Exploration of Inherited Enzymatic Deficiency by Perturbation of Reconstructed RBC Model

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Abstract

In this project, the RBC MASS model was used to study three case studies related to three pathological deficiencies: the PGI deficiency, PGK deficiency and GND deficiency. Those deficiencies are caused by mutation in part of the chromosome, and have a large effect on some specific populations. The RBC model contains four subsystems: glycolysis, pentose phosphate pathway(PPP), AMP metabolism, and hemoglobin modules. The merging of these subsystems alongside with the enzyme module provides insights of how the deficiencies impact the system as a whole. In the PGI case study, decrease of PGI forward rate was utilized to represent PGI deficiency, and the influence towards G6PDH2r and HEX1 was explored. In the PGK case study, the perturbation of increase and decrease ATP utilizations were made by increasing and decreasing the rate constant of the ADK1 enzyme, and their effects on healthy and PGK deficiency models were examined. In the GND case study, we examined the relationship between GND deficiency and G6PDH2r deficiency by comparing two mutated models under GSH utilization perturbation. We hope the case studies can provide insights for the treatment of inherited enzyme deficiency.

Introduction

The RBC MASS model was used to study three case studies related to three pathological deficiencies. This model is constructed using the Mass Action Stoichiometric python package (MASSpy), merging the glycolysis, pentose phosphate pathway(PPP), AMP metabolism, and hemoglobin modules. The glycolysis pathway degrades glucose and forms pyruvate or lactate as end products, during which redox potential is built in the form of NADH and high energy phosphate bonds are built in the form of ATP. Glycolysis also assimilates an inorganic phosphate group that is converted into a high-energy bond and then hydrolyzed in the ATP use reaction. Glycolysis has key factors ATP and NADH, whose behavior under different perturbation will be carefully examined in the case studies. Pentose phosphate pathway starts from G6P in glycolysis. Its function is to produce redox potential in the form of NADPH and pentose phosphate. The AMP salvage pathway is responsible for AMP synthesis and degradation. Hemoglobin is the oxygen carrier. It has four binding sites that exhibit regulatory functions. All four pathways have crucial physiological functions in the human red blood cell and the entire human body.

We built five enzyme modules to analyze the RBC model. Each module was added to the RBC model and validated by stimulating to steady state. We utilizes three case studies to closely explore the function of the enzymes and their regulatory relationships inside RBC. All three of the case studies focus on genetic diseases that cause enzyme deficiency. Most of the genetic diseases are inheritable and could be diagnosed by early screening. Thus, we hope the case study can explore the mechanism of enzyme deficiency and provide insights for its treatment. Each study contains one healthy model, which doesn't contain any modification, and at least one mutated model, which contains modifications that represent the deficiency based on published paper.

In the case studies, we explore three key enzymes in the glycolysis and pentose phosphate pathways, subsystems in the RBC model. The three enzymes are glucose phosphate isomerase (PGI), Phosphoglycerate kinase (PGK), and 6-Phosphogluconate dehydrogenase (GND). PGI is an enzyme in glycolysis. In category PGI is an isomerase, which catalyzes reactions involving a structural rearrangement of a molecule, in which it catalyzes the reaction of converting glucose-6-phosphate (G6P) to fructose-6-phosphate (F6P), involving an structural rearrangement of the sugar molecule. To model PGI reaction, three catalytic steps are defined as G6P binding, inner conversion of G6P to F6P, and release of F6P. PGK is another enzyme in glycolysis, which provides the first ATP molecules in a cycle of glycolysis. PGK in this category is a kinase, which involves the transfer of a high energy phosphate bond. PGK in glycolysis helps facilitate the high energy phosphate transfer from 1,3-bisphosphoglycerate (1,3-BPG) to ADP producing 3-phosphoglycerate (3-PG) and ATP. To model PGI reaction, PGI can either bind with 1,3-BPG or ADP first and then the other, and perform an inner conversion of the substrates, and release 3PG and ATP together. Inhibition steps are involved with competitive inhibition by 2,3-diphosphoglycerate (2,3DPG) and 3PG, also uncompetitive inhibition steps by ATP with respect to 1,3-BPG and ADP. GND is an enzyme in pentose phosphate pathway, which catalyzes the reaction to produce the second NADPH in a cycle of pentose phosphate pathway. GND in category is a dehydrogenase, which involves catalyzation of an oxidation-reduction reaction by providing a redox cofactor on themselves that can provide a hydrogen atom to carry redox functions. GND in pentose phosphate pathway catalyzes the reaction of 6-phosphogluconate (6PG) to ribulose-5-phosphate (RU5P), in the process a molecule of NADPH and CO2 is released. To model GND reactions, GND can either bind with NADP or 6PG first, then the other, and perform an inner conversion of its substrate to product. The product then gets released in order of CO2, RU5P, and NADPH. Inhibition process is described as uncompetitive inhibition by NADPH.

ATP is the energy source of the cell, which contains two high energy phosphate bonds that when broken release a great amount of energy, and is the main product of glycolysis. NADPH is the redox factor that is important to balance the red blood cell's oxidation level since red blood cells need to deliver oxygen and their oxylated factors need to be reduced. ATP and NADPH are two

key important molecules in the cell that have two pathways to make them specifically. So in the following case study we are interested to find out how the body will respond when the enzyme that relates to ATP or NADPH production gets mutated, and how will the concentration and the flux of the two key molecules behave. In light of that, we have examined mutants of Phosphoglycerate kinase (PGK), a kinase that synthesizes ATP in glycolytic pathway, 6-phosphogluconate dehydrogenase (GND), a dehydrogenase in pentose phosphate pathway that synthesizes NADPH, and glucose phosphate isomerase (PGI), that is on the crossroad of glycolysis and pentose phosphate pathway, because its substrate glucose-6-phosphate is also the substrate of glucose-6-phosphate dehydrogenase (G6PDH). We will examine how the mutated enzyme will cause a change in ATP or NADPH concentration and fluxes, and under different perturbations to the system how the healthy and mutated models will react. The three case studies are briefly introduced in the Method session.

Method

To simulate the cases, the RBC MASS model was used to relate three pathological deficiencies. All models used are constructed using the Mass Action Stoichiometric python package (MASSpy) (https://github.com/opencobra/MASS-Toolbox) (Zachary Haiman et al., 2020). Algorithms for defining and calculates energy charge, redox ratio, and active fraction are shown below:

$$Energy\ Charge = \frac{[ATP] + \frac{1}{2}[ADP]}{[ATP] + [ADP] + [AMP]}$$

$$Redox\ Ratio = \frac{[NADPH]}{[NADP] + [NADPH]}$$

$$Active\ Fraction = \frac{active\ enzyme}{total\ enzyme} = \frac{\sum_{i=0}^{n} R_i + R_{i,A} + R_{i,AS}}{E_{total}}$$

Where brackets symbols concentration. In active fraction calculation, n is the number of enzymatic binding sites, Ri is the unbound enzyme in the active state (i.e., not bound to inhibitors), Ri,A is the enzyme bound to the cofactor, Ri,AS is the enzyme bound to the substrate and cofactor, and Etotal is the total amount of enzyme. The subscript i represents the amount of activators bound to allosteric sites (Yurkovich, J. T. et al., 2018).

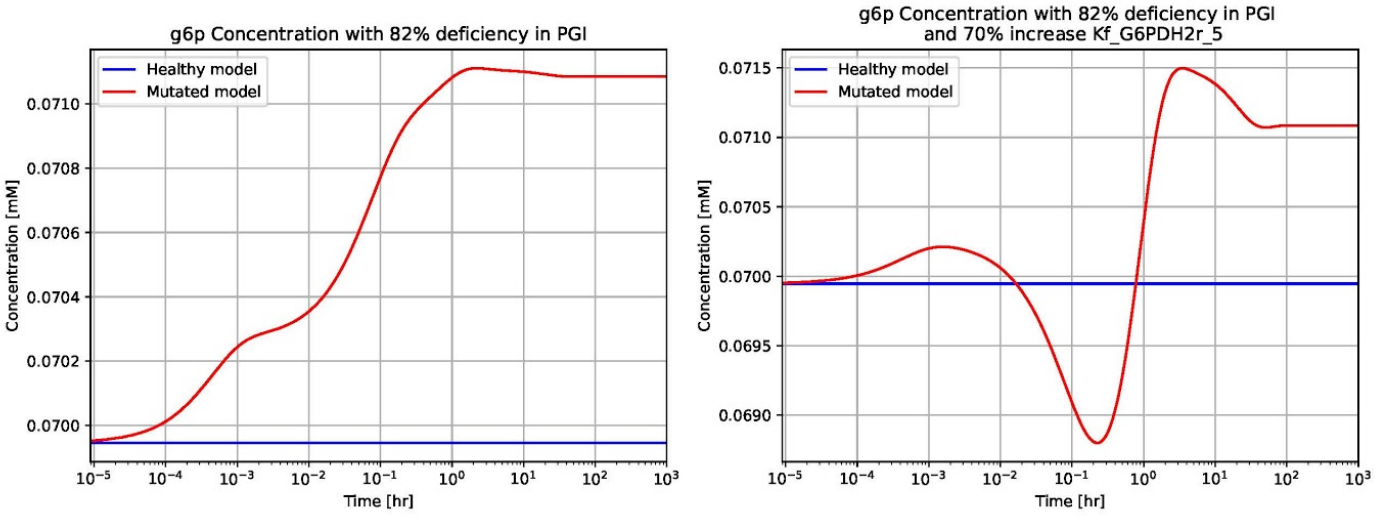
In case study of glucose phosphate isomerase (PGI), we examine a reported R346H mutation in PGI. Experimental result of the deficiency level was 82%, with a corresponding 70% increase in G6PDH activity (Ada Repiso et al., 2004). We merged the model with corresponding enzymes hexokinase (HEX) and glucose-6-phosphate dehydrogenase (G6PDH). Perturbation is used to lower all Kf's of PGI reaction by 82%, since PGI only carries one simple reaction of converting glucose-6-phosphate to fructose-6-phosphate. Perturbation is also used to demonstrate G6PDH

activity by increasing Kf of G6PDH binding reaction of G6P to be 70% higher. Since G6PDH has multiple reactions, without causing a disturbance in other reaction rates, only the reaction that relates to uptake of G6P is considered perturbing. In case study of Phosphoglycerate kinase (PGK), we examine a reported mutant form of PGK that has a Km constant for ADP to be 5-fold larger (Raymonde Rosa et al., 1982). We created a baseline model without any parameters change as control, and a mutated model with the update in 5 times Km. ADK1 enzyme is fused into both models and has its reaction forward rates perturbed to increase 50% to determine the effect of regulating ATP fluxes. Perturbations in various ATP utilization rates are also used in the study of the PGK mutant, and the perturbed values are 50% increase and 15% decrease, specified in conditions used in a previous simulation report using MASSpy (Yurkovich, J. T. et al., 2018). In the third case study, the relationship between two deficiency, 6-Phosphogluconate dehydrogenase (GND) deficiency and G6PDH deficiency, are explored. A reported Orissa variant of the enzyme displays the Km NADP is five-fold higher than that of normal enzyme due to the replacement of alanine to glycine, and glycine is a part of a putative co-enzyme binding site. Moreover, Km G6P reported an approximate two-fold change as well. The inhibitor constant Ki NADPH is reported as a threefold increase (J. S. Kaeda et al., 1995). In another paper by Beutler et al., patients with both deficiency have been identified, making the exploration of these two deficiency worthy to discuss (1985). The GND deficiency produces a 35% decrease in enzyme activity, which is represented by decreased rate in binding steps and catalization step, and increased rate in inhibition step (Caprari, 2001). We created a baseline model without any parameters change as control, a mutated model with modifications representing G6PDH2r deficiency, and a mutated model with both GND and G6PDH2r deficiency. The forward rate of GSH was applied as perturbation to explore the effect created by increased redox stress on three models.

Results

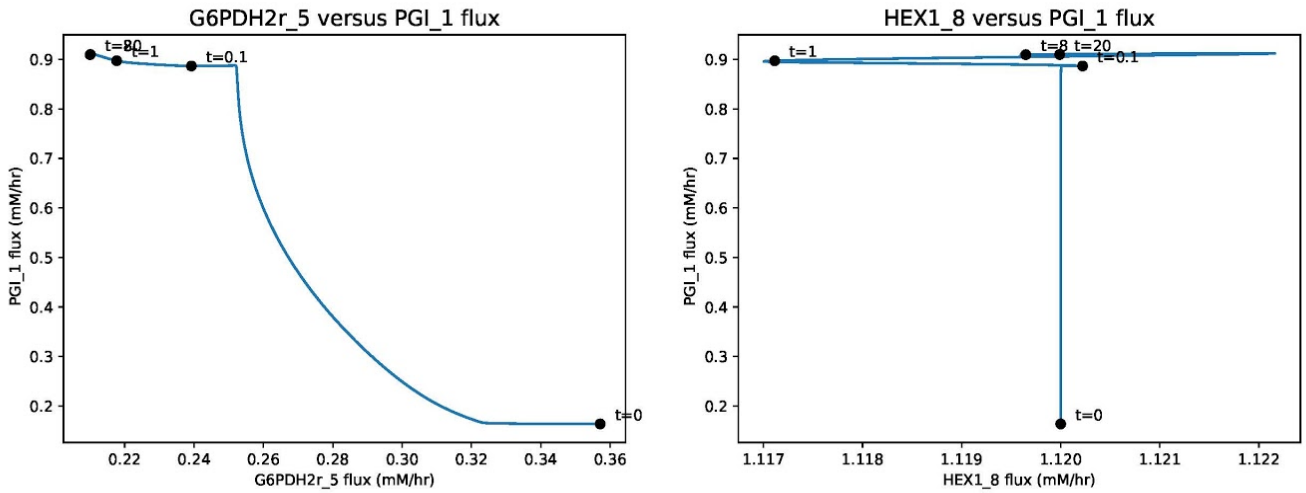
Case study 1:

After perturbed the dissociation constants of all PGI reactions to be 18% of its steady state value, the resulting graph of the time profile for G6P concentration shows an overall increase over a short amount of time, and regains steady state at approximately 0.0711mM. Besides perturbing PGI, when the dissociation constant of G6P binding reaction in G6PDH is perturbed to increase 70%, the time profile shows a decrease in G6P concentration at first, but then the system still regains to a steady state with a concentration value similar to that of perturbing PGI only.



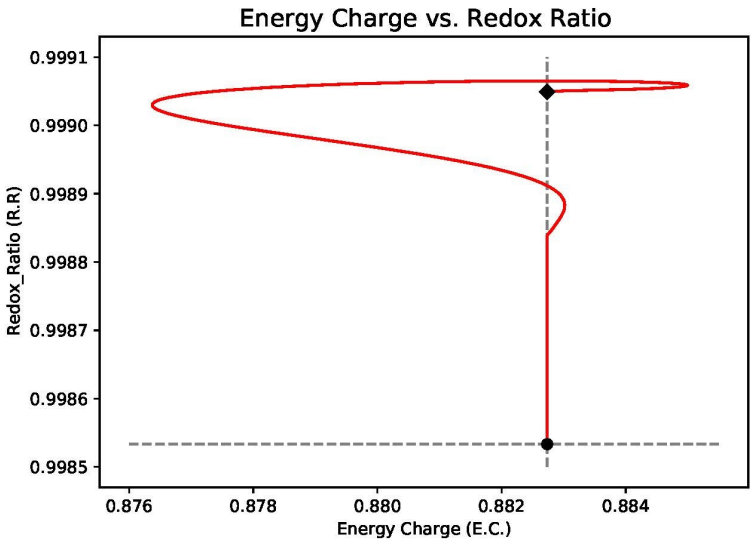
(Graph 1: The time profiles of: (left) G6P concentration when perturbed 82% decrease in Kd of all PGI reactions. (right) G6P concentration when perturbed 82% decrease in Kd of all PGI reactions and 70% increase in G6P binding reaction of G6PDH2r)

Taken the perturbation of both PGI and G6PDH, the following graphs shows the relationship of PGI binding activity with G6P with respect to G6PDH in flux (binding of G6P) and HEX1 out flux (production of G6P). Compared with G6PDH in flux, PGI flux was initially set low but regained over a very short amount of time, along with PGI flux which decreased, eventually back to steady state. When compared with HEX out flux, the PGI flux still increases flux fast, with HEX1 experiences some disturbance and is back to steady state value as well at last.



(Graph 2: The phase portrait of PGI in flux (binding reaction with G6P) versus (left) G6PDH2r in flux and (right) HEX1 out flux under perturbation of PGI and G6PDH2r. The final time points are the steady state value of the two fluxes)

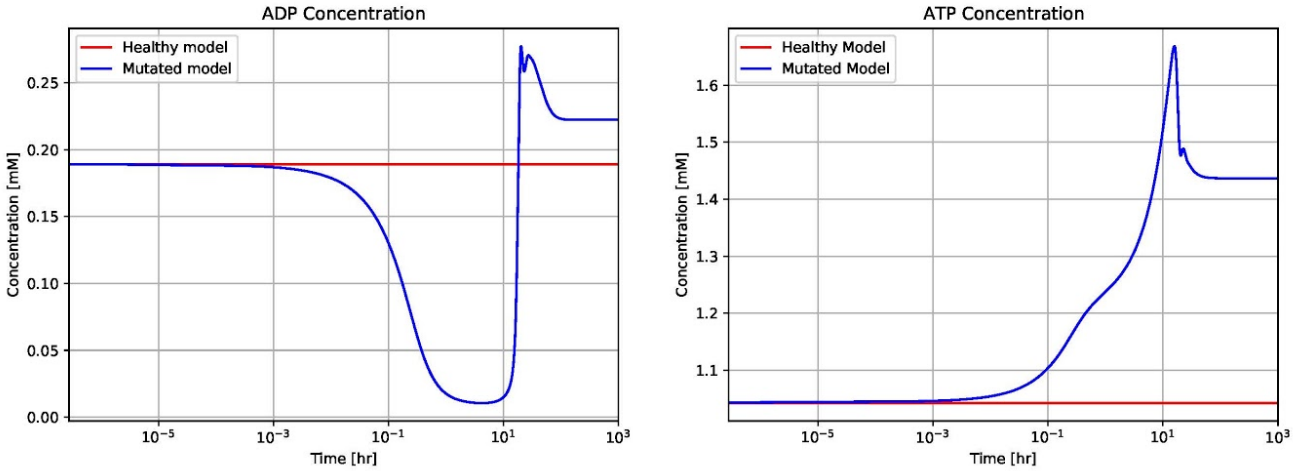
Compare the energy charge and redox ratio and drawn the two on a phase portrait, with the same perturbation condition of both PGI and G6PDH, the graph demonstrates a overall increase in redox ratio (0.99905 vs. 0.99853, approximated) while energy charge stays at steady state value at the endpoint.



(Graph 3: The phase portrait of system's redox ratio and energy charge under perturbation of PGI and G6PDH, grey dashed lines indicates steady state values)

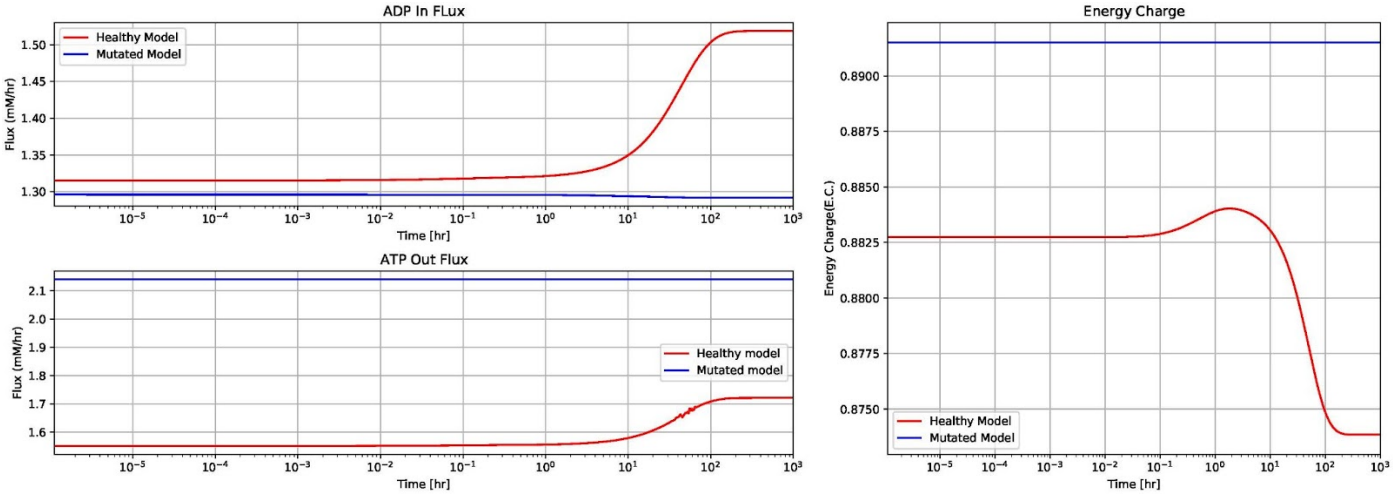
Case Study 2:

The following graph shows time profiles of ADP and ATP concentrations with respect to both healthy model and mutated model. While healthy model stays at steady state for both concentrations, mutated model in ADP concentration shows a slight decrease at first in a short amount of time, then it regains steady state with a value higher compared to that of healthy model; mutated model in ATP concentration shows an overall increase in its concentration and regains a steady state value still higher than the healthy model.



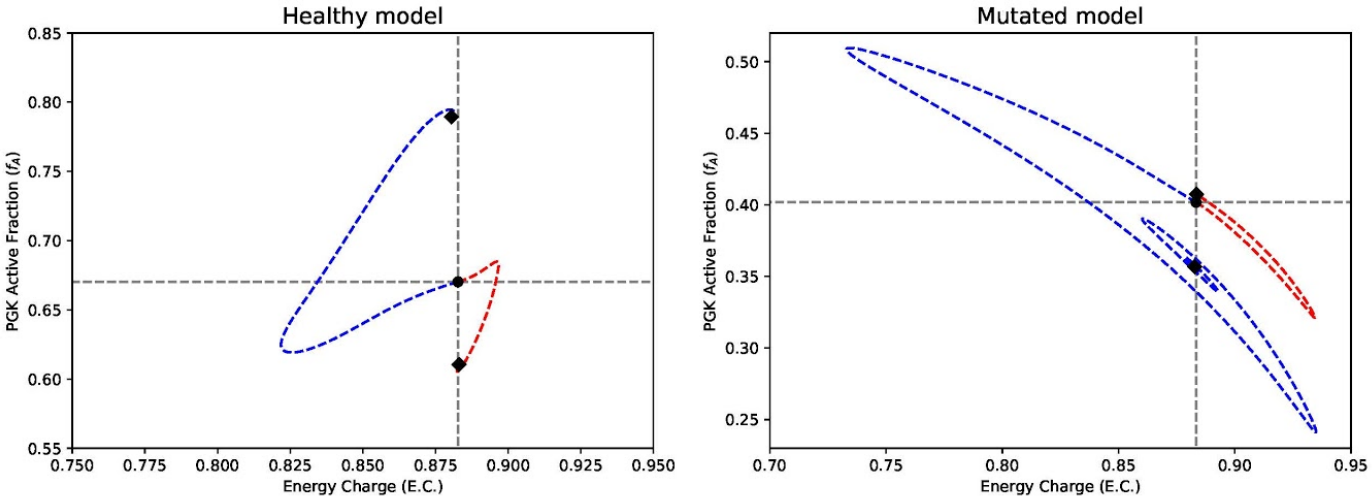
(Graph 4: time profile of ADP concentration and ATP concentration in the system, with respect to both models, healthy or mutated.)

In terms of ADP/ATP flow rate in the enzyme modules with ADK1 reactions' forward rates perturbed to be 50% increased, ADP influx and ATP outflux of the mutated enzyme is graphed in time profiles with respect to healthy model fluxes. ADP influx and ATP out flux are characterized as the flux of PGK binding reaction of ADP and ATP producing reaction. The graph on the right described the time profile of energy charge in the system over time. All mutated model fluxes display a stable state, the change in flux for the mutated model is not significant compared to the healthy model, except in ADP flux a minor decrease is observed. Compared to the steady state value before, the healthy state model shows an increase in ATP out flux and ADP in flux, and total energy charge is decreased.



(Graph 5: (upper left) time profile of ADP in flux of PGK, (lower left) time profile of ATP out flux of PGK, (right) time profile of the system's energy charge with 50% increase in ADK1 forward rates)

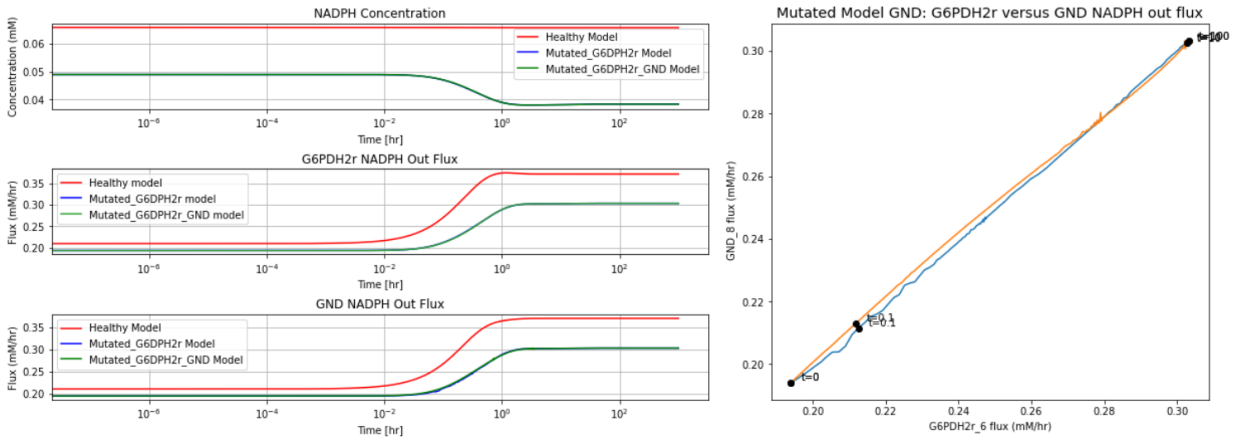
The following graphs demonstrate how the two models' active fraction and energy charge will behave when perturbations of 15% decrease in ATP utilization (red) and 50% increase in ATP utilization (blue). In a healthy model, when the utilization is increased, energy charge and active fraction will drop initially and then energy charge will regain to the steady state value and the active fraction will increase to higher than the original steady state, if the utilization is decreased then the trend is the reverse. For the mutated model, when ATP utilization is high, initially there is a overall trend that at first energy charge decreases while active fraction decreases, then active fraction would decrease and energy charge will increase and return to steady state value, while active fraction is lower than steady state value, and the trend is reversed when utilization of ATP is decreased.



(Graph 6: phase portraits of active fraction versus energy charge at perturbations of 50% increase in ATP utilization (blue) and 15% decrease in ATP utilization (red) with respect to healthy model(left) and mutated model(right).)

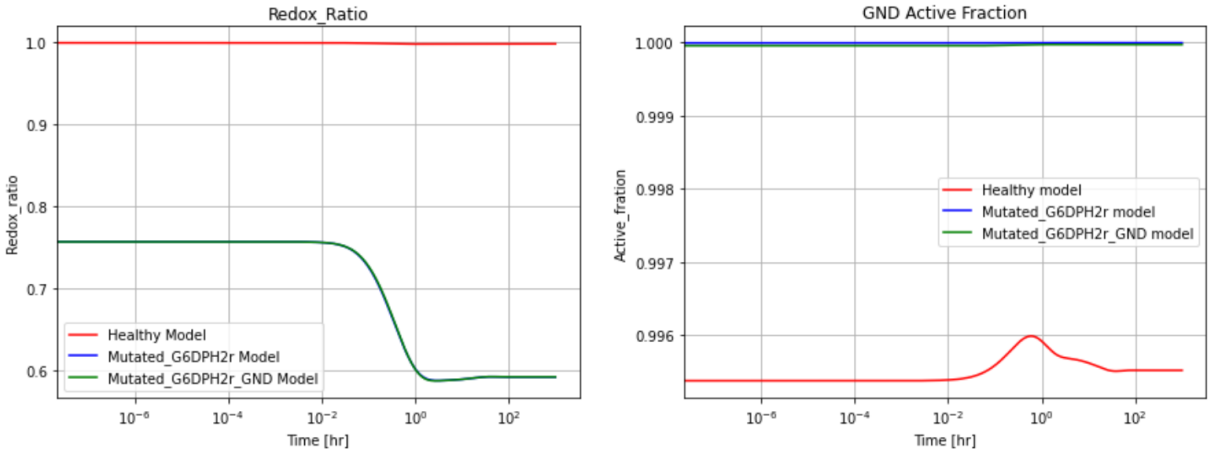
Case Study 3:

The following graph exhibits the behavior of the three models upon GSH utilization increased by two. The healthy model doesn't show any change in NADPH concentration. The output flux for the healthy model for both G6PDH2r and GND increased by about 50%, and reached their new steady state with a higher value than original. The two mutated models exhibited very similar behavior. The NADPH concentration experienced a rapid decrease by about 20%, and gradually achieved its new steady state that was lower than the original steady state value. The output flux for both mutated models for both G6PDH2r and GND in the original steady state are lower than the healthy model. After perturbation, they both increased by about 50%, and reached their new steady state with a value higher than original, but lower than the healthy model. In the graph on the right, we can see that both mutated models show a direct proportional relationship between G6PHD2r output flux and GND output flux. Both the output flux of G6PHD and GND increases from t=0 to t=100, and the increase amounts are similar.



(Graph 7: Time profile for NADPH concentration, the outflux of G6PDH2r and the outflux of GND. The phase portrait for GND vs G6PHD2r out flux for both mutated model)

The redox ratio, shown in the left graph, doesn't change by the perturbation, which is not surprising since the NADPH concentration doesn't change much. The GND active fraction shows a slight increase in the healthy model, but decreased very quick and achieved steady state. The new steady state value is slightly higher than original. Beginning by a lower redox ratio than the healthy model, the two mutated models showed a decrease in redox ratio. It reached a new steady state value even lower than original. The GND active fraction of the two mutated models was higher than healthy models, almost reaching 1 which means all GND enzymes are in active state before perturbation. After perturbation, no changes were observed in GND active fraction. It seems that the effect produced by GND deficiency is completely masked by G6PDH2r deficiency, as behavior for the model only contains G6PDH2r deficiency and model contains both deficiency are similar in all graphs.



(Graph 8: Time profile for redox ratio for all three models. GND active fraction for all three models.)

Discussion

Glucose-6-phosphate isomerase (PGI) deficiency is a major cause of the disease chronic nonspherocytic hemolytic anemia (CNSHA), a red blood cell autosomal recessive genetic disorder which causes severe metabolic alterations, and the experimental result of the deficiency level was 82%, with a corresponding 70% increase in G6PDH activity (Ada Repiso et al., 2004). The simulated result in either case all shows a same trend that the concentration of G6P increases approximately 0.0012mM than that of the steady state value of the unmutated model (Graph 1). Interestingly, the perturbation to increase the activity level of G6PDH to bind G6P helps decrease the level of G6P initially in a very short amount of time, but in the long term the two cases stay the same. Since G6PDH consumes G6P and is driven to Pentose Phosphate Pathway, then it is thought to help reduce the stacking of G6P if its binding affinity is boosted, but the simulation shows otherwise. To further investigate the cause, phase portrait of fluxes (Graph 2) shows that at first, the flux will be altered fast, for within 0.1hr PGI flux of binding G6P will be increased to a stable area where the flux will not change much, with G6PDH in flux kept decreasing, until a steady state is reached. This indicates that although the binding affinity for the two enzymes is being altered, the system would still want to make full use of the enzyme to proceed their function to get back to its original steady state. Hexokinase out flux behaves in a similar trend, as PGI flux would increase in a short amount of time, HEX1 activity starts to show some disturbance, which is probably due to the feedback inhibition of its product G6P and since G6P is stacking the activity will be that of a back-forth disturbance. So it takes more time for HEX1 flux to regain steady state compared to G6PDH. Comparison of the energy charge with redox ratio (Graph 3) gives decisive results. Since glycolysis is the energy production house of a red blood cell, and pentose phosphate pathway the redox factor production house, the system's energy fraction and the redox ratio would demonstrate the general behavior of glycolysis and pentose phosphate pathway. The end point shows that the energy charge of the system does not change while the redox ratio has increased compared to the steady state. This phenomenon indicates that NADPH concentration rises, thus pentose phosphate pathway is promoted, which indicates the increased activity of G6PDH, though flux are the same with steady state values, does relieves some pressure of the system by converting excess G6P to pentose phosphate pathway. The result finds correspondence to the in vivo experiment the paper produced, that the PGI mutant does not change the amount of ATP, ADP, and AMP, thus glycolysis is not affected much, this makes sense because PGI catalyzed reactions are not rate determining reactions. However, the stacking of G6P concentration is an important finding, though it does not impact glycolysis, but the amount of NADPH will rise. Thus, the result can make implication to the medical field that treatment with patients with this mutant will consider dealing with excessive redox factors and lower the activity of G6PDH.

Phosphoglycerate kinase (PGK) is an important enzyme that catalyzes the first ATP production mechanism in glycolysis converting 1,3-diphosphoglycerate to 3-phosphoglycerate. Mutations in

PGK have been found in various diseases, one specific mutant of PGK resulted in 5-folds increase in its Km of ADP, and resulting ADP to ATP forward to backward reaction rate ratio becomes larger (Raymonde Rosa et al., 1982). To study the effect of the change that brings to ATP and ADP concentrations in the system, a healthy control model with no parameters change and the mutated model with the change in Km, ADP is evaluated in the study. The concentration time profile reveals that the mutant form of PGK brings a change to both ADP and ATP, which elevates their concentrations (Gprah 4). While ADP concentration increase is approximately 18.4%, ATP concentration rises 38.8%. The concentration indicates the system is excessive in energy. The rise of the forward to backward rate gives excess ATP that can't be converted back very efficiently, thus it gives a corresponding rise in ADP since the excessive ATP can be regulated by converting back to ADP by Adenylate kinase (ADK). In light of that, we perturb the forward rates of the ADK enzyme to have a 50% boost and have the rate relationship graphed out with after the perturbation to study if the ADK activity can regulate the concentration of high energy compounds (Graph 5). It is expected that increased ADK activity would lower the system's energy charge by regulating the concentration of ATP since ATP can be converted to ADP and AMP. As observed in the healthy model, ADK activity lowers the system's energy charge ratio down, thus successfully regulates the system. The increase of ADP influx and ATP outflux for the healthy model proves that ATP synthesis is favored because of the perturbation. However, the perturbation does little effect on the mutated model. All mutated model fluxes and energy charge stays about the same, with little significance in change compared to healthy model's. This result indicates that the patient who has this mutation will experience an unregulated production of ATP by PGK. This might due to the efficiency of forward rate producing ATP is so high in PGK that ADK cannot regulate the mutated model's fluxes as it does in that of a healthy model. Since the level of ADP and ATP is different compared to normal enzymes, then we are interested to figure out how active the enzyme is compared to the system's energy charge. The graph to compare healthy model and mutated model under 50% increase in ATP utilization and 15% decrease in ATP utilization, perturbations proposed by Yurkovich, J. T. et al., gives decisive results (Graph 6). At steady state conditions, the mutated model's active fraction is significantly lower than that of the healthy model. This suggests that excessive ATP will inhibit the enzyme, thus, its active fraction would be lower. However, given the excess ATP, the energy charge does not deviate much, this might due to the amount of ADP and AMP also rises, thus the body will still behave in a normally charged state. In the healthy model, when the utilization of ATP is increased, the active fraction will increase in the end to produce more ATP for the body to be in the same energy charge. While when ATP utilization is low the trend is reversed. However, in the mutated model, when ATP utilization is high, then the active fraction would decrease. This might be due to the body being already in excess of ATP, when it needs more, it takes from the excessive pool and inhibits PGK to make more to let it be more excessive. On the other hand, when ATP utilization is low, excessive ATP may convert to ADP and AMP and thus the energy charge will decrease, so the body up regulates PGK to become active and turn the ADP back to ATP.

G6PDH2r and GND are both enzymes in PPP, and they are both responsible for reactions that produce NADPH. However, many more people are suffering from G6PDH2r deficiency and much more research study about it than GND deficiency. GND deficiency is an autosomal dominant disorder. Similar to G6PDH2r deficiency, it's an inherited genetic deficiency. However, the mutated sequences are on different chromosomes, meaning the two deficiencies happen independently. A published case study about a patient with both deficiencies showed the possibility of development of Hemolytic anemia (Beutler, 1985), which suggests that hemolysis in some other patients diagnosed with G6PDH2r deficiency might be explained by the interaction of these two deficiencies. We found another case study in an Italian family about GND deficiency, suggesting that the enzymatic activity decreased by 35%. The results shown above presented no difference between a model with G6PDH2r deficiency only and a model with both deficiencies. The increase in GSH utilization produces greater redox stress, which directly drives G6PDH2r reactions. This explained the decrease in NADPH concentration and increase in G6PDH2r output flux. The G6PDH2r becomes more active to meet the increased redox demand. GND reaction, however, is not directly driven by GSH utilization, which explained the unchanged behavior of the model with both deficiency compared to the one with only G6PDH2r deficiency. The model with both deficiency did show an increase in GND output flux, which was due to the increased amount of 6pg produced by increase of G6PDH2r output flux, as the GND reaction is preceded by G6PDH2r reaction. Both mutated models show a close to 1 ratio of GND activity ratio which didn't change by perturbation. This could be explained by when redox stress increases, less NADPH is presented in the cell, resulting in less inhibition of GND and more enzymes become active. The ratio was strived to increase upon perturbation, but since it already reached 1 it couldn't increase more, resulting in an ultimately decrease in NADPH concentration as the enzyme couldn't make up for the need.

The result of the study is limited to one reaction, but could still provide some insights for the affected populations. GND deficiency being so rarely diagnosed may be a result from the mask of G6PDH2r deficiency. Probably more people that are diagnosed with G6PDH2r deficiency are actually having both deficiencies. The current treatments are mostly for G6PDH2r, while GND deficiency is usually out of consideration. The ignorance of GND deficiency can result in delay of treatment, or less efficient treatment by only targeting G6PDH2r deficiency.

All three of the case studies focus on genetic diseases that cause enzyme deficiency. Most of the genetic diseases are inheritable and could be diagnosed by early screening. Thus, we hope the case study can explore the mechanism of enzyme deficiency and provide insights for their treatment.

References

Rosa, R., George, C., Fardeau, M., Calvin, M. C., & Rapin, M. (1982). A new case of phosphoglycerate kinase deficiency: PGK Creteil associated with rhabdomyolysis and lacking hemolytic anemia. Blood, 60(1), 84–91. https://doi.org/10.1182/blood.v60.1.84.84

Repiso, A., Oliva, B., Vives Corrons, J. L., Carreras, J., & Climent, F. (2005). Glucose phosphate isomerase deficiency: Enzymatic and familial characterization of Arg346His mutation. Biochimica et Biophysica Acta - Molecular Basis of Disease, 1740(3), 467–471. https://doi.org/10.1016/j.bbadis.2004.10.008

Morera, S., Chiarelli, L., Rovida, S., Bianchi, P., Fermo, E., Galizzi, A., Zanella, A., & Valentini, G. (2021). Phosphoglycerate Kinase Deficiency : Characterization of the. 1–2.

Van Zwieten, R., Verhoeven, A. J., & Roos, D. (2014). Inborn defects in the antioxidant systems of human red blood cells. Free Radical Biology and Medicine, 67, 377–386. https://doi.org/10.1016/j.freeradbiomed.2013.11.022

Yurkovich, J. T., Alcantar, M. A., Haiman, Z. B., & Palsson, B. O. (2018). Network-level allosteric effects are elucidated by detailing how ligand-binding events modulate utilization of catalytic potentials. PLoS Computational Biology, 14(8), 1–16. https://doi.org/10.1371/journal.pcbi.1006356

Kaeda, J. S., Chhotray, G. P., Ranjit, M. R., Bautista, J. M., Reddy, P. H., Stevens, D., Naidu, J. M., Britt, R. P., Vulliamy, T. J., Luzzatto, L., & Mason, P. J. (1995). A new glucose-6-phosphate dehydrogenase variant, G6PD Orissa (44 Ala→Gly), is the major polymorphic variant in tribal populations in India. American Journal of Human Genetics, 57(6), 1335–1341.

Haiman, Z. B., Zielinski, D. C., Koike, Y., Yurkovich, J. T., & Palsson, B. O. (2020). MASSpy: Building, simulating, and visualizing dynamic biological models in Python using mass action kinetics. BioRxiv. https://doi.org/10.1101/2020.07.31.230334

Caprari, P., Caforio, M., Cianciulli, P. et al. 6-Phosphogluconate dehydrogenase deficiency in an Italian family. Ann Hematol 80, 41–44 (2001). https://doi.org/10.1007/s002770000233

Beutler, E., Kuhl, W., & Gelbart, T. (1985). 6-Phosphogluconolactonase deficiency, a hereditary

erythrocyte enzyme deficiency: Possible interaction with glucose-6-phosphate dehydrogenase deficiency. Proceedings of the National Academy of Sciences of the United States of America, 82(11), 3876–3878. https://doi.org/10.1073/pnas.82.11.3876

Brewer GJ, Dern RJ (1964) A new inherited enzymatic deficiency of human erythrocytes: 6-phosphogluconate dehydrogenase deficiency. Am J Hum Genet 16:472–476