A MATHMATICAL MODEL OF PROMOTER METHYLATION IN THE CIRCADIAN CLOCK

by

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Abstract

This thesis studies the influence of DNA methylation on circadian rhythms in a mathematical model of the circadian clock. Building on the work of François and Hakim, we obtain dominant order contributions to the period of the clock arising from methylation. We then show that our model can be reduced to a Goodwin-type oscillator, similar to a model studied by Kim and Forger. In our reduced model, methylation can induce Hopf bifurcations, alter the period, and remove bistability from the system. We also expand upon Kim and Forger’s analysis of stoichiometry in the circadian clock by showing in detail how to obtain their model as an approximation of the mixed-feedback loop model of François and Hakim.
To my family, friends, and colleagues.
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Statement of Contributions

Chapters 3-4 are adapted for this thesis from joint work with Adam Stinchcombe and Edward Oh [1]. Appendix B is based on lecture notes written during the summer of 2022 which were co-authored with Adam Stinchcombe and Adam Morgan.
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Chapter 1

Introduction

1.1 Motivation

The mammalian circadian clock is made up of several genes that interact with one another to generate oscillations with a roughly (circa) 24 hour (daily) period, hence the name “circadian clock”. These oscillations act as cues for various other biological processes, and are essential for normal biological function. Viewed from a mathematical perspective, the circadian clock is an interesting example of a nonlinear oscillator. Part of this interest comes from the clock’s ability to strike a balance between robustness and plasticity.

The plasticity of the circadian clock is illustrated by a recent experiment on period compression [2]. In the experiment, mice were subjected to a 22 hour environment (11 hours of light then 11 hours of dark) for several days. As one would expect, the mice eventually exhibited 22hr circadian rhythms. Surprisingly, the 22hr period persisted even after the mice were left in constant darkness. Azzi et al. hypothesized that the retention of a compressed period was a consequence of epigenetic regulation of the circadian clock [2]. In this thesis we investigate this hypothesis from a mathematical perspective.

1.2 Thesis structure

Chapter 2 provides a brief overview of the concepts from genetics and mathematical modelling necessary for understanding this thesis. The final section of Chapter 2 is more technical than the rest of the thesis and is useful for context, but not essentially for understanding the subsequent results. In Chapter 3, we introduce the novel mathematical model studied in this thesis: the intermediate transcription rate mixed-feedback loop (IT-MFL) model. Stability analysis of the model is provided along with an estimate of the model’s period. This addresses the initial motivation
for this thesis: understanding the influence of promoter methylation on the period of the circadian clock.

Chapter 4 goes deeper into the structure of the IT-MFL model and its relationship to other models of the circadian clock. We show that under some timescale separation assumptions, the IT-MFL model can be approximated with a feedback loop, specifically a monotone cyclic feedback system. Analysis of the reduced model reveals that DNA methylation can cause a Hopf bifurcation, remove bistability from the clock, and is equivalent to changes in the other kinetic parameters describing the promoter states. Finally, a review of the main results is given in Chapter 5.
Chapter 2

Background

2.1 Genes, rhythms, and epigenetics

2.1.1 Gene expression

Around the turn of the twentieth century, the nascent field of genetics was mainly concerned with how differences in observable features propagate between generations [3]. Considerable progress was made by Gregor Mendel, who is well-known for postulating the three laws of genetic inheritance. In the decades that followed, the discovery of DNA and the central dogma of molecular biology came to provide a deeper explanation of genetic inheritance, consistent with Mendel’s laws.

It was discovered in the 1940’s that genes are found on chromosomes which are made up of deoxyribonucleic acid (DNA) [4, 5]. Roughly a decade later, the famous double-helix structure of DNA was discovered in the early 1950’s [6]. It is now understood that DNA is made up of two strands that are each comprised of smaller molecules called nucleotides. Each nucleotide is made up of a sugar-phosphate group and one of four possible bases: \( A \) (Adenine), \( T \) (Thymine), \( C \) (Cytosine), and \( G \) (Guanine). Nucleotides are paired or “complimentary” to one another in the sense that if \( A \) is found at a particular position on one strand of DNA, \( T \) must be found at the same position on the opposing strand. The same pairing is true of \( C \) and \( G \). Complementarity is crucial for faithfully transmitting the DNA sequence to future cells during cell division. Interestingly, nucleotide complementarity was predicted by Crick decades before its direct experimental observation [7]. See [8] for more discussion on the interplay of theory and experiment in biology.

Following the discovery of the structure of DNA in the 1950’s, additional experiments revealed that proteins are not created directly from the information stored in the DNA, but instead are produced in a two-step process known as gene expression. The first step of gene expression, known as transcription, occurs when a protein com-
plex called RNA polymerase moves along the DNA to create a shorter chain of similar molecules known as mRNA. The second stage, known as translation, involves the production of proteins from the mRNA. The mRNA interacts with a protein complex called a ribosome to produce the protein product associated with the gene. Each of the two steps in gene expression are made up of numerous sub-processes [9], however deterministic models of gene expression typically treat transcription and translation as elementary reactions. In the decades since the discovery of DNA, it has been shown that the vast majority of DNA does not contain genes, and is instead referred to as non-coding regions.

The rate at which transcription and translation occur is influenced by a variety of factors. A gene is said to be unregulated if the abundance of its protein product is affected only by its own transcription, translation, mRNA decay, and protein decay. If the protein product interacts with other chemical species (and therefore has a more complicated consumption rate) and the production rate is still unregulated, then the gene is said to be “constitutively expressed”. This is often used as an idealization in mathematical models when the expression of a protein is not well-understood. In many cases, the rate at which a gene is expressed is regulated by other proteins in the cell. This makes it possible for a protein to only be expressed at a certain time of day, or in response to a particular stimulus. The proteins responsible for this regulated expression are referred to as transcription factors, and can bind to specific regions of DNA that are close to the region that codes for the regulated gene. A transcription factor that increases (decreases) the transcription rate of a gene is referred to as an activator (repressor). The region of DNA to which an activator binds is referred to as its promoter.

Remark 1 (Nomenclature in genetics). The standard convention in genetics is to italicize the name of a gene (for instance per) and write the associated protein product (PER) in all capitals.

The term “gene regulatory network” refers to a group of genes that regulate one another’s expression. Gene regulatory networks exhibit a wide range of dynamic behaviours and can either naturally or synthetically be designed to perform specific functions such as: logical circuit operations [10], bistable switching [11], limit-cycle oscillations [12], and attenuation of molecular noise [13]. The idea of assigning a functional interpretation to networks builds on the classical view in genetics that individual genes serve unique functions [3]. Moreover, the interest in dynamic behaviour rather than steady state expression is a natural bridge between the worlds of molecular genetics and applied mathematics.
2.1.2 The structure of the circadian clock

A wide variety of cyclical biological processes known as “circadian rhythms” occur with a roughly 24 hour period, oscillate autonomously, and can be phase-shifted to match the phase of their environment. Over the last several decades, the gene regulatory networks responsible for producing circadian rhythms have been identified in many organisms. These networks are often referred to as “molecular circadian clocks”, or simply “circadian clocks”. In this thesis, we restrict our attention to the mammalian circadian clock.

Circadian rhythms in mammals are a consequence of a network of interacting genetic oscillators. The primary oscillator is located in the superchiasmatic nucleus (SCN), a region of the brain located in the anterior part of the hypothalamus. The SCN is made up of about 20,000 neurons which receive input from the visual system through the retinohypothalamic tract. In addition to the primary clock in the hypothalamus, many tissues also generate autonomous rhythms [14]. These so-called peripheral clocks have the same basic structure as the genetic oscillators found in the SCN, however there are differences in the expression patterns of certain genes [15]. For instance, the protein CLOCK is constitutively expressed in the SCN but not in peripheral tissues [16]. Despite these differences in dynamic behaviour, it is common to refer to the molecular architecture of the mammalian circadian clock because so many features are conserved between the primary and peripheral oscillators. A variety of signalling pathways transmit information from the SCN to the peripheral oscillators, including calcium channels, cyclic adenosine monophosphate (cAMP) secondary messenger kinases, and glucocorticoid mediated networks [17].

At a molecular level, the mammalian circadian clock is made up of two interacting transcriptional-translational feedback loops (TTFLs). In the main loop, activator proteins BMAL1 and CLOCK bind to one another and activate the expression of Period and Cryptochrome genes. The Period family is made up of 3 genes (Per1, Per2, and Per3) and the Cryptochrome family is made up of Cry1 and Cry2. After the protein products of the Per and Cry families undergo a sequence of post-translational modifications and bind to one another, the PER-CRY complex represses its own transcription by inhibiting CLOCK-BMAL1 activity in the nucleus. Due to this inhibitory activity, the PER TTFL constitutes a negative feedback loop.

The PER TTFL is generally accepted to be the primary driver of oscillatory behaviour in the circadian clock. A secondary feedback loop is responsible for generating the circadian expression of BMAL1. In this loop, BMAL1 activates the expression of ROR and REV-ERB proteins, which then either activate or repress BMAL1 expression. We do not include the secondary feedback loop in our model and assume instead that the complex CLOCK-BMAL1 is constitutively expressed.
2.1.3 Epigenetics, DNA methylation, and the circadian clock

The term “epigenetics” refers to any heritable phenotype (observable trait) that results from modifications of a chromosome that do not change the DNA sequence (the order of nucleotides in the DNA) [18, 19]. Some authors are more precise and instead use the terminology “molecular epigenetics” in reference to their field, because the term “epigenetics” has had looser definitions at other points in history. Readers interested in the history of the term are directed to [20, 21].

DNA methylation is the most well-studied form of epigenetic modification [22]. It refers to the attachment of an extra molecule (a methyl group) at a particular location on a cytosine molecule. This chemical modification is almost exclusively found at cytosines that are immediately followed by a guanine molecule, known as cytosine-phosphate-guanine (CpG) dinucleotides. The presence of the extra methyl group on the cytosine can increase or decrease the accessibility of the local DNA region to transcription factors, thereby altering transcriptional activity. In promoter regions, the methylation of CpG sites in promoter regions is generally associated with decreased transcriptomic activity, as will be assumed in the model presented in this thesis.

Unlike the DNA sequence which is almost perfectly copied between generations of cells, DNA methylation is only inherited in a probabilistic sense, see [23] for a recent mathematical model of Utsey and Keener. Since the density of epigenetic marks is stably propagated through cell division, they are an important aspect of the preservation of cellular identity. Moreover, these marks can be influenced by environmental stimuli and therefore serve as a form of cellular memory.

In experimental studies of DNA methylation, it is more common to encounter the term “DNA modification” rather than “DNA methylation”. This is because cytosines can be in states other than fully methylated or unmethylated. Since standard measurement techniques cannot distinguish the fully and partially methylated states, it is more appropriate to refer to anything other than an unmethylated state of the cytosine as simply “modified”. The term modification density refers to the fraction of modified cytosine nucleotides in a given region, and this is often the quantity reported in experimental papers. It also should be noted that DNA methylation is facilitated by two antagonistic groups of enzymes known as DNA methyltransferases and ten-eleven translocation methylcytosine dioxygenases. Since the model in this thesis approximates this as a linear process and this is equivalent to neglecting the enzyme catalysis of the reactions, we do not elaborate on the enzymes responsible for controlling methylation turnover.

In addition to cell-type-specific methylation patterns, average DNA modification levels also change over time. Over many years, the modification density undergoes
monotonic change. This pattern is so reliable that the modification status of a handful of CpGs can be sufficient for estimating an individual’s chronological age with a surprising amount of accuracy [24]. Beyond this monotonic change, it has been shown more recently that DNA modification dynamics have an oscillatory component [25, 26, 27]. In some cases, these oscillations occur with a 24hr periodicity. The post-translational consequences of cyclic methylation turnover are yet to be understood [28].

In this thesis, we explore a particular facet of the interaction between circadian and epigenetic systems. We focus on understanding the influence of DNA methylation on the primary TTFL in the mammalian circadian clock. This question is motivated by the work of Azzi et al. [2]. Azzi et al. entrained mice to a 22 hour day (11hr light, followed by 11hr dark) for 6 weeks. Following entrainment, mice were subjected to constant darkness for 10 days. Azzi et al. found that even in constant darkness, the mice retained a shortened circadian period. They also detected a difference in methylation of clock genes in the 22hr mice and chemically-induced inhibition of methylation suppressed period changes. From these observations, they hypothesized that DNA methylation of clock genes contributes to the plasticity of the mammalian circadian clock.

2.2 Mathematical models of biochemical systems

The biological mechanisms described in the previous section often lead to a diagrammatic understanding of biological regulation. To make predictions from such a description, it is generally useful to formulate the picture in terms of a mathematical model. This process is explained in this section, along with some standard techniques for approximating these models using timescale separation assumptions. Much of this section comes from [29], a textbook which provides an excellent introduction to mathematical biology.

2.2.1 From mass-action kinetics to models

Deterministic models of gene regulatory networks (the type of model studied in this thesis) are derived from the following two assumptions. First, one assumes that the chemical populations in the model are large enough that they can be described by continuous concentrations, rather than discrete copy numbers. This assumption is sometimes referred to as the continuum approximation. It is directly related to the deterministic nature of the model, because stochastic effects would otherwise have to be included when dealing with low population numbers. Second, it is common to assume that the reactant concentrations are homogeneous in space. Spatial homo-
geneity allows spatial degrees of freedom to be ignored in the model, and therefore the system can be described with a system of ODE rather than a system of reaction-diffusion PDE. There is a vast literature on differences between deterministic and stochastic models of biochemical systems [30, 31, 32, 33]. Removing the assumption of spatial homogeneity can also have interesting consequences on the model, see for instance [34].

The law of mass-action kinetics can be derived under the assumptions of spatial homogeneity and the continuum hypothesis. The law of mass-action states that the rate of a chemical reaction is proportional to the product of the concentrations of the reactants. For example, a reaction where two copies of a protein \( X \) come together and form a bond \( X + X \rightarrow X_2 \) would proceed at a rate \( k[X]^2 \), where \( k \in \mathbb{R}_{>0} \) is a constant and \([X]\) denotes the concentration of \( X \). Given a list of chemical reactions, mass-action kinetics provides a straightforward route for obtaining the rates at which the concentrations evolve. The time-evolution of a system governed by mass-action kinetics is typically expressed as

\[
\frac{dx(t)}{dt} = N\nu(x(t), t) \tag{2.1}
\]

where \( x(t) = (x_1(t), \ldots, x_n(t)) \) is a concentration vector, \( \nu \) is a nonlinear vector function of the concentrations referred to as the propensity, and the matrix \( N \) is referred to as the stoichiometry matrix. The columns of \( S \) correspond to distinct chemical reactions, and the rows correspond to distinct chemical species. Each entry \( S_{ij} \) is defined to be the number of molecules of species \( i \) consumed or produced by reaction \( j \). The use of a stoichiometry matrix is not only notationally convenient, but also tends to expose important features of the dynamical system. For instance, independent vectors in the left-nullspace of \( N \) correspond to the conserved quantities of Eq. (2.1). All the models studied in this thesis can be obtained from mass-action kinetics either directly, or from additional approximations.

### 2.2.2 Approximation techniques from timescale separation

The application of mass-action kinetics to a gene regulatory network often produces high-dimensional models. Fortunately, reactions in biology often take place on different timescales, and this can help in approximating a mathematical model. We review two standard approximations which are particularly useful: the rapid equilibrium and quasi-steady state approximations.

The rapid equilibrium approximation can be applied when a reversible reaction occurs on a faster timescale than the rest of the model. In this case, one may assume that the reversible reaction reaches equilibrium instantaneously and that this equilib-
rium is maintained at all future times. By assuming this reaction is at equilibrium, we can eliminate one dynamic variable from the model because its concentration is uniquely determined by the other variables in the model along with the equilibrium condition. For instance, suppose that we have a forward reaction rate $k_+ a(t)$ and reverse reaction rate $k_- b(t)$. At equilibrium, the concentrations satisfy $k_+ a = k_- b$, hence $\tilde{a}(t) = \frac{k_-}{k_+} \tilde{b}(t)$. The notation $\tilde{a}(t)$ is used in order to emphasize that $\tilde{a}(t)$ is the result of an approximation. Let $\tilde{r}(t)$ denote the concentration of the $a, b$ pool at time $t$

$$\tilde{r}(t) = a(t) + b(t).$$

Then, we have

$$\tilde{a}(t) = \frac{k_-}{k_+ + k_-} \tilde{r}(t),$$

$$\tilde{b}(t) = \frac{k_+}{k_- + k_+} \tilde{r}(t).$$

Hence, $\tilde{a}$ and $\tilde{b}$ can be recovered from the pooled concentration $\tilde{r}$ at all times, and the remaining governing equations can be rewritten in terms of $\tilde{r}$ alone, thereby reducing the dimension of the system. Reactions with more complicated kinetics are amenable to the same method, however the algebra becomes more involved.

The quasi-steady state approximation can be applied when a dynamic variable evolves on a fast timescale relative to the rest of the system. This is similar to the rapid-equilibrium assumption, however we are now applying this approximation to an individual dynamic variable in the model (which potentially participates in several reactions) rather than an individual reaction. Suppose our system is described by $x(t) = (x_1(t), \ldots, x_n(t))$ which evolves according to

$$\frac{d}{dt} x(t) = f(x(t), t)$$

and $x_k(t)$ is a fast variable relative to the other $n - 1$ components of $x$. The quasi-steady state approximation of $x_k(t)$, denoted by $x_k^{\text{qss}}(t)$ is the solution to the algebraic equation

$$0 = f(x_1(t), \ldots, x_{k-1}(t), x_k^{\text{qss}}(t), x_{k+1}(t), \ldots, x_n(t), (t)).$$

(2.2)

Equation (2.2) states that $x_k^{\text{qss}}(t)$ always maintains its equilibrium value relative to the remaining dynamic variables in the system. Since the remaining variables are not at equilibrium, the value $x_k^{\text{qss}}(t)$ changes over time, hence the name “quasi-steady state approximation”. See [29] for a more thorough introduction to these approximations.
and their applications.

### 2.3 Techniques for the analysis of feedback loops

Nonlinear feedback loops often give rise to oscillatory behaviour. The idea that nonlinearity and delay cooperate to generate oscillations can be seen in Brian Goodwin’s work on biochemical feedback loops [35]. At the time, it was unclear how a system of chemical reactions could be structured in such a way that they produce autonomous oscillations. Goodwin proposed a simple three-variable feedback loop of the form

\[
\frac{dx}{dt} = \frac{a}{k^n + z^n} - bx,
\]

\[
\frac{dy}{dt} = \alpha x - \beta y,
\]

\[
\frac{dz}{dt} = \gamma y - \delta z.
\]

The nonlinear term in Goodwin’s model is referred to as a Hill function. Goodwin demonstrated that his three-species model is oscillatory when the parameter \( n \) is sufficiently large. If Goodwin’s model is extended to form a longer feedback loop

\[
\frac{dx_1}{dt} = \frac{\alpha_1}{k^n + x_m^n} - \beta_1 x_1,
\]

\[
\frac{dx_2}{dt} = \alpha_2 x_1 - \beta_2 x_2,
\]

\[\vdots\]

\[
\frac{dx_m}{dt} = \alpha_m x_{m-1} - \beta_m x_m,
\]

then simulation reveals that increasing the length of the loop allows for oscillations at lower values of \( n \). This is consistent with the general idea that time-delay and nonlinearity work together to produce oscillations in feedback loops. In the decades since Goodwin’s model was proposed, theorems known as “secant conditions” have formalized this idea.

In order to introduce secant conditions, we focus our attention on a particularly well-behaved type of feedback loop known as a monotone cyclic feedback system.

**Definition 2.3.1** (Monotone cyclic feedback system). Let \( \mathbf{x} = (x_1, \ldots, x_n) \) be a solution to a dynamical system in \( \mathbb{R}^n \) of the form

\[
\dot{x}_i = f^i(x_i, x_{i-1})
\]
for \( i = 1, \ldots, n \), where we interpret \( x_0 := x_n \). Suppose all the \( f_i \) are defined on an open set \( \mathcal{D} \subset \mathbb{R}^n \) and denote the projection of \( \mathcal{D} \) onto the \((x_i, x_{i-1})\) plane by \( \mathcal{D}_i \). The system is referred to as a monotone-cyclic feedback system if for each \( i = 1, \ldots, n \)

- \( \mathcal{D}_i \) is convex,
- \( f_i \in C^1(\mathcal{D}_i) \),
- there exists \( \delta_i \in \{-1, 1\} \) such that

\[
\delta_i \frac{\partial f_i(x_i, x_{i-1})}{\partial x_{i-1}} > 0 \quad \text{for all } (x_i, x_{i-1}) \in \mathcal{D}_i.
\]

The pairwise interactions of the dynamic variables in an MCF system force the system to be effectively two-dimensional. This is made precise in Theorem 2.3.1, a generalization of the Poincaré-Bendixson theorem which first appeared in the work of Mallet-Paret and Smith.

**Theorem 2.3.1** (Main theorem of [36]). Let \( x(t) \) be the solution of a monotone cyclic feedback system with initial condition \( x(0) = x_0 \) and suppose \( \sup_{t>0} ||x(t)|| < \infty \). Then the omega-limit set \( \omega(x_0) \) is either

- a static equilibrium,
- a nonconstant periodic orbit, or
- a union of an equilibrium and a collection of homoclinic heteroclinic orbits between equilibria.

The statement of the theorem in [36] also provides a classification of the possibilities for heteroclinic orbits. We ignore this part of the classification because we only apply the theorem in settings where there is a unique equilibrium. Notice it follows from Theorem 2.3.1 that chaos is forbidden in MCF systems.

### 2.3.1 Local stability theory

The assumption of a single unstable equilibrium in a bounded MCF system implies the existence of an oscillatory solution by Theorem 2.3.1. Importantly, the converse reasoning does not hold (i.e. the stability of an equilibrium does not in general imply the absence of oscillations). Focusing on instability as a tool for detecting oscillations, linear stability analysis is perhaps the most elementary technique that comes to mind. Recall that if a dynamical system

\[
\dot{x}(t) = F(x(t))
\]
is at an equilibrium \( x_{eq} \in \mathbb{R}^n \), the eigenvalues of the Jacobian \( J_{ij} := \frac{\partial F_i(x_{eq})}{\partial x_j} \) determine the evolution of the system close to its equilibrium. This is because any perturbed solution \( x = x_{eq} + x_p \), the \( x_p \) must satisfy

\[
\frac{d}{dt} x_p = J x_p,
\]

and using the ansatz \( x_p(t) = x_p(0)e^{\lambda t} \) with \( \lambda \in \mathbb{C} \), we see that

\[
(\lambda I - J) x_p(0) = 0
\]

and hence \( \det(\lambda I - J) = 0 \), forcing \( \lambda \) to be an eigenvalue of the Jacobian. Consequently, the near-equilibrium stability is determined by checking if any of the eigenvalues have a non-negative real part.

Often one is interested in using stability analysis to determine which parameters promote or inhibit oscillations. This is sometimes possible when one calculates the eigenvalues directly, but typically these expressions are difficult to interpret. In some cases, the Routh-Hurwitz criterion is more informative for assessing the influence of parameters on stability.

**Theorem 2.3.2** (Routh-Hurwitz criterion, [37]). Let \( P_J(\lambda) \) be the characteristic polynomial of \( J \in \mathbb{R}^{n \times n} \)

\[
P_J(\lambda) = \lambda^k + a_1 \lambda^{k-1} + \cdots + a_k.
\]

Define the matrices

\[
H_1 = \begin{bmatrix} a_1 \end{bmatrix}, \quad H_2 = \begin{bmatrix} a_1 & 1 \\ a_3 & a_2 \end{bmatrix}, \\
H_3 = \begin{bmatrix}
a_1 & 1 & 0 & \cdots & 0 \\
a_1 & 0 & a_2 & a_1 & \cdots & \vdots \\
\vdots & \ddots & \ddots & \ddots & \ddots & \ddots \\
0 & \cdots & 0 & a_1 & \cdots & a_k
\end{bmatrix},
\]

with the \((l, m)\) element of matrix \( H_j \) given by

\[
H_{j,(l,m)} = \begin{cases} 
a_{2l-m} & 0 < 2l - m \leq k, \\
1 & 2l = m, \\
0 & \text{otherwise}. \end{cases}
\]

A necessary and sufficient condition for all roots of \( P_J(\lambda) = 0 \) to have negative real
parts is
\[ \det H_k > 0 \text{ for all } k = 1, \ldots, n. \]

For linear time-invariant dynamical systems, the Routh-Hurwitz criterion gives necessary and sufficient conditions for stability. In the nonlinear setting, the Routh-Hurwitz condition is only informative when the Hartman-Grobman theorem applies. This requires that none of the eigenvalues of the linearised system have real part equal to zero. A matrix satisfying the Routh-Hurwitz criterion is sometimes referred to as Hurwitz. In the special case of feedback systems, there are even simpler criteria for checking if a matrix is Hurwitz. The following secant condition is an example of this.

**Theorem 2.3.3** (Classical secant condition). Let \( M \in \mathbb{R}^{n \times n} \) be a matrix of the form

\[
M := \begin{pmatrix}
-\alpha_1 & 0 & \cdots & 0 & -\beta_1 \\
\beta_2 & -\alpha_2 & \cdots & 0 & 0 \\
\vdots & \vdots & \ddots & \vdots & \vdots \\
0 & 0 & \cdots & \beta_n & -\alpha_n
\end{pmatrix}
\]

where \( \alpha_i > 0 \) and \( \beta_i > 0 \) for \( i = 1, \ldots, n \). Then \( A \) is Hurwitz provided that

\[
\frac{\prod_{i=1}^{n} \beta_i}{\prod_{i=1}^{n} \alpha_i} < \sec(\frac{\pi}{n})^n.
\]

Notice from Eq. (2.3) that \( M \) has the correct form for being a linearization of a monotone cyclic feedback system. Theorem 2.3.3 appears in [38], but is almost certainly older than that paper. In any case, Theorem 2.3.3 can be obtained as a corollary of the more general secant condition developed by Thron [39], and Tyson and Othmer [40].

**Theorem 2.3.4** (Secant condition for end-product inhibition, [39]). Consider an \( n \)-variable system of the form

\[
\frac{dS_1}{dt} = F_1(S_n) - R_1(S_1), \\
\frac{dS_i}{dt} = F_i(S_{i-1}) - R_i(S_i), \quad i = 2, \ldots, n.
\]

Suppose that \( F_1 \) is non-increasing as a function of \( S_n \) and all other \( F_i \) are non-decreasing. Suppose further that the removal rates \( R_i \) are non-decreasing, with \( R_i(0) = 0 \), and all rates \( F_i \) and \( R_i \) are non-negative. Let \( \alpha_i \) and \( \beta_i \) be defined as the order of each reaction

\[
\alpha_i = \frac{\partial \ln R_i}{\partial \ln S_i}, \quad \beta_i = \frac{\partial \ln F_{i+1}}{\partial \ln S_i}.
\]
Finally let \( \phi = -\frac{\partial \ln F_1}{\partial \ln S_n} \). Then a necessary condition for oscillations is given by

\[
\sec\left(\frac{\pi}{n}\right)^n \leq \frac{\beta_1 \cdots \beta_n \phi}{\alpha_1 \cdots \alpha_n}.
\]

Thron’s secant condition is consistent with the intuitive discussion of necessary conditions for oscillation at the start of this section. The longer the feedback loop, the lower the right hand side of the inequality, and the more nonlinear the feedback loop, the greater the right hand side.

## 2.3.2 Global stability theory

In the decades since the work of Tyson, Othmer, and Thron, the secant condition has been generalized to more complex systems. A notable example of this is Sontag’s work on a secant condition for scalar cascades of passive systems \[38\]. The main result of his work is an inequality that generalizes the secant conditions discussed in the previous section. Importantly, Sontag’s result is global in the sense that it deals with \( L^2 \) stability rather than fixed point instability. Recently, Sontag’s work was extended by Forger \[41\] to derive another version of the secant condition. To discuss Forger’s global secant condition, consider a feedback loop of the form

\[
\dot{x}_i = p_{i-1}(c_{i-1}(x_{i-1})) - c_i(x_i) \quad \text{(for } i = 1, \ldots, n),
\]

(2.4)

where the index \( i = 0 \) is identified with \( i = n \). The functions \( p_i \) are referred to as production rates and the \( c_i \) are referred to as clearance rates. The dependence \( p_i(c_i(\cdot)) \) reflects the fact that chemicals in a feedback loop can undergo decay in addition to being transformed into the next species in the loop. For example, consider the following linear reaction network

\[
\begin{align*}
\dot{x}_1 &= \gamma x_3 - (\gamma + \delta)x_1, \\
\dot{x}_2 &= \gamma x_1 - (\gamma + \delta)x_2, \\
\dot{x}_3 &= \gamma x_2 - (\gamma + \delta)x_3,
\end{align*}
\]

where \( \gamma, \delta > 0 \). Since \( x_i \) decays at a rate \( \delta \) and is transformed into \( x_{i+1} \) at a rate \( \gamma \), it makes sense that the clearance rate of each species (in this case \( \gamma + \delta \)) is higher than the production rate of the next species.

Forger considers solutions to Eq. (2.4) in the extended space of square-integrable functions

\[
L^2_e = \{ u \in L^2([0,T]; \mathbb{R}^n) \text{ for all } T > 0 \}.
\]
and derives an inequality which holds true in the subspace $L^2_{e, \tau}$ of $\tau$-periodic functions

$$L^2_{e, \tau} = L^2_e \cap \{ u : \mathbb{R} \to \mathbb{R}^n | u(t + \tau) = u(t) \ \text{for all} \ t \in \mathbb{R} \}$$

that obey Eq. (2.4). The Hilbert space structure of $L^2(\mathbb{R})$ is naturally passed on to $L^2_{e, \tau}$ through the inner-product

$$\langle u, v \rangle : = \int_0^\tau u(s) \cdot v(s) \, ds,$$

defined for $u, v \in L^2_{e, \tau}$. Focusing on a nominal trajectory $x \in L^2_{e, \tau}$ Forger makes a final assumption for notational convenience that the production rates are mean-zero along this trajectory

$$\langle p_i \rangle : = \frac{1}{\tau} \int_0^\tau p_i(c_i(x_i(t))) \, dt = 0.$$

No generality has been lost since the average production rate can be added and subtracted from Eq. (2.4). Forger quantifies the nonlinearity in each stage of the feedback loop using a function we refer to as the Forger-gain or “F-gain” for short.

**Definition 2.3.2 (F-gain).** Given a nominal trajectory $x \in L^2_{e, \tau}$, the F-gain of the $i$-th stage of a feedback system relative with mean-zero production rates is

$$G_i(t) = \frac{p_i(c_i(x_i(t)))}{c_i(x_i(t)) - c_i^*}$$

where $c_i^*$ is chosen so that $p_i(c_i^*) = 0$.

Intuitively the F-gain is the average slope of $p_i$ between the points $c_i^*$ and $c_i(x_i(t))$. Forger works under the assumption that the F-gain is always well-defined, a condition known in control theory as passivity. Forger then defines the average F-gain as a weighted harmonic average.

**Definition 2.3.3 (Average F-gain).** Given a nominal trajectory $x \in L^2_{e, \tau}$, the average F-gain of the $i$-th step of a feedback system with mean-zero production rates is given by

$$\overline{G}_i = \frac{\int_0^\tau p_i(c_i(x_i(t)))^2 \, dt}{\int_0^\tau p_i(c_i(x_i(t)))^2 G_i(t) \, dt}.$$

The connection between the better-known secant gain used in Sontag’s work and the F-gain is discussed in the supplemental material of [41]. Forger uses the average F-gain to provide a necessary condition for the existence of periodic trajectories.

**Theorem 2.3.5 (Forger’s global secant condition).** Let $x \in L^2_{e, \tau}$ be a solution of
Eq. (2.4). Then the average F-gain must satisfy
\[
\sec \left( \frac{\pi}{n} \right)^n \leq \prod_{i=1}^{n} \sec(\theta_{i-1,i}) = \prod_{i=1}^{n} |G_i|
\]
where
\[
\theta_{i-1,i} = \frac{\langle x_{i-1}, x_i \rangle_\tau}{||x_{i-1}||_\tau ||x_i||_\tau}.
\]
Equality is achieved in Eq. (2.5) when \( \theta_{i-1,i} = \frac{\pi}{n} \).

The requirement that \( \theta_{i-1,i} = \frac{\pi}{n} \) for \( i = 1, \ldots, n \) suggests that a certain type of symmetry in a feedback loop promotes oscillations. We make use of this interpretation later in the report when considering feedback loops that only have nonlinearity in the first step
\[
\frac{dx_1}{dt} = \alpha_1 f(x_m) - \beta_1 x_1,
\]
\[
\frac{dx_2}{dt} = \alpha_2 x_1 - \beta_2 x_2,
\]
\[\vdots\]
\[
\frac{dx_m}{dt} = \alpha_m x_{m-1} - \beta_m x_m.
\]
In this special case, Forger’s condition suggests that setting
\[
\beta_1 = \beta_2 = \cdots = \beta_m,
\]
will be conducive for oscillations. We make this assumption in our analysis, and justify it using Theorem 2.3.5. We conclude this section with a brief proposition which clarifies why the F-gain is sometimes used in place of the average F-gain when applying Theorem 2.3.5.

**Proposition 2.3.1.** In Eq. (2.4), suppose that all production rates are monotonic and the clearance rates are of the form \( c_i(x) = \lambda_i x \) for \( \lambda_i > 0 \), and let \( x \in L^2_{\tau} \) be a solution to Eq. (2.4). Then for \( i = 1, \ldots, n \), the F-gain satisfies
\[
|\overline{G_i}| \leq \sup_{0 \leq t \leq \tau} |G_i(t)|.
\]

**Proof.** We show that each \( G_i(t) \) is of constant sign. First, consider the case where \( p_i \) is non-decreasing. We claim that this forces \( G_i(t) \geq 0 \) for all \( t \in [0, \tau] \). To see this,
fix $t^* \geq 0$. If $p_i(\lambda_i x_i(t^*)) > 0$, then $\lambda_i x_i(t^*) > c_i^*$ and so

$$G_i(t^*) = \frac{p_i(\lambda_i x_i(t^*))}{\lambda_i x_i(t^*) - c_i^*} > 0.$$  

If instead $p_i(\lambda_i x_i(t^*)) \leq 0$, then we must have $\lambda_i x_i(t^*) \leq c_i^*$ and so $G_i(t^*) \geq 0$. Next, consider the case where $p_i$ is non-increasing. The same reasoning shows that $G_i(t) \leq 0$ for all $t > 0$ because the numerator and denominator of $G_i(t)$ are always of opposite sign.

To verify Eq. (2.6), suppose that $\sup_{0 \leq t \leq \tau} |G_i(t)| = A$ for some $A > 0$. If $G_i$ is positive, we have

$$\frac{1}{\int_0^\tau \psi^2(t) \frac{1}{G_i(t)} dt} \leq A \frac{1}{\int_0^\tau \psi^2(t) dt}.$$  

Multiplying by $\int_0^\tau \psi^2(t) dt$ and setting $\psi(t) = p_i(\lambda_i x_i(t))$ we obtain

$$|G_i| = \left| \frac{\int_0^\tau p_i(\lambda_i x_i(t))^2 dt}{\int_0^\tau p_i(\lambda_i x_i(t))^2 \frac{1}{G_i(t)}} \right| = \frac{\int_0^\tau p_i(\lambda_i x_i(t))^2 dt}{\int_0^\tau p_i(\lambda_i x_i(t))^2 \frac{1}{G_i(t)}} \leq A.$$  

For the case where $G_i$ is negative, we have $|G_i(t)| = -G_i(t)$ and repeating the argument gives

$$|G_i| = \left| \frac{\int_0^\tau p_i(\lambda_i x_i(t))^2 dt}{\int_0^\tau p_i(\lambda_i x_i(t))^2 \frac{1}{G_i(t)}} \right| = -\frac{\int_0^\tau p_i(\lambda_i x_i(t))^2 dt}{\int_0^\tau p_i(\lambda_i x_i(t))^2 \frac{1}{G_i(t)}} \leq A$$  

since $|G_i(t)| \leq A$ implies $-G_i(t) \leq A$.

\qed
Chapter 3

Promoter methylation in a mixed-feedback loop model

This chapter is concerned with the influence of clock gene promoter methylation on the period of the circadian clock. We approach this question by focusing on the PER transcriptional-translational feedback loop (TTFL). Our interest in the PER TTFL comes from earlier work of Kim and Forger, which we review at the start of this chapter.

3.1 The Kim-Forger and mixed-feedback loop models

3.1.1 The role of stoichiometry in the Kim-Forger model

The Kim-Forger model consists of three dynamic variables: the concentrations of per mRNA $M(t)$, cytosolic PER protein $P_c(t)$, and nuclear PER protein $P(t)$. These evolve as follows

$$\frac{dM}{dt} = \alpha_1 f(P; A_{tot}, K_d) - \beta_1 M,$$

$$\frac{dP_c}{dt} = \alpha_2 M - \beta_2 P_c,$$

$$\frac{dP}{dt} = \alpha_3 P_c - \beta_3 P,$$

$$f(P; A_{tot}, K_d) = \frac{1}{2} \left( 1 - \frac{P}{A_{tot}} - \frac{K_d}{A} + \sqrt{\left( 1 - \frac{P}{A_{tot}} - \frac{K_d}{A} \right)^2 + 4 \frac{K_d}{A_{tot}}} \right).$$

The mRNA $M(t)$ is transcribed at a rate dependent on the abundance of an activator protein $A_{tot}$, and on the amount of PER protein $P(t)$. The function $f$ which gives the transcription rate can be viewed as an idealization of how the binding of PER with its
activator CLOCK-BMAL1 affects the transcription of per mRNA. The parameter $A_{\text{tot}}$ represents the concentration of CLOCK-BMAL1, since this is the main transcriptional activator of PER. The model also assumes that the total activator concentration $A_{\text{tot}}$ is constant in time. Translation and nuclear export of per mRNA are described in a single step, resulting in the concentration of cytosolic PER protein $P_c(t)$. Finally $P_c(t)$ re-enters the nucleus as $P(t)$. The rates $\alpha_1, \alpha_3, \beta_1, \beta_2, \beta_3 \in \mathbb{R}$ are assumed to be constant. The function $f(P; A_{\text{tot}}, K_d)$ is referred to as a sequestration function [42, 43]. Since $f$ is a decreasing function of $P$, the entire system is a negative feedback loop.

Analysis of the Kim-Forger model provides a heuristic argument that oscillations can only be seen if the stoichiometry ratio $S := P_{eq}/A_{\text{tot}}$ satisfies

$$\frac{8}{9} \leq S \leq \frac{8}{7}.$$ 

We reproduce only part of their argument here, there is an additional global secant condition and bound on the stoichiometry derived in the supplemental material of [44].

We begin by non-dimensionalizing the Kim-Forger model in order to reduce the parameters under consideration. We assume that $\beta_i = \beta$ and rescale time to $\tau = \beta t$ so that the Kim-Forger model can be rewritten as

$$\frac{d\tilde{M}}{d\tau} = f(\tilde{P}, \tilde{A}_{\text{tot}}, \tilde{K}_d) - \tilde{M},$$
$$\frac{d\tilde{P}_c}{d\tau} = \tilde{M} - \tilde{P}_c,$$
$$\frac{d\tilde{P}}{d\tau} = \tilde{P}_c - \tilde{P}.$$

Kim and Forger justify this assumption by arguing that balanced timescales are the ideal conditions for oscillations to occur, see Sec. 2.3. This non-dimensionalization is discussed in more detail in Chapter 4.

The solid curves in Fig. 3.1 show how the shape of $f$ changes for various values of $\tilde{K}_d$. As $\tilde{K}_d$ decreases, $f$ becomes closer to a piecewise linear function. The binding of PER and CLOCK-BMAL1 is known to be tight in the circadian clock, so it is reasonable to assume that the dissociation constant of this reaction $\tilde{K}_d$ is small. It follows that it would be reasonable to approximate $f$ with a piecewise linear function. Kim and Forger make a different approximation, they use a linear approximation to the left of the “knee” in the graph of $f$ and a higher order approximation to the right. This can be seen in Fig. 3.1 because the left approximation is independent of $\tilde{K}_d$ and the approximation used to the right of the knee is not. We derive the
Figure 3.1: A comparison of the transcription function from the Kim-Forger model and its approximations for various values of the dissociation constant $\tilde{K}_d$. For $\tilde{K}_d \ll 1$, the transcription function takes on a piecewise linear shape with a knee at $\tilde{P} = \tilde{A}_{\text{tot}}$. Dashed lines correspond to the two approximations given in Prop. 3.1.1. Parameters: $\tilde{A}_{\text{tot}} = 2$.

piecewise approximation by defining $\varepsilon = \tilde{K}_d / \tilde{A}_{\text{tot}}$ and $\delta = \frac{4\varepsilon \tilde{P}/A}{(1-\tilde{P}/A+\varepsilon)^2}$. Expanding in $\delta$, we obtain

$$f(\tilde{P}; \tilde{A}_{\text{tot}}, \tilde{K}_d) = \frac{1}{2} \left(1 - \tilde{P}/\tilde{A}_{\text{tot}} - \varepsilon + \sqrt{(1 - \tilde{P}/\tilde{A}_{\text{tot}} - \varepsilon)^2 + 4\varepsilon}\right)$$

$$= \frac{1}{2} \left(1 - \tilde{P}/\tilde{A}_{\text{tot}} - \varepsilon + \sqrt{(1 - \tilde{P}/\tilde{A}_{\text{tot}} + \varepsilon)^2 + 4\varepsilon \tilde{P}/\tilde{A}_{\text{tot}}}\right)$$

$$= \frac{1}{2} \left(1 - \tilde{P}/\tilde{A}_{\text{tot}} - \varepsilon + \frac{4\varepsilon \tilde{P}/\tilde{A}_{\text{tot}}}{(1 - \tilde{P}/\tilde{A}_{\text{tot}} + \varepsilon)^2}\right)$$

$$= \frac{1}{2} \left(1 - \tilde{P}/\tilde{A}_{\text{tot}} - \varepsilon + \frac{2\varepsilon \tilde{P}/\tilde{A}_{\text{tot}}}{1 - \tilde{P}/\tilde{A}_{\text{tot}} + \varepsilon}\right) + O(\delta^2).$$

Proposition (3.1.1) shows how Eq. (3.1) can be rewritten as a piecewise approximation of $f$.

**Proposition 3.1.1.** $f(\tilde{P}; \tilde{A}_{\text{tot}}, \tilde{K}_d)$ may be approximated to zeroth order in $\delta$ as

$$f(\tilde{P}; \tilde{A}_{\text{tot}}, \tilde{K}_d) \approx \begin{cases} 
1 - \frac{\tilde{P}}{\tilde{A}_{\text{tot}}} + \frac{\varepsilon \tilde{P}/\tilde{A}_{\text{tot}}}{\varepsilon(1+\varepsilon) - \tilde{P}/\tilde{A}_{\text{tot}} - \varepsilon} & \text{if } \delta \ll 1 \text{ and } 1 - \tilde{P}/\tilde{A}_{\text{tot}} + \varepsilon > 0, \\
1 - \frac{\tilde{P}}{\tilde{A}_{\text{tot}}} & \text{if } \delta \ll 1 \text{ and } 1 - \tilde{P}/\tilde{A}_{\text{tot}} + \varepsilon < 0,
\end{cases}$$

and if one expands the expression $\frac{\varepsilon(1+\varepsilon)}{\tilde{P}/\tilde{A}_{\text{tot}} - \varepsilon}$ to first order in $\varepsilon$,

$$f(\tilde{P}; \tilde{A}_{\text{tot}}, \tilde{K}_d) \approx \begin{cases} 
1 - \tilde{P}/\tilde{A}_{\text{tot}} & \text{if } \delta \ll 1 \text{ and } 1 - \tilde{P}/\tilde{A}_{\text{tot}} + \varepsilon > 0, \\
\frac{\varepsilon}{\tilde{P}/\tilde{A}_{\text{tot}} - 1 - \varepsilon} & \text{if } \delta \ll 1 \text{ and } 1 - \tilde{P}/\tilde{A}_{\text{tot}} + \varepsilon < 0.
\end{cases}$$
The conditions for the approximation in Eq. (3.3) can be rewritten as individual inequalities

\[ \delta \ll 1 \quad \text{and} \quad 1 - \frac{\tilde{P}}{\tilde{A}_{\text{tot}}} + \varepsilon > 0 \iff P/A \ll 1 - (2\sqrt{2\varepsilon^2 + \varepsilon - 3\varepsilon}), \]
\[ \delta \ll 1 \quad \text{and} \quad 1 - \frac{\tilde{P}}{\tilde{A}_{\text{tot}}} + \varepsilon < 0 \iff P/A \gg 1 + (2\sqrt{2\varepsilon^2 + \varepsilon + 3\varepsilon}), \]

which are expressed in terms of the stoichiometry \( S := \frac{\tilde{P}}{\tilde{A}_{\text{tot}}} \).

**Proof.** Eq. (3.1) reduces to Eq. (3.2) depending on if \( 1 - \frac{\tilde{P}}{\tilde{A}_{\text{tot}}} + \varepsilon > 0 \) or \( 1 - \frac{\tilde{P}}{\tilde{A}_{\text{tot}}} + \varepsilon < 0 \). Neglecting terms of order \( \varepsilon \) in the positive case and order \( \varepsilon^2 \) in the negative case, one obtains Eq. (3.3). To rewrite the conditions for approximation as a single inequality, notice that the equation

\[ \Phi(\frac{\tilde{P}}{\tilde{A}_{\text{tot}}}) := (1 - \frac{\tilde{P}}{\tilde{A}_{\text{tot}}} + \varepsilon)^2 - 4\varepsilon\tilde{P}/\tilde{A}_{\text{tot}} = 0 \]

has roots \( x_L = 1 - (2\sqrt{2\varepsilon^2 + \varepsilon - 3\varepsilon}) \) and \( x_R = 1 + (2\sqrt{2\varepsilon^2 + \varepsilon + 3\varepsilon}) \). Since \( 1 - \frac{\tilde{P}}{\tilde{A}_{\text{tot}}} + \varepsilon \) is a decreasing function of \( \tilde{P}/\tilde{A}_{\text{tot}} \), we can only have \( 1 - \frac{\tilde{P}}{\tilde{A}_{\text{tot}}} + \varepsilon > 0 \) and \( \delta \ll 1 \) if \( \frac{\tilde{P}}{\tilde{A}_{\text{tot}}} \ll x_L \). By the same reasoning, the condition \( 1 - \frac{\tilde{P}}{\tilde{A}_{\text{tot}}} + \varepsilon < 0 \) and \( \delta \ll 1 \) corresponds to \( \frac{\tilde{P}}{\tilde{A}_{\text{tot}}} \gg x_R \).

Equipped with an approximation of the transcription function which is valid when the stoichiometry is far from unity, Kim and Forger use the secant conditions of Sec. 2.3 to obtain conditions for the presence of oscillations. Thron’s secant condition given in Theorem 2.3.4 applied to the Kim-Forger model reduces to the necessary condition

\[ \left| \frac{\frac{\partial f(\tilde{P}; \tilde{A}_{\text{tot}}, K_d)}{\partial \tilde{P}}}{f(\tilde{P}; \tilde{A}_{\text{tot}}, K_d)} \right| > \left( \sec \left( \frac{\pi}{3} \right) \right)^3 = 8 \] (3.4)

for oscillations to occur. Kim and Forger apply Eq. (3.4) to the approximate transcription functions to obtain a heuristic for the stoichiometry necessary for oscillations to occur. Using the approximation which is valid for \( S < 1 - (2\sqrt{2\varepsilon^2 + \varepsilon - 3\varepsilon}) \), we obtain \( f(\tilde{P}; \tilde{A}_{\text{tot}}, K_d) \approx 1 - \frac{\tilde{P}}{\tilde{A}_{\text{tot}}} \) and so the equilibrium condition \( f(\tilde{P}_{\text{eq}}; \tilde{A}_{\text{tot}}, K_d) = \tilde{P}_{\text{eq}} \) implies \( \tilde{P}_{\text{eq}} \approx \tilde{A}_{\text{tot}}/(\tilde{A}_{\text{tot}} + 1) \). Substituting this into the secant condition, we obtain the condition

\[ S \approx \frac{1}{\tilde{A}_{\text{tot}} + 1} > \frac{8}{9} \]

necessary for oscillations to occur. The same reasoning applied under the assumption
that $S > 1 + (2\sqrt{2}\varepsilon^2 + \varepsilon + 3\varepsilon)$ gives an approximation

$$\left| \frac{df}{d\tilde{P}} \right| \approx \frac{\varepsilon \tilde{A}_{\text{tot}}}{(\tilde{P} - \tilde{A}_{\text{tot}})^2} > 8$$

or making the substitution $\frac{\tilde{P}_{\text{eq}}}{\tilde{A}_{\text{tot}} - 1} \approx f(\tilde{P}_{\text{eq}}, \tilde{A}_{\text{tot}}, \tilde{K}_d) = \tilde{P}$, we obtain

$$\frac{\tilde{P}_{\text{eq}}}{\tilde{A}_{\text{tot}}} - 1 > 8$$

hence, rephrasing in terms of the stoichiometry

$$S = \frac{\tilde{P}_{\text{eq}}}{\tilde{A}_{\text{tot}}} < \frac{8}{7}.$$

Taken together, these two inequalities suggest that $\frac{8}{9} < S < \frac{8}{7}$ is necessary for oscillations to occur. Again, this argument is only a heuristic since approximations of the transcription function were used in deriving the oscillation conditions.

### 3.1.2 Mixed-feedback loop models

We return to the biological question motivating our analysis. Since promoter methylation affects the transcription of PER, one would expect that adding promoter methylation to the Kim-Forger model would result in a different form of $f$, and that analysis of the modified transcription function would reveal the influence of promoter methylation on the clock. This turns out to be true, but it is not obvious if one starts only with the Kim-Forger model. It is instead necessary to start with mass-action kinetics, add methylation to this more detailed model, and then study an approximation that resembles the Kim-Forger model. The more detailed description of the clock obtained using mass action kinetics is known as the mixed-feedback loop (MFL) model, first proposed by François and Hakim.

In an *in silico* study of evolution, François and Hakim were interested in determining if a bistable switch can be created from two mutually repressing transcription factors. This study led to the mixed-feedback loop (MFL) model. In the MFL model, protein $A$ is constitutively expressed and acts as an activator for protein $B$. Hence when lots of $A$ is freely available in the system, it binds to the promoter of $B$ increasing its transcription rate. The system constitutes a negative feedback loop because the protein $B$ can bind to $A$, reducing the abundance of free $A$ available for activating $B$. Hence, a high concentration of $A$ causes an increase in the expression of $B$, accelerating the binding of $A$ and $B$ which in turn reduces the presence of free $A$, and consequently decreases the expression rate of $B$. 
Since its initial discovery, the MFL model has been found in a variety of biologically important networks: the circadian clocks of neuropsora [45] and drosophila [46], the p53-Mdm2 module [47], and the \emph{E. coli} lactose operon [48]. More recently, the MFL model has been used in several studies as a minimal model of the circadian clock [44, 49, 50, 51]. In the setting of the mammalian circadian clock, \(B\) and \(A\) represent \emph{PER} and \emph{CLOCK-BMAL1}, respectively. Figure 3.2 shows a detailed representation of the MFL model. The original MFL model is shown in black, and the reactions drawn in blue are unique to our extension of the MFL model, which we refer to as the intermediate transcription rate mixed-feedback loop (IT-MFL) model.

The IT-MFL model can be obtained from the MFL model by adding a new promoter state \(g : M\) to represent methylation of the \emph{PER} promoters. This introduces three additional parameters \(\alpha_2, \theta_2,\) and \(\rho_M\) to the model. The rate constants \(\alpha_2\) and \(\theta_2\) describe the methylation and demethylation rates of the promoter, and \(\rho_M\) is the transcription rate associated with the methylated promoter state. We assume that \(\rho_M\) lies between the transcription rates of the active and inactive states, \(\rho_b\) and \(\rho_f\). Although DNA methylation and demethylation are catalyzed by two families of enzymes, the IT-MFL model approximates these reactions with first-order kinetics.

Applying mass action kinetics to the reaction network for the IT-MFL model shown in Fig. 3.2 produces the following system of governing equations. For the \emph{PER}
promoters, we have
\[\frac{dg}{dt} = \theta_1[g : A] + \theta_2[g : M] - \alpha_1[g][A] - \alpha_2[g],\]
\[\frac{dg : M}{dt} = \alpha_2[g] - \theta_2[g : M],\]
\[\frac{dg : A}{dt} = \alpha_1[g][A] - \theta_1[g : A],\]
\[g_{\text{tot}} = [g] + [g : M] + [g : A].\]

The conservation \(g_{\text{tot}} = [g] + [g : M] + [g : A]\) lets us reduce the dimensionality of the system in our simulation and analysis. Next, the protein concentrations obey
\[\frac{dr_b}{dt} = \rho_f[g] + \rho_b[g : A] + \rho_M[g : M] - \delta_r[r_b],\]
\[\frac{d[B]}{dt} = \beta[r_b] - \delta_B[B] - \gamma_+[A][B] + \gamma_-[A : B],\]
\[\frac{d[A]}{dt} = \rho_A - \gamma_+[A][B] - \delta_A[A] + \theta_1[g : A]
- \alpha_1[g][A] + \gamma_-[A : B],\]
\[\frac{d[A : B]}{dt} = \gamma_+[A][B] - \gamma_-[A : B] - \delta_{AB}[A : B].\]

The mRNA of \(B\) is produced at a rate dependent on the concentration of the three promoter states, weighted by their transcription rates \(\rho_f, \rho_b, \text{ and } \rho_M\). The decay rate \(\delta_r\) of the mRNA will be crucial in our perturbative calculations later in the chapter. Translation proceeds at rate \(\beta\) and thus increases the concentration \([B](t)\).

The protein \(B\) can be lost to decay at rate \(\delta_B[B]\) and binding with \([A]\) at a rate \(\gamma_+[A][B]\). Since this binding is reversible, there also is a contribution \(\gamma_-[A : B]\) due to the unbinding of \(A\) and \(B\). Unlike the Kim-Forger model, we do not assume that the activator concentration is constant and instead describe the concentration of free activator \([A](t)\) explicitly. It is still constitutively expressed at rate \(\rho_A\), and can interact with \([B]\) and the promoter \([g]\) through reactions already described. Finally the abundance of the bound protein \([A : B]\) is produced through a reversible reaction with rate constants \(\gamma_+\) and \(\gamma_-\) and can also decay at a rate \(\delta_{AB}[A : B]\).

The influence of methylation is described in the IT-MFL model by the parameters \(\alpha_2, \theta_2, \text{ and } \rho_M\). The inclusion of reversible \(A\) and \(B\) binding, described by \(\gamma_-\) is also a novel feature of our model. The importance of reversible binding is emphasized in the work of Kim and Forger, since this corresponds to the parameter \(\tilde{K}_d\), whose behaviour was important in the previous section. Hence it is useful to include this in circadian applications. We maintain that \(\gamma_-\) is nonzero in Sec. 3.2 but assume
irreversibility ($\gamma_- = 0$) in Sec. 3.3 to simplify the calculations. The parameters and dynamic variables in the full IT-MFL model are summarized in Tables 3.1-3.2.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Meaning</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\alpha_1$</td>
<td>$E \rightarrow E : A$ reaction rate</td>
</tr>
<tr>
<td>$\theta_1$</td>
<td>$E : A \rightarrow E$ reaction rate</td>
</tr>
<tr>
<td>$\alpha_2$</td>
<td>$E \rightarrow E : M$ reaction rate</td>
</tr>
<tr>
<td>$\theta_2$</td>
<td>$E : M \rightarrow E$ reaction rate</td>
</tr>
<tr>
<td>$\rho_M$</td>
<td>$per$ transcription rate (methylated promoters)</td>
</tr>
<tr>
<td>$\rho_f$</td>
<td>$per$ transcription rate (inactive promoters)</td>
</tr>
<tr>
<td>$\rho_b$</td>
<td>$per$ transcription rate (active promoters)</td>
</tr>
<tr>
<td>$\beta$</td>
<td>rate of PER translation</td>
</tr>
<tr>
<td>$\rho_A$</td>
<td>activator expression rate</td>
</tr>
<tr>
<td>$\gamma_+$</td>
<td>$A + B \rightarrow A : B$ reaction rate</td>
</tr>
<tr>
<td>$\gamma_-$</td>
<td>$A : B \rightarrow A + B$ reaction rate</td>
</tr>
<tr>
<td>$\delta_A$</td>
<td>decay rate of activator</td>
</tr>
<tr>
<td>$\delta_B$</td>
<td>decay rate of B</td>
</tr>
<tr>
<td>$\delta_{AB}$</td>
<td>decay rate of $A : B$</td>
</tr>
<tr>
<td>$\delta_r$</td>
<td>decay rate of $r_b$</td>
</tr>
</tbody>
</table>

Table 3.1: Parameters in the dimensional IT-MFL model. When chemical species $A$ and $B$ are bound to one another, we denote this by $A : B$.

<table>
<thead>
<tr>
<th>Dynamic variable</th>
<th>Meaning</th>
</tr>
</thead>
<tbody>
<tr>
<td>$[g]$</td>
<td>inactive promoter concentration</td>
</tr>
<tr>
<td>$[g : A]$</td>
<td>active promoter concentration</td>
</tr>
<tr>
<td>$[g : M]$</td>
<td>methylated promoter concentration</td>
</tr>
<tr>
<td>$[r_b]$</td>
<td>$per$ mRNA concentration</td>
</tr>
<tr>
<td>$[B]$</td>
<td>PER protein concentration</td>
</tr>
<tr>
<td>$[A]$</td>
<td>activator protein concentration</td>
</tr>
<tr>
<td>$[A : B]$</td>
<td>$per$ activator concentration</td>
</tr>
</tbody>
</table>

Table 3.2: Dynamic variables in the dimensional IT-MFL model.

For the rest of this chapter, we will study a dimensionless form of the IT-MFL model, following the approach taken by François and Hakim in their study of the MFL model. Let $\tilde{t}$ be the dimensionless time $\tilde{t} := \delta_r t$ and write $\dot{u} := \frac{du}{d\tilde{t}}$ for $u \in C^1(\mathbb{R})$. We normalize the promoter states so that $g_{tot} = 1$ and obtain dimensionless equations for their time evolution

\[
\begin{align*}
\dot{g} &= \tilde{\theta}_1 \left( (1 - g - g_M) + \frac{\tilde{\theta}_2}{\tilde{\theta}_1} g_M - g \frac{A}{A_0} \right) - \tilde{\alpha}_2 g, \\
\dot{g}_M &= \tilde{\alpha}_2 g - \tilde{\theta}_2 g_M,
\end{align*}
\]

in which $g = [g]/g_{tot}$ and $g_M = [g : M]/g_{tot}$. The dimensionless active promoter
concentration $g_A = [g : A]/g_{tot}$ is given by $g_A = 1 - g - g_M$. We rescale the protein concentrations so that

$$A = \sqrt{\gamma_+/\rho_A}[A], \quad B = \sqrt{\gamma_+/\rho_A}[B], \quad A_B = \sqrt{\gamma_+/\rho_A}[A : B], \quad r = \sqrt{\gamma_+/\rho_A}[r_b],$$

and obtain

$$\dot{r} = \rho_0 g + \rho_1 (1 - g - g_M) + \rho_2 g_M - r,$$
$$\dot{A} = \frac{1}{\delta} (1 - A \cdot B) - d_a A + K_d A_B + \mu \tilde{\theta}_1 \left((1 - g - g_M) - g \frac{A}{A_0}\right),$$
$$\dot{B} = \frac{1}{\delta} (r - A \cdot B) - d_b B + K_d A_B,$$
$$\dot{A}_B = \frac{A \cdot B}{\delta} - d_{AB} A_B.$$

(3.6)

To summarize, the IT-MFL model consists of the parameters in Table 3.3 and the six dynamic variables $g, g_M, r, A, B, A_B$ that evolve according to Eqs. (3.5) - (3.6). Aside from the nonlinearity introduced by the sequestration of $A$ by $B$, the dynamics of the MFL and IT-MFL models are linear. This weak nonlinearity has made the MFL model attractive for stochastic extensions [52].

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Formula</th>
<th>Meaning</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\rho_0$</td>
<td>$\frac{\beta_{rl}}{\gamma_+}$</td>
<td>$B$ transcription rate (inactive promoters)</td>
</tr>
<tr>
<td>$\rho_1$</td>
<td>$\frac{\beta_{rA}}{\rho_A \delta_r}$</td>
<td>$B$ transcription rate (active promoters)</td>
</tr>
<tr>
<td>$\tilde{\theta}_1$</td>
<td>$\frac{\theta_1}{\delta_r}$</td>
<td>$g : A \rightarrow g$ rate / per mRNA decay</td>
</tr>
<tr>
<td>$\delta$</td>
<td>$\sqrt{\gamma_+}$</td>
<td>scaled $B$ mRNA decay</td>
</tr>
<tr>
<td>$d_a$</td>
<td>$\frac{\delta_A}{\delta_r}$</td>
<td>normalized activator decay</td>
</tr>
<tr>
<td>$d_b$</td>
<td>$\frac{\delta_B}{\delta_r}$</td>
<td>normalized $B$ decay</td>
</tr>
<tr>
<td>$d_{AB}$</td>
<td>$\frac{\delta_{AB}}{\delta_r}$</td>
<td>normalized $A : B$ decay</td>
</tr>
<tr>
<td>$\mu$</td>
<td>$\sqrt{\gamma_+}$</td>
<td>activator consumption / production</td>
</tr>
<tr>
<td>$A_0$</td>
<td>$\frac{\beta}{\alpha} \sqrt{\gamma_+}$</td>
<td>dimensionless critical binding scale</td>
</tr>
<tr>
<td>$K_d$</td>
<td>$\frac{\gamma_+}{\rho_A \delta_r}$</td>
<td>dissociation constant for $A, B$ binding</td>
</tr>
<tr>
<td>$\tilde{\alpha}_2$</td>
<td>$\frac{\alpha_2}{\delta_r}$</td>
<td>scaled rate of promoter demethylation</td>
</tr>
<tr>
<td>$\tilde{\theta}_2$</td>
<td>$\frac{\tilde{\theta}_2}{\delta_r}$</td>
<td>scaled rate of promoter methylation</td>
</tr>
<tr>
<td>$\rho_2$</td>
<td>$\frac{\beta_{rM}}{\rho_A \delta_r}$</td>
<td>per transcription rate (methylated promoters)</td>
</tr>
</tbody>
</table>

Table 3.3: Parameters for the dimensionless form of the MFL model. The final three parameters are new to the IT-MFL model.
3.2 Perturbative analysis of full model

We first show that uniqueness of the equilibrium is maintained provided that the new promoter state is intermediate. We then move on to a perturbative approach. A large portion of the analysis of François and Hakim is concerned with the behaviour of the MFL model in the small $\delta$ limit ($\delta \ll 1$). It is difficult to estimate $\delta$ from data because it involves the expression rate of $A$. Recall that the production of $A$ is a multi-step process, and therefore unlikely to occur at a constant rate unless additional assumptions are imposed. Hence, it is unclear if $\delta$ is small in our system. At the same time, these calculations give some insight into how the additional promoter state affects the dynamics. Maintaining the assumption that the new promoter state is intermediate so that the equilibrium is unique, we use perturbation theory to find dominant order contributions to the eigenvalues of the model near this equilibrium. Again, the linear stability theory of the new model coincides with the MFL model under this intermediate promoter state assumption.

3.2.1 Equilibrium uniqueness conditions

The assumption that $A$ is an activator forces the MFL model to be monostable. Expressed in terms of model parameters, $A$ functioning as an activator means that the promoter state $[g : A]$ should have a higher transcription rate than the unbounded promoter state $[g]$. Hence, we have $\rho_0 < \rho_1$ when $A$ is an activator. François and Hakim showed that this leads to monotonicity by first reducing the equilibrium equations of the MFL model to

$$1 = F_{\text{MFL}}(A) = \delta d_A A + \frac{A(\rho_1 A + \rho_0 A_0)}{(A + A_0)(A + \delta d_b)}. \quad (3.7)$$

Since $F_{\text{MFL}}$ is continuous, $F_{\text{MFL}}(0) = 0$, and $\lim_{A \to \infty} F_{\text{MFL}}(A) = \infty$, a solution to $F(A) = 1$ must exist by the intermediate value theorem applied on the interval $[0, \infty)$. This solution is unique because $F_{\text{MFL}}(A)$ is monotonic, and so there exists at most one solution to Eq. (3.7).

The same monotonicity argument applies to the IT-MFL model. We find that at steady-state, Eqs. (3.5)-(3.6) reduce to

$$1 = F_{\text{IT-MFL}}(A) = \delta d_A A + \frac{A \left(1 - \frac{\tilde{K}_2^\gamma}{d_{\tilde{A}B}}\right) \left(\rho_1 A + \left(\rho_0 + \frac{\rho_2 \tilde{\theta}_2}{\tilde{\theta}_2}\right) A_0\right)}{(A + A_0 \left(1 + \frac{\tilde{\theta}_2}{\tilde{\theta}_2}\right)) \left(A + \delta d_b \left(1 + \frac{\tilde{K}_2}{d_{\tilde{A}B}}\right)\right)}. \quad (3.8)$$

If we assume the additional promoter state has an intermediate transcription rate ($\rho_0 < \rho_2 < \rho_1$) then symbolic differentiation shows that $F_{\text{IT-MFL}}(A)$ is monotonic in
Moreover, clearly $F_{IT^{-MFL}}(0) = 0$ and $\lim_{A \to \infty} F_{IT^{-MFL}}(A) = \infty$, so there remains a unique non-negative solution to the system’s equilibrium equation. Also, notice that Eq. (3.7) is recovered from Eq. (3.8) in the limit of no methylation ($\tilde{\alpha}_2 \to 0$) and tight activator-target binding ($\tilde{K}_d \to 0$). This is to be expected, because the original governing equations are also recovered in this limit.

When the conditions for a unique non-negative equilibrium are not satisfied, a closed-form expression for the boundary in parameter space between unique and multiple equilibria can be derived for the MFL model [53, 54]. Alternatively one can approach the problem perturbatively, as in the work of François and Hakim. Notice that Eq. (3.8) can be rewritten as a polynomial of the form

$$0 = c_1 A^2 \delta^2 + c_2 A \delta^2 + c_3 A^3 \delta + c_4 A^2 \delta + c_5 A \delta + c_6 \delta + c_7 A^2 + c_8 A \quad (3.9)$$

We apply the method of dominant balance to solve Eq. (3.9) because the term of highest degree in $A$ vanishes as $\delta \to 0$. In this approach, one hypothesizes that two terms become largest as $\delta \to 0$, and then test the validity of this approximation by comparing the scaling to all other terms in the equation. The scaling is said to be “inconsistent” if one of the terms that was assumed to be lower order ends up dominating over the balanced terms. This is tedious to carry out by hand because there are so many possible pairings of terms, but it is easily done symbolically. The symbolic calculation reveals that the only consistent scaling options for $A$ are $A \sim 1/\delta$, $A \sim 1$, or $A \sim \delta$ as $\delta \to 0$. These are the same scalings found by François and Hakim in their analysis. Each scaling leads to an approximate solution for the corresponding root

$$A_1 := \delta \frac{d_b \left(1 + \frac{\tilde{\alpha}_2}{\theta_2}\right) \left(1 + \frac{\tilde{K}_d}{d_{AB}}\right)}{\rho_0 - 1 + \frac{\tilde{\alpha}_2}{\theta_2} (\rho_2 - 1)} + \mathcal{O}(\delta^2), \quad (3.10)$$

$$A_2 := \frac{A_0 \left(\rho_0 - 1 + \frac{\tilde{\alpha}_2}{\theta_2} (\rho_2 - 1)\right)}{1 - \rho_1} + \mathcal{O}(\delta), \quad (3.11)$$

$$A_3 := \frac{1 - \rho_1}{\delta d_a} + \mathcal{O}(1). \quad (3.12)$$

Once again, Eqs. (3.10)-(3.12) reduce to the analogous expressions found by François and Hakim in the no methylation and tight activator-target binding limit ($\tilde{K}_d \to 0$). We verify in Prop. 3.2.1 that the existence of three distinct roots to the equilibrium equation is consistent with the monostability conditions discussed in the previous section.

**Proposition 3.2.1.** If $\rho_0 < \rho_2 < \rho_1$ then exactly one of the steady-states given
3.2. PERTURBATIVE ANALYSIS OF FULL MODEL

in Eqs. (3.10)-(3.12) will be non-negative at dominant order. In the particular case where \( \rho_0 < 1 < \rho_2 \), it cannot be determined apriori which of the three steady-states is non-negative at dominant order.

Proof. If \( \rho_0 < \rho_2 < \rho_1 \) then there are four possible locations where the number 1 could go in this string of inequalities. For instance, \( \rho_0 < \rho_2 < \rho_1 < 1 \). In each case, the sign of the numerator and denominator of each steady-state is fully determined. We see in Table 3.4 that the only ambiguity is in the case where \( \rho_0 < 1 < \rho_2 < \rho_1 \). Fortunately it is clear that the numerator and denominator of \( A_1 \) and \( A_2 \) are always of opposite sign. Hence there still is a unique steady-state when \( \rho_0 < 1 < \rho_2 < \rho_1 \) even though it is not clear whether it is \( A_1 \) or \( A_2 \) apriori.

<table>
<thead>
<tr>
<th>( \rho_0 &lt; \rho_2 &lt; \rho_1 &lt; 1 )</th>
<th>( A_1 )</th>
<th>( A_2 )</th>
<th>( A_3 )</th>
</tr>
</thead>
<tbody>
<tr>
<td>( \rho_0 &lt; \rho_2 &lt; \rho_1 &lt; 1 )</td>
<td>+/-</td>
<td>-/+</td>
<td>+/-</td>
</tr>
<tr>
<td>( \rho_0 &lt; \rho_2 &lt; 1 &lt; \rho_1 )</td>
<td>+/-</td>
<td>-/-</td>
<td>-/+</td>
</tr>
<tr>
<td>( \rho_0 &lt; 1 &lt; \rho_2 &lt; \rho_1 )</td>
<td>+/-</td>
<td>+/-</td>
<td>-/+</td>
</tr>
<tr>
<td>( 1 &lt; \rho_0 &lt; \rho_2 &lt; \rho_1 )</td>
<td>+/-</td>
<td>+/-</td>
<td>+/-</td>
</tr>
</tbody>
</table>

Table 3.4: The sign of the dominant order contributions to the three steady-states in the IT-MFL model depends on inequalities of the form \( \rho_i < 1 \) or \( \rho_i > 1 \) for \( i = 0, 1, 2 \). The intermediate transcription rate assumption \( \rho_0 < \rho_2 < \rho_1 \) reduces this to only four possible cases, shown in each row of the table. The entries of the table indicate the sign of the numerator and denominator of the steady-states in each case, with non-negative entries highlighted in grey. The question marks in the \( \rho_0 < 1 < \rho_2 < \rho_1 \) row indicate that the sign is ambiguous in this case. Notice that the uniqueness of the steady-state still holds since \( A_1 \) and \( A_2 \) are forced to have opposite signs, so one and only one of them will be non-negative.

3.2.2 Linear stability analysis

Understanding the approximate near-equilibrium behaviour of the model is a natural next step in our analysis. As in the previous section, we assume the new promoter state is intermediate and that \( A \) is an activator. In terms of model parameters, these two assumptions give

\[
\rho_0 < \rho_2 < \rho_1.
\]

This simplifies the analysis because only one non-negative equilibrium exists in this case, and is reasonable for the biological system we are interested in. We know from Prop. 3.2.1 that the IT-MFL model has a single non-negative equilibrium \( A_{eq} := A_2 \) when \( \rho_0 < \rho_2 < \rho_1 \). Focusing on this equilibrium, we adopt the notation that a subscript eq refers to the steady-state value of a dynamic variable corresponding to the equilibrium \( A(t) = A_{eq} \).
The eigenvalues of the Jacobian evaluated at the unique equilibrium are conveniently expressed in terms of the equilibrium promoter and protein concentrations. In the MFL model, these concentrations are

\[
\begin{align*}
    g_{eq}^{\text{MFL}} &= \frac{A_0}{A_{eq} + A_0} \\
    B_{eq}^{\text{MFL}} &= \frac{\rho_1 A_{eq} + \rho_0 A_0}{(A_{eq} + A_0)(A_{eq} + \delta d_b)},
\end{align*}
\]

and in the IT-MFL model

\[
\begin{align*}
    g_{eq}^{\text{IT-MFL}} &= \frac{A_0}{A_{eq} + A_0 \left(1 + \frac{\tilde{\alpha}_2}{\tilde{\theta}_2}\right)}, \\
    B_{eq}^{\text{IT-MFL}} &= \frac{\left(1 + \frac{\tilde{K}_{d_{AB}}}{\rho_{1}}\right) \left(\rho_1 A_{eq} + \left(\rho_0 + \frac{\tilde{\rho}_2}{\tilde{\theta}_2}\right) A_0\right)}{(A_{eq} + A_0 \left(1 + \frac{\tilde{\alpha}_2}{\tilde{\theta}_2}\right)) \left(A_{eq} + \delta d_b \left(1 + \frac{\tilde{K}_{d_{AB}}}{\rho_{1}}\right)\right)}.
\end{align*}
\]

Using the expressions for the equilibrium concentrations, the eigenvalues in the MFL model may be written as

\[
\begin{align*}
    \lambda^{\text{MFL}}_i &= -\omega_i \left(\frac{\tilde{\theta}_1 g_{eq}^{\text{MFL}} A_{eq} (\rho_1 - \rho_0)}{\delta A_0 (A_{eq} + B_{eq}^{\text{MFL}})}\right)^{\frac{1}{3}} + O(1) \\
    \lambda^{\text{MFL}}_4 &= -(A_{eq} + B_{eq}^{\text{MFL}}) + O(1),
\end{align*}
\]

where \(\omega_i\) denotes the \(i\)-th cubic root of unity for \(i = 1, 2, 3\). Similar expressions hold when the IT-MFL model is linearised at its \(A_{eq}\) value

\[
\begin{align*}
    \lambda^{\text{IT-MFL}}_i &= -\omega_i \left(\frac{\tilde{\theta}_1 g_{eq}^{\text{IT-MFL}} A_{eq} (\rho_1 - \rho_0)}{\delta A_0 (A_{eq} + B_{eq}^{\text{IT-MFL}})}\right)^{\frac{1}{3}} + O(1) \\
    \lambda^{\text{IT-MFL}}_4 &= -(A_{eq} + B_{eq}^{\text{IT-MFL}}) + O(1),
\end{align*}
\]

however two additional eigenvalues are present

\[
\begin{align*}
    \lambda^{\text{IT-MFL}}_5 &= \left(1 + \frac{\tilde{\alpha}_2}{\tilde{\theta}_2}\right) \rho_1 - \left(\rho_0 + \frac{\tilde{\alpha}_2}{\tilde{\theta}_2}\rho_2\right) \frac{1}{\tilde{\theta}_2} (\rho_0 - \rho_1) + O(\delta) \\
    \lambda^{\text{IT-MFL}}_6 &= -d_{AB} + O(\delta)
\end{align*}
\]

Under the assumption that \(\rho_0 < \rho_2 \leq \rho_1\), the \(\lambda^{\text{IT-MFL}}_5\) eigenvalue is stable at dominant order.
Proposition 3.2.2. If $\rho_0 < \rho_2 \leq \rho_1$ then $\lambda_{5}^{IT-MFL} < 0$ to dominant order as $\delta \to 0$.

Proof. Let $\lambda \in \mathbb{R}$ such that $\lambda_{5}^{IT-MFL} = \lambda + \mathcal{O}(\delta)$ as $\delta \to 0$. We have

$$
\lambda = \frac{1 + \tilde{\alpha}_2}{\tilde{\theta}_2} \rho_1 - \left( \rho_0 + \frac{\tilde{\alpha}_2}{\tilde{\theta}_2} \rho_2 \right) = -\left( \rho_0 - \rho_1 \right) + \frac{\tilde{\alpha}_2}{\tilde{\theta}_2} \left( \rho_1 - \rho_2 \right) = -\tilde{\theta}_2 + \tilde{\alpha}_2 \left( \frac{\rho_1 - \rho_2}{\rho_0 - \rho_1} \right).
$$

Notice that $\lambda < 0$ if and only if

$$
\frac{\tilde{\alpha}_2}{\tilde{\theta}_2} \left( \frac{\rho_1 - \rho_2}{\rho_0 - \rho_1} \right) < 1. \tag{3.13}
$$

It is always the case that $\frac{\tilde{\alpha}_2}{\tilde{\theta}_2} > 0$ and $\frac{\rho_1 - \rho_2}{\rho_0 - \rho_1} \leq 0$ follows from the assumption that $\rho_0 < \rho_2 \leq \rho_1$. So as long as $\rho_0 < \rho_2 \leq \rho_1$, the left hand side of Eq. (3.13) is non-positive and the inequality holds true.

Clearly, the other new eigenvalue $\lambda_{6}^{IT-MFL}$ is stable at dominant since $d_{AB}$ is a reaction rate, hence $d_{AB} \geq 0$. Taken together, the equilibrium and linear stability analysis suggest the MFL and IT-MFL models are qualitatively similar provided that $\rho_0 < \rho_2 < \rho_1$. A natural extension of this work would be to see if this phenomenon persists in $n$-promoter state models, and to determine how different the models can be when the ordering of the promoter states is not satisfied.

### 3.3 Perturbative period estimation

The goal of this section is to determine how the period of the IT-MFL model is influenced by methylation. In order to simplify the boundary-layer calculations, we assume the binding of $A$ and $B$ is irreversible.

#### 3.3.1 Derivation of a zeroth order period estimate

The simulations in Fig. 3.3 show that it is possible to change the period of the IT-MFL model by only altering the methylation parameters. In essence this is consistent with the experiments that motivated this work. To analyze such solutions, we use the same technique as François and Hakim.

Oscillatory solutions of the MFL model alternate between phases of high-$A$/low-$B$ and low-$A$/high-$B$ concentration. Such phases are referred to as Phases I & II of the limit cycle, respectively. By solving the governing equations in each phase of the limit cycle and making some additional approximations, an $\mathcal{O}(\delta^0)$ approximation of the period can be obtained. The boundary layer structure of these oscillations –
Figure 3.3: Oscillatory solutions to the MFL and IT-MFL models. In both cases, the oscillatory solutions decompose into phases of high $A$ / low $B$ (Phase I) and high $B$ / low $A$ (Phase II) concentration. Promoter states are shown for both models. Simulation parameters: $\delta = 3 \times 10^{-3}$, $\rho_0 = 0$, $\rho_1 = 1.45$, $\tilde{\theta}_1 = 1.33$, $d_a = 0.33$, $d_b = 0.33$, $\mu = 0.31$, $A_0 = 4$, $\rho_2 = 0$, $\tilde{\alpha}_2 = 1$, $\tilde{\theta}_2 = 2$. 
schematized in Fig. 3.4 will become relevant in our derivation of a \( O(\delta^{1/2}) \) period estimate in the next section.

\[
\begin{array}{c|c|c|c}
O(\sqrt{\delta}) & O(\sqrt{\delta}) & O(\delta) & O(\delta) \\
\hline
\text{Phase I} & \text{Phase II} & \\
(t=0) & (t=t_1) & (t=t_2) & \\
\text{BL} & \text{BL} & \text{BL} & \text{BL} \\
\text{(High A, Low B)} & \text{(High B, Low A)} & \\
\end{array}
\]

Figure 3.4: Structure of the boundary layers in a limit-cycle solution to the MFL model. Boundary layers BL\(_2\) and BL\(_3\) form when the system transitions from a phase of high A to high B concentration. Boundary layers BL\(_1\) and BL\(_4\) appear as the quasi-steady state approximation for \( g \) breaks down.

Assuming that the binding of \( A \) with \( B \) is irreversible (\( \tilde{K}_d = 0 \)), the governing equations of the IT-MFL model reduce to

\[
\begin{align*}
\dot{g} &= \tilde{\theta}_1 \left(1 - g - g_M\right) + \frac{\tilde{\theta}_2}{\tilde{\theta}_1} g_M - g \frac{A}{A_0} - \tilde{\alpha}_2 g, \\
\dot{g}_M &= \tilde{\alpha}_2 g - \tilde{\theta}_2 g_M, \\
\dot{r} &= \rho_0 g + \rho_1 (1 - g - g_M) + \rho_2 g_M - r, \\
\dot{A} &= \frac{1}{\delta} (1 - A \cdot B) - d_a A + \tilde{\mu} \tilde{\theta}_1 \left(1 - g - g_M\right) - g \frac{A}{A_0}, \\
\dot{B} &= \frac{1}{\delta} (r - A \cdot B) - d_b B.
\end{align*}
\] (3.14)-(3.16)

We ignore the time evolution of \( A_B \) since it does not influence the dynamics of the other variables in the model under the irreversibly assumption. During Phase I, \( A \) is large so it is useful to rewrite Eqs. (3.14)-(3.16) in terms of a rescaled variable \( a := \delta A \). Following this change of variable, the leading order terms in Eqs. (3.14)-(3.16) are given by algebraic equations for \( g \) and \( B \)

\[
\begin{align*}
g &= \frac{\delta A_0}{a} \left(1 - \left(1 - \frac{\tilde{\theta}_2}{\tilde{\theta}_1}\right) g_M\right) \\
B &= \frac{\delta r_0}{a}.
\end{align*}
\]
and linear differential equations for $g_M(t), r(t), a(t)$

$$\dot{g}_M = -\tilde{\theta}_2 g_M$$  \hspace{1cm} (3.17)

$$\dot{r} = \rho_1(1 - g - g_M) + g_M \rho_2 - r$$

$$\dot{a} = 1 - r - d_a a.$$  \hspace{1cm} (3.18)

We obtain symbolic solutions to Eqs. (3.17)-(3.18) by imposing the initial conditions $r_I(0) = r_1, a_I(0) = 0,$ and $g_{M,I}(0) = g_{M1}$. This choice of initial conditions is consistent with $A$ being small at the start of the limit cycle. The constants $r_1, g_{M1} \in \mathbb{R}$ will be determined later when we derive a closed system of equations for the period of the oscillator. Since Eqs. (3.17)-(3.18) are linear, they are easily solved symbolically, or using standard ODE solution techniques.

Phase I ends and Phase II begins with $A$ falling to a low steady-state value and $B$ growing to the scale $\delta^{-1}$. We define another rescaled variable $b = \delta B$ and under this change of variable, the leading order terms in Eqs. (3.5)-(3.6) are given by an algebraic equation for $A$

$$A = \frac{\delta}{b}$$

and linear differential equations for $g(t), g_M(t), r(t),$ and $b(t)$

$$\dot{g} = \tilde{\theta}_1 \left(1 - g - g_M + \frac{\rho_2}{\tilde{\theta}_1} g_M\right) - \tilde{\alpha}_2 g,$$

$$\dot{g}_M = \tilde{\alpha}_2 g - \tilde{\theta}_2 g_M,$$

$$\dot{r} = \rho_0 g + \rho_1(1 - g - g_M) + g_M \rho_2 - r,$$

$$\dot{b} = r - bd_b - 1.$$

For Phase II, we impose initial conditions $g_{II}(0) = 0, g_{M,II}(0) = g_{M2}, r_{II}(0) = r_2,$ and $b_{II}(0) = 0$. We use the same coordinates as François and Hakim, where time $t = 0$ coincides with the start of the current phase of the limit cycle.

The period of the IT-MFL model can be estimated to zeroth order in $\delta$ by imposing that the solution is continuous across the two phases of the limit cycle. This results in the following system of equations

$$r_I(t_1) = r_2, \quad a_I(t_1) = 0, \quad g_{M,I}(t_1) = g_{M2},$$

$$r_{II}(t_2) = r_1, \quad b_{II}(t_2) = 0, \quad g_{M,II}(t_2) = g_{M1}.$$  \hspace{1cm} (3.19)

(3.20)

Eqs. (3.19)-(3.20) constitute a fully-determined nonlinear system of equations for the unknowns $\{t_1, t_2, r_1, r_2, g_{M1}, g_{M2}\}$. Such a system can be solved numerically, or
simplified to a two-dimensional system in the variables \( \{ t_1, t_2 \} \) using symbolic algebra.

To understand the nature of solutions to Eqs. (3.19)-(3.20), we make some additional assumptions. First, we assume the decay rate of \( B \) is smaller than the decay rate of its mRNA. Second, we assume the transcription rate corresponding to the active promoter state is larger than the expression rate of \( A \). When expressed in terms of the model parameters, these two assumptions give us \( d_b = 0 \) and \( \rho_1 \gg 1 \). Under the assumption \( \rho_1 \gg 1 \), it is justifiable to Taylor expand the system of implicit equations in powers of \( \frac{1}{\rho_1} \). In addition, it can be verified by simulation and later confirmed by the approximate formulas in Table 3.5 that \( t_1 \) decreases as \( \rho_1 \) increases. This allows us to also Taylor expand in powers of \( t_1 \). Finally, we also neglect terms that are exponentially small in \( t_2 \). When terms proportional to \( e^{-t_2} \) are neglected in the equation \( r_{II}(t_2) = r_1 \), it reduces to

\[
  r_1 \approx \frac{\tilde{\alpha} \rho_2 + \tilde{\theta} \rho_0}{\tilde{\alpha} + \tilde{\theta}}.
\]

Applying the same strategy to \( g_{M,II}(t_2) = g_{M,1} \) gives

\[
  g_{M,1} \approx \frac{\tilde{\alpha}}{\tilde{\theta} + \tilde{\alpha}}.
\]

Next, an approximation for \( t_1 \) can be obtained from the equation \( a_I(t_1) = 0 \). Since \( t_1 \) is small when \( \rho_1 \) is large, we may Taylor expand \( a_I(t_1) \) to second order in \( t_1 \) and obtain the approximate solution

\[
  t_1 \approx \frac{2(1 - \rho_0)}{\rho_1} + \frac{2(1 - \rho_2) \tilde{\alpha}}{\rho_1} \tilde{\theta}_2.
\]

Next, we obtain a value of \( g_{M,2} \) by expanding the equation \( g_{M,I}(t_1) = g_{M,2} \) to zeroth order in powers of \( \rho_1^{-1} \) to obtain

\[
  g_{M,2} \approx g_{M,1}.
\]

Approximating \( r_I(t_1) = r_2 \) to zeroth order in \( \rho_1^{-1} \) gives

\[
  r_2 = \frac{2 - \rho_0 + (2 - \rho_2) \tilde{\alpha} \tilde{\theta}_2}{1 + \tilde{\alpha} \tilde{\theta}_2}.
\]

Finally, an approximate expression for \( t_2 \) can be obtained by solving \( b_{II}(t_2) = 0 \) and
neglecting terms that decay exponentially fast in $t_2$ to obtain

$$t_2 \approx 2 + \frac{\rho_1 - \rho_0 + \tilde{\alpha}_2 (\rho_1 - \rho_2)}{\tilde{\theta}_1 \left(1 - \rho_0 + (1 - \rho_2) \tilde{\alpha}_2 \right) \left(1 + \frac{\tilde{\alpha}_2}{\tilde{\theta}_1} \right)}.$$

<table>
<thead>
<tr>
<th>Equation</th>
<th>MFL</th>
<th>IT-MFL</th>
</tr>
</thead>
<tbody>
<tr>
<td>$a_I(t_1) = 0$</td>
<td>$t_1 = \frac{2(1-\rho_0)}{\rho_1}$</td>
<td>$t_1 = \frac{2(1-\rho_0)}{\rho_1} + \frac{2(1-\rho_2) \tilde{\alpha}_2}{\tilde{\alpha}_2}$</td>
</tr>
<tr>
<td>$r_I(t_1) = r_2$</td>
<td>$r_2 = 2 - \rho_0$</td>
<td>$r_2 = \frac{2-\rho_0+(2-\rho_2) \tilde{\alpha}_2}{1 + \frac{\tilde{\alpha}_2}{\tilde{\theta}_1}}$</td>
</tr>
<tr>
<td>$r_{II}(t_2) = r_1$</td>
<td>$r_1 = \rho_0$</td>
<td>$r_1 = \frac{\rho_0+\rho_2 \tilde{\alpha}_2}{1 + \frac{\tilde{\alpha}_2}{\tilde{\theta}_1}}$</td>
</tr>
<tr>
<td>$b_{II}(t_2) = 0$</td>
<td>$t_2 = 2 + \frac{\rho_2-\rho_0}{\tilde{\theta}_1 (1-\rho_0)}$</td>
<td>$t_2 = 2 + \frac{\rho_1-\rho_0 + \tilde{\alpha}_2 (\rho_1-\rho_2)}{\tilde{\theta}_1 \left(1 - \rho_0 + (1 - \rho_2) \tilde{\alpha}_2 \right) \left(1 + \frac{\tilde{\alpha}_2}{\tilde{\theta}_1} \right)}$</td>
</tr>
<tr>
<td>$g_{M,I}(t_1) = g_{M2}$</td>
<td>N/A</td>
<td>$g_{M2} = \frac{\tilde{\alpha}_2}{\tilde{\alpha}_2 + \tilde{\theta}_2}$</td>
</tr>
<tr>
<td>$g_{M,II}(t_2) = g_{M1}$</td>
<td>N/A</td>
<td>$g_{M1} = \frac{\tilde{\alpha}_2}{\tilde{\alpha}_2 + \tilde{\theta}_2}$</td>
</tr>
</tbody>
</table>

Table 3.5: Limiting value of period estimate for MFL and IT-MFL models.

The results of this approximation in the MFL and IT-MFL settings are compared in Table 3.5. Figure 3.5 shows reasonable agreement between numerical period estimation and the approximate expressions from Table 3.5. Since we have Taylor expanded in powers of $\frac{1}{\rho_1}$, note that we expect to see agreement as $\rho_1$ gets large in Fig. 3.5.

![Figure 3.5: Comparison of numerically estimated period and the limiting values given in Table 3.5.](image)

We see $t_2^{IT-MFL} < t_2^{MFL}$ when $\rho_1$ is large, as proved in Remark 2. Simulation parameters: $\delta = 10^{-4}$, $\rho_0 = 0$, $\tilde{\theta}_1 = 1.33$, $d_a = 0.3$, $d_b = 0$, $\mu = 0.31$, $A_0 = 4$, $\rho_2 = 0.5$, $\tilde{\alpha}_2 = 1$, $\tilde{\theta}_2 = 1$.

Comparing the MFL and IT-MFL period estimates, it is immediate that $t_1$ is always larger in the IT-MFL model and that its contribution to the period vanishes.
as $\rho_1 \to \infty$. Hence in the large $\rho_1$ limit, the period is approximately equal to $t_2$. The following two remarks give some interpretation to the extra terms that appear in the expression for $t_2$ in the case of the IT-MFL model.

**Remark 2.** $t_2$ depends linearly on $\rho_1$ with a slope given by

\[
\frac{\partial t_2^\text{MFL}}{\partial \rho_1} = \frac{1}{\theta_1 (1 - \rho_0)},
\]

(3.21)

\[
\frac{\partial t_2^\text{IT-MFL}}{\partial \rho_1} = \frac{1}{\theta_1 \left(1 - \rho_0 + \frac{\tilde{\alpha}_2}{\tilde{\theta}_2} (1 - \rho_2)\right) \left(1 + \frac{\tilde{\alpha}_2}{\tilde{\theta}_2}\right)}.
\]

(3.22)

In the case that $\rho_0 < 1$ and $\rho_2 < 1$, both $t_2^\text{MFL}$ and $t_2^\text{IT-MFL}$ are monotonically increasing in $\rho_1$ with

\[
\frac{\partial t_2^\text{IT-MFL}}{\partial \rho_1} \leq \frac{\partial t_2^\text{MFL}}{\partial \rho_1}.
\]

An expression of the form $1 - \rho_0 > 0$ may be rewritten in dimensional parameters as $\delta_r \rho_A > \beta \rho_f$. So we see the sign and magnitude of our approximation for the slope are determined by how the timescales of activator and target production compare to one another. Also note that the right hand side of Eq. (3.22) becomes larger or smaller relative to the corresponding expression in the MFL model in Eq. (3.21) depending on if $\rho_2 < 1$ or $\rho_2 > 1$.

In the case where $\rho_0 < 1$ and $\rho_2 < 1$, we obtain a stronger result where the period of the IT-MFL model is controlled by the period of the MFL model up to a constant.

**Proposition 3.3.1.** If $\rho_0 < 1$, $\rho_2 < 1$, and $\rho_1 > \max(\rho_0, \rho_2)$ then

\[
t_2^\text{IT-MFL} \leq t_2^\text{MFL} + C
\]

with $C = \frac{\rho_1 - \rho_2}{\tilde{\theta}_1} \min\left(\frac{\tilde{\alpha}_2}{\tilde{\theta}_2 (1 - \rho_0)}, \frac{1}{1 - \rho_2}\right)$.

*Proof.* First, notice that $\left(1 + \frac{\tilde{\alpha}_2}{\tilde{\theta}_2}\right) \geq 1$, and since we have assumed $\rho_0 < \rho_1$ and $\rho_2 < \rho_1$

\[
t_2^\text{IT-MFL} = 2 + \frac{\rho_1 - \rho_0 + \frac{\tilde{\alpha}_2}{\tilde{\theta}_2} (\rho_1 - \rho_2)}{\tilde{\theta}_1 \left(1 - \rho_0 + (1 - \rho_2) \frac{\tilde{\alpha}_2}{\tilde{\theta}_2}\right) \left(1 + \frac{\tilde{\alpha}_2}{\tilde{\theta}_2}\right)} \leq 2 + \frac{\rho_1 - \rho_0 + \frac{\tilde{\alpha}_2}{\tilde{\theta}_2} (\rho_1 - \rho_2)}{\tilde{\theta}_1 \left(1 - \rho_0 + (1 - \rho_2) \frac{\tilde{\alpha}_2}{\tilde{\theta}_2}\right)}.
\]

(3.23)
Using $1 - \rho_0 + (1 - \rho_2) \frac{\delta_2}{\theta_2} \geq 1 - \rho_0$ and Eq. (3.23), we find that
\[
t_2^{\text{IT-MFL}} \leq 2 + \frac{\rho_1 - \rho_0}{\theta_1(1 - \rho_0)} + \frac{1}{\frac{\delta_1(1 - \rho_0)}{\theta_2(\rho_1 - \rho_2)} + \theta_1 \left(\frac{1 - \rho_2}{\rho_1 - \rho_2}\right)}.
\] (3.24)

Since we assumed $\rho_0 < 1$ and $\rho_2 < 1$, we know $(a + b)^{-1} \leq \min(a^{-1}, b^{-1})$ with $a = \frac{\delta_1(1 - \rho_0)}{\theta_2(\rho_1 - \rho_2)}$ and $b = \theta_1 \left(\frac{1 - \rho_2}{\rho_1 - \rho_2}\right)$ and so we obtain from Eq. (3.24) $t_2^{\text{IT-MFL}} \leq t_2^{\text{MFL}} + C$ with $C = \frac{\rho_1 - \rho_2}{\theta_1} \min \left(\frac{\theta_2}{\theta_2(1 - \rho_0)}, \frac{1}{1 - \rho_2}\right)$.

One can interpret these results as follows: in the current approximation, $t_2^{\text{IT-MFL}} < t_2^{\text{MFL}}$ when $\rho_1$ is large enough by Remark 2, and although we may find $t_2^{\text{MFL}} < t_2^{\text{IT-MFL}}$ for moderate $\rho_1$, this is controlled by the constant $C$ given in Prop. 3.3.1. Our finding that methylation generally compresses the period is consistent with the findings of Azzi et al., who showed that there was a greater difference between the 22hr and 24hr entrainment groups, rather than the 24hr and 26hr groups [2].

### 3.3.2 A higher order period estimate

We follow the same approach as François and Hakim to derive the $O(\sqrt{\delta})$ period estimate of the period in the IT-MFL model. Recall from Fig. 3.4 that two boundary layers appear at the Phase I-II boundary. The first layer denoted by BL\(_1\) occurs prior to $t_1$ when the quasi-steady state approximation of $g$ breaks down due to the decline in $A$ concentration. We assume BL\(_1\) begins when $g$ and $a$ are of the same magnitude so that $g \sim a \sim \frac{\delta}{a}$ and thus $a \sim g \sim \sqrt{\delta}$ within the boundary layer. This scaling suggests that BL\(_1\) is of thickness $t_1 - t \sim \sqrt{\delta}$. We verify this by determining the dominant order contributions to Eq. (3.5). Define the rescaled time $\tau := \frac{t}{\eta(\delta)}$ and rescaled protein concentrations $\hat{g} := \frac{g}{\sqrt{\delta}}$, $\hat{a} := \frac{a}{\sqrt{\delta}}$, and since we are in the high-$A$-phase, $a = \delta A$. Making the appropriate substitutions, Eq. (3.5) becomes
\[
\frac{\sqrt{\delta}}{\eta} \frac{d\hat{g}}{d\tau} = \tilde{\theta}_1 \left(1 - \sqrt{\delta}\hat{g} - g_M + \frac{\tilde{\theta}_2}{\theta_1} g_M - \frac{\hat{g} \hat{a}}{A_0}\right) - \sqrt{\delta}\hat{a}_2 \hat{g}.
\] (3.25)

If we assume $\eta(\delta) = \sqrt{\delta}$ then terms of the form $\sqrt{\delta}\hat{g}$ on the right hand side of Eq. (3.25) can be neglected. Transforming back to the original variables $(t, g, a)$, the dominant order terms in Eq. (3.25) become
\[
\dot{g} = \tilde{\theta}_1 \left(1 - g_M + \frac{\tilde{\theta}_2}{\theta_1} g_M - \frac{g a}{\delta A_0}\right).
\] (3.26)
Since the time-scaling factor $\eta$ is of order $\sqrt{\delta}$ we see that $\text{BL}_1$ is indeed of thickness $\sqrt{\delta}$. To solve Eq. (3.26), we approximate $a(t)$ by its linearization at the right endpoint of the boundary layer to obtain

$$a(t) = (1 - r_2)(t_1 - t) + o(\sqrt{\delta}) \quad (3.27)$$

More precisely, we have used the linearization of $a(t)$ with its derivative approximated to leading order in $\delta$ to obtain (3.27). Substitute Eq. (3.27) into Eq. (3.26) and integrate to obtain

$$g(t_1) - g(t)e^{-\kappa_2(t_1-t)^2} = (\tilde{\theta}_1 + (\tilde{\theta}_2 - \tilde{\theta}_1)g_{M2}) \int_{t-t_1}^{0} e^{\kappa_2 u^2} du.$$ 

where $\kappa_2 = \frac{\tilde{\theta}_1(1-r_2)}{2A_0\delta}$. Taking the $t \to -\infty$ limit gives the value of $g$ at the Phase I-II boundary

$$g_I(t_1) = g_{II}(0) = (\tilde{\theta}_1 + (\tilde{\theta}_2 - \tilde{\theta}_1)g_{M2}) \sqrt{\frac{\pi A_0\delta}{2\tilde{\theta}_1(r_2 - 1)}}.$$ 

The analysis of the $\text{BL}_4$ boundary layer is similar to the case of $\text{BL}_1$. We refer the reader to the appendix of the original MFL paper for a full derivation. Table 3.6 compares the entirety of our perturbative analysis to that of François and Hakim.
<table>
<thead>
<tr>
<th>Phase</th>
<th>MFL</th>
<th>IT-MFL</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Phase I</strong></td>
<td>( g = \frac{\delta A_0}{a} )</td>
<td>( g = \frac{\delta A_0}{a} \left(1 - \left(1 - \frac{\delta}{\theta_1}\right) g_M\right) )</td>
</tr>
<tr>
<td></td>
<td>( B = \frac{\delta r}{a} )</td>
<td>( B = \frac{\delta r_0}{a} )</td>
</tr>
<tr>
<td></td>
<td>( \dot{r} = \rho_1 - r )</td>
<td>( \dot{r} = \rho_1 (1 - g - g_M) + g_M \rho_2 - r )</td>
</tr>
<tr>
<td></td>
<td>( \dot{a} = 1 - r - d_\alpha a. )</td>
<td>( \dot{a} = 1 - r - d_\alpha a. )</td>
</tr>
<tr>
<td><strong>Phase II</strong></td>
<td>( A = \frac{\delta}{b} )</td>
<td>( A = \frac{\delta}{b} )</td>
</tr>
<tr>
<td></td>
<td>( \dot{g} = \tilde{\theta}_1 (1 - g) )</td>
<td>( \dot{g} = \tilde{\theta}_1 \left(1 - g - g_M + \frac{\delta}{\theta_1}g_M\right) - \tilde{\alpha}_2 g )</td>
</tr>
<tr>
<td></td>
<td>( \dot{r} = \rho_0 g + \rho_1 (1 - g) - r )</td>
<td>( \dot{r} = \rho_0 g + \rho_1 (1 - g - g_M) + g_M \rho_2 - r )</td>
</tr>
<tr>
<td></td>
<td>( \dot{b} = r - 1 - d_\beta b. )</td>
<td>( \dot{b} = r - 1 - b d_\beta. )</td>
</tr>
<tr>
<td><strong>BCs</strong></td>
<td>( r_I(0) = r_1, \ a_I(0) = 0, )</td>
<td>( r_I(0) = r_1, \ a_I(0) = 0, \ g_{M,I}(0) = g_{M1}, )</td>
</tr>
<tr>
<td>( O(\delta^0) )</td>
<td>( g_{II}(0) = 0, \ r_{II}(0) = r_2, )</td>
<td>( g_{II}(0) = 0, \ g_{M,II}(0) = g_{M2}, \ r_{II}(0) = r_2, )</td>
</tr>
<tr>
<td></td>
<td>( b_{II}(0) = 0 )</td>
<td>( b_{II}(0) = 0 )</td>
</tr>
<tr>
<td><strong>BCs</strong></td>
<td>( r_I(t_1) = r_2, \ a_I(t_1) = 0, )</td>
<td>( r_I(t_1) = r_2, \ a_I(t_1) = 0, \ g_{M,I}(t_1) = g_{M2}, )</td>
</tr>
<tr>
<td>( O(\sqrt{\delta}) )</td>
<td>( r_{II}(t_2) = r_1, \ b_{II}(t_2) = 0 )</td>
<td>( r_{II}(t_2) = r_1, \ b_{II}(t_2) = 0, \ g_{M,II}(t_2) = g_{M1} )</td>
</tr>
<tr>
<td></td>
<td>( g_{II}(0) = \sqrt{\frac{\pi A_0}{2(r_2 - 1)}}, \ r_{II}(0) = r_2, )</td>
<td>( g_{II}(0) = \left(\tilde{\theta}_1 + (\tilde{\theta}_2 - \tilde{\theta}<em>1)g</em>{M2}\right) \sqrt{\frac{\pi A_0}{2(\tilde{\theta}_1(r_2 - 1))}}, )</td>
</tr>
<tr>
<td></td>
<td>( b_{II}(0) = 0 )</td>
<td>( g_{M,II}(0) = g_{M2}, \ r_{II}(0) = r_2, )</td>
</tr>
<tr>
<td><strong>O(\sqrt{\delta})</strong></td>
<td>( r_{II}(t_2) = r_1 + g_1(\rho_1 - \rho_0) \sqrt{\frac{\pi}{4\kappa_1}}, )</td>
<td>( b_{II}(0) = 0, \ g_{M,II}(t_2) = g_{M1} )</td>
</tr>
</tbody>
</table>

Table 3.6: Summary of the nonlinear equations derived in the lowest order and dominant order period estimates. Phases I and II of the oscillations are defined in Fig. 3.4 and shown for a nominal simulation in Fig. 3.3. The rescaled variables are given by \( a := \delta A \) and \( b := \delta B \) and the parameters \( \kappa_1 := \frac{\delta_1(1 - \rho_1)}{2A_0 \delta}, \) and \( g_1 := 1 - e^{-\delta_1 t_2}. \)
3.3. PERTURBATIVE PERIOD ESTIMATION

Figure 3.6: Comparison of numerically and asymptotically estimated periods. The period is expressed as a ratio with its limiting value as $\delta \to 0$. Parameters for the intermediate promoter state have been selected so its influence is weak ($\rho_2 = 0, \tilde{\alpha}_2 \ll \tilde{\theta}_2$). Parameters: $\rho_0 = 0, \rho_1 = 10.45, \tilde{\theta}_1 = 1.33, d_a = 0.33, d_b = 0.33, \mu = 0.31, A_0 = 4, \rho_2 = 0, \tilde{\alpha}_2 = 1.0, \tilde{\theta}_2 = 10$. 

![Comparison of numerically and asymptotically estimated periods](image)
Chapter 4

Derivation and analysis of a reduced model

The Kim-Forger model and MFL model describe the same biological process, so it is natural to compare the two models. In this chapter, we show that the Kim-Forger model can be obtained as an approximation of the MFL model. Importantly, this approximation involves decoupling the equilibrium equations of the promoters and proteins. To consider the influence of fully-coupled equilibrium equations, we derive a more general approximation which we refer to as the reduced IT-MFL model. We find a nominal parameter set which shows that even in the absence of methylation, there can be substantial differences in the agreement of these two approximations with the full MFL model.

Focusing on the fully-coupled equilibrium equations, we analyze how the methylation parameters influence the presence and period of oscillations in the reduced IT-MFL model. Numerical bifurcation analysis reveals that the model becomes arrhythmic through a supercritical Hopf bifurcation that can be induced by changes in methylation parameters. Period sensitivity shows that a divergence in period occurs in the vicinity of these bifurcations, suggesting a mechanism for how methylation can affect the period. Finally, we investigate the extent to which the effects of methylation can be recovered in the no-methylation limit. We find that both of the parameters that describe the activator binding must be varied in order to compensate for methylation. This suggests that DNA methylation may be a mechanism for a cell to effectively operate with altered activator kinetics.
4.1 Details of model reduction

4.1.1 Recovery of Kim-Forger model from the MFL model

Recall the dimensional Kim-Forger model

\[
\begin{align*}
\frac{dM}{dt} &= \alpha_1 f(P; A, K_d) - \beta_1 M, \\
\frac{dP_c}{dt} &= \alpha_2 M - \beta_2 P_c, \\
\frac{dP}{dt} &= \alpha_3 P_c - \beta_3 P,
\end{align*}
\]

where \( f(P; A, K_d) \) is the transcription function

\[
 f(P; A, K_d) = \frac{1}{2} \left( 1 - \frac{P}{A} - \frac{K_d}{A} + \sqrt{(1 - \frac{P}{A} - \frac{K_d}{A})^2 + 4K_d/A} \right).
\]

The Kim-Forger model can be obtained as an approximation of the MFL model by applying the following approximations.

1. The promoters are at quasi-steady state.
2. Proteins \( B \) and \( A : B \) decay at the same rate \( (\delta_A = \delta_{AB}) \).
3. A rapid equilibrium approximation is applied to the reversible binding of \( A \) and \( B \).
4. Nuclear export is included as an explicit step in the model.
5. The quantity \( A_{\text{tot}} := [A] + [A : B] \) is constant in time.
6. The transcription rate corresponding to the inactive promoter state is identically zero.
7. The transcription rate of \( P \) is proportional to the fraction of free activator.

The first three approximations reduce the dimensionality of the system. Without nuclear export, the reduced model would only be a two-stage feedback loop. Adding the additional state extends the feedback loop and makes it easier for oscillations to occur. It should also be noted that the final assumption is equivalent to Taylor expanding the transcription function to first order and neglecting higher order terms.

We start with the QSS approximation. Assuming that the promoter states are at
quasi-steady state, the MFL model may be written as follows.

\[
\begin{align*}
\frac{d[r_b]}{dt} &= \rho_f[g]_{ss} + \rho_b[g : A]_{ss} - \delta_r[r_b], \\
\frac{d[B]}{dt} &= \beta[r_b] - \delta_B[B] - \gamma_+[A][B] + \gamma_-[A : B], \\
\frac{d[A]}{dt} &= \rho_A - \delta_A[A] - \gamma_+[A][B] + \gamma_-[A : B], \\
\frac{d[A : B]}{dt} &= \gamma_+ [A][B] - \gamma_-[A : B] - \delta_{AB}[A : B],
\end{align*}
\]

where the quasi-steady state values are given by

\[
\begin{align*}
[g : A]_{ss} &= \frac{[A]K_1g_{tot}}{1 + [A]K_1}, \\
[g]_{ss} &= g_{tot} - [g : A]_{ss}.
\end{align*}
\]

Next, we assume that the decay of the \(A : B\) state is the same as that of the \(B\) state (\(\delta_{AB} = \delta_B\)). Setting \(\delta_{AB} = \delta_B\) allows us to write the dynamic equations in terms of \([B_{tot}] := [B] + [A : B]\) at the end of the reduction. That is to say

\[
\frac{d}{dt}[B_{tot}] = \beta[r_b] - \delta_B[B] - \delta_B[A : B] = \beta[r_b] - \delta_B[B_{tot}]
\]

Applying the rapid equilibrium approximation to the binding of \(A\) with \(B\), we obtain

\[
\begin{align*}
\frac{d[r_b]}{dt} &= \rho_f[g]_{ss} + \rho_b[g : A]_{ss} - \delta_r[r_b], \\
\frac{d[B]}{dt} &= \beta[r_b] - \delta_B[B], \\
\frac{d[A]}{dt} &= \rho_A - \delta_A[A], \\
\frac{d[A : B]}{dt} &= -\delta_B[A : B].
\end{align*}
\]

We add an additional protein concentration \([B_c]\) which corresponds to nuclear export of \(B\). We define the variables \(A_{tot} := [A] + [A : B]\) and \([B_{tot}] := [B] + [A : B]\). We assume \(A_{tot}\) is constant, and hence write \(A_{tot}\) rather than \([A]_{tot}\) to emphasize that this is not a dynamic variable.

Next, we assume that the transcription rate of the inactive promoter state is
identically zero to obtain
\[
\frac{dr_b}{dt} = \rho_b [g:A]_{ss} - \delta_r [r_b],
\]
\[
\frac{dB_c}{dt} = \beta_1 [r_b] - \lambda_c [B],
\]
\[
\frac{d[B]}{dt} = \beta_2 [r_b] - \delta_B [B],
\]
\[
\frac{d[A]}{dt} = \rho_A - \delta_A [A],
\]
\[
\frac{d[A:B]}{dt} = -\delta_B [A:B].
\]

Since \([g:A]\) is an additional state that \(A\) could occupy, one should interpret \(A_{tot}\) as the total amount of activator present in the \(A-B\) reaction, not in the entire system. Since the binding of \(A\) and \(B\) is at steady-state and \(A\) and \(B\) satisfy the conservations stated above, we have a system of equations

\[
K_d = \frac{[A][B]}{[A:B]}, \quad (4.1)
\]
\[
A_{tot} = [A] + [A:B], \quad (4.2)
\]
\[
[B_{tot}] = [B] + [A:B], \quad (4.3)
\]

where the dissociation constant \(K_d := \gamma_- / \gamma_+\). Equations (4.1)-(4.3) can be solved to obtain

\[
f([B_{tot}]; A_{tot}, K_d) := \frac{[A]}{A_{tot}} = \frac{1}{2} \left( 1 - \frac{[B_{tot}]}{A_{tot}} - \frac{K_d}{A_{tot}} + \sqrt{\left( 1 - \frac{[B_{tot}]}{A_{tot}} - \frac{K_d}{A_{tot}} \right)^2 + 4 \frac{K_d}{A_{tot}}} \right).
\]

Eq. (4.4) is the transcription function that appears in the Kim-Forger model. Finally, notice that \([g:A]_{ss}\) may be approximated in terms of \(f\)

\[
[g : A]_{ss} = \frac{[A]K_1 g_{tot}}{1 + [A]K_1} \approx [A]K_1 g_{tot} = K_1 g_{tot} A_{tot} f([B_{tot}]; A_{tot}, K_d)
\]

where \(g_{tot} = [g] + [g : A]\) and \(K_1 = \alpha_1 / \theta_1\). This last approximation yields the following
4.1.2 Fully-coupled promoter states for MFL and IT-MFL

The approximation in the previous section involved decoupling the steady-state value of $[A]$ from the promoter concentrations in order to find a quadratic equation satisfied by $[A]$. The novelty of our reduction of the IT-MFL model is that it does not make this assumption. Instead, we use the fully-coupled set of equilibrium equations

$$K_d = \frac{[A][B]}{[A : B]}$$

$$A_{tot} = [A] + [g : A] + [A : B]$$

$$B_{tot} = [B] + [A : B]$$

$$[g : A] = (g_{tot} - [g : A] - [g : M]) K_1[A]$$

$$[g : M] = K_2(g_{tot} - [g : A] - [g : M]).$$

So by defining $A_{tot}$ in Eq. (4.9) to be the total amount of activator in the entire system – not just in the $A : B$ reaction – the promoter QSS gets coupled to the protein QSS. Comparing Eqs. (4.8)-(4.12) to Eqs. (4.1)-(4.3) should clarify what is meant by partially uncoupled versus fully-coupled QSS approximation.

Equations (4.8)-(4.12) can be converted to a single cubic equation $\Phi_{\text{dim}}([A]_{\text{qss}}) = 0$ for the QSS concentration $[A]_{\text{qss}}$, where $\Phi_{\text{dim}}$ is the polynomial

$$\Phi_{\text{dim}}([A]) = a_{\text{dim}}[A]^3 + b_{\text{dim}}[A]^2 + c_{\text{dim}}[A] + d_{\text{dim}}.$$
with coefficients

\[ a_{\text{dim}} = K_1, \]
\[ b_{\text{dim}} = 1 + K_2 + (g_{\text{tot}} + B_{\text{tot}} - A_{\text{tot}} + K_d) K_1, \]
\[ c_{\text{dim}} = (1 + K_2)(B_{\text{tot}} - A_{\text{tot}} + K_d) \]
\[ + (g_{\text{tot}} - A_{\text{tot}}) K_d K_1, \]
\[ d_{\text{dim}} = -(1 + K_2)A_{\text{tot}}K_d, \]

where the equilibrium constants are given by \( K_1 = \alpha_1/\theta_1 \) and \( K_2 = \alpha_2/\theta_2 \), the dissociation constant \( K_d = \gamma_-/\gamma_+ \), \( A_{\text{tot}} \) is constant by assumption, and \( B_{\text{tot}} = [B_{\text{tot}}] \). We emphasize that this polynomial is dimensional to distinguish it from its dimensionless form, \( \Phi(\tilde{A}) \) that appears in the next section.

Since the scalar equilibrium equation for \([A]\) is now cubic rather than quadratic, it is harder to prove that there is a unique non-negative real root. For the parameter sets considered in our work, we always found this to be the case. In future work, one should be cautious and verify this numerically before trusting that a given expression for a cubic root is unique. We denote our (supposedly unique) solution to \( \Phi_{\text{dim}}([A]) = 0 \) by \([A]_{\text{qss}}\). Rewriting the governing equations with \([A]_{\text{qss}}\) lets us obtain a reduced form of the IT-MFL model

\[ \frac{d[r_b]}{dt} = \rho_b[g : A]_{\text{qss}} + \rho_M[g : M]_{\text{qss}} - \delta_r[r_b], \]
\[ \frac{d[B_c]}{dt} = \beta_1[r_b] - \lambda_c[B_c], \]
\[ \frac{d[B_{\text{tot}}]}{dt} = \beta_2[B_c] - \delta_B[B_{\text{tot}}], \]

in which \([B_c]\) is the concentration of cytosolic protein and the promoter states \([g : A]_{\text{qss}}\) and \([g : M]_{\text{qss}}\) can be expressed in terms of \([A]_{\text{qss}}\) as

\[ [g : A]_{\text{qss}} = \frac{[A]_{\text{qss}}K_1g_{\text{tot}}}{1 + K_2 + [A]_{\text{qss}}K_1}, \]
\[ [g : M]_{\text{qss}} = \frac{K_2(g_{\text{tot}} - [g : A]_{\text{qss}})}{1 + K_2}. \]

It should be noted that although we include the additional methylated promoter state in this calculation, the main differences in the reduction are due to coupling the promoter and activators and not the additional promoter state.

Several assumptions and approximations have been made in deriving the reduced IT-MFL model. The assumption that \( A_{\text{tot}} \) is constant in time is the hardest to verify. We see in Fig. 4.1 that there is good agreement of the full and reduced models when
$A_{\text{tot}}$ is close to constant in time, but qualitative differences appear when this is not the case. Comparing the first column to the second, it may seem strange that a transient change in $A_{\text{tot}}(t)$ in the bottom right panel of Fig. 4.1 has a persistent effect on the reduced model. This is likely because the increased activator level was enough to remove oscillations from the system, however no such increase takes place in the reduced model because $A_{\text{tot}}$ is treated as a constant. Figure 4.2 provides a comparison of the full IT-MFL model, Kim-Forger model and reduced IT-MFL model. For the parameter set considered here, the only model to display oscillatory behaviour is the Kim-Forger model. Since no methylation is present in this simulation, we see that the improved quality of the approximation is a consequence of using fully-coupled equilibrium equations.

### 4.2 Analysis of reduced model

#### 4.2.1 General features

Following Kim and Forger, we define the dimensionless concentrations $\tilde{r}, \tilde{B}_c, \tilde{B}$ by

$$[r_b] = r_b^* \tilde{r}, \quad [B_c] = B_c^* \tilde{B}_c, \quad B_{\text{tot}} = B^* \tilde{B}_{\text{tot}}$$  \hspace{1cm} (4.13)
4.2. ANALYSIS OF REDUCED MODEL

Figure 4.2: A comparison of the full mixed-feedback loop model to the Kim-Forger and reduced IT-MFL models. The Kim-Forger model provides a worse approximation because it oscillates, even though the full model does not. Parameters: $\alpha_1 = 10^{-2}, \theta_1 = 10^2, \alpha_2 = 0, \rho_M = 0$ (no methylation), $\rho_f = 5 \times 10^{-3}, \rho_b = 10, \beta_1 = \beta_2 = \lambda_c = 10^{-2}, \rho_A = 10^{-1}, \gamma_+ = 10^2, \gamma_- = 10^{-2}, \delta_B = \delta_{AB} = \delta_r = 10^{-3}, \delta_A = 10^{-3}.$

with scaling factors

$$r_b^* = \frac{\rho_b g_{tot}}{\delta_r}, B_c^* = \frac{\beta_1 \rho_b g_{tot}}{\delta_r^2}, B^* = \frac{\beta_2 \beta_1 \rho_b g_{tot}}{\delta_r^3}.$$ 

Similarly, we define dimensionless parameters

$$\rho = \frac{\rho_M}{\rho_b}, K_2 = \frac{\alpha_2}{\theta_2}, \tilde{K}_1 = B^* K_1 = B^* \frac{\alpha_1}{\theta_1},$$

$$\tilde{K}_d = \frac{K_d}{B^*}, A_{tot} = B^* \tilde{A}_{tot}, g_{tot} = B^* \tilde{g}_{tot}. \quad (4.14)$$

Using the scaling factors and dimensionless parameters above, the reduced IT-MFL model can be transformed to a dimensionless form

$$\frac{d\tilde{r}}{d\tau} = T(\tilde{B}_{tot}) - \tilde{r}, \quad (4.15)$$

$$\frac{d\tilde{B}_c}{d\tau} = \tilde{r} - \tilde{B}_c, \quad (4.16)$$

$$\frac{d\tilde{B}_{tot}}{d\tau} = \tilde{B}_c - \tilde{B}_{tot}, \quad (4.17)$$

$$T(\tilde{B}_{tot}) = \left( \frac{\tilde{A}_{qss}(\tilde{B}_{tot}) \tilde{K}_1}{1 + K_2 + \tilde{A}_{qss}(\tilde{B}_{tot}) \tilde{K}_1} \right) \left( 1 - \frac{\rho K_2}{1 + K_2} \right) + \frac{\rho K_2}{1 + K_2}. \quad (4.18)$$

in which $\tau = t \delta_r$ and $[A]_{qss} = B^* \tilde{A}_{qss}(\tilde{B}_{tot})$. To reduce the number of parameters in the dimensionless model, we have also assumed all degradation rates are equal ($\delta_r = \lambda_c = \delta_B$). As discussed in the previous Chapter, this maximizes the chance of oscillations occurring in the system. Since our analysis in concerned with the
cessation of oscillations, we believe it is reasonable to choose conditions that are conducive to oscillations and happen to simplify the analysis by reducing the number of parameters.

The notation \( [A]_{\text{qss}} = B^* \tilde{A}_{\text{qss}}(\tilde{B}_{\text{tot}}) \) warrants some explanation. Notice that the equilibrium conditions given in Eqs. (4.8)-(4.12) are invariant under the rescaling \( K_1 \to \tilde{K}_1/B^* \) and \( x \to B^* \tilde{x} \) for all other dimensional parameters and dynamic variables, where \( B^* = \frac{\beta_2 \beta_1 \rho g_{\text{tot}}}{\tilde{K}_d} \). Hence we have a cubic equation for \( \tilde{A} \)

\[
0 = \Phi(\tilde{A}) = a \tilde{A}^3 + b \tilde{A}^2 + c \tilde{A} + d
\]

with coefficients

\[
\begin{align*}
a &= \tilde{K}_1 \\
b &= K_2 + \tilde{K}_1 \tilde{g}_{\text{tot}} - \tilde{A}_{\text{tot}} \tilde{K}_1 + \tilde{B}_{\text{tot}} \tilde{K}_1 + \tilde{K}_d \tilde{K}_1 + 1 \\
c &= \tilde{B}_{\text{tot}} - \tilde{A}_{\text{tot}} + \tilde{K}_d - \tilde{A}_{\text{tot}} K_2 + \tilde{B}_{\text{tot}} K_2 + K_2 \tilde{K}_d - \tilde{A}_{\text{tot}} \tilde{K}_d \tilde{K}_1 + \tilde{K}_d \tilde{K}_1 \tilde{g}_{\text{tot}} \\
d &= -\tilde{A}_{\text{tot}} \tilde{K}_d - \tilde{A}_{\text{tot}} K_2 \tilde{K}_d
\end{align*}
\]

As mentioned previously, we cannot guarantee that \( \Phi(\tilde{A}) \) has a unique real root, however we found in our numerical studies that there was always a unique non-negative real root for the parameter sets we considered. Since \( \tilde{B}_{\text{tot}} \) is the only dynamic variable appearing in the coefficients of \( \Phi(\tilde{A}) \), we denote the non-negative real root of \( \Phi(\tilde{A}) \) by \( \tilde{A}_{\text{qss}}(\tilde{B}_{\text{tot}}) \).

Written in its dimensionless form, our reduction of the IT-MFL model contains three dynamic variables \( \tilde{r}, \tilde{B}_{\text{c}}, \) and \( \tilde{B}_{\text{tot}} \) which evolve according to Eqs. (4.15)-(4.18) and six parameters \( \tilde{A}_{\text{tot}}, \tilde{g}_{\text{tot}}, \tilde{K}_d, \tilde{K}_1, \rho, \) and \( K_2 \). When the version of the Kim-Forger model stated in Eqs. (4.5)-(4.7) is non-dimensionalized using the same procedure as the reduced IT-MFL model, given in Eqs. (4.13)-(4.14), a factor of \( \tilde{K}_1 \tilde{A}_{\text{tot}} \) remains in front of the transcription function \( f(\tilde{B}_{\text{tot}}) \). As shown in Fig. 4.3, this factor can have a substantial effect on the transcription function. Moreover, even in the absence of methylation effects, there are substantial differences in the transcription functions of these two models. Importantly, the monotonicity of \( f(\tilde{B}_{\text{tot}}) \) which is crucial to the analysis of Kim and Forger appears to be preserved when one switches from \( f(\tilde{B}_{\text{tot}}) \) to \( T(\tilde{B}_{\text{tot}}) \) in Fig. 4.3. It is unclear if this is always the case, however by imposing the conditions listed below, we were able to verify monotonicity.

**Proposition 4.2.1** (Monotonicity of transcription function). If \( \tilde{B}_{\text{tot}} > (1 + K_d \tilde{K}_1) \tilde{A}_{\text{tot}} \) and \( \rho < 1 \) then the transcription function \( T(\tilde{B}_{\text{tot}}) \) is monotonically decreasing.
4.2. ANALYSIS OF REDUCED MODEL

Figure 4.3: Comparison of the transcription functions in the dimensionless Kim-Forger and reduced IT-MFL models. The analysis of Kim and Forger shows that $f(\tilde{B}_{\text{tot}})$ has a knee at the value $\tilde{B}_{\text{tot}} = \tilde{A}_{\text{tot}}$, indicated on the $\tilde{B}_{\text{tot}}$-axis. (Top panel) $\tilde{A}_{\text{tot}}$ is an order of magnitude larger than $\tilde{g}_{\text{tot}}$. As the equilibrium constant $\tilde{K}_1$ is varied, the transcription function $T(\tilde{B}_{\text{tot}})$ of the reduced IT-MFL model becomes increasingly nonlinear. (Bottom panel) The transcription function in the reduced IT-MFL model resembles a piecewise affine function when $\tilde{g}_{\text{tot}}$ and $\tilde{A}_{\text{tot}}$ are equal. Null methylation parameters were used in both plots ($\rho = 0 = K_2$) so any difference between the transcription functions should be attributed to the relaxation of Kim and Forger’s quasi-steady state assumption. Parameters: $\tilde{A}_{\text{tot}} = 2.20 \times 10^{-2}$, $\tilde{K}_d = 10^{-5}$, $\rho = K_2 = 0$. In the top panel $\tilde{g}_{\text{tot}} = 2.20 \times 10^{-3}$ and in the bottom $\tilde{g}_{\text{tot}} = 2.20 \times 10^{-2}$. 
Proof. Differentiate $\Phi(\tilde{A}) = 0$ with respect to $\tilde{B}_{\text{tot}}$ to find

$$0 = c_1 \frac{d\tilde{A}_{\text{qss}}}{d\tilde{B}_{\text{tot}}} + c_0$$

(4.19)

with coefficients

$$c_1 = \tilde{B}_{\text{tot}} - \tilde{A}_{\text{tot}} + \tilde{K}_d + 2\tilde{A}_{\text{qss}}(\tilde{B}_{\text{tot}}) \left( K_2 + \tilde{K}_1 \tilde{g}_{\text{tot}} + \tilde{B}_{\text{tot}} \tilde{K}_1 - \tilde{A}_{\text{tot}} \tilde{K}_1 + \tilde{K}_d \tilde{K}_1 + 1 \right)$$

$$+ 3\tilde{K}_1 \tilde{A}_{\text{qss}}(\tilde{B}_{\text{tot}})^2 + K_2 \left( B_{\text{tot}} - A_{\text{tot}} \left( 1 + \frac{\tilde{K}_d \tilde{K}_1}{K_2} \right) \right) + K_2 \tilde{K}_d + \tilde{K}_d \tilde{K}_1 \tilde{g}_{\text{tot}}$$

$$c_0 = (K_2 + 1) \tilde{A}_{\text{qss}}(\tilde{B}_{\text{tot}}) + \tilde{K}_1 \tilde{A}_{\text{qss}}(\tilde{B}_{\text{tot}})^2$$

Notice that $c_0 > 0$ and $c_1 > 0$ provided that $\tilde{B}_{\text{tot}} > (1 + \frac{\tilde{K}_d \tilde{K}_1}{K_2}) \tilde{A}_{\text{tot}}$. Hence we require $\tilde{A}'_{\text{qss}}(\tilde{B}_{\text{tot}}) < 0$ in order for Eq. (4.19) to hold true. Next notice that for $a_2 > 0$, the function

$$g(x; a_1, a_2, a_3) = a_1 + \frac{a_2 x}{1 + a_3 x}$$

is monotonically increasing on the positive real line and we may write

$$T(\tilde{B}_{\text{tot}}) = g(\tilde{A}_{\text{qss}}(\tilde{B}_{\text{tot}}); a_1, a_2, a_3)$$

(4.20)

for some $a_1, a_2, a_3 \in \mathbb{R}$. Inspection of the transcription function reveals

$$T(\tilde{B}_{\text{tot}}) = \left( \frac{\tilde{A}_{\text{qss}}(\tilde{B}_{\text{tot}}) \tilde{K}_1}{1 + K_2 + \tilde{A}_{\text{qss}}(\tilde{B}_{\text{tot}}) \tilde{K}_1} \right) \left( 1 - \frac{\rho K_2}{1 + K_2} \right) + \frac{\rho K_2}{1 + K_2}$$

so $\rho < 1$ implies $a_2 > 0$ in Eq. (4.20). Consequently $T'(\tilde{B}_{\text{tot}}) = \frac{d}{d\tilde{A}_{\text{qss}} d\tilde{B}_{\text{tot}}} < 0$. 

Assuming $\tilde{B}_{\text{tot}} > (1 + \frac{\tilde{K}_d \tilde{K}_1}{K_2}) \tilde{A}_{\text{tot}}$ and $\rho < 1$, it follows from the monotonicity of $T(\tilde{B}_{\text{tot}})$ that there is a unique non-negative equilibrium solution to Eqs. (4.15)-(4.18). It turns out that monotonicity also implies that solutions to Eqs. (4.15)-(4.18) are bounded. We show this by first proving a slightly more general result.

**Theorem 4.2.1 (Conditions for bounded MCF system).** Let $\frac{d}{dt} x(t) = F(x(t))$ be a $d$-dimensional monotone cyclic feedback system, with component equations

$$\frac{d}{dt} x_i(t) = f_i(x_{i-1}(t)) - g_i(x_i(t)),$$

where we identify $x_0(t) = x_d(t)$. Suppose that for $i = 1, \ldots, d$

1. $f_i \in C^1(\mathbb{R})$ is monotone decreasing,
2. \( g_i \in C^1(\mathbb{R}) \) is monotone increasing,

3. \( f_i(x) - g(0) > 0 \) for all \( x \geq 0 \),

and suppose the initial conditions satisfy

\[
0 \leq x_i(0) \leq g_i^{-1}(f_i(0)), \quad i = 1, \ldots, d.
\]

Then each \( x_i \) satisfies

\[
0 \leq x_i(t) \leq g_i^{-1}(f_i(0)) \quad \text{for all } t > 0.
\]

Proof. We prove this by constructing a bounding box in the phase space. Suppose the solution starts in the positive orthant and arrives at the \( x_i = 0 \) plane. We evaluate the normal derivative on this plane to find \( x_i = 0 \)

\[
e_i \cdot \frac{d}{dt} x \bigg|_{x_i=0} = f_i(x_{i-1}) - g_i(0) > 0,
\]

where \( e_i \) is the \( i \)-th standard basis vector. Equation (4.21) shows that solutions flow into the positive orthant, establishing a lower bound. Next, suppose that

\[
x_i = x^+ > g_i^{-1}(f_i(0)) > 0.
\]

Since \( g_i \) is monotone increasing, we can rearrange Eq. (4.22) to give

\[
0 > f_i(0) - g_i(x^+) > f_i(x_{i-1}) - g_i(x^+) = e_i \cdot \frac{d}{dt} x \bigg|_{x_i=x^+}
\]

for any \( x_{i-1} > 0 \). Thus, solutions starting in the region \( 0 \leq x_i \leq g_i^{-1}(f_i(0)) \) for \( i = 1, \ldots, d \) cannot exit this region. 

Corollary 4.2.1.1. The Kim-Forger model is bounded.

Corollary 4.2.1.2. Assuming there is a unique root to the equilibrium equation for \( \tilde{A} \) and the monotonicity conditions \( (\tilde{B}_{\text{tot}} > (1 + \frac{K_d K_1}{K_2}) \tilde{A}_{\text{tot}} \text{ and } \rho < 1) \) hold true, then the reduced IT-MFL model is bounded.

Taken together, these results show that if \( \tilde{B}_{\text{tot}} > (1 + \frac{K_d K_1}{K_2}) \tilde{A}_{\text{tot}}, \rho < 1 \), and there is a unique non-negative root to the cubic equation, then the IT-MFL model constitutes a bounded monotone cyclic feedback (MCF) system, so Theorem 2.3.1 applies. As mentioned in Chapter 2, MCF systems are capable of bistability, for example the coexistence of a stable equilibrium and a stable periodic solution. The top panel of Fig. 4.4 shows that the reduced IT-MFL model can display such behaviour. Bistability
can be eliminated by introducing a moderate amount of methylation into the system, as in the bottom panel of Fig. 4.4.

Figure 4.4: Methylation can remove bistability from the reduced IT-MFL model. The reduced IT-MFL model was simulated with initial conditions of the form $\sigma \tilde{B}_{\text{tot,eq}} = \dot{\tilde{b}}(0) = \tilde{B}_c(0) = \tilde{B}_{\text{tot}}(0)$ where $\tilde{B}_{\text{tot,eq}}$ is the equilibrium value of $\tilde{B}_{\text{tot}}$ and $\sigma$ takes 11 uniform values in $[1.01, 2]$. (Top) After an initial transient, solutions settle to either periodic or constant trajectories. (Bottom) All solutions settle to periodic trajectories due to the moderate amount of methylation. Parameters: $\tilde{A}_{\text{tot}} = 10^{-3}, \tilde{K}_d = 10^{-6.75}, \tilde{K}_1 = 10^2, \tilde{g}_{\text{tot}} = 10^{-4}, \rho = 0$. $K_2 = 0$ in the top panel and $K_2 = 10^{-1}$ in the bottom panel.

### 4.2.2 Bifurcation analysis

To better understand the qualitative influence of methylation in the reduced model, we fix parameters $\tilde{A}_{\text{tot}}, \tilde{K}_d$, and $\tilde{g}_{\text{tot}}$, and numerically compute the location of any Hopf bifurcations in the methylation parameters $\rho$ and $K_2$. Appendix A provides a brief overview of Hopf bifurcations, and Appendix B discusses methods for the detection and classification of Hopf bifurcations.

We use pseudo-arclength continuation [55] on the following system of nonlinear equations

\[
0 = \Phi(\tilde{A}_{\text{qss}}),
\]
\[
T(B_{\text{tot}}) = B_{\text{tot}},
\]
\[
0 = -\frac{1}{2} \sqrt{T''(B_{\text{tot}})} - 1.
\]

The first two equations enforce that the system is at equilibrium. The latter equation is a bifurcation condition, equivalent to imposing that a complex conjugate pair of eigenvalues of the equilibrium’s linearization are crossing the imaginary axis. This is a system in the unknowns $\tilde{A}_{\text{qss}}, B_{\text{tot}}, \rho$, and $K_2$. The results of numerical continuation
with $\tilde{K}_1$ is varied from $10^{-1}$ to $10^3$ and the result are shown in Fig. 4.5. These Hopf bifurcations are confirmed to be supercritical by numerically evaluating the first Lyapunov coefficient [56]. Therefore, to the left of each curve, there is a stable periodic solution and an unstable equilibrium. To the right of each curve, there is a single stable equilibrium. Notice the Hopf bifurcation curve disappears for $\tilde{K}_1$ below a specific value (11.6 for the parameters in Fig. 4.5) as $K_2 \to 0$. Also, the reduced IT-MFL exhibits oscillations for sufficiently high $\tilde{K}_1$ and for sufficiently low methylation parameters $\rho$ and $K_2$. These findings do not contradict Fig. 4.4 because they use different parameters.

Repeating the calculations shown in Fig. 4.5 with the parameters of Fig. 4.4, we found the Hopf bifurcations could be subcritical or supercritical depending on the value of $\tilde{K}_1$. Recall that a system can transition from bistable to monostable behaviour through a subcritical Hopf bifurcation. Hence our bifurcation analysis does not contradict our earlier observations of bistability in the reduced IT-MFL model.

![Figure 4.5: Each grayscale coloured curve corresponds to a Hopf bifurcation in $\rho$, $K_2$. The colour gives the value of $\tilde{K}_1$. Using the Lyapunov coefficient, we found these bifurcations are supercritical. To the left of each curve there is a stable periodic solution and to the right there is a single stable equilibrium. The other parameters are $\tilde{A}_{\text{tot}} = 1.31 \times 10^{-1}$, $\tilde{g}_{\text{tot}} = 1.31 \times 10^{-2}$, $\tilde{K}_d = 10^{-5}$. This shows that methylation can make the clock arrhythmic. Hopf bifurcations do not occur for $\tilde{K}_1$ below approximately 11.6 as the bifurcation value of $K_2$ goes to zero.](image-url)
4.2.3 Period sensitivity

The influence of the methylation parameters on the location of the Hopf bifurcation can also be observed when one performs parametric sensitivity analysis on the period. We use a method of sensitivity analysis intended for oscillating systems [57] which avoids some of the numerical difficulties encountered when studying period sensitivity. The sensitivities of the period with respect to $\tilde{A}_{\text{tot}}$ and $K_2$ are shown in Fig. 4.6. We see that variations in the methylation parameters $\rho$ and $K_2$ affect the sensitivity of the period with respect to other parameters in the model and these changes are most dramatic as the sensitivities diverge in the neighbourhood of the Hopf bifurcation. Since it would be risky for a biological clock to operate close to a Hopf bifurcation and the sensitivities are relatively unaffected by the methylation parameters away from the Hopf bifurcation, we see that the period is robust to changes in the methylation parameters. As in Fig. 4.5, where higher $\rho$ values cause the bifurcation to occur for lower values of $K_2$, Fig. 4.6 shows that higher values of $\rho$ cause the sensitivity curves to diverge for lower $K_2$. One could interpret this finding as saying that lower $\rho$ values allow the model to tolerate more methylation (larger $K_2$ value) before becoming arrhythmic.

![Figure 4.6: Numerically computed period sensitivities.](image)

$S(\hat{t}, p) := \frac{\rho \, \partial \hat{t}}{\partial p}$ denotes the sensitivity of the period $\hat{t}$ with respect to a parameter $p$, with $p = \tilde{A}_{\text{tot}}$ in the top panel and $p = K_2$ in the bottom panel. The dimensionless transcription rate $\rho$ was varied uniformly from 0 to 1 and this is represented by the transparency of each sensitivity curve. In general, the sensitivities of the period grow as $K_2$ is increased and the fixed point becomes stable, rendering the model arrhythmic. Simulation parameters: $\tilde{A}_{\text{tot}} = 7 \times 10^{-2}, \tilde{K}_d = 10^{-6}, \tilde{K}_1 = 10^3, \tilde{g}_{\text{tot}} = 10^{-3}$.
4.2.4 No methylation limit

In this section we explore the extent to which the influence of methylation in the reduced IT-MFL model can be reproduced in the no-methylation limit ($K_2 = 0 = \rho$). We investigate this by adjusting the transcription rate $\rho_f$ of the inactive promoter state. To make this comparison, we altered our derivation of the reduced IT-MFL model to allow a nonzero value of $\rho_f$. This results in the transcription function

$$T(\tilde{B}_{tot}) = \left( \frac{\tilde{A}_{qss}(\tilde{B}_{tot})\tilde{K}_1}{1 + K_2 + \tilde{A}_{qss}(\tilde{B}_{tot})\tilde{K}_1} \right) \left( 1 - \rho' - \frac{(\rho - \rho')K_2}{1 + K_2} \right) + \frac{(\rho - \rho')K_2}{1 + K_2} + \rho',$$

in which $\rho' = \rho_f/\rho_b$, and Eqs. (4.15)-(4.17) remain otherwise unchanged. We used numerical optimization to find the best approximation of the transcription function in the no-methylation limit and then assessed the quality of the approximation by comparing the limit-cycle trajectory $\tilde{B}_{tot}(t)$. Since $\rho_b$ is held constant and only the dimensionless ratio $\rho' = \rho_f/\rho_b$ affects the dynamics, we varied $\rho'$ instead of $\rho_f$. Figure 4.7 confirms that the influence of methylation on the model cannot be reproduced by altering the value $\rho'$ alone. By comparing a variety of methylation levels, we found this difference was most pronounced for high values of $K_2$ and low values of $\rho$. This is to be expected, since $K_2$ must be large for methylation to have a nontrivial effect, and a higher value of $\rho$ translates the transcription function upward, making it easier to approximate the function by simply increasing $\rho'$. A much better approximation can be found by varying $\tilde{K}_1$ and $\rho'$ simultaneously, as shown in the bottom two panels of Fig. 4.7.

The results of Fig. 4.7 suggest that methylation allows the system to operate with an effectively lowered value of $\tilde{K}_1$. In other words, methylation may be a mechanism for the clock to tune the value of $\tilde{K}_1$ which is crucial for generating the nonlinearity necessary for oscillatory behaviour. Since the equilibrium constants $\tilde{K}_1$ and $K_2$ are regulated by a variety of factors, some of which may be shared, it is difficult to argue that $K_2$ is more easily modified than $\tilde{K}_1$ in a real organism. At the same time, there is substantial experimental evidence that points to the integral role of methylation in the circadian clock [58]. Certainly our model is a vast simplification of this reality, however it is positive to see that the influence of methylation in our model is more complex than a mere vertical translation of the transcription function.
Figure 4.7: Varying $\rho'$ does not compensate for the effects of methylation, but it is possible to compensate for these effects by varying both $\rho'$ and $\tilde{K}_1$. (Top left) The transcription function of the reduced IT-MFL model with methylation is shown in blue, and the dashed black line shows the best approximation under the constraint that $K_2 = 0 = \rho$, $\rho' \in [0, 1]$, and all other parameters are fixed. Transcription functions with $\rho' \in [0, 1]$ and $K_2 = 0 = \rho$ are shown in grey, with darker lines representing higher levels of $\rho'$. (Top right) When only $\rho'$ is allowed to vary, there is a qualitative difference between the dynamics produced by the methylated transcription function shown in blue, and those produced by the best approximation shown with the dashed black line. (Bottom left) By allowing both $\rho'$ and $\tilde{K}_1$ to vary, we obtain a good approximation of the methylated transcription function, (bottom right) and good agreement in their trajectories $\tilde{B}_{tot}(t)$. Fixed parameters: $\tilde{K}_d = 10^{-7}$, $\tilde{A}_{tot} = 7 \times 10^{-2}$, $\tilde{g}_{tot} = 10^{-2}$. In the methylated case, $\rho' = 0$, $\rho = 3 \times 10^{-2}$, $K_2 = 3 \times 10^2$, and in the unmethylated case $\rho = K_2 = 0$ and $\rho' \in [0, 1]$. In the top row $\tilde{K}_1 = 3 \times 10^5$ and in the bottom row $\tilde{K}_1$ was optimized over the interval $[10^{-5}, 10^7]$ and an optimal value of $\tilde{K}_1 \approx 10^3$ was found. Transcription functions were optimized in the $L^\infty$ norm, similar results were obtained using the $L^2$ norm.
In this thesis, we have introduced and analyzed a mathematical model of promoter methylation in the PER transcriptional-translational feedback. By extending the perturbative analysis of François and Hakim, we found that adding methylation to the model preserved the uniqueness and stability of equilibria provided that the transcription rate of the new promoter state was between the inactive and active transcription rates. Under some additional restrictions on the parameters, we derived leading order estimates for the period in the IT-MFL model as well as bounds for the difference in period between the MFL and IT-MFL models. The main result in this section was that the period estimate for the MFL model is always larger in the limit of a high activated transcription rate.

Following our perturbative analysis of the full IT-MFL model, we compared two methods for approximating the IT-MFL model with a feedback loop. The first procedure reproduces the Kim-Forger model but requires decoupling the equilibrium equations in order to obtain their transcription function. The second procedure includes fully-coupled equilibrium equations, but has the disadvantage of producing a transcription function that is too cumbersome to be analyzed by hand. Hence, we were not able to reproduce the secant-condition analysis of Kim and Forger with the fully-coupled approximation. We were however able to show that although methylation influences the period and period sensitivity in the reduced IT-MFL model, excessive methylation can remove oscillations from the system through a Hopf bifurcation. We also identified a parameter set where oscillations were lost upon the removal of methylation and could not be restored by varying the other active and inactive transcription rates.
5.1 Future work

Several opportunities for future work are given below. First, since the assumptions for the reduced IT-MFL model are more general than those used in our derivation of the Kim-Forger model, it may be worthwhile to compare these two approximations in more detail.

Question 1. Under what conditions do the dynamics of the Kim-Forger model coincide with the reduced IT-MFL model?

The perturbative analysis in Chapter 3 revealed that the period estimates, monostability conditions, and linear stability of the IT-MFL model could all be phrased in terms of the corresponding behaviour in the MFL model. It would be interesting to see if this generalizes to extensions of the MFL model with \( n \)-promoter states.

Question 2. Does the promoter state with highest transcription rate always dictate qualitative behaviour in an \( n \)-promoter mixed-feedback loop model?

The enzymes which regulate promoter methylation are ignored in the IT-MFL model. These enzymes would provide an additional source of nonlinearity, and may allow the model to stay rhythmic at higher levels of methylation. Hence, adding a more detailed description of promoter methylation to the IT-MFL model would be a valuable extension of this work.

Question 3. How does the influence of methylation change when the enzymes that control methylation turnover are included?

Molecular noise is another biologically important factor [30, 32] which could be studied in future work. Recent work of Karapetyan and Buchler on a stochastic generalization of the MFL model [52] and the work of Wang and Peskin on the effects of molecular noise on entrainment in an MFL model of the circadian clock [51] provide useful starting points for extending our analysis to the stochastic setting. An example of a question one could pursue in this setting is given below.

Question 4. Does the presence of additional promoter states influence the attenuation of noise in the mixed-feedback loop model, or the role of methylation?


Appendix A

Hopf bifurcations

This chapter fills in some background information on Hopf bifurcations useful for understanding the bifurcation analysis in Chapter 4. The primary reference for this appendix is Kuznetsov’s textbook [59].

A.1 Intuitive discussion of Hopf bifurcations

A Hopf bifurcation is one of the ways an equilibrium can lose its stability. Other examples include transcritical, saddle-node, and pitchfork bifurcations. A Hopf bifurcation occurs when a pair of complex-conjugate eigenvalues of the Jacobian cross the imaginary axis. Before discussing generic properties of this type of bifurcation, we begin with two examples. Later in the chapter, it will be shown that these two examples are representative of all possible non-degenerate Hopf bifurcations (at least in a neighborhood) of the equilibrium. The first example is the following two-dimensional dynamical system

\[
\begin{align*}
\frac{dx_1}{dt} &= \alpha x_1 - x_2 - x_1(x_1^2 + x_2^2), \\
\frac{dx_2}{dt} &= x_1 + \alpha x_2 - x_2(x_1^2 + x_2^2),
\end{align*}
\]

or in polar coordinates

\[
\frac{dr}{dt} = (\alpha - r^2)r, \quad \frac{d\theta}{dt} = 1.
\]

When \(\alpha < 0\), there is a unique equilibrium at \(r = 0\) and this equilibrium is stable. At the critical value \(\alpha = 0\), the equilibrium \(r = 0\) becomes unstable. When \(\alpha > 0\), the \(r = 0\) equilibrium remains unstable and a stable equilibrium at \(r = \sqrt{\alpha}\) also exists. This is an example of a supercritical Hopf bifurcation.

We construct the second example by negating the sign of the nonlinear terms in
Eqs. (A.1)-(A.2) to obtain
\[
\frac{dx_1}{dt} = \alpha x_1 - x_2 + x_1(x_1^2 + x_2^2), \\
\frac{dx_2}{dt} = x_1 + \alpha x_2 + x_2(x_1^2 + x_2^2).
\]

Now in polar coordinates, we have
\[
\frac{dr}{dt} = (\alpha + r^2)r, \quad \frac{d\theta}{dt} = 1.
\]

As in the previous example, the equilibrium at \( r = 0 \) is stable when \( \alpha < 0 \) and then unstable for \( \alpha \geq 0 \). In contrast to the previous example, there also is an unstable orbit at \( r = \sqrt{-\alpha} \) which coexists when \( \alpha < 0 \) which disappears when \( \alpha \geq 0 \). This is an example of a subcritical Hopf bifurcation.

The two examples given above foreshadow a key difference between supercritical and subcritical Hopf bifurcations. Notice that in the first example (the supercritical case), the system transitions from a stable equilibrium at the origin to a low-amplitude limit-cycle when \( \alpha \) goes from negative to positive. Hence, the system could be returned to its original equilibrium by returning \( \alpha \) to a negative value. This is not true in the second example (the subcritical case). Since the basin of attraction of the stable equilibrium is bounded by the unstable orbit when \( \alpha < 0 \), the system could start at \( r = 0 \), escape the basin of attraction when \( \alpha > 0 \) and then remain outside this basin of attraction if \( \alpha \) is forced back to a negative value. As indicated by this example, systems that exhibit subcritical Hopf bifurcations are generally harder to control because reversing a parameter change may not bring the system back to its original (static) equilibrium.

In the context of the reduced IT-MFL model, the distinction between subcritical and supercritical Hopf bifurcations is mentioned in relation to bistability. This is illustrated by a system from Strogatz [60], given by

\[
\frac{dr}{dt} = \mu r + r^3 - r^5, \quad (A.3) \\
\frac{d\theta}{dt} = \omega + br^2, \quad (A.4)
\]

where \( \mu, \omega \in \mathbb{R} \) are fixed. When \( \mu < 0 \), Eqs. (A.3)-(A.4) have a stable equilibrium at \( r = 0 \) and a stable limit cycle separated by an unstable cycle. As \( \mu \) approaches zero from a negative value, the radius of the unstable cycle shrinks until it destabilizes the origin at the critical value \( \mu = 0 \). When \( \mu > 0 \), the origin remains unstable and the stable limit cycle remains present. As we will see later in this section, it can be
shown that the Hopf bifurcation at $\mu = 0$ is subcritical, illustrating how subcritical Hopf bifurcations can cause the loss of bistability.

### A.2 Generic Hopf bifurcations in two dimensions

Kuznetsov provides two derivations of the normal form of a two-dimensional system undergoing a Hopf bifurcation. The first derivation is more concrete and provides a direct connection to the Lyapunov coefficient, useful for determining the type of Hopf bifurcation. The second approach explains the logic behind some steps in the first approach through a general discussion of so-called “resonant terms”. We focus here only on the first approach and direct the reader to the Appendix of Chapter 3 of Kuznetsov’s textbook for the latter approach.

We consider a planar dynamical system

$$\frac{dx(t)}{dt} = f(x(t), \alpha),$$

where $x(t) = (x_1(t), x_2(t))^T \in \mathbb{R}^2$ is the state of the system at time $t > 0$ and $\alpha$ is the bifurcation parameter. We assume that $f(\cdot, \alpha)$ is a smooth function, and that there is an equilibrium $x = (0, 0)^T$ with eigenvalues $\pm i\omega_0$, when $\alpha = 0$. By the implicit function theorem, there is a unique equilibrium $x_0(\alpha)$ for all $\alpha$ in some neighborhood of the origin. We are free to make the coordinate change $x \to x - x_0(\alpha)$ for any $\alpha$ in this neighborhood. Making this coordinate change, we also rewrite the governing equation of the system as

$$\frac{dx}{dt} = A(\alpha)x + F(x, \alpha)$$

where $A(\alpha)$ is the Jacobian of $f(x, \alpha)$ evaluated at $x = 0$, and $F(x, \alpha) = f(x, \alpha) - A(\alpha)x$ is a function whose Taylor expansion starts at $O(||x||^2)$. We may express the characteristic equation as

$$P(\lambda) = \lambda^2 - \text{tr}A(\alpha)\lambda + \det A(\alpha) = 0.$$  

Making the change of variables

$$\mu(\alpha) = \frac{1}{2}\text{tr}A(\alpha), \quad \omega(\alpha) = \frac{1}{2}\sqrt{4\det A(\alpha) - (\text{tr}A(\alpha))^2},$$

the roots of $P(\lambda)$ may be expressed conveniently as a complex-conjugate pair

$$\lambda(\alpha) = \mu(\alpha) + i\omega(\alpha), \quad \bar{\lambda}(\alpha) = \mu(\alpha) - i\omega(\alpha).$$

**Lemma A.2.1 (First reparameterization).** Equation (A.5) can be rewritten as a com-
plex dynamical system of the form

\[
\frac{dz(t)}{dt} = \lambda(\alpha) z(t) + g(z(t), \bar{z}(t), \alpha)
\]

where \(z(t) \in \mathbb{C}^2\) for all \(t > 0\).

**Proof.** We derive this change of variable using the eigenvectors of \(A(\alpha)\). Suppose we have \(p(\alpha), q(\alpha) \in \mathbb{C}^2\) such that

\[
A(\alpha)q(\alpha) = \lambda(\alpha) q(\alpha), \quad A(\alpha)^T p(\alpha) = \bar{\lambda}(\alpha) p(\alpha).
\]

Then we may make the change of variable

\[
x = zq(\alpha) + \bar{z}q(\alpha),
\]

where the coefficient \(z \in \mathbb{C}\) is obtained by projecting Eq. (A.6) onto \(p\) to obtain\(^1\)

\[
z = \langle (p(\alpha), x) \rangle.
\]

Differentiation of the previous equation with respect to time reveals that

\[
\frac{dz}{dt} = \lambda(\alpha) z + \langle p(\alpha), F(zq(\alpha) + \bar{z}q(\alpha), \alpha) \rangle,
\]

from which we obtain \(g(z, \bar{z}, \alpha) = \langle p(\alpha), F(zq(\alpha) + \bar{z}q(\alpha), \alpha) \rangle \).

In order to find a convenient coordinate transformation where higher order nonlinearities are absent we must first expand the function \(g(z, \bar{z}, \alpha)\) with a formal Taylor series

\[
g(z, \bar{z}, \alpha) = \sum_{k+l \geq 2} \frac{1}{k! l!} g_{kl}(\alpha) z^k \bar{z}^l.
\]

This will allow us to derive reparameterizations of the dynamical system that have no quadratic and almost no cubic terms, as shown in the next two lemmas.

**Lemma A.2.2 (Removal of quadratic terms).** There exists an invertible parameter-dependent change of coordinates

\[
z = w + \frac{h_{20}}{2} w^2 + h_{11} w \bar{w} + \frac{h_{02}}{2} \bar{w}^2,
\]

\(^1\) The fact that \(p(\alpha)\) and \(q(\alpha)\) are necessarily orthogonal is used implicitly in this argument. This is quickly shown from the identity \(\langle p(\alpha), \bar{q}(\alpha) \rangle = \frac{\lambda(\alpha)}{\bar{\lambda}(\alpha)} \langle p(\alpha), q(\alpha) \rangle\), which can be rearranged to force \(\langle p(\alpha), \bar{q}(\alpha) \rangle = 0\).
such that if \( z \) satisfies
\[
\frac{dz}{dt} = \lambda z + \frac{1}{2} g_{20} z^2 + g_{11} z \bar{z} + \frac{1}{2} g_{02} \bar{z}^2 + O(|z|^3) \tag{A.7}
\]
where \( \lambda = \lambda(\alpha) = \mu(\alpha) + i\omega(\alpha) \), \( \mu(0) = 0 \), \( \omega(0) = \omega_0 > 0 \), \( g_{ij} = g_{ij}(\alpha) \), and \( \alpha \) is assumed to be small, then \( w \) obeys
\[
\frac{dw}{dt} = \lambda w + O(|w|^3).
\]

**Proof.** Start with the inverted transformation
\[
w = z - h_{20} \frac{z^2}{2} - h_{11} z \bar{z} - h_{02} \frac{\bar{z}^2}{2} + O(|z|^3)
\]
and differentiate with respect to time. The result can be simplified using Eq. (A.7) to obtain
\[
\frac{dw}{dt} = \lambda w + \frac{1}{2} (g_{20} - \lambda h_{20}) w^2 + (g_{11} + \frac{\lambda h_{11}}{\lambda}) w \bar{w} + \frac{1}{2} (g_{02} - (2\bar{\lambda} - \lambda) h_{02}) \bar{w}^2 + O(|w|^3)
\]
which forces the new coefficients to satisfy
\[
h_{20} = \frac{g_{20}}{\lambda}, \quad h_{11} = \frac{g_{11}}{\lambda}, \quad h_{02} = \frac{g_{02}}{2\bar{\lambda} - \lambda},
\]
in order to remove all quadratic terms. \( \square \)

Proceeding now to eliminate cubic terms

**Lemma A.2.3 (A resonant cubic term).** There exists a change of variables
\[
z = w + \frac{h_{30}}{6} w^3 + \frac{h_{21}}{2} w^2 \bar{w} + \frac{h_{12}}{2} w \bar{w}^2 + \frac{h_{03}}{6} \bar{w}^3
\]
that transforms the equation
\[
\frac{dz}{dt} = \lambda z + \frac{g_{30}}{6} z^3 + \frac{g_{21}}{2} z^2 \bar{z} + \frac{g_{12}}{2} z \bar{z}^2 + \frac{g_{03}}{6} \bar{z}^3 + O(|z|^4)
\]
into
\[
\frac{dw}{dt} = \lambda w + c_1(\alpha) w^2 \bar{w} + O(|w|^4)
\]
where \( \lambda = \lambda(\alpha) = \mu(\alpha) + i\omega(\alpha) \), \( \mu(0) = 0 \), \( \omega(0) = \omega_0 > 0 \), \( g_{ij} = g_{ij}(\alpha) \), and \( \alpha \) is taken to be sufficiently small.

**Proof.** The approach is the same as in Lemma A.2.2, except there is now one term
which cannot be removed. As before, we start with the inverted transformation

$$w = z - \frac{h_{30}}{6} z^3 - \frac{h_{21}}{2} z^2 \bar{z} - \frac{h_{12}}{2} z \bar{z}^2 - \frac{h_{03}}{6} \bar{z}^3 + \mathcal{O}(|z|^4)$$

and differentiate to obtain

$$\frac{dw}{dt} = \lambda w + \frac{1}{6} (g_{30} - 2\lambda h_{30}) w^3 + \frac{1}{2} (g_{21} - (\lambda + \lambda)h_{21}) w^2 \bar{w} + \frac{1}{2} (g_{12} + 2\lambda h_{12}) w \bar{w}^2 + \frac{1}{6} (g_{03} + (\lambda - 3\lambda)h_{03}) \bar{w}^3 + \mathcal{O}(|w|^4).$$

The $w^2 \bar{w}$ term is problematic because it requires us to set

$$h_{21} = \frac{g_{21}}{\lambda + \lambda},$$

but the denominator vanishes at $\alpha = 0$. Instead, we set $h_{21} = 0$ to remove any dependence on the eigenvalues in the nonlinear term. The remaining coefficients are forced to be

$$h_{30} = \frac{g_{30}}{2\lambda}, \quad h_{12} = \frac{g_{12}}{2\lambda}, \quad h_{03} = \frac{g_{03}}{3\lambda - \lambda}.$$\[\square\]

Ultimately, we have shown that the dynamics of our system in some neighborhood of the equilibrium are governed by

$$\frac{dw}{dt} = \lambda(\alpha) w + c_1(\alpha) w^2 \bar{w} + \mathcal{O}(|w|^4)$$ (A.8)

when $\alpha$ is small. The final step involved in constructing the topological normal form for a two-dimensional Hopf bifurcation is to show that the real part of the linear term in Eq. (A.8) and the factor on the nonlinear term can both be simplified through the following reparameterization.

**Lemma A.2.4 (Final reparameterization).** Consider the equation

$$\frac{dw}{dt} = (\mu(\alpha) + i\omega(\alpha)) w + c_1(\alpha) w^2 \bar{w} + \mathcal{O}(|w|^4)$$

where $\mu(0) = 0$ and $\omega_0(0) = \omega_0 > 0$. Suppose $\mu'(0) \neq 0$ and $\text{Re}(c_1(0)) \neq 0$. Then following a change of space and time coordinates, the equation can be recast as

$$\frac{du}{d\theta} = (\beta + i) u + su|u|^2 + \mathcal{O}(|u|^4),$$

where $s = \text{sign}(\text{Re}(c_1(0)))$, $\theta$ is the reparameterized time, and $\beta = \beta(\alpha)$. 
Proof. First, define the following quantities
\[ \tau = \omega(\alpha)t, \quad \beta(\alpha) = \frac{\mu(\alpha)}{\omega(\alpha)}, \quad d_1(\beta) = \frac{c_1(\alpha(\beta))}{\omega(\alpha(\beta))}. \]

Notice \( d_1(\beta) \) is well-defined because of the inverse function theorem, allowing us to locally define \( \alpha = \alpha(\beta) \). Substituting the new quantities into the governing equation, we obtain
\[ \frac{dw}{d\tau} = (\beta + i)w + d_1(\beta)|w|^2 + \mathcal{O}(|w|^4) \]

Next, define a rescaled time \( \theta \) by
\[ d\theta = (1 + e_1(\beta)|w|^2) \, d\tau \]

where \( e_1(\beta) = \text{Im}(d_1(\beta)) \). Substitute and find
\[ \frac{dw}{d\theta} = \frac{1}{1 + e_1(\beta)|w|^2} \left((\beta + i)w + d_1(\beta)|w|^2 + \mathcal{O}(|w|^4)\right) \]
\[ = (1 - e_1(\beta)|w|^2) \left((\beta + i)w + d_1(\beta)|w|^2\right) + \mathcal{O}(|w|^4) \]
\[ = (\beta + i)w + l_1(\beta)|w|^2 + \mathcal{O}(|w|^4) \]

where \( l_1(\beta) = \text{Re}(d_1(\beta)) - \beta e_1(\beta) \). Finally, we wish to simplify the coefficient on the nonlinear term in the model. To do this, we want to rescale \( w \) in such a way that we end with \( l_1(\beta)/|l_1(\beta)| = \text{sign}(l_1(\beta)) \). Scaling shows that this can be accomplished by defining
\[ w = \frac{u}{|\sqrt{l_1(\beta)}|}. \]

This rescaling is well-defined because \( l_1(0) \neq 0 \), and so there must exist some neighborhood of the origin such that \( l_1(\beta) \) has a fixed sign everywhere in this region, and in particular is nonzero. The new variable \( u \) satisfies
\[ \frac{du}{d\theta} = (\beta + i)u + \frac{l_1(\beta)}{|l_1(\beta)|} + \mathcal{O}(|u|^4). \tag{A.9} \]

Notice that Eq. (A.9) has the correct form since \( \frac{l_1(\beta)}{|l_1(\beta)|} = \text{sign}(c_1(0)) \).

Remark 3 (First Lyapunov coefficient).

The function \( l_1(\beta) \) is known as the first Lyapunov coefficient. We see now that after several coordinate changes, it is always possible to find a local smooth transformation from a two-dimensional dynamical system into a dynamical system resembling the two examples discussed at the start of this section. We also see how the sign of the Lyapunov coefficient determines whether the bifurcation will be subcritical or super-
critical. It is also worth noting that the value of the Lyapunov coefficient depends on one’s choice of coordinates, but the sign is invariant. The first Lyapunov coefficient can be expressed more explicitly as

\[ l_1(0) = \frac{\text{Re}(c_1(0))}{\omega(0)} = \frac{1}{2\omega^2_0} \text{Re}(ig_{20}g_{11} + \omega_0g_{21}). \quad (A.10) \]

The terms \( g_{jk} \) appearing in Eq. (A.10) can be easily computed using the following multilinear maps

\[
B_i(x, y) := \sum_{j,k=1}^{n} \frac{\partial^2 F_i(\xi, 0)}{\partial \xi_j \partial \xi_k} \bigg|_{\xi=0} x_j y_k,
\]

\[
C_i(x, y, z) := \sum_{j,k,\ell=1}^{n} \frac{\partial^3 F_i(\xi, 0)}{\partial \xi_j \partial \xi_k \partial \xi_\ell} \bigg|_{\xi=0} x_j y_k z_\ell.
\]

Using the notation \( q := q(0) \in \mathbb{C}^n \), we may evaluate \( B(zq + \overline{z}q, zq + \overline{z}q) \) to find

\[
B_i(zq + \overline{z}q, zq + \overline{z}q) = (zq + \overline{z}q)^T \nabla^2 F_i(\xi, 0) \bigg|_{\xi=0} (zq + \overline{z}q)
= z^2 B(q, q) + 2z\overline{z} B(q, \overline{q}) + \overline{z}^2 B(\overline{q}, \overline{q}) \quad (A.11)
\]

for \( i = 1, \ldots, n \). After projecting onto \( p = p(0) \in \mathbb{C}^n \), we see that the terms in Eq. (A.11) coincide with the \( g_{jk} \) terms in the Taylor expansion of \( g(z, \overline{z}, 0) \). More explicitly, we have

\[
\langle p, F(zq + \overline{z}q, 0) \rangle = \langle p, B((zq + \overline{z}q), (zq + \overline{z}q)) \rangle + \mathcal{O}(||(zq + \overline{z}q)||^3)
= z^2 \langle p, B(q, q) \rangle + 2z\overline{z} \langle p, B(q, \overline{q}) \rangle + \overline{z}^2 \langle p, B(\overline{q}, \overline{q}) \rangle + \mathcal{O}(||(zq + \overline{z}q)||^3)
= g_{20} z^2 + 2g_{11} z\overline{z} + g_{02} \overline{z}^2 + \mathcal{O}(||(zq + \overline{z}q)||^3).
\]

Which implies

\[
g_{20} = \langle p, B(q, q) \rangle, \quad g_{11} = \langle p, B(q, \overline{q}) \rangle, \quad g_{02} = \langle p, B(\overline{q}, \overline{q}) \rangle.
\]

Similar reasoning reveals that

\[ g_{21} = \langle p, C(q, q, \overline{q}) \rangle. \]

Altogether, we have obtained a way of evaluating the first Lyapunov coefficient directly from Hessian, and third derivative matrix of the original function \( f(x, \alpha) \).

When a system in \( n > 2 \) dimensions undergoes a Hopf bifurcation, there is a close analogy to the \( n = 2 \) case. The main idea is to find a way to divide the phase space
into a two-dimensional sub-manifold that resembles the planar theory, and an $n - 2$ dimensional sub-manifold with trivial dynamics. The third derivative term in the Lyapunov coefficient generalizes to

$$g_{21} = \langle p, C(q, q, \bar{q}) \rangle - 2\langle p, B(q, A^{-1}B(q, \bar{q})) \rangle + \langle p, B(\bar{q}, (2i\omega_0 I_n - A)^{-1}B(q, q)) \rangle$$

$$+ \frac{1}{i\omega_0} \langle p, B(q, q) \rangle \langle p, B(q, \bar{q}) \rangle - \frac{2}{i\omega_0} |\langle p, B(q, \bar{q}) \rangle|^2 - \frac{1}{3i\omega_0} |\langle p, B(\bar{q}, q) \rangle|^2,$$

whereas the quadratic terms coincide with the two-dimensional case. This results in a general expression for the Lyapunov coefficient

$$l_1(0) = \frac{1}{2\omega_0} \text{Re}\{\langle p, C(q, q, \bar{q}) \rangle - 2\langle p, B(q, A^{-1}B(q, \bar{q})) \rangle$$

$$+ \langle p, B(\bar{q}, (2i\omega_0 I_n - A)^{-1}B(q, q)) \rangle\}.$$

which remains valid in $n > 2$ dimensions. Numerical methods for evaluating this expression are discussed in Appendix B.
Appendix B

Numerical methods

B.1 Continuation for detecting Hopf bifurcations

The background on continuation methods in this section is of relevance to Fig. 4.5, which was obtained using a predictor-corrector method (an example of a continuation method). This section follows the introduction to numerical continuation provided in Kuznetsov’s textbook [59].

The identification of bifurcations in a mathematical model often involves solving an under-determined system of nonlinear equations

\[ F(x; \lambda) = 0 \]

where \( \lambda \in \mathbb{R} \) and \( x \in \mathbb{R}^m \) and \( F : \mathbb{R}^{m+1} \to \mathbb{R}^m \). Assuming the bifurcation takes place at an equilibrium of the model, we may assume \( F \) has the form

\[
F(x; \lambda) = \left( f_{eq}(x; \lambda) \right)^T g_{bif}(x; \lambda) = 0,
\tag{B.1}
\]

where \( f_{eq} \) is derived from the right-hand side of the differential equation and \( g_{bif} \) is a condition for bifurcations to occur. We keep \( g_{bif} \) generic since the technique described here is applicable to many types of bifurcations. We assume for simplicity that \( \lambda \) is a scalar, or in the language of bifurcation theory, we assume the bifurcation is of co-dimension 1. We use the notation \( y = (x, \lambda) \in \mathbb{R}^{m+1} \) to simplify later expressions.

Predictor-corrector methods start by finding a solution \( F(x_0; \lambda_0) = 0 \) and then alternate between prediction and correction steps. Predictions are generated by stepping a distance \( h_j \in \mathbb{R} \) in a pre-determined direction \( v_j \) to obtain

\[
y_{j+1}^{(p)} = y_j + h_j v_j.
\]
The prediction $y_{j+1}^{(p)}$ is then corrected using an augmented system

$$
\begin{pmatrix}
F(y) \\
G(y, y_{j+1}^{(p)})
\end{pmatrix} = 0,
$$

(B.2)

where $G : \mathbb{R}^{m+1} \times \mathbb{R}^{m+1} \to \mathbb{R}$ is an additional scalar condition. Notice that unlike Eq. (B.1), the augmented system in Eq. (B.2) is fully determined. Hence we are free to solve it using Newton’s method (for instance). In pseudo-arclength continuation (one example of a numerical continuation method), the additional equation $G(y, y_{j+1}^{(p)}) = 0$ takes the form of an orthogonality condition

$$
G(y, y_{j+1}^{(p)}) = \langle y - y_{j+1}^{(p)}, v_j \rangle = 0.
$$

Pseudo-arclength continuation is sufficient for the applications in Chap. 4, so all that remains is to specify the appropriate bifurcation condition. For detecting a Hopf bifurcation, one is interested in identifying when two eigenvalues cross the imaginary axis. A reasonable bifurcation condition is as follows

$$
g_{\text{bif}}(y) = \sum_{i>j} (\lambda_i(y) + \lambda_j(y)),
$$

(B.3)

where $\lambda_i(y)$ is the $i$-th eigenvalue of the Jacobian evaluated at $y$. Notice that

$$
g_{\text{bif}}(y) = 0
$$

when a Hopf bifurcation occurs. Equation (B.3) is not ideal for large systems because it requires explicit computation of the eigenvalues. Fortunately, the reduced IT-MFL model is only three-dimensional and exact expressions for the eigenvalues can be derived as follows. The dynamical system of interest takes the form

$$
\frac{d\tilde{r}}{d\tau} = T(\tilde{B}_{\text{tot}}) - \tilde{r}, \quad \frac{d\tilde{B}_c}{d\tau} = \tilde{r} - \tilde{B}_c, \quad \frac{d\tilde{B}_{\text{tot}}}{d\tau} = \tilde{B}_c - \tilde{B}_{\text{tot}}.
$$

Symbolic computation reveals that the eigenvalues of the Jacobian take the form

$$
\lambda_1 = T'(\tilde{B}_{\text{tot}})^{\frac{1}{3}} - 1, \\
\lambda_2 = -\frac{T'(\tilde{B}_{\text{tot}})^{\frac{1}{3}}}{2} - 1 - i \frac{\sqrt{3}}{2} T'(\tilde{B}_{\text{tot}})^{\frac{1}{3}}, \\
\lambda_3 = -\frac{T'(\tilde{B}_{\text{tot}})^{\frac{1}{3}}}{2} - 1 + i \frac{\sqrt{3}}{2} T'(\tilde{B}_{\text{tot}})^{\frac{1}{3}}.
$$

Notice that $T(\tilde{B}_{\text{tot}})$ is the only dynamic variable that influences the eigenvalues.
This makes intuitive sense because all other dynamic variables appear linearly in the governing equations. Since $\lambda_1$ is always real, it is reasonable to ignore it when constructing $g_{bif}$, which gives

$$g_{bif}(y) = \lambda_2 + \lambda_3 = -T'(\tilde{B}_{tot})^{\frac{1}{3}} - 2.$$  

As discussed in Appendix A, it is useful to compute the first Lyapunov coefficient $l_1(0)$ in order to determine if a Hopf bifurcation is subcritical or supercritical. For the reduced IT-MFL model, the derivative matrices are sparse and so it is reasonable to compute the terms in $l_1(0)$ manually to reduce the total amount of symbolic computation.

For higher dimensional systems, it can be worthwhile to use automatic differentiation (AD) for computing $l_1(0)$ [61, 62]. An implementation of the first Lyapunov coefficient using AD is available through the author’s github. It makes use of the MATLAB implementation of AD included in the Deep Learning Toolbox, and can easily be applied to other models which exhibit Hopf bifurcations.

### B.2 Period sensitivity for oscillating systems

In contrast to numerical bifurcation analysis where the emphasis is on sudden qualitative changes in dynamics, it can also be useful to study the influence of parameter changes when they have a smooth effect on the model. There are several reasons why one might want to determine how strongly a parameter influences an observed feature of a model:

- **Uncertainty in parameters** – If the value of a parameter cannot be reliably estimated from experimental data, it may still be reasonable to trust the model’s predictions provided that they are not sensitive to the uncertain parameter.

- **Determining optimality** – Suppose that we wish to maximize the steady-state of a dynamic variable. We will see that sensitivity analysis is closely related to studying gradients in parameter space, and hence useful for extremizing an objective function.

- **Experimental design** – Determining which parameters have the greatest influence on the model’s dynamic behaviour can help determine what kind of measurements to prioritize in future experiments.

- **Model validation** – Predicted sensitivities can be compared to data provided that there is an experimentally accessible control parameter.
- Simplifying the model - If the predictions of a model are insensitive to certain components, then these may be ignored when deriving a simplified version of the original model.

The analysis in Fig. 4.6 is an example of this, known as local sensitivity analysis. In this section we discuss a numerical method that was designed specifically for studying period sensitivity in oscillating systems [57]. This is a more challenging problem than the study of time-invariant equilibrium features, where finite-difference methods are often sufficient.

Given a scalar function \( \Phi(x; p) \) such as the steady-state value of a dynamic variable, the amplitude of an oscillation, or the period of an oscillation, local sensitivity analysis involves computing the derivative

\[
S_\Phi(p_0) = \frac{p_0}{\Phi(x; p_0)} \frac{\partial \Phi(x, p)}{\partial p} \bigg|_{p=p_0}.
\]

The normalization factor is included so that \( S_\Phi(p_0) \) can be viewed as a percentage change, that is to say “for a 1% change in parameter \( p \), the model predicts a \( S_\Phi(p_0) \)% change in \( \Phi \).

The numerical method studied in this section is most naturally expressed using the notation of stoichiometry matrices explained in Sec. 2.2. Hence, we suppose our model takes the form of a concentration vector \( s(t) = (s_1(t), \ldots, s_n(t)) \) made up of \( n \) chemical species that participate in \( m \) reactions, which evolves according to

\[
\frac{ds(t)}{dt} = Nv(s(t); p), \tag{B.4}
\]

where \( N \in \mathbb{R}^{n \times m} \) is the stoichiometry matrix, and the \( v(s; p) \in \mathbb{R}^m \) is the propensity vector. The dependence of the reaction rates on a parameter of interest is emphasized by writing \( v = v(s; p) \).

Since \( N \) need not be a square matrix, no generality has been lost by assuming the governing equation for our model has the form of Eq. (B.4). We assume for simplicity that the rows of \( N \) are linearly independent and that for all values of the distinguished parameter in some interval \( p \in [p_1, p_2] \subset \mathbb{R} \) the system exhibits stable limit-cycle trajectories. The method described below still applies if there is linear dependence in the rows, however it simplifies the notation and exposition if linear independence is assumed.

Let \( T : [p_1, p_2] \to \mathbb{R}_{>0} \) denote the period of the limit-cycle corresponding to parameter value \( p = \in [p_1, p_2] \subset \mathbb{R}_{>0} \) and assume that \( T \) is continuously differentiable in this interval.

**Definition B.2.1** (Unscaled sensitivity function). Let \( s(t; p_0) \) denote a solution to
Eq. (B.4) with parameters $p_0$ and initial condition $s(0) = s^0$, and define the unscaled sensitivity function by

$$R^s(t) = \frac{\partial s(t;p)}{\partial p} \bigg|_{p=p_0} = \lim_{\Delta p \to 0} \frac{s(t, p_0 + \Delta p) - s(t, p_0)}{\Delta p}.$$  

In the particular case where $R^s$ is associated with a periodic trajectory, we denote this by $R^s_*$ and refer to it as the asymptotic sensitivity function.

**Definition B.2.2 (Variation of sensitivity).** Suppose that $s(t; p_0) = s_{\text{per}}(t; p_0)$ is a periodic solution of Eq. (B.4) with period $T(p_0)$. We denote the variation of sensitivity over the limit-cycle by

$$\Delta R^s(t) = R^s(t + T(p_0)) - R^s(t).$$

To see why $\Delta R^s(t)$ is not identically zero, we write out $R^s(t + T(p_0))$ explicitly

$$R^s(t + T(p_0)) = \frac{\partial s}{\partial p} \bigg|_{p=p_0} (t + T(p_0)) = \frac{\partial}{\partial p} \bigg|_{p=p_0} s(t + T(p_0); p).$$  

Equation (B.5) is not necessarily equal to $R^s(t + T(p_0))$ because there may exist $p \in [p_1, p_2]$ such that

$$s(t + T(p_0); p) \neq s(t; p)$$

even though $s(t + T(p_0); p_0) = s(t; p_0)$. Hence, we should understand $\Delta R^s(t)$ to be a function with nontrivial time-dependence.

The crucial observation that allows us to determine $\frac{d}{dp} T(p)$ without applying a finite difference method to period estimates is as follows. The function $R^s(t)$ satisfies

$$\frac{d}{dt} R^s(t) = \left( N \frac{\partial v(t)}{\partial s} \right) R^s(t) + N \frac{\partial v(t)}{\partial p},$$

subject to the initial condition $R^s(0) = 0$ and $\Delta R^s(t)$ satisfies the homogeneous part of the equation above, under the initial condition $\Delta R^s(0) = R^s(T(p_0))$. The choice of initial condition in the former equation requires some justification discussed in [57].

The next result shows how to obtain the unscaled period sensitivity from the variation of sensitivity.

**Theorem B.2.1 (Estimation of period sensitivity, [63]).** Let $s_{\text{per}}(t; p)$ be a periodic solution to Eq. (B.4). The sensitivity of the oscillation period $T(p)$ can be estimated by

$$\frac{d}{dp} T(p) = \lim_{k \to \infty} \frac{-\Delta R^s_j(t_k)}{N_j \nu(s_{\text{per}}(t_k, p), p)},$$  

where $N_j \nu(s_{\text{per}}(t_k, p), p)$ is the Jacobian of the periodic solution. 

\[ \text{(B.6)} \]
where \( N_j \) is the \( j \)-th row of \( N \), \( \mathbf{s}_{\text{per}} \) is a limit-cycle solution of Eq. \((B.4)\), and \( \{t_k\}_{k=1}^{\infty} \) is an unbounded sequence of increasing times satisfying \( N_j \nu(\mathbf{s}_{\text{per}}(t_k, p_0), p_0) \neq 0 \).

**Proof.** Since \( \mathbf{s}_{\text{per}} \) is a periodic function of time, we have
\[
\mathbf{s}_{\text{per}}(t + T(p_0); p) = \mathbf{s}_{\text{per}}(t; p_0)
\]
for all \( t > 0 \) differentiation with respect to \( p \) gives
\[
\frac{\partial}{\partial t} \mathbf{s}_{\text{per}}(t + T(p_0), p_0) \frac{d}{dp} T(p) \bigg|_{p = p_0} + \frac{\partial}{\partial p} \mathbf{s}_{\text{per}}(t + T(p_0), p_0) = \frac{\partial}{\partial p} \mathbf{s}_{\text{per}}(t; p_0)
\]
the first term can be rewritten using Eq. \((B.4)\), and the latter two terms can be rewritten using the definitions \( \mathbf{R}^s(t) = \frac{\partial \mathbf{s}(t)}{\partial p} \) and \( \mathbf{R}^s_*(t) = \frac{\partial \mathbf{s}_{\text{per}}(t; p_0)}{\partial p} \) to obtain
\[
N \mathbf{v}(s, p_0) \frac{d}{dp} T(p) \bigg|_{p = p_0} = -\mathbf{R}^s_*(t + T(p_0)) + \mathbf{R}^s_*(t)
\]
\[
= -\lim_{t \to \infty} (\mathbf{R}^s(t + T(p_0)) + \mathbf{R}^s(t))
\]
\[
= -\lim_{t \to \infty} \Delta \mathbf{R}^s(t; p_0).
\]
Making equalities component-wise and dividing gives the desired result. \( \square \)

Hence, computing the period sensitivity can be reduced to first solving the system
\[
\frac{d}{dt} \begin{bmatrix} \mathbf{s}(t) \\ \mathbf{R}^s(t) \end{bmatrix} = \begin{bmatrix}
N \mathbf{v}(s; p) \\
N \frac{\partial \mathbf{v}(t)}{\partial s} \mathbf{R}^s(t) + N \frac{\partial \mathbf{v}(t)}{\partial p} \Delta \mathbf{R}^s(t)
\end{bmatrix}
\]
subject to the initial condition \( \mathbf{s}(0) = \mathbf{s}^0 \in \mathbb{R}^n \) and \( \mathbf{R}^s(0) = \mathbf{0} \). This produces an estimate of the period \( T(p_0) \) which is then used in constructing the initial conditions for the system
\[
\frac{d}{dt} \begin{bmatrix} \mathbf{s}(t) \\ \Delta \mathbf{R}^s(t) \end{bmatrix} = \begin{bmatrix}
N \mathbf{v}(s; p) \\
N \frac{\partial \mathbf{v}(t)}{\partial s} \Delta \mathbf{R}^s(t)
\end{bmatrix}
\]
subject to \( \mathbf{s}(0) = \mathbf{s}_\ell \), a point on the limit-cycle and \( \Delta \mathbf{R}^s(0) = T(p_0)1_n \) where \( 1_n \in \mathbb{R}^n \) is the vector of all ones. The period sensitivity then can be estimated using Eq. \((B.6)\).
Bibliography


[58] Jean-Michel Fustin, Shiqi Ye, Christin Rakers, Kensuke Kaneko, Kazuki Fuku-


