A Mathematical Model of Redox/Methylation Metabolism in Human Neuronal Cells

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A dissertation submitted to

The Faculty of
the College of Science of
Northeastern University
in partial fulfillment of the requirements
for the degree of Doctor of Philosophy

December 5, 2013

Dissertation directed by
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Acknowledgements

I would like to thank my advisor Professor Mikhail Malioutov and my co-advisor Professor Richard Deth for their patience and guidance throughout this work. I would like to thank Professor Deth for teaching me a lot about biochemistry related to my work and for our long discussions.

I would like to thank Professor Michael Reed from Duke University for being a committee member in my thesis and also for his detailed review of an earlier draft of this work. I would also like to thank Professor Aidong Ding and Doctor Hanai Sadaka for being in my committee.

I would like to thank all (former and current) members of the Department of Mathematics for their support during my studies.

I would like to thank members of Professor Deth’s Lab; Yiting Li, Malav Trivedi and especially Nate Hodgson for providing experimental results, on which this work was built on.

Finally, I would like to thank my wife Selva and my son Ahmet; without their moral support I could not have finished this work.
Abstract of Dissertation

It is vital for cells to control their state of reduction and oxidation (redox), and the metabolic pathways providing this crucial function intersect with pathways controlling hundreds of methylation reactions. It has been hypothesized that abnormal redox and methylation status contributes to a number of brain disorders, including autism or Alzheimer’s disease (AD) [8, 30]. Following in the footsteps of Reed et al.[35], who created a mathematical model of these pathways in liver cells, I built a mathematical model of redox and methylation metabolism for human neuronal cells, in order to explore the predictions of this hypothesis and to see if further insights can be gained based on this model. While redox and methylation metabolism exists in all human cells, in many regards the brain compartment provides a unique environment for its many aspects of regulation.

Among other findings, simulations with this neuronal model support the hypothesis that inhibition of selenoenzymes by mercury can alter the redox status of the cell to a significant extent, which can ultimately contribute to autism or AD, depending on age. In addition, inhibition of these enzymes could be essentially irreversible, in the sense that, no other treatment could restore the levels of key metabolites back to normal homeostatic levels. We further use the model to explore the behavior of neuronal cells under different metabolic circumstances.
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CHAPTER 1

Introduction

We will start with some basic definitions that we will be referring to throughout the text many times and let us also describe the significance of methylation and redox metabolism for a neuronal cell:

1.1. Some Preliminaries & Glossary

1.1.1. Methylation

In chemistry, methylation of a substrate is simply the transfer of a single carbon atom or methyl group (-CH3) into that substrate. There are more than 200 different methylation reactions within a neuronal cell. A couple of the important methylation reactions are briefly described.

1.1.2. DNA methylation

Human genomic information is encoded in DNA, which is stored in 46 chromosomes. The haploid human genome has roughly 3 billion DNA base pairs and there are about 23,000 protein-coding genes in this genome. The rest of the DNA consists of regulatory sequences, introns, non-coding RNA genes and non-coding DNA. The inactive DNA is wrapped around histones, a process controlled by their methylation. Expression of these protein-coding genes is essential for survival of a cell, but the frequency
of expression of a particular gene can change from tissue to tissue, from one human being to another or even from one time to another time for a single cell, depending on the metabolic conditions.

When a particular gene on DNA is methylated, it is simply silenced. The histones could be methylated as well. There are several locations on a histone where the methyl group could be attached. Depending on the location, this could result in expression or silencing of the gene that is wrapped around the histone. Therefore, the availability of methyl groups is of central importance for gene regulation of a cell.

1.1.3. Phospholipid Methylation

Another important methylation reaction in a neuronal cell is phospholipid methylation, i.e. methylation of the cell membrane. When the cell membrane is methylated, it affects the activity of membrane proteins. Phospholipid methylation activity may be especially important for synchronization of neuronal cells during times of attention and learning. It was shown by Sharma et al. that the neurotransmitter dopamine stimulates PLM([37]) while Waly et al.([43]) showed that thimerosal, some heavy metals and ethyl alcohol, inhibit the same process.

Some other important methylation reactions include protein methylation, RNA methylation and methylation of neurotransmitters such as dopamine.

1.1.4. Reduction, Oxidation and Redox

In a chemical reaction, oxidation of an atom or a molecule is simply a loss of (an) electron(s) for that atom or molecule. Similarly, reduction of an atom or a molecule is a gain
of electron(s). The term redox refers to all reduction and oxidation reactions in chemistry. For example, in the following reaction

$$H_2O_2 + 2NADPH \rightarrow 2H_2O + 2NADP^+$$

the two oxygen molecules in $H_2O_2$ are reduced while $NADPH$ is oxidized.

1.1.5. Reactive Oxygen Species

Oxygen is a highly reactive element. Chemically reactive molecules containing oxygen are called reactive oxygen species (ROS). In a neuronal cell, some well known examples of ROS are $H_2O_2$, hydrogen peroxide and $O_2^{-1}$, superoxide anion.

1.1.6. Redox Status and Oxidative Stress

A large proportion of metabolic reactions involve oxidation and reduction of molecules, and it is essential for cells to maintain the balance between oxidation and reduction within a useful range to facilitate reactions. The term redox status is meant to describe this balance, reflected as the redox equilibrium or the redox state of the cell. Like all living organisms, neuronal cells need a constant source of energy to survive. Mitochondria use oxygen to produce this required energy and ROS like $H_2O_2$ are byproducts of respiration in a neuronal cell. There are several indicators of the redox status of a cell. The concentration of ROS could be regarded as one of them (more indicators will be described later). When there is an imbalance between the production and reduction of ROS, the concentration of the latter starts increasing. This situation is called oxidative stress. As described earlier,
when the level of many ROS is high, i.e. more free radicals are present within the cell, these radicals are likely to interrupt many critical processes within the cell.

1.1.7. The importance of redox status for a cell

- First of all, there are many enzymes that are sensitive to the redox status of the cell. When the cell is under oxidative stress, many of them are going to be inhibited or even totally blocked while others may be activated, including enzymes that regulate survival or functionality of the cell. Both PLM and DNA methylation[28] are two examples of metabolic activities that are crippled by oxidative stress, since aggregate methylation slows down in the cell when redox status shifts towards oxidation.

- It is known that when a cell is more reduced, it becomes more responsive to survival and self renewal factors (mitogens), while when it is more oxidized, it becomes more responsive to differentiation and death factors [31]. This means that the redox status of a cell could be used as a modulator between several states, like from proliferation to survival and so on. In this manner, change in redox status can be regarded as a signaling activity.

- There is enough evidence in the literature to establish an association between the redox status of neuronal cells and many neurodegenerative diseases like autism [1], Alzheimers disease [7], attention-deficit hyperactivity disorder (ADHD)[23] and schizophrenia[9]. However, even though this association could be described as a strong one, the nature of this relation is not fully understood yet.
• Increasing frequency of some of the above diseases, especially autism, could be partially linked to changing environmental factors over decades. It is well known that many environmental toxicants, such as lead (Pb) and mercury (Hg), are potent pro-oxidants, i.e. exposure to such chemicals changes the redox status of a neuronal cell. This could explain the elevated levels of prevalence for these diseases in last couple of decades.

• As we age, there is an increased risk of oxidative stress[?, 10], which can be linked to late onset Alzheimers disease and other neurodegenerative disorders.

• Astrocytes and neuronal cells, which are two types of cells in brain, both develop from neuronal stem cells. It is known that, in early stages of brain development, when these stem cells are under oxidative stress, the proportion of astrocytes increases, while being in a more reduced state results in a larger proportion of neuronal cells. Again this proportion of neuronal cells vs. astrocytes could ultimately affect ones vulnerability to neurodegenerative diseases.

1.2. Developing The Model

1.2.1. The Model of Reed and Nijhout, Glutathione Metabolism in Hepatic Cells

Building upon a mathematical model of the methionine cycle of methylation, which consists of only 4 differential equations[34], Reed and Nijhout developed a much more sophisticated mathematical model for glutathione metabolism in liver[35]. In this model 34 differential equations were used to describe the rate of change of each substrate in glutathione metabolism. These differential equations were determined by the enzymes
that are essential for each reaction. My goal is to build a similar model for neuronal cells. The differences between two models will be specified later.

Now let us describe the glutathione metabolism in liver in a few words:

We will describe this metabolism starting with the amino acid methionine (Met). Met could be regarded as the first amino acid entering the cell. It is an essential amino acid (which means the human body cannot synthesize this amino acid) and comes from dietary proteins. It is uptaken from blood into liver cells. Methionine is converted into S-adenosylmethionine (SAM) by ATP and 2 iso-enzymes of methionine adenosyltransferase: methionine adenosyltransferase-1 (MAT1) and methionine adenosyltransferase-3 (MAT3).
SAM is simply the universal methyl donor of a cell in more than 200 methyltransferase reactions and is converted to S-adenosylhomocysteine (SAH) in these reactions, including DNA and phospholipid methylations. There are many different methyltransferase enzymes taking part in this action, and DNA methyltransferase (DNMT) and glycine N-methyltransferase (GNMT) could be counted as two examples. We should mention that the product of all these methylation reactions, SAH, is actually also an inhibitor of all these methyltransferase reactions, since it competes with SAM for binding to methyltransferase enzymes. Therefore, \([\text{SAM}] / [\text{SAH}]\), i.e. the ratio of concentration of SAM to SAH, could be regarded as an index of methylation for a cell. If we think of this as a fraction, when \([\text{SAM}]\) is fixed and \([\text{SAH}]\) increases, there will be less methylation. Similarly, when \([\text{SAH}]\) is fixed and \([\text{SAM}]\) increases, there will be more methylation in the cell.

SAH is subsequently hydrolyzed to homocysteine (Hcy) by the enzyme S-adenosylhomocysteine hydrolase (SAHH) through a balanced reaction, i.e. the reaction \(\text{SAH} \leftrightarrow \text{Hcy}\) is a reversible reaction. Adenosine is a byproduct of this reaction. Indeed the synthesis of SAH from Hcy and adenosine is thermodynamically favored, so removal of products (Hcy and adenosine) is essential for dynamic methylation activity. Therefore, accumulation of Hcy in a cell induces elevated levels of SAH and in turn less active methylation reactions. Hcy can be metabolized by two major reactions: (i) It can be remethylated into Met either by betaine homocysteine methyltransferase (BHMT) or the vitamin B12 dependent enzyme methionine synthase (MS), or (ii) It can be combined with serine to form cystathionine (Cyst), which is mediated by a vitamin B6-dependent enzyme cystathionine \(\beta\)-synthase (CBS). Synthesis of Met from Hcy closes the loop and this cycle containing four substrates
(MET, SAM, SAH and HCY) is also known as the Methionine Cycle. For re-methylation of Hcy by MS to form Met, 5-methyltetrahydrofolate (5mTHF) is also substrate. The concentration of 5mTHF is regulated by the folate cycle. We will not go into details of folate cycle for now, but we should indicate that the whole folate cycle can be summarized as the concentration of 5mTHF. Other than 5mTHF, there is no direct interaction between the folate cycle and the rest of the metabolic reactions we are describing.

When a cell is under oxidative stress, MS is inhibited which increases the concentration of HCY and its conversion to Cyst. As we mentioned earlier, this automatically slows down all methylation reactions due to SAH formation.

Cystathionine is broken down to cysteine (CYS) and 2-oxobutanoate in a reaction mediated by an enzyme called \( \gamma \)-cystathionase or cystathionine gamma lyase (CTGL). Conversion of HCY to Cyst and then to CYS is called transsulfuration. Cys can be uptaken from the extracellular space as well by the excitatory amino acid transporter 3 (EAAT3), which also transports glutamate (Glut) but with less efficiency than CYS. Glut and CYS can combine and form gamma-glutamyl-cysteine (Glc), via a reaction mediated by the enzyme \( \gamma \)-glutamylcysteine ligase (GCL) ( or glutamylcysteine synthetase-GCS). This reaction is rate limiting for GSH synthesis.

Finally, glycine (Gly) and Glc are condensed to form the main antioxidant of a cell, the tripeptide glutathione, via a reaction mediated by glutathione synthetase (GS). Glutathione can be present in two forms in a cell; a reduced form for which we usually say
simply glutathione (GSH) or in an oxidized dimeric form, glutathione disulfide (GSSG). The ratio of the concentration of reduced to oxidized glutathione, i.e. \([\text{GSH}]/[\text{GSSG}]\) could be as high as 100 in a cell. This ratio could be regarded as an indicator of the redox status for a cell. When the cell is under oxidative stress, this ratio could go down dramatically. Since GSH concentration is very high compared to other antioxidants, GSH could be regarded as the primary antioxidant in a cell. De novo synthesis of GSH is very important for the redox balance of a cell, since it cannot be uptaken from extracellular space. In the synthesis of GSH, availability of Cys is the rate limiting factor, together with the activity of the enzyme GCS.

Now let us take a moment to give an example of how GSH counteracts ROS in a liver cell. As we mentioned earlier, hydrogen peroxide, \(H_2O_2\) is an example of ROS. GSH reduces \(H_2O_2\) by the following reaction:

\[
2\text{GSH} + H_2O_2 \leftrightarrow \text{GSSG} + H_2O.
\]

This reaction is mediated by glutathione peroxidase. There are many other ROS, and many other enzymes oxidizing GSH. Here all such enzymes are summarized under the common name glutathione peroxidases (GPx). There are also enzymes reducing GSSG and therefore re-synthesizing GSH. One example of such enzymes is the glutathione reductase (GR). Again we will use glutathione reductase (GR) as a common name for all the enzymes reducing GSSG and forming GSH. These are the main variables in the Reed and Nijhout model that are concerned with
redox status. Every other substrate is assumed to be constant.

For each substrate a differential equation is given. Let us give one example. For the substrate SAM, we have the following differential equation:

\[
\frac{d}{dt}SAM = V_{MAT1} + V_{MAT3} - V_{GNMT} - V_{DNMT}.
\]

SAM is a product in two reactions mediated by two isoenzymes MAT1 and MAT3 and is a substrate in two reactions mediated by enzymes DNMT and GNMT. The independent variable is \( t \) (in hours) and concentrations of substrates are in \( \mu M \) (micromolar). The term stands for velocity of the reaction due to the enzyme MAT1. \( V_{MAT3}, V_{GNMT}, V_{DNMT} \) have similar meanings for the corresponding enzymes. Again, let’s explain with an example:

\[
V_{MAT1} = \frac{V_{max} Met}{K_m + Met} \left(0.23 + 0.8 * e^{-0.0026SAM}\right) \frac{2200.71}{2140 + GSSG},
\]

where \( V_{max} = 260 \) and \( K_m = 41 \).

Basically there are three factors in this expression, the first one is the Michaelis-Menten equation for one substrate for the enzyme MAT1, the second one represents inhibition of this reaction by the product SAM and the last one is inhibition of the enzyme by oxidative stress. There are two parameters here: \( V_{max} \) and \( K_m \) for the Michaelis-Menten equation. These two parameters and the additional inhibition equations are all derived through linear or nonlinear regression using various data from the literature. For all of the enzyme reactions, a form of Michaelis-Menten equations or the Hill equation (which could
be regarded as a special case of Michaelis-Menten) is used, depending on reversibility and number of substrates involved in that reaction. This is a quite complicated system and many enzymes could be affected by other substrates, either inhibited or activated. Such interactions are also accounted in those equations.

Let us describe the Michaelis-Menten Equation for an irreversible reaction with one substrate very briefly:

If we have the following reaction:

\[
E + S \leftrightarrow ES \rightarrow E + P
\]

where E denotes enzyme, S denotes substrate and P denotes product. Then

\[
\frac{dP}{dt} = \frac{V_{\text{max}}[S]}{K_m + [S]}
\]

For any enzyme there are two important parameters: \( V_{\text{max}} \) and \( K_m \). Those two parameters are defined in the following way: Assuming that we have a reaction like \( S \rightarrow P \), then \( V_{\text{max}} \) is simply the maximum reaction velocity when there is an extremely large concentration of substrate, and \( K_m \) is the concentration of \( S \) such that \( \frac{V_{\text{max}}}{2} \) is attained, e.g.:

Finding those two values for any reaction or any enzyme is not too hard and the literature provides many resources. However, we should indicate that, those parameters can change from tissue to tissue or even from time to time for the exact same cell.
This model was then used by Reed et al.\cite{35} to explore short term deviations in metabolism due to perturbations, especially to oxidative stress. Metabolic profiles of diseases such as Down syndrome and autism were successfully simulated.

1.2.2. Methylation and Redox Metabolism in Neuronal Cells

As we mentioned earlier, the model of Reed and Nijhout is specific to liver cells. Our model will be for neuronal cells, which could provide novel insights about the origin of brain disorders, especially those related to oxidative stress. The following graph will be used frequently throughout the text. It summarizes the the pathways we are interested in this work.

Now let us describe methylation and redox metabolism for neuronal cells. We will be pointing out main differences between the two cell types as well.

First of all, neuronal cells are surrounded by an extracellular fluid related to cerebrospinal fluid (CSF), unlike liver cells which are surrounded by extracellular fluid related to blood. There is a barrier between blood and CSF, which is called the blood brain barrier (BBB). BBB is a selective membrane that blocks many chemicals from entering
the CSF. Notably, CSF has much lower levels of CYS and GSH, as compared to blood, implying that the brain has limited antioxidant resources.

The whole metabolism in neuronal cells is very similar to the metabolism in liver cells. Instead of repeating all the previous work, let us point out some differences that are intrinsic to neuronal cells.

For the conversion of Met into SAM, the enzymes MAT1 and MAT3 are replaced by their iso-enzyme methionine adenosyltransferase-2 (MAT2). Then, for the second step of methionine metabolism, i.e. for all of the methylation reactions, the enzyme GNMT seems to be under-expressed in neuronal cells when compared with liver cells[26]. Another substrate in methionine metabolism, Hcy, can be remethylated into Met by only one enzyme in neuronal cells, MS, since neuronal cells are lacking BHMT which carries
out the same reaction in liver.

When a neuronal cell is under oxidative stress, MS is inhibited to favor the transsulfuration pathway, which increases concentration of HCY and promotes CYS and GSH formation. As mentioned earlier, this automatically slows down all methylation reactions. A novel mechanism for dopamine stimulated PLM was described by Sharma et al.\[37\], which is mediated by the D4 dopamine receptor and supported by MS. When a neuronal cell is under oxidative stress, inhibition of MS automatically inhibits dopamine-stimulated PLM. There is a competition for the enzyme MS in this respect; a single MS molecule can either be used for (i) re-methylation of HCY into MET or (ii) used in dopamine-stimulated PLM. When the external concentration of dopamine increases, the second type of PLM is favored. Thus higher concentrations of dopamine have an oxidation-like effect on MS activity and less HCY is remethylated into MET while CYS and GSH synthesis is increased. As mentioned earlier, dopamine stimulated PLM is thought to provide synchronization of neuronal cells, which may play a crucial role during times of attention and learning.

Notably, DNA methylation has recently been shown to be an important aspect of memory formation \[40\].

Another big difference for redox/methylation metabolism in neuronal cells when compared to liver cells is that the transsulfuration pathway is partially blocked in neuronal cells. For a long time, this pathway was believed to be totally blocked in neuronal cells. Recent findings showed that it was only partially blocked\[42\]. Therefore, CYS can be synthesized from cystathionine at a low rate in neuronal cells. Levels of cystathionine are remarkably higher in human cortex than any other tissue or species, because of partially
blocked transsulfuration pathway, increasing the importance of CYS uptake to support GSH synthesis.

CYS can be uptaken from the extracellular space by the excitatory amino acid transporter 3 (EAAT3), which also transports glutamate (Glut). EAAT3 is downstream of
a neurotrophic growth factor signaling pathway and growth factors stimulate CYS uptake in neuronal cells via PI3 kinase activation[21]. CYS and Glut combine and form glutamyl-cysteine (GLC), via a reaction mediated by the enzyme glutamylcysteine synthetase (GCS). Finally, just like in liver cells, the primary antioxidant of a neuronal cell, GSH, is synthesized by glutathione synthase. Again, we could regard the ratio of its reduced to oxidized form, i.e. \([\text{GSH}]/[\text{GSG}] \) as an index of the redox status of a neuronal cell. The kidneys and liver are primary organs for detoxification, and GSH concentration is highest in these two organs. In addition to its central role in redox balance, GSH also is involved in detoxification of any cell.

*De novo* synthesis of GSH is very important for the redox equilibrium of a cell, since it cannot be uptaken. In the synthesis of GSH, availability of Cys is the rate limiting factor. Compared to liver cells or other tissues, intracellular concentrations of GSH are very low in neuronal cells, making *de novo* synthesis of GSH even more important to maintain the redox balance in a neuronal cell.

There are two resources for CYS in a neuronal cell. It can either be synthesized from methionine, i.e. transsulfuration pathway or it can be uptaken from extracellular compartment by EAAT3. Since the transsulfuration pathway is partially blocked in neuronal cells, cys input by EAAT3 becomes of central importance for redox balance in a neuronal cell. It is known that dopamine and growth factors like IGF-1 stimulate EAAT3, while \(TNF-\alpha, Hg^{2+}, Al^{3+}\) and opiates such as morphine inhibit EAAT3.
Now let’s take a step back and take a look at the bigger picture for availability of extra-cellular cysteine for neuronal cells.

Cysteine is an amino acid that can be uptaken from the diet by GI epithelial cells. Surprisingly, for this uptake, the amino acid transporter EAAT3 plays a crucial role again. Once CYS becomes available in blood, it is oxidized into cystine (simply two oxidized cys molecules) by liver. CYS cannot pass the BBB, but its oxidized form cystine can do so. After cystine is in the CSF, astrocytes take it up and reduce it to CYS which is converted to GSH. GSH is released from astrocytes and CYS is released by the action of peptidase enzymes. Neuronal cells can then take up cys, which is of crucial importance for de novo synthesis of GSH.
There are various enzymes regulating the redox status using either GSH or GSSG as a substrate. When compared to liver cells, an almost 30-fold lower concentration of GSH is present in neuronal cells, showing that the enzymes that are regulating the redox status in neuronal cells have to be working much more efficiently than the ones in liver cells. That is why, in addition to GPx and GR, we will be identifying several other enzymes taking part in this regulation. A common characteristic possessed by many of these enzymes is that they are selenoenzymes, i.e. enzymes containing selenocysteine (Sec).

Selenium is believed to be one of the most prominent redox factors in brain, owing to provide electrons to many reducing reactions. In a recent paper it was shown that, fed by a selenium-deficient diet, rats had less than 2% of selenium in most of its tissues, while brain still had 60% of its regular concentration [2]. It was also reported that even small deviations from normal SE concentration results in loss of many cognitive functions for mice.

As mentioned earlier, some environmentally toxic materials, especially Hg2+, are known to be pro-oxidant. Selenoenzymes are much more sensitive to Hg exposures because of its high binding affinity ($10^{45}$) for Se. The binding affinity of Hg for Se is one million times greater than its binding affinity for cysteine. We will be giving more details on this later, but simply, if Hg binds to Se of these enzymes, some of these enzymes lose their functionality. If this inhibition lasts for an extended period of time, it is likely to change the redox and methylation status of a neuronal cell.
We will be more specific on selenoenzymes, but in simplest terms, GSH reduces ROS and GSSG is a byproduct. Some enzymes such as GR, reduce GSSG and GSH is synthesized again, but those reducing enzymes are oxidized themselves in this procedure.

Now let us list some of the selenoenzymes that are known to be regulating the redox status in neuronal cells[33]:

- Glutathione Peroxidase 4 (GPx4): This enzyme reduces phospholipid hydroperoxides, which is why it is sometimes referred as ph-GPx4. It is also considered to be a universal antioxidant for biomembranes. GPx4 knockouts are known to be lethal at an early embryonic age. Neuron-specific knockout of GPx4 results in a selective decrease in certain interneurons, interneurons that are critical for neural synchronization during attention.

- Thioredoxin Reductase 1 (TrxR1): This cytoplasmic enzyme has a pivotal involvement in the redox regulation and DNA synthesis. It reduces a number of oxidized substrates. Just like GPx4, deletion of the gene encoding this enzyme is lethal [3], indicating necessity of its functions. When Hg binds to the Se of this enzyme, not only does TrxR1 lose its functionality, but this loss can even initiate an apoptosis of the Se-deprived neuronal cell [4].

- Thioredoxin Reductase 2 (TrxR2): This enzyme is located in mitochondria, the main producer of ROS in any cell. It is a ubiquitous homodimeric pyridine nucleotide-disulfide oxidoreductase.
1.2.3. Main Differences of Glutathione Metabolism in Liver and Neuronal Cells

Some of these metabolic differences became apparent throughout the preceding text but let us list all these one by one:

(1) Extracellular Environment
To begin with, these two tissues have totally different extracellular environments. Neuronal cells are surrounded by a CSF-like fluid while liver cells lie within a blood-like fluid. In addition, astrocytes (which could be regarded as cells supporting neuronal cells) and neuronal cells are highly interdependent upon each other, while there are no such cells in liver.
As we mentioned earlier, GSH and cys are of central importance in redox metabolism. The concentration of cys in CSF is almost 10−fold lower than blood.

(2) Enzymes
There are several enzymes that exist in only one of these two tissues. The ones that exist in liver cells but not in neuronal cells, to the extent we are covering in these two tissues are: MAT1, MAT3, GNMT and BHMT.
Likewise, MAT2 is expressed more in neuronal cells while it is almost non-existent in liver cells.

(3) Enzyme Kinetics
Not all enzymes are equally expressed in these two tissues and as a result, almost all of these enzymes have cell type-specific kinetic parameters. There are countless examples for such differences in the literature.
(4) Substrate Concentrations

As a result of variation in enzyme kinetics in these two tissues, almost all of the substrates have different concentrations, but we will remark on the ones that are central to our model. As we mentioned earlier, the transsulfuration pathway is partially blocked in neuronal cells, which results in higher concentrations of cystathionine and therefore the concentrations of cys and GSH are lower in neuronal cells. The concentration of GSH in neuronal cells, the primary antioxidant, is almost 40-fold lower than in liver cells.

This low concentration of GSH requires a very efficient use of antioxidants. Apparently, because of this, the dynamic activity of selenoproteins is more critical in neuronal cells when compared to other tissues.
1.3. A Mathematical Model for Redox and Methylation Metabolism in Neuronal Cells

1.3.1. Structure

Our model will consist of differential equations describing the rate of change of each substrate in redox and methylation metabolism. These differential equations will be determined by the enzymes that are essential for each reaction.

1.3.2. Resources

There will be a lot of data mining for parameters of the model. The primary source of our experimental data will be Dr Deth’s lab, and a secondary source will be the literature. In this regard, BRENDA (www.brenda-enzymes.info) deserves particular recognition. BRENDA has an extremely useful and detailed database on enzyme kinetics for many different species and tissues. Some regression and a very good interpretation of the data from literature will be necessary. The software we will be using for developing the model is MATLAB and especially the toolbox Simbiology. Simbiology allows us to do parameter estimation for highly complicated biological systems and it has a user-friendly interface. All simulations will also be run on Simbiology.

1.3.3. Aims of The Model

If we were to itemize our specific aims in building this model:

(1) It has been hypothesized that abnormal redox and methylation status contributes to a number of brain disorders, including autism or Alzheimers disease (AD)
As we have mentioned earlier, GSH concentration is lower in brain, compared to other tissues. This makes a dynamic and more efficient utilization of GSH a must in order to maintain redox balance in the brain. We have itemized some of the selenoenzymes that are involved in maintaining redox balance in the cell. High affinity of mercury towards selenium is also known. We want to see the effects of inhibition of selenoenzymes on the whole system. We would like to explore the predictions of this hypothesis and to see if further insights can be gained based on this model.

(2) Can we quantify the redox status of a cell? This is one of the questions we are seeking with the help of this model. The ratio $GSH/GSSG$ is widely regarded as primary indicator of redox status. However, when we think of the kinetics, this ratio may not be functionally sufficient. As mentioned earlier, there are many enzymes that are sensitive to oxidative stress. Linking this sensitivity to the redox status of the cell is an important aspect of this work.

(3) There is also data giving short term deviations from this steady state under different circumstances. These deviations should be predicted by the model to some extent.

(4) The fourth goal is to incorporate the important role of EAAT3 into this model. As mentioned earlier, CYS is the rate limiting factor for synthesis of GSH, the primary antioxidant of neuronal cells. Since the transsulfuration pathway is partially blocked in neuronal cells, CYS uptake by EAAT3 becomes more important for neuronal cells.
(5) When compared with other tissues, brain has a much more limited GSH concentration (almost 40-fold lower). This makes efficient use of GSH extremely important. Selenoproteins seem to be really crucial in this efficiency. My fifth goal is to incorporate selenoproteins into my model.

(6) Some short term (i.e. less than 1 hour) and relatively longer term (around 24 hours) deviations in the concentrations of the metabolites in our model due changing environment is available through our lab data. We have data specifying changes in concentrations of metabolites due to presence of morphine and IGF-1 in media. An initial guess is, these changes might be due to changing redox status (since these chemicals affect activity of EAAT3) but we want to see if there are more changes in the parameters of the model due to changing conditions.

1.3.4. Main Differences between Two Models

We can classify the main differences between the model of Reed and Nijhout of liver cells and my model into two categories; (i) intrinsic differences, i.e. tissue specific differences and (ii) authentic differences, which are simply author based differences.

i. Tissue Specific Differences

All these differences have been listed above in a separate section. Needless to say, our mathematical model will be reflecting all of these differences.

ii. Authenticity

It is recognized that these metabolic pathways are regulated in a much more complicated manner than any model can express. One has to decide which features to suppress and which to express in order to achieve a manageable mathematical model.
For example, there are more than 200 methylation reactions in a neuronal cell. Identifying each reaction one by one, having an equation for every single one of them would be simply impossible.

Compared to the model of Reed and Nijhout, the following are the differences that we are incorporating into our model:

(a) First of all, the model of Reed and Nijhout has a lot of detail concerning folate metabolism and many reactions in mitochondria, which we will be summarizing as the concentration of 5-methyltetrahydrofolate.

(b) As we mentioned earlier, EAAT3 is responsible for transporting cys, whose availability is a rate limiting factor for de novo synthesis of GSH, into neuronal cells. EAAT3 is activated as a downstream of growth factors, growth factors stimulate cys uptake via activating PI3 kinase. Therefore, availability of EAAT3, concentrations of growth factors and PI3 kinase activity will all be incorporated into our model.

(c) As we mentioned earlier, some heavy metals (e.g. Al, Hg) and opiates (cocaine, morphine) inhibit EAAT3. The effects of these xenobiotics will be examined by this model. This will enable us to introduce xenobiotics as a component in our model.

(d) Finally, as mentioned earlier, Se seems to be playing an important role in brain. Activity of selected selenoenzymes and concentration of Se will be expressed in our model.
CHAPTER 2

The Model

2.1. Notation

As mentioned earlier, this dynamic model consists of 9 metabolites that we keep track of, which give rise to 9 differential equations. Here is a list of metabolites, enzymes, their abbreviations and then the associated differential equations.

2.1.1. Metabolites

\[ MET = \text{methionine} \]
\[ SAM = \text{S-adenosylmethionine} \]
\[ SAH = \text{S-adenosylhomocysteine} \]
\[ HCY = \text{homocysteine} \]
\[ CYST = \text{cystathionine} \]
\[ CYS = \text{cysteine} \]
\[ GLC = \text{glutamyl-cysteine} \]
\[ GSH = \text{glutathione} \]
\[ GSSG = \text{glutathione disulfide} \]
2.1.2. Enzymes

MAT-II = Methionine Adenosyl Transferase II
DNMT = DNA Methyltransferase
SAHH = S-Adenosylhomocysteine Hydrolase
MS = Methionine Synthase
CBS = Cystathionine β-synthase
CTGL = γ-cystathionase
GCS = γ-glutamylcysteine Synthase
GS = Glutathione Synthase
GPx = Glutathione Peroxidase
GR = Glutathione Reductase

2.2. Differential Equations

For any of the above enzymes, the velocity of a reaction mediated by that enzyme will be represented by \( V \) with a subscript referring to the enzyme, for example \( V_{\text{MS}} \) refers to the velocity of the reaction catalyzed by MS, methionine synthase. The units for all of the reaction velocities will be micromolar per hour, \( \mu M/h \). In addition to the reaction velocities catalyzed by enzymes, the transporters of two metabolites, methionine and cysteine, into the cell will also be represented by \( V_{\text{metin}} \) and \( V_{\text{EAAT3}} \).

The reduced and oxidized forms of glutathione, GSH and GSSG, are also exported from the cell. Especially, GSH is not used only as an antioxidant but it is also the main detoxifier of any cell. As a result, it may be exported from any cell in (relatively) larger
quantities. Velocities of these reactions will be represented by $V_{GSH_{out}}$ and $V_{GSSG_{out}}$.

In addition to being a precursor for GSH, CYS could be consumed for other purposes. Similarly, this loss will be represented by $V_{CY_{S_{out}}}$.

The differential equations follow from mass-balance reactions. Simply, each differential equation gives the rate of change of a metabolite per unit time. This change would be result of consumption and/or the production of that metabolite. For example, in the following equation

$$\frac{d(HCY)}{dt} = V_{SAHH} - V_{MS} - V_{CBS}$$

homocysteine is a product of the reaction mediated by S-adenosylhomocysteine hydrolase (SAHH) and it is used as a substrate by 2 enzymes, methionine synthase (MS) and cystathionine–$\beta$–synthase (CBS). This is how we derive the above differential equation.

Here is a list of differential equations for this work:
\[
\frac{d(MET)}{dt} = V_{\text{metin}} + V_{MS} - V_{MATH}
\]
\[
\frac{d(SAM)}{dt} = V_{MATH} - V_{DNMT}
\]
\[
\frac{d(SAH)}{dt} = V_{DNMT} - V_{SAH}
\]
\[
\frac{d(HCY)}{dt} = V_{SAH} - V_{MS} - V_{CBS}
\]
\[
\frac{d(CYST)}{dt} = V_{CBS} - V_{CTGL}
\]
\[
\frac{d(CYS)}{dt} = V_{EAAT3} + V_{CTGL} - V_{GCS} - V_{CYS_{out}}
\]
\[
\frac{d(GLC)}{dt} = V_{GCS} - V_{GS}
\]
\[
\frac{d(GSH)}{dt} = V_{GS} + 2 \cdot V_{GR} - 2 \cdot V_{GPx} - V_{GSH_{out}}
\]
\[
\frac{d(GSSG)}{dt} = V_{GPx} - V_{GR} - V_{GSSG_{out}}
\]

2.2.1. Enzymes and Associated Reaction Velocities

Now let us describe the reaction velocities for all of the reactions in this system. For most of these reaction velocities, general form of that reaction velocity will be taken from [35].

(1) Methionine Uptake (metin):

\[
V_{\text{metin}} = \frac{V_{\text{max}} \cdot MET_{\text{ext}}}{K_m + MET_{\text{ext}}} - MET
\]

where \( MET_{\text{ext}} \) represents the extracellular methionine concentration.

(2) MAT-II:

\[
V_{MATH-II} = \frac{V_{\text{max}} \cdot MET}{K_m + MET} \cdot \frac{A}{B + SAM}
\]
The first fraction is simply Michaelis-Menten kinetics, while the second fraction represents inhibition of MAT-II by its product SAM.

(3) DNMT:
There are more than 200 methylation reactions taking place in this step. We are taking this enzyme as a representative of aggregate methylation:

\[ V_{DNMT} = \frac{V_{\text{max}} \cdot SAM}{K_m + SAH + SAM} \]

(4) SAHH: This is a reversible reaction. We are taking the production of HCY from SAH as the positive direction.

\[ V_{SAHH} = \frac{V_{\text{max}1} \cdot SAH}{K_{m1} + SAH} - \frac{V_{\text{max}2} \cdot HCY}{K_{m2} + HCY} \]

(5) MS:

\[ V_{MS} = \frac{V_{\text{max}} \cdot HCY}{K_m + HCY} \]

Here the concentration of 5-methyltetrahydrofolate is taken as a constant.

(6) CBS:

\[ V_{CBS} = \frac{V_{\text{max}} \cdot HCY}{K_m + HCY} \cdot \frac{A(SAM + SAH)^2}{B + (SAM + SAH)^2} \]

(Concentration of serine is taken as a constant.)

(7) Cysteine Uptake (EAAT3):

\[ V_{EAAT3} = \frac{V_{\text{max}} \cdot CYS_{\text{ext}}}{K_m + CYS_{\text{ext}}} - CYS \]

where \( CYS_{\text{ext}} \) represents extracellular cysteine concentration.
(8) CTGL:

\[ V_{CTGL} = \frac{V_{max} \cdot CYST}{K_m + CYST} \]

(9) Loss of Cysteine (CYS\(_{out}\)):

\[ V_{CYS_{out}} = k \cdot [CYS]^2 \]

(10) GCS:

\[ V_{GCS} = \frac{V_{max}(CYS \cdot GLUT)}{K_{m,c}K_{m,g} + K_{m,c}GLUT + K_{m,g}CYS \left( 1 + \frac{GSH}{K_i} + \frac{GLUT}{K_{m,g}} \right) + \frac{GSH}{K_i}} \]

(11) GS:

\[ V_{GS} = \frac{V_{max}(GLY \cdot GLC)}{K_{m,glc}K_{m,gly} + K_{m,gly}GLC + K_{m,glc}GLY \left( 1 + \frac{GLC}{K_{m,glc}} \right)} \]

(12) GPx:

\[ V_{GPx} = \frac{V_{max}(GSH \cdot H_2O_2)}{(K_{m,g} + GSH) (K_{m,h} + H_2O_2)} \]

(13) GR:

\[ V_{GR} = \frac{V_{max}(GSSG \cdot NADPH)}{(K_{m,g} + GSSG) (K_{m,n} + NADPH)} \]

(14) GSH\(_{out}\):

\[ V_{GSH_{out}} = \frac{V_{max} \cdot GSH}{K_m + GSH} \]

(15) GSSG\(_{out}\):

\[ V_{GSSG_{out}} = \frac{V_{max} \cdot GSSG}{K_m + GSSG} \]
These are all the velocity reactions we will be using in the model. As mentioned earlier, the form of the equations are taken from [35]. We will be taking most of $K_m$ values as they are from the same article, but we will get the values for $V_{max}$ using the steady state approach.

2.3. Developing The Model Using Steady State Approach

Let us remember the diagram of the pathways we are modeling:

To begin with, we will assume that the right hand sides of all of the differential equations that we described before are 0, for finding the necessary parameters. When we do that, once we have a reaction velocity, we can use it to figure out most of the others. However, there are some critical steps in the process. These could be itemized as:

- The amount of MET uptake or MAT-II activity.
- The fraction of remethylated HCY.
- The amount of CYS uptake.

For all 3 items, we use some previously published data. Before doing that, let us mention an important point for taking data from literature:

An Important Issue: Unit Conversion

In this work, all of the concentrations of metabolites will be given in micromoles per liter i.e. $\mu M$. However, almost all of the data available in literature give these measurements in nano (pico etc.) moles per milligram protein, i.e. per unit weight rather than per unit volume. We need the concentrations for all of these calculations and these units have to be converted. For all the unit conversions, I will be using the following:

In our lab, GSH concentration in postmortem brain samples were found to be 16.01
nmol/mg protein. It is known that GSH concentration in brain cells is approximately 210 µM, which means

\[ 1 \text{ nmol/mg protein} = 13.12\mu M. \]

Let us begin this section by giving the experimental data from SY5Y cells from our lab, with the necessary conversions:

<table>
<thead>
<tr>
<th>Metabolite</th>
<th>nmol/mg pr</th>
<th>µM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cysteine</td>
<td>104.38</td>
<td>1368.42</td>
</tr>
<tr>
<td>Cystathionine</td>
<td>15.22</td>
<td>199.53</td>
</tr>
<tr>
<td>GSH</td>
<td>19.07</td>
<td>250.01</td>
</tr>
<tr>
<td>SAM</td>
<td>6.2</td>
<td>81.28</td>
</tr>
<tr>
<td>Homocysteine</td>
<td>1.57</td>
<td>20.58</td>
</tr>
<tr>
<td>Methionine</td>
<td>3.47</td>
<td>45.49</td>
</tr>
<tr>
<td>GSSG</td>
<td>0.42</td>
<td>5.51</td>
</tr>
<tr>
<td>SAH</td>
<td>1</td>
<td>13.11</td>
</tr>
</tbody>
</table>

In addition to some available data in literature, we will be using the values in the second column to find the reaction velocities and the related \( V_{max} \) values for each enzyme. Let us start with MAT-II.

- MAT-II Parameters: The enzyme MAT-II catalyzes the formation of SAM from methionine and ATP. In 2 articles, [15] and [39], it was shown that SAM inhibits MAT-II enzyme. Now let us try to find a relation between the percentage activity of MAT-II and the concentration of SAM (in µM). We will be using the data provided in [39] for finding the inhibition parameters. The following data is from [39], Figure 5:
Table 2.2. MAT-II inhibition by SAM

<table>
<thead>
<tr>
<th>SAM(µM)</th>
<th>MAT-II Act(%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>100</td>
</tr>
<tr>
<td>25</td>
<td>67.28</td>
</tr>
<tr>
<td>50</td>
<td>52.78</td>
</tr>
<tr>
<td>75</td>
<td>45.72</td>
</tr>
<tr>
<td>100</td>
<td>39.4</td>
</tr>
<tr>
<td>200</td>
<td>26.39</td>
</tr>
<tr>
<td>300</td>
<td>20.44</td>
</tr>
<tr>
<td>400</td>
<td>16.35</td>
</tr>
<tr>
<td>500</td>
<td>13.75</td>
</tr>
<tr>
<td>1000</td>
<td>7.43</td>
</tr>
</tbody>
</table>

Then using curve fitting toolbox on MATLAB, we get:

\[ f(x) = \frac{p_1}{x + q_1} \]

Coefficients (with 95% confidence bounds):

\[ p_1 = 65.04 \ (57.07, \ 73.01) \]
\[ q_1 = 66.74 \ (56.34, \ 77.14) \]

Goodness of fit:

SSE: 0.005248
R-square: 0.9929

Figure 2.1. MAT-II Inhibition by SAM
In [15], the kinetic parameters for MAT-II on SY5Y cells are given as $K_m = 9 \mu M$ methionine and $V_{max} = 105$(pmol SAM formed)/mg protein/min. When we look at the available literature data, we see that we have a wide range of values for $K_m$. For human cells, a range of values for $K_m$ is given as $6 - 3300 \mu M$([24, 45]).

Using the fact that this $V_{max}$ is measured under the presence of SAM, where $[SAM] = 81.24 \mu M$, then by above inhibition formula we get, $V_{max} = 105 \times (81.24 + 66.74)/65.04 = 238.9$ pm/mgp/min, and then using the unit conversion we mentioned earlier, $V_{max} = 238.9 \times 13.12 \times 60/1000 = 188.11 \mu M/h$. Then to summarize:

$$V_{MAT-II} = \frac{V_{max} \cdot MET}{K_m + MET} \cdot \frac{A}{B + SAM}$$

$V_{max} = 188.11, \ K_m = 9, \ A = 65.04, \ B = 66.74$.

Then, under the steady state, using the values $MET = 45.54$ and $SAM = 81.24$, $V_{MAT-II} = 69.00 \mu M/h$.

- DNMT Parameters: As mentioned earlier, there are around 200 methylation reactions in a cell, which take place at this step. I will be using DNMT as the representative of all of these methylation reactions. It is known that methylation reactions are inhibited by SAH. The general form of this equation, including the inhibition by SAH is taken from [35]. The form of the reaction velocity for
DNMT is:
\[
V_{DNMT} = \frac{V_{\text{max}} \cdot SAM}{K_m + SAH + SAM}
\]
where \(K_m = 1.4\) is given in [35]. Now, to find the \(V_{\text{max}}\) of DNMT, we will use the reaction velocity of MAT-II and concentrations for SAM and SAH. These concentrations are given as SAH=13.11 and SAM=81.28. Then using the fact that \(V_{\text{MAT-II}} = 69.00\), we get the following equation: 
\[
69 = \frac{V_{\text{max}} \cdot \text{SAM}}{K_m + \text{SAM} + \text{SAH}},
\]
which gives \(V_{\text{max}} = 81.32\). Just like MAT-II, \(V_{DNMT} = 69.00 \mu M/h\).

To summarize the parameters of DNMT:
\[
V_{DNMT} = \frac{V_{\text{max}} \cdot SAM}{K_m + SAH + SAM}
\]
\[K_m = 1.4, \ V_{\text{max}} = 81.32.\]

• SAHH Parameters: S-adenosyl homocysteine, SAHH, catalyzes a reversible reaction. We will take formation of HCY as the positive direction. Just like the previous two enzymes, because of the steady state, \(V_{SAHH} = 69.00 \mu M/h\). That will be the difference between the productions of HCY and SAH, i.e. the difference between the rate of production of these two metabolites have to be 69\(\mu M/h\). Here there is an important fact about the HCY concentration in SY5Y cells; since SY5Y cells are neuroblastoma cells, there is a significant HCY accumulation in these cells. For example, when we compare with liver cells, HCY concentration is almost 20 fold higher in SY5Y cells. As a result, when we use the \(V_{\text{max}}\) or \(K_m\)
values provided by [35], the net rate turns out to be negative, i.e., production of SAH far exceeds production of HCY. This could be the case in a cell only for a very short period of time; this can not be true especially for the homeostasis. We will use the following information to address this issue: in [35], the ratio of SAH production to HCY production, by SAHH, is about 1:8. In another article, a similar ratio, 1:8.6 is given by [5] for rat liver cells. In this we can take the same ratio for that fraction, i.e. for every SAH molecule produced by SAHH, 8 HCY molecules are produced. Then, denoting the reaction velocity of HCY formation by $V_f$ and SAH formation by $V_r$, we get

$$V_f = \frac{8}{7} \times 69 = 78.86, \text{ and } V_r = \frac{1}{7} \times 69 = 9.86$$

Then, using the following equation for $V_{SAHH}$:

$$V_{SAHH} = \frac{V_{max1} \cdot SAH}{K_{m1} + SAH} - \frac{V_{max2} \cdot HCY}{K_{m2} + HCY},$$

where $K_{m1} = 6.5$, $K_{m2} = 150$,

and solving this equation for $V_{max1}, V_{max2}$ we get

$$V_{max1} = 117.96 \mu M/h, \text{ } V_{max2} = 81.70 \mu M/h$$

• MS Parameters: The enzyme methionine synthase, (MS) plays a crucial role in this system because activity of MS affects both methylation reactions and redox
status of the cell. The fraction of HCY that is remethylated is also very important in this model to determine the necessary parameters for the enzymes MS, CBS and the uptake of MET into the cell.

Together with HCY, 5-methyltetrahydrofolate is also a substrate for MS. We are going to take the concentration of it as a constant. Then the remethylation of HCY becomes a reaction with just one substrate and then the form of the reaction velocity becomes Michaelis-Menten with one substrate. In this form, the $V_{max}$ would simply include the concentration of 5 – methyltetrahydrofolate as well.

If we were to measure effects of 5-methyltetrahydrofolate on MS, or as a result on the whole system, we can simply increase/decrease the $V_{max}$ value.

The form of the equation for the reaction velocity is given as

$V_{MS} = \frac{V_{max} \cdot HCY}{K_m + HCY}$

Here we will take $K_m = 1$ from [35]. To find $V_{max}$, we will use data from [43]. In this article, MS activity was measured as 29.1 pmol/min/mg, using the same conversion, that gives $V_{MS} = 29.1 \cdot 60 \cdot 13.12/1000 = 22.91 \mu M/h$. Then solving (2.10) for $V_{max}$, we get $V_{max} = 24.02 \mu M/h$.

- metin Parameters: By metin, we represent the amino acid transporter that is responsible from uptake of MET into the cell. For these transport kinetics, $V_{metin}$
and $V_{EAAT3}$, we will use the following equation from [35]:

\begin{equation}
V = \frac{V_{max} AA_{ext}}{K_m + AA_{ext}} - AA
\end{equation}

(2.11)

where $AA_{ext}$ represents the extracellular amino acid concentration and $AA$ is the intracellular concentration of the same amino acid. Then for metin, the equation will be

\begin{equation}
V_{metin} = \frac{V_{max} MET_{ext}}{K_m + MET_{ext}} - MET.
\end{equation}

(2.12)

We will take $MET_{ext} = 150$ and $K_m = 150$. Now since $V_{MAT-II} = 69\mu M/h$ and $V_{MS} = 22.91\mu M/h$, we get $V_{metin} = 69 - 22.91 = 46.09\mu M/h$. Then solving (2.12) for $V_{max}$, we get $V_{max} = 183.16\mu M/h$. To summarize the parameters of metin:

\begin{equation*}
V_{metin} = \frac{V_{max} MET_{ext}}{K_m + MET_{ext}} - MET, \text{ where } V_{max} = 183.16, \ K_m = 150.
\end{equation*}

- CBS Parameters: Again, since the system should be in balance, we need to have $V_{CBS} = 46.09\mu M/h$, where the reaction velocity is given as

\begin{equation}
V_{CBS} = \frac{V_{max} \cdot HCY}{K_m + HCY} \cdot \frac{A(SAM + SAH)^2}{B + (SAM + SAH)^2}
\end{equation}

(2.13)

In this equation, the second fraction represents the stimulation of CBS by SAM and SAH pool. This fraction will be simply one for the steady state, then since we know that $V_{CBS} = 46.09\mu M/h$, taking $K_m = 1000$ from [35] and solving (2.13) for $V_{max}$, we get $V_{max} = 2285.64\mu M/h$. 

39
• CTGL Parameters: Just like the preceding reaction, we should have $V_{CTGL} = 46.09\mu M/h$, where

$$V_{CTGL} = \frac{V_{max} \cdot CYST}{K_m + CYST}$$

(2.14)

$K_m$ for this enzyme was given as 500 $\mu M$ in [35]. In [47], for different variants of CTGL, $K_m$ was measured as 400 – 720$\mu M$. We will take $K_m = 500\mu M$, then using $V_{CTGL} = 46.09\mu M/h$ and solving (3.6) for $V_{max}$, we get $V_{max} = 161.59\mu M$.

• Cysteine Uptake, EAAT3 Parameters: It is known that availability of CYS is a rate limiting factor for the synthesis of GSH in the cell. Having a lower GSH availability in neuronal cells, compared to other tissues, makes CYS uptake even more important for these cells. CYS is transported into the cell by EAAT3 (Excitatory Amino Acid Transporter 3). As a result, factors affecting activity of EAAT3, like IGF-1, can affect the redox status of the cell directly. Again, the form the equation for this uptake is just like (2.11).

The CYS uptake in SY5Y cells was actually measured by Nate Hodgson in our lab. It was found that CYS uptake is 1.115 nmol/mg protein/5 min. Using the same conversion formula gives $V_{EAAT3} = 175.55\mu M/h$. The equation of the reaction velocity for EAAT3 is

$$V_{EAAT3} = \frac{V_{max} \cdot CYS_{ext}}{K_m + CYS_{ext}} - CYS$$

(2.15)
where \( CYS_{ext} \) represents the extracellular cysteine concentration (which is taken as a constant 186\( \mu M \)) and \( K_m = 2100\mu M \) from [35]. Then solving (2.15) for \( V_{max} \), we get \( V_{max} = 18975.89\mu M \).

- Loss of Cysteine, \( CYS_{out} \) Parameters: Cysteine is utilized in other reactions like production of sulfate and taurine. This loss of cysteine for similar reactions is represented by \( CYS_{out} \). The rate of cysteine lost to similar reactions is relatively low under steady state, but as the cysteine concentration starts increasing, this rate also goes up [38].

For the steady state, this loss may not seem very important for two reasons; first of all, this loss is relatively low under homeostasis and secondly, this loss could easily be encompassed into the \( V_{max} \) for the production of GLC, glutamyl-cysteine. However, when IGF-1, insulin like growth factor, is introduced into the media, EAAT3 activity increases and as a result the concentrations of both CYS and GSH both increase and the cell becomes more reduced within 2 hours. After 2 hours, even though the presence of IGF-1 does not change, the concentrations of CYS and GSH do not change too much. This could be interpreted as the “new steady state”. For this new state, CYS loss will be essential to keep CYS concentration low despite the increased uptake of CYS from the extracellular environment. Once CYS is stabilized, the concentrations of GSH and GSSH also stabilize.

For now, we will take this loss as only 5\% of the whole production of CYS, which
is $0.05 \cdot (V_{EAA T3} + V_{CTGL}) = 11.08\mu M/h$. Then if we let

\begin{equation}
V_{CYS_{out}} = k \cdot [CYS]^2
\end{equation}

that gives $k = 0.000006$. This will be adequate for now. However, once we start incorporating the redox status into the model, an adjustment may be necessary.

- GCS Parameters: This enzyme has rather complicated kinetics. In addition to CYS, glutamate is also a metabolite used in this reaction. Furthermore, there is competition between GSH and glutamate and as a result GSH is a competitive inhibitor of GCS. We will take the concentration of glutamate as a constant and in [11], it was measured that the concentration of glutamate in SY5Y cells is $100nm/mg$ protein, which is equivalent to $1312\mu M$. The concentration of GLC was not measured in our experiments, so we will take the GLC concentration as $9.8\mu M$ from [35]. The equation for $V_{GCS}$ is also from [35] and [29]:

\begin{equation}
V_{GCS} = \frac{V_{max}(CYS \cdot GLUT)}{K_{m,c}K_{m,g} + K_{m,c}GLUT + K_{m,g}CYS \left(1 + \frac{GSH}{K_i} + \frac{GLUT}{K_{m,g}}\right) + GSH} + \frac{GSH}{K_i}
\end{equation}

Here, we take $K_{m,c} = 100$, $K_{m,g} = 1900$ represent the $K_m$ values for CYS and glutamate respectively. $K_i = 8200$ reflects inhibition by GSH. Under a steady state, $V_{GCS} = V_{EAA T3} + V_{GLC} - V_{CYS_{out}} = 210.56\mu M$. Solving (2.17) for $V_{max}$, we get $V_{max} = 562.45\mu M$. 

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• GS Parameters: This is the final step for the production of GSH. Glycine is a substrate for the production of GSH, but since we are not going to keep track of glycine, its concentration will be taken as constant, $GLY = 924\mu M$ from [35]. The equation for $V_{GS}$ is from [35], [29] and [19]:

\[
V_{GS} = \frac{V_{max}(GLY \cdot GLC)}{K_{m,glc}K_{m,gly} + K_{m,gly}GLC + K_{m,glc}GLY \left(1 + \frac{GLC}{K_{m,glc}}\right)}
\]

where $K_{m,glc} = 22$, $K_{m,gly} = 300$ are $K_m$ values for GLC and GLY respectively for a bi-reactant Michaelis-Menten equation. Since $V_{GCS} = 210.56\mu M$, we will have $V_{GS} = 210.56\mu M/h$. Then solving (2.18) for $V_{max}$ we get $V_{max} = 905.28\mu M$.

• $GSH_{out}$ Parameters: In order to have a balance in the system, we need some transport from the cells. MET and CYS were inputs, our exports from the cell will be GSH and GSSH. Reduced and oxidized forms of glutathione could both be exported from the cell or utilized for various reactions. Some examples could be detoxifying reactions for GSH or transport of GSSG to maintain the redox status. All similar reactions will be represented by $GSH_{out}$ or $GSSG_{out}$ in our model. A total of $210.56\mu M$ of glutathione should be exported/utilized per hour in either form, since $V_{GS} = 210.56\mu M$. Note that 1 molecule of GSSG loss for the cell means loss of 2 GSH molecules. For the total of $210.56\mu M$ GSH we will use a similar ratio like [35], 14 : 1 for $GSH : GSSG$ transport ratio. That
means $V_{\text{GSH}_{\text{out}}} = \frac{14}{15} \times 210.56 = 196.52 \mu M$. We will use the one substrate Michaelis-Menten for the transports of GSH and GSSG. Then $V_{\text{GSH}_{\text{out}}}$ will be given as

$$V_{\text{GSH}_{\text{out}}} = \frac{V_{\text{max}} \cdot \text{GSH}}{K_m + \text{GSH}}$$

(2.19)

Here we will take $K_m = 150$ as in [35]. Then $V_{\text{GSH}_{\text{out}}} = 196.52 \mu M$ and solving (2.19) for $V_{\text{max}}$, we get $V_{\text{max}} = 314.43$

- **GSSG$_{\text{out}}$** Parameters: As we discussed in the previous enzyme, we need to have $V_{\text{GSSG}_{\text{out}}} = \frac{V_{\text{GS}} - V_{\text{GSH}_{\text{out}}}}{2} = 7.02 \mu M/h$. Then the equation that describes reaction velocity for GSSG$_{\text{out}}$ is given as

$$V_{\text{GSSG}_{\text{out}}} = \frac{V_{\text{max}} \cdot \text{GSSG}}{K_m + \text{GSSG}}$$

(2.20)

Here, we take $K_m = 1250$ as in [35], then solving (2.20) for $V_{\text{max}}$, where $V_{\text{GSSG}_{\text{out}}} = 7.02 \mu M/h$, we get $V_{\text{max}} = 1600$.

- **GPx** Parameters: First of all, we need to recognize that there are lots of enzymes utilizing GSH for reducing oxygen radicals and similarly there are many enzymes reducing GSSG. The symbols GPx and GR actually both represent a family of enzymes; GPx represents GSH-utilizing enzymes and GR represents the enzymes
that reduce GSSG.

Now, to determine the parameters for both GR and GPx, we will use the fact that $V_{GPx} - V_{GR} = 7.02 \mu M/h$, since $V_{GS} - V_{GSH_{out}} = 7.02 \mu M$ (or we could say $V_{GPx} - V_{GR} = V_{GSSG_{out}} = 7.02 \mu M/h$ under steady state). To determine the exact values for these two velocities in our model, we will follow [35] again. The ratio of $V_{GPx} : V_{GR}$ is around 7 : 6, which means $V_{GPx} = 7 \cdot 7.02 = 49.14 \mu M/h$ and $V_{GR} = 6 \cdot 7.02 = 42.12 \mu M/h$.

Hydrogen peroxide, $H_2O_2$ is a substrate for the enzyme GPx, which we will take as $H_2O_2 = .01 \mu M$ again from [35]. For the steady state, we will assume the concentration of $H_2O_2$ will not change, we are taking that as a constant. The form of the reaction velocity is Michaelis-Menten with 2 substrates:

$$V_{GPx} = \frac{V_{max}GSH \cdot H_2O_2}{(K_{m,g} + GSH)(K_{m,h} + H_2O_2)}$$

where $K_{m,g} = 1330 \ [6]$ and $K_{m,h} = 0.09 \ [35]$ are the $K_m$ values for GSH and $H_2O_2$ respectively. Then solving (2.21) for $V_{max}$ we get $V_{max} = 3046.58 \mu M$.

- GR Parameters: As we described in the preceding section, $V_{GR} = 42.12 \mu M/h$.

Nicotinamide adenine dinucleotide phosphate, NADPH is also a substrate for this enzyme. The concentration of (NADPH) in SY5Y cells is given in [12] as $200 \ pmol/mgprotein$ which is equivalent to $2.62 \mu M$ using the same conversion.
The reaction velocity is again Michaelis-Menten with 2 substrates:

\[
V_{GR} = \frac{V_{max} \cdot GSSG \cdot NADPH}{(K_{m,g} + GSSG) (K_{m,n} + NADPH)}
\]

where \( K_{m,g} = 72 \) [36] and \( K_{m,n} = 10.4 \) [25, 35] are the \( K_m \) values for GSSG and \( NADPH \) respectively. Then solving (2.22) for \( V_{max} \) we get \( V_{max} = 2944.45 \mu M \).

This completes the first step of our model.

2.4. Simulations with Steady State Parameters

We will be using MATLAB for all our parameter estimation, data fitting and simulations. Simbiology offers a nice graphical interface where you can enter reaction velocities, specify parameters, metabolites and define initial conditions. These are all transformed into differential equations and results are displayed with custom made graphs. Here is our first diagram for the steady state approach:
When we run the model with the specified parameters, here is the concentrations in µM both from the experiments and simulations:

It is not surprising that the model values and the experiments are so close since this is a result of the way we defined our parameters. We used the experimental results to define all the $V_{max}$ and $K_m$ values.
<table>
<thead>
<tr>
<th>Metabolite</th>
<th>Model (Simulated)</th>
<th>Exp (Observed)</th>
</tr>
</thead>
<tbody>
<tr>
<td>MET</td>
<td>45.48881</td>
<td>45.54414</td>
</tr>
<tr>
<td>SAM</td>
<td>81.28871</td>
<td>81.23823</td>
</tr>
<tr>
<td>HCY</td>
<td>20.58269</td>
<td>20.5827</td>
</tr>
<tr>
<td>SAH</td>
<td>13.11015</td>
<td>13.11</td>
</tr>
<tr>
<td>CYST</td>
<td>200.9358</td>
<td>199.5342</td>
</tr>
<tr>
<td>CYS</td>
<td>1368.55</td>
<td>1368.398</td>
</tr>
<tr>
<td>GLC</td>
<td>9.79927</td>
<td></td>
</tr>
<tr>
<td>GSH</td>
<td>250.7431</td>
<td>250.0059</td>
</tr>
<tr>
<td>GSSG</td>
<td>5.412585</td>
<td>5.468641</td>
</tr>
</tbody>
</table>

As we can see in the following 3 graphs, no matter at what value the metabolite concentrations start, the system comes to the steady state pretty quickly:

(the "Time" is in hours, while "States" represent the concentration of each metabolite, in \( \mu M \).)
CHAPTER 3

Redox Status

It is known that many enzymes used in this model are sensitive to the redox status of the cell. Depending on the enzyme, this sensitivity could be in either way; the enzyme could be inhibited or stimulated under oxidative stress. However, there are some enzymes which are not affected by the redox status of the cell directly. For each enzyme, we will talk about sensitivity later.

As we mentioned earlier, the ratio \([GSH]/[GSSG]\) could be regarded as an indicator of redox status of a cell. In this part, for all the enzymes that are sensitive to redox status, we will be revising all the related velocity reactions using this ratio. This revision will be simply adding a coefficient to the previously defined reaction velocities.

The availability of cysteine is a rate-limiting factor for GSH synthesis. As a result, any factor(s) affecting cysteine concentration affects the GSH concentration automatically. Therefore, cysteine uptake, which is mediated by EAAT3, directly affects the GSH concentration and also the redox status of the cell. We have some experimental data detailing the concentrations of metabolites as a result of change in EAAT3 activity. In these experiments, some known stimulants (like IGF-1) or inhibitors (like morphine, amyloid beta (ABeta), oligomeric peptides or the EAAT3 blocker LBTA) were added to the
media and thiol concentrations were measured. We have the following data about EAAT3 activity under the presence of indicated agents:

<table>
<thead>
<tr>
<th>Table 3.1. % Cysteine Uptake</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
</tr>
<tr>
<td>---------</td>
</tr>
<tr>
<td>100</td>
</tr>
</tbody>
</table>

As one would expect, these inhibitors/stimulators are concentration-dependent. These changes in cysteine uptake induce changes in metabolite concentrations. For instance, the metabolite concentrations after the addition of the above agents are as follows (These are experimental results provided by Dr. Deth’s lab):

<table>
<thead>
<tr>
<th>Table 3.2. Metabolite Concentrations After Changes in EAAT3 Activity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
</tr>
<tr>
<td>---------</td>
</tr>
<tr>
<td>Cysteine</td>
</tr>
<tr>
<td>Cystathionine</td>
</tr>
<tr>
<td>GSH</td>
</tr>
<tr>
<td>SAM</td>
</tr>
<tr>
<td>Homocysteine</td>
</tr>
<tr>
<td>Methionine</td>
</tr>
<tr>
<td>GSSG</td>
</tr>
<tr>
<td>SAH</td>
</tr>
</tbody>
</table>

The relation between cysteine and GSH can be clearly seen in this table and they are correlated. Actually, for the above data, the correlation coefficient $r$ between GSH and cysteine turns out to be .77, which indeed indicates a strong correlation between these two.

The fact that availability of cysteine is a rate limiting factor for the production of GSH is enough to explain the above correlation. However, as a whole the transsulfuration pathway is irreversible, i.e. how can we explain other apparent correlations? For example, $r = -.83$ for homocysteine and cysteine. Homocysteine is not a product of cysteine, but
IGF-1 simply increases the cysteine concentration, then why would the concentration of homocysteine decrease as the cysteine concentration is increasing? The fact that many enzymes are responsive to the redox status (i.e. GSH/GSSG) ratio seems to be the main reason for all of the above changes. For the cysteine-homocysteine pair, MS is stimulated when cell is more reduced (or equivalently when there is more GSH), then as a result the concentration of HCY, being a substrate for MS goes down. this is the main reason why more cysteine results in less HCY.

Now our main task is to quantify sensitivity of enzymes that respond to the redox status. Here is some more experimental data that I will be using to “train” the model (These experiments were conducted by Nate Hodgson):

<table>
<thead>
<tr>
<th>T(hours)</th>
<th>CYS</th>
<th>Cystathionine</th>
<th>GSH</th>
<th>HCY</th>
<th>MET</th>
<th>GSSG</th>
<th>SAM</th>
<th>SAH</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>1368.4</td>
<td>199.5</td>
<td>250</td>
<td>20.6</td>
<td>45.5</td>
<td>5.5</td>
<td>81.2</td>
<td>13.1</td>
</tr>
<tr>
<td>0.5</td>
<td>1438.2</td>
<td>203.7</td>
<td>251.5</td>
<td>21</td>
<td>61.2</td>
<td>5.5</td>
<td>91.4</td>
<td>13.6</td>
</tr>
<tr>
<td>1</td>
<td>1631.1</td>
<td>121.3</td>
<td>263.5</td>
<td>19.3</td>
<td>73.8</td>
<td>5.2</td>
<td>131.9</td>
<td>12.8</td>
</tr>
<tr>
<td>2</td>
<td>1814.5</td>
<td>117.1</td>
<td>311.8</td>
<td>18.1</td>
<td>164.4</td>
<td>4.8</td>
<td>170</td>
<td>11.9</td>
</tr>
<tr>
<td>4</td>
<td>1659.9</td>
<td>153.8</td>
<td>310.8</td>
<td>17.2</td>
<td>132.2</td>
<td>5.3</td>
<td>154.8</td>
<td>11.6</td>
</tr>
<tr>
<td>48</td>
<td>1661.2</td>
<td>175.6</td>
<td>297</td>
<td>18.9</td>
<td>103.8</td>
<td>5.5</td>
<td>145.1</td>
<td>13.4</td>
</tr>
</tbody>
</table>

This table gives the concentrations of the metabolites over a 48 hour period. For each sensitive enzyme, we will define some parameters related to the redox status of the cell and then try to fit those parameters to the above data set. Before we start talking about equations and fitting parameters into data, we need to make some simplifying assumptions. The main reason for that is, fitting the whole system at once is really difficult since we have observations at only 6 points.
We will consider this system as consisting of 2 parts; the chain of reactions until cysteine as the first part and then everything after the production of cysteine as the second part. Obviously both parts are dependent on each other but clearly second part has a greater affect on the first part. The reason for that is, about 20% of newly synthesised cysteine comes through homocysteine and this percentage goes down as the cell becomes more reduced. So we will be working on the second part first, fit the parameters and so on. Then we will work on the first part.

3.1. Cysteine Uptake and GSH Synthesis

In this part, the main activities are cysteine uptake and GSH synthesis. The metabolites included in this part are CYS, GLC, GSH and GSSG; the enzymes are CTGL, EAAT3, GCS, GS, GR and GPx.

Now this part starts with the enzyme CTGL. The enzyme CTGL is not redox sensitive. Its activity depends only on the concentration of cystathionine. We would like to express the reaction velocity since it affects the cysteine concentration. However, since cystathionine is not included in this part, we would like to express $V_{CTGL}$ in terms of one of the parameters that we use in this part. With the parameters we found in the previous chapter, here is the cystathionine vs $V_{CTGL}$ data:

As we mentioned earlier, we would like $V_{CTGL}$ to be a function of one of the variables that we will be keeping track of. Here, when we calculate the correlation of $V_{CTGL}$ with GSH, GSSG or GSH/GSSG; GSSG gives the largest correlation coefficient $r = 0.86$: 53
As a result, we will take the $V_{CTGL}$ as a linear function of GSSG. Then linear regression on the values of $V_{CTGL}$ and corresponding GSSG values gives $V_{CTGL} = 21.8 \cdot GSSG - 76.2$. We will use that instead of the actual $V_{CTGL}$. One could question the relation between $V_{CTGL}$ and GSSG since we have said that the enzyme CTGL is not redox sensitive. The reason behind this relation is, cystathionine is a product of homocysteine, whose concentration is highly redox sensitive because of MS and CBS. As a result, such a relation between $V_{CTGL}$ and GSSG would not be unreasonable.

We also need to indicate that some revisions on the parameters we found for the steady state model will be necessary as we do parameter optimization.

Now we start with the IGF-1 data. As IGF-1 is added into the cell media (3), the cysteine uptake by EAAT3 increases, which increases the cysteine concentration and also
the GSH concentration. This means the cell becomes more “reduced”, which affects activity of MS. Once activity of MS changes, this automatically changes the concentrations of methionine cycle metabolites.

Among the above mentioned enzymes, only GCS is sensitive to redox status of the cell. GCS is actually stimulated under oxidative stress. For all the other enzymes in this part, we will assume they are not redox sensitive.

For GCS, we have the following important point. When we change the EAAT3 activity, or in other words, when cysteine concentration increases, GSH concentration does not increase too much. Here is an example of EAAT3 activity versus CYS and GSH concentration:

<table>
<thead>
<tr>
<th>$V_{EAAT3}$(%)</th>
<th>CYS</th>
<th>GSH</th>
</tr>
</thead>
<tbody>
<tr>
<td>100</td>
<td>1375.3</td>
<td>251</td>
</tr>
<tr>
<td>150</td>
<td>2144.4</td>
<td>260.9</td>
</tr>
<tr>
<td>200</td>
<td>2907.5</td>
<td>265</td>
</tr>
<tr>
<td>250</td>
<td>3669.4</td>
<td>267.5</td>
</tr>
<tr>
<td>300</td>
<td>4441</td>
<td>270</td>
</tr>
</tbody>
</table>

The main reason for so little change in GSH concentration where amount of CYS is more than tripled is very low $K_m$, i.e., the reaction is almost saturated. GSH also inhibits this reaction, this can be the second reason. However, this inhibition is not so significant in this case. Here is the equation we derived for $V_{GCS}$ earlier:

$$V_{GCS} = \frac{V_{max}(CYS \cdot GLUT)}{K_{m,c}K_{m,g} + K_{m,c}GLUT + K_{m,g}CYS \left( 1 + \frac{GSH}{K_{i}} + \frac{GLUT}{K_{m,g}} \right) + \frac{GSH}{K_{i}}}$$
where $K_{m,c} = 100$, $V_{\text{max}} = 562.45$. Here $K_{m,c} = 100 \mu M$ is the $K_m$ value for CYS, while the CYS concentration is more than 1300 $\mu M$, which indicates that $K_m$ is too small compared to CYS concentration. In order to have a bigger change in GSH concentration for increased EAAT3 activity, we will need a much bigger $K_m$ for CYS in GCS.

We will simply define $K_m$ as one of the parameters of our model and make a parameter fit for this new $K_m$ as well. In the literature, we are given a wide range of values for $K_m$ of CYS in GCS ($50 - 800 \mu M$, [41, 46]) for human cells and much larger $K_m$ values for non-human cells (2700-4000 $\mu M$ [22, 20]).

When we take $K_m$ as a parameter, we have to modify the $V_{\text{max}}$ to get the desired concentrations in 2.3. Now let us take $V_{\text{max}}$ also as a parameter instead of a constant. We will define one more parameter. As we mentioned earlier, IGF-1 stimulates the activity of EAAT3 and therefore cysteine uptake increases. Let us define a new parameter $kIGF$ which gives the ratio of EAAT3 activity increase, so we will modify (2.15) as follows:

\[
V_{\text{EAAT3}} = kIGF \frac{V_{\text{max}} \cdot CYS_{\text{ext}}}{K_m + CYS_{\text{ext}}} - CYS.
\]

So all together we have 3 parameters for this part; $kIGF, K_m, V_{\text{max}}$. We will use the experimental values given in 3 for CYS, GSH and GSSG and fit data into that set of values. When we do the parameter estimation in Simbiology, here is the output:
We have to indicate that software output depends heavily on the initial estimate of these 3 parameters. We are taking $k_{IGF} = 1.23$, $K_m = 1000$ and $V_{max} = 500$ as our initial estimate. These initial estimates were taken as follows: $k_{IGF}$ was estimated in

---

**Figure 3.1. Parameter Estimation**

<table>
<thead>
<tr>
<th>#</th>
<th>Name</th>
<th>Estimated Value</th>
<th>Standard Error</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>$K_m$</td>
<td>411.09129485</td>
<td>17.22057369</td>
</tr>
<tr>
<td>2</td>
<td>$k_{IGF}$</td>
<td>1.23843167</td>
<td>0.02376004</td>
</tr>
<tr>
<td>3</td>
<td>$V_{max}$</td>
<td>705.33072691</td>
<td>33.55857904</td>
</tr>
</tbody>
</table>

**Figure 3.2. Observed vs Predicted Concentrations**

---

We have to indicate that software output depends heavily on the initial estimate of these 3 parameters. We are taking $k_{IGF} = 1.23$, $K_m = 1000$ and $V_{max} = 500$ as our initial estimate. These initial estimates were taken as follows: $k_{IGF}$ was estimated in
cysteine uptake experiments from our lab, $K_m$ was estimated based on literature data and $V_{max}$ was estimated taking $K_m = 1000$ in (2.17).

Here are the observed vs predicted concentrations for these 3 metabolites:

Table 3.4. Observed vs Predicted Concentrations

<table>
<thead>
<tr>
<th>T</th>
<th>CYS</th>
<th>GSH</th>
<th>GSSG</th>
<th>CYS</th>
<th>GSH</th>
<th>GSSG</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>1368.4</td>
<td>250</td>
<td>5.47</td>
<td>1365</td>
<td>250</td>
<td>5.45</td>
</tr>
<tr>
<td>0.5</td>
<td>1438.2</td>
<td>251.5</td>
<td>5.47</td>
<td>1504.82</td>
<td>254.21</td>
<td>5.46</td>
</tr>
<tr>
<td>1</td>
<td>1631.1</td>
<td>263.5</td>
<td>5.19</td>
<td>1588.91</td>
<td>259.72</td>
<td>5.56</td>
</tr>
<tr>
<td>2</td>
<td>1814.5</td>
<td>311.8</td>
<td>4.8</td>
<td>1670.49</td>
<td>270.61</td>
<td>5.77</td>
</tr>
<tr>
<td>4</td>
<td>1659.9</td>
<td>310.8</td>
<td>5.31</td>
<td>1713.72</td>
<td>286.96</td>
<td>6.09</td>
</tr>
<tr>
<td>48</td>
<td>1661.2</td>
<td>297</td>
<td>5.54</td>
<td>1731.82</td>
<td>310.6</td>
<td>6.54</td>
</tr>
</tbody>
</table>

We have relatively good estimates for CYS and GSH ($R^2 = 0.74$ for both), but the GSSG predictions are not very good. As one can see, the predictions for GSSG are almost all greater than the observed values.

Addition of one more feature to the model becomes essential in this case. Since the ratio GSH/GSSG is a key indicator of the redox status of the cell, an error of 10-20% in prediction of GSSG concentration would not be negligible. This new feature will have to limit the increase in GSSG.

First of all, the increase in GSSG would be unavoidable because of the way we have designed this model. There are two reactions between GSH and GSSG, regulated by the enzymes GR and GPx. Together these two reactions could be regarded as a single reversible reaction (i.e. a balance reaction between the two metabolites), so an increase in
GSH would induce an increase in GSSG concentration. The fact that GSSG is a product of \( H_2O_2 \) and GSH will be helpful in adding this new feature. Basically, the reason why GSSG does not increase when GSH increases is, the concentration of \( H_2O_2 \) is the rate limiting factor for GSSG production. An increase in GSH would result in decrease of \( H_2O_2 \), so even if there is an increase in GSSG production, that would be very limited. In our model, initially we took \( H_2O_2 \) concentration as a constant, but then when GSH goes up, so does GSSG.

Therefore, we will keep track of \( H_2O_2 \) concentration in our model. That way, it will limit the production of GSSG. Having \( H_2O_2 \) as a variable in the model actually enables us to simulate many additional changes in the conditions of the cell. For example, in a neuronal cell, when the neurons are firing, this results in a greater demand for ATP, which simply increases the \( H_2O_2 \) production by mitochondria. Or another example could be mitochondrial dysfunction, which also increases the \( H_2O_2 \) production. Or a third example could be increase in \( H_2O_2 \) production due to heavy metal presence in the cytoplasm, like methyl mercury. These all could be simulated in the model by changing the production rate of \( H_2O_2 \).

We will take the \( H_2O_2 \) concentration as 0.01 \( \mu M \) under steady state. The rate it is produced will also be taken as a constant for now. Then the rate it is consumed will come from GPx activity. These two rates have to be equal under steady state conditions. As we have shown in Chapter 2, we will take \( V_{GPx} = 49.14 \mu M/h \) which will also be taken as the amount of \( H_2O_2 \) reduced per hour by GSH. For now we will take this as a constant,
i.e. this reaction rate will not change over time.

To demonstrate the effect of adding low-concentration $H_2O_2$ into the model, let us increase the CYS uptake by 40% and watch the changes in metabolite concentrations in Simbiology. Here is the related output:

The concentrations of CYS and GSH increase by 40% and 20% respectively, while $H_2O_2$ concentration goes down by 20% and there is absolutely no change in GSSG concentration (The GSSG concentrations before and after increasing the CYS uptake are both 5.5098µM). Let us re-estimate the above parameters when we have $H_2O_2$ in the model. Here is the new set of parameters for $V_{max}$, $K_m$, $kIGF$: 
Figure 3.3. Parameter Estimation with $H_2O_2$

<table>
<thead>
<tr>
<th>#</th>
<th>Name</th>
<th>Estimated Value</th>
<th>Standard Error</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>$k_{f0}$</td>
<td>1.24473054</td>
<td>0.02446503</td>
</tr>
<tr>
<td>2</td>
<td>$V_{max}$</td>
<td>849.426003902</td>
<td>32.37646993</td>
</tr>
<tr>
<td>3</td>
<td>$K_m$</td>
<td>922.006279217</td>
<td>26.31304654</td>
</tr>
</tbody>
</table>

**Estimation Statistics**

<table>
<thead>
<tr>
<th>Property</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Error Model Parameters</td>
<td>$a = 48.06601583$</td>
</tr>
</tbody>
</table>

Figure 3.4. Observed vs Predicted Concentrations

And the observed vs predicted concentrations are:
Table 3.5. Observed vs Predicted Concentrations (with $H_2O_2$)

<table>
<thead>
<tr>
<th>T(hours)</th>
<th>CYS</th>
<th>GSH</th>
<th>GSSG</th>
<th>CYS</th>
<th>GSH</th>
<th>GSSG</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>1368.4</td>
<td>250</td>
<td>5.47</td>
<td>1368.00</td>
<td>250.00</td>
<td>5.51</td>
</tr>
<tr>
<td>0.5</td>
<td>1438.2</td>
<td>251.5</td>
<td>5.47</td>
<td>1512.73</td>
<td>252.45</td>
<td>5.51</td>
</tr>
<tr>
<td>1</td>
<td>1631.1</td>
<td>263.5</td>
<td>5.19</td>
<td>1597.98</td>
<td>257.54</td>
<td>5.51</td>
</tr>
<tr>
<td>2</td>
<td>1814.5</td>
<td>311.8</td>
<td>4.8</td>
<td>1677.45</td>
<td>269.61</td>
<td>5.51</td>
</tr>
<tr>
<td>4</td>
<td>1659.9</td>
<td>310.8</td>
<td>5.31</td>
<td>1713.15</td>
<td>290.04</td>
<td>5.51</td>
</tr>
<tr>
<td>48</td>
<td>1661.2</td>
<td>297</td>
<td>5.54</td>
<td>1718.60</td>
<td>326.05</td>
<td>5.51</td>
</tr>
</tbody>
</table>

As we can see, the predicted GSSG concentrations are much closer than before. Let us finish this section by summarizing some important points. In this part, we have considered part of our model instead of the whole thing. The reason behind that was to make parameter estimation an easier task. So here is the graphical design of that part in Simbiology:
Based on the experimental data, we have made the following changes compared to the steady state:

- In order to avoid CYS accumulation and to make GSH synthesis sensitive to CYS availability even when we have higher concentrations of CYS, we have changed $K_m$ and $V_{max}$ values for GCS.
- Since GSSG concentration is not really going up in our experimental data, taking $H_2O_2$ as a variable was essential.
- The effect of IGF-1 presence on CYS uptake was taken as a constant percentage increase.

Then with these changes here are the parameters we derived from data fitting task with Simbiology:

\[
K_{m,c} = 822 \, \mu M, \quad V_{max} = 843 \, \mu M, \quad kIGF = 1.24.
\]

3.2. Methionine Cycle and Transsulfuration Pathway

In this section, we will consider the whole model, however we will do data fit only for the enzymes between the metabolites MET and CYS. These enzymes are MAT-2, DNMT, SAHH, MS, CBS, CTGL and methionine uptake. Among these enzymes, CBS, MS, MAT-2 and possibly methionine uptake are sensitive to redox status of the cell. This redox sensitivity will be specified later. For the enzymes GCS, GS, GPx, GR and CYS uptake by EAAT3 we will use the parameters we found in the previous section.
The enzymes MS and MAT-2 are inhibited by oxidative stress while CBS is activated. For the methionine uptake; in [27] it is shown that IGF-1 concentration decreases with age and in [32] a negative association between age and methionine uptake is shown. So basically IGF-1 concentration and methionine uptake are associated. We will assume methionine uptake (just like CYS uptake) is stimulated under the presence of IGF-1.

Quantifying redox sensitivity has been quite a challenge for system biologists [14, 18, 17, 44]. The ratio $GSH/GSSG$ (or $GSH^2/GSSG$) is widely regarded as an indicator of redox status for a cell. However, using neither of those ratios seemed to be useful in our preliminary work (The changes in the ratio of GSH/GSSG were relatively low compared to some of the changes in other metabolites, rational or exponential functions of this ratio did not give a “good” fit to our experimental data). Now thanks to the previous section, we may have another option; expression of the concentration of $H_2O_2$ in our model was essential to limit GSSG increase when GSH was increasing. Now the concentration of $H_2O_2$ can potentially be a better alternate; it has a really low concentration in a cell and relative changes in its concentration could be more dramatic.

For each one of the enzymes in this part, we will modify the related reaction velocity by a factor that represents the sensitivity of the enzyme to the redox status of the cell. Please note that these factors are all 1 under steady state.

For the enzyme CBS, there is one more factor in addition to the redox sensitivity. CBS is activated by the (SAM-SAH) pool. We will also find parameters for this stimulation
based on experimental results. In addition to the redox sensitivity, adjustment of some
$K_m$ and $V_{max}$ values may be necessary.

Now, let us take a look at percentage change in metabolites over the 48 hour period,
when IGF-1 is added to the cell media. Then let us remember the parameters for the key
enzymes in this part. These two tables will give us an idea about possible changes that
is necessary for $K_m$ and (consequently) $V_{max}$ values.

| Table 3.6. % Changes in Metabolites due to IGF-1 |
|-----------------|-------------|-------------|-------------|-------------|-------------|
| T(hours)        | MET         | SAM         | SAH         | HCY         | Cyst        |
| 0               | 0           | 0           | 0           | 0           | 0           |
| 0.5             | 34.4        | 12.5        | 3.4         | 1.9         | 2.1         |
| 1               | 62          | 62.3        | -2.5        | -6.4        | -39.2       |
| 2               | 261         | 109.2       | -9.4        | -12.1       | -41.3       |
| 4               | 190.4       | 90.5        | -11.2       | -16.2       | -22.9       |
| 48              | 128         | 78.6        | 2           | -8          | -12         |

| Table 3.7. Enzymes, $K_m$ Values and Metabolite Conc. |
|-----------------|---------------|-------------|
| Enzyme          | $K_m$         | Metabolite  |
| MAT-2           | 9             | MET=45      |
| DNMT            | 1.4           | SAM=81      |
| SAHH_f          | 6.5           | SAH=13      |
| SAHH_r          | 150           | HCY=20      |
| MS              | 1             | HCY=20      |
| CBS             | 1000          | HCY=20      |
| CTGL            | 500           | Cyst=200    |

### 3.2.1. Enzymes MAT-2, DNMT and metin

For this part, in order to have a manageable system of parameters, we will consider only
the metabolites MET and SAM. Then we will need equations for 3 enzymes, metin, MAT-2 and DNMT. New MET comes from 2 sources; methionine uptake or methylation of HCY.
by MS. For now, we will assume $V_{MS} = 22.91\mu M$ (from chapter 2) is just constant and only the methionine uptake increases. Once we have the equations for other metabolites and enzymes, especially for MS, we will make adjustments on metin.

We need to have presence of one more metabolite for this system, which is SAH, since the reaction velocity of DNMT, $V_{DNMT}$ depends on the concentration of SAH. Now let us take a look at the following table:

Table 3.8. $V_{DNMT}$ with IGF-1

<table>
<thead>
<tr>
<th>SAM</th>
<th>SAH</th>
<th>SAH variable</th>
<th>SAH Constant</th>
<th>% change</th>
</tr>
</thead>
<tbody>
<tr>
<td>81.2</td>
<td>13.1</td>
<td>69</td>
<td>69.27</td>
<td>0.391304</td>
</tr>
<tr>
<td>91.4</td>
<td>13.6</td>
<td>69.86</td>
<td>70.43</td>
<td>0.815918</td>
</tr>
<tr>
<td>131.9</td>
<td>12.8</td>
<td>73.42</td>
<td>73.45</td>
<td>0.040861</td>
</tr>
<tr>
<td>170</td>
<td>11.9</td>
<td>75.42</td>
<td>75.08</td>
<td>-0.45081</td>
</tr>
<tr>
<td>154.8</td>
<td>11.6</td>
<td>75.02</td>
<td>74.52</td>
<td>-0.66649</td>
</tr>
<tr>
<td>145.1</td>
<td>13.4</td>
<td>73.79</td>
<td>74.1</td>
<td>0.420111</td>
</tr>
</tbody>
</table>

As we mentioned earlier, we do not want to have more than 2 variables for this step. For that, we will take the concentration of $SAH$ as $12.73 \mu M$, which is the average of SAH over these 6 observations. The above table shows $V_{DNMT}$ for two cases, first when we take SAH as a variable, and the second when we take SAH concentration as a constant, $12.73 \mu M$. As we can see, there is no significant change in the reaction velocity of DNMT (less than 1% for all these 6 points), so we will take SAH concentration as $12.73 \mu M$.

We can see a substantial change in the amount of MET which is followed by a similar change in SAM. Now for the enzyme MAT-2, we took $K_m = 9$ for MET, while the steady
state concentration of MET is 45. This implies that the reaction mediated by MAT-2 is almost saturated, so increasing the MET concentration would not increase the reaction velocity too much. As a result, having a much bigger $K_m$ value for MET could be a way of getting greater concentrations of SAM. The enzyme MAT-2 is also redox sensitive, it is stimulated when the cell is reduced more. We will add redox sensitivity as a factor. This redox sensitivity of MAT-2 could be a second explanation of increased concentration of SAM. For now, we will keep $K_m$ as it is. The reason for that is, the reaction velocity of MAT-2 was of fundamental importance in steady state solution and we used these $K_m$ and $V_{max}$ values to determine the reaction velocity of MAT-2. So, we will not change $K_m$ or $V_{max}$ values for MAT-2.

Please note that MAT-2 is inhibited by its own product SAM. We found that inhibition factor as $\frac{A}{B+SAM}$, where $A = 65.04$, $B = 66.74$. We may have to adjust $A$ and $B$ accordingly.

For methionine uptake (metin), we will define a parameter for a possible stimulation of this process by IGF-1. The related equations for these 3 reactions, after the introduction of new parameters are as follows:
\( V_{MAT-II} = \frac{V_{max} \cdot MET}{K_m + MET} \cdot \frac{A}{B + SAM} \cdot \left( \frac{0.02}{H_2O_2 + 0.01} \right)^{kM2} \)

where \( V_{max} = 188.11, \ K_m = 9, \ A = 65.04, \ B = 66.74 \)

\( V_{DNMT} = \frac{V_{max} \cdot SAM}{K_m + SAH + SAM} \)

where \( K_m = 1.4, \ V_{max} = 81.32, \ SAH = 12.73 \)

\( V_{metin} = kMET \cdot \frac{V_{max} \cdot MET_{ext}}{K_m + MET_{ext}} - MET \)

where \( V_{max} = 183.16, \ K_m = 150. \)

In order to find these parameters, we will need the dynamic concentration of \( H_2O_2 \).

We will use the system of equations and parameters we derived in previous section. In order to do this in Simbiology, we will have to use two systems parallel to each other.

Here is the related diagram from the software:

Figure 3.6. Parameter Estimation for MAT-2, metin and SAHH
The part we are doing estimation is on the left hand side of the graph. We will be using the experimental results in table 3.6. Here we will make a parameter estimation for the stimulation of methionine uptake by IGF-1, \( kMET \) and activation of MAT-2 by lower \( H_2O_2 \) concentration.

We will be using the experimental results in table 3.6. However, we will weight the measurements this time. Since 48 hours may be a long period of time, and gene expression in the cell is likely to change over such a long period of time, we will weigh only the observations at 48 hours by a factor of 0.5. Every other observation will be counted as 1. When we do data fit on the value of \( kMET \) and \( kM2 \), here is the software output:

Figure 3.7. Parameter Estimation for MAT-2, metin and SAHH

<table>
<thead>
<tr>
<th>Estimated Values</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>#</td>
<td>Name</td>
<td>EstimatedValue</td>
</tr>
<tr>
<td>1</td>
<td>kMET</td>
<td>2.07446637e0</td>
</tr>
<tr>
<td>2</td>
<td>kM2</td>
<td>4.44622501e0</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Estimation Statistics</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Property</td>
<td>Value</td>
</tr>
<tr>
<td>Error Model Parameters</td>
<td>a = 36.8148839</td>
</tr>
</tbody>
</table>

69
Here $k_{MET}$ is the net change of reaction velocity for newly produced MET.

### 3.2.2. Enzymes SAHH, CBS, MS and CTGL

The enzyme DNMT utilizes SAM as a substrate, whose concentration increases substantially with IGF-1 addition to the media. However, its product SAH decreases. The reaction between the two metabolites SAH and HCY is a balance reaction, so the reason why SAH decreases is the decrease in HCY concentration. There are two enzymes that use the SAH-HCY pool, CBS and MS. Looking at the experimental data, since the concentration of Cyst is going down, the activity of CBS has to go down with the addition of IGF-1 to the media. Then, the only explanation for the fact that both SAH and HCY go down could be the activation of MS under reduced conditions, i.e. a lower $H_2O_2$ concentration, or higher $GSH/GSSG$ ratio. This activation should dominate the activation of DNMT due to elevated concentrations of SAM.
The 3 enzymes, SAHH, CBS and MS together regulate the SAH and HCY concentration with DNMT. We have no evidence that DNMT is sensitive to oxidative stress directly, so we will keep the reaction velocity for DNMT as it is. We know that MS is highly sensitive to oxidative stress, it is stimulated when the cell is more reduced. For the enzyme CBS, we know it is inhibited when the cell is reduced. CBS is also activated by the SAM-SAH pool, in this case since SAH is decreasing while SAM is almost doubling, the net rate of this pool on CBS activity should be positive, i.e. it is simulated by SAM-SAH pool when IGF-1 is present. Again, since the cystathionine concentration is going down, the inhibition of CBS by reduced state of the cell should dominate the activation by SAM-SAH pool.

Now let us do a parameter estimation for SAH-HCY concentration, or in other words, for the enzymes SAHH-CBS-MS. For now we will ignore the stimulation of CBS by SAM-SAH pool, we will take only the inhibition of CBS by reduced concentration of \( H_2O_2 \), since the inhibition of CBS dominates the activation. Similarly, we will consider the activation of MS by \( H_2O_2 \).

The equations for these 3 enzymes, SAHH-CBS-MS, with the parameters to be optimized are as follows:

\[
V_{SAHH} = \frac{117.96 \cdot SAH}{6.5 + SAH} - \frac{81.7 \cdot HCY}{150 + HCY}
\]
\[
V_{CBS} = \frac{2285.64 \cdot HCY \cdot H_2O_2 + 0.01}{1000 + HCY \cdot 0.01 + 0.01}
\]
\[ V_{MS} = \frac{24.02 \cdot \text{HCY}}{1 + \text{HCY}} \left( \frac{0.01 + 0.01}{H_2O_2 + 0.01} \right)^k \]

For CBS, factor for activation by oxidative stress (which becomes inhibition for IGF-1 presence) is taken from [35]. Since the steady state concentration of \(H_2O_2\) is 0.01 \(\mu M\), when it starts increasing, enzyme activity increases, or similarly, once it starts decreasing, the enzyme is inhibited.

For MS, similarly we are taking the activation factor by reduced state from [35] again. However, we are introducing a new variable \(k\) as a power of this activation. We need a greater activation as explained earlier; this parameter should be large enough to estimate the decrease in SAH and HCY concentrations.

We need to have dynamic concentration of \(H_2O_2\) for the redox sensitive changes in the reaction velocities for MS and CBS. We will use the system of equations and parameters we derived in previous sections. Here is the related diagram from the software:

Figure 3.8. Parameter Estimation for MS
The part we are doing estimation is on the left hand side of the graph. For this step, we will do individual fits for the concentrations of SAH and HCY separately. Now when we try to fit the experimental results for SAH only, here is the output:

Figure 3.9. Parameter Estimation for MS

<table>
<thead>
<tr>
<th>#</th>
<th>Name</th>
<th>EstimatedValue</th>
<th>StandardError</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>k</td>
<td>7.99777872</td>
<td>1.25813429</td>
</tr>
</tbody>
</table>

**Estimation Statistics**

<table>
<thead>
<tr>
<th>Property</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Error Model</td>
<td>a = 1.2289757</td>
</tr>
<tr>
<td>Parameters</td>
<td></td>
</tr>
</tbody>
</table>
And when we try fitting the data for HCY only, the output is:

Figure 3.10. Parameter Estimation for MS

<p>| Estimated Values |
|------------------|-----------------|------------------|</p>
<table>
<thead>
<tr>
<th>#</th>
<th>Name</th>
<th>EstimatedValue</th>
<th>StandardError</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>k</td>
<td>5.99382801</td>
<td>0.73466317</td>
</tr>
</tbody>
</table>

| Estimation Statistics |
|-----------------------|------------------|
| Property             | Value            |
| Error Model Parameters | a = 1.42179956   |

Individual Fit
Group 1

OBS1 (HCY)
PRED1 (N2.HCY)
So for these 2 different metabolites, the \( k \) value are given as 6 or 8. We will take \( k = 7 \). Please note that this is with taking inhibition of CBS. Here we need to indicate that, in [43], the activity of MS with the presence of IGF-1 was estimated as 112% higher than normal. In our case, such a \( k \) would mean \((2/1.8)^7 = 2.09\) which is consistent with those findings.

Now we need to close the methionine cycle, as you can see in the above diagrams, we assumed there is an enzyme MS, which is only regarded as a loss in HCY. We assumed that the whole increase in MET comes from methionine uptake from extracellular environment. Since we know the additional amount coming from HCY, we can adjust the methionine uptake stimulation by IGF-1:

![Figure 3.11. Adjusting kMET](image)
Then here is the output when we try fitting the MET concentration to the experimental results:

Figure 3.12. Adjusting kMET
Now the net change of CBS activity after the addition of IGF-1 to the media is inhibition. We know that less $H_2O_2$ inhibits the enzyme while greater concentration of SAM-SAH pool activates. We will assume the inhibition is twice as big as the activation. For the inhibition, we will again use what we have found so far. For the above calculations, we already took the net inhibition of CBS by $H_2O_2$ as $(H_2O_2 + 0.01)/0.02$, which becomes in this case $0.018/0.02 = 0.9$. So we will assume inhibition by $H_2O_2$ at a concentration of 0.8 is actually $((H_2O_2 + 0.01)/0.02)^2 = 0.81$, while activation by SAM-SAH pool is $0.9/0.81 = 1.11$. For the factor of activation, we will again follow [35]. So we will assume the activation of CBS by SAM-SAH pool is

$$\frac{A(SAM + SAH)^2}{B + (SAM + SAH)^2}.$$  

This factor needs to be 1 under steady state, i.e. when SAM+SAH=94.3, and when $H_2O_2$ concentration reaches 0.8, i.e. when SAM+SAH=184.1, this factor needs to be 1.11. Then solving 2 equations for 2 unknowns, we get $A = 1.155$ and $B = 1380$.

Then we can rewrite the equation for $V_{CBS}$ as follows:

$$V_{CBS} = \frac{2285.64 \cdot HCY}{1000 + HCY} \left( H_2O_2 + 0.01 \right)^2 \frac{1.155(SAM + SAH)^2}{1380 + (SAM + SAH)^2}.$$  

Now as final step towards the completion of this section let us see if we need to change any parameters about CTGL. The equation for $V_{CTGL}$ was

(3.6) $$V_{CTGL} = \frac{V_{\text{max}} \cdot CYST}{K_m + CYST},$$ where $V_{\text{max}} = 161.59$, $K_m = 500$. 

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Again, let us use the same system of equations and add 1 more reaction to the previous diagram. Then when we run the simulation, we get the following values:

<table>
<thead>
<tr>
<th>T</th>
<th>Model</th>
<th>Observed</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>200</td>
<td>199.5</td>
</tr>
<tr>
<td>0.5</td>
<td>200.44</td>
<td>203.7</td>
</tr>
<tr>
<td>1</td>
<td>200.47</td>
<td>121.3</td>
</tr>
<tr>
<td>2</td>
<td>198.72</td>
<td>117.1</td>
</tr>
<tr>
<td>4</td>
<td>189.60</td>
<td>153.8</td>
</tr>
<tr>
<td>48</td>
<td>112.65</td>
<td>175.6</td>
</tr>
</tbody>
</table>

When we look at the table, especially in the first 2 hours, there is a very rapid decrease in the concentration of Cyst. There is roughly 80 $\mu M$ decrease in the Cyst concentration from $t = 0.5$ tp $t = 1$ hour, or in other words 160 $\mu M/h$. We calculated $V_{CTGL}$ around 46 $\mu M/h$ under homeostasis. This average reaction velocity is almost 3.5 times faster than the regular reaction. There may be some transcriptional factors affecting this reaction, which we are not representing in this model. For now, we will keep the parameters as they are for $V_{CTGL}$.

### 3.3. All Redox Parameters

We have estimated all the necessary parameters in this model for now. For all the redox sensitive enzymes, basically we have used the concentration of $H_2O_2$ as the factor of this sensitivity. Let us summarize all the related redox parameters for each enzyme:
(1) To begin with, we have estimated that CYS uptake by EAAT3, when IGF-1 is present, increases by 24%, i.e. $k_{IGF} = 1.24$, where

$$V_{EAAT3} = k_{IGF} \frac{V_{max} \cdot CY S_{ext}}{K_m + CY S_{ext}} - CY S$$

(3.7)

(2) Similarly, we have found that methionine uptake also increase around 63%, i.e. $k_{MET} = 1.63$, where

$$V_{metin} = k_{MET} \frac{V_{max} \cdot MET_{ext}}{K_m + MET_{ext}} - MET.$$ 

(3.8)

(3) For the enzyme MAT-2, the new velocity reaction was given as

$$V_{MAT-2} = \frac{V_{max} \cdot MET \cdot A}{K_m + MET \cdot (\frac{0.02}{H_2O_2 + 0.01})^{kM2}}$$

and we estimated $kM2 = 4.44$.

(4) For MS, the reaction velocity was given as

$$V_{MS} = \frac{24.02 \cdot HCY}{1 + HCY} \left(\frac{0.01 + 0.01}{H_2O_2 + 0.01}\right)^k$$

and we estimated $k = 7$.

(5) For CBS, the reaction velocity was given as

$$V_{CBS} = \frac{2285.64 \cdot HCY \left(\frac{H_2O_2 + 0.01}{0.01 + 0.01}\right)^2}{1380 + (SAM + SAH)^2} + 1.155(SAM + SAH)^2.$$ 

This equation gives the activation of CBS by both oxidative stress and the SAM-SAH pool.
For the enzyme GCS, it is known that t is activated by oxidative stress. However, since our data does not show any inhibition or decrease in CYS or GSH concentration, we were unable to estimate any parameters for this activation/inhibition.

Now when we bring all these equations and parameters together and when we simulate the model without any IGF-1 presence in the media, here are our new steady state concentrations for the metabolites:

Table 3.10. Steady State Values with Redox Parameters

<table>
<thead>
<tr>
<th></th>
<th>Predicted</th>
<th>Observed</th>
</tr>
</thead>
<tbody>
<tr>
<td>MET</td>
<td>45.59</td>
<td>45.49</td>
</tr>
<tr>
<td>SAM</td>
<td>82.21</td>
<td>81.28</td>
</tr>
<tr>
<td>SAH</td>
<td>13.13</td>
<td>13.11</td>
</tr>
<tr>
<td>HCY</td>
<td>20.52</td>
<td>20.58</td>
</tr>
<tr>
<td>CYST</td>
<td>198.45</td>
<td>199.53</td>
</tr>
<tr>
<td>CYS</td>
<td>1366.16</td>
<td>1368.42</td>
</tr>
<tr>
<td>GLC</td>
<td>9.93</td>
<td>-</td>
</tr>
<tr>
<td>GSH</td>
<td>256.78</td>
<td>250.01</td>
</tr>
<tr>
<td>$H_2O_2$</td>
<td>0.01</td>
<td>-</td>
</tr>
<tr>
<td>GSSG</td>
<td>5.51</td>
<td>5.51</td>
</tr>
</tbody>
</table>
CHAPTER 4

Simulations and Results

In this chapter we will be doing in silico simulations with the model developed in chapter 3. We will be mainly interested in methylation reactions and the redox status of the cell. For methylation reactions we will look at the SAM/SAH ratio as well as the changes in the flux from SAM to SAH. For the redox status, in addition to GSH/GSSH ratio we will compare the related $H_2O_2$ concentrations. We will be highlighting some features of the model related to the work done by our lab members.

4.1. Changes in Methionine Synthase (MS) Activity

As we mentioned earlier, the activity of MS is of central importance for methylation reactions. MS has a cobalt atom in its structure and cobalt can be easily oxidized. This makes MS highly sensitive to oxidative stress. The parameters we have determined in Chapter 3 represent this sensitivity; among all the enzymes that are redox sensitive, MS is quantitatively the most responsive enzyme.

Vitamin B12 (cobalamin) is a co-factor of MS, its availability affects the reaction rate. We are not keeping track of cobalamin concentration in our model. To see the effects of availability of cobalamin, we will simply change the value of $V_{max}$ in the related reaction rate for MS. To have an idea, we will take 50% lower and higher values of $V_{max}$ for MS. For other factors affecting the activity of MS like 5-methyltetrahydrofolate (5mthf), a
similar approach could be applied.

Keeping every other variable, MET and CYS uptake as they are in the steady state, here is the new concentrations of metabolites when the MS activity changes (for example depending on cobalamin availability):

Table 4.1. Metabolite Concentrations vs MS Activity (B12)

<table>
<thead>
<tr>
<th>Metabolite</th>
<th>50</th>
<th>75</th>
<th>100</th>
<th>125</th>
<th>150</th>
</tr>
</thead>
<tbody>
<tr>
<td>GSH/GSSG</td>
<td>51.203</td>
<td>48.803</td>
<td>46.93</td>
<td>45.406</td>
<td>44.126</td>
</tr>
<tr>
<td>V	extsubscript{DNMT}</td>
<td>69.674</td>
<td>69.575</td>
<td>69.332</td>
<td>69.01</td>
<td>68.637</td>
</tr>
<tr>
<td>H	extsubscript{2}O	extsubscript{2}</td>
<td>0.009</td>
<td>0.01</td>
<td>0.01</td>
<td>0.01</td>
<td>0.01</td>
</tr>
<tr>
<td>MET</td>
<td>37.564</td>
<td>42.182</td>
<td>45.908</td>
<td>49.025</td>
<td>51.698</td>
</tr>
<tr>
<td>SAM</td>
<td>105.538</td>
<td>93.677</td>
<td>84.132</td>
<td>76.277</td>
<td>69.673</td>
</tr>
<tr>
<td>HCY</td>
<td>25.008</td>
<td>22.361</td>
<td>20.403</td>
<td>18.89</td>
<td>17.69</td>
</tr>
<tr>
<td>Cyst</td>
<td>250.52</td>
<td>219.632</td>
<td>196.525</td>
<td>178.293</td>
<td>163.416</td>
</tr>
<tr>
<td>CYS</td>
<td>1491.789</td>
<td>1424.429</td>
<td>1371.928</td>
<td>1329.245</td>
<td>1293.456</td>
</tr>
<tr>
<td>GSH</td>
<td>281.503</td>
<td>268.311</td>
<td>258.013</td>
<td>249.636</td>
<td>242.599</td>
</tr>
<tr>
<td>GSSG</td>
<td>5.498</td>
<td>5.498</td>
<td>5.498</td>
<td>5.498</td>
<td>5.498</td>
</tr>
</tbody>
</table>
4.1.1. Results

4.1.1.1. Redox Status. As we can see, when MS activity increases from 50% to 150% gradually, the change in redox status of the cell is very limited. The change in GSH/GSSG ratio is around 1%. The reason for that limited change in redox status is, relatively a smaller fraction of CYS comes through transsulfuration pathway compared to uptake from the extracellular environment. So even if relatively less HCY is transsulfurated, it really does not change the redox status of the cell.

4.1.1.2. Methylation Metabolism. Unlike the redox status, we can see significant changes in the methylation metabolism as MS activity increases. The SAM/SAH ratio goes up from 5.4 to 7.3, approximately a 35% change in this ratio. This change is mainly due to the decrease in SAH concentration, which is a result of the decrease in HCY availability. However, if we look at the the flux from SAM to SAH, i.e. $V_{DNMT}$ this change becomes less dramatic (around 5%). Here we need to indicate that there are other methylation reactions with bigger $K_m$ values which carry a lot of the flux, like PEMT (phosphatidylethanolamine N-methyltransferase) and GAMT (guanidinoacetate N-methyltransferase). Due to this higher $K_m$, these reaction rates drop more and flux would decrease more than what we observed. However, even small changes in the methylation reactions may result in important changes in DNA methylation and as a result in gene expression.
4.2. Changes in Methionine Uptake

As we have mentioned earlier, age dependency of methionine uptake has been reported[32]. This dependence may be related to several factors, like redox status of neuronal cells or presence of some growth factors in CSF. In this section, regardless of the reason behind such a change, we will simply investigate the effects of this change. Again, we will simply let $V_{max}$ of metin to vary from 50% to 150%. We will also assume that the CYS uptake is fixed while metin activity changes.

Here is the corresponding metabolite concentrations as metin activity changes:

<table>
<thead>
<tr>
<th>MET Uptake (%)</th>
<th>50</th>
<th>75</th>
<th>100</th>
<th>125</th>
<th>150</th>
</tr>
</thead>
<tbody>
<tr>
<td>SAM/SAH</td>
<td>2.269</td>
<td>5.141</td>
<td>6.325</td>
<td>6.835</td>
<td>7.114</td>
</tr>
<tr>
<td>GSH/GSSG</td>
<td>42.585</td>
<td>46.095</td>
<td>46.746</td>
<td>46.965</td>
<td>47.073</td>
</tr>
<tr>
<td>$V_{DNMT}$</td>
<td>54.364</td>
<td>66.866</td>
<td>69.211</td>
<td>70.006</td>
<td>70.397</td>
</tr>
<tr>
<td>$H_2O_2$</td>
<td>0.0108</td>
<td>0.0101</td>
<td>0.0099</td>
<td>0.0099</td>
<td>0.0099</td>
</tr>
<tr>
<td>MET</td>
<td>8.723</td>
<td>24.181</td>
<td>45.732</td>
<td>68.164</td>
<td>90.821</td>
</tr>
<tr>
<td>SAM</td>
<td>25.387</td>
<td>64.644</td>
<td>83.082</td>
<td>91.37</td>
<td>95.965</td>
</tr>
<tr>
<td>SAH</td>
<td>11.188</td>
<td>12.574</td>
<td>13.136</td>
<td>13.367</td>
<td>13.489</td>
</tr>
<tr>
<td>Cyst</td>
<td>148.63</td>
<td>189.694</td>
<td>197.578</td>
<td>200.261</td>
<td>201.586</td>
</tr>
<tr>
<td>CYS</td>
<td>1250.295</td>
<td>1348.524</td>
<td>1366.758</td>
<td>1372.907</td>
<td>1375.928</td>
</tr>
<tr>
<td>GSH</td>
<td>234.127</td>
<td>253.42</td>
<td>257</td>
<td>258.207</td>
<td>258.799</td>
</tr>
<tr>
<td>GSSG</td>
<td>5.498</td>
<td>5.498</td>
<td>5.498</td>
<td>5.498</td>
<td>5.498</td>
</tr>
</tbody>
</table>
4.2.1. Results

4.2.1.1. Redox Status. Changes in MET uptake does not change the redox status too much, with one exception; when the MET uptake goes down by as much as 50%, this results in unexpectedly lower values for GSH/GSSG. Apparently, the reason for that exception is, MET concentration goes down a lot and this reduces SAM concentration as well. Then since CBS is sensitive to the SAM-SAH pool, which is going down, CBS activity decreases a lot. This can be seen from the high concentration of HCY and the low concentration of Cyst.

4.2.1.2. Methylation Metabolism. As expected, changes in MET uptake generates changes in SAM/SAH ratio. Again, the most significant change is observed when MET uptake goes down by 50%, and the reason for that significance is similar to the significance in redox status. An important implication of this significant change would be, even if there is no change in redox status of the cell, lower MET uptake has a potential to cripple the
methylation metabolism. If there is an abnormality in the methylation metabolism for a neuronal cell, this lower uptake could address that abnormality.
4.3. The Effects of EAAT3 Activity

EAAT3 activity plays a crucial role in redox and methylation metabolism. As a result, any factor that is affecting the EAAT3 activity would directly affect the same metabolism. Without specifying these factors we will simply assume the $V_{\text{max}}$ will change by 50% and investigate the related changes on the whole system. Similarly, we will assume the MET uptake does not change while we are varying the EAAT3 activity.

Extracellular cysteine availability could also be a parameter for the redox status of the cell. Compared to the plasma, CYS is very limited in CSF, so this could very well be an interesting feature for the model. However, we can explore this feature by changing EAAT3 activity as well, so we will not have a separate section on CYS availability.

Here is the corresponding metabolite concentrations as EAAT3 activity changes:

<table>
<thead>
<tr>
<th>EAAT3 Act(%)</th>
<th>50</th>
<th>75</th>
<th>100</th>
<th>125</th>
<th>150</th>
</tr>
</thead>
<tbody>
<tr>
<td>SAM/SAH</td>
<td>0.32</td>
<td>2.23</td>
<td>6.31</td>
<td>13.34</td>
<td>26.99</td>
</tr>
<tr>
<td>GSH/GSSG</td>
<td>20.71</td>
<td>33.99</td>
<td>46.72</td>
<td>59.01</td>
<td>71.11</td>
</tr>
<tr>
<td>$V_{\text{DNMT}}$</td>
<td>16.96</td>
<td>54.06</td>
<td>69.19</td>
<td>75.03</td>
<td>77.97</td>
</tr>
<tr>
<td>$H_2O_2$</td>
<td>0.0231</td>
<td>0.0135</td>
<td>0.0099</td>
<td>0.008</td>
<td>0.0069</td>
</tr>
<tr>
<td>MET</td>
<td>75.24</td>
<td>44.87</td>
<td>45.71</td>
<td>62.54</td>
<td>81.04</td>
</tr>
<tr>
<td>SAM</td>
<td>2.18</td>
<td>24.88</td>
<td>82.94</td>
<td>158.02</td>
<td>234.28</td>
</tr>
<tr>
<td>SAH</td>
<td>6.86</td>
<td>11.14</td>
<td>13.13</td>
<td>11.85</td>
<td>8.68</td>
</tr>
<tr>
<td>HCY</td>
<td>41.98</td>
<td>27.06</td>
<td>20.47</td>
<td>14.32</td>
<td>5.74</td>
</tr>
<tr>
<td>Cyst</td>
<td>55.96</td>
<td>202.79</td>
<td>197.72</td>
<td>109.17</td>
<td>34.61</td>
</tr>
<tr>
<td>CYS</td>
<td>636.13</td>
<td>1010.04</td>
<td>1366.05</td>
<td>1712.3</td>
<td>2059.71</td>
</tr>
<tr>
<td>GLC</td>
<td>6.17</td>
<td>8.38</td>
<td>9.93</td>
<td>11.09</td>
<td>12.01</td>
</tr>
<tr>
<td>GSH</td>
<td>113.85</td>
<td>186.88</td>
<td>256.86</td>
<td>324.43</td>
<td>390.95</td>
</tr>
<tr>
<td>GSSG</td>
<td>5.498</td>
<td>5.498</td>
<td>5.498</td>
<td>5.498</td>
<td>5.498</td>
</tr>
</tbody>
</table>
4.3.1. Results

As *de novo* synthesis of GSH depends on CYS availability, changing EAAT3 activity highly affects the redox status of the cell. Since two key enzymes for methylation reactions, MS and MAT-2 are both redox sensitive, EAAT3 activity affects methylation reactions to a great extent.

For SAM/SAH ratio we have a range of values from 0.3 to 27, a 90-fold change. For GSH/GSSG ratio, we have a change from 20 to 71, a 3.5-fold change. These changes, unlike many other simulations, are supported by our experimental data (3).

We will do some further simulations with EAAT3 activity later.
4.4. The Effects of Mitochondrial Efficiency and Changes in ROS Production

For any cell, mitochondria could be regarded as the “power plant” of the cell. Mitochondria produces the energy required for the survival of the cell and oxygen is essential for this energy production. As a result of these reactions, mitochondria releases a lot of oxygen compounds into the cell as a byproduct, especially reactive oxygen species (ROS). These oxygen compounds should be handled immediately in order to prevent oxidative damage for the cell. Any changes in the efficiency of this handling process may cause oxidative stress in the cell.

Consumption of oxygen by human brain accounts for 25% of the total consumption by the whole body. Compared to its weight, this is disproportionately big. Therefore ROS production, compared to other tissues, is much greater in neuronal cells. In that regard, any impairment of mitochondria makes neuronal cells more susceptible to oxidative stress. Mitochondrial dysfunction is very common in children with autism [13]. This could happen in several ways, but we will simply assume there is more ROS production in the cell. In addition to mitochondrial dysfunction, there may be a temporarily higher demand for energy in the cell, which would again increase the ROS production.

We are considering only the $H_2O_2$ concentration as a representative of ROS in the cell. We took its production rate as a constant in Chapter 3, so here we will simply change that production rate and observe changes in the metabolite concentrations. We will consider 20% lower and higher values of ROS production. For a greater range, the software gives
Here is the corresponding metabolite concentrations as ROS production changes:

Table 4.4. Metabolite Concentrations vs ROS Production

<table>
<thead>
<tr>
<th>ROS Production(%)</th>
<th>80</th>
<th>90</th>
<th>100</th>
<th>110</th>
<th>120</th>
</tr>
</thead>
<tbody>
<tr>
<td>GSH/GSSG</td>
<td>60.501</td>
<td>52.957</td>
<td>46.72</td>
<td>41.474</td>
<td>37.005</td>
</tr>
<tr>
<td>Vdnmt</td>
<td>76.208</td>
<td>72.995</td>
<td>69.194</td>
<td>64.48</td>
<td>58.367</td>
</tr>
<tr>
<td>$H_2O_2$</td>
<td>0.008</td>
<td>0.009</td>
<td>0.01</td>
<td>0.011</td>
<td>0.013</td>
</tr>
<tr>
<td>MET</td>
<td>68.901</td>
<td>54.356</td>
<td>45.708</td>
<td>42.135</td>
<td>42.822</td>
</tr>
<tr>
<td>SAM</td>
<td>182.147</td>
<td>125.184</td>
<td>82.938</td>
<td>53.005</td>
<td>33.034</td>
</tr>
<tr>
<td>HCY</td>
<td>11.567</td>
<td>17.489</td>
<td>20.474</td>
<td>22.423</td>
<td>24.964</td>
</tr>
<tr>
<td>Cyst</td>
<td>81.304</td>
<td>149.237</td>
<td>197.722</td>
<td>219.935</td>
<td>215.555</td>
</tr>
<tr>
<td>CYS</td>
<td>1344.515</td>
<td>1358.026</td>
<td>1366.046</td>
<td>1369.333</td>
<td>1368.648</td>
</tr>
<tr>
<td>GSH</td>
<td>262.652</td>
<td>260.329</td>
<td>256.861</td>
<td>252.472</td>
<td>247.369</td>
</tr>
<tr>
<td>GSSG</td>
<td>4.341</td>
<td>4.916</td>
<td>5.498</td>
<td>6.087</td>
<td>6.685</td>
</tr>
</tbody>
</table>
4.4.1. Results

ROS production affects the $H_2O_2$ concentration directly, i.e. the redox status of the cell. This automatically affects SAM/SAH ratio as expected.

As we can see, even though the $H_2O_2$ concentration is experiencing more than 50% change (from $0.008\mu M$ to $0.013 \mu M$), the change in GSH concentration is very limited. However, the GSH/GSSG ratio changes considerably since the GSSG concentration changes a lot ($\sim 50\%$). So far, this is the only case where the GSSG concentration is actually changing. This implies that if there is a temporary demand for greater ROS production, this would be reflected in elevated levels of GSSG but GSH levels would not change too much.

We can see that SAM/SAH ratio also changes a lot. The reason for that is basically the redox sensitivity of MAT-II. This enzyme is either inhibited or activated too much by the redox status (i.e. the $H_2O_2$ concentration of the cell) and this results in big deviation in the concentration of SAM. as a result, we can see these big changes in the SAM/SAH ratio.

Here is one more important point. We could change the $K_m$ value for MAT-II. Basically, if we had a bigger value for $K_m$, MAT-II would be less redox sensitive. However, since we have $K_m$ values taken from [15] and since they are also measuring these parameters on SY5Y cells, we prefer to keep the $K_m$ value as it is.
4.5. The importance of efficiency for GR and GPx, The Role of Selenium

The two enzymes GPx and GR play an important role in redox status of the cell, first one reduces ROS ($H_2O_2$ in particular) while the latter re-synthesizes GSH from GSSG. Enzyme GPx already has a selenium in its structure, while conversion of GSSG to GR depends on selenium availability indirectly. To see possible effects of selenium on this pair of enzymes, we will assume the $V_{max}$ values for both enzymes increase/decrease together by a factor of 0.85 to 1.15.

Here is the corresponding metabolite concentrations as selenium availability and GPx-GR efficiency changes:

Table 4.5. As The Efficiency of GPx/GR Pair Increases (Selenium Availability)

<table>
<thead>
<tr>
<th>GPx/GR Efficiency (%)</th>
<th>85</th>
<th>92.5</th>
<th>100</th>
<th>107.5</th>
<th>115</th>
</tr>
</thead>
<tbody>
<tr>
<td>SAM/SAH</td>
<td>3.85</td>
<td>5.25</td>
<td>6.6</td>
<td>7.96</td>
<td>9.39</td>
</tr>
<tr>
<td>GSH/GSSG</td>
<td>41.6</td>
<td>44.91</td>
<td>47.41</td>
<td>49.44</td>
<td>51.13</td>
</tr>
<tr>
<td>$V_{DNMT}$</td>
<td>63.08</td>
<td>67.15</td>
<td>69.64</td>
<td>71.4</td>
<td>72.73</td>
</tr>
<tr>
<td>$H_2O_2$</td>
<td>0.0116</td>
<td>0.0105</td>
<td>0.0098</td>
<td>0.0093</td>
<td>0.0088</td>
</tr>
<tr>
<td>MET</td>
<td>41.93</td>
<td>43.47</td>
<td>46.39</td>
<td>49.85</td>
<td>53.51</td>
</tr>
<tr>
<td>SAM</td>
<td>47.11</td>
<td>67.62</td>
<td>86.9</td>
<td>104.86</td>
<td>121.56</td>
</tr>
<tr>
<td>SAH</td>
<td>12.22</td>
<td>12.87</td>
<td>13.17</td>
<td>13.17</td>
<td>12.95</td>
</tr>
<tr>
<td>HCY</td>
<td>22.96</td>
<td>21.4</td>
<td>20.23</td>
<td>19.06</td>
<td>17.79</td>
</tr>
<tr>
<td>Cyst</td>
<td>221.21</td>
<td>211.47</td>
<td>193.66</td>
<td>173.65</td>
<td>153.68</td>
</tr>
<tr>
<td>CYS</td>
<td>1447.69</td>
<td>1426.25</td>
<td>1385.48</td>
<td>1338.15</td>
<td>1289.45</td>
</tr>
<tr>
<td>GLC</td>
<td>10.24</td>
<td>10.16</td>
<td>10.01</td>
<td>9.82</td>
<td>9.63</td>
</tr>
<tr>
<td>GSH</td>
<td>264.94</td>
<td>265.03</td>
<td>260.67</td>
<td>254.37</td>
<td>247.22</td>
</tr>
<tr>
<td>GSSG</td>
<td>6.37</td>
<td>5.9</td>
<td>5.5</td>
<td>5.15</td>
<td>4.83</td>
</tr>
</tbody>
</table>
4.5.1. Results

As we can see, GPx/GR efficiency and selenium availability plays a crucial role in redox balance of the cell. When these two enzymes are 15% more efficient than normal, simply the cell becomes more reduced ($H_2O_2$ concentration goes down and GSH/GSSG is greater) even if the concentration of GSH in the cell is actually going down. Similarly, when they are 15% less efficient, the cell becomes more oxidized with greater GSH concentration.

This fact may have important implications. It is known that neuronal cells have 10-20 fold lower GSH concentrations compared to other tissues. As we can see from the above table, it is possible to have less GSH concentration with the same levels of ROS.

We will return to simulations involving GPx/GR efficiency later.
4.6. Temporary Changes in ROS Production, How soon Can The Cell Normalize?

As we mentioned earlier, it is possible to have an increase in ROS production temporarily. For example when there is a greater demand for brain involvement, i.e. when the neurons are firing, ROS production would increase. Now let us see how soon could the neuronal cell go back to homeostasis after this temporary demand is over.

Let us assume there is 10% more ROS production for a period of 5 hours, then let us assume it is over after 5 hours. Here are the graphs giving changes in some key variables for our model:
Here is what happens when 5-hour increased ROS production ends:

The $H_2O_2$ concentration goes back to steady state value very rapidly (first graph). The reason for that is, $H_2O_2$ concentration, compared to GSH is very low. So once the additional ROS production is over, the available GSH quickly reduces the extra $H_2O_2$. 
SAM keeps decreasing for the first 5 hours and then it takes roughly another 5 hours to restore SAM levels (second graph). The GSSG concentration goes back to normal levels again very quickly. However, it takes a longer time for GSH (around 3 hours) to come back to normal values.

This simulation indicated that, once the circumstances creating oxidative stress change, the cell can quickly get rid of the ROS. However, restoring homeostasis concentrations for key metabolites like GSH and SAM could take longer.
4.7. Oxidative Stress: B12 Supplement, MET Uptake, CYS Uptake or Selenium Uptake?

For this section let us assume there is an increase in ROS concentration constantly; we will assume ROS production goes up by 10%. Then let us see the effects of additional MET, CYS or selenium uptake on the methylation and redox metabolism.

4.7.1. B12 Supplement

First of all, we already know that B12 supplement does not affect the redox status of the cell too much. Let us see to what extent can this be functional in restoring the methylation reactions. We already have the concentrations of the key metabolites when ROS production goes up by 10%. Now let us see how much B12 supplement would be necessary to restore SAM/SAH ratio back to normal:
This graph is somewhat surprising for us. According to this graph, under moderate oxidative stress, B12 supplement may not be ideal to restore SAM/SAH ratio, because it increases MS activity, which simply inhibits the transsulfuration pathway. Even though the available MET increases, since the oxidative stress also increases, it further inhibits the MAT-2 reaction and SAM concentration really does not increase.

4.7.2. MET Uptake

First of all, we already know that MET uptake does not affect the redox status of the cell too much. Let us see to what extent can this be functional in restoring the methylation reactions. We already have the concentrations of the key metabolites when ROS production goes up by 10%. Now let us see how much MET uptake would be necessary to restore SAM/SAH ratio back to normal:
As this graph indicates, increasing the MET uptake even by 500% would not restore the SAM/SAH ratio back to its steady state value. However, increasing MET uptake by 100% significantly improves the same ratio. (The changes in GSH/GSSG are not graphed since this change is negligible due to changes in MET uptake)

4.7.3. CYS Uptake

We know the effects of EAAT3 on the cell, let us see its effects when ROS production increases by 10%:
Based on these two graphs, we can say two things:

- The EAAT3 activity can be really effective in reducing the levels of ROS and restoring the steady state values.

- However, we are getting close to steady state values for almost 200% increase in EAAT3 activity. In our experimental data, we have seen an increase of EAAT3 up to 40%, so such a big increase may not be realistic for neuronal cells.

4.7.4. Selenium Uptake

As we have mentioned earlier, selenium availability affects the efficiency of GPx/GR enzyme pair and we already know that it has a great impact on the redox status of the cell. Again, when ROS production goes up by 10%, let us see how much increase in efficiency (i.e. selenium uptake, indirectly) we need to restore the homeostasis levels:
From the two graphs, it looks like an additional 10% efficiency for the GPx/GR would be enough to restore the $H_2O_2$ level, SAM/SAH and GSH/GSSG ratios. Apparently, among these 4 “treatments”, increasing GPx/GR efficiency is the most effective way of restoring the homeostasis levels.
CHAPTER 5

Conclusions

Biological systems are very complicated by nature. In order to have a manageable mathematical model, one has to simplify a lot of things. This simplification sometimes may include ignoring some important features of the system in consideration. This model is no different in that respect; I take a lot of variables like concentration of metabolites as constants, I ignore many other details related to kinetic properties of enzymes, I assume for the time course that I consider there are no substantial changes in protein levels and gene expression and so on.

In addition to the simplifications mentioned above, there is one more important fact about my model. My data fitting and parameter estimation is based on SH-SY5Y cells. These cells were derived from human neuroblastoma cells. Being a derivative of human neuronal cells makes these cells invaluable. However there are some big differences between actual neuronal cells and SH-SY5Y cells. First of all, these are cancer cells which divide, unlike actual human neuronal cells. Secondly, some metabolite concentrations in SH-SY5Y cells and actual neuronal cells may differ greatly like homocysteine or cysteine concentrations. Also, the SH-SY5Y cells are grown in cell culture media, whose composition is not the same as the cerebral spinal fluid (CSF). The lack of data on actual neuronal cells makes us dependent on these cell lines for many experiments. It is believed that even with these shortcomings, SH-SY5Y cells still provide a reasonable basis to begin
to understand many important phenomenon about human neuronal cells and brain.

I have used Reeds Model as an example in developing my model, however there are some differences between the two models. First of all, many parameters I have fitted for my model are different than the corresponding parameters for the liver. Secondly, all of the enzymes and metabolites related to folate metabolism have been summarized as the concentration of 5-methyltetrahydrofolate in my model. Finally, a dynamic concentration of $H_2O_2$ in my model is another difference between the two models. In the liver model, it is taken as a constant and this constant is changed for several experiments.

I did parameter estimation and data fitting in chapter 3. Working on the whole system at once is almost impossible from a mathematical point of view. There will be too many parameters and the model quickly becomes unmanageable for Simbiology. I had to approach the system step by step, making many assumptions and simplifications in the rest of the system. Furthermore, I had to ignore some possible temporal changes in data fitting. These changes can be due to many different reasons, like adaptive response of the cell or some transcriptional factors that are affected by the shift in the redox status of the cell. The main result of this thesis is related to the inhibition of selenoenzymes by several factors, especially heavy metals, including mercury. I have shown that minor changes in the efficiency of enzyme pair GPx/GR can affect the redox status of the cell to a significant extent. I have also shown that, if there is a constant oxidative stress in the neuronal cell, no other factor except the increased efficiency of GPx/GR enzyme pair (which may be possible by additional selenium supplement), can restore the levels of key
metabolites back to homeostatic levels. This result provides an example of the potential utility of a computational model for generating novel predictions.

I have employed the model to explore effects of some supplements on SAM/SAH ratio and GSH/GSSH ratio. In silico simulations suggest that increasing MS activity (by additional B12 supplement) when the cell is reduced causes an increase in the SAM/SAH ratio. However, if the cell is already in oxidative stress, increasing this activity backfires, and the cell goes into a bigger oxidative stress which inhibits SAM formation and decreases the SAM/SAH ratio. At this point, I have investigated the effects of MET uptake as well. Higher MET uptake, just like increased MS activity, increases the SAM/SAH ratio when there is no oxidative stress. However, under oxidative stress, unlike increased MS activity, MET uptake still increases the SAM/SAH ratio. In addition, MET uptake also reduces the cell, even if the level of reduction is not significant. I have investigated the effects of EAAT3 activity using the model as well, which gave results consistent with the observed experimental data.

This model can certainly be developed further to include many other aspects. One direction could be the incorporation of DNA methylation data which has been experimentally obtained by our lab. Another option could be adding dopamine D4 receptor to the model. A novel mechanism on involvement of D4 receptor in phospholipid methylation has been previously described [37]. Some significant changes in the thiol levels due to activation of the D4 dopamine receptor when dopamine is present have been measured in our lab as well. Utilizing the model to get further insight about this mechanism could be
the basis for a future study. A third direction, which may be interesting for many neuroscientists, would be building a model based on thiol results from actual brain samples. These have also been measured in our lab. However, we have very limited data or literature about the enzymes involved in human brain. Since making experiments on actual human neuronal cells is not an option for now, some other approach such as neuroimaging of key enzyme activities in human brain, like MAT-2, MS or CYS uptake from CSF would be necessary to build such a model. Studies with neurons derived from human stem cells could provide another interesting option to pursue.

In summary, I have successfully developed an initial model of redox and methylation pathways in a neuronal cell. The features of this model may make it useful for exploration of the behavior of metabolites in response to different conditions or during different disease states. Further enhancement of the model could improve its utility as well as its ability to mimic the characteristics of true neurons.
References


[38] Stipanuk MH, Coloso RM, Garcia RA, Banks MF. Cysteine concentration regulates cysteine metabolism to glutathione, sulfate and taurine in rat hepatocytes.


