STABILITY OF CONTROL SYSTEM OF INTRACELLULAR IRON HOMEOSTASIS: A MATHEMATICAL PROOF

ADRIANA MORALES AND MITCHELL EITHUN

ABSTRACT. Iron is a metal essential for cellular metabolism. Excess or lack of iron can cause serious health conditions. To deal with these difficulties, the intracellular levels of iron are tightly constrained by a complex control network of proteins. Recently, Chifman *et al.* developed and validated a mathematical model in the form of five differential equations, of the core control system of intracellular iron homeostasis in normal breast epithelial cells. Their work was motivated by the fact that intracellular iron homeostasis can play a role in the pathogenesis of breast cancer. For any choice of parameters, their dynamical system has a unique equilibrium, and Chifman *et al.*'s simulations suggest it is globally stable. Here we introduce a biologically reasonable simplification of the Chifman model. For this valid approximation, we show that it has a unique steady state and that it is locally asymptotically stable. We also give evidence that this model seems to approach global stability by using a geometric analysis. This reduced version gives us an insight on how the original model behaves.

1. Introduction

Iron is a metal fundamental to the biology of organisms that live in an oxygen-rich environment. The challenge for most organisms is to acquire adequate amounts of iron for critical biological processes while simultaneously avoiding the toxicity associated with free iron. Dysregulation of iron homeostasis has been implicated in a wide variety of diseases, one of them being breast cancer. Thus, iron uptake, utilization, and storage is highly regulated by a complex control network of proteins and other molecules.

We focus on a core control system in the form found in breast epithelial cells. This is motivated by the fact that intracellular iron homeostasis can play a role in the pathogenesis of breast cancer. Pinnix *et al.* have shown that some of the proteins involved are differentially regulated in breast cancer cells and can be used as prognostic markers [10]. It is believed that differences in iron metabolism may be key to both development and recurrence of breast cancer [5]. Numerous authors have shown that individuals who accumulate excess iron are prone to cancer [6, 9, 11]. In addition, Hann *et al.* have shown that the growth of breast tumors in animal models is accelerated by high levels of iron in their diet [2, 3].

Recently, Chifman *et al.* [1] introduced and validated a mathematical model for the core control system in normal breast epithelial cells. It is essential to first understand this core control system before being able to properly assess the effect of other components in the larger iron homeostasis network constructed earlier by Hower *et al.* in [4]. Because of Chifman *et al.* we know that the model has a unique positive steady state, and their extensive simulations suggest that the steady state is globally asymptotically stable.

Even though the simulations suggest that the positive equilibrium point might be asymptotically stable, achieving a proof that this model is globally stable has been elusive. Even

Date: July 20, 2016.

checking *local* stability is currently intractable due to the number of parameters. Therefore, we analyze a valid approximation of the Chifman model. For this simplification of the Chifman model we show that it has a unique positive steady state. More precisely, we show that the steady state is locally asymptotically stable by using the well-known Routh-Hurwitz criterion. We also use a geometric analysis to give evidence that seems to suggest this reduced model is globally stable. We look at the behavior of the equations in a specific region, and we note that as time increases the equations get closer to the steady state for any starting point.

This reduced version gives us an insight on how the original model behaves. We conjecture that the full system described by Chifman *et al.* has a globally stable steady state based on the dynamics of our reduced model and the heuristic approach established by the paper.

The paper is organized as follows. In Section 2, we present relevant background on stability analysis of differential equations. Section 3 is an overview of the mathematical model presented in Chifman *et al.* [1]. We introduce a reduced version of the original model in Section 4, along with the results on the stability analysis of this new reduced model. In Section 5 we study the behavior of the reduced system using a geometrical analysis. Finally, in Section 6 we discuss our results and what they say about the behavior of the Chifman model.

2. Background

2.1. **Stability Analysis of Differential Equations.** In this section, some preliminaries on the stability analysis of differential equations are presented. In this paper we consider autonomous differential equations:

$$\begin{aligned} \dot{x}_1 &= f_1(\mathbf{x}) \\ \dot{x}_2 &= f_2(\mathbf{x}) \\ &\vdots \\ \dot{x}_n &= f_n(\mathbf{x}), \end{aligned} \tag{1}$$

where $\mathbf{x} = (x_1, x_2, \ldots, x_n)$, $f_i \in \mathbb{R}[\mathbf{x}]$, $x_i = x_i(t)$, and $\dot{x}_i = dx_i/dt$.

Definition 2.1. A point $\bar{\mathbf{x}} = (\bar{x}_1, \bar{x}_2, \ldots, \bar{x}_n)$ in $\mathbb{R}^n$ is called a *steady state* (*fixed point* or *equilibrium*) of the differential system (1) if $f_i(\bar{\mathbf{x}}) = 0$ for all $i \in \{1, 2, \ldots, n\}$.

Let $x(t, x_0)$ denote the solution to (1) with initial value $x(0) = x_0$.

Definition 2.2 (Locally Stable)**.** An equilibrium point $\bar{\mathbf{x}} \in \mathbb{R}^n$ of (1) is said to be *locally stable* if, for each neighborhood U of $\bar{\mathbf{x}}$, there exists a neighborhood V of $\bar{\mathbf{x}}$ such that $x(t, x_0) \subset U$, for $x_0 \in V$ and all $t > 0$.

Definition 2.3. An equilibrium point $\bar{\mathbf{x}}$ is said to *attract points* in a neighborhood W if $x(t, x_0) \to \bar{\mathbf{x}}$ as $t \to \infty$ for each $x_0 \in W$. If a stable $\bar{\mathbf{x}}$ attracts points in a bounded set W, then the attraction is uniform with respect to $x_0 \in W$, in this case $\bar{\mathbf{x}}$ is said to attract W.

Definition 2.4 (Asymptotically Stable)**.** An equilibrium point $\bar{\mathbf{x}}$ is *locally asymptotically stable* if it is locally stable and attracts a neighborhood.

Definition 2.5. The *basin of attraction* of $\bar{\mathbf{x}}$ is the union of all points which it attracts.

Definition 2.6 (Globally Stable)**.** An equilibrium point $\bar{\mathbf{x}}$ is said to be *globally stable* with respect to an open set D if it is asymptotically stable and its basin of attraction contains D.

3. Chifman's Iron Model

Chifman's model represents the core control system of intracellular iron homeostasis in normal breast epithelial cells. It consists of five nonlinear differential equations describing the changes in the concentrations of the proteins that control the system with respect to time. The concentration of the labile iron pool is denoted by $x_1 = [\text{LIP}]$ and each protein is denoted by $x_2 = [\text{TfR1}]$, $x_3 = [\text{Fpn}]$, $x_4 = [\text{Ft}]$, and $x_5 = [\text{Active IRP's}]$. The mathematical model[1] is the following:

$$\begin{aligned}
\dot{x_1} &= \alpha_1 \text{Fe}_{\text{ex}} x_2 + \gamma_4 x_4 - \alpha_6 x_1 x_3 - \alpha_4 x_1 \frac{k_{54}}{k_{54} + x_5}, \\
\dot{x_2} &= \alpha_2 \frac{x_5}{k_{52} + x_5} - \gamma_2 x_2, \\
\dot{x_3} &= \alpha_3 \frac{k_{53}}{k_{53} + x_5} - (\gamma_3 + \hat{\gamma}_h \text{Hep}) x_3, \\
\dot{x_4} &= \alpha_4 x_1 \frac{k_{54}}{k_{54} + x_5} - \gamma_4 x_4, \\
\dot{x_5} &= \alpha_5 \frac{k_{15}}{k_{15} + x_1} - \gamma_5 x_5.
\end{aligned} \tag{2}$$

For this network, each x_i with $i \in \{1, \ldots, 5\}$ is an activating/inhibiting state variable and the k_{nj}'s are the activation thresholds for $n \in \{1, 5\}$ and $j \in \{2, 3, 4, 5\}$. The α_ℓ's are the maximum production rates of the regulated proteins for $\ell \in \{1, \ldots, 6\}$. Each protein undergoes self-degradation, thus each protein has a decay rate γ_j. Note that *Hep* and Fe_{ex} are control parameters, so they are considered constants fixed between 0 and 1. The final parameter is $\hat{\gamma_h}$ which is the maximum production rate of *Hep*.

An equilibrium point of a dynamical system represents a stationary state for the dynamics. If the differential equations from the model are set equal to zero, we find that the system has a unique steady state defined by the cubic polynomial

$$p(x_1) = a x_1^3 + b x_1^2 + c x_1 + d \tag{3}$$

in terms of x_1, where

$$\begin{aligned}
a &= \alpha_3 \alpha_6 \gamma_2 (\gamma_5^2) k_{52} k_{53}, \\
b &= \alpha_3 \alpha_6 \gamma_2 \gamma_5 k_{15} k_{53} (\alpha_5 + 2\gamma_5 k_{52}), \\
c &= \gamma_5 k_{15} (-\alpha_1 \alpha_2 \alpha_5 \text{Fe}_{\text{ex}} (\gamma_3 + \hat{\gamma_h} \text{Hep}) + \alpha_3 \alpha_6 \gamma_2 k_{15} (\alpha_5 + \gamma_5 k_{52})) k_{53}, \\
d &= -\alpha_1 \alpha_2 \alpha_5 Fe_{ex} (\gamma_3 + \hat{\gamma_h} \text{Hep}) (k_{15})^2 (\alpha_5 + \gamma_5 k_{53}).
\end{aligned}$$

It is important to mention that all parameters in the system are strictly positive real numbers. Thus, the coefficients of (3) are as follows: $a > 0$, $b > 0$ and $d < 0$, while c could be of either sign depending on the choice of parameters.

Theorem 3.1 (Descartes' Rule of Signs [7])**.** *The number of real positive roots of a single-variable polynomial is equal to the number of sign changes in the ordered sequence of coefficients or less than it by an even number.*

[1]More on this system and how every protein fits in the model can be found in [1].

By [1] we have the following corollary.

Corollary 3.2. *For any choice of parameters,* (3) *has exactly one positive solution, while the other two solutions are either negative real or complex.*

Proof. The two possible sign vectors of (3) are $(+,+,-,-)$ and $(+,+,+,-)$. In either case, there is one sign alteration. By Descartes' Rule of Signs, the system has one real positive root, thus proving the corollary. □

4. Reduced Model

In this section we reduce the amount of parameters of the Chifman model presented in Section 3 in a way that is biologically plausible. For this experimentally supported simplification of the Chifman model we show that it has a unique positive steady state and that it is locally asymptotically stable.

4.1. **Reduction Assumptions.** To understand our reduction we first need to understand the biology behind it. For the reduction to be biologically supported, we assume a constant amount of TfR1 and Fpn as explained bellow.

The Chifman model includes a protein called transferrin receptor 1 (TfR1), which is the primary conduit for iron import in breast epithelial cells. Recall that the concentration of this protein is denoted by $x_2 = [\text{TfR1}]$. We also have the parameter α_1 which represents the rate of extracellular iron that is being imported into the cell. Thus, we make x_2 a constant and combine it with α_1 to create a new iron import rate denoted by $\hat{\alpha_1} = \alpha_1 x_2$. Similarly, we have Ferroportin, which is the primary protein that exports the labile iron pool out of the cell. We make $x_3 = [\text{Fpn}]$ a constant and combine it with α_6 to make the new iron export rate $\hat{\alpha_6} = \alpha_6 x_3$.

Thus, the new reduced 3-variable model that is obtained from the original model (2) is the following:

$$\begin{aligned}
\dot{x_1} &= \hat{\alpha_1}\text{Fe}_{\text{ex}} + \gamma_4 x_4 - \hat{\alpha_6} x_1 - \alpha_4 x_1 \frac{k_{54}}{k_{54} + x_5} \\
\dot{x_4} &= \alpha_4 x_1 \frac{k_{54}}{k_{54} + x_5} - \gamma_4 x_4 \\
\dot{x_5} &= \alpha_5 \frac{k_{15}}{k_{15} + x_1} - \gamma_5 x_5.
\end{aligned} \tag{4}$$

The model now comprises the labile iron pool (x_1), Ferritin (x_4) and the iron regulatory proteins (x_5) shown in Figure 1.

4.2. **Steady-State Analysis.** The attractive behavior of the steady states defined in Definition 2.1 provides insight into the long-term dynamics of the system. By using the Jacobian matrix we show that the reduced model (4) has a unique, locally stable steady state.

Steady states are points $(x_1, x_4, x_5) \in \mathbb{R}^3_{>0}$ such that $\dot{x_1}$, $\dot{x_4}$ and $\dot{x_5}$ are all zero. By setting each equation in system (4) equal to zero, we can solve for x_1 by adding the equations for

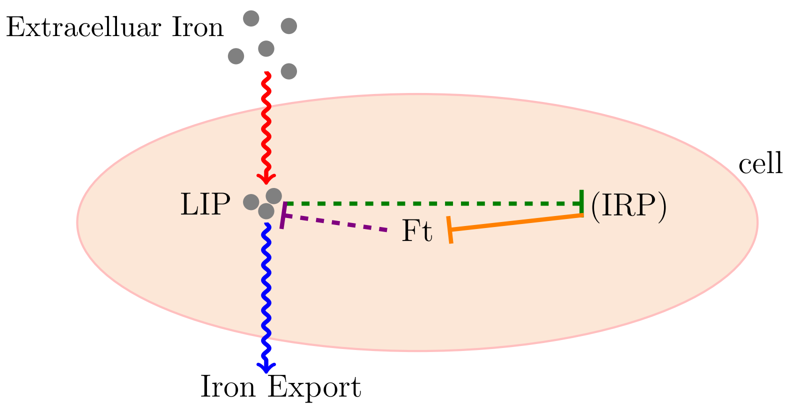

FIGURE 1. The resulting control system. The species that control the system are [LIP]= x_1, [Ft]= x_4, and [IRP's]=x_5. Solid lines indicate positive or negative regulation; dotted lines indicate reactions that consume or produce the indicated species. The red and blue "snake" arrows represent the iron import and export respectively.

$\dot{x_1}$ and $\dot{x_4}$ together as done below:

$$\left[\hat{\alpha_1}\mathrm{Fe_{ex}} + \gamma_4 x_4 - \hat{\alpha_6} x_1 - \alpha_4 x_1 \frac{k_{54}}{k_{54}+x_5}\right] + \left[\alpha_4 x_1 \frac{k_{54}}{k_{54}+x_5} - \gamma_4 x_4\right] = 0$$
$$\hat{\alpha_1}\mathrm{Fe_{ex}} - \hat{\alpha_6} x_1 = 0$$
$$x_1 = \frac{\hat{\alpha_1}\mathrm{Fe_{ex}}}{\hat{\alpha_6}}$$

Notice that $\dot{x_5}$ is linear in x_5 and so we can solve for x_5 by substituting in x_1. Likewise, we can substitute x_1 and x_5 in $\dot{x_4}$ to find x_4. Hence, we get the following proposition.

Proposition 4.1. *For any choice of parameters, system* (4) *has the following unique steady state:*

$$\begin{aligned}
\bar{x}_1 &= \frac{\hat{\alpha_1} Fe_{ex}}{\hat{\alpha_6}},\\
\bar{x}_4 &= \frac{Fe_{ex}^2 \alpha_4 \gamma_5 k_{54} \hat{\alpha_1}^2 + Fe_{ex} \alpha_4 \gamma_5 k_{15} k_{54} \hat{\alpha_1} \hat{\alpha_6}}{Fe_{ex} \gamma_4 \gamma_5 k_{54} \hat{\alpha_1} \hat{\alpha_6} + (\gamma_4 \gamma_5 k_{15} k_{54} + \alpha_5 \gamma_4 k_{15}) \hat{\alpha_6}^2},\\
\bar{x}_5 &= \frac{\alpha_5 k_{15} \hat{\alpha_6}}{Fe_{ex} \gamma_5 \hat{\alpha_1} + \gamma_5 k_{15} \hat{\alpha_6}}.
\end{aligned} \tag{5}$$

Since all the parameters are positive, the steady state is also positive.

4.3. **Local Stability of the System.** Now we appeal to stability theory of system (4) to prove that the steady state of the system is locally asymptotically stable in Theorem 4.4.

Theorem 4.2 (Stability Criterion)**.** *If the eigenvalues of the Jacobian matrix of a dynamical system all have negative real part at an equilibrium point, the point is asymptotically stable. If at least one eigenvalue has positive real part, the equilibrium is unstable.*

The Jacobian matrix of system (4) is given by

$$J_x = \begin{pmatrix} -\dfrac{\alpha_4 k_{54}}{k_{54}+x_5} - \hat{\alpha_6} & \gamma_4 & \dfrac{\alpha_4 k_{54} x_1}{(k_{54}+x_5)^2} \\ \dfrac{\alpha_4 k_{54}}{k_{54}+x_5} & -\gamma_4 & -\dfrac{\alpha_4 k_{54} x_1}{(k_{54}+x_5)^2} \\ -\dfrac{\alpha_5 k_{15}}{(k_{15}+x_1)^2} & 0 & -\gamma_5 \end{pmatrix} \tag{6}$$

Due to the difficulty of determining the signs of the eigenvalues of (6) we use a condition that is equivalent to the one given for local stability in Theorem 4.2. This is the well-known *Routh-Hurwitz criterion* [8].

Definition 4.1. Given a real polynomial

$$\mathcal{P}(\lambda) = a_0\lambda^n + a_1\lambda^{n-1} + \cdots + a_{n-1}\lambda + a_n,$$

the $n \times n$ square matrix

$$H_n = \begin{pmatrix} a_1 & a_3 & a_5 & \dots & 0 & 0 \\ a_0 & a_2 & a_4 & \dots & 0 & 0 \\ 0 & a_1 & a_3 & \dots & 0 & 0 \\ \vdots & \vdots & \vdots & \ddots & \vdots & \vdots \\ 0 & 0 & 0 & \dots & a_{n-1} & 0 \\ 0 & 0 & 0 & \dots & a_{n-2} & a_n \end{pmatrix}$$

is called the *Hurwitz matrix* corresponding to the polynomial $\mathcal{P}(\lambda)$.

Definition 4.2. The *leading principal minors* are the determinants of the upper left $1 \times 1, 2 \times 2, \dots, n \times n$ submatrices of H_n, the upper left $k \times k$ minors are denoted Δ_k for $k = 1, 2, ...n$. For example, the leading principal minors of the Hurwitz matrices H_1, H_2, and H_3 respectively are

$$\Delta_1 = |a_1|, \quad \Delta_2 = \begin{vmatrix} a_1 & a_3 \\ a_0 & a_2 \end{vmatrix}, \text{and } \Delta_3 = \begin{vmatrix} a_1 & a_3 & a_5 \\ a_0 & a_2 & a_4 \\ 0 & a_1 & a_3 \end{vmatrix}.$$

Theorem 4.3 (Routh-Hurwitz Criterion)**.** *The eigenvalues of a Jacobian matrix J_x all have negative real part if and only if all of the coefficients of the characteristic polynomial of J_x are positive and all leading principal minors of the Hurwitz matrix corresponding to the characteristic polynomial are also positive.*

Using `Sage`, we computed that the characteristic polynomial of the 3×3 Jacobian matrix (6) is:

$$\mathcal{P}(\lambda) = a_0\lambda^3 + a_1\lambda^2 + a_2\lambda + a_3, \tag{7}$$

where

$$
\begin{aligned}
a_0 &= 1 \\
a_1 &= \gamma_4 + \gamma_5 + \hat{\alpha_6} + \frac{\alpha_4 k_{54}}{k_{54} + x_5} \\
a_2 &= \gamma_4\gamma_5 + \gamma_4\hat{\alpha_6} + \gamma_5\hat{\alpha_6} + \frac{\alpha_4\gamma_5 k_{54}}{k_{54} + x_5} + \frac{\alpha_4\alpha_5 k_{15} k_{54} x_1}{(k_{15} + x_1)^2 (k_{54} + x_5)^2} \\
a_3 &= \gamma_4\gamma_5\hat{\alpha_6}.
\end{aligned}
\tag{8}
$$

Now, we can prove the asymptotic stability of (4) using the Routh-Hurwitz criterion.

Theorem 4.4. *The simplified system* (4) *has a unique steady state and it is asymptotically stable.*

Proof. The system has a unique steady state given by (5). By Theorems 4.3 and 4.2, we need to show that

(1) the coefficients $a_0, \ldots, a_3 > 0$ and
(2) the leading principal minors of the Hurwitz matrix are positive.

First, we must verify that the coefficients of the characteristic polynomial of the Jacobian matrix, displayed in (8), are all positive. Notice that the coordinates of the steady state are all positive because all parameters are restricted to be positive. This implies that $a_0, ..., a_3 > 0$.

Next, we need to verify that the principal minors $\Delta_1, \Delta_2, \Delta_3$ of the Hurwitz matrix corresponding to the characteristic polynomial, evaluated at the steady state, are positive. Notice that $\Delta_1 = a_0$, and $\Delta_3 = a_3\Delta_2$ and so it suffices to show that $\Delta_2 > 0$ or, equivalently, $a_2 a_1 > a_0 a_3$. By inspection, we have

$$
\begin{aligned}
a_2 a_1 &> (\gamma_4 + \gamma_5 + \hat{\alpha_6})(\gamma_4\gamma_5 + \gamma_4\hat{\alpha_6} + \gamma_5\hat{\alpha_6}) \\
&> \hat{\alpha_6}\gamma_4\gamma_5 \\
&= a_0 a_3.
\end{aligned}
$$

The conditions of Theorem 4.3 are satisfied and so the result follows. □

5. Geometric Analysis: Towards a proof of global stability

In this section we analyze the global stability of the reduced system (4) by using a geometrical approach. By looking at the vector fields in Figure 2 we can see that the system has unique a steady state as previously shown by Proposition 2.1.

To explain what is meant by geometrical approach we have the following example.

Example 5.1. First, if we look at the reduced system in (4) we notice that

$$\dot{x}_1 + \dot{x}_4 = \hat{\alpha}_1 \mathrm{Fe}_{\mathrm{ex}} - \hat{\alpha}_6 x_1.$$

Consider the case: $\mathbf{x_1} < \bar{\mathbf{x}}_\mathbf{1}$. Let $\bar{x}_1 = \dfrac{\hat{\alpha}_1 \mathrm{Fe}_{\mathrm{ex}}}{\hat{\alpha}_6}$, then

$$\dot{x}_1 + \dot{x}_4 = \hat{\alpha}_1 \mathrm{Fe}_{\mathrm{ex}} - \hat{\alpha}_6 x_1 > \hat{\alpha}_1 \mathrm{Fe}_{\mathrm{ex}} - \hat{\alpha}_6 \bar{x}_1 > 0.$$

Thus, we conclude that if $x_1 < \bar{x}_1$, then $\dot{x}_1 + \dot{x}_4 > 0$. Similarly, if we look at $\mathbf{x_1} > \bar{\mathbf{x}}_\mathbf{1}$, then $\dot{x}_1 + \dot{x}_4 < 0$. A diagram of this analysis can be seen in Figure 3.

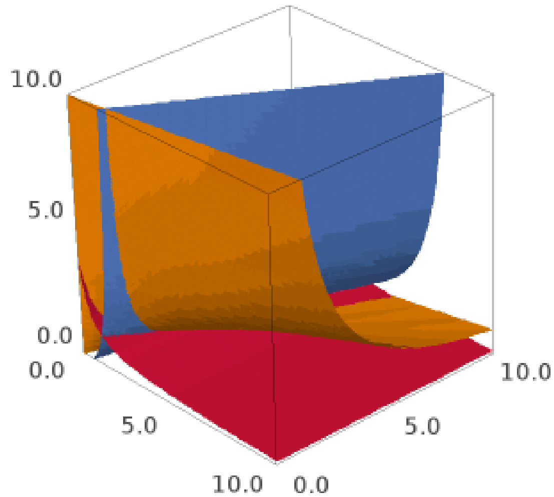

FIGURE 2. Vector fields of our system: $\dot{x}_1 = 0$ is the blue surface, $\dot{x}_4 = 0$ is the orange surface, and $\dot{x}_5 = 0$ is the red surface, with parameters $\hat{\alpha}_1 = 0.5$, $\hat{\alpha}_6 = 0.3$, $\alpha_4 = 0.7$, $\alpha_5 = 0.7$, $\gamma_4 = 0.2$, $\gamma_5 = 0.2$, $k_{54} = 0.4$, $k_{15} = 0.5$, and $\mathrm{Fe_{ex}} = 1$.

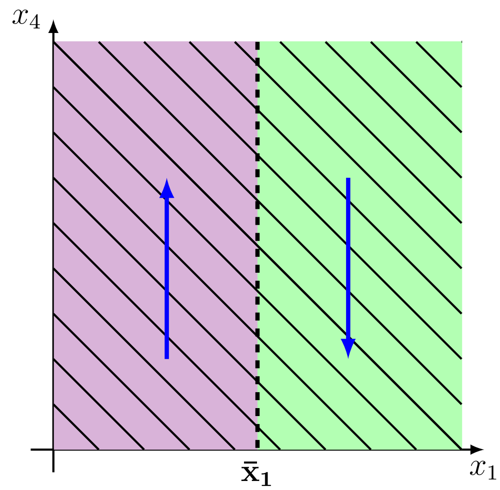

FIGURE 3. The diagonal black lines represent the $\dot{x}_1 + \dot{x}_4$ equation. The dashed black line represents the equilibrium point $\bar{x}_1$. The purple region is when $x_1 < \bar{x}_1 \implies \dot{x}_1 + \dot{x}_4 > 0$, hence the blue arrow pointing upwards. Similarly the green region is when $x_1 > \bar{x}_1 \implies \dot{x}_1 + \dot{x}_4 < 0$, hence the blue arrow pointing downwards.

By doing the same analysis as in Example 5.1 for all of the equations in (4), we get the regions depicted in Figure 4. The plane is divided into 6 regions, in which we determine the behavior of the equations.

The behaviors of the equations in the color-coded regions of Figure 4 can be seen in Table 1. Everything in the table may be found by analyzing the system (4) using the constraints of its respective regions. See Appendix A for the case-by-case analysis of each of the equations in (4).

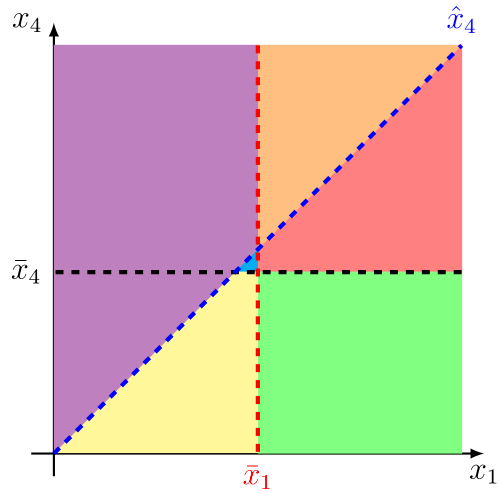

FIGURE 4. The six color-coded regions. The red vertical dashed line is the equilibrium point $\bar{x}_1$ and the black horizontal dashed line is the equilibrium point $\bar{x}_4$. The blue diagonal dashed line $\hat{x}_4$ is what we call the *cutoff point* for x_4. More precisely, it is the point at which x_4 gets so big that the equation $\dot{x}_4$ starts decreasing.

TABLE 1. Behavior of equations in the color coded regions

Color of region	Behavior of equations in the region
(purple)	$\dot{x}_1 + \dot{x}_4 > 0$ & $\dot{x}_4 < 0$ & $\dot{x}_1 > 0$
(yellow)	$\dot{x}_1 + \dot{x}_4 > 0$ & If $x_5 < \bar{x}_5$, then $\dot{x}_5 > 0$
(blue)	$\dot{x}_5 > 0, \dot{x}_4 < 0$, and $\dot{x}_1 > 0$. This region exists iff $x_5 < \bar{x}_5$
(orange)	$\dot{x}_1 + \dot{x}_4 < 0$ & $\dot{x}_4 < 0$
(red)	$\dot{x}_1 + \dot{x}_4 < 0$ & If $x_5 > \bar{x}_5$, then $\dot{x}_5 < 0$
(green)	$\dot{x}_1 + \dot{x}_4 < 0$ & If $x_5 > \bar{x}_5$, then $\dot{x}_5 < 0$. Otherwise, $\dot{x}_4 > 0$ and $\dot{x}_1 < 0$

Remark. If you look at Table 1 the behavior of the equations of the reduced system (4) tell us that no matter where we start in a region we approach the equilibrium point as time increases. Thus, the system seems to be globally stable, but a few details remain to be worked out. It is important to note that there are still cases in which we can not determine the behavior of the equations. For example, the behavior for the equation $\dot{x}_1$ can not be determined when $x_1 > \bar{x}_1$ and $x_5 > \bar{x}_5$.

6. DISCUSSION

For any choice of parameters, the dynamical system (2) has a unique positive steady state, and Chifman *et al.*'s simulations suggest it is globally stable. This recapitulates biology: in healthy cells, iron levels are tightly controlled. Proving the global stability of this model is yet to be done. Even *local* stability is currently intractable due to the number of unknown parameters. This is why we use a reduced to model to gain more insight on how the Chifman model behaves.

We showed that our approximation of the Chifman model has a unique positive steady state and it is locally stable. We also use a geometrical analysis to provide evidence that the behavior of the model points toward global stability. Since our model is a biologically reasonable simplification of the Chifman model, the analysis on the simplificated model can serve as a guide to proving the global stability of the Chifman model. We conjecture that the full system described by Chifman *et al.* in [1] has a globally stable steady state based on the dynamics of our reduced model and the heuristic approach established by the paper. However, the high number of parameters and non-linear nature of the model make it difficult to use symbolic computation to establish stability of any kind.

Can the same geometrical analysis be done in the Chifman model? What if we reduced the system into four differential equations instead of three? Is there another way to simplify the model and still be close to the original Chifman model? What happens for a model without any iron export? What if we get rid of the IRP's and let the LIP directly control the rates? What if we include the regulation of iron import in the simplified model? Is there a way to find the actual values of the parameters by experimentation?

There are still many questions that need to be answered. It is important to carry out a more extensive validation of the model. Proving the global stability of the system would mean that the system could potentially be used for medical purposes. The model can be expanded to make it a good model for identifying the key regulators in the iron metabolism network that are modified as breast epithelial cells transition into malignancy.

Acknowledgements

We would like to thank our mentor, Dr. Anne Shiu. This research experience would not have been possible if not for her mentorship and guidance. We would also like to thank Robert Williams for his assistance and Dr. Paul Lindhal for helping with the biological aspects of the research.

This research was conducted as part of the NSF-funded REU program in Mathematics at Texas A&M University (DMS-1460766), Summer 2016.

References

[1] J. Chifman, A. Kniss, P. Neupane, I. Williams, B. Leung, Z. Deng, P. Mendes, V. Hower, F.M. Torti, S.A. Akman, S.V. Torti, and R. Laubenbacher. The core control system of intracellular iron homeostasis: A mathematical model. *Journal of Theoretical Biology*, 300:91–99, 2012.

[2] H.W. Hann, M.W. Stahlhut, and B.S. Blumberg. Iron nutrition and tumor growth: decreased tumor growth in iron-deficient mice. *Cancer Res.*, 48(15):4168–4170, 1988.

[3] H.W. Hann, M.W. Stahlhut, and H. Menduke. Iron enhances tumor growth: observation on spontaneous mammary tumors in mice. *Cancer*, 68(11):2407–2410, 1991.

[4] V. Hower, P. Mendes, F.M. Torti, R. Laubenbacher, S. Akman, V. Shulaev, and S.V. Torti. A general map of mammalian iron metabolism and tissue-specific subnetworks. *Mol. BioSyst.*, 5:422–443, 2009.

[5] X. Huang. Does iron have a role in breast cancer? *Lancet Oncol.*, 9(8):803–807, 2008.

[6] S.P. Hussain, K. Raja, P.A. Amstad, M. Sawyer, L.J. Trudel, G.N. Wogan, L.J. Hofseth, P.G. Shields, T.R. Billiar, C. Trautwein, T. Hohler, P.R. Galle, D.H. Phillips, R. Markin, A.J. Marrogi, and C.C. Harris. Increased p53 mutation load in nontumorous human liver of wilson disease and hemochromatosis: oxyradical overload diseases. *Proc. Natl. Acad. Sci.*, 97(23):12770–12775, 2000.

[7] Granino A. Korn and Theresa M. Korn. Mathematical handbook for scientists and engineers. 2nd ed.(4-5):17–18, 1968.

[8] J.D. Murray. Mathematical biology i: An introduction. 17:507–508, 2002.

[9] C. Niederau, R. Fischer, A. Sonnenberg, W. Stremmel, H.J. Trampisch, and G. Strohmeyer. Survival and causes of death in cirrhotic and in noncirrhotic patients with primary hemochromatosis. *Proc. Natl. Acad. Sci.*, 313(20):1256–1262, 1985.
[10] Z.K. Pinnix, L.D. Miller, W. Wang, R. Jr. D'Agostino, T. Kute, M.C Willingham, H. Hatcher, L. Tesfay, G. Sui, X. Di, S.V. Torti, and F.M. Torti. Ferroportin and iron regulation in breast cancer progression and prognosis. *Sci. Transl. Med.*, 2(43):43–56, 2010.
[11] R. Stevens, Y. Jones, M. Micozzi, and P. Taylor. Body iron stores and the risk of cancer. *N. Engl. J. Med.*, 319(16):1047–1052, 1988.

Appendix A. Geometric Analysis of the Regions

This appendix contains the various cases that were analyzed in order to find the behavior of the differential equations found in system (4).

A.1. The equation $\dot{x}_4$.

Here we analyze

$$\dot{x_4} = \alpha_4 x_1 \frac{k_{54}}{k_{54} + x_5} - \gamma_4 x_4.$$

Proposition A.1. *If $x_1 < \bar{x}_1$, $x_4 > \bar{x}_4$, and $x_5 > \bar{x}_5$, then $\dot{x}_4 < 0$.*

Proof. Assume $x_1 < \bar{x}_1, x_4 > \bar{x}_4$, and $x_5 > \bar{x}_5$. Then,

$$\dot{x}_4 = \alpha_4 x_1 \frac{k_{54}}{k_{54} + x_5} - \gamma_4 x_4 < \alpha_4 \bar{x}_1 \frac{k_{54}}{k_{54} + \bar{x}_5} - \gamma_4 \bar{x}_4 = 0$$

Therefore $\dot{x}_4 < 0$. □

Proposition A.2. *Let $x_4 < \bar{x}_4$, $x_1 > \bar{x}_1$, and $x_5 < \bar{x}_5$, then $\dot{x}_4 > 0$.*

Proof. Assume $x_4 < \bar{x}_4$, $x_1 > \bar{x}_1$, and $x_5 < \bar{x}_5$. Then,

$$\dot{x}_4 = \alpha_4 x_1 \frac{k_{54}}{k_{54} + x_5} - \gamma_4 x_4 > \alpha_4 \bar{x}_1 \frac{k_{54}}{k_{54} + \bar{x}_5} - \gamma_4 \bar{x}_4 = 0$$

Therefore $\dot{x}_4 > 0$. □

A.2. The equation $\dot{x}_5$.

Here we analyze the equation

$$\dot{x_5} = \alpha_5 \frac{k_{15}}{k_{15} + x_1} - \gamma_5 x_5.$$

Proposition A.3. *If $x_1 > \bar{x}_1$ and $x_5 > \bar{x}_5$, then $\dot{x}_5 < 0$.*

Proof. Assume $x_1 > \bar{x}_1$ and $x_5 > \bar{x}_5$. Then,

$$\dot{x_5} = \alpha_5 \frac{k_{15}}{k_{15} + x_1} - \gamma_5 x_5 < \alpha_5 \frac{k_{15}}{k_{15} + \bar{x}_1} - \gamma_5 \bar{x}_5 = 0$$

Therefore $\dot{x}_5 < 0$. □

Proposition A.4. *If $x_1 < \bar{x}_1$ and $x_5 < \bar{x}_5$, then $\dot{x}_5 > 0$.*

Proof. Assume $x_1 < \bar{x}_1$ and $x_5 < \bar{x}_5$. Then,

$$\dot{x_5} = \alpha_5 \frac{k_{15}}{k_{15} + x_1} - \gamma_5 x_5 > \alpha_5 \frac{k_{15}}{k_{15} + \bar{x}_1} - \gamma_5 \bar{x}_5 = 0$$

Therefore $\dot{x}_5 > 0$. □

A.3. **The equation $\dot{x}_1$.** Here we analyze the equation

$$\dot{x_1} = \hat{\alpha}_1 \mathrm{Fe}_{\mathrm{ex}} + \gamma_4 x_4 - \hat{\alpha}_6 x_1 - \alpha_4 x_1 \frac{k_{54}}{k_{54} + x_5}. \tag{9}$$

Proposition A.5. *If $x_1 < \bar{x}_1$, $x_4 > \bar{x}_4$, and $x_5 > \bar{x}_5$, then $\dot{x}_1 > 0$.*

Proof. Assume $x_1 < \bar{x}_1$, $x_4 > \bar{x}_4$, and $x_5 > \bar{x}_5$. Then,

$$\dot{x_1} = \hat{\alpha}_1 \mathrm{Fe}_{\mathrm{ex}} + \gamma_4 x_4 - \hat{\alpha}_6 x_1 - \alpha_4 x_1 \frac{k_{54}}{k_{54} + x_5} > \hat{\alpha}_1 \mathrm{Fe}_{\mathrm{ex}} + \gamma_4 \bar{x}_4 - \hat{\alpha}_6 \bar{x}_1 - \alpha_4 \bar{x}_1 \frac{k_{54}}{k_{54} + \bar{x}_5} = 0$$

Therefore $\dot{x}_1 > 0$. □

Proposition A.6. *If $x_1 > \bar{x}_1$, $x_4 < \bar{x}_4$, and $x_5 > \bar{x}_5$, then $\dot{x}_1 < 0$.*

Proof. Assume $x_1 > \bar{x}_1$, $x_4 < \bar{x}_4$, and $x_5 > \bar{x}_5$. Then,

$$\dot{x_1} = \hat{\alpha}_1 \mathrm{Fe}_{\mathrm{ex}} + \gamma_4 x_4 - \hat{\alpha}_6 x_1 - \alpha_4 x_1 \frac{k_{54}}{k_{54} + x_5} < \hat{\alpha}_1 \mathrm{Fe}_{\mathrm{ex}} + \gamma_4 \bar{x}_4 - \hat{\alpha}_6 \bar{x}_1 - \alpha_4 \bar{x}_1 \frac{k_{54}}{k_{54} + \bar{x}_5} = 0$$

Therefore $\dot{x}_1 < 0$. □

Department of Mathematics, University of Puerto Rico, Río Piedras Campus, San Juan, PR 00931
E-mail address: adriana.morales@upr.edu

Department of Mathematical Sciences, Ripon College, Ripon, WI 54971
E-mail address: eithunm@ripon.edu