The production rates and dissociation constants are assumed to be different in general for each thermodynamic state. Specifically, r_{iG} denotes the production rate of gene G when i number of transcription factor are bound. Further, $K_{G_1G_2}$ denotes the dissociation constant of transcription factor G_1 binding to gene G_2 . ω is the cooperativity, which takes into account the extra interaction energy between X and Y when bound to the DNA. Finally, the probabilities $p_{\text{act}}^X(c_X)$ and $p_{\text{act}}^Y(c_Y)$ scale the activity of transcription factors X and Y . Note also that we consider different effectors c_X and c_Y for the two genes that can be varied independently. These functions may be distinct in principle, and the analytic discussions here make no assumption regarding their nature. For numerical results, however, we assume, for simplicity, that these probability functions, and thus effector activity functions, are the same regardless of target transcription factor.

We non-dimensionalize the equations by using $1/\gamma$ as our unit of time and K_{XY} as our measure of concentration. In light of these conventions, we arrive at

$$\frac{d\bar{Y}}{d\bar{t}} = -\bar{Y} + \frac{\bar{r}_{0Y} + \bar{r}_{1Y} p_{\text{act}}^X(c_X) \bar{X}}{1 + p_{\text{act}}^X(c_X) \bar{X}}, \quad (4.23)$$

and

$$\frac{d\bar{Z}}{d\bar{t}} = -\bar{Z} + \frac{\bar{r}_{0Z} + \bar{r}_{1Z}(\mathcal{X} + \mathcal{Y}) + \omega \bar{r}_{2Z} \mathcal{X} \mathcal{Y}}{1 + \mathcal{X} + \mathcal{Y} + \omega \mathcal{X} \mathcal{Y}}. \quad (4.24)$$

To make subsequent analysis less cumbersome, we further introduce $\mathcal{X} = p_{\text{act}}^X(c_X) \bar{X} / \bar{K}_{XZ}$ and $\mathcal{Y} = p_{\text{act}}^Y(c_Y) \bar{Y} / \bar{K}_{YZ}$ as simpler notation for the effective regulatory contributions of $\bar{X}$ and $\bar{Y}$ to $\bar{Z}$ expression. The bar indicates quantities where time is measured in units of $1/\gamma$, and where concentration and dissociation constants are measured in units of K_{XY} . Specifically, we define dimensionless dissociation constants $\bar{K}_{XZ} = K_{XZ}/K_{XY}$ and $\bar{K}_{YZ} = K_{YZ}/K_{XY}$. The rates are then in units of γK_{XY} . Note that this model fixes the concentration of $\bar{X}$ for simplicity, such that its activity regulating $\bar{Y}$ and $\bar{Z}$ depends entirely on effector concentration c_X .

4.3.1 Characterizing delay responses in coherent feed-forward loops

Previous work has shown that coherent feed-forward loops can delay a system's response to an input signal [47]. Here, we demonstrate from our thermodynamic

modeling perspective the analytic origins of this delay. In particular, we rigorously define how the introduction of an indirect but coherent path for regulation of output $\bar{Z}$ affects its response.

Suppose that we keep the effector concentration c_Y fixed, and that at time $\bar{t} = 0$ the effector concentration c_X changes sharply from an initial concentration c_X^i to a final concentration c_X^f such that input $\bar{X}$ activity either increases (an “ON” step) or decreases (an “OFF” step). This then means that

$$\mathcal{X}(\bar{t}) = \begin{cases} \mathcal{X}_i = \frac{p_{\text{act}}^X(c_X^i)\bar{X}}{\bar{K}_{XZ}} & \text{if } \bar{t} \leq 0, \\ \mathcal{X}_f = \frac{p_{\text{act}}^X(c_X^f)\bar{X}}{\bar{K}_{XZ}} & \text{if } \bar{t} > 0. \end{cases} \quad (4.25)$$

In the coherent feed-forward loop, since all the regulatory relations are activation, both $\bar{Y}$ and $\bar{Z}$ increase in response to an ON step, and decrease in response to an OFF step.

To understand intuitively how exactly the feed-forward loop regulatory structure responds to such a switch in input signal, let us first consider a simpler scheme in which $\bar{X}$ no longer regulates $\bar{Y}$, leaving $\bar{X}$ and $\bar{Y}$ to regulate $\bar{Z}$ independently with $\bar{Y}(\bar{t}) = \bar{Y}$ fixed at a value. Since we are keeping effector concentration c_Y fixed constant, this then also means that $\mathcal{Y}$ is constant. We refer to this setting as “simple regulation.”

Before the switch in c_X , the simple regulation system is at steady state, with initial output expression $\bar{Z}_s^i$ where subscript s denotes simple regulation. After the switch, the system relaxes to a new steady state with final expression $\bar{Z}_s^f$. For $\bar{t} > 0$, the production term in Eqn. 4.24 is simply a constant, yielding the following differential equation for $\bar{Z}$.

$$\begin{aligned} \frac{d\bar{Z}}{d\bar{t}} &= -\bar{Z} + \frac{\bar{r}_{0Z} + \bar{r}_{1Z}(\mathcal{X}_f + \mathcal{Y}) + \omega \bar{r}_{2Z} \mathcal{X}_f \mathcal{Y}}{1 + \mathcal{X}_f + \mathcal{Y} + \omega \mathcal{X}_f \mathcal{Y}} \\ &\equiv -\bar{Z} + \bar{Z}_s^f. \end{aligned} \quad (4.26)$$

Integrating Eqn. 4.26, we thus determine that under simple regulation, output $\bar{Z}$ evolves after the switch in input $\bar{X}$ signal by a standard exponential behavior defined as

$$\bar{Z}_s(\bar{t}) = \bar{Z}_s^i e^{-\bar{t}} + \bar{Z}_s^f (1 - e^{-\bar{t}}). \quad (4.27)$$

By contrast, in the coherent feed-forward loop, the concentration of $\bar{Y}$ directly depends on $\bar{X}$ as seen in Eqn. 4.23. As a result, additional time is needed for $\bar{Y}$ to

evolve from its initial steady-state value $\bar{Y}_i$ to a new value $\bar{Y}_f$ following a change in $\bar{X}$. Expressed mathematically, since $\bar{Y}$ itself is a function of time, the solution of $\bar{Z}$ to Eqn. 4.24 is not strictly an exponential relaxation to steady state. More formally, output expression for the coherent feed-forward loop evolves by a function of the form

$$\bar{Z}(\bar{t}) = \bar{Z}_i e^{-\bar{t}} + \bar{Z}_f (1 - e^{-\bar{t}}) + \Theta(\bar{t}), \quad (4.28)$$

where $\bar{Z}_i$ is the initial steady state in the feed-forward setting before the change in c_X , and $\bar{Z}_f$ is the final steady state after the change to which $\bar{Z}(\bar{t})$ eventually relaxes. We derive Eqn. 4.28 explicitly from Eqns. 4.23 and 4.24 in Appendix 4.K. Note that the sum of the first two terms in Eqn. 4.28 describes behavior of the same form as simple regulation in Eqn. 4.27. Therefore, by rescaling Eqn. 4.27 we can treat the exponential portion of Eqn. 4.28 as an equivalent $\bar{Z}_{\text{simple}}$ with the same relaxation dynamics as observed for simple regulation. We can also then express output response for the feed-forward loop as

$$\bar{Z}(\bar{t}) = \bar{Z}_{\text{simple}}(\bar{t}) + \Theta(\bar{t}). \quad (4.29)$$

We thus observe that the feed-forward loop's output response differs from behavior in simple regulation by a function $\Theta(\bar{t})$. Solving Eqns. 4.23 and 4.24 results in

$$\Theta(\bar{t}) = -\frac{\Phi \Delta \mathcal{Y}}{S^2} e^{-\bar{t}} \log \left(\frac{S e^{\bar{t}} - \Delta \mathcal{Y} (1 + \omega \mathcal{X}_f)}{S - \Delta \mathcal{Y} (1 + \omega \mathcal{X}_f)} \right). \quad (4.30)$$

Here, $\Theta(\bar{t})$ depends on the three quantities $\Delta \mathcal{Y}$, Φ , and S . First, the quantity

$$\Delta \mathcal{Y} = \mathcal{Y}_f - \mathcal{Y}_i = \frac{p_{\text{act}}^Y(c_Y)}{\bar{K}_{YZ}} (\bar{Y}_f - \bar{Y}_i) \quad (4.31)$$

denotes the total change in quantity $\mathcal{Y}$ in response to the change in input effector concentration c_X . $\Delta \mathcal{Y}$ thus contains implicit information about regulation of $\bar{Y}$ by $\bar{X}$ from its evolution as defined in Eqn. 4.23. $\Theta(\bar{t})$ also depends on the coefficient

$$\begin{aligned} \Phi = & \omega(\mathcal{X}_f)^2 (\bar{r}_{2Z} - \bar{r}_{1Z}) + \omega \mathcal{X}_f (\bar{r}_{2Z} - \bar{r}_{0Z}) \\ & + (\bar{r}_{1Z} - \bar{r}_{0Z}), \end{aligned} \quad (4.32)$$

which encodes how the rates and cooperativity regulate output $\bar{Z}$ expression as a function of input signal $\mathcal{X}_f$. Interestingly, we note that all types of feed-forward loops have the same solution as Eqn. 4.30, except with a potentially different Φ . Appendix 4.K.4 discusses this in more detail. Finally, the quantity

$$S = 1 + \mathcal{X}_f + \mathcal{Y}_f + \omega \mathcal{X}_f \mathcal{Y}_f \quad (4.33)$$

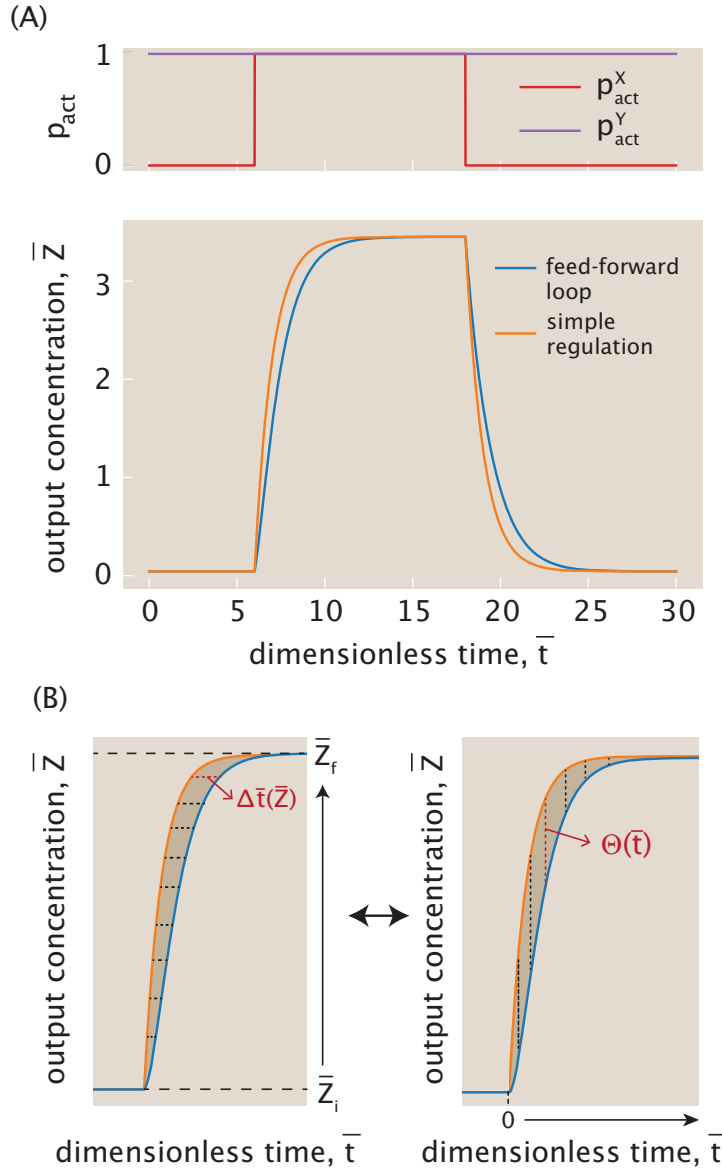


Figure 4.20: Delay in coherent feed-forward loop response compared to simple regulation. (A) The input signal is applied by tuning c_X as a step function: from high (10^{-4} M) to low (10^{-7} M) at $\bar{t} = 6$, and back to high at $\bar{t} = 18$. The effector concentration c_Y is held constant at 10^{-7} M. These inputs determine the activation probabilities p_{act}^X and p_{act}^Y , shown in red and purple, respectively. The second panel plots the time evolution of the dimensionless output concentration $\bar{Z}(\bar{t})$ under feed-forward and simple regulation schemes, with $\bar{r}_{0Y} = \bar{r}_{0Z} = 0$, $\bar{r}_{1Y} = \bar{r}_{1Z} = 2$, $\bar{r}_{2Z} = 10$, $\omega = 1$, and $\bar{K}_{XZ} = \bar{K}_{YZ} = 1$. (B) Schematic demonstrating the two ways to equivalently quantify the delayed response of the feed-forward loop compared to simple regulation, captured by the shaded area between the two curves. One can either integrate over individual time delay measurements $\Delta \bar{t}(\bar{Z})$ as a function of $\bar{Z}$, or equivalently integrate the difference in responses $\Theta(\bar{t})$ as a function of $\bar{t}$.

is the sum of the dimensionless weights for all possible regulatory states with zero, one, or two transcription factors bound, at final concentrations of active $\bar{X}$ and $\bar{Y}$.

Fig. 4.20(A) highlights the coherent feed-forward loop's delayed response to changes in input signal for a specific set of parameters. In our simulations, we set the rates $\bar{r}_{0G}$ to zero, indicating that this system requires activator(s) to be bound to express output $\bar{Z}$. In the top plot of Fig. 4.20(A), the effector concentration c_Y is fixed such that $p_{\text{act}}^Y(c_Y)$ is always at high activity, and the effector concentration c_X jumps such that $p_{\text{act}}^X(c_X)$ reaches high activity as a step function (ON step), and then back to low activity (OFF step). The bottom plot of Fig. 4.20(A) shows how the output $\bar{Z}$ evolves over time in response to a step function input $\bar{X}$ activity, evolving and stabilizing to a high $\bar{Z}$ value before the OFF step in $p_{\text{act}}^X(c_X)$ causes the output concentration to decay back down to steady state value zero. We observe that, compared to simple regulation, the change in output concentration $\bar{Z}$ is slower when responding to both an increase and a decrease in input activity, matching our expectation.

Fig. 4.20(B) visualizes how to quantify this delay in the time it takes the feed-forward system to reach a given output concentration as it responds to an input pulse. The diagram on the left illustrates the response to the ON step, in which the output starts to increase from $\bar{Z}_i$ to $\bar{Z}_f$ at (dimensionless) time $\bar{t} = 0$. If we choose a value of $\bar{Z}$ in this time frame, we observe that it takes longer to reach this value on its way to steady state $\bar{Z}_f$ in the feed-forward loop setting than in simple regulation. We highlight one such horizontal distance between the two curves as the time delay $\Delta\bar{t}(\bar{Z})$. Explicitly, we define $\Delta\bar{t}(\bar{Z})$ to be the difference between the time it takes for simple regulation to reach a given $\bar{Z}$ and that for the feed-forward loop. $\Delta\bar{t}(\bar{Z}) < 0$ indicates a delay and $\Delta\bar{t}(\bar{Z}) > 0$ indicates acceleration. We can then compute the average time difference observed between the two curves by integrating $\Delta\bar{t}(\bar{Z})$ over the range of output $\bar{Z}$, and normalizing by this range. Therefore, for a given step function change in input $\bar{X}$ activity, the average delay during the system's evolution toward its new final steady state is

$$\langle \Delta\bar{t} \rangle = \frac{1}{|\bar{Z}_f - \bar{Z}_i|} \int_{\bar{Z}_i}^{\bar{Z}_f} \Delta\bar{t}(\bar{Z}) d\bar{Z}. \quad (4.34)$$

Note, however, that it is not straightforward to derive an expression for $\Delta\bar{t}(\bar{Z})$. Instead, since this integral geometrically captures the area between the two curves, we can equivalently evaluate this area as shown in the second diagram of Fig. 4.20(B) by integrating vertical slices through this shaded region. At a given time $\bar{t}$, the

vertical dotted line corresponds to the difference in output response between the two curves, defined by the function $\Theta(\bar{t})$ previously derived in Eqn. 4.30.

From this description, we can then also derive the average time delay from the offset $\Theta(\bar{t})$, and thus arrive at the equivalent definition

$$\langle \Delta \bar{t} \rangle = \frac{1}{\bar{Z}_f - \bar{Z}_i} \int_0^\infty \Theta(\bar{t}) d\bar{t}. \quad (4.35)$$

Notice that the absolute value on $\bar{Z}_f - \bar{Z}_i$ is dropped to match the sign of $\langle \Delta \bar{t} \rangle$ in Eqn. 4.34. Substituting $\Theta(\bar{t})$ from Eqn. 4.30 and evaluating the integral, the average time delay becomes

$$\langle \Delta \bar{t} \rangle = \frac{\Phi(\bar{Z}_f - \bar{Z}_i)^{-1}}{S(1 + \omega \mathcal{X}_f)} \log \left(\frac{1 + \mathcal{X}_f + \mathcal{Y}_i + \omega \mathcal{X}_f \mathcal{Y}_i}{S} \right). \quad (4.36)$$

This result highlights that $\langle \Delta \bar{t} \rangle$ depends on both the initial and final states of $\mathcal{X}$ and $\mathcal{Y}$. In fact, it is this dual dependence that causes the delays in response to ON and OFF steps to differ in Fig. 4.20(A). Switching from ON to OFF and vice versa simply swaps the initial and final expression states of $\mathcal{X}$ and $\mathcal{Y}$. Applying these transformations $\mathcal{X}_i \leftrightarrow \mathcal{X}_f$ and $\mathcal{Y}_i \leftrightarrow \mathcal{Y}_f$ in Eqn. 4.36 shows that the magnitude of the average delay $\langle \Delta \bar{t} \rangle$ is in general not conserved under the exchange of initial and final states. It is therefore this asymmetric dependence on initial and final states that directly leads to differences in output responses to ON and OFF steps in the coherent feed-forward loop.

From Eqn. 4.36, we can analytically deduce whether a feed-forward loop delays or accelerates output response from the sign of $\langle \Delta \bar{t} \rangle$. In Appendix 4.K.2, we prove that in general $\langle \Delta \bar{t} \rangle \leq 0$ for the coherent feed-forward loop, leading to delay for both the ON and OFF steps as seen in the example of Fig. 4.20(A).

4.3.2 Robustness of delayed response for different coherent logic gates

While Fig. 4.20(A) demonstrates delays in both the ON and OFF step of the feed-forward loop for an arbitrary choice of the dimensionless rates, dissociation constants, and cooperativity, distinct choices for this set of parameters lead to different magnitudes of delay. Returning to Fig. 4.19, the thermodynamic states and weights listed here are defined generally such that all of the states can contribute to transcription factor expression, and this is reflected in the example feed-forward loop shown in Fig. 4.20(A). Certain alternative choices for parameters, however, can carry physical significance because they restrict the regulatory states allowing expression to only a subset of those depicted in Fig. 4.19. How would the feed-forward

loop's behavior differ, for example, if expression could only be enhanced when both activators are bound? We use the framework of *logic gates* to define such unique categories for regulatory conditions.

Specifically, we highlight three commonly-encountered logic gates—the AND, XOR, and OR gates. We will assume here that all gates can express output $\bar{Z}$ at a basal level, as defined by rate $\bar{r}_{0Z}$ for the state. Each logic gate is then characterized by a different set of parameters for the states in which one or both activators can be bound, and these parameters determine whether a given state's expression is enhanced or remains unaffected at the basal level.

In the AND gate, Z expression is enhanced only when both X and Y are bound. From the description in Fig. 4.19, this corresponds to systems in which cooperativity ω is nonzero and $\bar{r}_{2Z} > \bar{r}_{1Z} = \bar{r}_{0Z}$, such that X and Y have no activating effect on basal expression unless simultaneously bound. In the XOR gate, X and Y cannot be bound at the same time ($\omega = 0$), and single-activator bound states enhance Z expression ($\bar{r}_{1Z} > \bar{r}_{0Z}$). Finally, the OR gate allows enhanced expression when either X , Y , or both are bound, and broadly applies to systems for which $\bar{r}_{2Z} \geq \bar{r}_{1Z} > \bar{r}_{0Z}$. Note that the expression rates when one or both transcription factors bind can differ.

In previous work, XOR and AND gates have been reported to exhibit sign-sensitive delays in response to a signal change in c_X : the XOR gate feed-forward loop delays the OFF step but not the ON step, while the AND gate delays the ON step but not the OFF step [47]. While these descriptions hold for certain parameter choices, it remains unclear how robust these patterns are across parameter space as the conditions governing regulatory interactions change. We will now examine the conditions under which the feed-forward loop response delays both ON and OFF steps, delays only one, or delays neither as we compare the different types of logic gates.

To evaluate the robustness of time delays across different types of logic gates, we sweep across parameter space and find regions with high average delay, $\langle \Delta \bar{t} \rangle$. For now, we choose to fix rate and cooperativity parameters and sweep across the two-dimensional space $(\bar{K}_{XZ}, \bar{K}_{YZ})$. The motivation is to find the suitable dissociation constants given a logic gate, in the case in which the production rates and cooperativity must satisfy certain requirements. For example, in the XOR gate, $\omega = 0$ is fixed and is not a tunable parameter.

In Fig. 4.21, we show a parameter sweep for three sets of rates and cooperativity

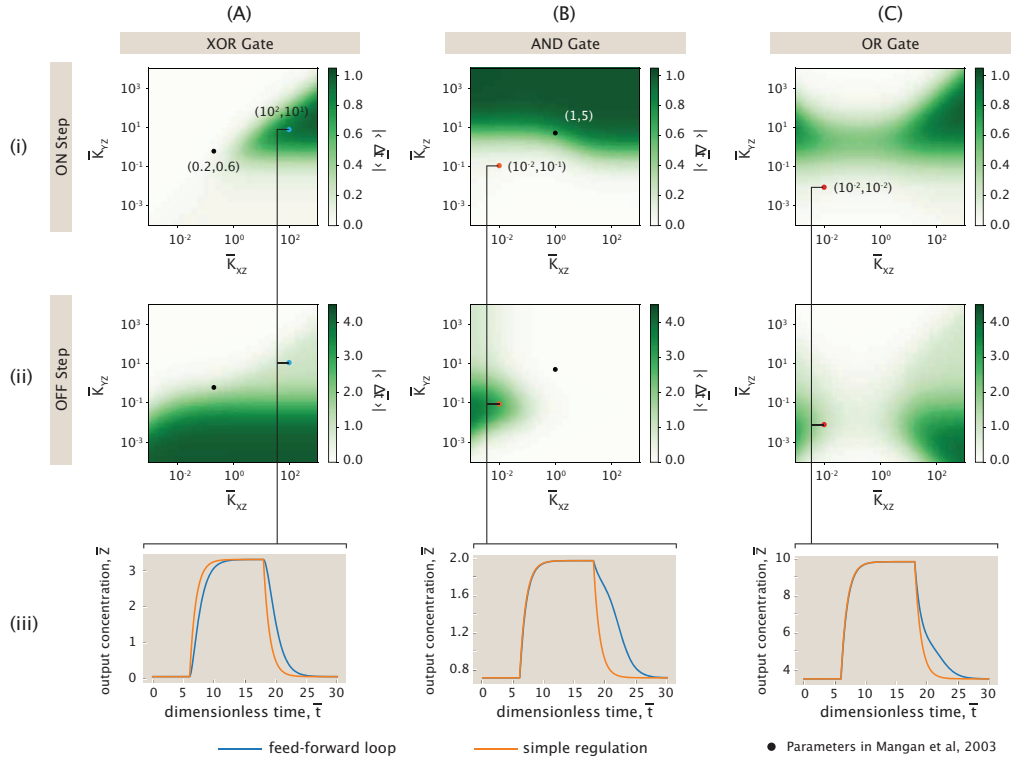


Figure 4.21: Magnitude of average time delay observed across parameter space in the ON and OFF steps of different coherent feed-forward logic gates. Each colorplot shows $\Delta \bar{t}$ as a function of $\bar{K}_{XZ}$ and $\bar{K}_{YZ}$. ON (row (i)) and OFF (row (ii)) steps are defined by the same c_X step function as in Fig. 4.20. Each panel represents a different logic gate — (A) the XOR gate, (B) the AND gate, and (C) the OR gate. For each gate, we select a set of $(\bar{K}_{XZ}, \bar{K}_{YZ})$ that exhibit unexpected behaviors, and in row (iii) plot the corresponding feed-forward and simple regulation trajectories observed from numerical integration. The c_X and c_Y signal for these trajectories are the same as in Fig. 4.20. The XOR gate parameters are $\bar{r}_{0Y} = \bar{r}_{0Z} = 0$, $\bar{r}_{1Y} = \bar{r}_{1Z} = 2$, and $\omega = 0$. The AND gate parameters are $\bar{r}_{0Y} = \bar{r}_{0Z} = \bar{r}_{1Z} = 0$, $\bar{r}_{1Y} = \bar{r}_{2Z} = 2$, and $\omega = 10$. The OR gate parameters are $\bar{r}_{0Y} = \bar{r}_{0Z} = 0$, $\bar{r}_{1Y} = \bar{r}_{1Z} = 2$, $\bar{r}_{2Z} = 10$, and $\omega = 1$, which are the same as in Fig. 4.20.

parameters that correspond to three different logic gates. For each set of parameters, both ON and OFF steps are studied. These calculations inspire several observations. First, for a given step undergone by $p_{\text{act}}^X(c_X)$, we can computationally identify a maximum $\langle \Delta \bar{t} \rangle$ with respect to all other parameters. Further, this maximum is different for ON and OFF steps. For example, in Fig. 4.21(i), specific to the input step chosen here, we observe that in all logic gates the maximal $\langle \Delta \bar{t} \rangle$ achievable is about 1 for the ON step and 4.5 for the OFF step. To tie this back to units of time, we consider *E. coli*. Here, proteins tend to be stable over the timescale of a cell cycle and hence the dilution resulting from cell division becomes the effective degradation rate. Taking a rate of degradation of order $\gamma = 10^{-2} \text{min}^{-1}$, we then get a maximum delay of 500 min, which is more than 8 hours. The asymmetry between the maximal $\langle \Delta \bar{t} \rangle$ of the ON and OFF steps resonates with the analytic discussion in the previous section. We show computational evidence in Appendix 4.L that sweeps across all other parameters indicate the existence of a global maximum $\langle \Delta \bar{t} \rangle$. Note that the exact value and existence of this maximum is a result specific to function $p_{\text{act}}(c_X)$ and the allosteric parameters we chose to describe effector activity in Fig. 4.5.

Second, both the XOR and AND gates exhibit the expected sign-sensitive delay across a substantial portion of the $(\bar{K}_{XZ}, \bar{K}_{YZ})$ parameter space, though not uniformly. In particular, Fig. 4.21(A)(i,ii) shows that for the XOR gate, the lower half of this space yields negligible ON-step delay but a pronounced OFF-step delay. Conversely, Fig. 4.21(B)(i,ii) reveals that for the AND gate, the upper half of the space yields negligible OFF-step delay and a pronounced ON-step delay. Nevertheless, in specific regions of the $(\bar{K}_{XZ}, \bar{K}_{YZ})$ parameter space—namely, the upper left quadrant for the XOR gate and the lower right quadrant for the AND gate—neither the ON nor the OFF step exhibits any appreciable delay. More intriguingly, certain extreme choices of dissociation constants contradict the expected behavior: the XOR gate can show delay on both ON and OFF steps, and the AND gate can only delay the OFF step. Example trajectories corresponding to these atypical regimes are shown in Fig. 4.21(A,B)(iii).

Finally, the OR gate can produce sizable delays, but only for more extreme values of the dissociation constants. Near the region where $\bar{K}_{XZ} \approx \bar{K}_{YZ} \approx 1$ —which corresponds to similar binding strengths between X , Y , and Z with the DNA—the delays for both ON and OFF steps are minimal. This suggests that in some biologically relevant regimes, where dissociation constants are typically of the same order of magnitude, the OR gate is the least effective at generating a temporal delay.

We observe that the average delay $\langle \Delta \bar{t} \rangle$ in coherent feed-forward loops depends strongly on the dissociation constant $\bar{K}_{YZ}$, which sets the binding affinity of Y to the promoter of Z —one of the interactions that distinguishes feed-forward loops from simple regulation. A clear trend emerges: ON steps (Fig. 4.21(A-C)(i)) exhibit stronger delays when Y binding is weak (large $\bar{K}_{YZ}$), whereas OFF steps (Fig. 4.21(A-C)(ii)) show stronger delays when Y binding is strong (small $\bar{K}_{YZ}$).

Since the feed-forward loop ultimately aims to control Z activation, another relevant feature is the output amplitude $\Delta \bar{Z} = |\bar{Z}_f - \bar{Z}_i|$ for a given change in input c_X . A small $\Delta \bar{Z}$ would imply that $\bar{Z}$ remains nearly constant, making the response uninformative. However, we show in Appendix 4.L that $\Delta \bar{Z}$ scales linearly with the production rates. Although the dependence of $\Delta \bar{Z}$ on other parameters is non-trivial, globally the effect of the rates dominates. We thus reserve a detailed discussion of the optimization of $\Delta \bar{Z}$ values for Appendix 4.L and remain focused here on the average delay $\langle \Delta \bar{t} \rangle$.

4.3.3 Existence of pulse in incoherent feed-forward loop

We now turn attention to the incoherent feed-forward loop depicted in Fig. 4.22. This architecture is a commonly observed motif in *E. coli*, where X activates both Y and Z , while Y represses Z [68, 69]. This motif gives rise to a qualitatively distinct dynamical behavior from the coherent feed-forward loop: a pulse in the output Z . A pulse is conventionally defined as a transient trajectory of $\bar{Z}(\bar{t})$ such that there exists a time $\bar{t}$ where $\bar{Z}(\bar{t}) > \bar{Z}_f$ if the response of $\bar{Z}(\bar{t})$ is increasing, or $\bar{Z}(\bar{t}) < \bar{Z}_f$ if the response of $\bar{Z}(\bar{t})$ is decreasing. In incoherent feed-forward loops, an ON step signal where $p_{\text{act}}^X(c_X)$ increases does not necessarily produce an increasing response in $\bar{Z}(\bar{t})$, as activation induced by increasing $\bar{X}$ competes with repression induced by increasing $\bar{Y}$. This is precisely the incoherence in the name of such circuits.

Referring to the states, weights, and rates shown in Fig. 4.22, the non-dimensional dynamics of the system are described by

$$\frac{d\bar{Y}}{d\bar{t}} = -\bar{Y} + \frac{\bar{r}_{0Y} + \bar{r}_{1Y} p_{\text{act}}^X \bar{X}}{1 + p_{\text{act}}^X \bar{X}} \quad (4.37)$$

and

$$\frac{d\bar{Z}}{d\bar{t}} = -\bar{Z} + \frac{\bar{r}_{0Z} + \bar{r}_{1Z} \mathcal{X}}{1 + \mathcal{X} + \mathcal{Y} + \omega \mathcal{X} \mathcal{Y}}, \quad (4.38)$$

with $\mathcal{X} = p_{\text{act}}^X(c_X) \bar{X} / \bar{K}_{XZ}$ and $\mathcal{Y} = p_{\text{act}}^Y(c_Y) \bar{Y} / \bar{K}_{YZ}$. Again the bar indicates the time in units of $1/\gamma$, concentrations and dissociation rates in units of K_{XY} and rates in units of γK_{XY} .

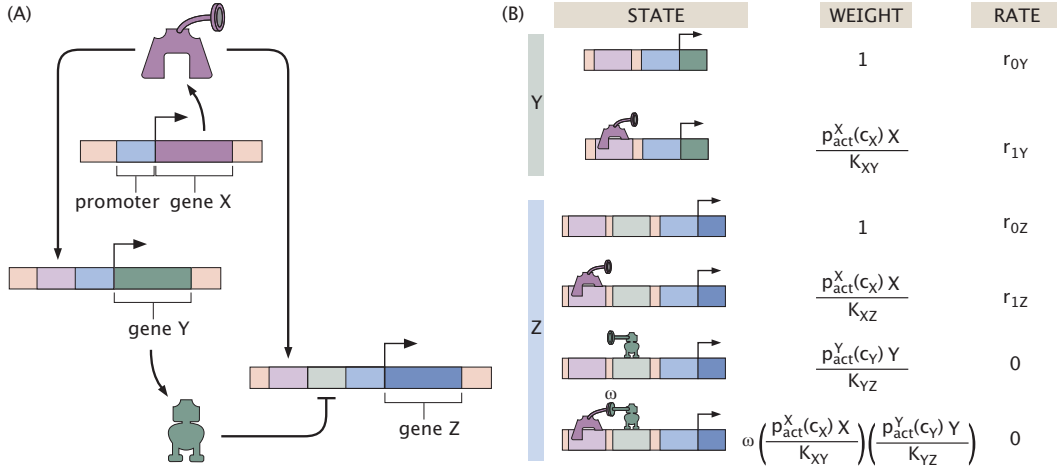


Figure 4.22: The incoherent feed-forward loop. (A) Schematic representation. Expression of output protein Z is controlled by expression of protein X , either by direct activation or indirectly, first activating expression of Y which then represses Z . The regulatory circuit is incoherent because the pathways have opposing effects on Z . (B) Thermodynamic states, weights, and rates for expression of repressor Y and output protein Z . X and Y interact with cooperativity ω , but bound repressor suppresses expression regardless of activator presence.

Intuitively, pulses emerge because of the delay in the repression exerted by Y . Following an ON step in c_X , $\bar{Y}$ increases exponentially to its final value. In the early phase of the response, X already activates Z strongly, but Y has not yet accumulated enough to exert repression. As a result, $\bar{Z}$ temporarily overshoots its final steady state. This behavior is depicted in Fig. 4.23(D), where the blue curve exhibits a pronounced pulse, in contrast to the monotonic exponential relaxation of the simple regulation output (orange).

The accelerating nature of the incoherent feed-forward loop can also be captured analytically by repeating the calculation leading to Eqn. 4.30 but adapted to this new setting. The modified prefactor

$$\Phi = -(\bar{r}_{0Z} + \bar{r}_{1Z}\chi_f)(1 + \omega\chi_f) \quad (4.39)$$

is always negative. As a result, the average time difference $\langle \Delta \bar{t} \rangle$ as defined in Eqn. 4.35 is always positive, both for ON and OFF steps, meaning that the incoherent feed-forward loop accelerates the response for both transitions. In Appendix 4.M, we remark that the definition and interpretation of $\langle \Delta \bar{t} \rangle$ is subtle when $\bar{Z}$ exhibits a pulse. Thus, we only consider $\langle \Delta \bar{t} \rangle$ when a pulse does not exist, and focus on the difference between the peak of the pulse and final steady state of $\bar{Z}$ for the pulses.

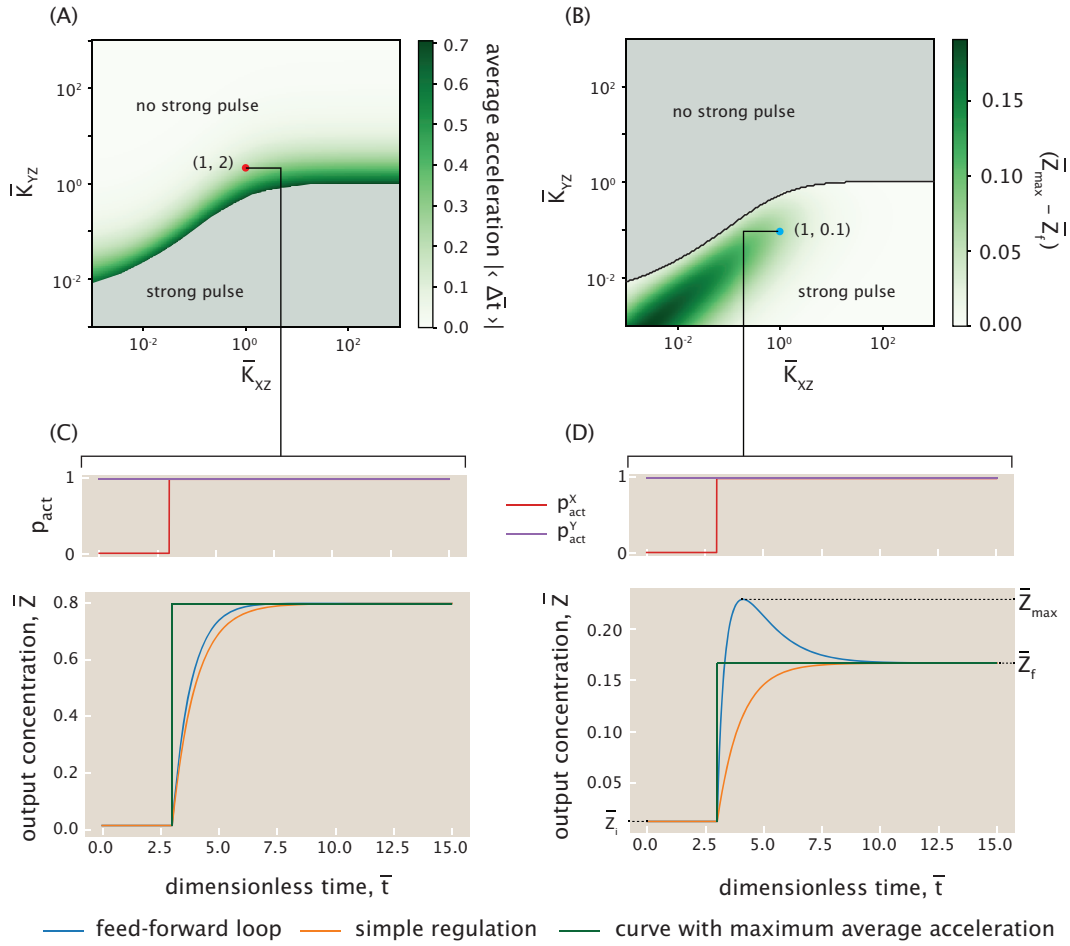


Figure 4.23: Existence of a pulse and acceleration in the incoherent feed-forward loop. Parameters used here are $\bar{r}_{0Y} = \bar{r}_{0Z} = 0$, $\bar{r}_{1Y} = \bar{r}_{1Z} = 2$, and $\omega = 0$. (A) Average acceleration $|\langle \Delta \bar{t} \rangle|$ during ON step across the phase space $(\bar{K}_{YZ}, \bar{K}_{XZ})$, computed only for parameter sets where no strong pulse is observed. For the parameters we chose, the ON step in $p_{\text{act}}^X(c_X)$ coincides with $\bar{Z}(\bar{t})$ having an increasing response. (B) Pulse amplitude $\bar{Z}_{\max} - \bar{Z}_f$ across the same phase space, quantifying the transient overshoot above steady state. (C) Example trajectories corresponding to $(\bar{K}_{XZ}, \bar{K}_{YZ}) = (1, 2)$. No strong pulse is observed, but the feed-forward loop response is accelerated compared to the simple regulation response. The green curve is the trajectory with the largest $\langle \Delta \bar{t} \rangle$ without a pulse. The blue and orange curves are the feed-forward loop and simple regulation trajectories, respectively. (D) Example trajectories corresponding to $(\bar{K}_{XZ}, \bar{K}_{YZ}) = (1, 0.1)$, where a strong pulse is observed.

To further quantify this acceleration, we again compute the average time difference $\langle \Delta \bar{t} \rangle$ between the feed-forward loop and simple regulation trajectories with Eqn. 4.35. Fig. 4.23(A) shows $\langle \Delta \bar{t} \rangle$ across the phase space defined by $(\bar{K}_{XZ}, \bar{K}_{YZ})$, when the output does not present a strong pulse. The definition of “strong” is described in Appendix 4.M. We observe that acceleration is limited to a maximum value of 1. This upper bound arises because the most accelerated trajectory would consist of an instantaneous jump to the final steady state (green curves in Fig. 4.23(C) and (D)), corresponding to $\langle \Delta \bar{t} \rangle = 1$ as shown in Appendix 4.M. An example of a feed-forward trajectory that does not exhibit a strong pulse is shown in Fig. 4.23(C). The region of highest acceleration lies near the boundary separating the strongly pulsed and not strongly pulsed regimes. Interestingly, acceleration is only substantial in a restricted region of parameter space. For example, we see in Fig. 4.23(A) that high values of $\bar{K}_{YZ}$ —corresponding to weak binding of Y —lead to negligible acceleration.

In addition to acceleration, the presence and magnitude of a pulse is another hallmark of the studied network. In Fig. 4.23(B), we map the pulse height, defined as the maximum deviation of $\bar{Z}(t)$ above its steady state value, across the $(\bar{K}_{XZ}, \bar{K}_{YZ})$ space. Pulses are observed only in a portion of this space—specifically, for small enough values of $\bar{K}_{XZ}$, where X binds strongly. The highest pulses occur when both $\bar{K}_{XZ}$ and $\bar{K}_{YZ}$ are small, indicating that strong binding of both regulators enhances the transient overshoot in this case.

4.3.4 Continuous signal

To conclude our discussion of feed-forward loops, we now consider the system’s response to a continuously tuned effector concentration, rather than an abrupt step change. When the timescale of effector concentration variation, denoted $\bar{t}_c$, is much shorter than the system’s relaxation timescale, the dynamics resemble those observed under step function inputs. Conversely, when $\bar{t}_c$ is much longer than the relaxation time, the system remains quasi-stationary when the input is evolving, effectively tracking steady state values of the output $\bar{Z}$ at each time step.

To analyze this quantitatively, we compare $\bar{t}_c$ to the intrinsic relaxation timescales of both the simple regulation case and the coherent feed-forward loop. In coherent feed-forward loops, we have shown that the output response is consistently delayed relative to simple regulation. The precise relaxation timescale of this architecture, however, is not straightforward and depends strongly on the biochemical parameters. As a result, we use the relaxation timescale of the simple regulation system—which

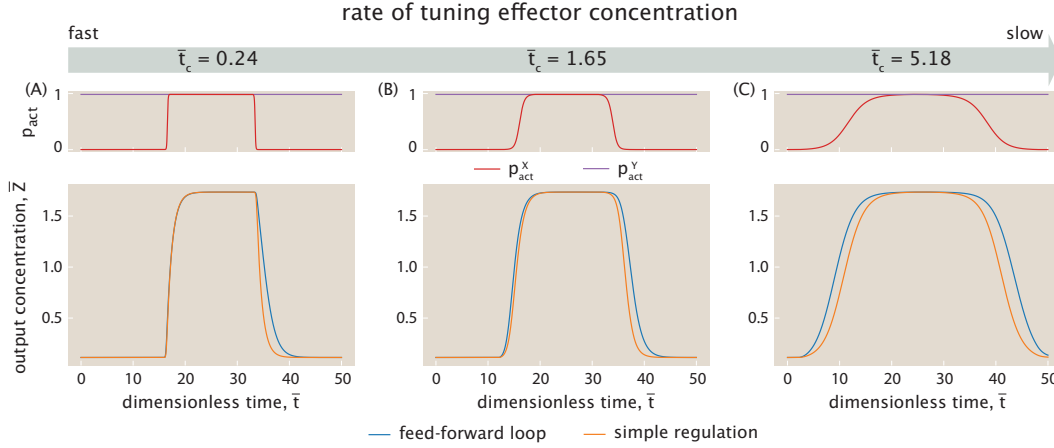


Figure 4.24: Feed-forward loop response to the rate of continuous tuning of effector concentration. From left to right, the rate of tuning effector concentration slows down while every other parameter is held constant. Each ON step is a loglinearly increasing function from $c_X^{\min} = 10^{-4}$ M to $c_X^{\min} = 10^{-7}$ M across some time; an OFF step is the reverse. From left to right, the timescales of effector concentration variation are $\bar{t}_c = 0.24$, $\bar{t}_c = 1.65$, $\bar{t}_c = 5.18$. As defined in the main text, $\bar{t}_c$ is the time it takes for $p_{\text{act}}^X(c_X(\bar{t}))$ to increase from 0.2 to 0.8. $\bar{Y}$ is set to be 1 at all times for simple regulation. Parameters are the XOR gate parameters used in Fig. 4.21.

is 1 in units of $1/\gamma$ —as a reference estimate for the order of magnitude of the feed-forward loop’s relaxation time. We can thus define two limiting regimes: a fast tuning regime where $\bar{t}_c \ll 1$, and a slow tuning regime where $\bar{t}_c \gg 1$.

Before proceeding to the analysis of the delay, we must first clarify the definition of simple regulation in the case of a continuous signal. We adopt the same definition as the step function case, where we fix concentration $\bar{Y} = 1$. Further, in our previous analysis, when $c_X(\bar{t})$ is a step function, the choice of $\bar{Y}$ does not affect the dynamics of relaxation of the simple regulation output. However, when $c_X(\bar{t})$ is a continuous function, the choice of $\bar{Y}$ changes these dynamics slightly. Fortunately, the effect of $\bar{Y}$ is small and does not qualitatively change our observations in the rest of the section, allowing us to continue with the comparison between feed-forward loops and simple regulation (for a more detailed discussion, see Appendix 4.N).

We numerically integrate the dynamical equations under a continuously changing $c_X(\bar{t})$ and illustrate the result in Fig. 4.24, where we analyze the response of a coherent feed-forward loop operating as an XOR gate. In this setting, we numerically define the timescale of effector concentration variation t_c as the time it takes for $p_{\text{act}}^X(c_X)$ to change from 0.2 to 0.8 or vice versa. t_c indicates the time it takes for the switch to be flipped on or off, as regulated by the effector concentration c_X .

When the effector concentration $c_X(\bar{t})$ is tuned rapidly as shown in Fig. 4.24(A), with $\bar{t}_c \approx 0.24$, we observe a large delay in the output $\bar{Z}(\bar{t})$ on the OFF step and almost no delay on the ON step, consistent with the step function dynamics discussed previously. In contrast, when $c_X(\bar{t})$ varies slowly, as depicted in Fig. 4.24(C) with $\bar{t}_c \approx 5.18$, both the feed-forward loop and the simple regulation trajectories become dominated by their respective steady states. They simply track their steady states dictated by $c_X(\bar{t})$. As a consequence, the responses to ON and OFF steps must be symmetric, as there is exactly one steady state corresponding to a specific c_X . Interestingly, the OFF delay is preserved, while for the ON step the feed-forward loop response is accelerated compared to simple regulation. The magnitude of the ON step acceleration is similar to that of the OFF step delay, as required by symmetry. We note that the magnitude of the acceleration/delay depends on the choice of $\bar{Y}$ in simple regulation.

Nevertheless, for all choices of $\bar{Y}$, the feed-forward loop accelerates the ON step and delays the OFF step when the concentration of effector c_X is slowly tuned. Between the two limits, the feed-forward loop response smoothly transitions as the tuning rate of c_X decreases. An example in this regime is shown in Fig. 4.24(B) with $\bar{t}_c \approx 1.65$, where we begin to observe the ON step acceleration, despite its magnitude being smaller than in Fig. 4.24(C). These results demonstrate that the dynamics of feed-forward loops under continuously varying effector concentrations are governed by the timescale of input change: rapid tuning reproduces the asymmetric ON- and OFF-step delays seen with step inputs, whereas slow tuning yields symmetric responses where ON-step acceleration balances OFF-step delay.

4.4 Discussion

The history of modern molecular biology has been a dazzlingly successful exploration of the ways in which genes dictate the function and dynamics of the cells making up organisms of all kinds. One of the greatest success stories of that history is the development of our understanding of how genes are connected together in genetic circuits [70], giving rise to an array of stereotyped regulatory motifs such as switches, oscillators, double negative networks and feed-forward networks, to name but a few examples [68]. We note that despite all of this progress, there remain gaping holes in our knowledge of how most genes are regulated. Even in our best understood organisms such as *E. coli*, we lack any knowledge of how more than 60% of its genes are regulated [71]. As a result, we expect that with the advent of the high-throughput era in biology, ever more genetic circuits like those we discussed

here will be discovered.

In addition to our ignorance of the genetic circuits themselves, our understanding of the proteins that mediate those circuits is also very limited. In particular, we often don't know how effector molecules alter the activity of the transcription factors that control these genes [72]. The key point here is that the action of proteins such as transcription factors is often altered through the binding of effector molecules, which induce allosteric conformational changes that in turn change the state of activity of those transcription factors. For many genes, however, we still remain ignorant of which effector molecules effect those changes. The central thesis of this chapter is that, in fact, most gene circuits have their activity tuned by precisely these kinds of effector molecules and as a result, we need to revisit the theoretical analysis of such circuits to account for the effect of allosteric induction.

In parallel with the impressive progress in molecular biology and the dissection of the rules of regulation, huge progress was made in the study of the behavior of dynamical systems in contexts of all kinds [63], and with special reference to genetic circuits themselves [5]. However, when theorists have used dynamical systems frameworks to explore the behavior of such circuits, they have largely adopted an approach in which those circuits are tuned in abstract terms using model parameters such as degradation rates γ , mRNA production rates r and binding constants K_d . As was so importantly discovered in the 1960s, typically these parameters are in fact “tuned” in the context of living cells through the action of allosteric transitions of transcription factors between active and inactive conformations as a result of the binding of effector molecules [23, 39–45]. The history of dynamical systems in these problems largely leaves the all-important effector molecules out of this story, only considering them implicitly. In this study we have undertaken a systematic analysis of the role of such effectors in governing the function and dynamics of a variety of fundamental genetic regulatory motifs.

The overarching theme of the work described here is that whereas typical dynamical systems approaches to genetic networks feature the number of transcription factors such as $A(t)$ for activator concentrations and $R(t)$ for repressor concentrations, the variable that the cell actually “cares about” is the active number of activators and repressors. There are a variety of well-defined statistical mechanical approaches that allow us to compute this active fraction by multiplying the total number of transcription factors by the function $p_{\text{act}}(c)$ as dictated by the Monod-Wyman-Changeux model, for example, and highlighted in Eqn. 4.4. The power of this approach is that

now the parameters governing properties such as bistability in genetic circuits will be tuned by experimentally and biologically accessible parameters such as the concentrations of effector molecules.

Throughout the chapter, we have shown how the tuning variable of effector concentration makes it possible for the dynamical systems describing genetic circuits to range across their phase portraits. We began with perhaps the simplest of such circuits, the auto-activation motif, and showed how tuning effector concentration narrows the range of possible behaviors relative to those found in an unconstrained dynamical system perspective. We also carefully analyzed the temporal relaxation of these genetic circuits to their terminal steady states. That discussion revealed that for initial conditions that are not “far” from the stable fixed points, the relaxation to steady state is exponential with a time constant dictated by the derivative of the nonlinear protein degradation/production function evaluated at the fixed point. For initial conditions that start near to the unstable fixed point, the dynamics are richer.

With the description of the induced auto-activation circuit in hand, we turned to the mutual repression motif, which is ubiquitous in prokaryotes and eukaryotes alike. Here, again, the capacity to independently tune the effector concentration for each repressor revealed a large flexibility in how cells and synthetic biologists alike can decide to tune the dynamical behavior of this genetic circuit, from dictating its steady state behavior to the dynamics of the repressors as they converge to those steady state values.

Finally, our analysis of feed-forward loops shows that the dynamic behavior typically associated with these motifs in response to input effector signals is more nuanced and parameter-dependent than previously appreciated [47]. For the coherent feed-forward loop, we analytically confirm the presence of delay in output response compared to simple regulation. We show, however, that both the magnitude and the *sign-sensitivity* of these delays depend on system parameters such as dissociation constants, production rates and cooperativity. This rich range of qualitatively distinct behavior remains true even within the different categories of logic gates that can emerge from special combinations of these parameters. Conversely, incoherent feed-forward loops accelerate output responses compared to simple regulation and can generate transient pulses—again only in certain regions of parameter space.

Within this analysis of feed-forward loops, we demonstrate the crucial roles of effector concentration in our models. The leakiness of $p_{\text{act}}(c)$, for example, influences key metrics such as delay time. We also highlight how the sigmoidal shape of

$p_{\text{act}}(c)$ enables a continuous change in effector concentration to be translated to a sharp signal when tuning the probability of transcription factors being active. Overall, while the dynamical behaviors of feed-forward loops can be rich, they are not always guaranteed. This emphasizes how behavior emerges from a delicate interplay of biochemical parameters rather than rigid circuit logic alone, and underscores the need for further experimental and theoretical efforts toward understanding the functions and dynamics of feed-forward loops.

All told, our efforts demonstrate that there is great flexibility inherent in the endogenous signaling modalities adopted by living cells to be contrasted with the way in which model parameters are artificially tuned in many dynamical systems approaches to these same problems. We are excited for experimental efforts to make a substantial push to solve the huge puzzle of the allosterome, opening the door to more realistic analyses of genetic circuits from a dynamical systems perspective.

Appendices

4.A Thermodynamic model equivalence to description with polymerase

In this section, we demonstrate the equivalence of the thermodynamic models used throughout the chapter, in which we do not directly account for the presence of RNA polymerase (RNAP), to those that explicitly incorporate interaction with RNAP. To illustrate, we begin by examining the auto-activation switch described in Section 4.2.1 .

4.A.1 Coarse-graining the auto-activation model

The statistical mechanical model for auto-activation depicted in Fig. 4.2 implicitly accounts for interaction between the activator and polymerase. Fig. 4.25 represents the complete set of thermodynamic states, weights and rates, now explicitly including all of the possible polymerase bound states and denoting the interaction energy between polymerase and activator as ε_{ap} . In light of this, we can write the dynamical equation for the number of activators as

$$\frac{dA}{dt} = -\gamma A + \frac{\frac{P}{K_P} [r_0 + 2r_1 e^{-\beta \varepsilon_{ap}} \frac{p_{\text{act}}(c)A}{K_d} + r_2 e^{-2\beta \varepsilon_{ap}} \omega(\frac{p_{\text{act}}(c)A}{K_d})^2]}{Z}, \quad (4.40)$$

where P is the number of copies of polymerase present and K_P is the corresponding dissociation constant. We define the partition function Z , obtained by summing the

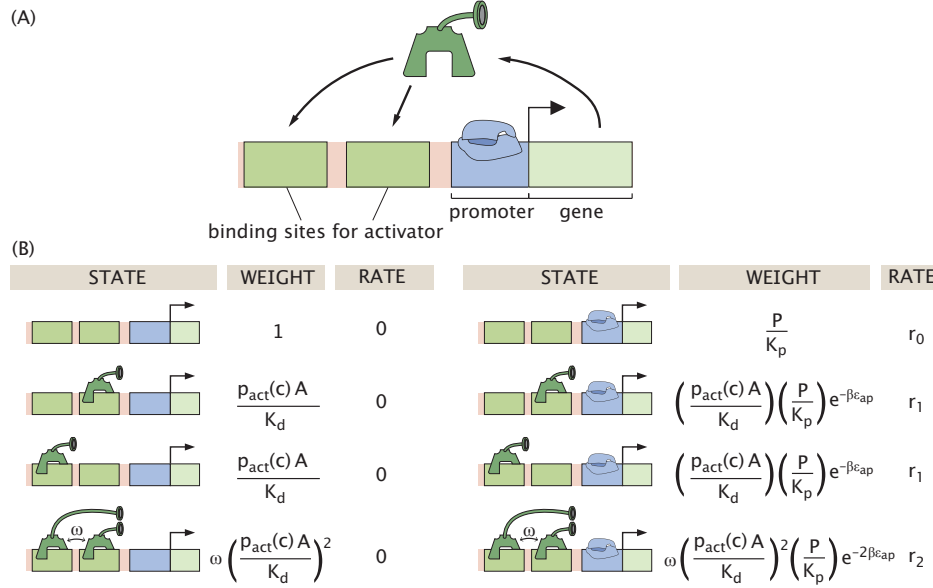


Figure 4.25: The auto-activation regulatory circuit. (A) A schematic of the circuit operation. Polymerase binding at the promoter (blue) transcribes the gene (encoded in the green region), producing a protein that can activate its own expression at a sufficient concentration. In our model, an activator can bind at two possible sites to enhance gene transcription. (B) The thermodynamic states, weights, and rates for the auto-activation motif including polymerase binding explicitly. The parameter ω denotes the binding cooperativity between two activators.

statistical weights of all of the states in Fig. 4.25, as

$$\begin{aligned}
 Z &= \frac{P}{K_p} \left[1 + 2e^{-\beta\epsilon_{ap}} \frac{p_{act}(c)A}{K_d} + e^{-2\beta\epsilon_{ap}} \omega \left(\frac{p_{act}(c)A}{K_d} \right)^2 \right] \\
 &\quad + \left[1 + 2 \frac{p_{act}(c)A}{K_d} + \omega \left(\frac{p_{act}(c)A}{K_d} \right)^2 \right] \\
 &= 1 + \frac{P}{K_p} + 2 \frac{p_{act}(c)A}{K_d} \left(1 + \frac{P}{K_p} e^{-\beta\epsilon_{ap}} \right) \\
 &\quad + \omega \left(\frac{p_{act}(c)A}{K_d} \right)^2 \left(1 + e^{-2\beta\epsilon_{ap}} \frac{P}{K_p} \right) \\
 &= \left(1 + \frac{P}{K_p} \right) \left[1 + 2 \frac{p_{act}(c)A}{K_d} \frac{\left(1 + \frac{P}{K_p} e^{-\beta\epsilon_{ap}} \right)}{1 + \frac{P}{K_p}} \right. \\
 &\quad \left. + \omega \left(\frac{p_{act}(c)A}{K_d} \right)^2 \frac{\left(1 + e^{-2\beta\epsilon_{ap}} \frac{P}{K_p} \right)}{1 + \frac{P}{K_p}} \right] \\
 &\equiv \left(1 + \frac{P}{K_p} \right) Z_0.
 \end{aligned} \tag{4.41}$$

Note that by defining the various “constants” that appear in Eqn. 4.40, we can show that it is equivalent to Eqn. 4.8, in which we omit polymerase as a variable. In particular, we seek effective versions of the parameters K_d , ω , r_0 , r_1 and r_2 that have all the polymerase dependence embedded within them. To re-express Z_0 as a sum of states with implicit dependence on polymerase, we note that if we define

$$K_d^{\text{eff}} = \frac{1 + \frac{P}{K_P}}{1 + \frac{P}{K_P} e^{-\beta \varepsilon_{ap}}} K_d, \quad (4.42)$$

and

$$\omega^{\text{eff}} = \frac{(1 + \frac{P}{K_P})(1 + \frac{P}{K_P} e^{-2\beta \varepsilon_{ap}})}{(1 + \frac{P}{K_P} e^{-\beta \varepsilon_{ap}})^2} \omega, \quad (4.43)$$

the denominator will have the same form as the denominator of Eqn. 4.8. Next, if we redefine the rate parameters as

$$r_0^{\text{eff}} = \frac{\frac{P}{K_P}}{1 + \frac{P}{K_P}} r_0, \quad (4.44)$$

$$r_1^{\text{eff}} = \frac{\frac{P}{K_P} e^{-\beta \varepsilon_{ap}}}{1 + \frac{P}{K_P} e^{-\beta \varepsilon_{ap}}} r_1, \quad (4.45)$$

$$r_2^{\text{eff}} = \frac{\frac{P}{K_P} e^{-2\beta \varepsilon_{ap}}}{1 + \frac{P}{K_P} e^{-2\beta \varepsilon_{ap}}} r_2, \quad (4.46)$$

we recover an equation that is equivalent to Eqn. 4.8. Note that for convenience, in Eqn. 4.8 we have dropped the superscript “eff” to simplify the notation, but the two approaches are formally equivalent.

It is also important to bear in mind that the rate parameters, cooperativity, and dissociation constant used in our reduced representation are effective parameters that implicitly depend on the concentration of polymerase present (P), the strength of polymerase binding to the DNA (K_P), and the strength of interaction between activator and RNAP (ε_{ap}). This leads to a description of the auto-activation switch in which the polymerase serves as a hidden variable, showing that there is a rigorous way to analyze dynamics without explicit treatment of the polymerase.

4.A.2 Coarse-graining the mutual repression model

In the main body of the chapter, we also examine the auto-activation motif, we treated the mutual repression motif without making explicit reference to RNA polymerase. We now consider the full set of states, weights and rates illustrated in Fig. 4.26, in contrast to those shown in Fig. 4.13, where all reference to RNA polymerase is

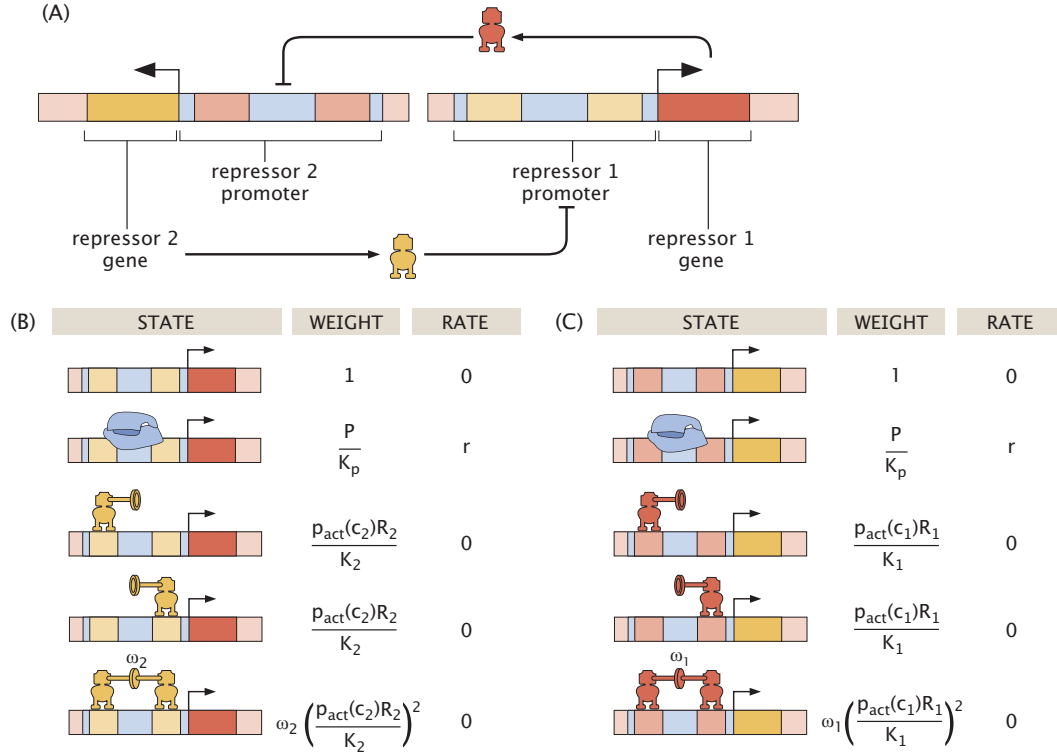


Figure 4.26: The mutual repression regulatory circuit. (A) Schematic of the operation of the circuit. When the gene for repressor 1 is expressed, the resulting protein downregulates the expression of the gene for repressor 2. Repressor 2, in turn, downregulates the expression of the gene for repressor 1. (B) Thermodynamic states, weights, and rates for expression of repressor 1 including the action of the inducer, which tunes the number of active repressors. In our model, a repressor can bind non-exclusively at one of two possible sites within the target promoter region to suppress gene transcription. The parameter ω_2 denotes the cooperative strength between two bound repressors R_2 . (C) Thermodynamic states, weights, and rates for expression of repressor 2 including the action of the inducer, which tunes the number of active repressors. These states and weights are analogous to those shown for repressor 1. However, the dissociation constant of repressor 1 in this case is given by K_1 , and the cooperativity term for the interaction of two repressor 1 molecules bound to the DNA is ω_1 .

absent. We now demonstrate the equivalence of these two descriptions of the mutual repression switch following the strategy used in the context of auto-activation.

Under this expanded thermodynamic model, the governing equations for the dynamics of R_1 and R_2 prior to non-dimensionalization can be read directly from Fig. 4.26,

yielding

$$\frac{dR_1}{dt} = -\gamma_1 R_1 + \frac{r \frac{P}{K_P}}{1 + \frac{P}{K_P} + 2 \frac{p_{\text{act}}(c_2)R_2}{K_2} + \omega_2 \left(\frac{p_{\text{act}}(c_2)R_2}{K_2} \right)^2} \quad (4.47)$$

and

$$\frac{dR_2}{dt} = -\gamma_2 R_2 + \frac{r \frac{P}{K_P}}{1 + \frac{P}{K_P} + 2 \frac{p_{\text{act}}(c_1)R_1}{K_1} + \omega_1 \left(\frac{p_{\text{act}}(c_1)R_1}{K_1} \right)^2}. \quad (4.48)$$

These equations can be algebraically manipulated by factoring out $(1 + P/K_P)$ from the denominator, resulting in the forms

$$\frac{dR_1}{dt} = -\gamma_1 R_1 + \frac{r \frac{\frac{P}{K_P}}{1 + \frac{P}{K_P}}}{1 + 2 \frac{p_{\text{act}}(c_2)R_2}{K_2(1 + \frac{P}{K_P})} + \omega_2 (1 + \frac{P}{K_P}) \left(\frac{p_{\text{act}}(c_2)R_2}{K_2(1 + \frac{P}{K_P})} \right)^2}, \quad (4.49)$$

$$\frac{dR_2}{dt} = -\gamma_2 R_2 + \frac{r \frac{\frac{P}{K_P}}{1 + \frac{P}{K_P}}}{1 + 2 \frac{p_{\text{act}}(c_1)R_1}{K_1(1 + \frac{P}{K_P})} + \omega_1 (1 + \frac{P}{K_P}) \left(\frac{p_{\text{act}}(c_1)R_1}{K_1(1 + \frac{P}{K_P})} \right)^2}. \quad (4.50)$$

This formulation reveals that the original equations given in Eqns. 4.16 and 4.17 can be recovered through a simple transformation of parameters in which we once again define effective parameters. The effective mRNA production rate is given by

$$r^{\text{eff}} = \frac{\frac{P}{K_P}}{1 + \frac{P}{K_P}} r, \quad (4.51)$$

the two K_d s for transcription factor-DNA binding are given by

$$K_1^{\text{eff}} = (1 + \frac{P}{K_P}) K_1 \quad (4.52)$$

and

$$K_2^{\text{eff}} = (1 + \frac{P}{K_P}) K_2 \quad (4.53)$$

and the two cooperativities are written in effective form as

$$\omega_1^{\text{eff}} = (1 + \frac{P}{K_P}) \omega_1 \quad (4.54)$$

and

$$\omega_2^{\text{eff}} = (1 + \frac{P}{K_P}) \omega_2. \quad (4.55)$$

This demonstrates that polymerase binding can be absorbed into effective parameters, yielding a reduced model equivalent to the one presented in the main text, with

renormalized production rate, dissociation constants, and cooperativities. Once again, the polymerase copy number P and binding strength K_P are hidden variables in the context of the bare model, but the results are exact - this is not an approximation valid only in the limit of weak promoters, for example. Note also that as in the case of the auto-activation switch, in the main body of the chapter we do *not* retain the “eff” notation, electing instead to simply use the parameters r , K_1 , K_2 , ω_1 and ω_2 , with the convention that those parameters include the hidden variables associated with polymerase.

4.B Oligomerization and DNA looping in auto-activation

In the main text, transcription factors (TFs) are treated as single binding units that occupy one site at a time, with cooperative interactions between TFs bound at two neighboring sites on the DNA. This description, however, omits two important possibilities: (i) TFs may oligomerize before binding DNA (for example as dimers, like the transcription factor PhoP in *E. coli* [73], or as tetramers, like the transcription factor ComK [74] in *B. subtilis*); and (ii) they may bridge distant sites to form DNA loops [32, 75–77]. Here we consider how these features would change the behavior of an auto-activating transcription factor, with similar analysis possible for mutual repression and other regulatory motifs.

To isolate the effects of oligomerization, the following discussion does not include inducers, though in general they could interact differently with monomers and oligomers. To extend the model, Eqn. 4.8 in Section 4.2.1, we now allow the transcription factor to dimerize, and each protein copy to exist as a free monomer or as part of a dimer. We track the total concentration of monomer subunits as

$$A_{\text{tot}} = A + 2A_2, \quad (4.56)$$

where A_2 denotes dimers. Protein synthesis increases A_{tot} , and assuming rapid dimerization the newly produced monomers are instantly redistributed between monomers and dimers according to equilibrium. We assume that dilution and degradation act at the same effective rate γ on both monomers and dimers, which is reasonable for stable TFs in fast-growing *E. coli* where dilution dominates, as a few candidates of auto-activating feedback loops are present in this organism [78, 79].

Assuming the reaction $2A \leftrightarrow A_2$ is much faster than synthesis or degradation, the dimer concentration is given by

$$A_2 = \frac{A^2}{K_{\text{dim}}}. \quad (4.57)$$

Mass balance then gives

$$A_{\text{tot}} = A + \frac{2A^2}{K_{\text{dim}}}, \quad (4.58)$$

which, solving for A , yields an explicit definition for the free monomer concentration as a function of A_{tot} ,

$$A(A_{\text{tot}}) = \frac{K_{\text{dim}}}{4} \left(\sqrt{1 + \frac{8A_{\text{tot}}}{K_{\text{dim}}}} - 1 \right), \quad (4.59)$$

and, given Eqn. 4.60, the corresponding free dimer concentration as a function of A_{tot} ,

$$A_2(A_{\text{tot}}) = \frac{A(A_{\text{tot}})^2}{K_{\text{dim}}}. \quad (4.60)$$

The transcription factor can bind DNA either as a monomer, with dissociation constant K_d^m , or as a dimer, with dissociation constant K_d^d . We now consider a promoter with two identical binding sites. The binding of two proteins can be cooperative, described by cooperativity parameters ω_{mm} (monomer–monomer), ω_{md} (monomer–dimer), and ω_{dd} (dimer–dimer). When both sites are occupied by dimers, they can also bridge the two sites to form a DNA loop, with a dissociation constant K_d^l describing the dimer bound to DNA in its looped state. Each promoter state is associated with a transcription rate r_0 , r_1 , or r_2 , corresponding to zero, one, or two bound proteins, respectively. Given the statistical weights of all promoter states as presented in Fig. 4.27, the mean transcription rate is then

$$\begin{aligned} F(A, A_2) = & \frac{r_0}{Z} + r_1 \frac{2 \frac{A}{K_d^m} + 2 \frac{A_2}{K_d^d}}{Z} \\ & + r_2 \frac{\omega_{mm} \left(\frac{A}{K_d^m} \right)^2 + 2\omega_{md} \frac{A}{K_d^m} \frac{A_2}{K_d^d} + \omega_{dd} \left(\frac{A_2}{K_d^d} \right)^2}{Z} \\ & + r_2 \frac{\frac{A_2}{K_d^l}}{Z}, \end{aligned} \quad (4.61)$$

with a partition function

$$\begin{aligned} Z = & 1 + 2 \frac{A}{K_d^m} + 2 \frac{A_2}{K_d^d} + \omega_{mm} \left(\frac{A}{K_d^m} \right)^2 + 2\omega_{md} \frac{A}{K_d^m} \frac{A_2}{K_d^d} \\ & + \omega_{dd} \left(\frac{A_2}{K_d^d} \right)^2 + \frac{A_2}{K_d^l}, \end{aligned} \quad (4.62)$$

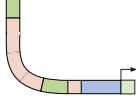
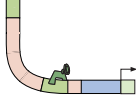
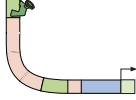
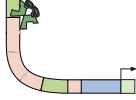
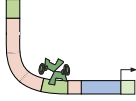
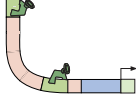
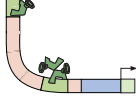
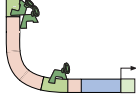
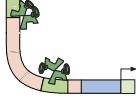
STATE	WEIGHT	RATE
	1	r_0
	$\frac{A}{K_d^m}$	r_1
	$\frac{A}{K_d^m}$	r_1
	$\frac{A_2}{K_d^d}$	r_1
	$\frac{A_2}{K_d^d}$	r_1
	$\omega_{mm} \left(\frac{A}{K_d^m} \right)^2$	r_2
	$\omega_{md} \frac{A}{K_d^m} \frac{A_2}{K_d^d}$	r_2
	$\omega_{md} \frac{A}{K_d^m} \frac{A_2}{K_d^d}$	r_2
	$\omega_{dd} \left(\frac{A_2}{K_d^d} \right)^2$	r_2
	$\frac{A_2}{K_d^l}$	r_2

Figure 4.27: Promoter states for a dimerizing auto-activating transcription factor. Each row shows a possible promoter occupancy state when two binding sites are available. States differ by the number and type of bound transcription factor (monomer or dimer) and by whether the two dimers form a DNA loop. Each state is associated with a statistical weight determined by its binding configuration and a transcription rate (r_0 , r_1 , or r_2) that depends on the number of bound molecules.

and the corresponding dynamics of the system become

$$\frac{dA_{\text{tot}}}{dt} = -\gamma A_{\text{tot}} + F(A(A_{\text{tot}}), A_2(A_{\text{tot}})). \quad (4.63)$$

Eqn. 4.63 shows that once oligomerization is included, the system must be described in terms of A_{tot} rather than the free monomer alone. Importantly, the dynamics become more nonlinear, allowing new behaviors to emerge.

We now consider an alternative setting in which several oligomeric forms coexist in solution, but only one can bind to DNA. As an example, suppose that monomers and dimers are found in solution with equilibrium constant K_{dim} , but only monomers can bind to the gene promoter site. We then have

$$\frac{dA_{\text{tot}}}{dt} = -\gamma A_{\text{tot}} + \frac{r_0 + 2r_1 \frac{A}{K_d^m} + r_2 \omega_{mm} \left(\frac{A}{K_d^m}\right)^2}{1 + 2 \frac{A}{K_d^m} + \omega_{mm} \left(\frac{A}{K_d^m}\right)^2}. \quad (4.64)$$

Substituting $A_{\text{tot}} = A + 2A^2/K_{\text{dim}}$ and using the chain rule, we can ultimately arrive at an ordinary differential equation with additional terms beyond those of the simple monomer case,

$$\begin{aligned} \frac{dA}{dt} = & -\gamma \frac{K_{\text{dim}}A + 2A^2}{K_{\text{dim}} + 4A} \\ & + \frac{K_{\text{dim}}}{K_{\text{dim}} + 4A} \frac{r_0 + 2r_1 \frac{A}{K_d^m} + r_2 \omega_{mm} \left(\frac{A}{K_d^m}\right)^2}{1 + 2 \frac{A}{K_d^m} + \omega_{mm} \left(\frac{A}{K_d^m}\right)^2}. \end{aligned} \quad (4.65)$$

The presence of multiple oligomeric states could therefore alter the number and stability of steady states, although in practice usually only one oligomeric form is dominant.

We next consider looping when only the dimeric form of the transcription factor is present. If a dimer both binds and loops DNA, looping can be seen as adding binding configurations that strengthen effective binding and cooperativity. The production term of a looping dimer A_2 can then be written as

$$\frac{r_0 + r_1 \left(2 + \frac{K_d^d}{K_d^l}\right) p_{\text{act}} \frac{A_2}{K_d^d} + r_2 \omega_{dd} \left(\frac{p_{\text{act}} A_2}{K_d^d}\right)^2}{1 + \left(2 + \frac{K_d^d}{K_d^l}\right) p_{\text{act}} \frac{A_2}{K_d^d} + \omega_{dd} \left(\frac{p_{\text{act}} A_2}{K_d^d}\right)^2}, \quad (4.66)$$

which is algebraically equivalent to the standard model using renormalized parameters

$$\tilde{K}_d^d = \frac{2K_d^d}{2 + \frac{K_d^d}{K_d^l}} \quad (4.67)$$

and

$$\tilde{\omega}_{dd} = \frac{4\omega_{dd}}{(2 + \frac{K_d^d}{K_d^l})^2}. \quad (4.68)$$

Looping therefore does not change the functional form of the model but simply rescales K_d^d and ω_{dd} .

Finally, when dimers form from monomers with equilibrium constant K_{dim} , and there is no looping and all cooperativity terms are equal ($\omega_{mm} = \omega_{md} = \omega_{dd} = \omega$), the production term can be reduced exactly to the monomer-only functional form as

$$f_{mono}(x) = \frac{r_0 + 2r_1x + r_2\omega x^2}{1 + 2x + \omega x^2}, \quad (4.69)$$

where

$$x = \frac{A}{K_d^m} + \frac{A_2}{K_d^d}. \quad (4.70)$$

The dynamical equation can then be written as

$$\frac{dA_{tot}}{dt} = -\gamma A_{tot} + f_{mono}(x(A_{tot})). \quad (4.71)$$

At steady state this means that

$$\gamma(x)x = f_{mono}(x), \quad (4.72)$$

with

$$\gamma(x) = \frac{2K_d^d \left(K_d^d - K_d^m \left(K_{dim} + \sqrt{K_d^d \left(\frac{K_d^d}{(K_d^m)^2} + 4x \right)} \right) \right)}{K_d^d (1 - 2K_{dim}) - K_d^m \sqrt{K_d^d \left(\frac{K_d^d}{(K_d^m)^2} + 4x \right)}}. \quad (4.73)$$

Thus, the production curve retains the same shape as in the monomeric model, but the effective degradation rate becomes concentration-dependent.

The various settings discussed in this appendix demonstrate how incorporating oligomerization alters the equations of the dynamical system. Specifically, accounting for oligomerization introduces additional molecular states, and makes the effective production term a function of the total protein pool rather than only the active monomer. This transforms the dynamics from a simple one-variable system into a more nonlinear one. By contrast, looping does not change the mathematical structure of the model but can be absorbed into effective parameters that strengthen binding and cooperativity. When only a single oligomeric form binds DNA, the production term can still be written in the same functional form as the monomer-only model, but the degradation term becomes effectively concentration-dependent.

4.C Minimal bounds on cooperativity and rates for the existence of bistability in auto-activation

The auto-activation system can exhibit bistability, meaning that it can reach different steady states depending on the initial condition. However, this behavior arises only within a restricted range of parameter values, shown in Fig. 4.11 as the red region. To investigate the conditions for which multiple different steady states are possible, we derive analytic bounds in parameter space. Setting $d\bar{A}/d\bar{t} = 0$ and re-expressing in standard polynomial form, $\bar{A}$ must satisfy

$$\omega p_{\text{act}}^2 \bar{A}^3 + p_{\text{act}}(2 - \omega \bar{r}_2 p_{\text{act}}) \bar{A}^2 + (1 - 2\bar{r}_1 p_{\text{act}}) \bar{A} - \bar{r}_0 = 0. \quad (4.74)$$

If the system exhibits bistability, the corresponding polynomial must have three real roots, as a third-order polynomial cannot have exactly two. Physically, these roots correspond to two stable steady states and one unstable steady state. Further, the presence of only one real root indicates that the system is monostable, as discussed in Appendix 4.D.

To identify conditions for bistability, we search for combinations of ω , $\bar{r}_0$, $\bar{r}_1$, $\bar{r}_2$, and effector concentration c that produce three positive real roots of the polynomial—corresponding to the red region in Fig. 4.11. We may bound this bistable region of parameter space analytically using Descartes' rule of signs, which states that for a single-variable polynomial with real coefficients, the number of positive roots of the polynomial is equal to the number of sign changes between consecutive non-zero coefficients minus an even number. In our case, the polynomial in Eqn. 4.74 must then have either one or three sign changes. Therefore, three sign changes are necessary for the system to allow bistability. Evaluating Eqn. 4.74, we observe that the coefficient of $\bar{A}^3$ is strictly positive and the constant term is strictly negative. Thus, three (consecutive) coefficient sign changes are only possible if the second term of Eqn. 4.74 is negative, and the third term of that same equation is positive. Specifically, the condition on the third term implies that

$$p_{\text{act}}(c) \left(2 - \omega \bar{r}_2 p_{\text{act}}(c) \right) < 0 \implies p_{\text{act}}(c) > \frac{2}{\omega \bar{r}_2}, \quad (4.75)$$

while the condition on the second term leads to

$$1 - 2\bar{r}_1 p_{\text{act}}(c) > 0 \implies p_{\text{act}}(c) < \frac{1}{2\bar{r}_1}. \quad (4.76)$$

Thus, these two conditions can be combined to yield

$$\frac{2}{\omega \bar{r}_2} < p_{\text{act}}(c) < \frac{1}{2\bar{r}_1}. \quad (4.77)$$

4.C.1 Necessary condition for bistability at some effector concentration c

Note that if the above condition were true at all possible effector concentrations c , the system would always be bistable. Rather, we are more specifically interested in the conditions that would allow bistability for at least one value of effector concentration c_0 . In other words, there exists a concentration such that

$$\frac{2}{\omega \bar{r}_2} < p_{\text{act}}(c_0) \quad (4.78)$$

and

$$\frac{1}{2\bar{r}_1} > p_{\text{act}}(c_0). \quad (4.79)$$

If the inequality in Eqn. 4.78 holds true, then we also know that

$$\frac{2}{\omega \bar{r}_2} < p_{\text{act}}(c_0) \leq \max_{c \in [0, \infty]} [p_{\text{act}}(c)]. \quad (4.80)$$

This result itself then directly requires the existence of some effector concentration for which Eqn. 4.78 is true. We can prove this by considering two possible cases. First, if

$$\frac{2}{\omega \bar{r}_2} < \min_{c \in [0, \infty]} [p_{\text{act}}(c)] < \max_{c \in [0, \infty]} [p_{\text{act}}(c)], \quad (4.81)$$

then we know that Eqn. 4.78 holds true for all concentrations $c \geq 0$. Otherwise, if

$$\min_{c \in [0, \infty]} [p_{\text{act}}(c)] < \frac{2}{\omega \bar{r}_2} < \max_{c \in [0, \infty]} [p_{\text{act}}(c)], \quad (4.82)$$

then Eqn. 4.78 is true for all non-negative effector concentrations smaller than a threshold concentration

$$c^* = p_{\text{act}}^{-1}\left(\frac{2}{\omega \bar{r}_2}\right), \quad (4.83)$$

(derived from the inverse of Eqn. 4.78) because p_{act} is a continuous and monotonically decreasing function.

Applying similar logic to the inequality in Eqn. 4.79, we may thus rewrite the necessary conditions for bistability in Eqns. 4.78 and 4.79 as

$$\frac{2}{\omega \bar{r}_2} < \max_{c \in [0, \infty]} (p_{\text{act}}(c)) = \frac{1}{1 + e^{-\beta \varepsilon}}, \quad (4.84)$$

$$\frac{1}{2\bar{r}_1} > \min_{c \in [0, \infty]} (p_{\text{act}}(c)) = \frac{1}{1 + e^{-\beta \varepsilon} \bar{K}_c^2}, \quad (4.85)$$

and

$$\omega \bar{r}_2 > 4\bar{r}_1, \quad (4.86)$$

recalling the saturation (maximum) and leakiness (minimum) of $p_{\text{act}}(c)$ defined in Eqns. 4.6 and 4.7. This fixes the values of saturation and leakiness, which would otherwise be switched if $K_c < 1$. After some algebra, we can re-express Eqns. 4.84 and 4.85 such that the necessary conditions for bistability are given by

$$\frac{\omega \bar{r}_2}{2} > 1 + e^{-\beta \varepsilon}, \quad (4.87)$$

$$1 + e^{-\beta \varepsilon} \bar{K}_c^2 > 2 \bar{r}_1, \quad (4.88)$$

and

$$\omega \bar{r}_2 > 4 \bar{r}_1. \quad (4.89)$$

In the next section, we derive a necessary condition for bistability that depends on the concentration of effector c . For fixed parameter values, this condition defines a bounded range of effector concentrations outside of which the system is guaranteed to be monostable.

4.C.2 Necessary condition for bistability for a fixed concentration of effector

c

We consider the case where the activation probability $p_{\text{act}}(c)$ is a decreasing function of the effector concentration c , as seen in Fig. 4.5. This monotonicity condition, which requires the derivative of the probability function to be negative for all possible c , depends on the parameters of the model, particularly the ratio of dissociation constants $\bar{K}_c$. The derivative of $p_{\text{act}}(c)$ with respect to c is given from Eqn. 4.4 by

$$\frac{dp_{\text{act}}}{dc} = -\frac{2(1 + c/K_A)e^{\beta \varepsilon}(-1 + \bar{K}_c)(1 + c/K_I)}{\left((1 + c/K_A)^2 e^{\beta \varepsilon} + (1 + c/K_I)^2\right)^2}, \quad (4.90)$$

which is negative for all $c > 0$ if and only if $\bar{K}_c > 1$.

Recalling the previously-derived necessary condition for bistability,

$$\frac{2}{\omega \bar{r}_2} < p_{\text{act}}(c) < \frac{1}{2 \bar{r}_1}, \quad (4.91)$$

we now investigate what constraint this condition imposes on the effector concentration c , assuming the parameters of the system are fixed. First, the inequality

$$p_{\text{act}}(c) < \frac{1}{2 \bar{r}_1} \quad (4.92)$$

can be re-expressed equivalently using the explicit expression of $p_{\text{act}}(c)$ in Eqn. 4.4 as

$$g(c) = \left(\frac{c}{K_A}\right)^2 \frac{1}{1 + e^{-\beta \varepsilon}} \left[\frac{1}{2 \bar{r}_1} - \frac{1}{1 + e^{-\beta \varepsilon} \bar{K}_c^2} \right]$$

$$\begin{aligned}
& + 2 \frac{c}{K_A} \frac{\frac{1}{2\bar{r}_1}(1 + e^{-\beta\varepsilon}\bar{K}_c) - 1}{(1 + e^{-\beta\varepsilon})(1 + e^{-\beta\varepsilon}\bar{K}_c^2)} \\
& + \left[\frac{1}{2\bar{r}_1} - \frac{1}{1 + e^{-\beta\varepsilon}} \right] \frac{1}{1 + e^{-\beta\varepsilon}\bar{K}_c^2} > 0.
\end{aligned} \tag{4.93}$$

Similarly, the condition

$$p_{\text{act}}(c) > \frac{2}{\omega\bar{r}_2} \tag{4.94}$$

is equivalent to requiring that

$$\begin{aligned}
h(c) = & \left(\frac{c}{K_A} \right)^2 \frac{1}{1 + e^{-\beta\varepsilon}} \left[\frac{2}{\omega\bar{r}_2} - \frac{1}{1 + e^{-\beta\varepsilon}\bar{K}_c^2} \right] \\
& + 2 \frac{c}{K_A} \frac{\frac{2}{\omega\bar{r}_2}(1 + e^{-\beta\varepsilon}\bar{K}_c) - 1}{(1 + e^{-\beta\varepsilon})(1 + e^{-\beta\varepsilon}\bar{K}_c^2)} \\
& + \left[\frac{2}{\omega\bar{r}_2} - \frac{1}{1 + e^{-\beta\varepsilon}} \right] \frac{1}{1 + e^{-\beta\varepsilon}\bar{K}_c^2} < 0.
\end{aligned} \tag{4.95}$$

We can now apply Descartes' Rule of Signs to the polynomials $g(c)$ and $h(c)$ to determine when the inequalities are satisfied. Since we are working under the assumption that $\bar{K}_c > 1$, this means that,

$$p_{\text{act}}^{\text{max}} = \frac{1}{1 + e^{-\beta\varepsilon}} > \frac{1}{1 + e^{-\beta\varepsilon}\bar{K}_c} > \frac{1}{1 + e^{-\beta\varepsilon}\bar{K}_c^2} = p_{\text{act}}^{\text{min}}. \tag{4.96}$$

For the polynomial $g(c)$, three cases then arise. First, if

$$\frac{1}{2\bar{r}_1} > \frac{1}{1 + e^{-\beta\varepsilon}}, \tag{4.97}$$

then all coefficients of $g(c)$ are positive and $g(c) > 0$ for all $c \geq 0$, so Eqn. 4.92 is always satisfied. Second, if

$$\frac{1}{2\bar{r}_1} > \frac{1}{1 + e^{-\beta\varepsilon}\bar{K}_c^2}, \tag{4.98}$$

then all coefficients are negative, and the condition is never satisfied for any c . Finally, if the intermediate case

$$\frac{1}{1 + e^{-\beta\varepsilon}\bar{K}_c} > \frac{1}{2\bar{r}_1} > \frac{1}{1 + e^{-\beta\varepsilon}\bar{K}_c^2} \tag{4.99}$$

holds, then the coefficient of the term proportional to c^2 in $g(c)$ is positive, while those of the remaining terms proportional to c^1 and c^0 are negative. This results in exactly one sign change, so by Descartes' Rule of Signs, the polynomial $g(c)$

has exactly one positive root. This defines the minimal concentration for bistability, denoted $c_{\text{bistab}}^{\min}(\bar{r}_1)$, and given by

$$c_{\text{bistab}}^{\min}(\bar{r}_1) = K_A \frac{e^{-\beta\varepsilon} \bar{K}_c + 2\bar{r}_1 - 1 + \sqrt{e^{-\beta\varepsilon}(1 + \bar{K}_c)^2(2\bar{r}_1 - 1)}}{e^{-\beta\varepsilon} \bar{K}_c^2 - 2\bar{r}_1 + 1}. \quad (4.100)$$

Under these conditions, the inequality $g(c) > 0$ holds for all $c > c_{\text{bistab}}^{\min}(\bar{r}_1)$.

We now turn to the polynomial $h(c)$. If

$$\frac{2}{\omega\bar{r}_2} > \frac{1}{1 + e^{-\beta\varepsilon}}, \quad (4.101)$$

then all coefficients are positive and the polynomial is strictly positive for all c , meaning that the condition in Eqn. 4.94 is never satisfied. Conversely, if

$$\frac{2}{\omega\bar{r}_2} < \frac{1}{1 + e^{-\beta\varepsilon} \bar{K}_c^2}, \quad (4.102)$$

then all coefficients are negative and the condition is always satisfied. Lastly, in the intermediate case

$$\frac{1}{1 + e^{-\beta\varepsilon} \bar{K}_c} > \frac{2}{\omega\bar{r}_2} > \frac{1}{1 + e^{-\beta\varepsilon} \bar{K}_c^2}, \quad (4.103)$$

Descartes' Rule of Signs again implies exactly one positive root of $h(c)$, corresponding to the upper bound of the bistable region. This upper concentration threshold is denoted $c_{\text{bistab}}^{\max}(\omega\bar{r}_2)$ and given by

$$c_{\text{bistab}}^{\max}(\omega\bar{r}_2) = K_A \frac{e^{-\beta\varepsilon} \bar{K}_c + \frac{\omega\bar{r}_2}{2} - 1 + \sqrt{e^{-\beta\varepsilon}(1 + \bar{K}_c)^2 \left(\frac{\omega\bar{r}_2}{2} - 1 \right)}}{e^{-\beta\varepsilon} \bar{K}_c^2 - \frac{\omega\bar{r}_2}{2} + 1}. \quad (4.104)$$

Under these conditions, the inequality $h(c) < 0$ holds for all $c < c_{\text{bistab}}^{\max}(\omega\bar{r}_2)$.

Summing up the case-by-case analysis, we derive an effector concentration-dependent necessary condition for bistability. The full set of conditions allowing for bistability in different ranges of effector concentrations is given by

$$\left\{ \begin{array}{ll} c_{\text{bistab}}^{\max} > c > c_{\text{bistab}}^{\min} & \text{if } \frac{1}{1 + e^{-\beta\varepsilon} \bar{K}_c^2} < \frac{2}{\omega\bar{r}_2} < \frac{1}{2\bar{r}_1} < \frac{1}{1 + e^{-\beta\varepsilon}}, \\ c_{\text{bistab}}^{\max} > c & \text{if } \frac{1}{1 + e^{-\beta\varepsilon} \bar{K}_c^2} < \frac{2}{\omega\bar{r}_2} < \frac{1}{1 + e^{-\beta\varepsilon}} < \frac{1}{2\bar{r}_1}, \\ c > c_{\text{bistab}}^{\min} & \text{if } \frac{2}{\omega\bar{r}_2} < \frac{1}{1 + e^{-\beta\varepsilon} \bar{K}_c^2} < \frac{1}{2\bar{r}_1} < \frac{1}{1 + e^{-\beta\varepsilon}}, \\ c \geq 0 & \text{if } \frac{2}{\omega\bar{r}_2} < \frac{1}{1 + e^{-\beta\varepsilon} \bar{K}_c^2} < \frac{1}{1 + e^{-\beta\varepsilon}} < \frac{1}{2\bar{r}_1}, \\ \text{no bistability} & \text{if } \frac{2}{\omega\bar{r}_2} > \frac{1}{1 + e^{-\beta\varepsilon}} \text{ or } \frac{1}{1 + e^{-\beta\varepsilon} \bar{K}_c^2} > \frac{1}{2\bar{r}_1}. \end{array} \right. \quad (4.105)$$

As noted, the parameter $\bar{r}_0$ does not enter into these Descartes-based bounds and thus does not influence the existence of bistability in this analysis. From these

expressions, we recover the necessary conditions for bistability, stated in the previous section as

$$\frac{2}{\omega \bar{r}_2} < \frac{1}{1 + e^{-\beta \varepsilon}}, \quad (4.106)$$

$$\frac{1}{1 + e^{-\beta \varepsilon} \bar{K}_c^2} < \frac{1}{2 \bar{r}_1}, \quad (4.107)$$

and

$$\frac{2}{\omega r_2} < \frac{1}{2 \bar{r}_1}. \quad (4.108)$$

and re-expressed in Eqns. 4.10 - 4.12.

These conditions can equivalently be rewritten as

$$\omega \bar{r}_2 > \max \left(2(1 + e^{-\beta \varepsilon}), 4 \bar{r}_1 \right) \quad (4.109)$$

and

$$\bar{r}_1 < \min \left(\frac{1 + e^{-\beta \varepsilon} \bar{K}_c^2}{2}, \frac{\omega \bar{r}_2}{4} \right). \quad (4.110)$$

From Eqn. 4.105, we identify necessary conditions under which the system displays bistability for all effector concentrations above a minimal threshold. This corresponds to being in either the third or fourth case of Eqn. 4.105, captured by the inequality

$$\omega \bar{r}_2 > 2(1 + e^{-\beta \varepsilon} \bar{K}_c^2), \quad (4.111)$$

which implies that, for a sufficiently large product $\omega \bar{r}_2$, the system permits bistability across a semi-infinite range of effector concentrations.

To complement the analytical results summarized in Eqn. 4.105, we compare the derived necessary conditions for bistability with numerically computed bistability regions across different parameters. As shown in Figure 4.28, the analytically predicted bounds—represented in orange—are in close agreement with the numerically determined region of bistability—shown in red—near the onset of bistability. For larger values of the cooperativity parameter ω or the activation rate $\bar{r}_2$, as well as for smaller values of the intermediate rate $\bar{r}_1$, the analytical bounds significantly overestimate the true bistable region. This discrepancy arises because the analytical bounds are necessary but not sufficient conditions, and therefore do not capture the full behavior of the system. Nevertheless, these bounds offer a valuable predictor of the minimal and maximal effector concentrations that can support bistability under a given set of parameters.

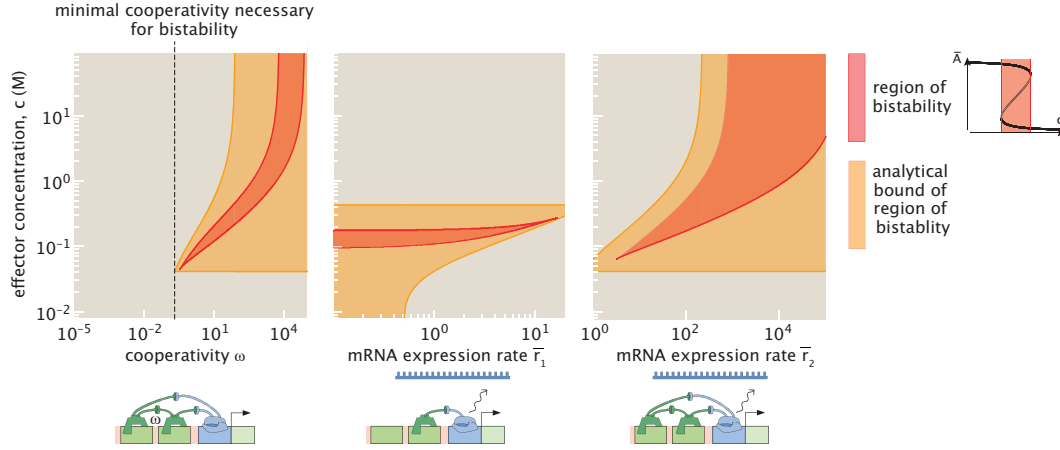


Figure 4.28: Minimal and maximal values of effector concentration between which the system is bistable. Given baseline parameter values $\omega = 7.5$, $\bar{r}_0 = 0.1$, $\bar{r}_1 = 1$, $\bar{r}_2 = 20$, each figure varies a different parameter, keeping all others fixed. For each panel, the shaded region in red is the region of effector concentration for which there is bistability. The shaded region in orange denotes the analytically-bounded region of bistability. The dotted line in the first figure is an analytical lower bound for the minimal cooperativity required for bistability. Note that the analytic approach discussed here, summed up in Eqn. 4.105, invokes a necessary but not sufficient condition for bistability, and thus always encompasses a larger region of parameter space than the system's observed region in red.

4.D Fixed point structure of the auto-activation system as a gradient flow

The auto-activation dynamical system defined in Eqn. 4.9 is a dynamical system that derives from a gradient. Indeed, we can write this equation as

$$\frac{d\bar{A}}{d\bar{t}} = -\frac{dV}{d\bar{A}}, \quad (4.112)$$

with

$$-\frac{dV}{d\bar{A}} = \frac{P(\bar{A})}{1 + 2p_{\text{act}}(c)\bar{A} + \omega(p_{\text{act}}(c)\bar{A})^2}, \quad (4.113)$$

and

$$\begin{aligned} P(\bar{A}) &= r_0 + (r_1 2p_{\text{act}}(c) - 1)\bar{A} \\ &\quad + (r_2 \omega (p_{\text{act}}(c))^2 - 2p_{\text{act}}(c))\bar{A}^2 - \omega (p_{\text{act}}(c))^2 \bar{A}^3 \\ &= \omega p_{\text{act}}^2(c) (\bar{A} - \bar{A}_1)(\bar{A} - \bar{A}_2)(\bar{A} - \bar{A}_3), \end{aligned} \quad (4.114)$$

where $(\bar{A}_1, \bar{A}_2, \bar{A}_3) \in \mathbb{R}$, if there is bistability.

Given Eqn. 4.113, our goal now is to determine the landscape $V(A)$ itself. To that end, we need to integrate Eqn. 4.113. We invoke the strategy of separation of

variables, resulting in

$$-dV = d\bar{A} \cdot \frac{1 + 2p_{\text{act}}(c)\bar{A} + \omega(p_{\text{act}}(c)\bar{A})^2}{\omega p_{\text{act}}^2(c)(\bar{A} - \bar{A}_1)(\bar{A} - \bar{A}_2)(\bar{A} - \bar{A}_3)}. \quad (4.115)$$

To make progress with this integral, we express the right-hand side using partial fraction decomposition. This yields

$$\frac{1 + 2p_{\text{act}}(c)\bar{A} + \omega(p_{\text{act}}(c)\bar{A})^2}{\omega p_{\text{act}}^2(c) \prod_{i=1}^3 (\bar{A} - \bar{A}_i)} = \sum_{i=1}^3 \frac{C_i}{\bar{A} - \bar{A}_i}. \quad (4.116)$$

We find the coefficients C_1 , C_2 , and C_3 by multiplying through by the common denominator and evaluating at $\bar{A} = \bar{A}_i$, for $i \in \{1, 2, 3\}$. The resulting expressions are

$$\begin{cases} C_1 = \frac{\frac{1}{p_{\text{act}}^2(c)\omega} + \frac{2}{p_{\text{act}}(c)\omega}\bar{A}_1 + \bar{A}_1^2}{(\bar{A}_1 - \bar{A}_2)(\bar{A}_1 - \bar{A}_3)}, \\ C_2 = \frac{\frac{1}{p_{\text{act}}^2(c)\omega} + \frac{2}{p_{\text{act}}(c)\omega}\bar{A}_2 + \bar{A}_2^2}{(\bar{A}_2 - \bar{A}_1)(\bar{A}_2 - \bar{A}_3)}, \\ C_3 = \frac{\frac{1}{p_{\text{act}}^2(c)\omega} + \frac{2}{p_{\text{act}}(c)\omega}\bar{A}_3 + \bar{A}_3^2}{(\bar{A}_3 - \bar{A}_1)(\bar{A}_3 - \bar{A}_2)}. \end{cases} \quad (4.117)$$

We can then write the potential function $V(\bar{A})$ as

$$V(\bar{A}) = C_1 \ln |\bar{A} - \bar{A}_1| + C_2 \ln |\bar{A} - \bar{A}_2| + C_3 \ln |\bar{A} - \bar{A}_3|. \quad (4.118)$$

Since the auto-activation system derives from a gradient, we can apply classical results from one-dimensional gradient dynamics: namely, that the number of stable steady states is equal to the number of unstable steady states plus one [63]. Indeed let's assume that

$$\frac{dV(\bar{A})}{d\bar{A}} \Big|_{\bar{A}=\bar{A}^i} = 0 \quad (4.119)$$

at finitely many points $i \in [1, n]$ and

$$\frac{d^2V(\bar{A})}{d\bar{A}^2} \Big|_{\bar{A}=\bar{A}^i} \neq 0 \quad (4.120)$$

at those points (the stable points are not degenerate), with $\bar{A}_1 < \dots < \bar{A}_n$. With two minima in V , the function must then reach a local maximum between the two to transition between these minima. We therefore see that the local minima and maxima of V must alternate. As a final key point, the first and last extrema of V must be minima. If the first extremum of V were a maximum—corresponding to

an unstable steady state—a small perturbation toward smaller $\bar{A}$ would drive the system toward the boundary of the domain, where no minimum of V exists and no steady state is defined. This would render the system ill-posed. A similar reasoning can be applied to understand why the last steady state also has to be a minimum. Intuitively, imagining our dynamical system as a one-dimensional energy landscape, two stable steady state “valleys” must be connected by an unstable steady state “hill.” Therefore, bistability implies that our system has three steady states, two stable and one unstable.

4.E Auto-activation: No bistability at high cooperativity and rate $\bar{r}_2$.

As shown in Fig. 4.11, for sufficiently large values of ω and $\bar{r}_2$, the system does not exhibit bistability for any effector concentration c . In this section, we support this observation using bi-dimensional numerical parameter sweeps and provide analytical arguments explaining its origin.

In Fig. 4.29, we report the maximal cooperativity ω above which the system is monostable for all values of effector concentration. The rate parameters are varied two at a time while keeping the third fixed. For each triplet $(\bar{r}_0, \bar{r}_1, \bar{r}_2)$, we sample all effector concentrations by sweeping over values of p_{act} between leakiness and saturation. We then determine the maximal value of ω for which the system is bistable for at least one value of c . These parameter sweeps reveal a finite—but potentially large—upper bound on cooperativity beyond which bistability is lost. The yellow regions in Fig. 4.29(A–B) indicate that no upper bound was found within our sampled cooperativity range (10^1 to 10^9); this absence does not imply the bound does not exist, but rather reflects the limits of our numerical exploration, which did not extend beyond 10^9 due to sampling choices and diminishing biophysical relevance. However, since a finite bound exists in other parts of parameter space, we hypothesize that such a bound also exists in these regions. In the next section, we confirm this analytically. Interestingly, the appearance of yellow regions in Fig. 4.29(A–B)—where no numerical upper bound on cooperativity is observed—correlates with increasing values of $\bar{r}_2$, consistent with the fact that raising $\bar{r}_2$ initially promotes bistability. While these bounds appear only at very high cooperativities (typically $\omega > 10^2$), and may exceed biologically plausible values, they nonetheless depend on the system’s rate parameters and could be lower in other settings.

We explain this behavior analytically. As discussed in Appendix 4.C.2, bistability can be assessed by examining the number of non-negative roots of the steady-state

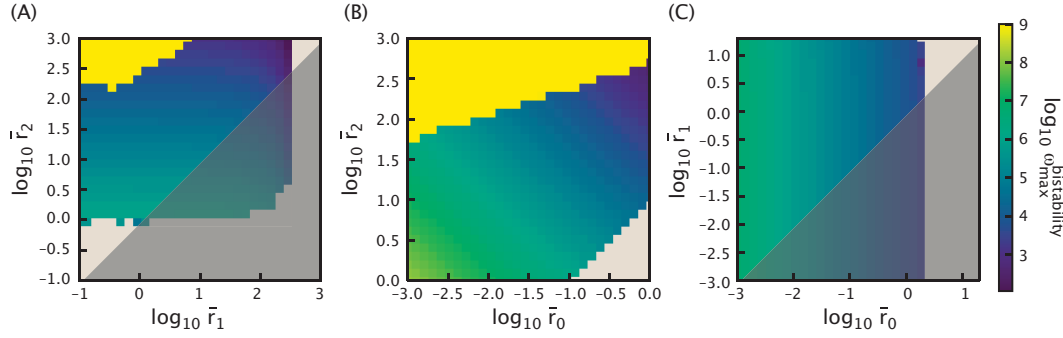


Figure 4.29: Parameter space exploration of the maximal cooperativity $\omega_{\max}^{\text{bistable}}$ above which the system becomes monostable for all effector concentrations. The cooperativity ω is sampled over the interval $\omega \in [10^1, 10^9]$. Effector concentrations are effectively scanned from 0 to ∞ by sweeping p_{act} between its biologically constrained bounds: the leakiness level $p_{\text{act}}^{\min} = \frac{1}{1+e^{-\beta\epsilon\bar{K}_c^2}}$ and the saturation level $p_{\text{act}}^{\max} = \frac{1}{1+e^{-\beta\epsilon}}$, with fixed parameters $\beta\epsilon = 4.5$ and $\bar{K}_c = 2.6 \times 10^2$. Three two-dimensional parameter sweeps are performed. In panel (A), $(\bar{r}_1, \bar{r}_2)$ are varied in $[\bar{r}_0, 10^5] \times [\bar{r}_0, 10^5]$ with $\bar{r}_0 = 0.1$ held constant. In panel (B), $(\bar{r}_0, \bar{r}_2)$ are varied in $[10^{-5}, \bar{r}_1] \times [\bar{r}_1, 10^5]$ with $\bar{r}_1 = 1$ fixed. In panel (C), $(\bar{r}_0, \bar{r}_1)$ are varied in $[10^{-5}, \bar{r}_2] \times [10^{-5}, \bar{r}_2]$ with $\bar{r}_2 = 20$ fixed. Regions shaded in gray correspond to parameter combinations that violate the auto-activation condition $\bar{r}_0 \leq \bar{r}_1 \leq \bar{r}_2$, and for which the system no longer functions as an auto-activating unit. In regions where no maximal cooperativity values for bistability are reported, the system remains monostable across the entire range of cooperativity values sampled.

polynomial Eqn. 4.74. To be bistable, the system has to admit more than one steady state, which corresponds to the polynomial having three real non-negative roots. A necessary condition for this, is that the polynomial has three real roots, regardless of their sign. While this condition does not guarantee bistability—since some of the roots may be negative—it is nonetheless governed directly by the sign of the polynomial's discriminant. For a general cubic polynomial

$$Q(x) = ax^3 + bx^2 + cx + d, \quad (4.121)$$

the discriminant is given by

$$\Delta = b^2c^2 - 4ac^3 - 4b^3d - 27a^2d^2 + 18abcd. \quad (4.122)$$

If $\Delta > 0$, the polynomial has three distinct real roots; if $\Delta < 0$, it has only one real root; and if $\Delta = 0$, it has at least one repeated root.

For the auto-activation system, letting $p_{\text{act}}(c) = p$, the discriminant of the steady-state polynomial can be written as

$$\Delta = p^2 \left[4 + 32p\bar{r}_0 + 16p\bar{r}_1(-1 + p\bar{r}_1) \right] \quad (4.123)$$

$$\begin{aligned}
& + 4\omega(-1 + 2p\bar{r}_1)(1 + 9p\bar{r}_0 + 4p\bar{r}_1(-1 + p\bar{r}_1)) \\
& - 4\omega p(1 + 4p(3\bar{r}_0 + \bar{r}_1(-1 + p\bar{r}_1)))\bar{r}_2 \\
& - 4\omega^3 p^4 \bar{r}_0 \bar{r}_2^3 \\
& + \omega^2 p^2 \left[-27\bar{r}_0^2 + (1 - 2p\bar{r}_1)^2 \bar{r}_2^2 + 6\bar{r}_0 \bar{r}_2 (3 - 6p\bar{r}_1 + 4p\bar{r}_2) \right].
\end{aligned}$$

We now study the asymptotic behavior of this discriminant in the limits of infinite ω , $\bar{r}_2$, and $\bar{r}_0$. We do not consider the limit of infinite $\bar{r}_1$, since, according to the bounds in Eqns. 4.10 - 4.12, a necessary condition for bistability is that $\bar{r}_1$ remains below a threshold set by the other system parameters. Therefore, in this limit, the system is necessarily monostable.

In each of the asymptotic limits, we derive the leading-order term of the discriminant and infer the discriminant diverges negatively. For ω ,

$$\begin{cases} \Delta \underset{\omega \rightarrow \infty}{\sim} -4p^6 \bar{r}_0 \bar{r}_2^3 \omega^3, \\ \lim_{\omega \rightarrow \infty} \Delta = -\infty, \end{cases} \quad (4.124)$$

for $\bar{r}_2$,

$$\begin{cases} \Delta \underset{\bar{r}_2 \rightarrow \infty}{\sim} -4p^6 \bar{r}_0 \bar{r}_2^3 \omega^3, \\ \lim_{\bar{r}_2 \rightarrow \infty} \Delta = -\infty, \end{cases} \quad (4.125)$$

and for $\bar{r}_0$,

$$\begin{cases} \Delta \underset{\bar{r}_0 \rightarrow \infty}{\sim} -27\omega^2 p^4 \bar{r}_0^2, \\ \lim_{\bar{r}_0 \rightarrow \infty} \Delta = -\infty. \end{cases} \quad (4.126)$$

These asymptotic results indicate that bistability becomes impossible in the limit of arbitrarily large ω , $\bar{r}_2$, or $\bar{r}_0$.

Since a negative discriminant implies that the polynomial admits only one real root, the system is necessarily monostable in these asymptotic regimes. This analytically supports the existence of upper bounds on ω and $\bar{r}_2$ observed in Fig. 4.29 and Fig. 4.11(A,D), consistent with the scaling behaviors shown in Eqns. 4.124 and 4.125. Moreover, the symmetric structure of these leading-order terms highlights the seemingly interchangeable roles of ω and $\bar{r}_2$ in promoting bistability. The loss of bistability for large $\bar{r}_0$, as seen in Fig. 4.11(B), is similarly explained by the negative divergence of the discriminant in Eqn. 4.126.

4.F Conditions for activation in auto-activation circuit

We define the range of parameters on which we will focus in the study of auto-activation. In this framework, the production term in Eqn. 4.9, which we will refer to as $y(\bar{A})$, must be monotonically increasing. In other words, we want $dy/d\bar{A} \geq 0$ for all $\bar{A} \geq 0$. To simplify the computation, let $x = p_{\text{act}}(c)\bar{A}$. We then have

$$\frac{dy}{dx} = \frac{1}{p_{\text{act}}(c)} \frac{dy}{d\bar{A}}, \quad (4.127)$$

and the condition thus becomes $dy/dx \geq 0$ for all $x \geq 0$.

Writing down the expression of $y(x)$,

$$y(x) = \frac{\bar{r}_0 + 2\bar{r}_1x + \omega\bar{r}_2x^2}{1 + 2x + \omega x^2}, \quad (4.128)$$

we compute the derivative

$$\frac{dy}{dx} = \frac{2(\bar{r}_1 - \bar{r}_0 + x\omega(\bar{r}_2 - \bar{r}_0) + x^2\omega(\bar{r}_2 - \bar{r}_1))}{(1 + x(2 + x\omega))^2}. \quad (4.129)$$

For this expression to be non-negative for all x , it must be non-negative for $x = 0$ and for $x \rightarrow \infty$. This then requires that $\bar{r}_2 \geq \bar{r}_1$ and $\bar{r}_1 \geq \bar{r}_0$, further implying that $\bar{r}_2 \geq \bar{r}_0$. This is enough to assert that $y(x)$ is an increasing function of x as its derivative is always non-negative for all $x \geq 0$.

4.G Auto-activation: Bistability is possible for noncooperative $\omega = 1$

4.G.1 Definition of the effective Hill coefficient

We first recall how to compute the Hill coefficient of an activating Hill function with constitutive expression, denoted $g(x)$, defined by

$$g(x) = \frac{\bar{r}_0 + \bar{r}_2x^n}{1 + x^n}. \quad (4.130)$$

Its log-derivative is

$$\frac{d \ln g}{d \ln x} = n \cdot \frac{x^n(\bar{r}_2 - \bar{r}_0)}{(1 + x^n)(\bar{r}_0 + \bar{r}_2x^n)}. \quad (4.131)$$

Let us define x^* such that

$$g(x^*) = \frac{\bar{r}_2 + \bar{r}_0}{2}. \quad (4.132)$$

This holds for $x^* = 1$. Evaluating the derivative at this value then gives

$$\left. \frac{d \ln g}{d \ln x} \right|_{x=x^*} = \frac{1}{2}n \cdot \frac{\bar{r}_2 - \bar{r}_0}{\bar{r}_2 + \bar{r}_0}, \quad (4.133)$$

and solving for n , we obtain

$$n = \left. \frac{d \ln g}{d \ln x} \right|_{x=x^*} \cdot 2 \frac{\bar{r}_2 + \bar{r}_0}{\bar{r}_0 - \bar{r}_2}. \quad (4.134)$$

We now define a similar expression for a thermodynamic model $w(x)$ given by

$$w(x) = \frac{\bar{r}_0 + 2\bar{r}_1 p_{\text{act}} x + \omega \bar{r}_2 p_{\text{act}}^2 x^2}{1 + 2p_{\text{act}} x + \omega p_{\text{act}}^2 x^2}. \quad (4.135)$$

We observe that $w(x)$ can be written as $\hat{w}(p_{\text{act}} x)$ with

$$\hat{w}(\hat{x}) = \frac{\bar{r}_0 + 2\bar{r}_1 \hat{x} + \omega \bar{r}_2 \hat{x}^2}{1 + 2h\hat{x} + \omega \hat{x}^2}, \quad (4.136)$$

where $h = 1$ in our case. Since $d \ln w / d \ln x = d \ln \hat{w} / d \ln \hat{x}$ when $h = 1$, the effective Hill coefficient does not depend on p_{act} . The derivative of $\hat{w}$ is

$$\frac{d \ln \hat{w}}{d \ln \hat{x}} = \frac{2(1 + h\hat{x})}{1 + \hat{x}(2h + \omega\hat{x})} - \frac{2(\bar{r}_0 + \bar{r}_1 \hat{x})}{\bar{r}_0 + \hat{x}(2\bar{r}_1 + \omega \bar{r}_2 \hat{x})}. \quad (4.137)$$

Letting $\hat{x}^*$ be defined by $\hat{w}(\hat{x}^*) = \frac{\bar{r}_2 + \bar{r}_0}{2}$ yields

$$\hat{x}^* = \frac{-h\bar{r}_0 + 2\bar{r}_1 - h\bar{r}_2}{(\bar{r}_0 - \bar{r}_2)\omega} + \sqrt{S}, \quad (4.138)$$

where

$$S = \frac{h^2 \bar{r}_0^2 - 4h\bar{r}_0\bar{r}_1 + 4\bar{r}_1^2 + 2h^2 \bar{r}_0\bar{r}_2 - 4h\bar{r}_1\bar{r}_2 + h^2 \bar{r}_2^2 + \bar{r}_0^2 \omega - 2\bar{r}_0\bar{r}_2 \omega + \bar{r}_2^2 \omega}{(\bar{r}_0 - \bar{r}_2)^2 \omega^2}. \quad (4.139)$$

This then gives the derivative at $\hat{x}^*$ as

$$\left. \frac{d \ln \hat{w}}{d \ln \hat{x}} \right|_{\hat{x}=\hat{x}^*} = \frac{\bar{r}_2 - \bar{r}_0}{\bar{r}_2 + \bar{r}_0} \cdot \frac{(h(\bar{r}_0 + \bar{r}_2) - 2\bar{r}_1)u + t}{(2h\bar{r}_2 - 2\bar{r}_1)u + t}, \quad (4.140)$$

with

$$\begin{cases} \alpha = \sqrt{\frac{(-2\bar{r}_1 + h(\bar{r}_0 + \bar{r}_2))^2}{(\bar{r}_0 - \bar{r}_2)^2} + 4\omega}, \\ u = -2\bar{r}_1 + h(\bar{r}_0 + \bar{r}_2) + (-\bar{r}_0 + \bar{r}_2)\alpha, \\ t = 4(\bar{r}_0 - \bar{r}_2)^2 \omega. \end{cases} \quad (4.141)$$

We defined $\hat{w}$ with an extra parameter h to allow a mapping between the thermodynamic model and the Hill function. For the Hill case, we set $h = 0$, $\bar{r}_1 = 0$, and $\omega = 1$. In our thermodynamic model, $h = 1$ and the expression of Eqn. 4.140 becomes

$$\left. \frac{d \ln \hat{w}}{d \ln \hat{x}} \right|_{\hat{x}=\hat{x}^*} = \frac{\bar{r}_2 - \bar{r}_0}{\bar{r}_2 + \bar{r}_0} \cdot \frac{(\bar{r}_0 + \bar{r}_2 - 2\bar{r}_1)u + t}{(2\bar{r}_2 - 2\bar{r}_1)u + t}, \quad (4.142)$$

with

$$\begin{cases} \alpha = \sqrt{\frac{(-2\bar{r}_1 + \bar{r}_0 + \bar{r}_2)^2}{(\bar{r}_0 - \bar{r}_2)^2} + 4\omega}, \\ u = -2\bar{r}_1 + \bar{r}_0 + \bar{r}_2 + (-\bar{r}_0 + \bar{r}_2)\alpha, \\ t = 4(\bar{r}_0 - \bar{r}_2)^2\omega. \end{cases} \quad (4.143)$$

To ensure consistency with the Hill model case, we therefore define the effective Hill coefficient as

$$n_{\text{eff}} = \left. \frac{d \ln w}{d \ln x} \right|_{x=x^*} \cdot 2 \frac{\bar{r}_2 + \bar{r}_0}{\bar{r}_2 - \bar{r}_0} \quad (4.144)$$

for

$$w(x) = \frac{\bar{r}_0 + 2\bar{r}_1 p_{\text{act}} x + \omega \bar{r}_2 p_{\text{act}} x^2}{1 + 2p_{\text{act}} x + \omega p_{\text{act}} x^2} \quad (4.145)$$

and x^* defined such that

$$w(x^*) = \frac{\bar{r}_2 + \bar{r}_0}{2}. \quad (4.146)$$

We note that for the auto activation system, in which $\bar{r}_0 \leq \bar{r}_1 \leq \bar{r}_2$, having an effective Hill coefficient larger than unity ($n_{\text{eff}} > 1$) is equivalent to

$$\omega > \frac{(\bar{r}_1 - \bar{r}_0)(\bar{r}_2 - \bar{r}_1)}{(\bar{r}_2 - \bar{r}_0)^2}. \quad (4.147)$$

Therefore, depending on the parameters of the system, the effective Hill coefficient can be larger or smaller than one, despite the activator having two binding sites.

4.G.2 Numerical sweep for minimal cooperativity above which there is bistability.

To better understand how the parameters of the auto-activation system constrain the emergence of bistability, we explore the minimal cooperativity $\omega_{\text{min}}^{\text{bistable}}$ required to observe bistability across a broad range of rate parameters. Specifically, we perform numerical parameter sweeps over $(\bar{r}_0, \bar{r}_1, \bar{r}_2)$, systematically enforcing the auto-activation condition $\bar{r}_0 \leq \bar{r}_1 \leq \bar{r}_2$. Regions where this condition is violated are shaded in gray. For each valid triplet, we determine the minimal value of ω for which bistability occurs over a finite range of effector concentrations.

Fig. 4.30(A) illustrates the numerical method used to identify this minimum cooperativity. The resulting values are displayed in Fig. 4.30(B), where we observe that the required cooperativity varies significantly across parameter space. Notably, bistability can be achieved even in the case where $\omega \leq 1$, particularly where $\bar{r}_2$ is sufficiently large relative to $\bar{r}_1$ and $\bar{r}_0$. This includes cases where $\omega < 1$, which corresponds to anti-cooperative behavior—i.e., where the binding of the first activator

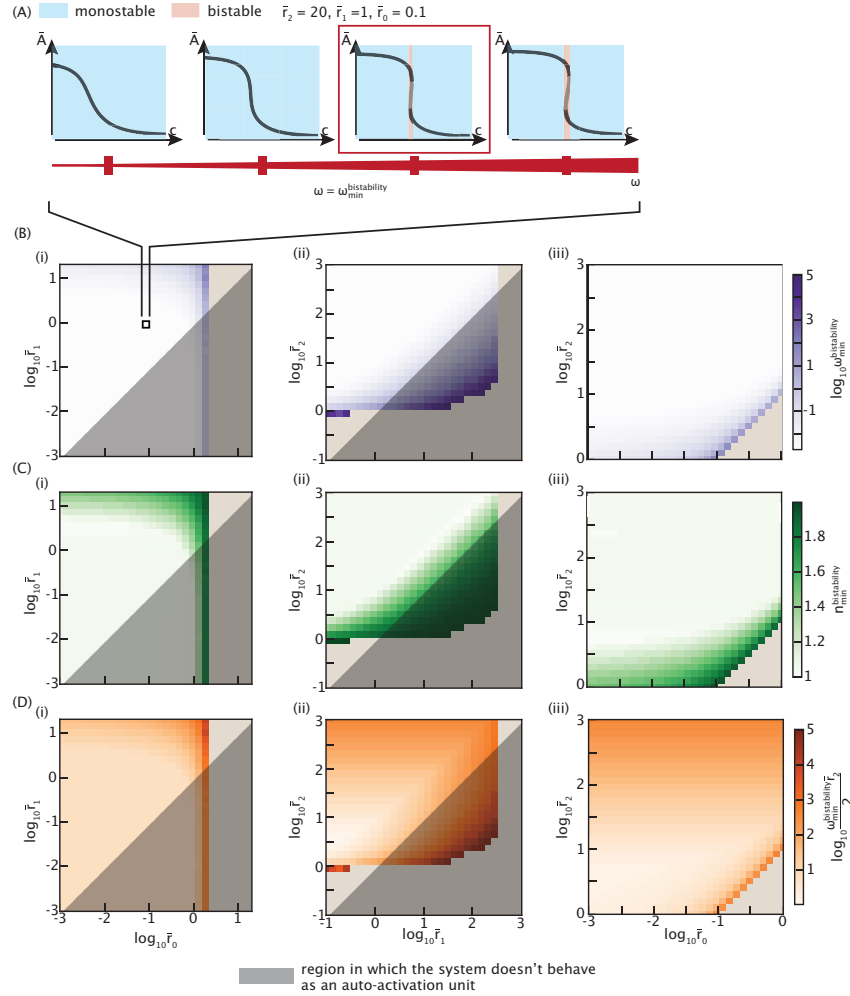


Figure 4.30: Parameter space exploration tracking the minimal cooperativity required for bistability over a range of effector concentrations. The cooperativity ω is sampled over the interval $\omega \in [1, 10^5]$. The condition $\bar{r}_0 \leq \bar{r}_1 \leq \bar{r}_2$ is imposed to ensure auto-activation behavior; regions where this is not satisfied are shaded in gray. (A) Illustration of the method used to determine the minimal cooperativity required for bistability. (B) Minimal cooperativity values identified using the approach in (A). (C) Corresponding effective Hill coefficient. (D) Effective cooperativity estimated from the a necessary condition for bistability $\omega \bar{r}_2 / 2 > 1$. In panels (B–D), subpanels (i)–(iii) show different slices of parameter space: (i) $(\bar{r}_0, \bar{r}_1) \in [10^{-5}, \bar{r}_2]^2$ with $\bar{r}_2 = 20$ fixed; (ii) $(\bar{r}_1, \bar{r}_2) \in [\bar{r}_0, 10^5]^2$ with $\bar{r}_0 = 0.1$ fixed; (iii) $(\bar{r}_0, \bar{r}_2) \in [10^{-5}, \bar{r}_1] \times [\bar{r}_1, 10^5]$ with $\bar{r}_1 = 1$ fixed. In regions where no minimal cooperativity values for bistability are reported, the system remains monostable across the entire range of cooperativity values sampled.

decreases the likelihood of a second one binding—and cases where $\omega = 1$, which corresponds to no cooperativity.

While cooperativity in the strict thermodynamic sense may not be required, the system still exhibits an effective nonlinearity sufficient to support bistability. To assess this, we compute the effective Hill coefficient of the production term, derived with a thermodynamical model, shown in Fig. 4.30(C). When the system is bistable, the effective Hill coefficient always exceeds 1, consistent with theoretical expectations [59]. Furthermore, in Fig. 4.30(D), we evaluate an effective cooperativity based on the inequality $\omega\bar{r}_2/2 > 1$, which serves as a necessary (though not sufficient) condition for bistability. The consistency of this bound with the numerically determined $\omega_{\min}^{\text{bistable}}$ highlights its predictive value.

Together, these analyses reveal that bistability is not strictly dependent on cooperative binding in the classical sense, but rather emerges from the combined effects of system parameters—particularly the balance between production rates. This underscores the importance of kinetic tuning in biological systems and the potential for bistable behavior even in regimes of weak or anti-cooperative interactions.

4.H Relaxation timescale to equilibrium for the auto-activation system

We examine the relaxation timescales to steady state in the auto-activation system as a function of the initial concentration of activator A , denoted $\bar{A}_0$. To define the timescale, we employ two different methods. The first method, referred to as the *threshold approach*, involves measuring the time it takes for the system to evolve from the initial condition to a fixed fraction of its steady state. We track the time-dependent trajectory $\bar{A}(\bar{t})$ and define the relaxation timescale as the time $\bar{t}^*$ such that

$$\frac{\bar{A}(\bar{t}^*) - \bar{A}_i}{\bar{A}_f - \bar{A}_i} = \epsilon, \quad (4.148)$$

where ϵ is the chosen threshold. In practice, since the system is simulated numerically over N discrete time points, the relaxation time is computed as the earliest sampled time $\bar{t}_j$ for which the normalized deviation exceeds ϵ ,

$$\bar{t}^* = \min_{j \in [1, N]} \left\{ \bar{t}_j \left| \frac{\bar{A}(\bar{t}_j) - \bar{A}_i}{\bar{A}_f - \bar{A}_i} > \epsilon \right. \right\}. \quad (4.149)$$

We report the relaxation timescale obtained using various values of ϵ in Fig. 4.31. All resulting curves exhibit similar behavior, indicating that the precise value of the threshold does not significantly affect the overall system dynamics.

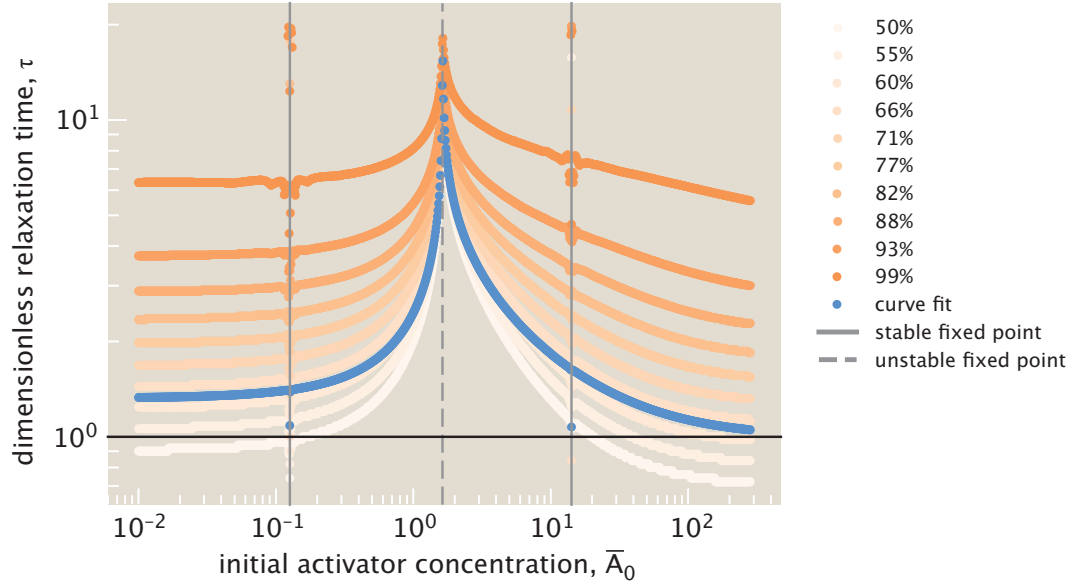


Figure 4.31: Relaxation timescales as a function of the initial concentration of gene A ($\bar{A}_0$). The parameters of the system are fixed at the following values: $\omega = 7.5$, $\bar{r}_0 = 0.1$, $\bar{r}_1 = 1$, $\bar{r}_2 = 20$, and $c = 2 \cdot 10^{-5}\text{M}$. Timescales are computed using two approaches: a threshold-based method (orange curves) and exponential curve fitting (blue curve). Each orange curve corresponds to a different threshold value ϵ indicated as percentages in the legend. The threshold timescale $\bar{t}^*$ is the time required for $\bar{A}(\bar{t})$ to reach a fraction ϵ of the total change from initial condition $\bar{A}_0$ to steady state $\bar{A}_\infty$. The blue curve represents the relaxation time obtained from exponential fits to $\bar{A}(\bar{t})$ trajectories. Vertical lines mark positions of stable (solid lines) and unstable (dashed line) fixed points. The horizontal black line indicates the unity timescale as a reference for comparison.

We compare these threshold-based relaxation timescales with those obtained by fitting an exponential function to the trajectory $\bar{A}(\bar{t})$. If $\bar{A}(\bar{t})$ followed a purely exponential decay—as it does near stable fixed points—then the timescale from the exponential fit would match the threshold-based timescale for $\epsilon \approx 0.63$. As shown in Fig. 4.31, the curve corresponding to the exponential fit closely matches the threshold-based curve with $\epsilon = 0.63$, even when the initial condition is far from the stable fixed points and the system is not strictly exponential, as illustrated in Fig. 4.12(A). Nevertheless, near the stable fixed points, we observe that the threshold method reports a longer timescale. This is because the initial condition is very close to that of the stable fixed point, and the system takes longer to cross the relative threshold. Therefore, we rely on the timescale obtained from the exponential fitting, as shown in Fig. 4.12(B).

4.I Bistability regimes in the mutual repression circuit

4.I.1 A necessary condition for bistability

In the mutual repression system at steady state, we set the time derivatives to zero, leading to the equations

$$\bar{R}_1 = \frac{\bar{r}}{1 + 2p_2\bar{R}_2 + \omega_2(p_2\bar{R}_2)^2} \quad (4.150)$$

and

$$\bar{R}_2 = \frac{\bar{r}}{1 + 2p_1\frac{\bar{R}_1}{\bar{K}} + \omega_1\left(p_1\frac{\bar{R}_1}{\bar{K}}\right)^2}, \quad (4.151)$$

where for convenience we define $p_1 = p_{\text{act}}(c_1)$ and $p_2 = p_{\text{act}}(c_2)$. Substituting the expression for $\bar{R}_1$ of Eqn. 4.150 into Eqn. 4.151 for $\bar{R}_2$, and rewriting in standard polynomial form, $M(\bar{R}_2) = 0$, we obtain

$$\begin{aligned} M(\bar{R}_2) = & p_2^4\omega_2^2\bar{R}_2^5 + p_2^3\omega_2(4 - \bar{r}\omega_2p_2)\bar{R}_2^4 \\ & + 2p_2^2(2 + \omega_2(1 + \bar{r}(\frac{p_1}{\bar{K}} - 2p_2)))\bar{R}_2^3 \\ & + 4p_2(1 + \bar{r}(\frac{p_1}{\bar{K}} - p_2 - \frac{\omega_2p_2}{2}))\bar{R}_2^2 \\ & + (1 + \frac{2p_1\bar{r}}{\bar{K}} + \frac{\omega_1p_1^2\bar{r}^2}{\bar{K}^2} - 4p_2r)\bar{R}_2 - \bar{r}. \end{aligned} \quad (4.152)$$

To assess whether the system is monostable, we examine the number of non-negative roots of the polynomial $M(\bar{R}_2)$. If the second derivative of $M(\bar{R}_2)$ does not change sign, then the polynomial can have at most two real roots. In particular, if the polynomial is convex for all non-negative values of $\bar{R}_2$, i.e., $M''(\bar{R}_2) > 0$, then the system cannot be bistable. The second derivative of $M(\bar{R}_2)$ is given by

$$\begin{aligned} M''(\bar{R}_2) = & 20p_2^4\omega_2^2\bar{R}_2^3 + 12p_2^3\omega_2(4 - \bar{r}\omega_2p_2)\bar{R}_2^2 \\ & + 12p_2^2\left(2 + \omega_2\left(1 + \bar{r}\left(\frac{p_1}{\bar{K}} - 2p_2\right)\right)\right)\bar{R}_2 \\ & + 8p_2\left(1 + \bar{r}\left(\frac{p_1}{\bar{K}} - p_2 - \frac{\omega_2p_2}{2}\right)\right). \end{aligned} \quad (4.153)$$

To guarantee that $M''(\bar{R}_2) > 0$ for all non-negative values of $\bar{R}_2$, we require that all coefficients in the polynomial expression of $M''(\bar{R}_2)$ remain strictly positive. This condition translates into three distinct inequalities. First, the positivity of the quadratic term, requires that

$$4 - \bar{r}\omega_2p_2 > 0. \quad (4.154)$$

Next, positivity of the linear term imposes the constraint

$$2 + \omega_2 \left(1 + \bar{r} \left(\frac{p_1}{\bar{K}} - 2p_2 \right) \right) > 0. \quad (4.155)$$

Finally, the positivity of the constant term yields

$$1 + \bar{r} \left(\frac{p_1}{\bar{K}} - p_2 - \frac{\omega_2 p_2}{2} \right) > 0. \quad (4.156)$$

These conditions can be equivalently rewritten in terms of upper bounds on p_2 and combinations of p_1 and p_2 , yielding

$$p_2 < \frac{4}{\bar{r}\omega_2}, \quad (4.157)$$

$$2p_2 - \frac{p_1}{\bar{K}} < \frac{1}{\bar{r}} \left(\frac{2}{\omega_2} + 1 \right), \quad (4.158)$$

$$\left(1 + \frac{\omega_2}{2} \right) p_2 - \frac{p_1}{\bar{K}} < \frac{1}{\bar{r}}. \quad (4.159)$$

To ensure that the system remains monostable for all values of effector concentrations c_1 and c_2 , we require that these inequalities hold for the maximum possible values for the different functions of c_1 and c_2 . Thus, we obtain the sufficient conditions

$$p_{\max} < \frac{4}{\bar{r}\omega_2}, \quad (4.160)$$

$$2p_{\max} - \frac{p_{\min}}{\bar{K}} < \frac{1}{\bar{r}} \left(\frac{2}{\omega_2} + 1 \right), \quad (4.161)$$

$$\left(1 + \frac{\omega_2}{2} \right) p_{\max} - \frac{p_{\min}}{\bar{K}} < \frac{1}{\bar{r}}, \quad (4.162)$$

Finally, in the special case where $\bar{K} = 1$, the sufficient conditions under which the system remains monostable for all effector concentrations simplify, using Mathematica, to

$$\bar{r} < \frac{1}{p_{\max} - p_{\min} + \omega_2 p_{\max}/2}. \quad (4.163)$$

Taking the contrapositive, we obtain a necessary condition for the system to exhibit bistability at some value of the effector concentration

$$\bar{r} > \frac{1}{p_{\max} - p_{\min} + \omega_2 p_{\max}/2} \quad (4.164)$$

The bound stated in Eqn. 4.164 depends on both ω_2 and $\bar{r}$, again, similarly to the auto-activation system, acting together to determine whether bistability can be accessed or not.

For $\bar{K} = 1$ the two cooperativities play a symmetric role. Therefore, necessary conditions for bistability are

$$\bar{r} > \frac{1}{p_{\max} - p_{\min} + \omega_2 p_{\max}/2} \quad (4.165)$$

and

$$\bar{r} > \frac{1}{p_{\max} - p_{\min} + \omega_1 p_{\max}/2}. \quad (4.166)$$

From Eqns. 4.166 and 4.165 a necessary condition for bistability for $K = 1$ is that

$$\bar{r} > \max\left(\frac{1}{p_{\max} - p_{\min} + \omega_2 p_{\max}/2}, \frac{1}{p_{\max} - p_{\min} + \omega_1 p_{\max}/2}\right). \quad (4.167)$$

which simplifies to

$$\bar{r} > \frac{1}{p_{\max} - p_{\min} + \min(\omega_2, \omega_1) p_{\max}/2}. \quad (4.168)$$

4.1.2 Effective Hill coefficient of the production terms

In the case of auto-activation, analyzing the effective Hill coefficient provided insight into how bistability can arise even in non-cooperative systems— $\omega > 1$ is not a necessary condition for bistability, but from our numerical sweeps displayed in Fig. 4.30, $n_{\text{eff}} > 1$ is. Motivated by this, we now examine whether a similar criterion might help explain the restriction of bistability to specific zones of parameter space in mutual repression circuits.

We defined (Eqn. 4.144, Eqn. 4.146) and derived an analytical formula (Eqn. 4.143, Eqn. 4.142) for the effective Hill coefficient for a general production term

$$w(x) = \frac{\bar{r}_0 + 2\bar{r}_1 p_{\text{act}} x + \omega \bar{r}_2 p_{\text{act}} x^2}{1 + 2p_{\text{act}} x + \omega p_{\text{act}} x^2}, \quad (4.169)$$

in Appendix 4.G.1. In the mutual repression system, the production terms of interest are the production of R_1 driven by promoter 1 and regulated by R_2

$$f_1(\bar{R}_2) = \frac{\bar{r}}{1 + 2(p_{\text{act}}(c_2)\bar{R}_2) + \omega_2(p_{\text{act}}(c_2)\bar{R}_2)^2} \quad (4.170)$$

and the production of R_2 driven by promoter 2 and regulated by R_1

$$f_2(\bar{R}_1) = \frac{\bar{r}}{1 + 2(p_{\text{act}}(c_1)\frac{\bar{R}_1}{K}) + \omega_1(p_{\text{act}}(c_1)\frac{\bar{R}_1}{K})^2}. \quad (4.171)$$

With $\bar{r}_0 = \bar{r}$, $\bar{r}_1 = 0$, and $\bar{r}_2 = 0$, $p_{\text{act}} \equiv p_{\text{act}}(c_2)$ for $f_1(\bar{R}_2)$ and $p_{\text{act}} \equiv p_{\text{act}}(c_1)/K$ for $f_2(\bar{R}_1)$; we see that those two production terms fall in to the more general form

$w(x)$ of Eqn. 4.169. We can therefore apply the reasoning and algebra derived in Appendix 4.G.1. The corresponding effective Hill coefficients are given by

$$n_1 = 2 - \frac{\sqrt{1 + 4\omega_2} - 1}{2\omega_2}, \quad (4.172)$$

for the production term $f_1(\bar{R}_2)$ and

$$n_2 = 2 - \frac{\sqrt{1 + 4\omega_1} - 1}{2\omega_1}, \quad (4.173)$$

for the production term $f_2(\bar{R}_1)$. We see that the two expressions of the effective Hill coefficients have the same functional form. Therefore showing that $n_1 > 1$ for all $\omega_2 > 0$, implies that $n_2 > 1$ for all $\omega_1 > 0$. We therefore establish that $n_1 > 1$ for all $\omega_2 > 0$, and $n_2 > 1$ for all $\omega_1 > 0$ then follows. This amounts to showing

$$2\omega_2 > \sqrt{1 + 4\omega_2} - 1. \quad (4.174)$$

Adding 1 to each side and squaring both sides gives

$$(2\omega_2 + 1)^2 > 1 + 4\omega_2. \quad (4.175)$$

Expanding the square and canceling identical terms leaves

$$4\omega_2^2 > 0, \quad (4.176)$$

which is true for every $\omega_2 > 0$.

We therefore showed that the effective Hill coefficients of the production terms are always greater than one in this system. As a result, while this observation is consistent with conventional expectations [59], it provides little discriminatory power for identifying regions of bistability, since the condition is satisfied across parameter space.

4.I.3 Effect of cooperativity on the bistability region

Fig. 4.32(A)(i) shows how the geometry of the bistable region evolves as the cooperativity of repressor R_2 (ω_2) is varied, while $\omega_1 = 7.5$, $\bar{K} = 1$, and $\bar{r} = 2$ are held fixed. This corresponds to a symmetric case where both repressors bind their respective promoters with equal affinity. At low ω_2 , the system is monostable for all inducer concentrations, which is consistent with the known requirement for a minimal degree of nonlinearity to enable bistability. As ω_2 increases beyond a threshold, bistability emerges, but initially in a constrained region where the tunability is mostly limited by c_1 . When ω_2 lies approximately between 2 and 5, a

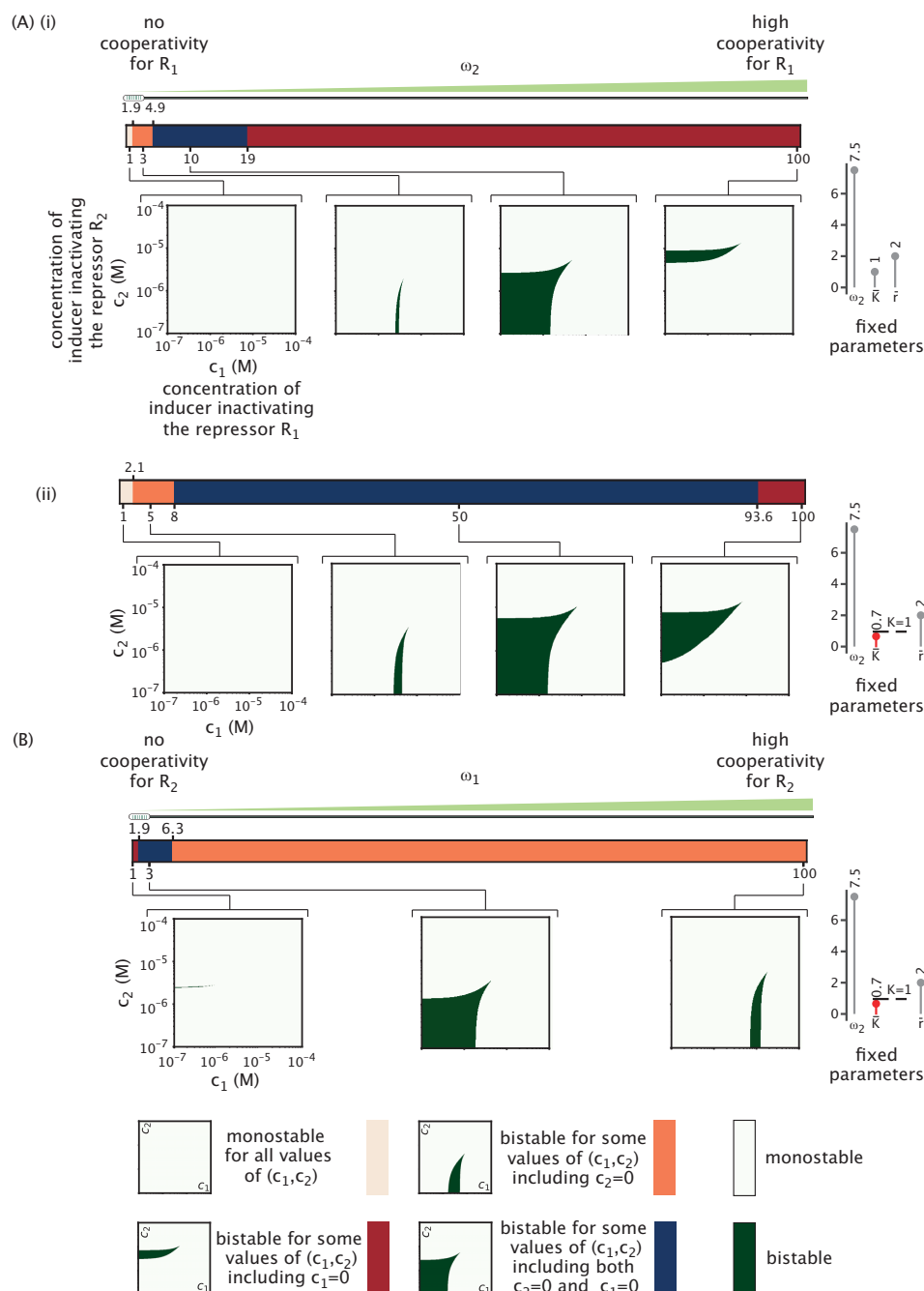


Figure 4.32: Bistability regimes in mutual repression as a function of cooperativity. Colored regions denote distinct bistable phase space geometries, defined by whether bistability occurs at very small c_1 ($c_1 = 10^{-7}$ M), very small c_2 ($c_2 = 10^{-7}$ M), both, or neither. (A) Evolution of the geometry of the bistability phase space, sweeping on inducer concentrations (c_1, c_2) , for fixed $\omega_1 = 7.5$ and $\bar{r} = 2$ and respectively $\bar{K} = 1$ and $\bar{K} = 0.7$ for (i) and (ii), when the parameter ω_2 is varied. (B) Evolution of the geometry of the bistability phase space, sweeping on inducer concentrations (c_1, c_2) , for fixed $\omega_2 = 7.5$, $\bar{r} = 2$ and $\bar{K} = 0.7$, when the parameter ω_1 is varied.

nonzero concentration of c_1 is required to inactivate a portion of the repressors R_1 , thereby reducing their ability to bind DNA efficiently. In this regime, we are still in a setting where $\omega_1 > \omega_2$, meaning that R_1 binds more strongly to the DNA than R_2 —as they have equal binding constants, the difference in binding arises solely from the cooperativity parameters. To support two distinct expression states—one with high R_1 and low R_2 , and another with the reverse—the binding strength of R_1 must be reduced. This enables a more balanced competition between the two repressors, making it possible for both stable states to coexist. When ω_2 becomes large (e.g., $\omega_2 \gtrsim 20$), the situation reverses: the bistable region in the (c_1, c_2) phase space becomes constrained along the c_2 axis, as higher concentrations of inducer are required to counteract the strong DNA binding of R_2 .

For intermediate values of cooperativity, approximately between 5 and 20, the (c_1, c_2) phase space is less constrained. In this regime, the concentrations of c_1 and c_2 need to be small enough to maintain repression by R_1 and R_2 . If either inducer concentration becomes too high, the system is not repressed anymore and only has a unique steady state with high concentrations of both repressors.

While increasing ω_2 initially expands the bistable region and enhances its robustness, we find that beyond a certain threshold, further increases in cooperativity begin to shrink the bistable domain. This reflects a general principle also observed for the parameter $\bar{K}$: pushing the system too far in one direction strongly constrains bistability. In the case of ω_2 , overly strong cooperativity amplifies the binding of R_2 and therefore the repression of R_1 , so the system commits to one state, thereby reducing the range of inducer concentrations for which multiple steady states coexist.

Fig. 4.32(A)(ii) explores the impact of tuning ω_2 in an asymmetric setting, where $\bar{K} = 0.7$, $\omega_1 = 7.5$, and $r = 2$ are fixed. In this case, repressor R_1 binds more tightly to its promoter than R_2 does, breaking the symmetry observed in Fig. 4.32(A)(i). At low values of ω_2 , the system is monostable, consistent with insufficient nonlinearity to support multiple steady states. As ω_1 increases, bistability appears, but the geometry of the bistable region is notably skewed. Compared to Fig. 4.32(A)(i), it is interesting to note that the bistability region at higher cooperativity is larger than in the symmetric case for the range of inducer concentrations considered. Finally in Fig. 4.32(B), we tune the cooperativity ω_1 instead of ω_2 as was done in the previous panel. Therefore the roles of c_1 and c_2 are mirrored.

4.J Separatrix for mutual repression

We recall the differential equations governing the mutual repression system,

$$\begin{aligned}\frac{d\bar{R}_1}{d\bar{t}} &= -\bar{R}_1 + \bar{r} \frac{1}{1 + 2p_{\text{act}}(c_2)\bar{R}_2 + \omega_2 [p_{\text{act}}(c_2)\bar{R}_2]^2} \\ &= F(\bar{R}_1, \bar{R}_2),\end{aligned}\quad (4.177)$$

$$\begin{aligned}\frac{d\bar{R}_2}{d\bar{t}} &= -\bar{R}_2 + \bar{r} \frac{1}{1 + 2p_{\text{act}}(c_1)\frac{\bar{R}_1}{\bar{K}} + \omega_1 \left[p_{\text{act}}(c_1)\frac{\bar{R}_1}{\bar{K}} \right]^2} \\ &= G(\bar{R}_1, \bar{R}_2).\end{aligned}\quad (4.178)$$

The separatrix is defined as the curve $\bar{R}_2(\bar{R}_1)$ that satisfies the differential equation

$$\frac{d\bar{R}_2}{d\bar{R}_1} = \frac{G(\bar{R}_1, \bar{R}_2)}{F(\bar{R}_1, \bar{R}_2)}, \quad (4.179)$$

which tracks the trajectory along which the system transitions between the basins of attraction of the two stable steady states.

4.K Coherent feed-forward loop response to a step function signal

4.K.1 Analytical solution for the output $\bar{Z}(\bar{t})$

We now rewrite the dynamical equations of the coherent feed-forward loop. In particular, we introduce a simplifying notation for the activation terms for gene products Y and Z . The reason such a definition is useful is that these terms are independent of Y and Z themselves and depend only upon X itself and the concentration of inducer. To that end, we write the dynamical equations for Y and Z as

$$\frac{d\bar{Y}}{d\bar{t}} = -\bar{Y} + f_Y(\bar{t}) \quad (4.180)$$

$$\frac{d\bar{Z}}{d\bar{t}} = -\bar{Z} + f_Z(\bar{t}), \quad (4.181)$$

with the simplifying notation

$$f_Y(\bar{t}) = \frac{\bar{r}_{0Y} + \bar{r}_{1Y} p_{\text{act}}^X(c_X(\bar{t}))\bar{X}}{1 + p_{\text{act}}^X(c_X(\bar{t}))\bar{X}}, \quad (4.182)$$

and

$$f_Z(\bar{t}) = \frac{\bar{r}_{0Z} + \bar{r}_{1Z}(\bar{X}(\bar{t}) + \bar{Y}(\bar{t})) + \omega \bar{r}_{2Z} \bar{X}(\bar{t}) \bar{Y}(\bar{t})}{1 + \bar{X}(\bar{t}) + \bar{Y}(\bar{t}) + \omega \bar{X}(\bar{t}) \bar{Y}(\bar{t})}. \quad (4.183)$$

We recall that the bar indicates quantities where time is measured in units of $1/\gamma$, and where concentration and dissociation constants are measured in units of K_{XY} .

The rates are then in units of γK_{XY} . The notations $\mathcal{X}$ and $\mathcal{Y}$ and are defined as $\mathcal{X}(\bar{t}) = p_{\text{act}}^X(c_X(\bar{t}))\bar{X}/\bar{K}_{XZ}$ and $\mathcal{Y}(\bar{t}) = p_{\text{act}}^Y(c_Y)\bar{Y}(\bar{t})/\bar{K}_{YZ}$. We study the response of the coherent feed-forward loop to a step function in effector concentration acting on X , namely,

$$c_X(\bar{t}) = \begin{cases} c_X^i & \text{if } \bar{t} \leq 0, \\ c_X^f & \text{if } \bar{t} > 0. \end{cases} \quad (4.184)$$

The step in the active concentration of X and the rescaled concentration $\mathcal{X}$ are themselves subject to a step and can be written as

$$\mathcal{X}(\bar{t}) = \begin{cases} \mathcal{X}_i = \frac{p_{\text{act}}^X(c_X^i)\bar{X}}{\bar{K}_{XZ}} & \text{if } \bar{t} \leq 0, \\ \mathcal{X}_f = \frac{p_{\text{act}}^X(c_X^f)\bar{X}}{\bar{K}_{XZ}} & \text{if } \bar{t} > 0. \end{cases} \quad (4.185)$$

The concentration of effector acting on Y , $c_Y(\bar{t})$ is taken to be constant $c_Y(\bar{t}) = c_Y^0$. Our goal here is to solve for the feed-forward dynamics analytically and obtain insights into the system on the basis of such a solution. Such a solution is possible because Eqns. 4.23 and 4.24 have a simple form, the time derivative of a variable equals the negative of itself plus a function of time,

$$\frac{dG(t)}{dt} = -G(t) + f(t). \quad (4.186)$$

Such equations can be solved in their most general form as

$$G(t) = e^{-t} \left(G(0) + \int_0^t e^{t'} f(t') dt' \right). \quad (4.187)$$

In the cases of interest here, $G(t)$ is either $\bar{Y}(\bar{t})$ or $\bar{Z}(\bar{t})$, and $f(t)$ correspondingly is either $f_Y(\bar{t})$ or $f_Z(\bar{t})$, the activation terms for Y and Z , respectively, in Eqns. 4.181 and 4.180.

We first solve Eqn. 4.180, because its dynamics are not coupled to $\bar{Z}(\bar{t})$. Notice that for $\bar{t} > 0$, $f_Y(\bar{t})$ is constant. Referring to Eqn. 4.187, we see that $\bar{Y}$ evolves from initial to final state purely exponentially according to the time evolution

$$\bar{Y}(\bar{t}) = -\Delta\bar{Y}e^{-\bar{t}} + \bar{Y}_f. \quad (4.188)$$

Here $\Delta\bar{Y} = \bar{Y}_f - \bar{Y}_i$ is the difference between the final and initial concentration of $\bar{Y}$. We write the explicit expression of those initial and final steady states hereafter,

$$\bar{Y}_i = \frac{\bar{r}_{0Y} + \bar{r}_{1Y} p_{\text{act}}^X(c_X^i)\bar{X}}{1 + p_{\text{act}}^X(c_X^i)\bar{X}} \quad (4.189)$$

$$\bar{Y}_f = \frac{\bar{r}_{0Y} + \bar{r}_{1Y} p_{\text{act}}^X(c_X^f) \bar{X}}{1 + p_{\text{act}}^X(c_X^f) \bar{X}}. \quad (4.190)$$

As $p_{\text{act}}^Y(c_Y(\bar{t})) = p_{\text{act}}^Y(c_Y^0)$ is constant, $\bar{Y} \rightarrow \mathcal{Y}$ is a proportional mapping. Therefore, like $\bar{Y}(t)$, $\mathcal{Y}(\bar{t})$ also evolves exponentially in time by the similar form

$$\mathcal{Y}(\bar{t}) = -\Delta \mathcal{Y} e^{-\bar{t}} + \mathcal{Y}_f, \quad (4.191)$$

with $\mathcal{Y}_{f/i} = p_{\text{act}}^Y(c_Y^0) \bar{Y}_{f/i} / \bar{K}_{YZ}$ and $\Delta \mathcal{Y} = \mathcal{Y}_f - \mathcal{Y}_i$. Given this expression for $\mathcal{Y}(\bar{t})$, we now know the full time dependence of $f_Z(\bar{t})$ in Eqn. 4.181. Next, we can solve for the dynamics of $\bar{Z}$ by substituting $f_Z(\bar{t})$ into the general solution Eqn. 4.187. By evaluating the integral, we find that

$$\begin{aligned} \bar{Z}(\bar{t}) &= \bar{Z}_i e^{-\bar{t}} + \left(e^{-\bar{t}} \int_0^{\bar{t}} e^{t'} f_Z(t') dt' \right) \\ &= \bar{Z}_i e^{-\bar{t}} + \left(\bar{Z}_f (1 - e^{-\bar{t}}) + \Theta(\bar{t}) \right) \\ &= \bar{Z}_{\text{simple}}(\bar{t}) + \Theta(\bar{t}), \end{aligned} \quad (4.192)$$

with

$$\begin{aligned} \Theta(\bar{t}) &= -\frac{\Phi \Delta \mathcal{Y}}{S^2} e^{-\bar{t}} \log \left(\frac{S e^{\bar{t}} - \Delta \mathcal{Y} (1 + \omega \mathcal{X}_f)}{S - \Delta \mathcal{Y} (1 + \omega \mathcal{X}_f)} \right), \\ \Phi &= \omega \mathcal{X}_f^2 (\bar{r}_{2Z} - \bar{r}_{1Z}) + \omega \mathcal{X}_f (\bar{r}_{2Z} - \bar{r}_{0Z}) \\ &\quad + (\bar{r}_{1Z} - \bar{r}_{0Z}), \end{aligned} \quad (4.193)$$

$$S = 1 + \mathcal{X}_f + \mathcal{Y}_f + \omega \mathcal{X}_f \mathcal{Y}_f,$$

as shown in the main text. The solution of $\bar{Z}(\bar{t})$ cleanly splits into two parts. The first two terms describe the exponential behavior one expects from simple regulation

$$\bar{Z}_{\text{simple}}(\bar{t}) = \bar{Z}_i e^{-\bar{t}} + \bar{Z}_f (1 - e^{-\bar{t}}) \quad (4.194)$$

with $\bar{Z}_i$ and $\bar{Z}_f$, respectively the initial and final steady state concentration of the output $\bar{Z}$. Their explicit expression is given by

$$\bar{Z}_i = \frac{\bar{r}_{0Z} + \bar{r}_{1Z} (\mathcal{X}_i + \mathcal{Y}_i) + \omega \bar{r}_{2Z} \mathcal{X}_i \mathcal{Y}_i}{1 + \mathcal{X}_i + \mathcal{Y}_i + \omega \mathcal{X}_i \mathcal{Y}_i} \quad (4.195)$$

$$\bar{Z}_f = \frac{\bar{r}_{0Z} + \bar{r}_{1Z} (\mathcal{X}_f + \mathcal{Y}_f) + \omega \bar{r}_{2Z} \mathcal{X}_f \mathcal{Y}_f}{1 + \mathcal{X}_f + \mathcal{Y}_f + \omega \mathcal{X}_f \mathcal{Y}_f}. \quad (4.196)$$

We see that $\Theta(\bar{t})$ accounts for the difference between the feed-forward trajectory $\bar{Z}$ and the simple regulation trajectory $\bar{Z}_{\text{simple}}$. As a sanity check, we see $\Theta(\bar{t}) = 0$ when $\bar{t} = 0$ and $\bar{t} \rightarrow \infty$, confirming that the feed-forward loop and the simple regulation trajectory have the same initial and final state, as expected.

4.K.2 Derivation and sign of the average delay $\langle \Delta \bar{t} \rangle$

From the analytical expression of $\bar{Z}(\bar{t})$, we can then also derive the average time delay from the offset $\Theta(\bar{t})$

$$\langle \Delta \bar{t} \rangle = \frac{1}{\bar{Z}_f - \bar{Z}_i} \int_0^\infty \Theta(\bar{t}) d\bar{t}. \quad (4.197)$$

With Eqn. 4.193, we can analytically evaluate the integral and obtain the following expression for the average delay

$$\langle \Delta \bar{t} \rangle = \frac{\Phi(\bar{Z}_f - \bar{Z}_i)^{-1}}{S(1 + \omega X_f)} \log \left(\frac{1 + X_f + Y_i + \omega X_f Y_i}{S} \right). \quad (4.198)$$

As a reminder, $\langle \Delta \bar{t} \rangle$ signifies the average time difference between the feed-forward loop response and the simple regulation response. The sign of $\langle \Delta \bar{t} \rangle$ indicates whether the feed-forward loop delays ($\langle \Delta \bar{t} \rangle < 0$) or accelerates ($\langle \Delta \bar{t} \rangle > 0$). From Eqn. 4.198, we can analytically determine whether the feed-forward loop delays or accelerates by treating the contribution from each component. To begin with, we have $S > 0$ and $1 + \omega X_f > 0$ as concentrations are strictly non-negative. For the coherent feed-forward loop, we have $\Phi \geq 0$ as $\bar{r}_{2Z} \geq \bar{r}_{1Z} \geq \bar{r}_{0Z}$ since both X and Y activate Z . The term $(\bar{Z}_f - \bar{Z}_i)$ depends on the direction of the step. For an ON step, $(\bar{Z}_f - \bar{Z}_i) > 0$ and for an OFF step, $(\bar{Z}_f - \bar{Z}_i) < 0$. Finally, the logarithm also depends on the direction of the step. For an ON step, we have

$$1 + X_f + Y_i + \omega X_f Y_i \leq 1 + X_f + Y_f + \omega X_f Y_f = S \quad (4.199)$$

$$\implies \log \left(\frac{1 + X_f + Y_i + \omega X_f Y_i}{S} \right) \leq 0, \quad (4.200)$$

since $X_f \geq X_i$ and $Y_f \geq Y_i$. Combined with the effect of other terms, we find $\langle \Delta \bar{t} \rangle \leq 0$ for an ON step. Similarly, for the OFF step, we have

$$1 + X_f + Y_i + \omega X_f Y_i \geq 1 + X_f + Y_f + \omega X_f Y_f = S \quad (4.201)$$

$$\implies \log \left(\frac{1 + X_f + Y_i + \omega X_f Y_i}{S} \right) \geq 0, \quad (4.202)$$

and we thus find that $\langle \Delta \bar{t} \rangle \leq 0$ for the OFF step as well.

We have shown that the average time difference $\langle \Delta \bar{t} \rangle$ is negative for both the ON and OFF steps. To complete the analysis, we will further demonstrate that the time difference $\Delta \bar{t}(\bar{Z})$, defined in Section 4.3.1, has the same sign for any concentration $\bar{Z}$. This amounts to saying that the feed-forward trajectory and the simple regulation trajectory never cross each other. We can show this by proving that $\Theta(\bar{t})$ has the

same sign for any $\bar{t} > 0$. In Eqn. 4.193, we observe that the time dependence in $\Theta(\bar{t})$ appears in $e^{-\bar{t}}$ and in $Se^{\bar{t}}$ inside the logarithm. $e^{-\bar{t}} > 0$ for any $\bar{t}$, thus only the logarithm term might change its sign as time evolves. However, we observe that

$$\begin{aligned} S - \Delta\mathcal{Y}(1 + \omega\mathcal{X}) &= 1 + \mathcal{X}_f + \mathcal{Y}_i \\ &+ \omega\mathcal{X}_f\mathcal{Y}_i > 0. \end{aligned} \quad (4.203)$$

In other words, $S > \Delta\mathcal{Y}(1 + \omega\mathcal{X})$. Since $e^{\bar{t}} > 1$ for any $\bar{t} > 0$, it is always true that $Se^{\bar{t}} > \Delta\mathcal{Y}(1 + \omega\mathcal{X})$. Therefore, the sign of the logarithm does not change with time. Thus, we have proven that $\Delta\bar{t}(\bar{Z})$ has the same sign as $\langle\Delta\bar{t}\rangle$ for any concentration $\bar{Z}$.

4.K.3 Logic gate analysis in coherent feed-forward loop

In this appendix section, we will prove that for the XOR gate in the coherent feed-forward loop, the OFF step delay is always greater than the ON step delay. In the XOR limit, $\omega = 0$. Eqn. 4.36 simplifies to

$$\langle\Delta\bar{t}\rangle = \frac{1}{(\bar{Z}_f - \bar{Z}_i)} \frac{\bar{r}_{1Z} - \bar{r}_{0Z}}{1 + \mathcal{X}_f + \mathcal{Y}_f} \log\left(\frac{1 + \mathcal{X}_f + \mathcal{Y}_i}{1 + \mathcal{X}_f + \mathcal{Y}_f}\right). \quad (4.204)$$

The time delay $\langle\Delta\bar{t}\rangle$ is different for ON and OFF steps because $\mathcal{X}$ and $\mathcal{Y}$ values at $\bar{t} = 0$ and $\bar{t} = \infty$ are different for ON and OFF steps. Specifically, for ON step, $\mathcal{X}_f = \mathcal{X}_{\max}$ and $\mathcal{Y}_f = \mathcal{Y}_{\max}$; while for OFF step $\mathcal{X}_f = \mathcal{X}_{\min}$ and $\mathcal{Y}_f = \mathcal{Y}_{\min}$. Let's now compare the time delay $\langle\Delta\bar{t}\rangle$ for ON and OFF steps by taking their ratio

$$\begin{aligned} \left| \frac{\langle\Delta\bar{t}\rangle_{\text{OFF}}}{\langle\Delta\bar{t}\rangle_{\text{ON}}} \right| &= \left| \frac{1 + \mathcal{X}_{\max} + \mathcal{Y}_{\max}}{1 + \mathcal{X}_{\min} + \mathcal{Y}_{\min}} \frac{\log\left(\frac{1 + \mathcal{X}_{\min} + \mathcal{Y}_{\max}}{1 + \mathcal{X}_{\min} + \mathcal{Y}_{\min}}\right)}{\log\left(\frac{1 + \mathcal{X}_{\max} + \mathcal{Y}_{\min}}{1 + \mathcal{X}_{\max} + \mathcal{Y}_{\max}}\right)} \right| \\ &= \frac{1 + \mathcal{X}_{\max} + \mathcal{Y}_{\max}}{1 + \mathcal{X}_{\min} + \mathcal{Y}_{\min}} \frac{\log\left(\frac{1 + \mathcal{X}_{\min} + \mathcal{Y}_{\max}}{1 + \mathcal{X}_{\min} + \mathcal{Y}_{\min}}\right)}{\log\left(\frac{1 + \mathcal{X}_{\max} + \mathcal{Y}_{\max}}{1 + \mathcal{X}_{\max} + \mathcal{Y}_{\min}}\right)}. \end{aligned} \quad (4.205)$$

Note that $1 + \mathcal{X}_{\max} + \mathcal{Y}_{\max} \geq 1 + \mathcal{X}_{\min} + \mathcal{Y}_{\min}$, and that

$$\frac{1 + \mathcal{X}_{\min} + \mathcal{Y}_{\max}}{1 + \mathcal{X}_{\min} + \mathcal{Y}_{\min}} \geq \frac{1 + \mathcal{X}_{\max} + \mathcal{Y}_{\max}}{1 + \mathcal{X}_{\max} + \mathcal{Y}_{\min}}, \quad (4.206)$$

because for any fraction a/b where $a \geq b > 0$, $a/b \geq (a + c)/(b + c)$ for any $c \geq 0$. Here, $a = 1 + \mathcal{X}_{\min} + \mathcal{Y}_{\max}$, $b = 1 + \mathcal{X}_{\min} + \mathcal{Y}_{\min}$, and $c = \mathcal{X}_{\max} - \mathcal{X}_{\min}$. As logarithm is an increasing function, this means

$$\log \frac{1 + \mathcal{X}_{\min} + \mathcal{Y}_{\max}}{1 + \mathcal{X}_{\min} + \mathcal{Y}_{\min}} \geq \log \frac{1 + \mathcal{X}_{\max} + \mathcal{Y}_{\max}}{1 + \mathcal{X}_{\max} + \mathcal{Y}_{\min}}. \quad (4.207)$$

Therefore, the ratio $|\langle \Delta \bar{t} \rangle_{\text{OFF}} / \langle \Delta \bar{t} \rangle_{\text{ON}}| \geq 1$. We have demonstrated that for the XOR gate, the OFF step delay is larger than the ON step delay. We cannot say much analytically about the magnitude of the ratio of delay. As we see in Fig. 4.21(A), this ratio can range anywhere from close to 1 to a large number.

From here, we might be tempted to argue that for the AND gate, the ratio always satisfies $|\langle \Delta \bar{t} \rangle_{\text{OFF}} / \langle \Delta \bar{t} \rangle_{\text{ON}}| \leq 1$. However, Fig. 4.21(B)(iii) provides a counter example. The statement for AND gate is thus false.

4.K.4 Analytic solutions of other feed-forward loop networks.

In any feed-forward loop, X regulates Y and Z , and Y regulates Z . As there are three regulatory interactions, there exist $2^3 = 8$ different regulatory logics. For the dynamical equation of $\bar{Y}$, if X activates Y , then

$$\frac{d\bar{Y}}{d\bar{t}} = -\bar{Y} + \frac{\bar{r}_{0Y} + \bar{r}_{1Y} p_{\text{act}}^X(c_X) \bar{X}}{1 + p_{\text{act}}^X(c_X) \bar{X}}. \quad (4.208)$$

Otherwise, if X represses Y , then

$$\frac{d\bar{Y}}{d\bar{t}} = -\bar{Y} + \frac{\bar{r}_{0Y}}{1 + p_{\text{act}}^X(c_X) \bar{X}}. \quad (4.209)$$

There exist 4 different possibilities for how X and Y regulate Z . In Section 4.3, we showed the case where X and Y both activate Z , and the case where X activates but Y represses Z . If X represses and Y activates Z , we have

$$\frac{d\bar{Z}}{d\bar{t}} = -\bar{Z} + \frac{\bar{r}_{0Z} + \bar{r}_{1Z} \mathcal{Y}}{1 + \mathcal{X} + \mathcal{Y} + \omega \mathcal{X} \mathcal{Y}}. \quad (4.210)$$

If both X and Y repress Z , then

$$\frac{d\bar{Z}}{d\bar{t}} = -\bar{Z} + \frac{\bar{r}_{0Z}}{1 + \mathcal{X} + \mathcal{Y} + \omega \mathcal{X} \mathcal{Y}}. \quad (4.211)$$

Using the procedures described in the main text, we can similarly find analytic solutions describing all the other regulatory logics of the feed-forward architectures. Note that for a step function signal in c_X and c_Y ,

$$\mathcal{Y}(\bar{t}) = -\Delta \mathcal{Y} e^{-\bar{t}} + \mathcal{Y}_f. \quad (4.212)$$

Thus, we can compute the analytic expression of $\bar{Z}$ without worrying about how Y is regulated. We find that the solutions all have the same form, except with a different Φ for each architecture of logic. Specifically, we have

$$\Phi_{XY} = \omega \mathcal{X}^2 (\bar{r}_{2Z} - \bar{r}_{1Z}) + \omega \mathcal{X} (\bar{r}_{2Z} - \bar{r}_{0Z}) \quad (4.213)$$

$$+ (\bar{r}_{1Z} - \bar{r}_{0Z})$$

$$\Phi_X = -(\bar{r}_{1Z}\mathcal{X} + \bar{r}_{0Z})(1 + \omega\mathcal{X}) \quad (4.214)$$

$$\Phi_Y = \bar{r}_{1Z}(1 + \mathcal{X}) - \bar{r}_{0Z}(1 + \omega\mathcal{X}) \quad (4.215)$$

$$\Phi_0 = -\bar{r}_{0Z}(1 + \omega\mathcal{X}), \quad (4.216)$$

where the subscript on Φ indicates which TF activates Z . $\mathcal{X} = \mathcal{X}_f$ is the value of $\mathcal{X}$ after the step function jump. Interestingly, we can write these expressions as

$$\Phi_{XY} = \omega\mathcal{X}(1 + \mathcal{X})\bar{r}_{2Z} + \Phi_X + \Phi_Y - \Phi_0 \quad (4.217)$$

$$\Phi_X = -\bar{r}_{1Z}\mathcal{X}(1 + \omega\mathcal{X}) + \Phi_0 \quad (4.218)$$

$$\Phi_Y = \bar{r}_{1Z}(1 + \mathcal{X}) + \Phi_0 \quad (4.219)$$

$$\Phi_0 = -\bar{r}_{0Z}(1 + \omega\mathcal{X}). \quad (4.220)$$

A way to interpret this is that Φ gets a new term associated with a weight of a state when that state changes from no expression to expression.

4.L Functionality condition comparison

In Section 4.3.2, we mentioned that besides the time delay, another helpful criterion for a functional feed-forward loop is the existence of a large difference between the maximum and minimum steady state values of Z . Let's define $\Delta\bar{Z} = |\bar{Z}_f - \bar{Z}_i|$. We want this to be big so that the dynamical change between low and high concentrations is meaningful. The steady state concentrations $\bar{Z}_f$ and $\bar{Z}_i$ have expressions given by

$$\bar{Z}_i = \frac{\bar{r}_{0Z} + \bar{r}_{1Z}(\mathcal{X}_i + \mathcal{Y}_i) + \omega\bar{r}_{2Z}\mathcal{X}_i\mathcal{Y}_i}{1 + \mathcal{X}_i + \mathcal{Y}_i + \omega\mathcal{X}_i\mathcal{Y}_i} \quad (4.221)$$

$$\bar{Z}_f = \frac{\bar{r}_{0Z} + \bar{r}_{1Z}(\mathcal{X}_f + \mathcal{Y}_f) + \omega\bar{r}_{2Z}\mathcal{X}_f\mathcal{Y}_f}{1 + \mathcal{X}_f + \mathcal{Y}_f + \omega\mathcal{X}_f\mathcal{Y}_f}. \quad (4.222)$$

We observe that both $\bar{Z}_i$ and $\bar{Z}_f$ scale linearly with the rate parameters $\bar{r}_{iZ}$ for $i = 0, 1, 2$. For this reason, in the theoretical setting, a large $\Delta\bar{Z}$ can always be obtained by tuning the rate parameters high. Therefore, we did not include the discussion of this criterion in the main text. Nevertheless, it might be worthwhile to demonstrate the dependence of $\Delta\bar{Z}$ on the dissociation constants $\bar{K}_{XZ}$ and $\bar{K}_{YZ}$. We perform similar sweeps as Fig. 4.21, except we plot $\Delta\bar{Z}$ for each choice of $(\bar{K}_{XZ}, \bar{K}_{YZ})$. The result is shown in Fig. 4.33. We employ the same logic gate parameters as in Fig. 4.21. We observe that the region of large relative $\Delta\bar{Z}$ tends to have an L-shape. The XOR and AND gate have regions of large $\Delta\bar{Z}$ that extend in

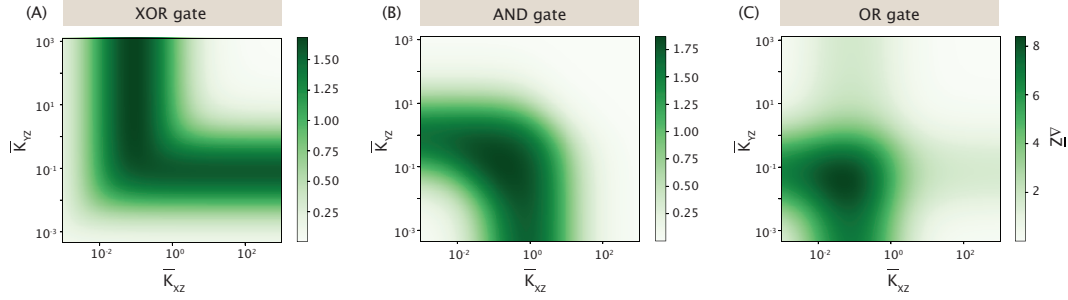


Figure 4.33: Systematic sweep to find region of large $\Delta\bar{Z} = \bar{Z}_{\max} - \bar{Z}_{\min}$. For every logic gate, we sweep across $(\bar{K}_{XZ}, \bar{K}_{YZ}) \in [10^{-3}, 10^3] \times [10^{-3}, 10^3]$ and inspect the region of large relative $\Delta\bar{Z}$. (A) XOR gate. (B) AND gate. (C) OR gate. The XOR gate parameters are $\bar{r}_{0Y} = \bar{r}_{0Z} = 0$, $\bar{r}_{1Y} = \bar{r}_{1Z} = 2$, and $\omega = 0$. The AND gate parameters are $\bar{r}_{0Y} = \bar{r}_{0Z} = \bar{r}_{1Z} = 0$, $\bar{r}_{1Y} = \bar{r}_{2Z} = 2$, and $\omega = 10$. The OR gate parameters are $\bar{r}_{0Y} = \bar{r}_{0Z} = 0$, $\bar{r}_{1Y} = \bar{r}_{1Z} = 2$, $\bar{r}_{2Z} = 10$, and $\omega = 1$. These are the same as in Fig. 4.21.

opposite directions. While XOR gate tends to prefer weak binding, the AND gate benefits from strong binding. The OR gate shape is a superposition of the XOR and AND gate, which is perhaps not surprising since XOR and AND gates are in a sense the limit cases of OR gate. Note that the difference between the magnitude of $\Delta\bar{Z}$ across logic gates is artificial. The absolute magnitude of OR gate $\Delta\bar{Z}$ is large only because $\bar{r}_{2Z} = 10$. We verify the previous claim that $\Delta\bar{Z}$ scales linearly with production rates.

Regarding the average time delay $\langle\Delta\bar{t}\rangle$, we demonstrate computational evidence for the existence of an upper bound on $\langle\Delta\bar{t}\rangle$, given a step in $p_{\text{act}}^X(c_X)$ (fix the high and low values of p_{act}^X). The full set of tunable parameters of the system is $\bar{r}_{iY}, \bar{r}_{jZ}, \omega, \bar{X}, \bar{K}_{XZ}, \bar{K}_{YZ}$, where $i \in \{0, 1\}$ and $j \in \{0, 1, 2\}$. They span a semi-infinite 9-dimensional parameter space. To sweep across this entire space is computationally prohibitive. As a result, we instead take a few 2-dimensional slices to illustrate the existence of the upper bound on $\langle\Delta\bar{t}\rangle$. Specifically, we pair-wise tune 5 different parameters: $\bar{r}_0 = \bar{r}_{0Y} = \bar{r}_{0Z}$, $\bar{r}_1 = \bar{r}_{1Y} = \bar{r}_{1Z}$, $\bar{r}_{2Z}$, ω , and $\bar{X}$. For each parameter combination, we search in K -subspace (as in Fig. 4.21, $(\bar{K}_{XZ}, \bar{K}_{YZ}) \in [10^{-3}, 10^3] \times [10^{-3}, 10^3]$) and find the combination of $(\bar{K}_{XZ}, \bar{K}_{YZ})$ that generates the maximum $\langle\Delta\bar{t}\rangle$ and record the value of the largest time delay as $\langle\Delta\bar{t}\rangle_{\max}$. In Fig. 4.34, we plot $\langle\Delta\bar{t}\rangle_{\max}$ as a function of 10 different pair-wise parameters. We find that all parameter combinations yield $\langle\Delta\bar{t}\rangle < 5$. $\langle\Delta\bar{t}\rangle$ remains finite when any parameter (when possible) is tuned towards ∞ .

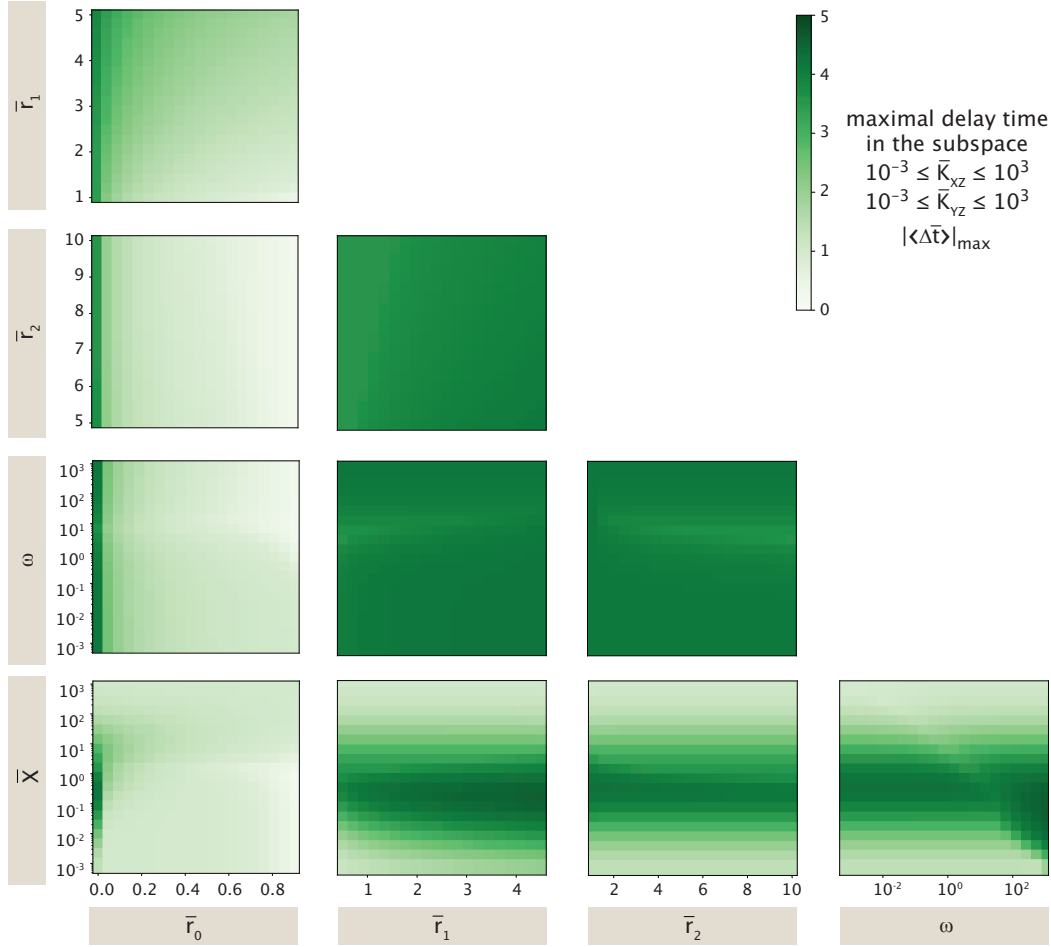


Figure 4.34: Systematic sweep across all tunable parameters in coherent feed-forward loops to find the largest $\langle \Delta \bar{t} \rangle$ for the step in $p_{\text{act}}^X(c_X)$ used throughout the feed-forward loop section, where $c_X^{\max} = 10^{-4}$ and $c_X^{\min} = 10^{-7}$. We pick a default set of parameters: $\bar{r}_{0Y} = \bar{r}_{0Z} = \bar{r}_0 = 0$, $\bar{r}_{1Y} = \bar{r}_{1Z} = \bar{r}_1 = 1$, $\bar{r}_{2Z} = 5$, $\omega = 5$, $\bar{X} = 1$. Note that we set $\bar{r}_{0Y} = \bar{r}_{0Z}$ and $\bar{r}_{1Y} = \bar{r}_{1Z}$ to decrease the number of degrees of freedom in the parameter space without losing too much information. For every colormap, we select a pair of parameters, and perform sweeps on a grid of values, while the other parameters remain the default value. For each value pair, they are used to search in the space $(\bar{K}_{XZ}, \bar{K}_{YZ}) \in [10^{-3}, 10^3] \times [10^{-3}, 10^3]$, and the maximum $\langle \Delta \bar{t} \rangle$ is recorded.

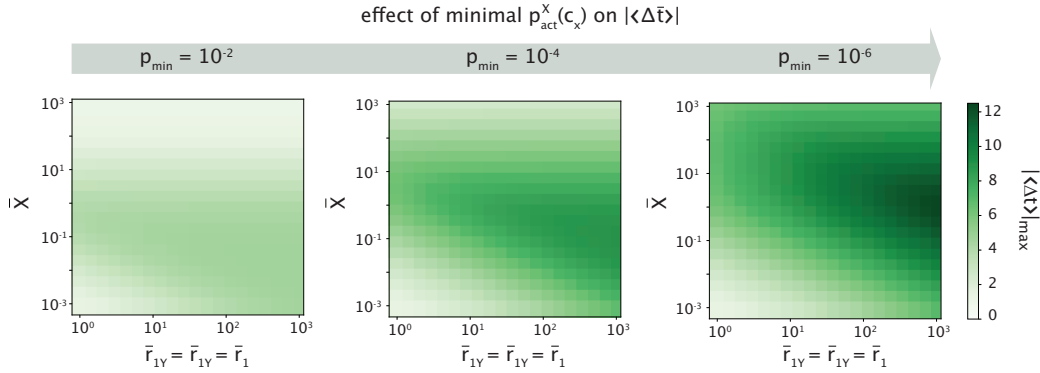


Figure 4.35: The effect of leakiness of $p_{\text{act}}^X(c_X)$ on $\langle \Delta \bar{t} \rangle$. We examine the dependence $\langle \Delta \bar{t} \rangle$ on minimal $p_{\text{act}}^X(c_X)$ in a step, $p_{\min}$, in the XOR gate setting. Parameters used are $\bar{r}_{0Y} = \bar{r}_{0Z} = 0$, $\omega = 0$. The value of $\bar{r}_{1Y} = \bar{r}_{1Z} = \bar{r}_1$ and $\bar{X}$ are tuned to find the largest $\langle \Delta \bar{t} \rangle$ in that parameter subspace. The detailed sweep process is identical to that in Fig. 4.34.

Finally, we address the dependence of $\langle \Delta \bar{t} \rangle$ on the leakiness and saturation of $p_{\text{act}}^X(c_X)$. Here, we denote the minimal $p_{\text{act}}^X(c_X)$ in a step as $p_{\min}$ and the maximal $p_{\text{act}}^X(c_X)$ as $p_{\max}$. After many experimentation with the numerics, we find that the saturation $p_{\max}$ plays a smaller role in determining $\langle \Delta \bar{t} \rangle_{\max}$. The limit $p_{\max} = 1$ yields a similar $\langle \Delta \bar{t} \rangle_{\max}$ as $p_{\max} = 0.95$, and decreasing $p_{\max}$ only makes $\langle \Delta \bar{t} \rangle_{\max}$ smaller. Contrary to $p_{\max}$, $p_{\min}$ plays a much bigger role. We demonstrate this dependency in Fig. 4.35. Here, we examine the OFF step delay in the XOR coherent feed-forward loop, as from previous sweeps in Fig. 4.21 and Fig. 4.34, we see that this tends to be the setting that generates the largest amount of delay. For a given $p_{\min}$, we sweep across $\bar{r}_{1Y} = \bar{r}_{1Z} = \bar{r}_1$, $\bar{X}$. For each combination of $\bar{r}_1$ and $\bar{X}$, we again perform another sweep in $(\bar{K}_{XZ}, \bar{K}_{YZ}) \in [10^{-3}, 10^3] \times [10^{-3}, 10^3]$ to find largest $\langle \Delta \bar{t} \rangle$. We see from Fig. 4.35 that as $p_{\min}$ decreases, the maximal $\langle \Delta \bar{t} \rangle_{\max}$ increases. We note, however, that $\langle \Delta \bar{t} \rangle_{\max}$ still converges computationally for finite $p_{\min}$. Due to the constraint of the explicit effector function $p_{\text{act}}^X(c_X)$, for any biological parameter $p_{\min}$ is finite. For this reason, we demonstrate the maximum delay corresponding to a biologically sensible set of parameters, and investigate its significance in the main text.

4.M Technical details in incoherent feed-forward loop

In this appendix, we will return to some technical details regarding pulses in the incoherent feed-forward loop, as presented in Section 4.3.3. To begin with, we discuss the quantity $\langle \Delta \bar{t} \rangle$ in incoherent feed-forward loops. In the coherent feed-

forward loop, the definition of $\langle \Delta \bar{t} \rangle$ in Eqn. 4.34 and Eqn. 4.35 are equivalent, allowing us to interpret it as the average time delay across concentrations. Here, when $\bar{Z}$ does not exhibit a pulse, there is no difference from the coherent case. However, Eqn. 4.34 becomes ill-defined when $\bar{Z}$ exhibits a pulse. This is because the feed-forward loop response can reach $\bar{Z}$ in a way that the simple regulation curve cannot. A value can still be computed for $\langle \Delta \bar{t} \rangle$ using Eqn. 4.35, but it contains information about the relative size of the pulse rather than acceleration. Because it has unit of time, we regard the direct magnitude of the pulse $\bar{Z}_{\max} - \bar{Z}_f$ for an increasing $\bar{Z}$ response in the main text.

Next, let's derive the maximal average time acceleration $\langle \Delta \bar{t} \rangle_{\max}$ when a pulse does not exist. The green curve shown in Fig. 4.23(C) and (D) is the trajectory that has the largest acceleration; denote this trajectory to be $\bar{Z}_{\text{step}}(\bar{t})$. Mathematically, $\bar{Z}_{\text{step}}(\bar{t}) = \bar{Z}_f$ for $\bar{t} > 0$. The simple regulation curve is again

$$\bar{Z}_{\text{simple}}(\bar{t}) = \bar{Z}_i e^{-\bar{t}} + \bar{Z}_f (1 - e^{-\bar{t}}). \quad (4.223)$$

From Eqn. 4.35, we can then compute the maximal average time acceleration as

$$\begin{aligned} \langle \Delta \bar{t} \rangle_{\max} &= \frac{1}{\bar{Z}_f - \bar{Z}_i} \int_0^{\infty} d\bar{t} (\bar{Z}_{\text{step}}(\bar{t}) - \bar{Z}_{\text{simple}}(\bar{t})) \\ &= \frac{1}{\bar{Z}_f - \bar{Z}_i} \int_0^{\infty} d\bar{t} (\bar{Z}_f - \bar{Z}_i e^{-\bar{t}} - \bar{Z}_f (1 - e^{-\bar{t}})) \\ &= \frac{1}{\bar{Z}_f - \bar{Z}_i} \int_0^{\infty} d\bar{t} (\bar{Z}_f - \bar{Z}_i) e^{-\bar{t}} \\ &= \frac{\bar{Z}_f - \bar{Z}_i}{\bar{Z}_f - \bar{Z}_i} = 1. \end{aligned} \quad (4.224)$$

Finally, we discuss the definition of strong pulse used in Fig. 4.23. Numerically, we characterize a pulse to be strong when the maximum concentration that the transient $\bar{Z}(\bar{t})$ reaches, $\bar{Z}_{\max}$, satisfies $(\bar{Z}_{\max} - \bar{Z}_f)/(\bar{Z}_f - \bar{Z}_i) > 0.05$ for an increasing $\bar{Z}$ response. The threshold of 0.05 is an arbitrary choice. Due to this threshold, there exists a region of trajectories that are technically pulses but are not strong enough to be considered. The trajectory in Fig. 4.23(C) in fact belongs to this region. It exhibits a pulse strictly speaking. Nevertheless, the magnitude of the pulse is vanishingly small. Functionally, it acts in the same manner as the trajectories that do not possess a pulse.

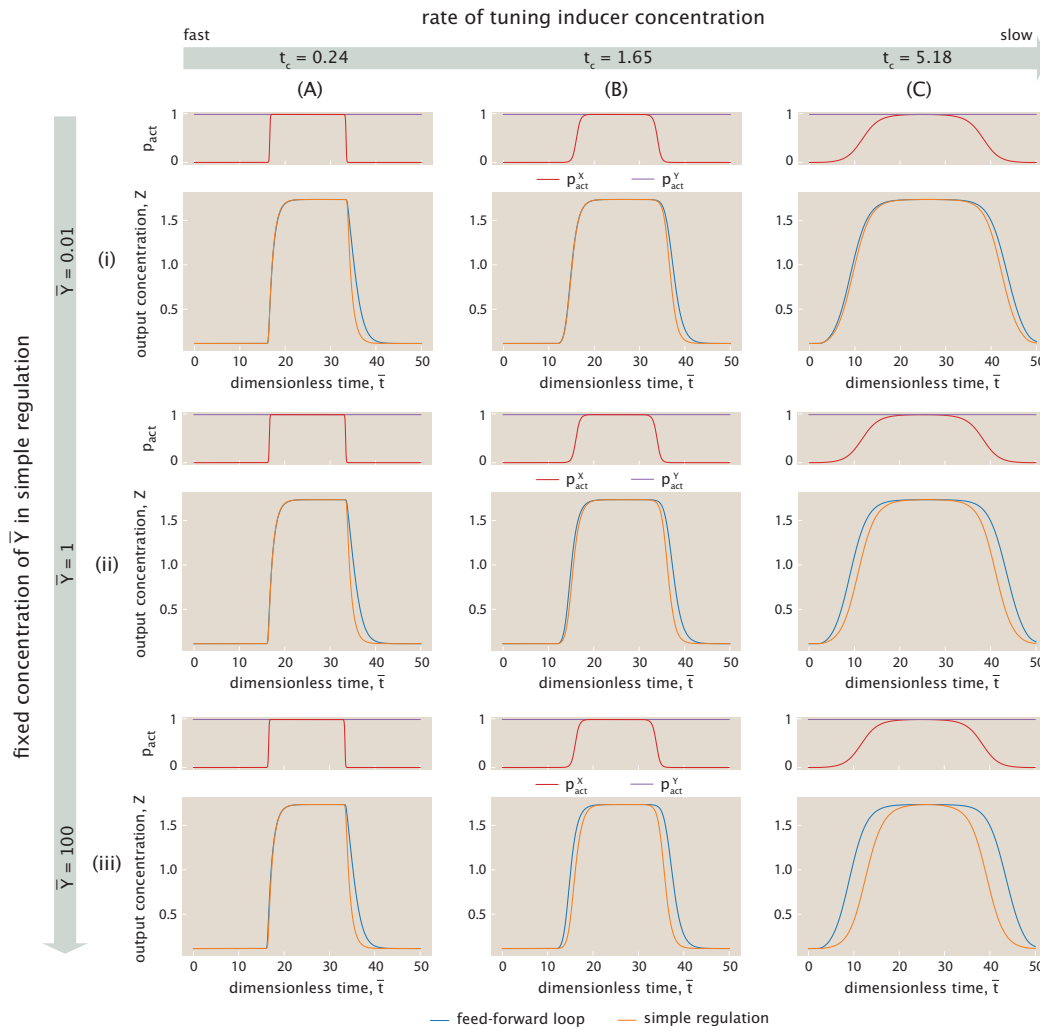


Figure 4.36: Demonstrating the how the arbitrary choice of $\bar{Y}$ affects the simple regulation dynamics. We replicate Fig. 4.24 with different choices of $\bar{Y}$. From top to bottom, $\bar{Y}$ is set to be 0.01 in (i), 1 in (ii), which matches the setting in Fig. 4.24, and 100 in (iii). From left to right, the rate of concentration is tuned the same way as in Fig. 4.24. System parameters are also identical to those in Fig. 4.24.

4.N Effect of $\bar{Y}$ in continuous tuning

In Section 4.3.4, we discussed the scenario where the signal $c_X(\bar{t})$ is no longer a step function, but a continuous function in time. We mentioned that the comparison with simple regulation in this case is subtle, as the arbitrary choice of value $\bar{Y}$ affects the shape of simple regulation response. Here, we expand on Fig. 4.24 where we repeat the numerical integration for three distinct values of $\bar{Y}$, as shown in Fig. 4.36. For the fast tuning case, different choices of $\bar{Y}$ have no effect on the simple regulation trajectory. This is expected since in the limit case of a step function signal in c_X , the value of $\bar{Y}$ strictly has no effect on the shape of the trajectory. As the rate of tuning c_X slows down, the effect of $\bar{Y}$ becomes more and more pronounced. Specifically, as $\bar{Y}$ increases, the rescaled simple regulation curve “shrinks”. As a result, the magnitude of apparent delay/acceleration between the feed-forward loop and simple regulation response increases. Due to the artificial nature of the choice of $\bar{Y}$ in simple regulation, we cannot make a claim about the magnitude of delay/acceleration in the slow tuning limit. Nevertheless, our qualitative results stand. The choice of $\bar{Y}$ does not change the type of response to a step. For example, even though $\bar{Y}$ affects the magnitude of delay on the OFF step, the feed-forward loop in this case always delays on the OFF step.

4.O Code availability

All Jupyter notebooks used to generate graphs in figures throughout this chapter are available².

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²Supporting Python code is available on GitHub at github.com/RPGroup-PBoC/2025_inducers

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