The Mechanics and Thermodynamics of Amyloid Beta Protein Aggregation in Competing Pathways

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Abstract

The primary purpose of this paper is to investigate the mechanics of \( A\beta \) protein aggregation within the brain through mathematical modeling and simulation. Aggregation of \( A\beta \) is the cause of plaques within the brain of Alzheimer’s Disease sufferers. Because the pathways of aggregation from monomer to oligomer to polymer are numerous and complex, we have had to simplify our model to a limited number of species. Of great concern, too, is the process by which \( A\beta \) can form as “off-pathway” species, which is when \( A\beta \) reacts with fatty acid micelles. It is this species of \( A\beta \), which due to its toxicity to neurons, that is now believed to cause Alzheimer’s Disease.

Although the precise mechanism of \( A\beta \) aggregation continues to be heavily debated, evidence suggests a rate-limiting mechanism. Thus we will use Mass Action Kinetics to write a system of differential equations for the purpose of simulating aggregation of the \( A\beta \) protein in its different forms. We will analyze the stability of the system under different reaction rate regimes, as well as the system’s preferences for particular equilibrium states. Finally, we will examine the thermodynamics of the system.
MONTCLAIR STATE UNIVERSITY

The Mechanics and Thermodynamics of Amyloid Beta Protein Aggregation in Competing Pathways

By

Edward Steen

A Master's Thesis Submitted to the Faculty of Montclair State University In Partial Fulfillment of the Requirements For the Degree of

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In dedication to my mother, Hildegard B. Steen.

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## Contents

1 Introduction 8  
1.1 Motivation 8  
1.2 History of AD 9  
1.3 The Biophysics of AD 9  

2 Four Species Model 13  
2.1 The Model 13  
2.2 Equilibrium points 14  
2.3 Linear Stability Analysis 15  
2.4 Numerical Results 19  
  2.4.1 Nash Equilibrium 19  

3 Six Species Model 24  
3.1 The Model 24  
3.2 Equilibrium Points 27  
3.3 Linear Stability Analysis 34  
3.4 Numerical Results 42  
  3.4.1 Nash Equilibrium of the Six Species Model 47  
3.5 Fluxes and Forces 53  
3.6 Entropy Production 55  

4 Conclusions 57  
4.1 Four Species Model 57
4.2 Six Species Model ........................................ 58
4.3 Future Study ............................................. 60
List of Figures

1.1 Plaque Deposits in AD Brain. ............................................. 10

2.1 Schematic of the system with backward and forward rates. .............. 14

2.2 The eigenvalues of matrix $B$ as a function of $\alpha_3$. Case (a) shows the switching event at a critical value of $\alpha_3$ while panel (b) shows the eigenvalues to the on- and off-pathways. .............................................. 17

2.3 The eigenvalues of matrix $B$ as a function of $\alpha_3$. Case (a) shows the switching event at a critical value of $\alpha_3$ while panel (b) shows the eigenvalues to the on- and off-pathways. .............................................. 18

2.4 Eigenvalues as a function of backward rates (first frame) and forward rates (second frame). .............................................. 21

2.5 Eigenvalues as $n$ is varied. .............................................. 22

2.6 Solution of Model with $B_1(0) = 1$, $B_1'(0) = B_n(0) = B_n'(0) = 0$, $\alpha_1 = \beta_1 = .001$, $\alpha_2 = \beta_2 = 1$ and $n = 4$. .............................................. 22

2.7 Time evolution of the Base Case model as $\alpha_3$ varies. The red region is strictly on-pathway, blue is strictly off-pathway, and yellow is where $B_1 < B_1'$ and $B_n > B_n'$ or mixed on-/off-pathway. Thus there are 2 possible equilibrium states, where a mixed state can persist in the short run. The second graph is a zoomed-in version of the first, showing the persistence of yellow over long periods of time for $\alpha_3$ close to 1. .............................. 23

3.1 Schematic of System with forward and backward rates. ................. 26

3.2 Solutions of the Base Model and Model 2. ................................ 29
3.3 Absolute percentage change of the concentration of $B_1$ and $B_m$ as a function of time over identical time spans. Model 2 achieves equilibrium more quickly.

3.4 Solution of the System (Base Case) with $t = 0 : 2200$ and zoomed in concentration size. Note the slow but continued growth of $B_m$ and $B_m'$.

3.5 Plots showing percentage change of $B_1$ and $B_m'$ over different timespans for the Base Model.

3.6 Equilibrium time as a function of the ratio of backward to forward rates.

3.7 Fluctuations in the size of $B_1$ and $B_m'$ under Model 2 conditions, after equilibrium is achieved.

3.8 Percentage change in concentration of $B_1$ and $B_m'$ with high backward rates.

3.9 The first figure is $\lambda_5$ as a function of $\alpha_3$ and the second graph is $\lambda_5$ as a function of $\alpha_4$. Note that the scale is $10^{-16}$.

3.10 $\lambda_i$ as a function of $\alpha_3$ and $\alpha_4$.

3.11 $\lambda_i$ as a function of $\alpha_3$ and $\alpha_4$.

3.12 Switching Behavior of $\lambda_2$, $\lambda_3$ and $\lambda_4$ with forward rates doubled.

3.13 Switching of eigenvalues in Base Model as $n$ and $m$ are varied. The white region in both plots indicates a zero value for the eigenvalue for any value of the parameters.

3.14 Switching of eigenvalues in Model 2 as $n$ and $m$ are varied.

3.15 Switching and crossing of $\lambda_1$, $\lambda_2$ as a function of $\alpha_3$.

3.16 Concentration levels of Base Model.

3.17 Concentration levels of Base Model with $\alpha_3$, $\alpha_4$ =10.

3.18 Concentration of $B_1'$ and $B_n'$ as $\alpha_3$ and $\alpha_4$ are varied.

3.19 Progression of Species Concentrations. Over time, the spike in concentration of $B_1'$ is reduced as monomers of that species aggregate into $B_n'$.

3.20 Concentration ratios as $\lambda_3$ and $\lambda_4$ vary.

3.21 Concentration ratios as $\lambda_3$ varies, with $\lambda_4$ held constant.

3.22 By increasing the forward reaction rates in the direction of $B_m'$, we were able to get $B_m'$ to outperform all other species on an absolute basis.
3.23 The four equilibrium states of the system. Clockwise from top left, the first figure has all on-pathway species outperforming off-pathway. The second figure has all off-pathway species outperforming on-pathway, and the remaining two are mixed on-/off-pathway.

3.24 Aggregation pathways as a function of $\alpha_3$ and $\alpha_4$ with step size of .02. Colors correspond to the equilibrium states from Figure 3.22.

3.25 Aggregation pathways as a function of $\alpha_3$ and $\alpha_4$. The blue region continues to “win” as $\alpha_3$ and $\alpha_4$ increase without limit.

3.26 Aggregation pathways as a function of $\alpha_3$ and $\alpha_4$ with backward and forward rates equal.

3.27 Dominance of on-pathway $n$ and $m$ species for low $\alpha_4$ and high $\alpha_3$.

3.28 Species formation by iteration when successively adding one unit of $B_1$ at each iteration. Note that with the exception of $B_m$ and $B'_m$ in the Base Model, prime and non-prime species are right on top of each other.

3.29 A schematic of the reaction pathways.

3.30 A contour plot of entropy production along the four different pathways as a function of $\alpha_3$ and $\alpha_4$. The red regions correspond to large values, while blue points to smaller values of entropy production. For this calculation, the forward rate constants along the purely on and off pathways are taken to be equal to 1.0 while he backward constants are 0.001.
List of Tables

2.1 A sample game-theoretical payoff matrix to depict the on- and off-pathway competition ........................................... 21

3.1 Table of reaction rates between species ........................................... 26
3.2 Time to equilibrium as a function of the ratio of backward to forward rates. 32
3.3 Time to equilibrium of the Base Model and Model 2 versus fsolve. ....... 33
3.4 Table of time to equilibrium values as $n$ and $m$ are varied. ................. 34
3.5 Eigenvalues of the Base Model and Model 2. ................................ 35
3.6 Switch and cross points of $\alpha_3$ as a function of $\alpha_4$. ...................... 41
3.7 Table of equilibrium states as a function of $\alpha_3$ and $\alpha_4$. ................. 49
Chapter 1

Introduction

1.1 Motivation

According to the Fisher Center for Alzheimer’s Research, 30 million people live with Alzheimer’s Disease (AD) worldwide, and the number is expected to reach 60 million people by 2030 [1]. Alzheimer’s Disease can occur in people as young as their early 40’s (Early Onset), but these cases represent only 5% of the total. Most AD cases occur in people in their mid-60’s and on, with approximately 10% of the population at risk of contracting the disease in that age group. By some estimates, by the time a person reaches their mid-80’s, there is a 50% chance of having AD. Thus have improvements in health and longevity been a mixed blessing. According to the Alzheimer’s Association, the average duration of the disease is 7 years, and once contracted progressively worsens, ultimately resulting in death. There is currently no cure.

The Alzheimer’s Association recently reported that the cost of care for people with AD in the US will consume 25% of Medicare spending by the middle of the century. In 2010 alone, the total, direct cost of AD care in the US was $157 billion, or approximately $50,000 per patient. These figures, while of concern on their own, don’t count the indirect burden of AD care exacted upon loved ones and caregivers. For those who cannot afford nursing home care, looking after a family member with AD can be an all-consuming job. Therefore, the number of people affected by AD is much larger than the 30 million who actually have the disease. In many developed countries, with the aging of populations, these social and
financial costs will only continue to increase.

Thus, from both a public health and a fiscal health perspective, Alzheimer’s Disease is a major challenge worldwide. The need for research is therefore vitally important. While we are enabling people to live longer, this increased longevity comes at a great cost in terms of caring for a significant proportion of the elderly population who are incapacitated, not to mention the diminished quality of life of those suffering from AD. It is these concerns that have motivated our research.

1.2 History of AD

The identification of the causes of dementia is credited to Alois Alzheimer. In 1907, while performing an autopsy on a woman who had died from a progressive behavioral and cognitive disorder, Alzheimer noted the presence of two pathologies: neurofibrillary tangles and neuritic plaques [2]. Neurons are cells that transmit nerve impulses, or information, throughout the body, and the tangles were essentially deformed neurons. The plaques were extra-cellular deposits found throughout the brain. Subsequent research down the years has shown that these tangles are related to the protein tau and the neuritic plaques are aggregations of the amyloid-β peptide (Aβ) [2]. In our research, we will be modeling the latter pathology: the formation of Aβ plaques.

1.3 The Biophysics of AD

At the outset it should be noted that much of what we “know” about the role of Aβ or the causes of AD are heavily debated. In fact, scientists have long been puzzled by the weak link between the amount of Aβ deposition and the degree of dementia, not to mention the uncertainty as to which form of Aβ causes AD [3]. AD sufferers all have the plaques, however not all people with the plaques necessarily had AD. The process of aggregation from monomer to oligomer is also poorly understood [4]. This lack of understanding is due in part to two major problems with AD research: first, early diagnosis at this point is inexact and unreliable, and secondly, measuring the amount of Aβ plaque deposition occurs only post mortem [3]. Thus, accurately tracking the progression of the disease in humans is virtually impossible at this time. With that said, the following is a brief outline of what can best be described as the consensus view.
Alzheimer’s Disease is one in a class of protein folding disorders called amyloidosis [1]. Amyloid Precursor Protein (APP) is produced in large quantities in the brain, and is believed to play an important role in healthy cell functioning. APP is broken down into monomers (single units) of amino acids by the action of beta and gamma secretase enzymes. It is these amino acids that can aggregate to form Aβ peptides (or oligomers), which are strings of these amino acids. These peptides form extracellular deposits, or plaques, which are believed to be toxic to neurons. Aβ40 is the most abundant, but it is Aβ42, which due to its hydrophobic and fibrillogenic nature, that is responsible for plaque deposition in AD brains. Recent evidence, however, seems to implicate an intermediate form of Aβ, which is soluble, as the cause of AD rather than the plaques themselves. It is this “off-pathway” aggregation of the soluble form of Aβ that is now thought to be the neurotoxic agent[3].

Thus, aggregation of Aβ, in some form, is believed to constitute the central process in the AD pathology. Brains of AD patients contain large amounts of plaques that are mainly comprised of insoluble Aβ fibril deposits.

Monomeric Aβ peptides (Aβ40 and Aβ42 ) spontaneously undergo self-assembly towards large fibrils in a nucleation dependent manner. Although the precise mechanism of nucleation continues to be heavily debated, substantial evidence suggests a rate-limiting mechanism for the formation of nucleus or nuclei [5; 6; 7; 8; 9]. The dynamics associated with reactions leading up to nucleation is critical for aggregation. Energetically, the pre-nucleation phase is nebulous with monomers and oligomers (dimers, trimers etc.) being in dynamic flux involving stochastic interactions [10; 11; 12]. Intrinsic disorder and amphipathic nature of monomeric Aβ also facilitate significant phase transitions and heterogeneous interactions during nucleation, making the process particularly sensitive to environmental factors such as pH, ionic strength, temperature and other interacting partners [13; 15; 16; 17; 18]. This is sig-
significant because smaller, soluble aggregates have emerged as the primary neurotoxic agents responsible for memory loss in AD \[19; 20; 21; 22\]. Furthermore, it is clear that oligomers may not be obligatory intermediates to fibril formation, and that the oligomers can also be populated along alternate pathways of aggregation (off-pathways) \[23; 24; 25; 26; 27; 28\]. As previously mentioned, off-pathway oligomers may differ from those formed along the on-pathway resulting in multiple conformational variants with distinct biochemical and cellular properties. Given the conformational diversity among oligomers, it is imperative to determine the factors that affect dynamics involved in their formation to establish a framework of molecular mechanisms that better defines amyloid progression.

Some of such biologically significant interacting partners that could affect A$\beta$ pre-nucleation dynamics are anionic surfactants such as fatty acids and lipids \[29; 30; 31; 32; 33; 34; 35\]. Interfaces of lipids and fatty acids are of profound interest in physiological contexts as they are abundant in both cerebral vasculature and CSF \[36; 37\]. Amphipathic A$\beta$ peptide is known to have strong affinity for membranes and hence, these interactions may affect early steps of aggregation. Several reports also show the effects of phospholipids on A$\beta$ aggregation \[38; 39; 40\]. Similarly, polyunsaturated (PUFAs) as well as saturated fatty acids are also known to have a significant effect on AD brain \[41; 42\]. In previous publications, our collaborators have also reported the generation of 4-5mers and 12-24mers from lauric acid interfaces along a non-fibril formation pathway \[18\]. More importantly, using carbon chain lengths of C9-C12 fatty acids, we established that below (non-micellar), near (pseudo-micellar) and above (micellar) their critical micelle concentrations, they generate A$\beta$ oligomers or fibrils via distinct pathways \[18\].

There are 3 main sections to this report. In the first section, we examine a reduced-order 4 species model. Using Mass Action Kinetics, we formulate a system of differential equations that model the chemical reactions between these four species. We then examine the stability of the system to verify the validity of the model and the appropriateness of our parameters. We also study the evolution of the model and its equilibria under various rate regimes. Our collaborators at Virginia Commonwealth University have created a much larger and more
complicated version of our system. Our approach has been to simplify this system so that we can focus on a limited number of parameters that determine the pathways of Aβ aggregation. Other recent work along the lines of mathematical modeling of Aβ aggregation includes that done by Murphy and Knowles. What sets our work apart from these is our analysis of stability, modeling of species evolution over time, and our use of a game theoretic approach to competition between species.

In the second part, we will look at a six species model where we will again analyze the stability and numerical results. By adding two more species to the model, we add another path leading to off-pathway aggregation, thus adding more complexity to the system while keeping its essential simplicity. In the third part, we will analyze the thermodynamics of the system.

What follows is an investigation into mathematically modeling the aggregation process of Aβ. The major obstacle to investigating AD is the lack of understanding of the progression of the disease. Therefore, comparing our model to actual data is impossible at this point. Thus we have had to content ourselves with a purely mathematical analysis. Although we are not presuming to recommend therapies or direct courses of action to cure Alzheimer’s, the hope is to gain a greater understanding of what could be happening inside the brain of an AD sufferer. Our models are simplified versions consisting of 4 and then 6 species of Aβ with the objective of understanding the dynamics of the system on a rudimentary level. Even with just 6 species, however, there are infinitely many rate regimes that we could investigate, most of which would be physically meaningless. Thus, we have not made an exhaustive sweep of all parameter values, but have chosen to experiment with physically meaningful rate regimes about which we have varied our key parameters. The goal is to gain a greater understanding of how to mathematically model Aβ aggregation and hopefully make a contribution to solving what is becoming a serious health care crisis.
Chapter 2

Four Species Model

2.1 The Model

We examine the sets of on- and off-pathway reactions shown below. The model system is as follows:

\[ A_1 + L \rightleftharpoons_{k_1^+}^{k_1^-} A_1' \]

\[ n \cdot A_1 \rightleftharpoons_{k_2^+}^{k_2^-} A_n \]

\[ n \cdot A_1' \rightleftharpoons_{k_3^+}^{k_3^-} A_n' \]

We use the Law of Mass Action Kinetics to formulate our system of differential equations. For example, if we take the second chemical reaction that describes the aggregation of molecules of the species \( A_1 \) into an \( n \)-mer, \( A_n \),

\[ n \cdot A_1 \rightleftharpoons_{k_2^+}^{k_2^-} A_n \]

and consider just the forward reaction,

\[ n \cdot A_1 \xrightarrow{k_2^+} A_n \]

the left side are the reactants, and the right side are the products, with \( k_2^+ \) being the reaction rate constant. In a reaction, the stoichiometric coefficients, in this case \( n \), become exponents [44]. If we add the backward reaction, this coupled system would be expressed as:

\[
\frac{dA_1}{dt} = nk_2^- A_n - nk_2^+ A_1^n \quad (2.1)
\]

\[
\frac{dA_n}{dt} = -k_2^- A_n + k_2^+ A_1^n \quad (2.2)
\]
In similar fashion, the entire system of the above reactions would be expressed as,

\[ \frac{dA_1}{dt} = nk_2^{-} A_n - nk_2^{+} A_1^n + k_1^{-} A_1^l - k_1^{+} A_1 L \]  
(2.3)

\[ \frac{dA_1'}{dt} = nk_3^{-} A_n' - nk_3^{+} A_1'^n - k_1^{-} A_1^l + k_1^{+} A_1 L \]  
(2.4)

\[ \frac{dA_n}{dt} = -k_2^{-} A_n + k_2^{+} A_1^n \]  
(2.5)

\[ \frac{dA_n'}{dt} = -k_3^{-} A_n' + k_3^{+} A_1'^n \]  
(2.6)

that are better suited for analysis in non-dimensional form. If we chose the characteristic concentration and time as \( A_0 \) (the equilibrium concentration of monomers) and \( \frac{1}{k_1} \), respectively, then the dimensionless variables can be defined as,

\[ B_1 = \frac{A_1}{A_0}; B_1' = \frac{A_1'}{A_0}; B_n = \frac{A_n}{A_0}; B_n' = \frac{A_n'}{A_0} \]

\[ \alpha_1 = \frac{k_2^{-}}{k_1^{-}}; \alpha_2 = \frac{k_3^{+} A_0^{n-1}}{k_1^{-}}; \alpha_3 = \frac{k_1^+ L}{k_1^{-}}; \beta_1 = \frac{k_3^{-}}{k_1^{-}}; \beta_2 = \frac{k_3^{+} A_0^{n-1}}{k_1^{-}}; \]
(2.7)

the corresponding dimensionless differential equations of which can now be written as,

\[ \frac{dB_1}{ds} = n\alpha_1 B_n - n\alpha_2 B_1^n + B_1' - \alpha_3 B_1 \]  
(2.8)

\[ \frac{dB_1'}{ds} = n\beta_1 B_n' - n\beta_2 B_1'^n + \alpha_3 B_1 - B_1' \]  
(2.9)

\[ \frac{dB_n}{ds} = \alpha_2 B_1^n - \alpha_1 B_n \]  
(2.10)

\[ \frac{dB_n'}{ds} = \beta_2 B_1'^n - \beta_1 B_n' \]  
(2.11)

Figure 2.1: Schematic of the system with backward and forward rates.

2.2 Equilibrium points

In the equations (2.3)-(2.6), if we consider the limit \( (k_1^+, k_1^-, L) \rightarrow (0, 0, 0) \), then the off-pathway dynamics is turned off, leaving only the reaction below.

\[ n \cdot A_1 \xleftrightarrow{k_2^+} A_n \]
However, the on-pathway cannot be switched off and always persists. In this case the equilibrium solution is given by the pair \( (A_{1,e}, (\frac{k^+_2}{k^-_2}A_{1,e})^{1/n}) \). In the more general case of equations (2.8)-(2.11), the equilibrium points, \( B_e = (B_{1,e}, B'_{1,e}, B_{n,e}, B'_{n,e}) \) can be obtained by the vanishing of the equations 2.8-2.11. Solving for these four simultaneous equations, we obtain the following relations, in terms of the equilibrium concentration \( B_{1,e} \):

\[
\begin{align*}
B_{n,e} &= \frac{\alpha_2}{\alpha_1} B^n_{1,e}; \\
B'_{1,e} &= \alpha_3 B_{1,e}; \\
B'_{n,e} &= \frac{\beta_2 \alpha_3^n}{\beta_1} B^n_{1,e}.
\end{align*}
\] (2.12)

The subscript \( e \) indicates that these species are in equilibrium.

### 2.3 Linear Stability Analysis

When formulating a system of differential equations, it’s important to analyze the stability of the system. Specifically, we are interested in the effect upon the system of small changes in initial conditions. Do these changes have a negligible effect on the solutions, or do they result in dramatic changes? The method used to determine this is linear stability analysis. The idea is to perturb the system with a small change \( \epsilon > 0 \) to the initial conditions and then linearize the perturbed system at our equilibrium point to determine the trajectories at that point. The method involves writing the Jacobian matrix of the system evaluated at the fixed, or equilibrium, point. The eigenvalues of the Jacobian are the solutions of the linearized system at this point, those being exponentials whose powers are the eigenvalues. Negative eigenvalues mean that the perturbation decays to zero, or converges to the equilibrium point, while positive eigenvalues mean that the system moves infinitely away from the equilibrium point over time.

There are three general classifications of fixed points for a system of differential equations: a fixed point is **asymptotically stable** if all of the eigenvalues are negative, **unstable** if just one of the eigenvalues are positive, and **neutrally stable** if the eigenvalues are complex with real parts less than or equal to zero. When the eigenvalues all have the same sign, the fixed point is a **node** which can be stable or unstable depending on the sign of the eigenvalues. When the eigenvalues differ in sign, the fixed point is a **saddle point** which is unstable. Complex eigenvalues just add oscillations and are called **spiral points**, which again can be stable or
unstable depending upon the sign of the real part. In the case when there are zero real parts (with none being positive), the fixed point is a center, and is considered neutrally stable: small perturbations neither decay to the equilibrium point nor do they diverge infinitely away from the equilibrium point.

To study the stability of the four species, we linearly perturb the system which is mathematically represented by

\[
B_1 = B_{1,e} + \epsilon X_1 \quad (2.13)
\]

\[
B'_1 = B'_{1,e} + \epsilon Y_1 \quad (2.14)
\]

\[
B_n = B_{n,e} + \epsilon X_n \quad (2.15)
\]

\[
B'_n = B'_{n,e} + \epsilon Y_n \quad (2.16)
\]

then the linearized set of differential equations for the perturbations, at \( o(\epsilon) \) becomes

\[
\frac{dX_1}{dt} = n\alpha_1 X_n - n^2\alpha_2 B_1^{n-1} X_1 + Y_1 - \alpha_3 X_1 \quad (2.18)
\]

\[
\frac{dY_1}{dt} = n\beta_1 Y_n - n^2\beta_2 \alpha_3 B_1^{n-1} Y_1 - Y_1 + \alpha_3 X_1 \quad (2.19)
\]

\[
\frac{dX_n}{dt} = n\alpha_2 B_1^{n-1} X_1 - \alpha_1 X_n \quad (2.20)
\]

\[
\frac{dY_n}{dt} = \beta_2 \alpha_3 B_1^{n-1} Y_1 - \beta_1 Y_n \quad (2.21)
\]

which can be expressed in operator form with the perturbation matrix \( B \) given by

\[
B = \begin{pmatrix}
-n^2\alpha_2 B_1^{n-1} - \alpha_3 & 1 & n\alpha_1 & 0 \\
\alpha_3 & -n^2\beta_2 \alpha_3 B_1^{n-1} - 1 & 0 & n\beta_1 \\
-n\alpha_2 B_1^{n-1} & 0 & -\alpha_1 & 0 \\
0 & \beta_2 \alpha_3 B_1^{n-1} & 0 & -\beta_1
\end{pmatrix}
\]

The matrix \( B \) is the Jacobian which is evaluated at the equilibrium point given by 2.12. The stability of the equilibrium is found from computing the eigenvalues, \( \lambda_i \ (i = 1, 2, 3, 4) \) of the matrix \( B \). This is best done numerically. In the figures below we discuss the results.
of the computations. In particular, the term \( \alpha_3 \) is varied throughout the analysis since it captures a significant dynamical feature of this problem; the switching from on-pathway to off-pathway. Over all, we choose the following ranges for our parameters: \( 0 \leq \alpha_3 \leq 2 \) for \( i = 1, 2 \); \( 2 \leq n \leq 20 \); and \( 10^{-3} \leq \alpha_i, \beta_i \leq 2 \times 10^3 \) for \( i = 1, 2 \). We begin with the base case defined by the choice \( \alpha_1 = \beta_1 = 0.001 \), \( \alpha_2 = \beta_2 = 1 \), \( n = 4 \) and \( B_1 = 1 \) and then study the sensitivity of the system and our results to each of the parameters.

Figure 2.2: The eigenvalues of matrix \( B \) as a function of \( \alpha_3 \). Case (a) shows the switching event at a critical value of \( \alpha_3 \) while panel (b) shows the eigenvalues to the on- and off-pathways.

The figure 2.3 shows a significant event in the dynamics of this system. As \( \alpha_3 \) is increased from zero, the real part of the eigenvalues \( \lambda_i \) are all negative, indicating that the equilibria are stable. When \( \alpha_3 < 1.0 \), we see that \( \lambda_2 > \lambda_1 \). However, when \( \alpha_3 > 1.0 \), \( \lambda_2 < \lambda_1 \) indicating an exchange in the magnitude of the stability. For \( \alpha_3 > 1.0 \), it appears that the species \( B_1 \) become more stable than \( B'_1 \), indicating a transcritical-like bifurcation at \( \alpha_3 = 1.0 \). The switching of stabilities is clear from the meaning of \( \alpha_3 \) which encompasses the ratio of the forward to backward rate constants for this reaction and also contains the concentration of the micelles which enhances the formation of the primed-species. The figure 2.3(b) shows the rate of reformation of the species \( B_n \) and \( B'_n \) which is seen to be a bit slower than the monomeric species. The figure 2.3(a) shows a second switching at around \( \alpha_3 = 1.87 \) beyond which could be indicative of the effect of the strong nonlinearity of the system, which needs to be experimentally verified. However, this could be a mathematical artifact and may

\footnote{We note that the traditional definition of trans-critical bifurcation involves a stable equilibrium and one unstable equilibrium which exchange stabilities.}
Figure 2.3: The eigenvalues of matrix $B$ as a function of $\alpha_3$. Case (a) shows the switching event at a critical value of $\alpha_3$ while panel (b) shows the eigenvalues to the on- and off-pathways.

point to the invalidity of $\alpha_3 > 1.87$. The latter case can be helpful in selecting appropriate, physically meaningful rate constants which remains a significant confounding aspect of this approach.

Next we varied the backward and forward parameters, $\alpha_i, \beta_i$ for $i = 1, 2$ (see figure 2.4). When we increase the backward rates from our Base Case, the model exhibits an increase in stability as represented by the magnitude of the eigenvalues (left image in figure 2.4). Again, however, $\lambda_3$ is a marginal case being just barely in negative territory. When we increase forward rates ($\alpha_2, \beta_2$) $\lambda_1$ and $\lambda_2$ become more negative while $\lambda_3$ and $\lambda_4$ become less so (right image in figure 2.4). Thus, when backward and forward rates are more in line with each other, the model exhibits greater stability.

The effect of $n$ on the system was also explored (for $2 \leq n \leq 20$) and seen to result in some very significant changes to the stability of the system (see figure 2.5). In particular, the eigenvalues $\lambda_1$ and $\lambda_2$ showed dramatic changes as $n$ varies. As $n$ increases, we see an increase in the magnitude of stability of both $\lambda_1$ and $\lambda_2$, however, $\lambda_3$ and $\lambda_4$ exhibit a
switching in stability as \( n \) increases, with \( n = 4 \) being the most stable environment for this parameter, albeit marginally so. Both \( \lambda_3 \) and \( \lambda_4 \) are just barely in negative territory.

Overall, the stability of our model was very sensitive to the rate constants. The Base Case model was seen to be *asymptotically stable*, but marginally so (\( \lambda_3 \) and \( \lambda_4 \)). In other words, perturbations to initial conditions decay but only gradually. At worst, when the forward rates \( \alpha_2 \) and \( \beta_2 \) are increased for instance, the model was *neutrally stable*. The non-linearity of the system and the number of dimensions made analyzing the stability of the system difficult. Perhaps the use of a non-linear method to analyze this problem in the future may bear fruit.

### 2.4 Numerical Results

The system of equations (2.3)-(2.6) were solved using the Matlab ode45 function. A sample solution is shown in Fig. 2.6 corresponding to initial conditions: \( B_1(0) = 1, B_1'(0) = B_n(0) = B_n'(0) = 0, \alpha_1 = \beta_1 = .001, \alpha_2 = \beta_2 = 1 \) and \( n = 4 \).

At equilibrium, equations (2.8)-(2.11) vanish, and (2.10) and (2.11) imply that,

\[
\alpha_2 B_{1,e}^n = \alpha_1 B_{n,e} \quad (2.22)
\]
\[
\beta_2 B_{1,e}^n = \beta_1 B_{n,e}' \quad (2.23)
\]

Substituting (2.22) and (2.23) into equations (2.8) and (2.9) we have \( \alpha_3 B_{1,e} = B_{1,e}' \). Thus, if \( \alpha_3 = 1 \), we have \( B_{1,e} = B_{1,e}' \), and using this in (2.22) and (2.23) means that \( B_{n,e} = B_{n,e}' \).

As can be seen from figure 2.6, at the limit it must be that we have

\[
B_{n,e}^* = \frac{B_{1,e}^n}{\alpha_1} = B_{1,e}^*
\]

where * indicates both on- and off-pathway species. The high forward rates of the Base Case strongly favor formation of \( n \) species, and as the model evolves it will seek equality of species size in spite of the initial “head start” that both on- and off-pathway monomers have. At \( \alpha_3 = 1 \) the system is such that \( B_{1,e} = B_{1,e}' = B_{n,e} = B_{n,e}' \), or the Nash equilibrium.

#### 2.4.1 Nash Equilibrium

Figure 2.7 shows the time evolution of species for the Base Case for different values of \( \alpha_3 \). The horizontal line \( \alpha_3 = 1 \) entirely determines on- or off-pathway species formation over the long term for the Base Case. The yellow portion is where \( B_1 < B_1' \) and \( B_n > B_n' \). For \( \alpha_3 > 1 \)
we do see dominance of $B_n$ over $B'_n$ for a time, but over the long term, both off-pathway species eventually dominate. The second figure shows the same graph, zoomed-in over the time period $t = 3000 - 4000$. For $\alpha_3$ slightly above 1, we continue to see a thin band of yellow, however at $\alpha_3 = 1$, over time we do have equality of all species. Interestingly, we were unable to force $B_1 > B'_1$ and $B_n < B'_n$, which would seem to be a result of not having a direct pathway between $B_1$ and $B'_n$; a limitation of the model as constructed.

If viewed as a “competition” in the vernacular of Game Theory, who are the players, what are the rules, and what are the payoffs? In the case of our model, the “players” are the competing on- and off-pathways, the rules of the game are the reaction rate constants, and the payoffs are the different equilibrium states. In the case of a competition with different strategic options, the Nash equilibrium is the particular set of strategies that the players would adopt regardless of how the competition behaved. In the biophysical context, it is the set of parameters from which neither competing species would deviate if view as sentient “competitors”. For our model, then the equilibrium $B_{e}|_{\alpha_3=1} = B_{Nash,e}$ is nothing but the Nash equilibrium of the system given by

$$B_{Nash,e} := \left( B_{1,e}, B_{1,e}, \frac{\alpha_2}{\alpha_1} B_{1,e}^{n}, \frac{\beta_2}{\beta_1} B_{1,e}^{n} \right)$$

(see figure 2.6 for an example of such an equilibrium). This equilibrium exists only at $\alpha_3 = 1$ which has been seen to have special significance in terms of the stability of the system. It follows that when $\alpha_3 > 1$, then the off-pathway species dominate creating more equilibrium concentrations of $B'_1,e$ and $B'_n,e$, and for $\alpha_3 < 1$, on-pathway dominates resulting in greater production of $B_{1,e}$ and $B_{n,e}$.

We define $k^+ = k_1^+ L$ and $k^- = k_1^-$ so that the former leads to off-pathway and the latter to on-pathway. The table below points to a sample case of the various strategies in the competition between the two pathways with the pay-off for each “strategy” given by the equilibrium concentrations.
Table 2.1: A sample game-theoretical payoff matrix to depict the on- and off-pathway competition.

<table>
<thead>
<tr>
<th>ON-PATHWAY</th>
<th>OFF-PATHWAY</th>
</tr>
</thead>
<tbody>
<tr>
<td>$k^- = 10$</td>
<td>Equality on-pathway</td>
</tr>
<tr>
<td>$k^- = 1$</td>
<td>off-pathway Equality on-pathway</td>
</tr>
<tr>
<td>$k^- = 0.1$</td>
<td>off-pathway off-pathway Equality</td>
</tr>
</tbody>
</table>

Figure 2.4: Eigenvalues as a function of backward rates (first frame) and forward rates (second frame).
Figure 2.5: Eigenvalues as $n$ is varied.

Figure 2.6: Solution of Model with $B_1(0) = 1$, $B'_1(0) = B_n(0) = B'_n(0) = 0$, $\alpha_1 = \beta_1 = .001$, $\alpha_2 = \beta_2 = 1$ and $n = 4$. 
Figure 2.7: Time evolution of the Base Case model as $\alpha_3$ varies. The red region is strictly on-pathway, blue is strictly off-pathway, and yellow is where $B_1 < B'_1$ and $B_n > B'_n$ or mixed on-/off-pathway. Thus there are 2 possible equilibrium states, where a mixed state can persist in the short run. The second graph is a zoomed-in version of the first, showing the persistence of yellow over long periods of time for $\alpha_3$ close to 1.
Chapter 3

Six Species Model

3.1 The Model

We wanted to increase the complexity of our system by increasing the number of species to six. The idea behind increasing the model size was to add another pathway between on- and off-pathway species. In this model, oligomers can react with the fatty acid \( L \) to create off-pathway oligomers. The system of chemical reactions in our model consist of the following,

\[
\begin{align*}
A_1 + L &\quad {k_1^+ \atop k_1^-} \quad A_1' \\

n \cdot A_1 &\quad {k_2^+ \atop k_2^-} \quad A_n \\
n \cdot A_1' &\quad {k_3^+ \atop k_3^-} \quad A_n' \\
A_n + nL &\quad {k_4^+ \atop k_4^-} \quad A_n' \\

\frac{m}{n} \cdot A_n &\quad {k_5^+ \atop k_5^-} \quad A_m \\
\frac{m}{n} \cdot A_n' &\quad {k_6^+ \atop k_6^-} \quad A_m'
\end{align*}
\]

As with the 4 Species Model, the non-prime species, \( A_1, A_n, \) and \( A_m \) represent on-pathway \( A\beta \) monomers and oligomers; whereas the prime species, \( A_1', A_n', \) and \( A_m' \), are the off-pathway species which are created through a reaction with the fatty acid micelles, \( L \). For each species,
Using the Law of Mass Action Kinetics, we formulate the system of differential equations as follows.

\[
\frac{dA_1}{dt} = nk_2 A_n - nk_2^+ A_1^n + k_1^- A_1 - k_1^+ A_1 L \tag{3.1}
\]

\[
\frac{dA_1'}{dt} = nk_3 A_n' - nk_3^+ A_1'^n + k_1'^+ A_1 L - k_1'^- A_1' \tag{3.2}
\]

\[
\frac{dA_n}{dt} = k_2^+ A_1^n + \frac{m}{n} k_5^- A_m + k_4^+ A_n' - k_2^- A_n - k_4^+ A_n L^n - \frac{m}{n} k_5^+ A_n^m \tag{3.3}
\]

\[
\frac{dA_n'}{dt} = k_3^+ A_1'^n + k_4^+ A_n L^n + \frac{m}{n} k_6^- A_m' - k_3^- A_n' - \frac{m}{n} k_6^+ A_n'^m - k_4^- A_n' \tag{3.4}
\]

\[
\frac{dA_m}{dt} = k_5^+ A_m^m - k_5^- A_m \tag{3.5}
\]

\[
\frac{dA_m'}{dt} = k_6^+ A_m'^m - k_6^- A_m' \tag{3.6}
\]

This system is then put into non-dimensional form. We let \( A_0 \) be the characteristic concentration of monomers and \( \frac{1}{k_1} \) be time, and define the dimensionless species as follows:

\[
B_1 = \frac{A_1}{A_0}; B_n = \frac{A_n}{A_0}; B_m = \frac{A_m}{A_0}; B_1' = \frac{A_1'}{A_0}; B_n' = \frac{A_n'}{A_0}; B_m' = \frac{A_m'}{A_0}
\]

The reaction constants are defined as follows:

\[
\alpha_1 = \frac{k_2^-}{k_1^-}; \alpha_2 = \frac{k_2^+ A_2^{n-1}}{k_1^-}; \alpha_3 = \frac{k_1^+ L}{k_1^-}; \alpha_4 = \frac{k_4^+ L^n}{k_1^-}; \alpha_5 = \frac{k_5^-}{k_1^-}; \alpha_6 = \frac{k_6^+ A_6^{m-1}}{k_1^-}
\]

\[
\beta_1 = \frac{k_1^-}{k_1^-}; \beta_2 = \frac{k_1^+ A_2^{n-1}}{k_1^-}; \beta_3 = \frac{k_4^+ A_4^{m-1}}{k_1^-}; \beta_4 = \frac{k_4^-}{k_1^-}; \beta_5 = \frac{k_5^-}{k_1^-}
\]

Note that both \( \alpha_3 \) and \( \alpha_4 \) have a factor \( L \) which is responsible for off-pathway aggregation. These will be the bridge variables between on- and off-pathway species in the analysis which
follows. The dimensionless system is

\[
\frac{dB_1}{ds} = n\alpha_1 B_n - n\alpha_2 B_1^n + B'_1 - \alpha_3 B_1 \quad (3.7)
\]

\[
\frac{dB'_1}{ds} = n\beta_1 B'_n - n\beta_2 B'_1^n + \alpha_3 B_1 - B'_1 \quad (3.8)
\]

\[
\frac{dB_n}{ds} = \alpha_2 B_1^n - \alpha_1 B_n + \frac{m}{n} \alpha_5 B_m + \beta_4 B'_n - \alpha_4 B_n - \frac{m}{n} \beta_3 B_n^n \quad (3.9)
\]

\[
\frac{dB'_n}{ds} = \beta_2 B'_1^n - \beta_1 B'_n + \alpha_4 B_n + \frac{m}{n} \beta_5 B'_m - \frac{m}{n} \alpha_6 B'_n^n - \beta_4 B'_n \quad (3.10)
\]

\[
\frac{dB_m}{ds} = \beta_3 B_n^n - \alpha_5 B_m \quad (3.11)
\]

\[
\frac{dB'_m}{ds} = \alpha_6 B'_n^n - \beta_5 B'_m \quad (3.12)
\]

Figure 3.1: Schematic of System with forward and backward rates.

Below is a table of the non-dimensional reaction variables that we will be referring to in our work. Reaction variables between on- and off-pathway species will be referred to as “bridge” variables.

<table>
<thead>
<tr>
<th>On/Off Pathway Reaction Rate Variables</th>
<th>Forward</th>
<th>Backward</th>
</tr>
</thead>
<tbody>
<tr>
<td>On-Pathway</td>
<td>(\alpha_2)</td>
<td>(\beta_3)</td>
</tr>
<tr>
<td>Off-Pathway</td>
<td>(\beta_2)</td>
<td>(\alpha_6)</td>
</tr>
<tr>
<td>Bridge</td>
<td>(\alpha_3)</td>
<td>(\alpha_4)</td>
</tr>
</tbody>
</table>

Table 3.1: Table of reaction rates between species

Throughout this part of our work, we will primarily be analyzing two models which will be referred to as the Base Model and Model 2. Our choices of the parameters in these two
models are guided by prior work with our collaborators in the belief that they capture the essential dynamics of the system, without loss of generality. In our Base Model, we set all forward rates and bridge rates equal to 1 and all backward rates to .001, as previous mathematical models and experimental data have indicated [43]. In the context of our model, a forward rate is defined as one that converts a smaller molecular structure into a larger structure, and backward being the reverse process. In Model 2, we set all forward and backward rates equal. We used Matlab’s \textit{ode 45} for our numerical solutions.

3.2 Equilibrium Points

In order to study the stability of the system we have had to rely on experimental data to determine our rate constants which in turn determine our equilibrium, or fixed point, concentration levels [43]. With the Four Species Model, we were able to analytically solve for the equilibrium concentration sizes by simultaneously solving the system of ODEs in terms of a single species. Because the Six Species model is so highly interdependent, we were unable to use this approach. The presence of two bridge reactions between on- and off-pathway meant that each species was dependent on two other species in a circular fashion.

Given the above discussion, our first problem was how to determine the equilibrium points for the system, with the stipulation that all species sizes be greater than zero. Equilibria where any species size is zero would be of little interest in the context of our problem. Because our system is non-linear and 6 dimensional, there are many possible fixed points. We weren’t interested in classifying all of these points, but only those corresponding to our models.

In general, equilibrium for our model is the set of points that would make (3.7)-(3.12) vanish for a given set of parameter values. We knew from the numerical solutions of the model that the system appeared to settle at equilibrium, or more accurately, that each species exhibited asymptotic behavior. In the absence of being able to solve our system, we wanted to approximate a limiting value for each species. The non-analytical analog of this would be where the change in concentration size for each species gets reasonably close to zero as \( t \rightarrow \infty \), knowing that absolute zero would most likely be unachievable due in part to numerical error. One solution to this problem would be to just use very large values of \( t \)
and take the ending concentration levels as our equilibrium points. The necessity of running a large number of simulations under different rate environments, however, meant that we needed to limit how big \( t \) could be.

To solve the problem of having to limit computation time, we ran both models through a code that would identify the time \( T \) when the change in all species sizes was less than 0.1% of the previous level using time increments of 5. For instance, we computed the change in concentrations sizes for \( t = 0 : 5, t = 0 : 10 \), etc. This would be a proxy for solving our system for 0, and the concentration levels at time \( T \) would be our equilibrium concentration sizes. As it turns out, concentration sizes continue to fluctuate over time for both models, but in all cases these fluctuations were within 0.1% of previous levels for \( t > T \), which we considered acceptable. To confirm our equilibrium values, we ran the results through Matlab’s \textit{fsolve} as will be discussed shortly. In the following simulations we set initial conditions to be \( B_1 = 1 \) with all other species at 0. Below are the graphs of the numerical solutions of the Base Model and Model 2.

As can be seen from the graphs in figure 3.2, as time increases the concentration levels exhibit asymptotic behavior and equilibrium appears to be achieved. We found, however, that the “look” of the graph was deceptive. Because the absolute size of the concentration of \( B_m \) and \( B'_m \) were so small, the fluctuations weren’t showing up on the solution graphs. Figure 3.3 shows graphs of the Base Model and Model 2 showing the percentage change in concentration sizes as a function of time using representative species. \( B_m \) is shown as that is one of the species that tended to have greater fluctuations over time.

It’s clear from 3.3 that there continues to be relatively large percent changes in concentration levels in the Base Model while the concentration levels in Model 2 “settle” down more quickly. As our working definition of equilibrium, we therefore wanted to see the absolute percentage change in concentration of any species to be no greater than 0.1% over a period of 5 units of time. As it turned out, in order to achieve our definition of equilibrium for the Base model, we needed to use a time span of \( t = 0 : 2200 \), whereas for Model 2 we needed a time span of just \( t = 0 : 20 \). We had therefore found that the type of rate regime used has a dramatic effect on the time it takes for the system to reach equilibrium. Figure 3.4 is a graph of the Base Case model with \( t = 0 : 2200 \), and a zoomed in concentration size window.
While it appears that it takes longer for $B_1$, $B'_1$, $B_n$, and $B'_n$ to settle, it is in fact $B_m$ and $B'_m$ (in green) that continue to grow until our definition of equilibrium is achieved at $t = 2200$. To confirm our hypothesis, we ran the model through Matlab’s numerical non-linear system solver, \textit{fsolve}. As our initial “guess”, we used the final concentration size at $t = 2200$ and with just 1 iteration, \textit{fsolve} returned an equilibrium value for each species that was within 2% of our guess. By increasing the time span to 2500, we were able to get this difference under 1%. We considered these to be reasonably accurate equilibrium values for our Base Case model with which to work. Below are plots of the percentage change of $B_1$ and $B'_m$ over different timespans. $B'_m$ continues to grow slowly as $B_1$ fluctuates over $t = 350 - 450$. In the second plot, $B'_m$ is approximately at 0% change while $B_1$ continues fluctuating.

Under the Model 2 regime shown below, equilibrium is achieved more quickly. Using a time span of just $t = 0 : 20$, and taking the ending concentration sizes as an initial guess,
Figure 3.3: Absolute percentage change of the concentration of $B_1$ and $B_m$ as a function of time over identical time spans. Model 2 achieves equilibrium more quickly.

$f\text{solve}$ returned a value that was at worst, 0.08% different from our equilibrium value. The difference in time it takes to achieve equilibrium seems to be a function of the ratio of backward to forward rates: the closer that ratio is to 1, the more quickly the system reaches equilibrium. We ran a power regression of the data and found that the time to equilibrium is $TE \approx 25r^{-0.615}$ (figure 3.6 and table) where $r$ is the ratio of backward to forward rates. Intuitively speaking, the direction that has a much smaller reaction rate will tend to become overwhelmed until that species has a critical concentration mass where, despite the lower reaction rate, it’s greater relative concentration size will “push back”. This will create a cycle of over-shoot that will take longer to dissipate. Interestingly, in the case of Model 2 as our definition of equilibrium is achieved it is $B'_m$ that has the greater fluctuations while $B_1$ is more stable (see figure 3.7), the opposite of the Base Model. It would appear that increasing backward rates, while enabling the model to settle to equilibrium more quickly
Figure 3.4: Solution of the System (Base Case) with $t = 0 : 2200$ and zoomed in concentration size. Note the slow but continued growth of $B_m$ and $B'_m$.

Figure 3.5: Plots showing percentage change of $B_1$ and $B'_m$ over different timespans for the Base Model.

does increase fluctuations in the concentration size of $n$ and $m$ species relative to monomers.

As increasing backward rates from the Base Model to Model 2 had greatly reduced the time it took to achieve equilibrium, it made sense to look at a regime where backward rates are greater than forward rates. While not necessarily physically meaningful in the context of our problem, for comparisons sake, we wanted to know the time horizon over which equilibrium was achieved. For this model, we first set forward rates to be 0.01, and then 0.001, with backward rates equal to 1 in both cases. In this scenario, the model did not settle down to our definition of equilibrium even over a time span of $t = 0 : 5000$. In fact, large (percentage-wise) fluctuations in the concentration of $B'_m$ became greater over time. Figure 3.8 is the graph of this simulation. Note that the scale of the graph is $\pm 150\%$.  

31
<table>
<thead>
<tr>
<th>Backward/Forward Ratio</th>
<th>Equilibrium Time</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.0001</td>
<td>5,190</td>
</tr>
<tr>
<td>0.001</td>
<td>2,200</td>
</tr>
<tr>
<td>0.01</td>
<td>595</td>
</tr>
<tr>
<td>0.1</td>
<td>105</td>
</tr>
<tr>
<td>1</td>
<td>20</td>
</tr>
</tbody>
</table>

Table 3.2: Time to equilibrium as a function of the ratio of backward to forward rates.

Due to low forward rates, the concentration size of $B_1$ stays high and stable throughout, but there are periodic, large percentage changes in the size of $B'_m$. We analyzed the concentration pattern of both $B_1$ and $B'_m$ over this period and their growth and decline patterns are a mirror image of each other: periods of increase in $B_1$ were accompanied by declines in $B'_m$. Because the relative concentration sizes are so different, percentage-wise changes in $B_1$ were on a much smaller scale, as can be seen from the graph.

From our equilibrium analysis of these three models we found when the ratio of backward to forward rates is close to 1, the model settles at equilibrium more quickly than when there are large differences in the magnitude of backward and forward rates. Also, the higher we put backward rates the more volatility in the concentration size of $n$ and $m$ species relative
Figure 3.7: Fluctuations in the size of $B_1$ and $B'_m$ under Model 2 conditions, after equilibrium is achieved.

to monomers, whereas higher forward rates had the opposite effect.

Below is a table of the analysis of equilibrium.

<table>
<thead>
<tr>
<th>Analysis of Equilibrium</th>
<th>Base Model</th>
<th>Model 2</th>
<th>High Backward</th>
</tr>
</thead>
<tbody>
<tr>
<td>Time $T$ to Equilibrium</td>
<td>$T = 2200$</td>
<td>$T = 20$</td>
<td>-</td>
</tr>
<tr>
<td>Max. Difference Model/fsolve</td>
<td>1.7%</td>
<td>0.08%</td>
<td>-</td>
</tr>
</tbody>
</table>

Table 3.3: Time to equilibrium of the Base Model and Model 2 versus $fsolve$.

We also looked at both models with various sizes of oligomers by varying $n$ and $m$. In both cases, increasing $n$ and $m$ had the effect of increasing the time it took for each model to reach equilibrium. Below is a table of results. It could be that the higher the power of the non-linear terms, the greater the potential for over- and under-shoot as the model evolves over time, thus taking longer to achieve equilibrium.
Figure 3.8: Percentage change in concentration of $B_1$ and $B'_m$ with high backward rates.

<table>
<thead>
<tr>
<th>Oligomer Size</th>
<th>Base Model</th>
<th>Model 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>$n = 2, m = 10$</td>
<td>780</td>
<td>25</td>
</tr>
<tr>
<td>$n = 4, m = 20$</td>
<td>2,200</td>
<td>20</td>
</tr>
<tr>
<td>$n = 6, m = 30$</td>
<td>3,650</td>
<td>30</td>
</tr>
<tr>
<td>$n = 8, m = 40$</td>
<td>4,020</td>
<td>35</td>
</tr>
</tbody>
</table>

Table 3.4: Table of time to equilibrium values as $n$ and $m$ are varied.

3.3 Linear Stability Analysis

Like the 4 species model, we linearize the dimensionless system as shown below.

\[
B_1 = B_{1,e} + \epsilon X_1
\]  
\[
B'_1 = B'_{1,e} + \epsilon Y_1
\]  
\[
B_n = B_{n,e} + \epsilon X_n
\]  
\[
B'_n = B'_{n,e} + \epsilon Y_n
\]  
\[
B_m = B_{m,e} + \epsilon X_m
\]  
\[
B'_m = B'_{m,e} + \epsilon Y_m
\]
Here, for instance, $B_{1,e}$ is the equilibrium concentration of the species $B_1$, and $\epsilon X_1$ is the perturbation. For our equilibrium concentrations, we used the values derived in the previous section.

We substitute these values into equations (3.7)-(3.12) and keep only the linear terms of order $\epsilon$. The linearized system is

$$
\frac{dX_1}{ds} = n\alpha_1 X_n - n^2\alpha_2 B_{1,e}^{n-1} X_1 + Y_1 - \alpha_3 X_1 \quad (3.19)
$$

$$
\frac{dY_1}{ds} = n\beta_1 Y_n - n^2\beta_2 B_{1,e}' Y_1 + \alpha_3 X_1 - Y_1 \quad (3.20)
$$

$$
\frac{dX_n}{ds} = n\alpha_2 B_{1,e}^{n-1} X_1 - \alpha_1 X_n + \frac{m}{n}\alpha_5 X_m + \beta_4 Y_n - \alpha_4 n X_n = (\frac{m}{n})^2\beta_3 B_{n,e}^{m+1} X_n \quad (3.21)
$$

$$
\frac{dY_n}{ds} = \beta_2 B_{1,e}^{n-1} - \beta_1 Y_n + n\alpha_4 X_n + \frac{m}{n}\beta_5 Y_m - (\frac{m}{n})^2\alpha_6 B_{n,e}' \frac{m}{n} Y_n - \beta_4 Y_n \quad (3.22)
$$

$$
\frac{dX_m}{ds} = \frac{m}{n}\beta_3 B_{n,e}^{m+1} X_n - \alpha_5 X_m \quad (3.23)
$$

$$
\frac{dY_m}{ds} = \frac{m}{n}\alpha_6 B_{n,e}' \frac{m}{n} Y_n - \beta_5 Y_m \quad (3.24)
$$

Which is then put into operator matrix form using the appropriate rate constants and equilibrium values found in the previous section.

$$
B = \begin{pmatrix}
-n^2\alpha_2 B_{1,e}^{n-1} + \alpha_3 & 1 & n\alpha_1 & 0 & 0 & 0 \\
\alpha_3 & -n^2\beta_2 B_{1,e}^{n-1} + 1 & 0 & n\beta_1 & 0 & 0 \\
n\alpha_2 B_{1,e}^{n-1} & 0 & -(\alpha_1 + \alpha_4 + \frac{m}{n}\beta_3 B_{n,e}^{m+1}) & \beta_4 & \frac{m}{n}\alpha_5 & 0 \\
0 & n\beta_2 B_{1,e}^{n-1} & \alpha_4 & -(\beta_1 + \frac{m}{n}\alpha_6 B_{n,e}' \frac{m}{n}) + \beta_4 & 0 & \frac{m}{n}\beta_5 \\
0 & 0 & \frac{m}{n}\beta_3 B_{n,e}^{m+1} & 0 & -\alpha_5 & 0 \\
0 & 0 & 0 & \frac{m}{n}\alpha_6 B_{n,e}' \frac{m}{n} & 0 & -\beta_5
\end{pmatrix}
$$

We then compute the eigenvalues of $B$, the Jacobian, which is shown in the table below. Because we were not able to determine equilibrium for the high backward rate regime, we have excluded it from the stability analysis. Notice that all of the eigenvalues are negative with the exception of $\lambda_5 = 0$ for the Base Model and $\lambda_2 = 0$ for Model 2.

<table>
<thead>
<tr>
<th>Eigenvalues of Base Model and Model 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\lambda_1$</td>
</tr>
<tr>
<td>Base Model</td>
</tr>
<tr>
<td>Model 2</td>
</tr>
</tbody>
</table>

Table 3.5: Eigenvalues of the Base Model and Model 2.

Below are plots of the Base Model $\lambda_5$ as a function of both $\alpha_3$ and $\alpha_4$. 

35
Figure 3.9: The first figure is $\lambda_5$ as a function of $\alpha_3$ and the second graph is $\lambda_5$ as a function of $\alpha_4$. Note that the scale is $10^{-16}$.

$\lambda_5$ is zero out to the 15th decimal place. In the case of Model 2, $\lambda_2$ was on a similar scale. $\lambda_5$ is fluctuating between $\pm 2.5 \times 10^{-16}$, depending on parameter values, or effectively zero if we make allowance for round-off error in the numerics. This would mean that neither model is asymptotically stable, but rather neutrally stable. While perturbations of initial conditions won’t blow up, nor will they decay to equilibrium.

As the rate regime had a significant impact on time to equilibrium, so too did it impact stability. We therefore ran simulations of each model while varying the rate regime. Of key interest was the effect of the bridge parameters, $\alpha_3$ and $\alpha_4$, and their effect on the stability
of each model. Figures 3.10 and 3.11 are contour plots of the eigenvalues of the Base Model and Model 2 with $0 \leq \alpha_3, \alpha_4 \leq 2$. We incremented the two parameters by 0.1.

The stability picture of each system is significantly different. In the Base Model, $\lambda_5$ is zero for all values of $\alpha_3$ and $\alpha_4$, while for Model 2, there was some switching between $\lambda_2$ and $\lambda_4$, as seen in the upper left hand corner of the contour plots for these two eigenvalues in figure 3.11.

We ran simulations for other reaction rate regimes and found that over a range of values for $\alpha_3$ and $\alpha_4$, there was consistent switching of eigenvalues. Figure 3.12 shows Model 2 with forward rates doubled. Note the switching behavior between $\lambda_2$, $\lambda_3$, and $\lambda_4$. 
Figure 3.11: $\lambda_i$ as a function of $\alpha_3$ and $\alpha_4$.

Switching of Eigenvalues

Figure 3.12: Switching Behavior of $\lambda_2$, $\lambda_3$ and $\lambda_4$ with forward rates doubled.

Figure 3.12 is a good example of the switching behavior we observed as we varied reaction rates. Note how $\lambda_3 = 0$ for low $\alpha_3, \alpha_4$, and that as we increase these two parameters, $\lambda_2$ is zero and then finally $\lambda_4$ is zero. This was the case for all rate environments that we ran, albeit not always as pronounced. Thus, for any rate environment, the stability of the system
near the point of equilibrium was neutrally stable.

We then ran contour plots varying $n$ and $m$ which are shown below. We ran two cases for each model: $n = 2$, $m = 4$, and $n = 8$, $m = 40$. These would represent oligomer sizes both less than and greater than our standard designation for $n$ and $m$ of 4 and 20 respectively.

Figure 3.13: Switching of eigenvalues in Base Model as $n$ and $m$ are varied. The white region in both plots indicates a zero value for the eigenvalue for any value of the parameters.
In the case of the Base Model, there was no switching of eigenvalues for a given species size environment as $\alpha_3$ and $\alpha_4$ are varied. For the Base Model, $\lambda_5$ is zero for all values of $\alpha_3$ and $\alpha_4$ when $n = 2$ and $m = 4$, and when $n = 8$ and $m = 40$, $\lambda_1$ is zero. For Model 2, there is switching between $\lambda_2$, $\lambda_3$, and $\lambda_4$ when $n = 2$ and $m = 4$, whereas when $n = 8$ and $m = 40$, there is switching between $\lambda_1$, $\lambda_2$ and $\lambda_3$. In general, as with the Four Species Model, as $n$ and $m$ are increased, the overall magnitude of stability increases.
Finally, we then looked to analyze the effect on $\lambda_1$ and $\lambda_2$ of varying the bridge variables $\alpha_3$ and $\alpha_4$. We observed a pattern of switching and crossing of the eigenvalues as the two bridge parameters are varied. Below is a graph of where we have set $\alpha_4 = 2$ and $0 \leq \alpha_3 \leq 4$. Note the switching at $\alpha_4 = 2$ and $\alpha_3 \approx 0.9$ and crossing at $\alpha_3 = \alpha_4 = 2$. The table below shows switching and crossing points for $\lambda_1$ and $\lambda_2$ as $\alpha_3$ and $\alpha_4$ vary.

<table>
<thead>
<tr>
<th>$\alpha_4$</th>
<th>$\alpha_3$</th>
<th>$\alpha_3$</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>0.9</td>
<td>2</td>
</tr>
<tr>
<td>4</td>
<td>1.525</td>
<td>4</td>
</tr>
<tr>
<td>8</td>
<td>2.35</td>
<td>8</td>
</tr>
<tr>
<td>16</td>
<td>4.05</td>
<td>16</td>
</tr>
<tr>
<td>32</td>
<td>6.65</td>
<td>32</td>
</tr>
<tr>
<td>64</td>
<td>8.05</td>
<td>64</td>
</tr>
</tbody>
</table>

Table 3.6: Switch and cross points of $\alpha_3$ as a function of $\alpha_4$.

As can be seen, there is crossing where $\alpha_3 = \alpha_4$, whereas switching has an exponential relationship between the two parameters. We ran a regression model of the data and found that $\alpha_4 \approx 2.04 \times 1.55^{\alpha_3}$ modeled the switch points with a $r^2 = 0.953$.

We have found that varying reaction rates and oligomer size affected the stability of
the model. As with the 4 Species Model, given the non-linear nature and the number of dimensions, analyzing the stability of the model has proved difficult. We were unable to employ standard methods such as using phase plots and determining bifurcation points that are used with two dimensional models. Perhaps our analysis of equilibrium, which has shown that certain rate regimes result in the model not being able to reach equilibrium, explains the borderline nature of the stability of the model. Using just linear stability analysis, however, we have shown that over a range of parameter values the model is neutrally stable.

3.4 Numerical Results

The key parameters in our model are \( \alpha_3 \) and \( \alpha_4 \) as they are the bridge variables governing the reaction between on- and off-pathway. We therefore wanted to see the effect of varying both on species formation while holding all other reaction rates constant. Figure 3.16 shows the species concentrations as a function of time for the Base Model. For the purposes of this topic, we are showing a shorter time span, our previous discussion of equilibrium notwithstanding: the essential look of the graph to the naked eye is indistinguishable.

![Base Model Solution](image)

Figure 3.16: Concentration levels of Base Model.
The first graph in figure 3.16 shows the solution of the system for the Base Model, and the second shows the solution of the model with increased $\alpha_3$ and $\alpha_4$. Under the Base Model reaction rate regime, the prime and non-prime species are approximately equal, whereas when we increase the bridge variables we see an outperformance of all prime species as would be expected.

Figure 3.17: Concentration levels of Base Model with $\alpha_3$, $\alpha_4 = 10$. 
Figure 3.18: Concentration of $B'_1$ and $B'_n$ as $\alpha_3$ and $\alpha_4$ are varied.

Figure 3.18 shows the solution with just $B'_1$ and $B'_n$ as we vary $\alpha_3$ and $\alpha_4$. Note that as we increase both $\alpha_3$ and $\alpha_4$, we get an initial spike in the concentration of $B'_1$ before settling back to equilibrium, whereas $B'_n$ exhibited no such initial spike. This reflects the fact that over time, the concentration of $B'_1$ is reduced in the forward reaction $B'_1 \rightarrow B'_n$. Figure 3.19 shows just this part of the system.

Figure 3.19: Progression of Species Concentrations. Over time, the spike in concentration of $B'_1$ is reduced as monomers of that species aggregate into $B'_n$.

We now look at the relationship between $\alpha_3$ and $\alpha_4$, the bridge rates, and the ratio of
off-pathway to on-pathway species concentrations. When we increase both \( \alpha_3 \) and \( \alpha_4 \), we see a direct increase in the ratio of off-pathway species to on-pathway species. The relationship is linear and can be written as: \( \frac{B'_1}{B_1} = \alpha_3 \), and \( \frac{B'_n}{B_n} = \alpha_4 \). Because \( \frac{B'_m}{B_m} \) is not directly governed by the bridge variables, it is slower to react to an increase, but eventually exhibits what appears to be exponential or power growth at higher values of the bridge variables. This is most likely due to the fact that \( B'_m \) formation is dependent upon \( \alpha_3 \) and \( \alpha_4 \), so that increasing both eventually impacts \( B'_m \) in a greater manner.

Figures 3.20 and 3.21 underscore the importance of the bridge variables. If we leave \( \alpha_4 \) unchanged and just increase \( \alpha_3 \), there is limited flow through from \( B'_1 \) to \( B'_n \) and \( B'_m \); their ratios to the non-prime species increase slightly above 1, but cease to grow from there even as \( \alpha_3 \) continues to increase. Therefore, the bridge reaction \( B_n \rightleftharpoons B'_n \) is critical in the formation of \( n \) and \( m \) species. For our model, oligomer to oligomer reactions are more significant than monomer to monomer in terms of overall aggregation.

Figure 3.20: Concentration ratios as \( \lambda_3 \) and \( \lambda_4 \) vary.
We then wanted to test the model to make sure that we could force any species to outperform by varying the rate constants. Forcing $B_1$ to outperform, for instance, is just a matter of greatly reducing or shutting off all the forward reactions, and therefore easily done. For species further down the reaction “chain”, we are required to increase forward reactions to get the desired outperformance. In the case of $B'_m$, we were able to get outperformance of this species in absolute terms by dramatically increasing the forward reaction rates $\alpha_3$, $\beta_2$, and $\alpha_6$ (see figure 3.22). Putting these reaction rates at $5 \times 10^4$ we were able to achieve this. Outperformance by $B_m$ was achieved in a similar manner. Doing this has little physical relevance, but does demonstrate the ability of the model to force a particular species to outperform in absolute terms.
Figure 3.22: By increasing the forward reaction rates in the direction of $B'_m$, we were able to get $B'_m$ to outperform all other species on an absolute basis.

3.4.1 Nash Equilibrium of the Six Species Model

As with the Four Species Model, we wanted to look at our model from a game theoretic point of view. Below are schematics of the 4 equilibrium states that our model can achieve.

Figure 3.23: The four equilibrium states of the system. Clockwise from top left, the first figure has all on-pathway species outperforming off-pathway. The second figure has all off-pathway species outperforming on-pathway, and the remaining two are mixed on-/off-pathway.

From top-left, clockwise, the first schematic highlighted in red is strictly on-pathway,
with non-prime species “winning”. The next highlighted in blue is strictly off-pathway, with all prime species winning. Yellow and green are a mixture of on/off-pathway.

Using our Base Model reaction rates, we ran simulations varying $\alpha_3$ and $\alpha_4$ to determine what values of those parameters would cause the different configurations of species dominance. For the simulation below we varied $\alpha_3$ and $\alpha_4$ between 0 and 2 in increments of .02 which resulted in 10,000 discrete points. Figure 3.25 has $0 \leq \alpha_3, \alpha_4 \leq 10$.

Figure 3.24: Aggregation pathways as a function of $\alpha_3$ and $\alpha_4$ with step size of .02. Colors correspond to the equilibrium states from Figure 3.22.

Figure 3.25: Aggregation pathways as a function of $\alpha_3$ and $\alpha_4$. The blue region continues to “win” as $\alpha_3$ and $\alpha_4$ increase without limit.

For the Base Model, the point $(\alpha_3, \alpha_4) = (1, 1)$ is critical. At $(1, 1)$, the concentration
of on- and off-pathway species are equal. As we vary $\alpha_3$ and $\alpha_4$ from this point we begin to see dominance of one species type over another. Notable too is the fact that the boundaries between the different equilibrium states are so linear: the line $\alpha_4 = 1$ determines the switching between on- and off-pathway dominance of $n$ and $m$ species, and the line $\alpha_3 = 1$ determines the switching between on- and off-pathway dominance of monomers.

Table 3.7 shows the equilibrium states as a function of $\alpha_3$ and $\alpha_4$ in the form of a payoff matrix. The Nash Equilibrium, in boldface, is where on-pathway species concentrations are equal to off-pathway species concentrations.

Next, we prove that the point $\alpha_3 = \alpha_4 = 1$ is the Nash Equilibrium of both the Base Model and Model 2.

**Theorem 1.** The Nash Equilibrium of the Base Model and Model 2 occur at the point $\alpha_3 = \alpha_4 = 1$.

**Proof.** In both the Base Model and Model 2, we set all non-bridge forward rates equal to each other, and all backward rates equal to each other. We let $\delta$ be forward rates and $\mu$ be backward rates, and assume that the system is in equilibrium. We then simultaneously solve equations (3.7)-(3.12) for zero. Equations (3.11) and (3.12) can be rewritten as

$$\delta B^m_n = \mu B_m$$

(3.25)

$$\delta B'_n^m = \mu B'_m$$

(3.26)

Substituting these expressions into equations (3.09) and (3.10) and then simplifying, we

<table>
<thead>
<tr>
<th>Payoff Matrix</th>
<th>$\alpha_3 &lt; 1$</th>
<th>$\alpha_3 = 1$</th>
<th>$\alpha_3 &gt; 1$</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\alpha_4 &lt; 1$</td>
<td>On</td>
<td>Mixed</td>
<td>Mixed</td>
</tr>
<tr>
<td>$\alpha_4 = 1$</td>
<td>Mixed</td>
<td>Off</td>
<td>Mixed</td>
</tr>
<tr>
<td>$\alpha_4 &gt; 1$</td>
<td>Mixed</td>
<td>Mixed</td>
<td>Off</td>
</tr>
</tbody>
</table>
rewrite the original system (3.7)-(3.12) as

\begin{align}
& n\mu B_n + B'_1 - n\delta B'^n_1 - \alpha_3 B_1 = 0 \quad (3.27) \\
& n\mu B'_n + \alpha_3 B_1 - n\delta B'^n_1 - B'_1 = 0 \quad (3.28) \\
& \delta B'^n_1 + \delta B'_n - \mu B_n - \alpha_4 B_n = 0 \quad (3.29) \\
& \delta B'^n_1 + \alpha_4 B_n - \mu B'_n - \delta B'_n = 0 \quad (3.30) \\
& \delta B'^n_m - \mu B_m = 0 \quad (3.31) \\
& \delta B'^n_n - \mu B'_n = 0 \quad (3.32)
\end{align}

We then solve (3.27) and (3.28) for $B'_1$ and find that

$$
\delta B'^n_1 - \mu B_n = \mu B'_n - \delta B'^n_1 \quad (3.33)
$$

Let $\delta B'^n_1 - \mu B_n = \mu B'_n - \delta B'^n_1 = \gamma$. If $\gamma = 0$, equations (3.27)/(3.28) simplify to $\alpha_3 B_1 = B'_1$ and $\alpha_4 B_n = B'_n$, and therefore equality of off-pathway and on-pathway species when $\alpha_3 = \alpha_4 = 1$. Assume, on the contrary, that $\gamma \neq 0$, and that w.l.o.g., $\gamma > 0$. If we let $\alpha_3 = \alpha_4 = \delta = 1$, this implies that

$$
B'_1 = B_1 + n\gamma \quad (3.34) \\
B_n = B'_n + \gamma \quad (3.35)
$$

which means that $B'_1 > B_1$ and $B_n > B'_n$. But $\gamma > 0$ and equation (3.33) mean that

$$
\frac{\delta}{\mu} B'^n_1 > B_n \quad \text{and} \quad B'_n > \frac{\delta}{\mu} B'^n_1,
$$

which by transitivity further implies that $B'^n_1 > B'^n_1$. This, however, contradicts our earlier assertion that $B'_1 > B_1$. Thus, $\gamma = 0$, and when $\alpha_3 = \alpha_4 = 1$, both models are at Nash Equilibrium with the concentration of off-pathway species equal to on-pathway species.

It’s notable that unlike with the Four Species Model, we do not get equality of all species types with one another at the point $\alpha_3 = \alpha_4 = 1$. There is undoubtedly at least one unique set of parameters that would get equality of all 6 species, but finding that would be difficult to say the least. As shown with forcing the outperformance of $B'_m$, we would need to increase forward rates dramatically to get equality of species.

We then ran the same code for Model 2, which is the graph below.
Figure 3.26: Aggregation pathways as a function of $\alpha_3$ and $\alpha_4$ with backward and forward rates equal.

Once again, the point $(\alpha_3, \alpha_4) = (1, 1)$ is critical, however, unlike with the Base Model case it doesn’t strictly define outperformance of $B_1$ over $B'_1$ and vice versa: we still see $B_1$ outperform $B'_1$ for $\alpha_3 > 1$ and low $\alpha_4$, and $B'_1$ outperform $B_1$ for $\alpha_3 < 1$ and high $\alpha_4$. The major difference is that the Red and Blue regions (strictly on-pathway and strictly off-pathway respectively) increase at the expense of Green and Yellow (Mixed). More importantly, the range of points $(\alpha_3, \alpha_4)$ over which on-pathway wins, gets bigger when backward rates are at parity with forward rates. For $0 \leq \alpha_3 < 1$, by increasing $\beta_1$ and $\beta_5$ we are effectively shutting off the $B_1 \rightarrow B'_1$ bridge towards off-pathway aggregation, hence the increase in red in the upper left of the graph in 3.26. The reverse happens in the lower right for $0 \leq \alpha_4 < 1$ as we get greater off-pathway aggregation up to a point. Despite increasing $\alpha_3$ above 2, however, if we reduce $\alpha_4$ we continue to see a thin band of dominance (see Fig. 3.27) of $B_n$ and $B'_m$ over their primes. Once again, this shows that the $B_n \rightarrow B'_n$ bridge is more critical for off-pathway aggregation of $n$ and $m$ species than $B_1 \rightarrow B'_1$. Figure 3.27 shows the lower right corner of 3.26 with higher values of $\alpha_3$, showing that if we reduce $\alpha_4$ we still get on-pathway dominance of $n$ and $m$ species even for high $\alpha_3$. Thus, it is difficult to force off-pathway aggregation of $n$ and $m$ species by just forcing over the $B_1 \rightarrow B'_1$ bridge.
Figure 3.27: Dominance of on-pathway \( n \) and \( m \) species for low \( \alpha_4 \) and high \( \alpha_3 \).

Figure 3.28: Species formation by iteration when successively adding one unit of \( B_1 \) at each iteration. Note that with the exception of \( B_m \) and \( B'_m \) in the Base Model, prime and non-prime species are right on top of each other.
Lastly, we wanted to look at our model in a more dynamic way. We decided to continuously add new \( B_1 \) to the system and then watch how each model evolved (figure 3.28). For both models, the amount of on-pathway and off-pathway species was virtually equal (for the Base Model, \( B_m \) does outperform \( B'_m \)). Also, the amount of \( B_m \) and \( B'_m \) eventually outstrips all other species, followed by the \( n \) species. In the case of Model 2, the outperformance of the \( m \) species takes longer to happen, which is the result of backward rates being much higher than those for the Base Model. It seems that both models favor the formation of oligomers over monomers.

### 3.5 Fluxes and Forces

Based on the system of equations corresponding to the 6-species aggregation model, we can compute certain thermodynamic quantities such as fluxes, forces and entropy production for the different pathways that we have identified. The pathways \( (P_i, i = 1, 2, 3, 4) \) are equations 3.36-3.39, where \( P_{42}^{(-)} \) refers to the reverse of \( P_{42} \). The fluxes corresponding to these pathways is given by \( J_i = R^f_i - R^b_i \) \( (i = 1, 2, 3, 4) \) where \( R^f \) refers to the forward reaction flux and \( R^b \) is the backward reaction flux. As a result the individual fluxes are given by equations 3.40-3.43.

![Figure 3.29: A schematic of the reaction pathways.](image-url)
On-Pathway  \( P_1 = P_{11} \cup P_{12} \)  \hspace{1cm} (3.36)

Off-Pathway  \( P_2 = P_{21} \cup P_{22} \cup P_{23} \)  \hspace{1cm} (3.37)

Off/On-Pathway  \( P_3 = P_{31} \cup P_{32} \cup P_{33} \cup P_{34} = P_{21} \cup P_{22} \cup P_{42}(-) \cup P_{12} \)  \hspace{1cm} (3.38)

On/Off-Pathway  \( P_4 = P_{41} \cup P_{42} \cup P_{43} = P_{11} \cup P_{42} \cup P_{23} \)  \hspace{1cm} (3.39)

\[
J_1 = J_{11} + J_{12} \\
= (\alpha_2 B_1^n - \alpha_1 B_n) + (\beta_3 B_{n/m} - \alpha_5 B_m) \]

\[
J_2 = J_{21} + J_{22} + J_{23} \\
= (\alpha_3 B_1 - B'_1) + (\beta_2 B_{1/n} - \beta_1 B'_n) + (\beta_4 B_{n/m} - \beta_5 B'_m) \]

\[
J_3 = J_{31} + J_{32} + J_{33} + J_{34} = J_{21} + J_{22} - J_{42} + J_{12} \\
= (\alpha_3 B_1 - B'_1) + (\beta_2 B_{1/n} - \beta_1 B'_n) + (\beta_4 B_{n/m} - \alpha_4 B_n) + (\beta_3 B_{n/m} - \alpha_5 B_m) \]

\[
J_4 = J_{41} + J_{42} + J_{43} = J_{11} + J_{42} + J_{23} \\
= (\alpha_2 B_1^n - \alpha_1 B_n) + (\alpha_4 B_n - \beta_4 B'_n) + (\beta_6 B_{n/m} - \beta_5 B'_m) \]

Similarly, the forces corresponding to the different pathways can be given by the reaction
affinities or forces which can be given by the expression \( F_i = RT \ln \left( \frac{R_i}{R_i^0} \right) \).

\[
F_1 = F_{11} + F_{12} = RT \ln \left( \frac{\alpha_2 B_1^n}{\alpha_1 B_n} \right) + RT \ln \left( \frac{\beta_3 B_n^m}{\alpha_5 B_m} \right) \\
= RT \ln \left( \frac{\alpha_2 \beta_3 B_1^n B_n^m}{\alpha_1 \alpha_5 B_n B_m} \right) \tag{3.44}
\]

\[
F_2 = F_{21} + F_{22} + F_{23} = RT \ln \left( \frac{\alpha_3 B_1}{B_1'} \right) + RT \ln \left( \frac{\beta_2 B_1^m}{\beta_1 B_1'} \right) + RT \ln \left( \frac{\alpha_4 B_n}{\beta_4 B_n'} \right) + RT \ln \left( \frac{\beta_6 B_n^m}{\beta_5 B_m'} \right) \\
= RT \ln \left( \frac{\alpha_3 \beta_2 \alpha_4 B_1 B_1^m}{\beta_1 \beta_5 \beta_4 B_1' B_n B_m'} \right) \tag{3.45}
\]

\[
F_3 = F_{31} + F_{32} + F_{33} + F_{34} = F_{21} + F_{22} - F_{42} + F_{12} \\
= RT \ln \left( \frac{\alpha_3 B_1}{B_1'} \right) + RT \ln \left( \frac{\beta_2 B_1^m}{\beta_1 B_1'} \right) - RT \ln \left( \frac{\alpha_4 B_n}{\beta_4 B_n'} \right) + RT \ln \left( \frac{\beta_6 B_n^m}{\beta_5 B_m'} \right) \\
= RT \ln \left( \frac{\alpha_3 \beta_2 \beta_3 \alpha_4 B_1 B_1^m}{\beta_1 \beta_5 \alpha_4 \beta_3 B_1' B_n B_m'} \right) \tag{3.46}
\]

\[
F_4 = F_{41} + F_{42} + F_{43} = F_{11} + F_{42} + F_{23} \\
= RT \ln \left( \frac{\alpha_2 B_1^n}{\alpha_1 B_n} \right) + RT \ln \left( \frac{\alpha_4 B_n}{\beta_4 B_n'} \right) + RT \ln \left( \frac{\beta_6 B_n^m}{\beta_5 B_m'} \right) \\
= RT \ln \left( \frac{\alpha_2 \alpha_4 \beta_6 B_1^n B_n^m}{\alpha_1 \beta_4 \beta_3 B_1' B_n B_m'} \right) \tag{3.47}
\]

The reaction affinities are related to the Gibbs free energy of the system by

\[
-\Delta G_i = F_i = RT \ln K_{eq} \tag{3.48}
\]

where \( K_{eq} \) is the equilibrium constant for the reaction. The Gibbs energy function is also related to the entropy, \( S \), in the system by

\[
\Delta G_i = -T \Delta S \tag{3.49}
\]

Therefore, if the change in \( \Delta G \) is negative (i.e. change in entropy is positive), then the chemical reaction occurs spontaneously at constant pressure and temperature.

### 3.6 Entropy Production

The entropy production corresponding to the various pathways is given by

\[
\sigma_i = \frac{1}{T} \sum_k F_{ik} J_{ik} \tag{3.50}
\]
where $i = 1, 2, 3, 4$. Expanding this sum yields:

$$\sigma_1 = \frac{1}{T} (F_{11} J_{11} + F_{12} J_{12})$$ (3.51)

$$\sigma_2 = \frac{1}{T} (F_{21} J_{21} + F_{22} J_{22} + F_{23} J_{23})$$ (3.52)

$$\sigma_3 = \frac{1}{T} (F_{31} J_{31} + F_{32} J_{32} + F_{33} J_{33} + F_{34} J_{43})$$ (3.53)

$$\sigma_4 = \frac{1}{T} (F_{41} J_{41} + F_{42} J_{42} + F_{43} J_{43})$$ (3.54)

where each of the component terms can be obtained from the expressions above. We think that computing the entropy production rate for each of the pathways can reveal significant information about the underlying energetics of the different pathways. To begin with, we first compute the values of $\sigma_i$s at the equilibrium concentrations of the oligomers. The results are presented in the form of contour plots of $\sigma_i$ as a function of the order parameters $\alpha_3$ and $\alpha_4$.

Figure 3.30: A contour plot of entropy production along the four different pathways as a function of $\alpha_3$ and $\alpha_4$. The red regions correspond to large values, while blue points to smaller values of entropy production. For this calculation, the forward rate constants along the purely on and off pathways are taken to be equal to 1.0 while the backward constants are 0.001.
Chapter 4

Conclusions

4.1 Four Species Model

An important consideration in any numerical simulation such as ours is error. Because we are dealing with a relatively large, non-linear system it was necessary to use numerical methods for our solutions. Our choice of numerical solution method was ode45 in Matlab, which is the Runge-Kutta method of order 4/5. ode45 is considered the method of choice for non-stiff systems of equations. Stiff equations occur when the solution has a transient exponential. When this happens, the error term, as represented by the \(n\)th derivative, can get very large in relation to the solution itself [46]. Determining whether a system is stiff in Matlab is a matter of running the code through ode 45 and seeing if it returns a solution. If it does, the problem is non-stiff, and if it doesn’t, or run times are exceedingly long, the problem could potentially be stiff. Our system did not encounter this problem. Nonetheless, any method employed will involve truncation error. The Runge-Kutta Method of order 4 has local truncation error of \(O(h^4)\). Thus, error related to numerical methods is impossible to avoid for problems such as ours, and so must be taken into account when considering whether a model accurately represents the behavior of a physical system. Our limitation with respect to comparing our results with empirical data makes this an enduring problem in the study of modeling \(A\beta\) aggregation.

Our linear stability analysis of the model showed that the system is stable for a broad range of parameters. For the Base Case, all eigenvalues were negative indicating asymptotic
Increasing backward rates relative to forward rates increased the magnitude of stability as represented by the eigenvalues. $\lambda_i$ for $i = 1, 2, 3, 4$ all became more negative as backward rates were increased relative to forward rates. We had mixed results when we increased forward rates. $\lambda_1$ and $\lambda_2$ become more negative, but $\lambda_3$ and $\lambda_4$ become less negative. The system is still asymptotically stable, but marginally less so. Varying $n$ also had mixed results. All eigenvalues become more negative except $\lambda_3$ which becomes less negative.

In conclusion, our analysis of linear stability determined that the model was asymptotically stable, with some variation in the magnitude of stability depending on parameter values.

Equilibrium analysis also revealed some interesting results. The parameter $\alpha_3$ determines switching between on- and off-pathway species formation, given that it contains the fatty acid micelle term $L$. For $\alpha_3 < 1$ formation of on-pathway species exceeds that of off-pathway species, with $\alpha_3 > 1$ leading to an outperformance of off-pathway species formation. At $\alpha_3 = 1$, the system is at the Nash Equilibrium where we will have equality of all species over the long term. While $\alpha_3 = 1$ is the line of demarcation between on- and off-pathway species formation, for $\alpha_3 > 1$ we did still see outperformance of $B_n$ over $B'_n$ for a period of time. As $\alpha_3$ approaches 1 from the right, this phenomenon persists for increasingly longer periods of time. Eventually, however, the concentration of $B'_n$ exceeds that of $B_n$. We were unable to force a situation where $B_1 > B'_1$ and $B_n < B'_n$. This is most likely due to the fact that $B_n$ has a direct reaction with $B_1$ whereas $B'_m$ is one step removed.

4.2 Six Species Model

Unlike with the Four Species model, we were unable to analytically solve for equilibrium. We therefore had to use our numerical solutions in determining equilibrium, which were then verified with Matlab’s fsolve and found to be within 1% of our values. We found that the time the system took to achieve equilibrium was very dependent upon the reaction rate regime. When the ratio of forward to backward rates is close to 1, our model achieves equilibrium very quickly. When forward rates are considerably higher than backward rates, or vice versa, the system takes longer to achieve equilibrium due to a cycle of over-shooting of species sizes.
resulting from a large difference in reaction rates. In the case of high backward rates, the 
model didn’t achieve equilibrium at all. Thus, Model 2 achieved equilibrium over a much 
shorter time span than the Base Model. The inability of the model to achieve equilibrium 
under the conditions of high backward rates is no more than a curiosity as high backward 
rates would indicate no aggregation. Increasing $n$ and $m$ resulted in the model taking longer 
to achieve equilibrium, which seems to mean that higher exponents increase fluctuations in 
the model before it is able to settle down.

In terms of stability, both of the 6 species models we experimented with were neutrally 
stable. For all sets of parameter values, there was consistently one eigenvalue with zero real 
part, indicating neutral stability: neither do perturbations die out over time nor do they 
blow up. This was in contrast to the Four Species model which is asymptotically stable over 
a broad range of parameter values. This is possibly a result of the greater complexity of the 
Six Species model and the addition of a higher degree oligomer size, $m$. Further study of 
the problem using non-linear methods would be the next step in analyzing stability.

We varied $\alpha_3$ and $\alpha_4$ between 0 – 2, and while we saw switching of stabilities, there was 
always at least one eigenvalue with zero real part. Doubled forward rates and varying $n$ and 
$m$ saw the same phenomenon: switching of eigenvalues, but neutral stability in all cases.

The greater complexity of the Six Species model yielded very interesting results as we 
performed our simulations. As $\alpha_3$ and $\alpha_4$ increase we saw a direct increase in ratio of off-
pathway to on-pathway species. The relationship is linear and explained by $B'_1/B_1 = \alpha_3$, 
and $B'_n/B_n = \alpha_4$. The ratio of $m'/m$ is very stable, but when $\alpha_3, \alpha_4$ exceeds 7, it appears 
to undergo exponential growth. When we increased only $\alpha_3$, we saw that there is an initial 
increase in the ratio of off-pathway to on-pathway oligomers, but the curve can be described 
as sigmoidal: no matter how high $\alpha_3$ goes, this ratio ceases to grow any higher than 1. 
This shows the $B_n$ to $B'_n$ bridge is critical for formation of $n$ and $m$ species, and can’t be 
forced only over the $B_1$ to $B'_1$ bridge. This was confirmed by our examination of the Nash 
Equilibrium using Model 2.

We were also able to show that we could get any species to outperform on an absolute 
basis. Doing this for $n$ and $m$ species required us to increase forward rates to levels, that 
weren’t necessarily realistic, but confirmed the ability of the system to be in any particular
equilibrium state.

Lastly, dynamic simulations of both models, done by increasing the amount of $B_1$ in the system at regular intervals, saw both the Base Model and Model 2 favoring the formation of $n$ and $m$ species. With backward and forward rates equal, Model 2 took longer for this to happen, but even here, the higher order species eventually dominate. It appears that both of the models have a preference for oligomers over monomers.

4.3 Future Study

There are a few recommendations for future study. Firstly, analyzing stability with the use of Lyapunov functions might bear some fruit and help to clear up the stability picture of the 6 Species models. The number of dimensions of the model and the non-linearity could mean that another approach to stability analysis is needed. It would be a vindication of the model to be able to show global stability.

Secondly, analyzing a more dynamical model with increased pathways would be of great interest. The analysis we have made of the Four and Six Species models has given us a solid understanding of the behavior of the model. Increasing the number of pathways to aggregation should be the next step in modeling. Currently, we have just $n$-mers to $n$-mers, but this is almost certainly not the case in vivo, where there can be $n$-mers to $m$-mers.

Also, our attempt at a more dynamical model in the Numerical Results section of Chapter 3 was rudimentary: we added discrete amounts of new monomers of $B_1$ in set intervals to simulate an evolving system. The reality of the process is that the formation of new $A\beta$ from APP is most likely stochastic. Adding randomness to this part of the model would be a good addition.

Finally, our look at the thermodynamics of the system in chapter 4 was just the beginning of what we would like to achieve. This area of our study will be expanded upon in future work.
Bibliography


