

# Mitochondrial Iron: a Mathematical Model for Iron Regulatory Disease

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## Abstract

Friedreich's ataxia (FRDA) is a genetic disease that leads to deficiency in the mitochondrial protein, Frataxin. In turn an accumulation of iron begins to propagate within the mitochondria resulting in cell death by oxidative stress. In this work we present a mechanistic mathematical model that incorporates both cytoplasm and mitochondria key processes responsible for bring iron balance to a healthy cell and permanent imbalance in an unhealthy cell. We hope to gain insight into the still unclear biological course of the FRDA within a human cell. Through sensitivity analysis we investigate components that are the most critical to the system and that can lead to effective treatments. Numerical simulations show that the model captures some biological properties necessary to model the role of iron and the interplay of various cellular processes within the cell. Our research seeks to understand and construct a biologically relevant robust model of iron homeostasis that can be used to in silico test the effects of Frataxin deficiency while incorporating both mitochondrial and cytoplasmic iron processes.

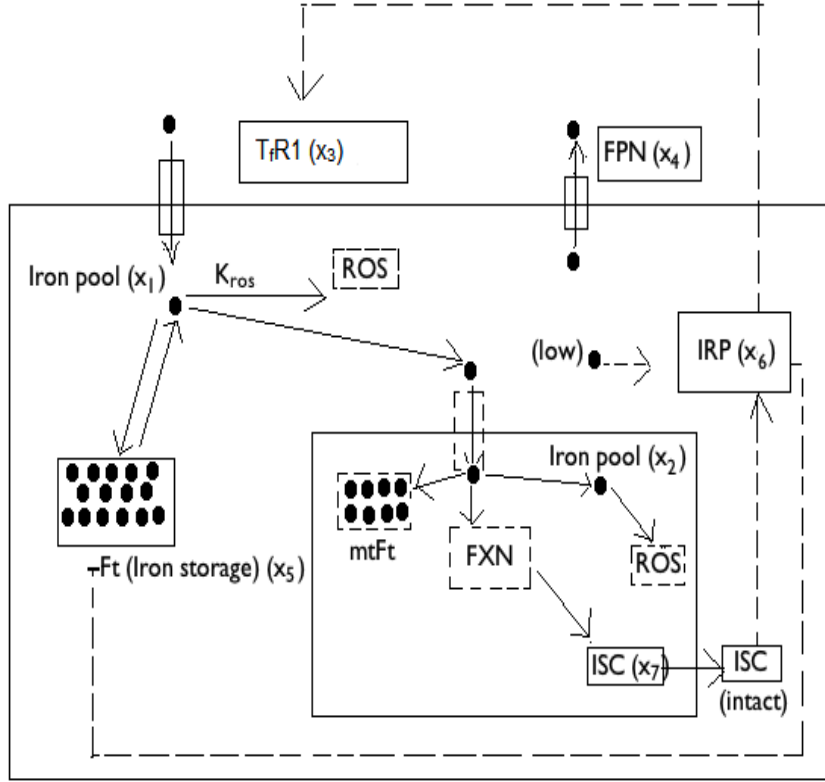
## 1 Introduction to Cellular iron Homeostasis

Iron homeostasis has been targeted as a mechanism for diseases ranging from cancer to diabetes. Some aspects of iron regulation take place within the mitochondria, whereas others occur in the cytoplasm [13]. Iron regulatory proteins (IRP) are vital in this process, as they are designed to respond to iron needs within the cell. Frataxin is a protein thought to deliver iron to aid in assembling Iron Sulphur Clusters (ISC's). A deficiency of Frataxin causes the citric acid cycle to fail. With too little ISC to carry out cellular respiration, ISC redirects itself towards the cytoplasm, where it signals the iron regulatory proteins to prompt the influx of more iron into the cell [13]. Biologists have noted that a deficiency of Frataxin causes an explosion of iron concentration in the mitochondria of the cell, Thus causing excess iron buildup, such that free iron particles will combine with reactive oxygen species (ROS) to produce damaging hydroxyl free radicals in a process known as oxidative stress [14, 13, 32].

Frataxin protein deficiency was targeted as the disease mechanism of Friedreich's Ataxia, an autosomal recessive hereditary disorder. Over time of Friedreich's Ataxia severely degrades an individual's motor coordination and often leads to premature death [1]. However, hereditary ataxias affect only a small minority in the world population (with a prevalence of approximately 10 per 100,000 affected). As a result, few treatments have been developed to address the biological causes of these diseases [10]. Additionally, many Friedreich's Ataxia patients are plagued with other diseases such as scoliosis, cardiomyopathy or diabetes. Most physical symptoms are due to a cellular iron buildup over long periods of time. [10]. Existing treatments have traditionally focused on rehabilitating parts of the body affected by disease symptoms. [1]. The genetic aspect of the disease, iron homeostasis as it pertains to other disease pathways [7], cellular respiration and oxidative phosphorylation have all been modeled in either a biological or mathematical context [22][8], [11], [12]. However, a mathematical model of iron homeostasis that incorporates both the mitochondrial and cytoplasmic iron levels has not yet been introduced. Such a the cellular model can be manipulated from normal levels of Frataxin to a diseased state in a manner proportional to the Frataxin levels. [32] [24]. Our research seeks to understand and construct a biologically relevant robust model of iron homeostasis that can be used to in silico test the effects of Frataxin deficiency while incorporating both mitochondrial and cytoplasmic iron processes.

## 2 The Model

As shown in the Figure 1, the pathway of regulation in iron homeostasis is essential to modeling diseases. Previous work by mathematicians has focused on iron levels within the cell and mainly on the relationship of the iron levels in the cytoplasm [14], [35]. Recent work in biology has studied the interplay between the cytoplasm and mitochondria.



----- Functional relation, — Flux, —| Inhibition, —> Activation

Figure 1: **A Diagram of Iron Homeostasis in the Cell.** Iron is traced from the Transferrin Receptors,  $T_fR1$  ( $x_3$ ), to the cytoplasm iron pool ( $x_1$ ), to either Ferritin,  $F_t$  ( $x_5$ ), sequestration or to the mitochondrial iron pool ( $x_2$ ), where Frataxin delivers the iron for Iron-Sulphur Cluster,  $ISC$  ( $x_7$ ), production. Upon export into the cytoplasm, the pathway is a feedback loop monitored by  $IRP$ 's ( $x_6$ ) and dependent on the level of  $ISC$ 's present. Feedback regulates the import and export of iron through the Ferroportin pumps,  $FPN$  ( $x_4$ ), and the Transferrin Receptors.

To generate a model that captures the process of iron homeostasis we have to map the relationship between cellular processes while considering these two components. Extra cellular iron is brought into the cell by the membrane bound protein Transferrin Receptors ( $T_fR1$ ,  $x_3$ ), a process limited by the availability of extracellular iron ( $Fe_0$ ) Upon arrival into the cytoplasm iron weakly binds to cellular elements (labile iron pool,  $x_1$ ) another three paths, 1) iron is sequestered by Ferritin ( $F_t$ ,  $x_5$ ) a storage protein 2) removed by reactions with other elements, such as contributing to the Reactive Oxygen Species (ROS) the Fenton Reaction or 3) the amount left (usually very small) can be circulated into the

mitochondria. The mechanisms by which iron enters into the mitochondria are not well understood at this time, so we use carrier-like transporters to model iron uptake by the mitochondria. Once in the mitochondrial iron pool ( $x_2$ ), similar to the cytoplasm, iron is stored by mitoferritin ( $m_t F_t$ ) and removed by Reactive Oxygen Species (ROS). We model the proposed role for Frataxin as to remove iron from the mitochondrial iron pool ( $x_2$ ) and deliver to the Iron-Sulphur Clusters. Once fully processed the Iron-Sulphur Clusters ISC, ( $x_7$ ) are removed to the cytoplasm where they can communicate with IRP's. This pathway is based on the level of ISC's available in the cytoplasm. There are two main Iron Regulatory Proteins (IRP1 and IRP2) involved in iron regulation but for the purpose of this work we consider them together and treat them as a variable (IRP,  $x_6$ ). IRP responds to the presence or absence of ISC and iron levels in the cytoplasm. Intact ISC's causes IRP's to behave like cytoplasmic aconitase. On the other hand, low levels of ISC causes IRP's to activate the Transcription of mRNA to bring more iron into the cell and inhibit FPN and Ft, which increases iron in the cytoplasm.

In order to accurately describe the processes that occur between each step of the iron regulatory pathway there are multiple processes described through various parameters in our model. See Table 1 on Section 1. In general, our parameters  $\beta$ ,  $\kappa$ ,  $\mu$  and  $\alpha$  are designed to model total flux, threshold levels, degradation rates, and per capita rates, respectively. A detail description of specific parameters and their functions (i.e. role in the system) is found in Table 2 of Section 2. Our mathematical model is an extension of previous work by [7], [35].

Thus, the model includes seven regulatory mechanisms, that we assume have a hyperbolic behavior as is common in modeling in metabolic routes to model activation and inactivation relations we use:

$$\frac{\beta x_i}{\kappa + x_i} \quad \text{and} \quad \frac{\beta \kappa}{\kappa + x_i}, \quad (1)$$

Where  $x_i$  is an activating/inhibiting state variable and  $\kappa$  is the activation threshold for  $i \in 1, 3, 5, j \in 2, 3, 4, 5$  and  $\beta$  is the maximum flux of the associated regulatory mechanism. Each protein undergoes self-degradation, thus the linear term  $\mu x_i$ , where  $\mu$  is a constant decay rate (as is explained above). In summary, our model is a representation of the following physiological phenomena:

- a) The extracellular iron is the only source and a limiting factor for iron movement into the cytoplasm.
- b) The amount of Ferritin is regulated by the feedback of the IRP's available for iron buffering.
- c) The iron uptake by the mitochondria is limited by the availability of iron in the cytoplasm.

- d)  $T_fR1$  expression (amount) is regulated by the IRP feedback loop. Activation of Transferrin Receptors by IRP's.
- e) FPN expression (amount) is regulated by the IRP feedback loop. Inactivation of Ferroportin by IRP's.
- f) IRP's are inactivated by the accumulation of iron in the cytoplasm.
- g) IRP's are inactivated by increased levels of intact ISC's.

Parameter	Definition	Units	Values
$x_1$	Iron concentration in cytoplasm	$\mu M$	10.08
$x_2$	Iron concentration in mitochondria	$\mu M$	7.56
$x_3$	Level of expression of $T_fR1$ receptors	<i>unitless</i>	0.06
$x_4$	Level of expression of $FPN$ pump	<i>unitless</i>	0.08
$x_5$	Ferritin concentration in the cytoplasm	$\mu M$	9.29
$x_6$	IRP concentration in cytoplasm	$\mu M$	6.85
$x_7$	ISC concentration in cytoplasm	$\mu M$	7.60

Table 1: **Initial Conditions.** All the units described above were based upon those reported by [35], [36], the steady state values of the state variables with parameters as shown, were obtained by evaluating equilibrium points using Maple 16. ??

$$\begin{aligned}
\dot{x}_1 &= \beta_{T_fR1}^1 \frac{Fe_O}{\kappa_{T_fR1}^1 + Fe_O} x_3 - \alpha_{FPN} x_1 x_4 + \mu_{F_t} x_5 - \beta_{F_t} \frac{1}{\kappa_{F_t} + x_6} x_1 - \beta_{mi} \frac{x_1}{\kappa_{mi} + x_1} - \kappa_{ROS} x_1 \\
\dot{x}_2 &= \beta_{mi} \frac{x_1}{\kappa_{mi} + x_1} - x_2 (\mu_{FXN} + \mu_{mtF_t} + \kappa_{ROS}) \\
\dot{x}_3 &= \alpha_{T_fR1} \frac{x_6}{\kappa_{T_fR1}^2 + x_6} - \mu_{T_fR1} x_3 \\
\dot{x}_4 &= \beta_{FPN} \frac{1}{\kappa_{FPN} + x_6} - \mu_{FPN} x_4 \\
\dot{x}_5 &= \beta_{F_t} \frac{1}{\kappa_{F_t} + x_6} x_1 - \mu_{F_t} x_5 \\
\dot{x}_6 &= \beta_{IRP} \frac{\kappa_{IRP}^1}{\kappa_{IRP}^1 + x_1} - \mu_{IRP} x_6 + \beta_{ISC} \frac{\kappa_{IRP}^2}{\kappa_{IRP}^2 + x_7} \\
\dot{x}_7 &= \rho \mu_{FXN} x_2 - \mu_{ISC} x_7
\end{aligned}$$

Model Parameters Units and Values.			
Parameter	Definition	Units	Values
$\rho$	conversion from Frataxin to ISC	unitless	1
$Fe_O$	Iron concentration in the Blood	$\mu M$	17
$\beta_{T_fR1}^1$	maximum rate of flux of $Fe^{2+}$ through $T_fR1$	$\mu M(sec^{-1})$	800
$\kappa_{T_fR1}^1$	concentration of $Fe_O$ that produces half maximum activity of $T_fR1$	$\mu M$	1
$\alpha_{T_fR1}$	maximum per capita rate of $T_fR1$ assembly modulated by IRP's	$sec^{-1}$	0.3
$\mu_{T_fR1}$	degradation rate of $T_fR1$	$sec^{-1}$	1
$\kappa_{T_fR1}^2$	concentration of $IRP$ that produces half maximum per capita rate of $T_fR1$ assembly	$\mu M$	5
$\mu_{F_t}$	degradation rate for Ferritin	$sec^{-1}$	0.5
$\beta_{F_t}$	maximum rate of flux of iron sequestration by Ferritin	$\mu M(sec^{-1})$	5
$\kappa_{F_t}$	concentration of Ferritin that produces half maximum sequestration rate	$\mu M$	4
$\beta_{mi}$	maximum rate of flux $Fe^{2+}$ moved into the mitochondria by iron transporters in mitochondria membrane	$\mu M(sec^{-1})$	5
$\kappa_{mi}$	concentration of cytoplasmic iron that produces half maximum flux of mitochondrial uptake	$\mu M$	3
$\mu_{FXN}$	reaction rate of Frataxin on iron binding	$sec^{-1}$	0.1
$\kappa_{ROS}$	reaction rate of $ROS$	$sec^{-1}$	0.5

Model Parameters Units and values (cont.).			
Parameter	Definition	Units	Values
$\mu_{m_t F_t}$	reaction rate of $m_t F_t$ binding	$sec^{-1}$	0.1
$\kappa_{FPN}$	concentration of IRP that produces half maximum rate of FPN assembly	$\mu M$	5
$\mu_{FPN}$	degradation rate of FPN	$sec^{-1}$	1
$\alpha_{FPN}$	per capita transportation rate of $Fe^{2+}$ via FPN	$sec^{-1}$	10
$\beta_{FPN}$	maximum rate of flux of FPN assembly modulated by cytoplasmic $Fe^{2+}$	$\mu M(sec^{-1})$	10
$\mu_{IRP}$	degradation rate	$sec^{-1}$	0.1
$\beta_{IRP}$	maximum rate of flux of IRP assembly modulated by cytoplasmic $Fe^{2+}$	$\mu M(sec^{-1})$	1
$\kappa_{IRP}^1$	concentration of $Fe^{2+}$ that produces half maximum rate of IRP assembly modulated by $Fe^{2+}$	$\mu M$	4
$\kappa_{IRP}^2$	concentration of ISC that produces half maximum rate of IRP assembly modulated by ISC	$\mu M$	5
$\mu_{ISC}$	degradation rate of ISC	$sec^{-1}$	0.05
$\beta_{ISC}$	maximum rate of flux of IRP assembly modulated by ISC	$\mu M(sec^{-1})$	0.05

All the parameters described above were estimated by modifying those reported by [35],[36]

### 3 Results

Studies have shown an inverse relationship between Frataxin levels and repeat length of a gene (GAA). To model the effect of Frataxin induced  $Fe^{2+}$  levels,  $\mu_{FXN}$  was lowered by 50 percent, reflecting non functional Frataxin levels at  $t = 60$  seconds and allowed to run until  $t = 180$  when equilibrium was once again achieved. At that point the Frataxin levels were brought back to normal levels. The system returned to its previous steady state after 60 seconds. It has been showed that Frataxin levels can be inhibited up to 40% without symptomatic expression. In our simulation a decrease of 20%  $\sim$  40% Frataxin levels causes an increase of iron less than  $< 50\%$ . Frataxin deficiencies displayed a quasi-exponential behavior in relation to intracellular iron, jumping as high as 26.09% and 48.7% for Frataxin deficiencies of 60% and 80% respectively. In contrast the iron level in the mitochondria had an almost linear relationship with Frataxin levels, changing by about 5% for each 20% decrease in Frataxin. The non-linear cytoplasmic iron response can be explained by a feedback loop that includes changing Ferroportin levels regulated by the IRP's. In contrast the mitochondrial iron levels are not out of the feedback loop.

$T_f R1$  expression displayed an exponential increase whereas Ferroportin expression decreased in a slightly more linear fashion. The actual change in these levels was quite small compared to the change in iron levels. This indicates that the movement of iron across



## Perturbation of Cellular Frataxin Levels

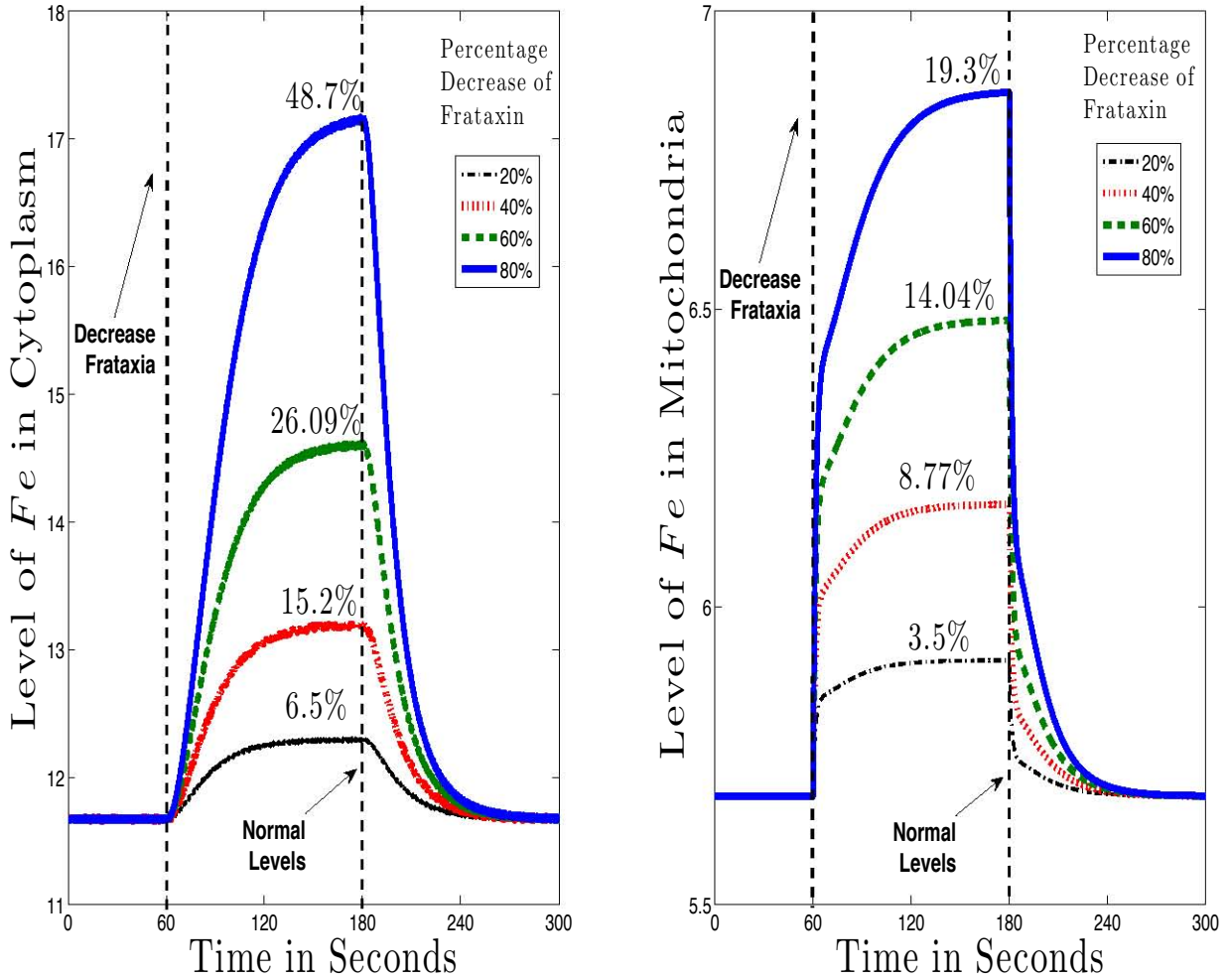


Figure 2: From steady state Frataxin levels are decreased up to 80% less than normal levels. This results in quasi-exponential increase in cytoplasmic iron levels and a linear increase in mitochondrial iron.

the cell membrane can be accomplished by just a small variation on the Transferrin and Ferroportin Levels of expression. This confirms the expected behavior under Frataxin deficient conditions and shows that the iron pathway is disregulated because iron levels are not deficient but the change in Frataxin triggers IRP's activity and thus  $Fe^{2+}$  mobilization into the cell.

From above we conclude that the model captures the disease mechanism of Frataxin deficiency. However they maybe other parameters that have a strong effect upon the sys-

tem. We proceed with a sensitivity analysis to determine what parameters contribute to variations in homeostatic iron levels. As shown in Figure 3 we plot the mitochondrial and cytoplasmic iron levels dependence between each other. The parameters are perturbed using the Central-Difference Formula such that the sensitivity can be calculated. The sensitivities of each parameter with respect to mitochondrial and cytoplasmic concentrations were plotted. The level of sensitivity can be inferred as a distance from the origin.

### Percentage Change of Iron in the Mitochondria and Cytoplasm

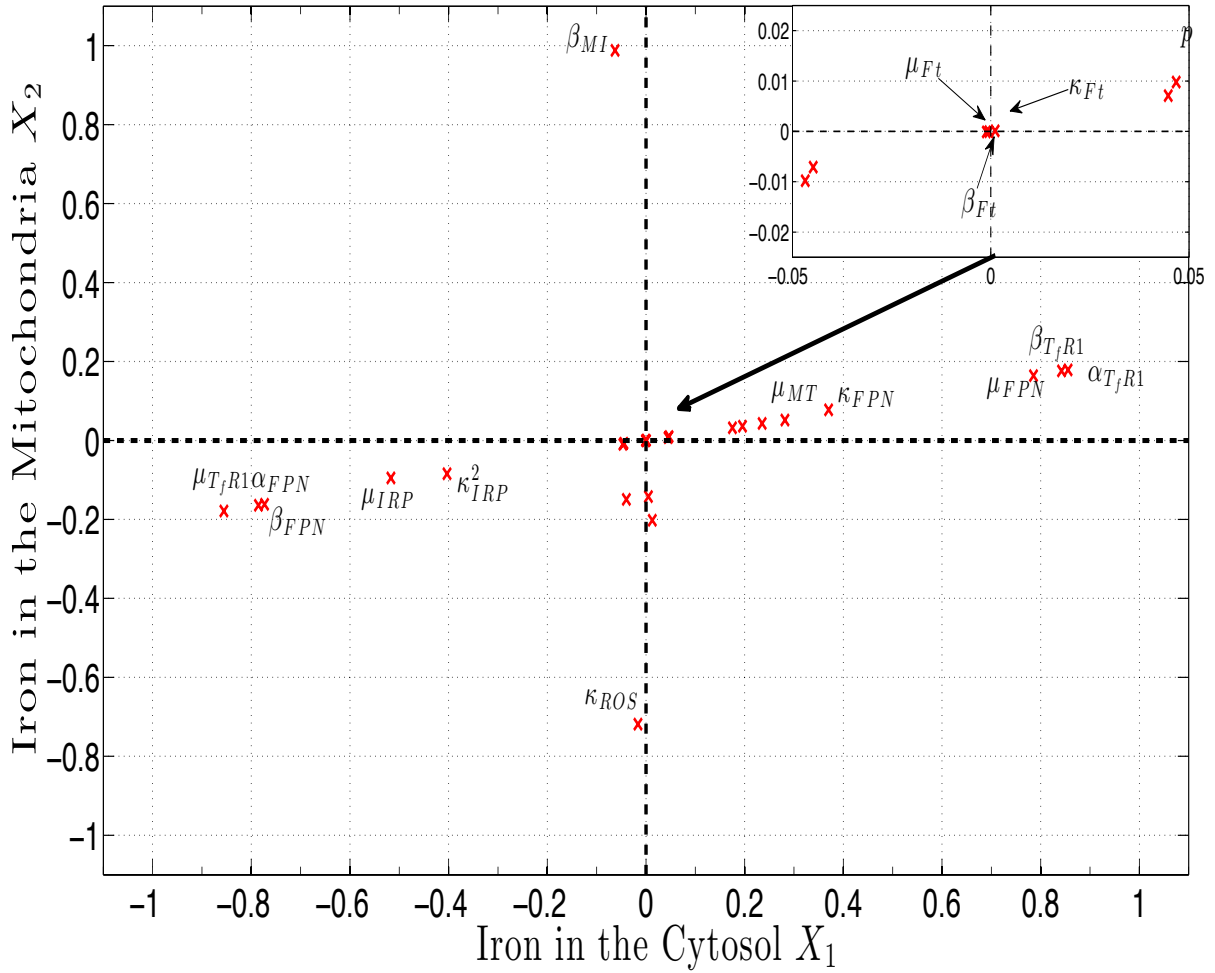


Figure 3: **Sensitivity of mitochondrial and cytoplasmic iron to parameters.** *The sensitivity of the iron pools to parameters is highest for perturbations in  $\mu_{TfR1}$ ,  $\beta_{FPN}$ ,  $\alpha_{FPN}$ ,  $\beta_{TfR1}$ ,  $\alpha_{TfR1}$ , and  $\mu_{FPN}$ .*

By looking at Figure 3 one can see that an increase of 1% in  $\alpha_{TfR1}$ ,  $\beta_{TfR1}$  or  $\mu_{FPN}$  leads

to an approximate 0.78%  $\sim$  0.9% increase in the amount of iron in the cytosol.

$T_fR1$  expression is regulated by IRP's, thus a positive correlation can be seen between transferrin levels and the IRP concentration. In the other hand, an increase in  $\mu_{T_fR1}$ ,  $\alpha_{FPN}$ , or  $\beta_{FPN}$  leads to an approximate 0.78%  $\sim$  0.85% decrease in the amount of iron in the cytosol. An increase in transferrin degradation,  $\mu_{T_fR1}$ , yields the decrease of iron in the cytosol; less  $Fe^{2+}$  is moving into the cell.

In the mitochondrial pool, an increase of 1% change in  $\beta_{mi}$  leads to an approximate 1% increase in iron. With  $\kappa_{ROS}$ , an increase of 1% leads to an approximate 0.7% decrease of iron, and represents the relative amount of  $Fe^{2+}$  removed from the pool by Fenton Reaction.

The sensitivity of the iron pools to parameters is highest for perturbations in  $\mu_{T_fR1}$ ,  $\beta_{FPN}$ ,  $\alpha_{FPN}$ ,  $\beta_{T_fR1}$ ,  $\alpha_{T_fR1}$ , and  $\mu_{FPN}$ . Thus it appears that manipulation of parameters that influence Ferroportin and Transferrin receptors may be good targets for instituting change in the system. In the context of Friedreich's Ataxia it may prove helpful to target a form of treatment that either shuts down the receptors or that increases the activity of Ferroportin pump activity or  $F_t$  buffering.

Following the work of Szabolcs [35] it is important to consider the effect intracellular iron Figure ??, Figure 4. Initially the iron levels outside the cell ( $Fe_O$ ) were considered to be at normal homeostasis levels (17  $\mu M$ ). At time  $t = 60$  the iron level outside of the cell was manipulated by increasing the level of  $Fe_O$  to twice its normal levels (34  $\mu M$ ). The responses of the state variables were graphed and recorded as shown in Figure ?? panel A (left). In contrast, in panel B (right) at time  $t = 60$  the iron level outside of the cell was decreased to reflect anemic conditions (1  $\mu M$ ). After  $t = 120$  in both panels the iron levels are returned to normal.

The highest levels of change, when extracellular iron increases, are found in the Cytoplasmic iron concentration ( $x_1$ ) and Ferritin ( $x_5$ ). During  $Fe_O$ , during anemic conditions Ferritin level drops from 12 to 7  $\mu M$  and the Level of iron in the cytoplasm drops about the same amount. Biologically, this appears to lessen the effect of iron loss in the mitochondria, since the level of mitochondrial iron only decreases from 6 to 5  $\mu M$ . It is interesting to note that the larger response occurs when iron levels are low, as opposed to when iron is high. High iron conditions affect the Cytoplasmic iron concentration ( $x_1$ ) and Ferritin ( $x_5$ ) levels by less than 1  $\mu M$ . Perhaps this is a throwback to biological and evolutionary survival implications such that the body will work harder to obtain the nutrients it needs when none are available, rather than to work towards conservation of nutrients in nutrient rich environments.

Note also in Figure 4 the Mitochondrial Iron levels ( $x_2$ ), and the Iron Regulatory Proteins ( $x_6$ ) (Center) have an antagonistic effect upon each other, however, they affect each other in equal magnitude. Under iron rich conditions, the concentration of Iron Regulatory Proteins decreases approximately .1  $\mu M$  and the level of mitochondrial iron increases sharply

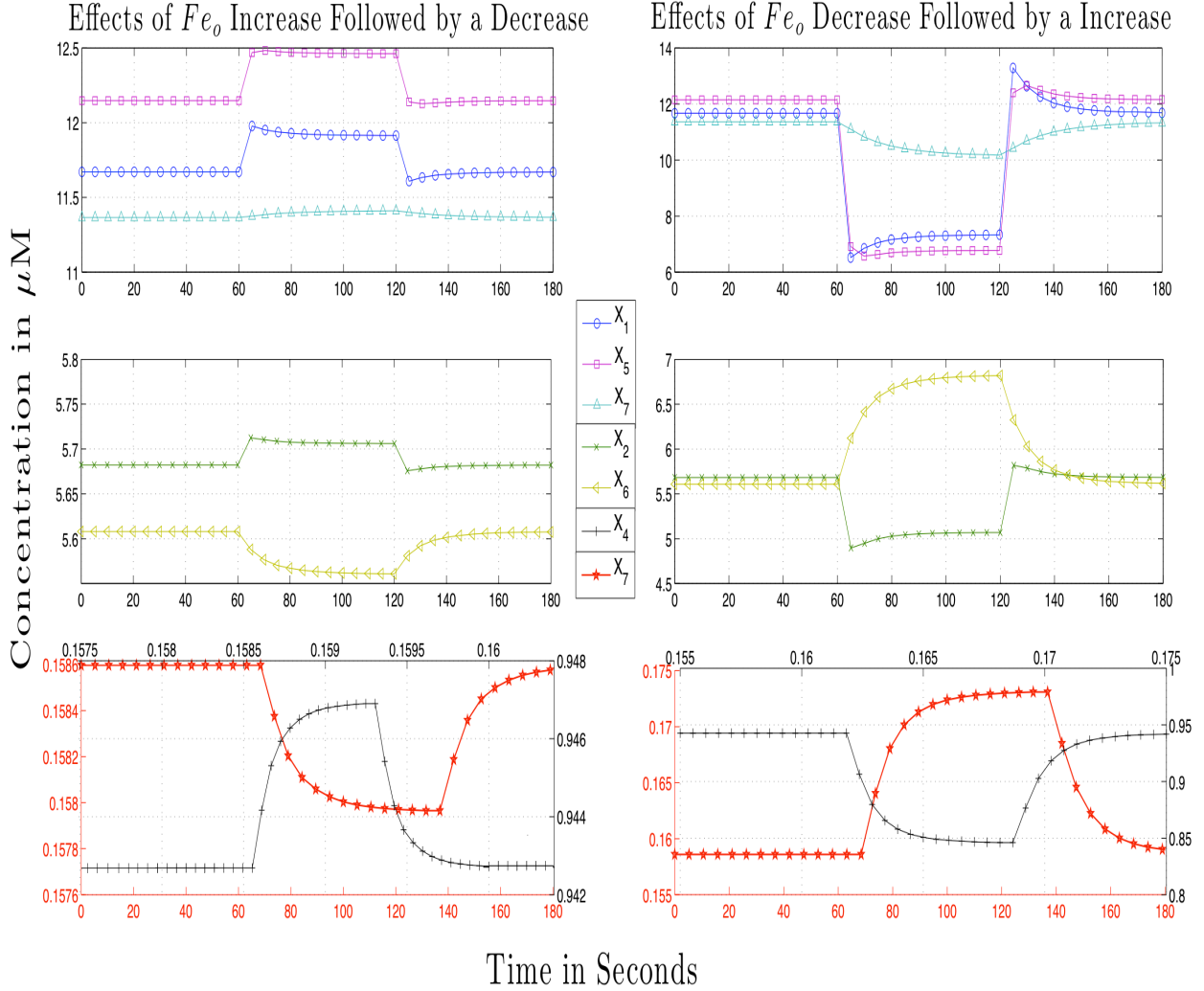


Figure 4: Manipulation of Extracellular Iron Levels. Magnification of ?? yields relative rection to  $Fe_0$  perturbation. The relationship between the Cytoplasmic Iron concentration ( $x_1$ ), Ferritin Receptors ( $x_3$ ), and Iron Sulphur Cluster ( $x_7$ ) production is shown for excess extracellular iron (top left), and then under anemic conditions (top right). The center graphs map the the reallionship between mitochondrial iron and the prevalence of IRP's. A .1 increase in mitochondrial iron triggers a gradual decrease of the same magnituse in the expression of IRP's. The lower graphs show that a decrease in Iron Sulphur Cluster Production yeilds an increase in ISC production of about .006  $\mu M$

by the same amount. Under iron poor conditions, both behave in the same manner but at a greater magnitude of about 1.5  $\mu M$ . The Production of Iron Sulphur Clusters decreases in iron poor conditions by a very small amount, about .015  $\mu M$ . The concentration of Ferroportin increases by about .005  $\mu M$ . Under iron rich conditions, however the Ferro-

portin decreases by about  $.002 \mu M$  and the Iron Sulphur Cluster production increases by the same amount.

These trends and differences under iron poor conditions indicate that the mitochondrial iron pathway remains conserved as much as possible, and that iron is always delivered to the mitochondria for use. This involves shutting down Ferroportin and increasing iron intake. Under iron rich conditions, there are some processes such as ferroportin activity that increase, but only slightly. Others, such as mitochondrial iron uptake actually shut down or decrease. This indicates that the mitochondrial iron process is conserved and varies little under extreme conditions outside of the cell. It also shows that the cytoplasm serves as a buffer to protect damage to the mitochondria when homeostasis does not occur.

Sensitivity analysis earlier revealed that the parameters involved in regulating Transferrin Receptors and Ferroportin pumps are likely to have the greatest effect upon the system. A disease state was also induced by manipulating levels of Frataxin protein. This gives rise to the potential to experiment with numerical simulation of treatment options. Proceeding in a fashion similar to the previous results, a healthy cell was induced to a diseased state at  $t = 60$  and then allowed to reach new homeostasis levels. At  $t = 220$  the cell was perturbed with a form of treatment. It was hypothesized that the manipulation of  $\alpha_{TFR1}$  would decrease the iron entering the cell, and that a similar but opposite effect would occur by increasing ferroportin  $\alpha_{FPN}$  activity. These results were confirmed when the diseased state was altered with treatment. The decrease in transferrin by 50 percent, from  $17 \mu M$  originally resulted in a new homeostasis that was lower in cytoplasmic iron ( $10 \mu M$ ) and slightly higher in mitochondrial iron levels ( $6 \mu M$ ) than before. Increasing the Ferroportin resulted in elevated levels of both mitochondrial and cytoplasmic iron levels, but it was still an improvement over the disease state. By potentially changing the flux mechanisms through a gene therapy could also provide a method for treatment. Such a therapy is currently being considered as a form of treatment [39],[41]. The results obtained from this method do not appear to reduce the system from the diseased state when both  $\beta_{ISC}$  and  $\beta_{FPN}$  are manipulated to increase their activity. However, when  $\beta_{ISC}$  is reduced by 50 percent and  $\beta_{FPN}$  is increased by 50 percent, the treatment does show some effectiveness, but not as well as reducing the Transferrin Receptors. Neither treatment had a significant effect upon the mitochondrial iron.

## 4 Conclusions

To summarize, our systematic extensive numerical investigation shows that we have a unique physiologically relevant and stable steady state in our model. Our model captures the feedback mechanism of the cellular iron regulation process proposed in the biological literature. This work shows that iron control system in the cell is more robust under iron deficiency, decrease levels of extracellular iron activating the post translational cell response that produces an iron intracellular increase. Our model reveals that decreasing the

## Gene Therapy Treatments

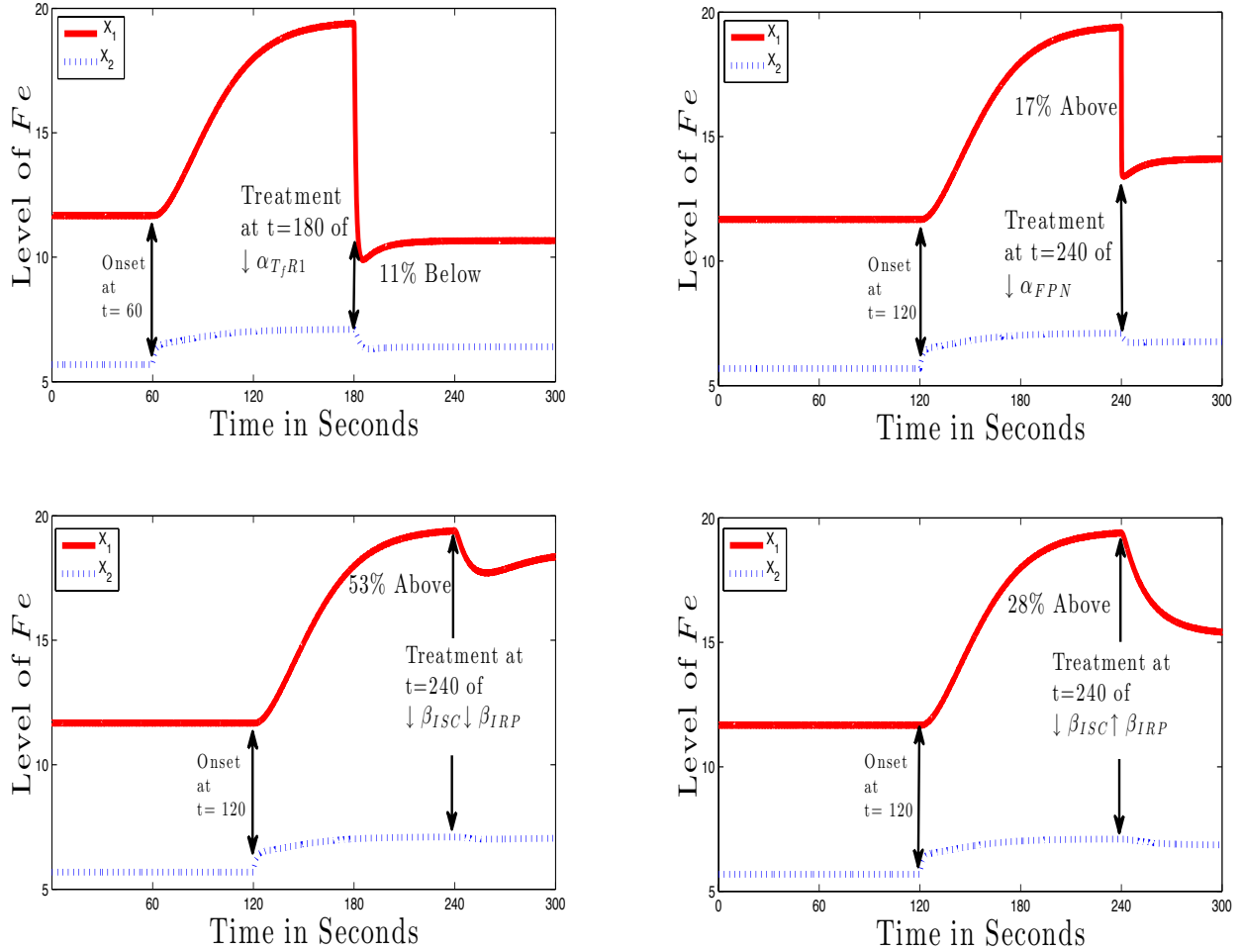


Figure 5:

levels of FXN, as is suggested in Friedreich's Ataxia patients shows a significant increase in both the cytoplasm and the mitochondria. However, the percent change in the cytoplasm is approximately 30% higher compared to the mitochondria. In this work possible treatments included affecting the level of expression of  $TfR1$ , FPN, IRP's.  $TfR1$  had the most robust response in iron level changes. Interestingly we found that an increase in  $\beta_{IRP}$  combined with a decreased in  $\beta_{ISC}$  was more efficient than lowering both at the same time.

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