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Robustness in bacterial chemotaxis

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Abstract

Applying mathematics on biological system is an emerging science and the main goal is to model and discover emergent properties. Within this science the aim is to examine interactions within a given biological system, to understand the behaviour and functions for these interactions that the system exhibits. Biological systems are complex chemical networks that often operate under a variety of external stimuli, therefore it is of interest to analyse the robustness of these systems to know how and why they are able to function in different environments. In this thesis, the focus lies on understanding the robustness of the biological system bacterial chemotaxis. The basis for the mathematical approach is control theory and it is used to try to characterise the mechanism robustness that the bacterial chemotaxis exhibits. The robustness analysis was done by studying the stability of the bacterial chemotaxis, using several stability criteria. In addition the robustness was analysed by examining the mechanism adaptation that the bacterial chemotaxis exhibits. Adaptation is analysed by plotting the steady state that the bacterial chemotaxis presents and by a control theory technique called integral feedback. Thus, by using different techniques from control theory, it was possible to display that the bacterial chemotaxis is a robust mechanism.

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1 Introduction

Since the beginning of evolution, a bacteria has evolved to survive in a variety of changes in the external environment. To be able to function in different environments the bacteria must be able to sense environmental changes and respond appropriately. One way for the bacteria to detect any external changes is by a mechanism called two-component signaling pathway. Two-component signaling pathway refers to when extracellular molecules attach on a transmembrane receptor protein and trigger a cellular response inside the bacteria. A bacteria can sense a variety of different external environmental changes and the response to these changes involves adjustments in the bacteria behaviour. The two-component signaling pathway has been identified in many different bacterial species and there is a wide range of variation to which role these pathways serve [8].

In this thesis we will look closer into a specific two-component signaling pathway, namely bacterial chemotaxis of the bacteria *Escherichia coli* (commonly known as *E.Coli*). Bacterial chemotaxis is one of the best-characterised sensory systems and it is a process that describes when an *E.coli* senses chemical species in its surrounding and moves along gradients of these specific chemical species [2]. The movement of an *E.coli* means that it either swims toward higher concentrations of nutrients (called chemoattractants) or away from toxin substances (called chemorepellents) [8] [1]. A study over an *E.Coli* chemotaxis when the *E.coli* senses chemoattractants in its surrounding, will be done in this thesis.

The mathematical approach in this study will have its focus on control theory. Control theory is a mathematical branch that deals with the behaviour of a dynamical system with inputs and outputs. It is mainly applied in engineering systems, where the dynamical behaviour of technical systems must be controlled. In common with engineering systems, biological systems (such as bacterial chemotaxis) must also function with the presence of internal and external uncertainty. This makes biological systems a good target for control theory. Within control theory an analysis of a biological system can be done with time-domain analysis and frequency-domain analysis. With help from both fields a robustness analysis of bacterial chemotaxis will be done.

2 Background

2.1 The Model

A simplified model from the textbook 'Mathematical modeling in systems biology' ([8], page 192) will be used in this thesis and can be seen in Figure 1. The model is a chemical reaction network and it provides a useful overview of the bacterial chemotaxis. As mentioned in the introduction, bacterial chemotaxis is a two-component signaling pathway and the mechanism describes the process when information is transferred across the membrane and trigger the *E.coli* to respond in an appropriate way. Our model (Figure 1) illustrates when chemoattractants binds to a transmembrane receptor protein and causes the bacteria

to swim towards higher concentration of chemoattractants. An E.coli has two ways to control its own movement, which are running and tumbling. Running refers to when the bacteria keeps a fairly constant direction and tumbling refers to when the bacteria stops and randomly changes direction [1]. When an E.coli is in a homogeneous environment the bacteria alternates between tumbling and running, and the movement results in a random walk. If instead the environment should contain chemoattractants or chemorepellents, the bacteria responds by tumbling less frequently when moving into a 'good' environment and tumbling more frequently when moving into a 'bad' environment. When the bacteria again senses that it is in a homogeneous environment it returns to random walk [8].

One key factor in the bacteria movement is adaptation. If an E.coli is in a homogeneous environment and chemoattractants are evenly added so that the environment again is homogeneous, but has a higher concentration of chemoattractants. Then at first the bacteria will start running, since it assumes that it is going into a 'good' direction. The period of time that it takes for the bacteria to comprehend it is in a homogeneous environment and starts random walking is called adaptation time [8]. The whole process, when the E.coli becomes insensitive to homogeneous concentration levels, is called perfect adaptation [6].

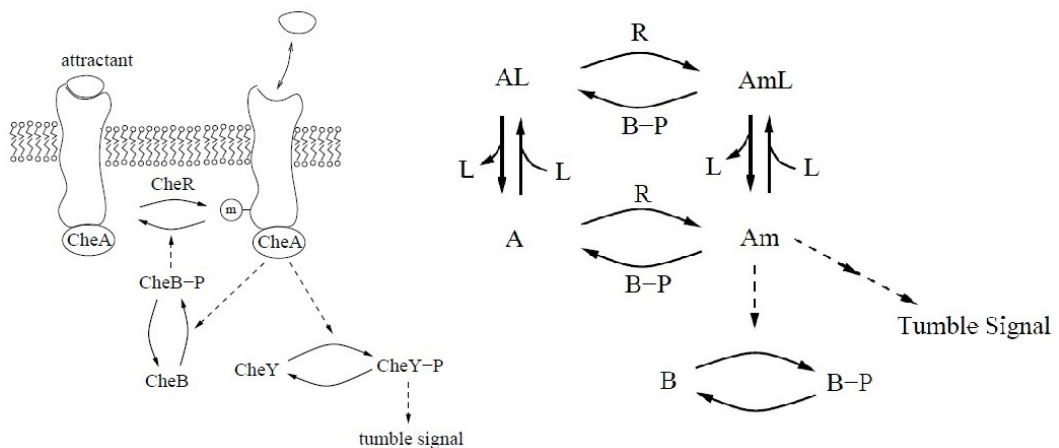


Figure 1: A chemical reaction network over an E.coli chemotaxis. The left image shows the anatomy of the reaction network whereas the right image illustrates the reaction scheme. The image is taken from the book 'Mathematical modeling in systems biology: an introduction' [8]

The left image in Figure 1 shows a chemical reaction network and the reaction starts when a chemoattractant is bound to the transmembrane receptor. The receptor-bound kinase CheA activates the protein CheY-P and in turn this protein induces tumbling. In addition to the tumbling response, this reaction is the basis for the E.Coli adaptation mechanism. However, the ability for the E.coli to adapt is somewhat more complex. On each receptor there is a site that can

bind methyl groups on CheA. The methylation, when a kinase CheA binds to a methyl group, is catalysed by the enzyme CheR. The demethylation, when a kinase CheA separates to a methyl group, is catalysed by the enzyme CheB. Tumbling is induced when CheA is methylated. A negative feedback loop is also provided by a catalytic function of the methylated CheA, which activates the enzyme CheB-P and it is this enzyme that creates the negative feedback loop. The feedback loop reduces tumbling and it is this mechanism that makes the system return to its nominal level, after an increased chemoattractant concentration. So, the mechanism adaptation is caused by the reactions between the methylated CheA, CheB, CheB-P and CheR [8]. Due to the ability for an E.Coli to change the tumbling frequency, the bacteria is not only able to adapt to its environment but also able to change its own motion [3].

The right image in Figure 1 shows the reaction scheme and it is a further simplification of the system. The species A is CheA, R is CheR, B is CheB, B-P is CheB-P, m indicates methylation and L is the chemoattractant [8]. It is this system of reactions that is used in this thesis. Note that the left image in Figure 1 consists of two more kinases than the right image, namely CheY and CheY-P. They can be left out from the model without loss of generality [2].

2.2 The robustness principle

An E.coli chemotaxis depends on the different biochemical species (CheA, CheR, CheB, CheB-P, CheY and CheY-P) and the parameters of their components, such as the concentration of the different chemical species and their rate constants. In real life, these parameters often vary from bacteria to bacteria, due to stochastic effects, even though they are genetically identical and are in the same environment [1]. Despite these internal differences between the bacteria, it seems that the mechanism chemotaxis is able to function correctly. A question that naturally comes up, how can bacterial chemotaxis function despite different disparities and how sensitive is the system to various changes in the parameters?

To be able to answer the question, the concept robustness is introduced. Robustness is the E.coli chemotaxis ability to function correctly in the presence of internal and external uncertainties [6]. Variations of the parameters, that is the rate's constants, is an example of internal uncertainty. If it can be shown that the bacterial chemotaxis is insensitive towards changes in the rate constants, then robustness is shown for internal uncertainty. Variations of the chemoattractants is an example of external uncertainty. If it can be shown that the bacterial chemotaxis is insensitive towards chemoattractant levels, then robustness is shown for external uncertainty. When robustness is discussed in a system, it is important to clarify which property is robust and with respect to which parameter. If a property is not robust, it is called fine-tuned, meaning that the system is sensitive to variations of the biochemical parameters. If a property is fined-tuned, then the properties can changes significantly if variations of the biochemical parameters occurs [1].

Mathematically, robustness analysis on a biological system can be performed by several methods and many of them come from the control theory [6]. In this thesis the method for robustness analysis will be done with robust stability.

Robust stability refers to the analysis of the stability of a system and see to what degree the stability holds, that is, how much internal or external variations must occur for the system to become unstable [6]. In addition to robust stability, a control technique called integral feedback will be applied. It refers to analysing the system towards external uncertainty and see if the system is insensitive to external stimuli [15].

3 Reduction and linearisation of the model

3.1 The non-linear system of bacterial chemotaxis

As described in the previous section, the model consists of a simplified reaction scheme. In order to proceed with the analysis, a dynamical mathematical model of these chemical reactions must be constructed. The mathematical model will take the form of ordinary differential equations. To obtain these differential equations, one must apply a law called 'law of mass action'. By this law, every chemical reaction in the model will correspond to one differential equation. Every differential equation describes the rate change of the specific chemical species that was in the corresponding chemical reaction [8].

Definition, Law of mass action [8]:

The rate of a chemical reaction is proportional to the product of the concentration of the reactants.

In addition, when CheB-P is demethylated into CheB it follows the Michaelis-Menten rate kinetics [8]. To better understand how the differential equations are generated, Figure 2 is provided and it illustrates the reaction scheme, but with the rate constants inserted.

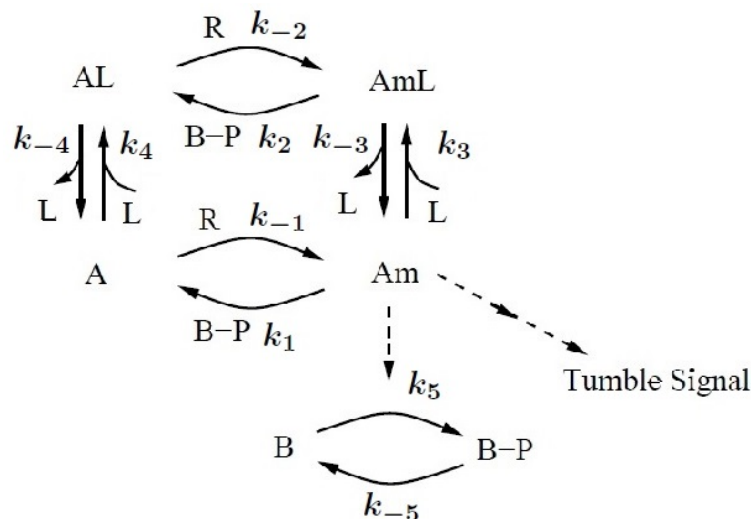


Figure 2: An illustration of the reaction scheme, with the rate constants inserted.

The following differential equation was generated by applying the law of mass action and Michaelis-Menten kinetics into the reaction network. The differential equations are taken from the book 'Mathematical modeling in systems biology: an introduction', [8].

$$\begin{aligned}
\frac{d}{dt}[Am] &= k_{-1} * [R] - \frac{k_1 * [BP](t) * [Am](t)}{k_{M1} + [Am](t)} - k_3 * [Am](t) * [L] + k_{-3} * [AmL](t) \\
\frac{d}{dt}[AmL] &= k_{-2} * [R] - \frac{k_2 * [BP](t) * [AmL](t)}{k_{M2} + [AmL](t)} + k_3 * [Am](t) * [L] - k_{-3} * [AmL](t) \\
\frac{d}{dt}[A] &= -k_{-1} * [R] + \frac{k_1 * [BP](t) * [Am](t)}{k_{M1} + [Am](t)} - k_4 * [A](t) * [L] + k_{-4} * [AL](t) \\
\frac{d}{dt}[AL] &= -k_{-2} * [R] + \frac{k_2 * [BP](t) * [AmL](t)}{k_{M2} + [AmL](t)} + k_4 * [A](t) * [L] - k_{-4} * [AL](t) \\
\frac{d}{dt}[B] &= -k_5 * [Am](t) * [B](t) + k_{-5} * [BP](t) \\
\frac{d}{dt}[BP] &= k_5 * [Am](t) * [B](t) - k_{-5} * [BP](t)
\end{aligned}$$

where every chemical species, written as [...], is represented by its concentration. k_{M1} , k_{M2} , k_i and k_{-i} , $i = 1, \dots, 5$ are rate constants for the different chemical reactions. See Figure 2 to better understand which rate constant belongs to which chemical reaction.

These differential equations form a six-dimensional non-linear dynamical system over the model. It is mathematically difficult to handle such a large system, therefore it is desirable to reduce this system into a smaller dimension. A system of this kind can be reduced with help from conservation laws. In our case, the conservation laws describe the behaviour for the different chemical species of the model. If the total concentration of two or more chemical species is conserved, meaning that the total concentration will not increase or decrease as time increases, then these chemical species together form a conservation law. In chemistry this is referred to as 'moiety conservations'. Mathematically this means that if certain chemical species correspond to a conservation law, then the sum of the chemical species concentration is a constant that holds for all time t . Also, if a conservation law exists then the sum of the differential equations that construct a conservation must be zero [8].

There are several methods to construct conservation laws. A common method is to use 'Noether's theorem'. This theorem makes it possible to construct a conservation law explicitly by a formula, but the knowledge of a suitable Lagrangian function for the system is required [12]. Since our system does not have a known Lagrangian function, another method called the direct method will be applied. This method is used by simply inspecting the system and see if there are conservation laws. By inspecting our system, it is clear that the system contains two conservation laws. The first conservation law corresponds to the first four differential equations and the second conservation law corresponds to the last two differential equations. We test the observation and see if it holds:

$$\begin{aligned}\frac{d}{dt}[Am] + \frac{d}{dt}[AmL] + \frac{d}{dt}[A] + \frac{d}{dt}[AL] &= 0 \\ \frac{d}{dt}[B] + \frac{d}{dt}[BP] &= 0\end{aligned}$$

Since the sum of the differential equations is zero, the observation is accurate. So without loss of generality we can take these two conservation laws:

$$\begin{aligned}[Am] + [AmL] + [A] + [AL] &= [A_T] \\ [B] + [BP] &= [B_T]\end{aligned}$$

where $[A_T]$ stands for the total concentration of $[Am]$, $[AmL]$, $[A]$ and $[AL]$, and $[B_T]$ stands for the total concentration of $[B]$ and $[BP]$. Both $[A_T]$ and $[B_T]$ are determined by the initial values of the chemical species. Namely when $t = 0$, meaning that the starting concentrations of the chemical species determine the total concentrations $[A_T]$ and $[B_T]$:

$$\begin{aligned}[Am](0) + [AmL](0) + [A](0) + [AL](0) &= [A_T] \\ [B](0) + [BP](0) &= [B_T]\end{aligned}$$

Since we do not have any information about the initial values, both $[A_T]$ and $[B_T]$ are set to arbitrary values in the simulation (see Appendix A).

The conservation laws allow us to reduce two equations from the system. By applying the first conservation law, we can substitute one of the first four equations, and applying the second law allows us to substitute one of the last two equations. In this study the equations $\frac{d}{dt}[B]$ and $\frac{d}{dt}[A]$ are eliminated. These result leaves us with a four dimensional system.

$$\begin{aligned}\frac{d}{dt}[Am] &= k_{-1} * [R] - \frac{k_1 * [BP](t) * [Am](t)}{k_{M1} + [Am](t)} - k_3 * [Am](t) * [L] + k_{-3} * [AmL](t) \\ \frac{d}{dt}[AmL] &= k_{-2} * [R] - \frac{k_2 * [BP](t) * [AmL](t)}{k_{M2} + [AmL](t)} + k_3 * [Am](t) * [L] - k_{-3} * [AmL](t) \\ \frac{d}{dt}[AL] &= -k_{-2} * [R] + \frac{k_2 * [BP](t) * [AmL](t)}{k_{M2} + [AmL](t)} + k_4 * ([A_T] - [Am](t) - [AmL](t) \\ &\quad - [AL](t)) * [L] - k_{-4} * [AL](t) \\ \frac{d}{dt}[BP] &= k_5 * [Am](t) * ([B_T] - [BP](t)) - k_{-5} * [BP](t)\end{aligned}$$

3.2 Equilibrium points

Biological systems often operate when the concentration of each chemical species remains approximately constant. This is referred to as the system being in steady state or in equilibrium. The point where the system exhibits steady state is called an equilibrium point. Since a biological system can be perturbed by unpredicted events, the system can exhibit several steady states and an equal number of equilibrium points. The equilibrium points are found by setting all the differential equations to zero and they are constant solutions. That means, if the state of a system starts in an equilibrium point it will remain in the equilibrium point for all $t > 0$ [6] [14].

For our existing system, we find the equilibrium points by setting the right hand-side of all the differential equations to zero and calculate the points where $[Am]$, $[AmL]$, $[AL]$ and $[BP]$ stay constant. We set:

$$\frac{d}{dt}[Am] = 0, \quad \frac{d}{dt}[AmL] = 0, \quad \frac{d}{dt}[AL] = 0, \quad \frac{d}{dt}[BP] = 0$$

The parameters are set to arbitrary values [8] and can be found in appendix A, as well as the calculations. Since we have a four dimensional system, four equilibrium points were calculated. Note that equilibrium points are sensitive to changes of the values for the parameters [6]. The sensitivity will be further studied in the section 'Time-domain analysis'. The following equilibrium points were obtained:

$$\begin{aligned} [Am] &= -501.1, [AmL] = -0.995, [AL] = 48.614, [BP] = 50.01 \\ [Am] &= -0.002, [AmL] = -0.591, [AL] = 48.21, [BP] = -1.072 \\ [Am] &= 0.0038, [AmL] = 0.4796, [AL] = 47.1395, [BP] = 1.8416 \\ [Am] &= -0.007, [AmL] = 3.681, [AL] = 43.938, [BP] = -3.579 \end{aligned}$$

These points correspond to a state where the reaction rate is zero and the concentration of each species remains constant. By examining the results we immediately see that only the third equilibrium point is possible in reality. It is due to the fact that the concentration of each species must be larger or equal to zero. Therefore the analysis will continue with the system being in the third equilibrium point and it is at this point that the system is said to be in steady state.

3.3 Linearisation around the equilibrium point

3.3.1 Input-output representation

Since it is hard to analyse a non-linear dynamical system it is desirable to linearise the system around an equilibrium point. It is possible with a linearised model to analyse the trajectories around the neighbourhood of the steady state,

because a linearised model approximates the systems behaviour in the neighbourhood of the equilibrium point. So far, we have only discussed our non-linear model with the general representation of the form without consideration of input and output [6]:

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

where $\mathbf{x}(t) = ([Am], [AmL], [AL], [BP])$

This representation can be extended into an alternative form, namely input-output representation, which is written as two sets of equations. One consists of ordinary differential equations and the other is algebraic:

$$\begin{cases} \frac{d}{dt}\mathbf{x}(t) = \mathbf{f}(\mathbf{x}(t), \mathbf{u}(t)) \\ \mathbf{y}(t) = \mathbf{h}(\mathbf{x}(t), \mathbf{u}(t)) \end{cases}$$

where $\mathbf{x}(t)$ is called a state vector, $\mathbf{y}(t)$ is called an output vector and $\mathbf{u}(t)$ is called an input vector. Together they form a state space model [6].

This representation is common in control engineering, because it provides a convenient and compact way to model high order dynamical systems that have several inputs and outputs. However, it is also a useful tool in several other applications, including mathematical biology. The state variables in mathematical biology are often represented by the concentration of different chemical species, which are changing over time. It is useful to present a model in state space form due to the fact that if the state of a system is known at time t_0 , then it is possible to compute the systems evolution for all $t > t_0$. This is possible even if information about the systems input ($\mathbf{u}(t)$) is not known at time $t < t_0$ [6].

To rewrite our system into an input-output model we first need to define the state variables for our system. By inspecting the different chemical species and understanding what role they have, it is not hard to see what the different state variables are. The input signal is the ligand level, $\mathbf{u}(t) = [L]$, the output function is the concentration of the methylated CheA, $\mathbf{h}(\mathbf{x}(t), \mathbf{u}(t)) = ([Am], [AmL], [AL], [BP], [L]) = [Am]$ and the state vector is $\mathbf{x}(t) = ([Am], [AmL], [AL], [BP])$. The state space model becomes:

$$\begin{aligned}
\frac{d}{dt}x_1(t) &= f_1([Am], [AmL], [AL], [BP], [L]) = \\
& k_{-1} * [R] - \frac{k_1 * [BP](t) * [Am](t)}{k_{M1} + [Am](t)} - k_3 * [Am](t) * [L] + k_{-3} * [AmL](t) \\
\frac{d}{dt}x_2(t) &= f_2([Am], [AmL], [AL], [BP], [L]) = \\
& k_{-2} * [R] - \frac{k_2 * [BP](t) * [AmL](t)}{k_{M2} + [AmL](t)} + k_3 * [Am](t) * [L] - k_{-3} * [AmL](t) \\
\frac{d}{dt}x_3(t) &= f_3([Am], [AmL], [AL], [BP], [L]) = \\
& -k_{-2} * [R] + \frac{k_2 * [BP](t) * [AmL](t)}{k_{M2} + [AmL](t)} + k_4 * ([A_T] - [Am](t) - [AmL](t) \\
& - [AL](t)) * [L] - k_{-4} * [AL](t) \\
\frac{d}{dt}x_4(t) &= f_4([Am], [AmL], [AL], [BP], [L]) = \\
& k_5 * [Am](t) * ([B_T] - [BP](t)) - k_{-5} * [BP](t) \\
y(t) &= [Am](t)
\end{aligned}$$

3.3.2 Linearisation and state space representation

Linearisation of a non-linear state space model is done with Taylor expansion around the equilibrium point.

Definition, Taylor expansion series [6]:

Taylor series expansion for the scalar case of $f(x)$ in the neighbourhood of x_e , where x_e is an equilibrium point.

$$f(x) = \sum_{n=0}^{\infty} \frac{f^{(n)}(x_e)}{n!} (x - x_e)^n$$

This can be translated to cases with higher dimensions, as we have.

Definition, Taylor expansion series for two variables [13]:

Let $f(x, y)$ be a C^3 -function in the open set M , and suppose that the point (a, b) is in M . Then

$$\begin{aligned}
f(a+h, b+k) &= f(a, b) + f'_x(a, b)h \\
& + f'_y(a, b)k + \frac{1}{2}(f''_{xx}(a, b)h^2 + 2f''_{xy}(a, b)hk + f''_{yy}(a, b)k^2) \\
& + (h^2 + k^2)^{3/2}B(h, k)
\end{aligned}$$

where $B(h, k)$ is a bounded function in the neighbourhood of origin.

By applying Taylor series expansion on a non-linear input-output model, the linearised form becomes a linear time invariant state space model:

$$\begin{cases} \frac{d}{dt} \mathbf{x}(t) = A\mathbf{x}(t) + B\mathbf{u}(t) \\ \mathbf{y}(t) = C\mathbf{x}(t) + D\mathbf{u}(t) \end{cases}$$

where $A = \frac{\partial \mathbf{f}}{\partial \mathbf{x}}$ is the systems Jacobian, $B = \frac{\partial \mathbf{f}}{\partial \mathbf{u}}$ is the input mapping, $C = \frac{\partial \mathbf{h}}{\partial \mathbf{x}}$ is the output mapping and $D = \frac{\partial \mathbf{h}}{\partial \mathbf{u}}$ is called the feed-through term. Together they are called the state space matrices [8]. All state space matrices are evaluated at the equilibrium point for our system. Linearisation of our input-output model gives the following state space matrices:

$$A^{SS} =$$

$$\begin{pmatrix} -\frac{k_1 * k_{M1} * [BP]^{SS}}{(k_{M1} + [Am]^{SS})^2} - k_3 * [L]^{SS} & k_{-3} & 0 & -\frac{k_1 * [Am]^{SS}}{k_{M1} + [Am]^{SS}} \\ k_3 * [L]^{SS} & a_{22} & 0 & -\frac{k_2 * [AmL]^{SS}}{k_{M2} + [AmL]^{SS}} \\ -k_4 * [L]^{SS} & a_{32} & -k_{-4} - k_4 * [L]^{SS} & \frac{k_2 * [AmL]^{SS}}{k_{M2} + [AmL]^{SS}} \\ k_5 * [B_T] - k_5 * [BP]^{SS} & 0 & 0 & -k_5 * [Am]^{SS} - k_{-5} \end{pmatrix}$$

$$B^{SS} = \begin{pmatrix} -k_3 * [Am]^{SS} \\ k_3 * [Am]^{SS} \\ k_4 * ([A_T]^{SS} - [AL]^{SS} - [Am]^{SS} - [AmL]^{SS}) \\ 0 \end{pmatrix}$$

$$C^{SS} = (1 \ 0 \ 0 \ 0) \quad \text{and} \quad D^{SS} = 0$$

where

$$a_{22} = -k_{-3} - \frac{k_2 * k_{M2} * [BP]^{SS}}{(k_{M2} + [AmL]^{SS})^2}$$

$$a_{32} = -k_4 * [L]^{SS} - \frac{k_2 * k_{M2} + [BP]^{SS}}{(k_{M2} + [AmL]^{SS})^2}$$

and SS stands for steady state and the steady states concentrations $[Am]^{SS}$, $[AmL]^{SS}$, $[A]^{SS}$ and $[BP]^{SS}$ are the concentrations at the equilibrium point. The detailed computations of the linearisation are shown in appendix A.

If the state matrices are put into state space representation, then we have a model that gives us a useful and compact basis for our analysis. However, when we will analyse our system in frequency domain, this representation will not be sufficient. In frequency domain, a system must be represented in the complex domain. Therefore, we give another representation called a transfer function, which is a complex valued function.

3.4 Transfer function

A transfer function is an additional way to represent the system. This representation is useful when it comes to analysing a model in frequency domain, seeing that it is a complex valued function. This will be further discussed in the section 'Frequency domain analysis'. It is possible to convert a system from a linear time invariant state space representation into a transfer function representation [16]. Our focus will be on a scalar transfer function, since the system under consideration is a single input-output model.

Definition, Transfer function [16]:

A transfer function $G(s)$ is the ratio of the Laplace transform of the systems output $y(t)$ to the Laplace transform of the systems input $u(t)$, when all of the initial conditions are set to zero, that is

$$G(s) = \frac{Y(s)}{U(s)}$$

Given a linear state space representation, the first step is to apply Laplace transform on the system.

Definition, Laplace transform [14]:

$$L[f](p) = \int_0^{\infty} e^{-px} f(x) dx \quad \text{for } p > 0$$

Laplace transform makes it possible to transform a function $f(x)$ into a new function $L[f](p)$, which often can be calculated by algebraic means. This transformation is often used in the field of differential equations. The advantage with Laplace transformation is that it can transform a differential equation into an algebraic equation, which makes it easier to calculate and solve a problem [14]. Note that we do not discuss the convergence issue. Taking the Laplace transform of:

$$\begin{cases} \frac{d}{dt} \mathbf{x}(t) = A\mathbf{x}(t) + B\mathbf{u}(t) \\ \mathbf{y}(t) = C\mathbf{x}(t) + D\mathbf{u}(t) \end{cases}$$

It becomes:

$$\begin{cases} s\mathbf{X}(s) = A\mathbf{X}(s) + B\mathbf{U}(s) \\ \mathbf{Y}(s) = C\mathbf{X}(s) + D\mathbf{U}(s) \end{cases}$$

Note that the Laplace transform is taken component wise. To obtain a transfer function, the next step is to simplify the first equation for $\mathbf{X}(s)$:

$$(sI - A)\mathbf{X}(s) = B\mathbf{U}(s) \iff \mathbf{X}(s) = (sI - A)^{-1}B\mathbf{U}(s)$$

Where I is the identity matrix with the same dimension as A . Substitute the result in the second equation above gives:

$$\mathbf{Y}(s) = C((sI - A)^{-1}B\mathbf{U}(s)) + D\mathbf{U}(s) \iff \mathbf{Y}(s) = (C((sI - A)^{-1}B) + D)\mathbf{U}(s)$$

Since the transfer function $G(s)$ is defines as $G(s) = \frac{Y(s)}{U(s)}$, it is clear that the transfer function is [16]

$$G(s) = C(sI - A)^{-1}B + D$$

As stated earlier, a transfer function is a complex valued function and it is represented as a fraction. The denominator, $D(s)$, is called the characteristic polynomial (more details about a characteristic polynomial is found under the section 'Lyapunov stability') and the roots of $D(s)$ is called the poles of $G(s)$. The roots of the nominator, $N(s)$, are called the zeros of $G(s)$. Assuming that $D(s)$ and $N(s)$ does not have any common factors, then $G(s)$ have n poles and m zeros that lies in the complex plane. $G(s)$ is defined as strictly proper if the $\deg N(s) = m < \deg D(s) = n$. A strictly proper transfer function is characterised by

$$\lim_{s \rightarrow \infty} G(s) = 0$$

if and only if the state matrix $D = 0$. Any transfer function that is strictly proper can be transformed back to a state space form directly from the transfer function using realisation theory. This will not be examined any further in this study, yet it is important to state, due to the fact that the resulting state space model is guaranteed to be controllable or observable depending on which realisation approach that is used [16]. More theory about controllable and observable system will be addressed in the following section. The transfer function for our system can be seen in appendix A.

Both state space representation and a transfer function give us a solid basis to continue the analysis. Remember that the results from the analysis will only be local, meaning that we will only look at the behaviour in the neighbourhood of the equilibrium point. If a global behaviour is preferred, the focus of the analysis must be on the non-linear system.

4 Controllability and observability

Within control theory, a dynamical system must maintain certain criteria to be able to stabilise the system and two fundamental concepts are controllability and observability. These dual concepts are a main objective in control theory and it answers the question, if it is possible to control and observe a dynamical system over finite time interval. If the answer would be no, then it would be pointless to continue on with an analysis, since the analysis may result in wrong conclusions [5]. The concepts will be introduced using a state space model.

4.1 Controllability

The term controllable has its focus on the input function $u(t)$. The question that arises when talking about controllability is, can a control function $u(t)$ be found over the period $[t_0, t_f]$ which will transfer the initial state $x(t_0) = x_0$ of a system to a desired final state $x(t_f) = x_f$? If that control function $u(t)$ exists, then the system is controllable [5].

Definition, controllability [5]:

A system $\dot{x} = Ax + Bu$, or the pair (A, B) , is called controllable if and only if there is a continuous control function $u(t)$ on $[t_0, t_f]$, that transform the initial state $x(t_0) = x_0$ to a specific state $x(t_f) = x_f$

This definition can be described by an integral called the controllability gramian $W_c(t_0, t_f)$, which is defined for the pair (A, B) as,

$$W_c(t_0, t_f) = \int_{t_0}^{t_f} e^{-At} B B^T e^{-A^T t} dt$$

$W_c(t_0, t_f)$ can be used to determine controllability [9].

Theorem [9]:

The pair (A, B) is controllable on $[t_0, t_f]$ if and only if the controllability gramian is positive definite.

If the controllability gramian is definite, x_0 is the initial state and x_f is the desired final state, then we can write:

$$x(t_f) = e^{At_f} x(t_0) + \int_{t_0}^{t_f} e^{A(t_f-\tau)} B u(\tau) d\tau$$

From this it is possible to calculate for which $u(t)$ that the pair (A, B) is controllable, when $x(t_f) = x_f$. However, when studying what properties the controllability gramian must take to be definite, an easier way to check controllability arises [9].

Corollary [9]:

The controllability gramian $W_c(t_0, t_f)$ is positive definite if and only if the controllability matrix \mathfrak{C} has full rank, where

$$\mathfrak{C} = [B, AB, A^2B, \dots, A^{n-1}B]$$

and it is $n \times nm$, when A is $n \times n$ and B is $n \times m$.

From this, a last theorem that determines controllability can be stated.

Theorem [9]:

The pair (A, B) is controllable if and only if the controllability matrix \mathfrak{C} have full rank, $\text{rank}(\mathfrak{C}) = n$.

The last theorem provides a useful way to determine controllability without tedious calculations. For our system, the controllability matrix were calculated (appendix A) and the rank calculated to four, for all parameters involved. Since our system is four-dimensional, the system is controllable.

4.2 Observability

The term observability has its focus on the output of the system. The question that arises when talking about observability is, can the state of the system be determined by measuring the system output $y(t)$ over a finite time? If the answer is yes, then the system is observable [5].

Definition, observability [10]:

The system

$$\begin{cases} \dot{x}(t) = Ax(t) + Bu(t) \\ y(t) = Cx(t) \end{cases}$$

or the pair (A, C) is called observable on $[t_0, t_f]$ if any initial state $x(t_0) = x_0$ can be uniquely determined from $y(t)$ on $[t_0, t_f]$. It is said to be re-constructable on $[t_0, t_f]$ if any final state $x(t_f) = x_f$ can be uniquely determined from $y(t)$ on $[t_0, t_f]$.

In other words, if a systems output $y(t)$ would not be influenced by the state variables $x(t)$, then the system is not observable [5]. A criterion for observability can be created and it is done in a similar manner as for controllability.

The definition can be described by an integral called the observability gramian $W_o(t_0, t_f)$, which is defined for the pair (A, C) as,

$$W_o(t_0, t_f) = \int_{t_0}^{t_f} e^{A^T t} C^T C e^{At} dt$$

$W_o(t_0, t_f)$ can be used to determine observability [10].

Theorem [10]:

The pair (A, C) is said to be observable on $[t_0, t_f]$ if and only if the observability gramian $W_o(t_0, t_f)$ is positive definite.

In the same way as for controllability, when examine the properties that the observability gramian must take to be positive definite an easier way to check observability arises.

Corollary [10]:

The observability gramian $W_o(t_0, t_f)$ is positive definite if and only if the observability matrix O has full rank, where

$$O = \begin{bmatrix} C \\ CA \\ \vdots \\ CA^{n-1} \end{bmatrix}$$

and it is $np \times n$, when A is $n \times n$ and C is $p \times n$.

From this, a theorem that determines observability can be stated.

Theorem [10]:

The pair (A, C) is observable if and only if the observability matrix O has full rank, $\text{rank}(O) = n$.

As for controllability, this last theorem provides a useful way to calculate observability. The observability matrix was calculated for our system (appendix A). The rank for the observability matrix is three and therefore our system is not observable. Thus, we have a controllable but an unobservable model. When this case arises, decomposition of the system can be done to locate which part of the system is observable.

4.3 Decomposition

It is clear that four cases may arise when studying controllability and observability for a dynamical system. The system can be:

1. Controllable and observable
2. Controllable but unobservable
3. Uncontrollable but observable
4. Uncontrollable and unobservable

In all cases, except for the first, decomposition of the system can be done in order to obtain controllability and observability parts [5]. In this thesis the focus will only be on the second case, since it is the case for our system. When a system is unobservable, is it possible to separate the observable parts from the

unobservable parts.

Theorem, Decomposition of unobservable systems [11]:

Let O be an observability matrix of the pair (A, C) , where A is $n \times n$ and C is $p \times n$. If $\text{rank}(O) = q < n$, then there exist a non-singular matrix P such that

$$\hat{A} = P^{-1}AP = \begin{bmatrix} \hat{A}_{11} & 0 \\ \hat{A}_{21} & \hat{A}_{22} \end{bmatrix}$$

$$\hat{C} = CP = [\hat{C}_1 \quad o]$$

where \hat{A}_{11} is $q \times q$ and \hat{C}_1 is $p \times q$ and the pair $(\hat{A}_{11}, \hat{C}_1)$ is observable.

The eigenvalues of \hat{A}_{11} are called the observable eigenvalues of A and the eigenvalues for \hat{A}_{22} are called unobservable eigenvalues of A . With this theorem it is possible to rewrite the system into two subsystems, one which is observable and one which is unobservable [11].

Decomposition was done for our system with help from Mathematica and the decomposed state space matrices can be seen below.

$$A^{SS} = \begin{pmatrix} -\frac{k_1 * k_{M1} * [BP]^{SS}}{(k_{M1} + [Am]^{SS})^2} - k_3 * [L]^{SS} & k_{-3} & -\frac{k_1 * [Am]^{SS}}{k_{M1} + [Am]^{SS}} \\ k_3 * [L]^{SS} & -k_{-3} - \frac{k_2 * k_{M2} * [BP]^{SS}}{(k_{M2} + [AmL]^{SS})^2} & -\frac{k_2 * [AmL]^{SS}}{k_{M2} + [AmL]^{SS}} \\ k_5 * [B_T] - k_5 * [BP]^{SS} & 0 & -k_5 * [Am]^{SS} - k_{-5} \end{pmatrix}$$

$$B^{SS} = \begin{pmatrix} -k_3 * [Am]^{SS} \\ k_3 * [Am]^{SS} \\ 0 \end{pmatrix}, \quad C^{SS} = (1 \quad 0 \quad 0) \quad \text{and} \quad D^{SS} = 0$$

where SS stands for steady state and the steady states concentrations $[Am]^{SS}$, $[AmL]^{SS}$ and $[BP]^{SS}$ is the concentrations in equilibrium points.

From this decomposed system a new transfer function is calculated and can be found in appendix A.

To understand which equation that was reduced after decomposition, we need to go back to the system of differential equations. Then it is easy to see that the chemical species that was removed is $[AL]$. This makes sense if we analyse the differential equations a bit closer. We see that when calculating the equilibrium points, the differential equation for $[AL]$ does not influence the other equations. Thus, we only need the other three differential equations for the chemical species $[Am]$, $[AmL]$ and $[BP]$ to determine the equilibrium points.

New test for controllability and observability was done on the decomposed system and it gave positive results (appendix A). The system we have is now

controllable and observable, the analyse of stability and robustness can now be done.

5 Stability and robustness

The final system is showing the bacterial chemotaxis when it is in steady state. The properties that the steady states holds can mathematically be analysed by examine the stability of the equilibrium point. When analysing the stability of an equilibrium point, information about the robustness around the neighbourhood of the equilibrium point can be displayed. Several stability tests have been constructed and can be applied to biological systems, but it is important to note that different stability tests include different properties of the system. In control theory stability and robustness of a system can be studied in time domain and frequency domain. Both domains present a great deal of the robustness, but target different properties in the system. In this analysis both domains will be analysed to give a broader survey of the robustness [6].

5.1 Time domain analysis

As time passes, the input, output and state of a dynamical system varies. Consequently it is a natural approach to analyse a system with respect to time t . Presenting a system in time domain, is the same as presenting the systems response with respect to time [8].

5.1.1 Stability in time domain analysis

To analyse the stability of the equilibrium point in time domain, the eigenvalues of the state matrix A is examined. In time domain analysis, there are various stability test that can be used. In this study two tests will be applied, namely Lyapunov stability test and Routh-Hurwitz stability criterion. Both hold information about the stability but yield different information about the properties and robustness of the system. In addition plots of the steady state will also be shown when system is perturbed with increasing and decreasing ligands levels. The plots will give further insight into the robustness of the system.

5.1.2 Lyapunov stability

Lyapunov stability discusses stability around the neighbourhood of an equilibrium point.

Definition, stability in the sense of Lyapunov [6]:

The origin of the state space $x = 0$ is a stable equilibrium point x_e at $t = 0$ for the system $\dot{x} = f(x)$, if for each $\epsilon > 0$ and any $t_0 \geq 0$ there is a $\delta > 0$ such that

$$\|x(t_0)\| < \delta \implies \|x(t)\| < \epsilon$$

This means that if a point near the equilibrium point is chosen as an initial condition, then the trajectories of the system will always stay near the equilibrium point. In addition, if all the trajectories of the system converge to the equilibrium point, that is

$$\|x(t_0)\| < \delta \implies \lim_{t \rightarrow \infty} x(t) = x_e$$

where we for simplicity assume that $x_e = 0$. Then the equilibrium point is said to be asymptotically stable. The set of the initial conditions that are sufficiently close to the equilibrium point, so that they converge to the equilibrium point is designated as the region of attractants (or domain) [6].

When analysing the stability of an equilibrium point with Lyapunov theory, it also provides some useful tools to determine how far away the trajectories can be from x_e and still converge to x_e as $t \rightarrow \infty$, that is the region of attraction [6].

Theorem, Lyapunov's direct method [6]:

Let $x = 0$ be an equilibrium point for the system $\dot{x} = f(x)$ and $D \subset \mathbb{R}^n$ be a domain and let $x \in D$. Further let $V : D \rightarrow \mathbb{R}$ be a continuously differentiable function such that

$$\begin{cases} V(0) = 0 \\ V(x) > 0, & \forall x \in D \setminus \{0\} \\ \dot{V}(x) \leq 0, & \forall x \in D \end{cases}$$

Then x is stable. Moreover, if

$$\dot{V}(x) < 0, \quad \forall x \in D \setminus \{0\}$$

then x is asymptotically stable.

The function $V(x)$ is called a Lyapunov function. The advantages of the above theorem are when the stability is determined, there is no need to compute the trajectories for the system. But there is a disadvantage, to find the Lyapunov function can be tedious work and is not always possible. Unfortunately there is no systematic way to find a suitable $V(x)$, but the most common approach is to construct a quadratic function, that have the form:

$$V(x) = x^T P x$$

where P is a square symmetric matrix. From this, it is possible to determine positive or negative definiteness, since the eigenvalues of P correspond to either being negative or positive [6].

Fortunately, Lyapunov's theory does not end here. If it is not possible to find a suitable Lyapunov function, then another more simple method can be applied. This method gives information about the stability of the equilibrium point and is called Lyapunov's indirect method [6]. Before Lyapunov's indirect method is stated, information about eigenvalues is provided.

Definition, Eigenvalues [16]:

An eigenvalue of any square, $n \times n$, matrix A is any scalar λ such that,

$$Av = \lambda v$$

where v is an $n \times 1$ eigenvector of A

Note that this definition can be rewritten into [16],

$$(\lambda I - A)v = 0$$

where v is a non zero vector if and only if the determinant of $(\lambda I - A)$ is zero. This determinant is defined as the characteristic equation of A , namely:

$$|\lambda I - A| = \lambda^n + a_{n-1}\lambda^{n-1} + \dots + a_1\lambda + a_0 = 0$$

with the characteristic polynomial of matrix A defined as,

$$a(\lambda) = \lambda^n + a_{n-1}\lambda^{n-1} + \dots + a_1\lambda + a_0$$

Theorem, Lyapunov's indirect method [6]:

Let $x_e = 0$ be an equilibrium point for the non linear system $\dot{x} = f(x)$, where $f : D \rightarrow \mathbb{R}^n$ is continuously differentiable and D is a neighbourhood of the origin and let

$$A = \left. \frac{\partial f}{\partial x}(x) \right|_{x=0}$$

then,

- (i) The origin is asymptotically stable if $Re(\lambda_i) < 0$ for all eigenvalues λ_i , $i = 1, \dots, n$ of A
- (ii) The origin is unstable if $Re(\lambda_i) > 0$ for at least one eigenvalue of A .

Note that this method does not give information about the stability if one or more eigenvalues lie on the imaginary axis, that is $Re(\lambda_i) = 0$ [6].

If we apply Lyapunov's theory on our system, we immediately come to the conclusion that finding a Lyapunov function is hard, if not impossible. Therefore,

to avoid tedious calculations we turn to Lyapunov's indirect method. To ensure stability for our system, all of the eigenvalues of the A matrix must lie in the stable half-plane, that is the left half complex plane.

The eigenvalues of the state matrix A was calculated for our system, which was implemented with the initial data around the equilibrium point (appendix A) and they are $\{-385.564, -1.78784, -0.0112275\}$. According to Lyapunov indirect method, our system is locally asymptotically stable. In terms of robustness this is useful information. Hence, biologically this means that if the bacterial chemotaxis is in steady state and is perturbed by increasing chemoattractants, then at first, the reaction will be disturbed and not be in steady state. But as time passes the biochemical reaction will recover and return to steady state. Off course, this does not imply for large perturbations. To be able to determine to what range the perturbation can take, we need to calculate the region of attraction.

Definition, Region of attraction [6]:

Let the origin $x = 0$ be an asymptotically stable equilibrium point for the non linear system $\dot{x} = f(x)$ and let $\Phi(t, x_0)$ be the solution of $\dot{x} = f(x)$ that starts at initial state x_0 at time $t = 0$. The region of attraction of the origin, denoted by R_A , is defined as

$$R_A = \{x_0 : \Phi(t, x_0) \rightarrow 0 \text{ as } t \rightarrow \infty\}$$

Note that the boundary of R_A is always formed by trajectories of the system. This makes it possible to determine region of attraction for a two-dimensional system on the phase-plane [6]. However, for larger systems it is more difficult to determine region of attraction. Burchardt and Ratschan [4] gives a thorough tutorial how to solve the region of attraction using a classical approach. This approach uses the Lyapunov's direct method and therefore a Lyapunov function is required. Due to lack of time, we have not made an attempt to find a Lyapunov function for our system and therefore the region of attraction cannot be solved. As a conclusion, this raises questions for future research.

5.1.3 Routh-Hurwitz stability criterion

In Routh-Hurwitz stability criterion it is the characteristic polynomial that is used to determine stability. The criterion does not depend on finding the eigenvalues of the characteristic polynomial, it depends on inspecting the coefficients and determine the range of variation the coefficients can take, under the condition that the roots must lie in the stable half-plane. The criterion is useful when talking about robustness, due to the fact that it is possible to calculate what conditions the parameter must take for the system to be asymptotically stable. From this, conclusions about the robustness can be drawn [5].

As stated in previous section, to ensure asymptotically stability all the eigenvalues must be negative and if there exist complex eigenvalues, then the real part must be negative. If this is satisfied, then the coefficients a_1, a_2, \dots, a_n of the

characteristic polynomial

$$a(\lambda) = \lambda^n + a_{n-1}\lambda^{n-1} + \cdots + a_1\lambda + a_0$$

must all be presented and positive.

The Routh-Hurwitz stability criterion involves the construction of a square matrix, where the elements depend on the coefficients of the characteristic polynomial and it is called the Hurwitz matrix [5]. A Hurwitz matrix is defined as [16]:

$$\begin{pmatrix} 1 & a_{n-2} & a_{n-4} & a_{n-6} & \cdots \\ a_{n-1} & a_{n-3} & a_{n-5} & a_{n-7} & \cdots \\ b_{n-2} & b_{n-4} & b_{n-6} & \cdots & \\ c_{n-3} & c_{n-5} & c_{n-7} & \cdots & \\ \vdots & \vdots & \vdots & & \\ d_1 & \cdots & & & \\ e_0 & \cdots & & & \end{pmatrix}$$

In the first two rows, the coefficient for the characteristic polynomial is inserted. When the coefficient 'run-out', then zeros are inserted. The third rows element are generated from its preceding two rows as follows [16]:

$$\begin{aligned} b_{n-2} &= -\det \begin{bmatrix} a_n & a_{n-2} \\ a_{n-1} & a_{n-3} \end{bmatrix} \div a_{n-1} \\ b_{n-4} &= -\det \begin{bmatrix} a_n & a_{n-4} \\ a_{n-1} & a_{n-5} \end{bmatrix} \div a_{n-1} \\ b_{n-6} &= -\det \begin{bmatrix} a_n & a_{n-6} \\ a_{n-1} & a_{n-7} \end{bmatrix} \div a_{n-1} \\ &\vdots \end{aligned}$$

The fourth row's element are generated from its preceding two rows, the same way as for the third row [16],

$$\begin{aligned} c_{n-3} &= -\det \begin{bmatrix} a_{n-1} & a_{n-3} \\ b_{n-2} & b_{n-4} \end{bmatrix} \div b_{n-2} \\ b_{n-2} &= -\det \begin{bmatrix} a_{n-1} & a_{n-5} \\ b_{n-2} & b_{n-6} \end{bmatrix} \div b_{n-2} \\ &\vdots \end{aligned}$$

The following rows of elements in the Hurwitz matrix follow the same procedure. If any element is undefined, then zeros are inserted. The complete Hurwitz matrix will be triangular, with the final two rows having only one element each, which was defined as d_1 and e_0 . With this information, the Routh-Hurwitz stability criterion can now be stated [16].

Theorem, Routh-Hurwitz stability criterion [5]:

- (i) *The roots of the characteristic polynomial all have negative real parts if and only if the elements of the first column of the Hurwitz matrix are positive.*
- (ii) *The number of roots with positive real parts is equal to the number of sign changes of the elements of the first column.*

Since we already have stated that our system is asymptotically stable, we use Routh-Hurwitz stability criterion not to determine stability but for determine what range the values for certain parameters can take. We utilise the fact that the elements in the first column of the Hurwitz matrix must be larger than zero to ensure asymptotically stability. This gives us the opportunity to set up inequality equations that can answer for the variation the parameters can take. The characteristic polynomial for our system has the form:

$$Polynomial(s) = ds^3 + cs^2 + bs + a$$

The Hurwitz matrix for our system takes the form of:

$$\begin{pmatrix} 1 & b & 0 & 0 \\ c & a & 0 & 0 \\ b_{n-2} & 0 & 0 & 0 \\ c_{n-3} & 0 & 0 & 0 \end{pmatrix}$$

The calculations are found in appendix A. From Routh-Hurwitz stability criterion we obtain that $c > 0$, $b_{n-2} > 0$ and $c_{n-3} > 0$. Since we have twelve parameters and three inequality equations, it is not possible to calculate the values the parameters must take when they stand in relation to each other. In addition, the parameters are arbitrary and taken from the book 'Mathematical modeling in systems biology: an introduction' [8], so we do not know the biological meaning for these rate constants. But we notice that all of them are set to one except for three, $k_1 = 200$, $k_5 = 0.05$ and $k_{-5} = 0.005$. This attracts attention and therefore we examine the variation these three parameters can take when all the others are fixed to its arbitrary values. The result are:

- $k_1 > -4.98968$
- $k_5 > -0.0401974$
- $k_{-5} > -0.00621931$

The first conclusion is that a rate constant cannot be negative in real life. Hence, all these three parameters must be > 0 for the system to be asymptotically stable. The mathematical conclusion that is drawn is that the system is robust, due to the fact that the rate constants can take any number > 0 . The stability is therefore insensitive towards changes of the values for the parameters k_1 , k_5 and k_{-5} . Biologically, the conclusion is more unclear, as we lack deeper knowledge within biology. But biological results can be built on our mathematical results. Since the conclusion was that the system is robust towards changes in the rate constants, then the biological model must also be robust.

5.2 Steady state analysis

When stability of the system has been discussed, the ligand concentration has been set to a fixed value of 20. Since $[L]$ is the input of the system, it is of interest to analyse the robustness toward changes of the ligand levels. Changing the ligand level can be viewed as external uncertainties [6]. This is best examined by plotting the chemical species $[Am]$ and $[Aml]$, when they converge to steady state and are perturbed by different ligand levels. For the first test (Figure 3), the ligand level is at first set to 20, then the level is changed to seven different levels and each perturbation last for one time-unit. For the second test (Figure 4) we first set the ligand level to 20 and at time $t = 10$ the ligand level is doubled for the rest of the time. The initial values are set to arbitrary units, see Appendix A.

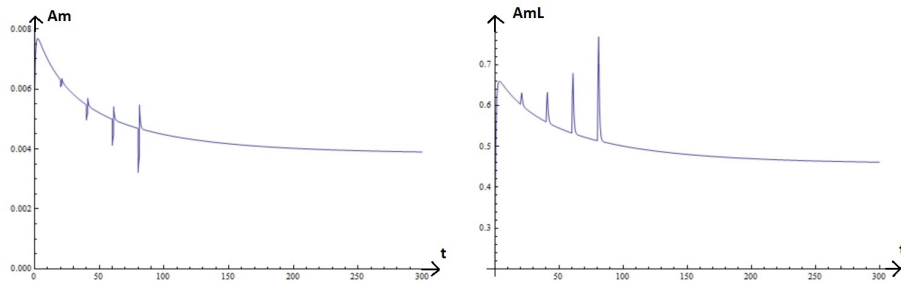


Figure 3: Plots exhibiting the convergence to steady state for each chemical species as the system is implemented with different ligand levels at different time-units. The time scale is arbitrary.

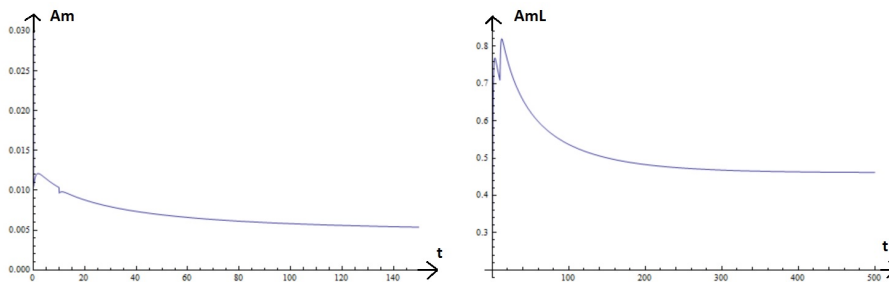


Figure 4: Plots exhibiting the convergence to steady state for each chemical species when the ligand concentration is doubled at time $t = 10$. The time scale is arbitrary.

As we can see, the plot exhibits the chemical species as they converge to the steady state. If we consider Figure 3, it is clear that both chemical species converge to the steady state, even though they were perturbed with increasing and decreasing ligand levels at different times. Together, both plots in Figure 3 indicate that the steady state is robust, even towards external changes.

If Figure 4 is considered, we see that although the ligand level was doubled at $t = 10$, the convergence to steady state recovers and the chemical species goes to the equilibrium point as $t \rightarrow \infty$. This presents the mechanism adaptation. As described in the beginning of the study, adaptation is the mechanism when the bacteria first responds to an input signal but then 'shuts' the response down when it comprehends that the input signal is constant and adapt to the signal's continued presence [8]. This means that the steady state is independent towards ligands changes [1] and thus is a robust mechanism. This will be further analysed in section 'Integral feedback'.

The chemical species $[BP]$ is not plotted against ligand changes. That depends on that the differential equation for $[BP]$ is not influenced by the ligand concentration.

5.3 Frequency domain analysis

A key property for our system is its dynamical response and an analytic tool to analyse the systems behaviour is through frequency response analysis. By analysing the system in frequency domain, it provides a deep insight into the dominant processes, which gives an overall response of the system. The analyse allows us as well to us to look into the long-term response of the system [6] [8].

5.3.1 Stability in frequency domain analysis

To analyse the stability of the equilibrium point in frequency domain the Nyquist stability criterion will be used. The stability test is restricted to linear time-invariant systems, which ours is. A time-invariant system is a system whose

output does not depend on the time that the input is applied, meaning if the same input is applied on the system at time t_0 or at time t_1 , the output will be the same except for the time delay. This can be stated mathematically as [6]:

$$y(t_0, u(t)) = y(t_1, u(t)) \quad \forall t_0, t_1 \in \mathbb{R}$$

As mentioned in the section 'the model', a negative feedback loop is implemented in the model. From a mathematical perspective this means that the system is not only a linear time-invariant system but also a negative feedback system. Negative feedback is a mechanism that allows the system to self-regulate by changing and controlling the dynamics of a system [6]. This means from a biological perspective that the E.coli is able to adapt to its surrounding, see the section 'background'. Nyquist stability criterion is a useful tool when it comes to analyse the stability of a linear feedback system [6].

5.3.2 Nyquist stability criterion

Nyquist stability criterion is based on Cauchy's argument principle, which comes from the field of complex analysis. The advantage of examining the stability with Nyquist stability criterion is that it gives more insight into the degree of the stability of a closed-loop negative feedback control system [6]. Before the Nyquist stability criterion can be stated, more knowledge is required.

Cauchy's argument principle concerns to the mapping of a known rational function and can be stated as follows. Let $F(s)$ be a differentiable function in the whole complex plane s except at its poles. Let Γ_s be a closed continuous contour on the s -plane that does not intersect any poles or zeros of $F(s)$. If the values of s travels around the contour Γ_s in a clockwise direction, the function $F(s)$ encircles the origin in the $(\text{Re}\{F(s)\}, \text{Im}\{F(s)\})$ -plane (which is called the ω -plane) N times, where $N = Z - P$. Z and P is the number of zeros and poles (including the multiples) of the function $F(s)$ and they lie inside the contour Γ_s . The new encirclement in the ω -plane is a new continuous contour Γ_ω , that is $\Gamma_\omega = F(\Gamma_s)$. This is called mapping from Γ_s into Γ_ω . The results can be written as $\arg\{F(s)\} = (Z - P)2\pi = 2\pi N$, [16] [6].

Now let $G(s) = \frac{N(s)}{D(s)}$ be a transfer function for a closed-loop system in the complex plane s . Nyquist stability criterion is obtained by applying Cauchy's argument principle on $1 + G(s)$ and that produces a polar plot called 'Nyquist plot'. The contour that is used is called 'the Nyquist contour' and it covers the whole unstable half right-hand side of the complex plane s . When applying the argument principle on $1 + G(s)$, the origin in the s -plane becomes the point $(-1, 0)$ in the ω -plane. According to Cauchy's argument principle $1 + G(s)$ must be analytic at every point on the Nyquist contour. If there are poles on the imaginary axis, then they need to be encircled by infinitesimally small semicircles, since the contour must encircle the poles. From Cauchy's argument principle, the number of clockwise encirclements of the point $(-1, 0)$ must be the number

of zeros of $1 + G(s)$ in the right-half complex plane s minus the poles of $1 + G(s)$ in the right-half complex plane s , that is $N = Z - P$. Note that the zeros of $1 + G(s)$ are the poles of the closed-loop system and that the poles of $1 + G(s)$ are the same as the poles of $G(s)$. The Nyquist stability criterion is now ready to be stated [16].

The Nyquist stability criterion [16]:

Given a Nyquist contour Γ_s , let P be the number of poles of $G(s)$ encircled by Γ_s and let Z be the number of zeros of $1 + G(s)$, also encircled by Γ_s . Then the number of clockwise encirclement, N , of the point $(-1, 0)$ by the Nyquist plot of $1 + G(s)$ is equal to $Z - P$, that is

$$N = Z - P$$

The criterion states that a stable closed-loop system can only become unstable if the number of encirclements of the point $(-1, 0)$ is not equal to $Z - P$.

In Figure 5 the Nyquist plot for our system is shown.

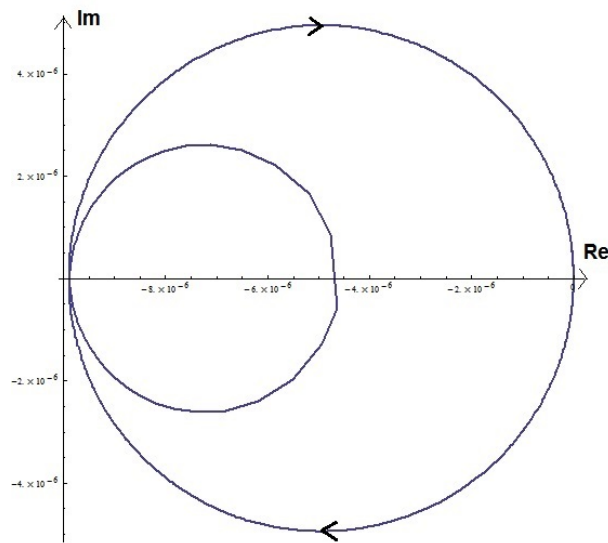


Figure 5: Nyquist plot for our system. Calculations is found in appendix A.

As the plot shows, the system does not enclose the point $(-1, 0)$, that is $N = 0$. The plot indicates that the system is stable. To be certain that the system is stable, we need to make sure that the Nyquist stability criterion holds. The zeros and the poles for our transfer function is,

zeros: -0.841243 and -0.0051912

poles: -385.564 , -1.78784 and -0.0112275

This gives us the information that $P = 0$ and $Z = 0$ and as a consequence $N = Z - P \implies 0 = 0 + 0$. The Nyquist stability criterion holds and our system is thus stable. But as mentioned in the beginning, an analysis over the degree that the stability holds for can be done and this is done by calculating gain and phase margins.

5.3.3 Gain and phase margins

There are two important measures of stability that can be defined, gain margins and phase margins. They measure the amount of uncertainty required to destabilise a stable closed-loop system and it is based on the closeness with which the Nyquist plot passes the point $(-1, 0)$. For instance, to determine closed loop stability on a stable open-loop system, the Nyquist plot need to cross the negative real axis between -1 and the origin [6].

To determine gain and phase margins, consider a system with $P = 0$, $Z = 0$ and that its corresponding Nyquist plot does not encircle the point $(-1, 0)$. This system is stable according to Nyquist stability criterion. Take the unit circle on the Nyquist plot given from the system and from here analyse the closeness of the Nyquist plot to the point $(-1, 0)$. Figure 6 is provided to visualise the problem [6].

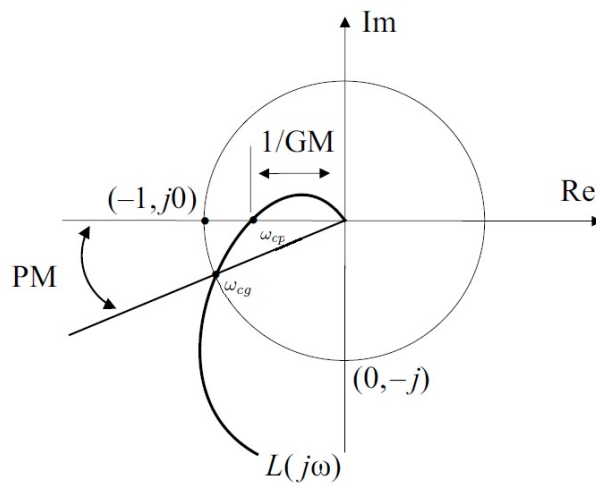


Figure 6: Nyquist plot for a stable closed-loop system with gain margins and phase margins [6].

From this, the phase and gain margins can be defined.

Definition, Phase margin (PM) [6]:

The angle that the frequency response would have to change to move to the point $(-1, 0)$, that is

$$PM = 180 + \arg\{G(i * \omega_{cg})\}$$

where ω_{cg} is the gain crossover frequency, that is the frequency at which the magnitude of the loop gain equals 1. In other words when the gain of $G(i * \omega_{cg}) = 1$.

Definition, Gain margin (GM) [6]:

The amount that the frequency response would have to increase to move to the point $(-1, 0)$, that is

$$GM = 20 \log \frac{1}{\|G(i * \omega_{cp})\|} [dB]$$

where ω_{cp} is the phase crossover frequency, that is the frequency at which the phase of $G(i * \omega_{cp}) = 180$. If the phase crossover should move to the left of the point $(-1, 0)$, then the system would become unstable.

Gain and phase margins are mutually independent but they should be compared to each other when analysing the degree of stability. Together they provide a measure of the closeness to the critical point $(-1, 0)$. The greater the distance is to the critical point, the stability is more robust. If the margins would be close to the critical point it would not take much for the system to become unstable [16].

If we analyse our Nyquist plot (Figure 5), note that the closed circles is very small and are close to the origin. The gain and phase margins and their corresponding phase and gain crossover frequency were calculated as follows (appendix A):

Gain margins and corresponding phase crossover frequency:

$$GM_1 = 101293, \omega_{cp1} = 19 \text{ and } GM_2 = 463445, \omega_{cp2} = 0$$

Phase margin and the corresponding gain crossover frequency:

$$PM = \infty, \omega_{cg} \text{ is undefined.}$$

If we first analyse the gain margins. They have both a large number and that tells us that the stability is very robust. The $\omega_{cp1} = 19$ is relative near zero and the frequency that it would take to come to the critical point $(-1, 0)$ is 101293. This is a high frequency and therefore it corresponds to a robust stability. The same argument holds for $\omega_{cp2} = 0$, where the frequency that it would take to come to the critical point $(-1, 0)$ is 463445. This frequency is even higher and shows that the stability is robust.

If we consider the phase margin, we see that it goes to infinity and that the ω_{cg} is undefined. This shows an excellent phase margin, since there are no frequencies that can be implemented to the system and make the phase margin cross the

critical point $(-1, 0)$. Thus the phase margin presents a robust stability. The reason that ω_{cg} is undefined can be explained by looking at the Nyquist plot. As we can see the Nyquist plot does not cross the unity circle, and therefore it is not possible for a gain crossover frequency to exist. Thus the phase margin goes to infinity [16].

By analysing the gain and phase margins we come to the conclusion that the stability is robust towards external inputs. Biologically this means that an E.coli can be located in an environment with external uncertainty and still function normally.

6 Integral feedback

So far, the robustness have been analysed by examine the stability around the equilibrium. The results for all stability tests have showed that an E.coli chemotaxis is robust towards external and internal stimuli. However, as mentioned in the beginning of the study, a key mechanism called adaptation is responsible for the robustness. We have examined this mechanism and showed through plotting the steady state, that it is insensitive toward external changes (section 'steady state analysis'). However, by using techniques from control theory it is possible to mathematically show that adaptation is robust and independent towards ligand changes. This is referred to as asymptotic tracking in control theory [15].

Integral feedback control is a common technique in control theory and it is used to ensure that an output from a dynamical system is independent of stimuli or variations in the systems parameters. Yi, Huang, Simon and Doyle [15] have showed that the robustness of adaptation is a result of the system possessing the property of integral feedback and that it is a necessary condition for robust implementation of adaptation. For that reason, we will show that our system have an integral feedback mechanism to ensure that the adaptation mechanism is robust in our model. We will use the same method they use, but our system is different from theirs, even though we both are analysing an E.Coli chemotaxis. They have used another approach to produce the mathematical system and applied different techniques to reduce their system.

A system in control theory is often represented by a block diagram, which is a schematic illustration showing the significant properties in blocks. For feedback control system it is an useful representation and below a block diagram over a system that possess the property integral feedback is provided (Figure 7) [5].

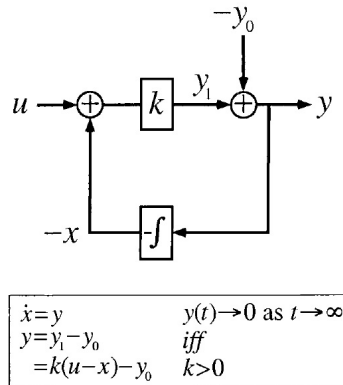


Figure 7: Block diagram over integral feedback control. The variable u represents the input, y the normalised system output error, the gain $k > 0 \in \text{Re}$ represents some process that take u as an input and produces the output y_0 and x is a time integral of the systems error that are feed back to the system. The condition $k > 0$ ensures stability [15].

Our model is a system having one input, $u = [L]$, and one output, $y = [Am]$, thus the above representation is suitable. If it is possible to show that such a feedback integral exist for our system, then we know that the system is insensitive to changes in the ligand levels and are therefore robust towards external stimuli [17]. The term integral feedback control refers to the systems error, more precisely the difference between the actual output y_1 and the desired output y_0 that is feed back to the system (see Figure 7) and the difference is referred to as the normalised output or error y . This type of control system ensures that the steady state error approaches zero as $t \rightarrow \infty$ despite changes in the process parameters. Yet, there is a required condition, the system must be stable at the steady state [15]. As shown earlier, this is the case for our system.

In order to find out if our system have an integral feedback as a structural property we examine our linearised system, with the state space matrices A , B and C . As we saw in section 'Lyapunov stability', our model is asymptotically stable and as a consequence the matrix A is non-singular. Consider our system in state space representation,

$$\begin{cases} \dot{\mathbf{x}}(t) = A\mathbf{x}(t) + B\mathbf{u}(t) \\ \mathbf{y}(t) = C\mathbf{x}(t) \end{cases}$$

At steady state the system is

$$\dot{\mathbf{x}}(t) = 0 \iff A\mathbf{x} + B\mathbf{u} = 0 \iff -A^{-1}B\mathbf{u} = \mathbf{x}$$

Inserting this results in the output equation we get

$$\mathbf{y} = -CA^{-1}B\mathbf{u}$$

The dynamics of the system can be ignored and therefore treatment of the problem can be purely algebraic [15]. Then $\forall \mathbf{u}$ that is constant, $\mathbf{y} = 0$ if and only if

$$CA^{-1}B = 0 \iff \begin{vmatrix} A & B \\ C & 0 \end{vmatrix} = 0$$

This equivalence is proven as,

$$\begin{aligned} \begin{vmatrix} A & B \\ C & 0 \end{vmatrix} &= \begin{vmatrix} I & 0 \\ -CA^{-1} & I \end{vmatrix} \begin{vmatrix} A & B \\ C & 0 \end{vmatrix} = \begin{vmatrix} A & B \\ 0 & -CA^{-1}B \end{vmatrix} = |A|(-CA^{-1}B) \\ &\implies (-CA^{-1}B) = 0, \quad \text{since } |A| \neq 0 \end{aligned}$$

The lower row, $[C \ 0]$ is linearly dependent on the upper row $[A \ B]$, and as a result there exists a non-zero real vector \mathbf{m} such that

$$[C \ 0] = \mathbf{m} [A \ B]$$

Therefore, the following is defined

$$\mathbf{z} = \mathbf{m}\mathbf{x}$$

from this we have

$$\dot{\mathbf{z}} = \mathbf{m}\dot{\mathbf{x}} = \mathbf{m}(A\mathbf{x} + B\mathbf{u}) = C\mathbf{x} = \mathbf{y}$$

If $\mathbf{y} = 0$ for all changes in the parameters, then $\dot{\mathbf{z}} = \mathbf{y}$ for all changes in the parameters. In other words, the normalised system output error is insensitive to changes in the parameters, and it follows that the condition

$$\begin{vmatrix} A & B \\ C & 0 \end{vmatrix} = 0$$

and it is equivalent to integral control being a structural property for the system. As stated earlier this is a necessary condition for robust asymptotic tracking, meaning that the system is robust towards external stimuli [15]. Note that this only deals with systems being in steady state.

If we turn to our model, we need to find \mathbf{m} such that $\begin{bmatrix} C & 0 \end{bmatrix} = \mathbf{m} \begin{bmatrix} A & B \end{bmatrix}$ to ensure that our model have integral control as a structural property. \mathbf{m} was calculated to (appendix A)

$$m_1 = \frac{([Am]^{SS} + K_{M_1})^2([Am]^{SS}k_5 + k_{-5})}{k_1(([Am]^{SS})^2[BP]^{SS}k_5 - ([Am]^{SS})^2B_Tk_5 - [Am]^{SS}B_Tk_5K_{M_1} - [BP]^{SS}K_{M_1} + k_{-5})}$$

$$m_2 = \frac{([Am]^{SS} + K_{M_1})^2([Am]^{SS}k_5 + k_{-5})}{k_1(([Am]^{SS})^2[BP]^{SS}k_5 - ([Am]^{SS})^2B_Tk_5 - [Am]^{SS}B_Tk_5K_{M_1} - [BP]^{SS}K_{M_1}k_{-5})}$$

$$m_3 = -\frac{([Am]^{SS}([Am]^{SS} + K_{M_1}))}{([Am]^{SS})^2[BP]^{SS}k_5 - [Am]^{SS})^2B_Tk_5 - [Am]^{SS}B_Tk_5K_{M_1} - [BP]^{SS}K_{M_1}k_{-5}}$$

Note that $\mathbf{m} \neq 0$ and does not depend on k_2 or $[L]$. The vector \mathbf{m} gives us the conditions under which the system has the property that the output is independent from the input level in steady state. As a conclusion, it is this property that makes the adaptation robust [15].

To make sure that $\mathbf{m} \neq 0$ for our system, we include the values of the parameters and calculate \mathbf{m} . The result is:

$$\mathbf{m} = (-0.000014167, -0.00139098, 0.20493)$$

According to this results and the previous calculations, we can now say with certainty that our system have an integral feedback as a structural property and that the adaptation is robust and insensitive towards ligand changes.

7 Conclusions and discussion

The aim for this study was to determine the robustness of the E.Coli chemotaxis. Biologically, the mechanism that is responsible for robustness is the ability for an E.Coli to adapt to its surrounding. Adaptation for an E.coli chemotaxis has been characterised in several studies that have a biochemical approaches [2] [3]. However, for this thesis the interest lies in trying to characterise the robustness property with help from mathematical control theory. The mathematical approach for the robustness analysis was to study the equilibrium point using stability tests and characterising the robustness of the adaptation mechanism using integral feedback.

The stability tests that were used was Lyapunov's stability test, Routh-Hurwitz stability criterion and the Nyquist stability criterion. All three tests examine different properties behind the stability. Yet, all the tests came to the same conclusion, the system is robust. In addition to stability tests, we showed plots over the convergence to steady state when the system was perturbed with different ligand levels. The plots also presented robustness in the system.

The mechanism adaptation was analysed using the control theory technique integral feedback. Integral feedback analysis exposed that the system does not depend on the input level when it is in steady state and consequently the mechanism adaptation is robust.

According to the robustness analysis, the system is robust. However, the study raises questions for further research. One proposition for further research on our system is to try to reduce the system once more, so that a two-dimensional system is obtained. This has been done in other studies [3] [15] and their approach to obtain a smaller dimension was to set the enzyme CheB-P to a constant. If CheB-P is considered to be a constant, then quasi-steady state can be assumed and the differential equation for CheB-P can be reduced. According to Cosentino and Bates [6] this reduction is possible since it has been shown experimentally that CheB-P is not responsible for the robustness. If the system is reduced to a two-dimensional system, then it is possible to do an attempt to calculate the region of attraction using a phase-plot. This would give further insight into the degree of the robustness for an E.Coli chemotaxis.

Nevertheless, with our broad robustness analysis it is clear that the E.coli chemotaxis is a robust biological mechanism.

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Appendix A

Showing the programming codes from wolfram Mathematica 9. The name of the parameters and the chemical species in the code are identical to the actual programming, but are not identical to the ones in the thesis. This is a conscious choice, as it facilitates for the reader to copy the codes from the appendix into wolfram Mathematica. It should not be difficult to understand what corresponds from the programming codes to the thesis.

The parameters that was used

```
R = 1; L = 20;  
k1 = 200; k2 = 1; k3 = 1; k4 = 1; k5 = 0.05;  
km1 = 1; km2 = 1; km3 = 1; km4 = 1; km5 = 0.005;  
KM1 = 1;  
KM2 = 1;
```

```
BT = 50; AT = 50;
```

```
Clear[R, L, k1, k2, k3, k4, k5, km1, km2, km3, km4, km5, KM1, KM2]
```

```
Clear[AT, BT]
```

The four-dimensional system in an nonlinear input-output representation (after removal of [A] and [B])

```
A = AT - Am - AmL - AL; B = BT - BP;  
f1[Am_, AmL_, AL_, BP_, L_] := km1 * R - k1 * BP * Am / (KM1 + Am) - k3 * Am * L + km3 * AmL  
f2[Am_, AmL_, AL_, BP_, L_] := km2 * R - k2 * BP * AmL / (KM2 + AmL) + k3 * Am * L - km3 * AmL  
f3[Am_, AmL_, AL_, BP_, L_] := - km2 * R + k2 * BP * AmL / (KM2 + AmL) + k4 * A * L - km4 * AL  
f4[Am_, AmL_, AL_, BP_, L_] := k5 * Am * B - km5 * BP
```

Calculations for the equilibrium points, when L=20

```
Chop[Solve[km1 * R - k1 * BP * Am / (KM1 + Am) - k3 * Am * L + km3 * AmL == 0 &&  
km2 * R - k2 * BP * AmL / (KM2 + AmL) + k3 * Am * L - km3 * AmL == 0 &&  
- km2 * R + k2 * BP * AmL / (KM2 + AmL) + k4 * A * L - km4 * AL == 0 &&  
k5 * Am * B - km5 * BP == 0, {Am, AmL, AL, BP}]]
```

```
Am = 0.0038240223826950933;
```

```
AmL = 0.4795700822816385;
```

```
AL = 47.13947753676598;
```

```
BP = 1.8415884373077727;
```

```
Clear[Am, AmL, AL, BP]
```

Linearisation from the input output system to state space representation, where L is the input and Am is the output. The state space matrices have the following arrangement, A, B and C. (D=0)

```

Amatrix[Am_, AmL_, AL_, BP_, L_] =
{{D[f1[Am, AmL, AL, BP, L], Am], D[f1[Am, AmL, AL, BP, L], AmL], D[f1[Am, AmL, AL, BP, L], AL],
D[f1[Am, AmL, AL, BP, L], BP]}, {D[f2[Am, AmL, AL, BP, L], Am], D[f2[Am, AmL, AL, BP, L], AmL],
D[f2[Am, AmL, AL, BP, L], AL], D[f2[Am, AmL, AL, BP, L], BP]},
{D[f3[Am, AmL, AL, BP, L], Am], D[f3[Am, AmL, AL, BP, L], AmL], D[f3[Am, AmL, AL, BP, L], AL],
D[f3[Am, AmL, AL, BP, L], BP]}, {D[f4[Am, AmL, AL, BP, L], Am], D[f4[Am, AmL, AL, BP, L], AmL],
D[f4[Am, AmL, AL, BP, L], AL], D[f4[Am, AmL, AL, BP, L], BP]}}//Simplify;
Amatrix[Am_, AmL_, AL_, BP_, L_]//MatrixForm
Bmatrix[Am_, AmL_, AL_, BP_, L_] =
{{D[f1[Am, AmL, AL, BP, L], L]}, {D[f2[Am, AmL, AL, BP, L], L]}, {D[f3[Am, AmL, AL, BP, L], L]},
{D[f4[Am, AmL, AL, BP, L], L]}}//Simplify;
Bmatrix[Am_, AmL_, AL_, BP_, L_]//MatrixForm
Cmatrix[Am_, AmL_, AL_, BP_, L_] = Transpose[{{1}, {0}, {0}, {0}}];
Cmatrix[Am_, AmL_, AL_, BP_, L_]//MatrixForm

```

$$\begin{pmatrix}
-\frac{k1KM1BP_-+k3(KM1+Am_-)^2L_-}{(KM1+Am_-)^2} & km3 & 0 & -\frac{k1Am_-}{KM1+Am_-} \\
k3L_- & -\frac{km3(KM2+AmL_-)^2+k2KM2BP_-}{(KM2+AmL_-)^2} & 0 & -\frac{k2AmL_-}{KM2+AmL_-} \\
-k4L_- & \frac{k2KM2BP_- - k4(KM2+AmL_-)^2L_-}{(KM2+AmL_-)^2} & -km4 - k4L_- & \frac{k2AmL_-}{KM2+AmL_-} \\
k5(BT - BP_-) & 0 & 0 & -km5 - k5Am_-
\end{pmatrix}$$

$$\begin{pmatrix}
-k3Am_- \\
k3Am_- \\
-k4(-AT + AL_- + Am_- + AmL_-) \\
0
\end{pmatrix}$$

$$\begin{pmatrix}
1 & 0 & 0 & 0
\end{pmatrix}$$

To obtain state space representation in Mathematica, the following code is used.

```

SSmodel = StateSpaceModel[{Amatrix[Am, AmL, AL, BP, L], Bmatrix[Am, AmL, AL, BP, L],
Cmatrix[Am, AmL, AL, BP, L]};

```

To obtain a transfer function and its zeros and poles in Mathematica, the following code is used. Where the transfer function below is on the same solidus.

SSTrans = TransferFunctionModel[SSmodel]

$$\frac{\text{Am k3}}{(-\text{BP} + \text{BT})\text{k5}(\text{Am} + \text{KM1})(-\text{AmL k2}(\text{Am} + \text{KM1})(\text{AmL} + \text{KM2})\text{km3} - \text{Am k1})}$$

$$\frac{(\text{Am} + \text{KM1})^2}{(\text{BP k2 KM2} + (\text{AmL} + \text{KM2})^2\text{km3} + (\text{AmL} + \text{KM2})^2\text{s}) + (-\text{Am k5} - \text{km5} - \text{s})}$$

$$\frac{(\text{Am k5} + \text{km5} + \text{s})}{(-\text{k3}(\text{Am} + \text{KM1})^2(\text{AmL} + \text{KM2})^2\text{km3 L} + (\text{BP k1 KM1} + \text{k3}(\text{Am} + \text{KM1})^2\text{L} + (\text{Am} + \text{KM1})^2\text{s})}$$

$$\frac{(\text{BP k2 KM2} + (\text{AmL} + \text{KM2})^2\text{s})}{(\text{BP k2 KM2} + (\text{AmL} + \text{KM2})^2\text{km3} + (\text{AmL} + \text{KM2})^2\text{s})}$$

For the calculation of the zeros and poles, the parameters were set to their values.

TransferFunctionZeros[SSTrans]

{{{-21., -0.841243, -0.0051912}}}

TransferFunctionPoles[SSTrans]

{{{-385.564, -21., -1.78784, -0.0112275}}}

Analysing Controllability and Observability

The controllability matrix is seen below.

ContMatrix = ControllabilityMatrix[SSmodel];

ContMatrix//MatrixForm

$$\begin{pmatrix} -0.00382402 & 1.47805 & -569.889 & 219729. \\ 0.00382402 & -0.0835214 & 29.7177 & -11453.7 \\ 2.37713 & -49.9165 & 1020.28 & -10596.3 \\ 0 & -0.00920794 & 3.55907 & -1372.27 \end{pmatrix}$$

Testing to see if the controllability matrix is of full rank.

```
ControllableModelQ[SSmodel]
```

True

The observability matrix is seen below.

```
ObsMatrix = ObservabilityMatrix[SSmodel];
```

```
ObsMatrix//MatrixForm
```

$$\begin{pmatrix} 1 & 0 & 0 & 0 \\ -385.517 & 1. & 0. & -0.761891 \\ 148641. & -387.358 & 0. & 293.402 \\ -5.73108 \times 10^7 & 149355. & 0. & -113125. \end{pmatrix}$$

Testing to see if the observability matrix is of full rank.

```
ObservableModelQ[SSmodel]
```

False

```
Det[ObsMatrix]
```

0

It turns out that the system is controllable but not observable. Decomposition must be done. This was also confirmed when calculating the poles and zeros from the transfer function. A pole and zero can be cancelled, since they are the same. The denominator should be of degree three, therefore the system can be reduced to a three-dimensional system.

Decomposition of the state space model

To decompose the state space model the following code was used.

```
SSminModel = MinimalStateSpaceModel[SSmodel];
```

The following codes gives the minimised state space matrices in order A, B and C.

```
Amin = SSminModel[[1]][[1]];
```

```
Amin//MatrixForm
```

```
Bmin = SSminModel[[1]][[2]];
```

```
Bmin//MatrixForm
```

```
Cmin = SSminModel[[1]][[3]];
```

```
Cmin//MatrixForm
```

$$\begin{pmatrix} -\frac{BPk1KM1+k3(Am+KM1)^2L}{(Am+KM1)^2} & km3 & -\frac{Amk1}{Am+KM1} \\ k3L & -\frac{BPk2KM2+(AmL+KM2)^2km3}{(AmL+KM2)^2} & -\frac{AmLk2}{AmL+KM2} \\ (-BP + BT)k5 & 0 & -Amk5 - km5 \end{pmatrix}$$

$$\begin{pmatrix} -Amk3 \\ Amk3 \\ 0 \end{pmatrix}$$

$$\begin{pmatrix} 1 & 0 & 0 \end{pmatrix}$$

Checking Controllability and Observability on decomposed system.

minContMatrix = ControllabilityMatrix[SSminModel];

minContMatrix//MatrixForm

$$\begin{pmatrix} -0.00382402 & 1.47805 & -569.889 \\ 0.00382402 & -0.0835214 & 29.7177 \\ 0 & -0.00920794 & 3.55907 \end{pmatrix}$$

ControllableModelQ[SSminModel]

True

minObsMatrix = ObservabilityMatrix[SSminModel];

minObsMatrix//MatrixForm

$$\begin{pmatrix} 1 & 0 & 0 \\ -385.517 & 1. & -0.761891 \\ 148641. & -387.358 & 293.402 \end{pmatrix}$$

ObservableModelQ[SSminModel]

True

The minimised model is controllable and observable.

Calculations for the new transfer function. The transfer function below is on the same solidus.

SSminTrans = TransferFunctionModel[SSminModel, s]

$$\frac{\frac{\text{Am } k3}{(-\text{BP} + \text{BT})k5(\text{Am} + \text{KM1})(-\text{AmL } k2(\text{Am} + \text{KM1})(\text{AmL} + \text{KM2})km3 - \text{Am } k1)}}{\frac{(\text{Am} + \text{KM1})^2}{(\text{BP } k2 \text{ KM2} + (\text{AmL} + \text{KM2})^2(km3 + s) + (-\text{Am } k5 - km5 - s))}}}{\frac{(\text{Am } k5 + km5 + s)}{(-k3(\text{Am} + \text{KM1})^2(\text{AmL} + \text{KM2})^2km3 \text{ L} + (\text{BP } k2 \text{ KM2} + (\text{AmL} + \text{KM2})^2(km3 + s)))}}}{\frac{(\text{BP } k2 \text{ KM2} + (\text{AmL} + \text{KM2})^2s)}{(\text{BP } k1 \text{ KM1} + (\text{Am} + \text{KM1})^2(k3 \text{ L} + s))}}$$

Calculations for the equilibrium points in the decomposed system.

Chop[Solve[km1 * R - k1 * BP * Am/(KM1 + Am) - k3 * Am * L + km3 * AmL == 0 && km2 * R - k2 * BP * AmL/(KM2 + AmL) + k3 * Am * L - km3 * AmL == 0 && k5 * Am * B - km5 * BP == 0, {Am, AmL, BP}]]

Am = 0.0038240223826950933;

AmL = 0.4795700822816385;

BP = 1.8415884373077727;

Clear[Am, AmL, BP]

Time domain analysis

Lyapunov's theory. Eigenvalues for the minimal A matrix, here we set the parameters to values.

Eigenvalues[Amin]

{-385.564, -1.78784, -0.0112275}

This ensure asymptotically stability due to Lyapunov's indirect method.

Routh-Hurwitz stability criterion

Calculations of the characteristic polynomial $a(s) = a + bs + cs^2 + ds^3$, according to Routh-Hurwitz stability criterion, $d = 1$ and $a, b, c > 0$

```
minPoly[s_] = Det[s * IdentityMatrix[3] - Amin]//Simplify;
```

The coefficients:

```
a = Coefficient[minPoly[s], s, 0]//Simplify;  
b = Coefficient[minPoly[s], s, 1]//Simplify;  
c = Coefficient[minPoly[s], s, 2]//Simplify;  
d = Coefficient[minPoly[s], s, 3]//Simplify;
```

We can calculate the Hurwitz matrix to find out for each element the parameter must be calculated for:

```
bn2matrix = {{1, b}, {c, a}};  
bn2 = -Det[bn2matrix]/c;
```

```
cn3matrix = {{c, a}, {bn2, 0}};  
cn3 = -Det[cn3matrix]/bn2;
```

Our Hurwitz matrix is

```
Hurwitzmatrix = {{1, b, 0, 0}, {c, a, 0, 0}, {bn2, 0, 0, 0}, {cn3, 0, 0, 0}};  
Hurwitzmatrix//MatrixForm
```

$$\begin{pmatrix} 1 & b & 0 & 0 \\ c & a & 0 & 0 \\ bn2 & 0 & 0 & 0 \\ cn3 & 0 & 0 & 0 \end{pmatrix}$$

We see that $c > 0$, $bn2 > 0$ and $cn3 > 0$ according to Routh-Hurwitz stability criterion and we look at the parameter $k1$, $k5$ and $km5$.

```
Reduce[c > 0 && bn2 > 0 && cn3 > 0, k1]  
k1 > -4.98968
```

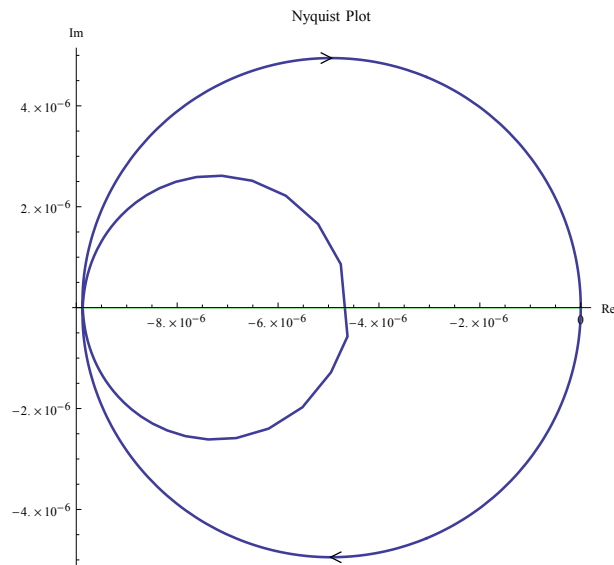
```
Reduce[c > 0 && bn2 > 0 && cn3 > 0, k5]  
k5 > -0.0401974
```

```
Reduce[c > 0 && bn2 > 0 && cn3 > 0, km5]  
km5 > -0.00621931
```

Frequency domain analysis

Nyquist stability criterion

```
NyquistPlot[SSminModel, PlotStyle → Thick, StabilityMargins → True,  
StabilityMarginsStyle → {{Green, Red}}, AxesLabel → {Re, Im}, PlotLabel → NyquistPlot]
```



```
TransferFunctionModel[SSminModel]
```

Analysing the zeros and poles for the transfer function to see if they lie in the right-half complex plane.

```
TransferFunctionZeros[TransferFunctionModel[SSminModel]]
```

```
TransferFunctionPoles[TransferFunctionModel[SSminModel]]
```

```
{{{-0.841243, -0.0051912}}}  
{{{-385.564, -1.78784, -0.0112275}}}
```

Calculations for the gain and phase margins.

```
{gm, pm} = GainPhaseMargins[TransferFunctionModel[SSminModel]];
```

```
{{19.1262, 101293.}, {0., 463445.}}  
{{None, ∞}}
```

Plots for steady states

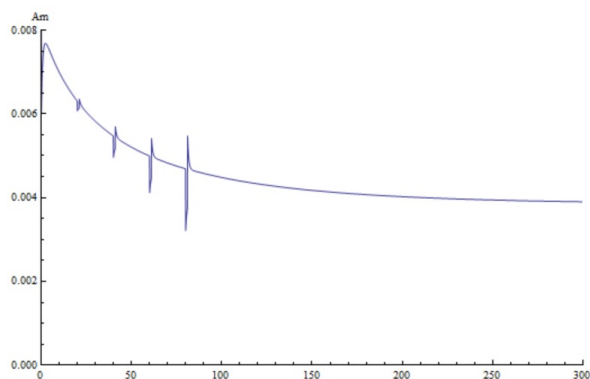
When the ligand levels are set to different values at different time.

```
LL[t_] = Piecewise[{{10, 0 < t < 10}, {20, 20 < t < 21}, {40, 40 < t < 41}, {80, 60 < t < 61},
{160, 80 < t < 81}, {320, 100 < t < 101&&120 < t < 121&&140 < t < 141}, {640, 160 < t < 161}}, 10]
```

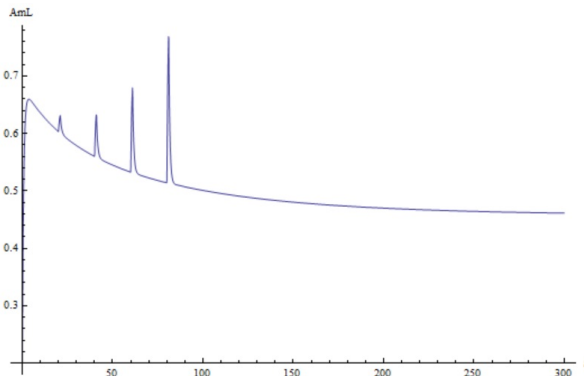
```
10  0 < t < 10
20  20 < t < 21
40  40 < t < 41
80  60 < t < 61
160 80 < t < 81
320 100 < t < 101&&120 < t < 121&&140 < t < 141
640 160 < t < 161
```

```
Sol = NDSolve[{Am'[t]==km1 * R - k1 * BP[t] * Am[t]/(KM1 + Am[t])
-k3 * Am[t] * LL[t] + km3 * AmL[t],
AmL'[t]==km2 * R - k2 * BP[t] * AmL[t]/(KM2 + AmL[t]) + k3 * Am[t] * LL[t] - km3 * AmL[t],
AL'[t]== - km2 * R + k2 * BP[t] * AmL[t]/(KM2 + AmL[t])
+k4 * (AT - Am[t] - AmL[t] - AL[t]) * LL[t] -
km4 * AL[t], BP'[t]==k5 * Am[t] * (BT - BP[t]) - km5 * BP[t], Am[0] == .1, AmL[0] == .2,
AL[0] == 1, BP[0] == 1}, {Am, AmL, AL, BP}, {t, 0, 500}]
```

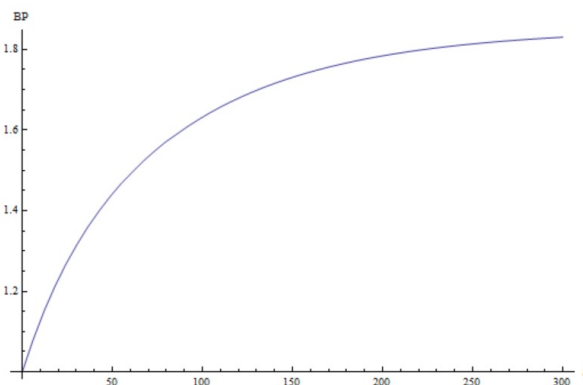
```
plotAm1 = Plot[Evaluate[Am[t]/.Sol], {t, 0, 300}, AxesLabel -> {t, Am}]
```



```
plotAmL1 = Plot[Evaluate[AmL[t]/.Sol], {t, 0, 300}, PlotRange -> All, AxesLabel -> {t, AmL}]
```



```
plotBP1 = Plot[Evaluate[BP[t]/.Sol], {t, 0, 300}, AxesLabel -> {t, BP}]
```



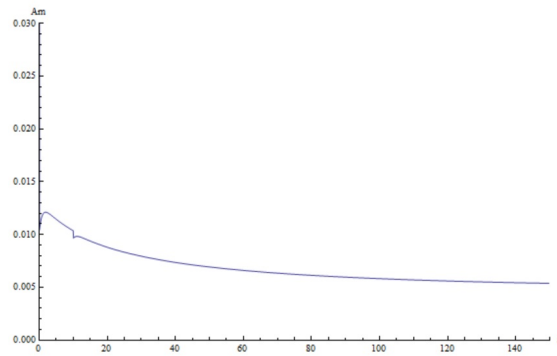
Plots for steady state when the ligand levels are doubled at time 10.

```
L[t.]:=20 + 20 * HeavisideTheta[t - 10];
```

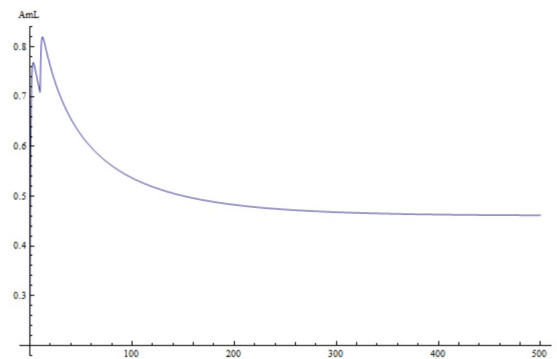
```
eqns = {Am'[t] == k11 * R - ((k1 * BP[t] * Am[t])/(kM1 + Am[t])) + k22 * R
-k3 * Am[t] * L + k33 * AmL[t],
AmL'[t] == k22 * R - ((k2 * BP[t] * AmL[t])/(kM2 + AmL[t])) + k3 * Am[t] * L - k33 * AmL[t],
AL'[t] == -k22 * R + ((k2 * BP[t] * AmL[t])/(kM2 + AmL[t]))
+k4 * (AT - Am[t] - AmL[t] - AL[t]) * L -
k44 * AL[t], BP'[t] == k5 * Am[t] * (BT - BP[t]) - k55 * BP[t]};
eqEqn = {};
icEqn = {Am[0]==0.1, AmL[0]==0.2, AL[0] == 1, BP[0] == 1};
params = {L -> L[t], R -> 1, k1 -> 200, k2 -> 1, k3 -> 1, k4 -> 1, k5 -> 0.05, k11 -> 1, k22 -> 1, k33 -> 1,
k44 -> 1, k55 -> 0.005, kM1 -> 1, kM2 -> 1};
```

```
sol = NDSolve[{eqns, eqEqn, icEqn}/.params, {Am[t], AmL[t], AL[t], BP[t]}, {t, 0, 500}]
```

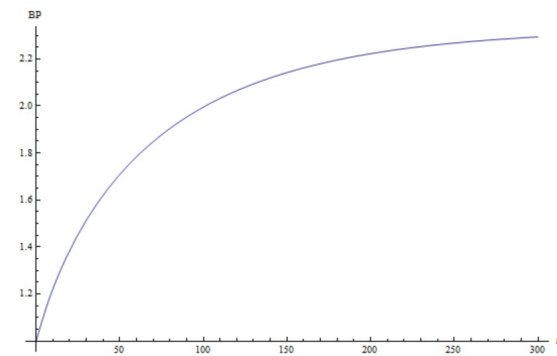
`plotAm2 = Plot[Evaluate[Am[t]]/.sol, {t, 0, 300}, PlotRange -> All, AxesLabel -> {t, Am}]`



`plotAmL2 = Plot[Evaluate[AmL[t]]/.sol, {t, 0, 500}, PlotRange -> All, AxesLabel -> {t, AmL}]`



`plotBP2 = Plot[Evaluate[BP[t]]/.sol, {t, 0, 300}, PlotRange -> All, AxesLabel -> {t, BP}]`



Robust asymptotic tracking

Calculations to solve the m -vector to ensure that the system have the integral feedback property.

$AB = \text{Join}[A_{\text{min}}, B_{\text{min}}, 2];$

$AB // \text{MatrixForm}$

$C0 = \{\{1\}, \{0\}, \{0\}\};$

$$\begin{pmatrix} -\frac{BPk1KM1+k3(Am+KM1)^2L}{(Am+KM1)^2} & km3 & -\frac{Amk1}{Am+KM1} & -Amk3 \\ k3L & -\frac{BPk2KM2+(AmL+KM2)^2km3}{(AmL+KM2)^2} & -\frac{AmLk2}{AmL+KM2} & Amk3 \\ (-BP + BT)k5 & 0 & -Amk5 - km5 & 0 \end{pmatrix}$$

$m = \{m1, m2, m3\};$

$mAB = m.AB;$

$\text{Solve}[C0 == mAB, \{m1, m2, m3\}]$

$$m1 = ((Am + KM1)^2(Am * k5 + km5))/(k1(Am^2 * BP * k5 - Am^2 * BT * k5 - Am * BT * k5 * KM1 - BP * KM1 + km5))$$

$$m2 = ((Am + KM1)^2 * (Am * k5 + km5))/(k1(Am^2 * BP * k5 - Am^2 * BT * k5 - Am * BT * k5 * KM1 - BP * KM1 * km5))$$

$$m3 = -(Am(Am + KM1))/((Am^2 * BP * k5 - Am^2 * BT * k5 - Am * BT * k5 * KM1 - BP * KM1 * km5))$$