



Constructing the Methionine Cycle and Its Related Disease Model with Hybrid Functional Petri Nets

Zhi En. Chen

Graduate Institute of Medical Informatics,
Tzu-chi University
chen.real@gmail.com

Austin H. Chen*

Graduate Institute of Medical Informatics,
Tzu-chi University
*The Corresponding Author:
achen@mail.tcu.edu.tw

Abstract

Methionine is a kind of essential amino acid, and it must be provided by the diet. Its derivatives play significant roles in human kinds. It has been proven that malfunctions in the methionine cycle have a relation with many severe diseases. As a result, we choose this mechanism to model for its indispensable status in human body. Petri Net is a promising tool to model metabolic pathway since we can establish a model based on biological knowledge intuitively.

In this paper, we construct a model of methionine cycle based on Hybrid Functional Petri Net (HFPN). Our results showed that several important effects of methionine cycle on severe diseases. Among them are excess methionine intakes result in a drastically increasing in Cystathionine that leads to disease like Down Syndrome, hepatic cancer, and liver disease. When the cycle with low CBS activity, cystathionine synthesis is stopped which causes cardiovascular disease, however, when CBS activity is too high, cystathionine increases and result in Down Syndrome.

Keywords: metabolic pathway, methionine cycle, Petri Net, HFPN.

1. Introduction

Methionine is a kind of essential amino acid, meaning that it cannot be produced by the body, and must be provided by the diet. Its derivatives play significant roles in human kinds. In our body, methionine and its downstream metabolites accrue a cycle, in brief, comprising methionine, S-adenosylmethionine (AdoMet), S-adenosyl-homocysteine (AdoHcy), homocysteine, and cystathionine. The Methionine cycle, in fact, is highly complicated and supplies sulfur and other compounds required by the body for normal metabolism and growth. Normal functioning of the methionine cycle is essential for growth and development. If the methionine cycle is broken or temperately blocked, many diseases may take place. It has been proven that malfunctions in the methionine cycle have a relation with Alzheimer's disease [7], Down Syndrome [14], cardiovascular disease [17], liver disease [6], neural tube defects [4], and cancers [8, 15]. As a result, we choose this mechanism to model for its indispensable status in human body.

The mechanism of the methionine cycle

Foods, such as fruits, meat, vegetables, nuts and legumes all contain methionine. There are several enzymes exist in the cycle, including MAT, SM, GNMT, AH, CBS, BHMT, and MS. When we take diet, methionine can first be change to AdoMet by an enzyme with isoforms, MATI and MATIII. After this reaction, SM and GNMT work to transfer methyl group and then produce AdoHcy. The enzyme called AH helps a reversible conversion of AdoHcy to Homocysteine, which has alternative fate in vivo. Homocysteine can be converted back to methionine, consequently, makes a close loop, or undergo transulfuration catalyzed by CBS to make cystathionine. In the operation of this cycle, positive and negative feedback make it more sophisticated than one considers. The first intermediate, AdoMet is served as an activator for MATIII and a repressor for MATI, however, its activity is not limited to the upstream reaction. AdoMet can also activate CBS and repress BHMT in the cycle. Furthermore, AdoHcy performs both activate and repressive properties, it can repress GNMT, SM, BHMT, however, also activate CBS.

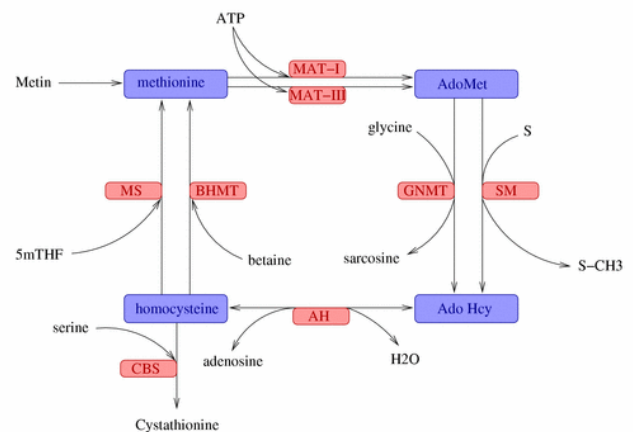


Figure 1. Components of the methionine metabolism modelled by Reed et al.. The main metabolites are shown in blue boxes and the enzymes in red boxes. Abbreviations: 5mTHF=5-methyl tetrahydrofolate; AdoHcy= S-adenosyl- homocysteine; AdoMet= S-adenosyl- methionine; AH=S-adenosylhomocysteine hydrolase; BHMT = betaine:homocysteine methyltransferase; CBS=cystathionine bsynthase; GNMT=glycine N-methyltransferase; MAT= methionine adenosyl transferase; Metin=rate of methionine input;





MS=methionine synthase; S=substrates for methylation; SM=S-adenosyl-methionine-dependent methyltransferases. (http://www.cellml.org/examples/images/reed_model_2004/reaction_diagram.gif)

A promising theory for metabolic pathway modeling

Petri Net is a theory employed in many fields. Many extensions to the simple Petri Nets model have been developed for various modeling and simulation purposes. The major categories of Petri Net extensions are listed: (a) Hierarchical Petri Nets, which allow the previously defined net to present in a new net as an entity or process. (b) Hybrid Petri Nets, which allow the component to deal with continuous values instead of integer numbers of tokens. (c) Timed Petri Nets, which introduce the concept of deterministic time delays. (d) Stochastic Petri Nets, in which entity and process may be assigned delays which are given by a probability distribution. (e) Colored Petri Nets, which allow more complex firing rules in the processes.

However, it is until recently that Petri Net makes its huge influence on biological application, especially the metabolic pathway and other networks in biology. A novel notion of Petri net called hybrid functional Petri net (HFPN) and the enhanced version, hybrid functional Petri net with extension (HFPNe), have been developed by Masao Nagasaki and his colleague. Both methods extend and combine the notions of different kinds of Petri Nets, making it suitable for solving biological problems. As a result, in this paper, we choose Genomic Object Net, a software tool with HFPNe basis development by Nagasaki [12], to construct and simulate the complicated mechanisms of the methionine cycle.

2. Materials and Methods

In this section, we try to elucidate the magnificent role of Petri Net in modern simulation work. We will discuss the important roles of Petri Net, introduce the basic elements in HFPN, discuss how to construct our model, and introduce the useful tool called Genomic Object Net, or Cell Illustrator 2.0, developed by Masao Nagasaki and his colleague [2, 13].

The important role of Petri Net in biological simulation

Many theories and tools have been demonstrated for biological pathways or metabolite pathways simulation, such as E-Cell [9, 20], BioSpice [18] and so forth. Many useful simulating systems are built on the basis of ordinary differential equations (ODEs). Unfortunately, it is rather complicated to use E-Cell for modeling a simple biological pathway. Someone majors in biology won't prefer to learn complicated mathematical equations to construct their own models. It is really a chore for

biologists. Nevertheless, Petri Net holds its advantage for constructing model intuitively. In brief, Petri nets is suitable than other mathematical descriptions while simulating biological phenomena.

Basic elements in Hybrid Functional Petri Net and their characteristics

HFPN contains several key components: entity (place), process (transition), connector (arc), and tokens (markings). Entity can represent protein, gene, metabolite, and any signal factor. Processes stand for reactions, binding, separation, transcription, translation and ordinary biochemical reactions. Connectors play the roles of connecting each component and making a network. Tokens, or markings, indicate the value held by each entity. Different kind of components and reactions are showed in Figure 2 and 3.

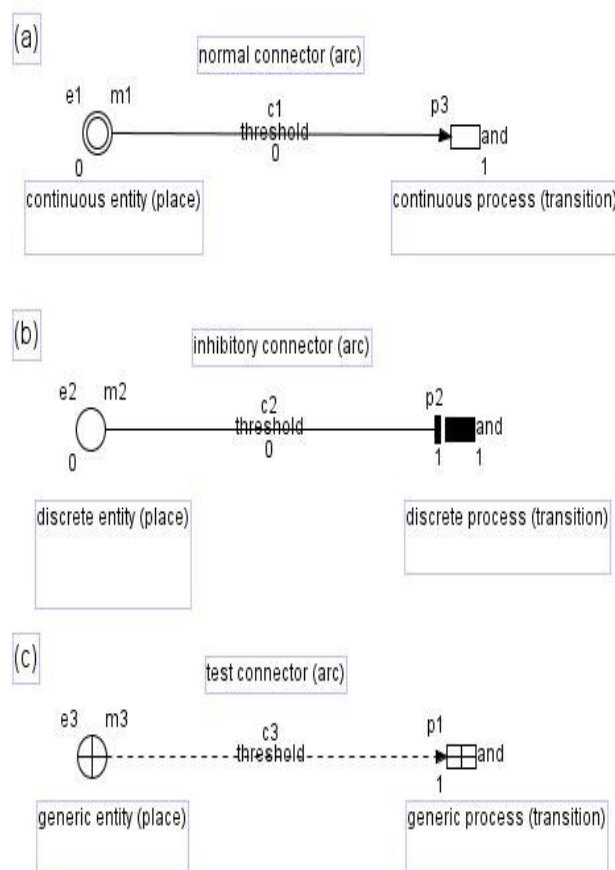


Figure 2. The different kinds of entities, processes, and connectors in HFPN. (a) continuous entity, normal connector, and continuous process. (b) discrete entity, inhibitory connector, and discrete process. (c) generic entity, test connector, generic process.



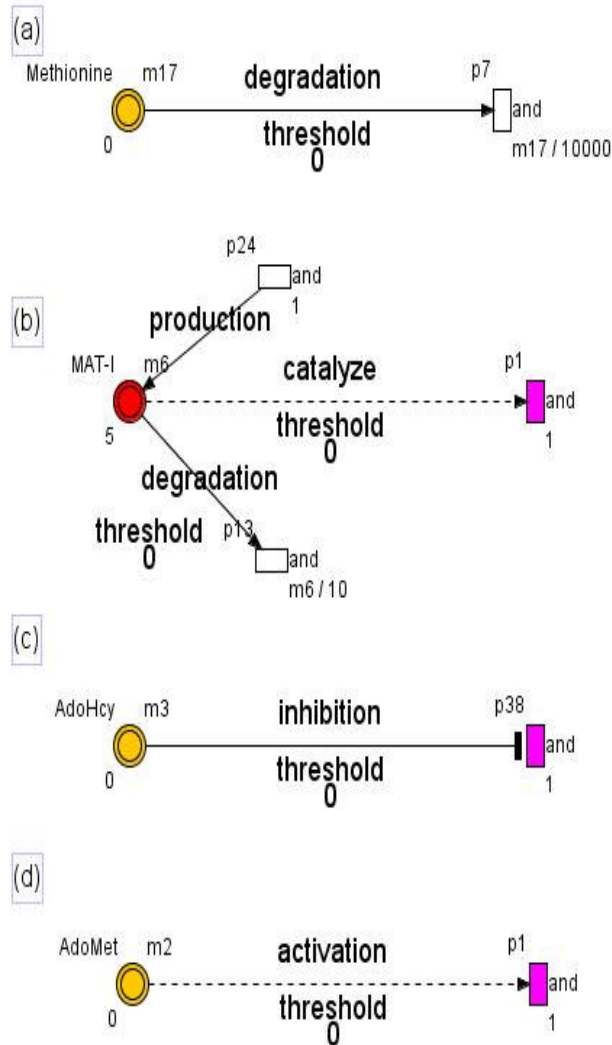


Figure 3. Various reactions in the model we established are expressed in (a) continuous entity, process and normal connector that represent degradation in metabolic cycle. (b) test connector that stands for enzyme catalysis and normal connector for protein production. (c) Inhibitory connectors that are used in inhibition reaction during the pathway. (d) test connectors that are used in activation.

Constructing methionine cycle with Genomic Object Net

We construct a model of methionine cycle and apply the key parameters in the model to simulate those diseases which may be caused by abnormal methionine cycle. Entities represent enzymes and metabolites that are participating in this pathway. Continuous processes, however, represent the specific reactions occurred in this pathway. As an example, we have shown a simple structure in Figure 4. After the main structure has been established, we apply parameters provided by Reed [16] and Martinov [11], as listed in Table 1 and Table 2, to complete the whole model of the methionine cycle. As can be seen in Figure 5, we have finished a complete model of methionine cycle with all the firing rules.

Methionine Cycle Simulation

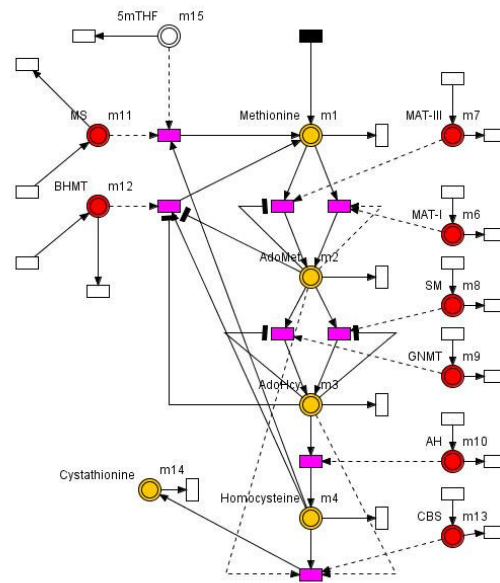


Figure 4. A simple structure of methionine cycle established by HFPN.

Table 1. Properties of each entry in our constructed model.

Name	Type	Variable	Initial Value
Methionine	Double	m1	3.33
AdoMet	Double	m2	0
AdoHcy	Double	m3	0
Homocysteine	Double	m4	0
Cystathionine	Double	m5	0
MAT-III	Double	m6	5
MAT-I	Double	m7	5
SM	Double	m8	5
GNMT	Double	m9	5
AH	Double	m10	5
MS	Double	m11	5
BHMT	Double	m12	5
CBS	Double	m13	5
5mTHF	Double	m14	5.2
	Double	a1	100
	Double	a2	10
	Double	b1	1.7
	Double	b2	30
	Double	VmaxMATI	0.155833333
	Double	VmaxMATIII	6.352777778
	Double	VmaxSM	1.255833333
	Double	VmaxGNMT	2.944444444
	Double	VAH	$a1*(m3-a2*m4)/3600$
	Double	VCBS	$(b1*(m2+m3)-b2)*m4/3600$
	Double	VmaxMS	0.138888889
	Double	VmaxBHMT	0.694444444
	Double	KmMATI	41
	Double	KmMATIII	$2000/(1+5.7*(m2/(m2+600))*(m2/(m2+600)))$
	Double	KmSM	$1.0*(1+(m3/4))$
	Double	KmGNMT	4500
	Double	KmBHMT	12
	Double	KmMS	0.1



**Table 2.** Firing rules and properties of each process

Name	Type	Firing Style	Kinetic Script
p1	continuous	and	$V_{\max}MATI/(1+(K_mMATI*(1+(m2/50))/m1))$
p2	continuous	and	$V_{\max}MATIII/(1+(K_mMATIII*21.1)/(m1*m1+m1*21.1))$
p3	continuous	and	$V_{\max}SM/(1+(K_mSM/m3)+10*10*K_mSM/m3)$
p4	continuous	and	$(1/(1+(m3/20)))*(V_{\max}GNMT/(1+(K_mGNMT/m2)*(K_mGNMT/m2)))$
p5	continuous	and	$a1*(m3-a2*m4)/3600$
p6	continuous	and	$(b1*(m2+m3)-b2*m4)/3600$
p7	continuous	and	$V_{\max}MS*m15*m4/(1*K_mMS+K_mMS*m15+25*m4+m15*m4)$
p8	continuous	and	$(0.7-(0.025*(m2+m3-150)))*V_{\max}BHMT*m4/(K_mBHMT+m4)$
p9	continuous	and	$m1/10000$
p10	continuous	and	$m2/10000$
p11	continuous	and	$m3/10000$
p12	continuous	and	$m4/10000$
p13	continuous	and	$m5 / 10000$
p14	continuous	and	$m14/10000$
p15	continuous	and	$m6 / 10$
p16	continuous	and	$m7 / 10$
p17	continuous	and	$m8 / 10$
p18	continuous	and	$m9 / 10$
p19	continuous	and	$m10 / 10$
p20	continuous	and	$m11 / 10$
p21	continuous	and	$m12 / 10$
p22	continuous	and	$m13 / 10$
p23	continuous	and	1
p24	continuous	and	1
p25	continuous	and	1
p26	continuous	and	1
p27	continuous	and	1
p28	continuous	and	1
p29	continuous	and	1
p30	continuous	and	1
p31	discrete	and	3.33

3. Results and Discussion

Now we can make a simulation on our methionine cycle set up by GON. We consider that the methionine in diet will be taken in gradually and we assume that the rate of methionine input (Metin) is $3.33\mu\text{m}/\text{min}$ (about $200\mu\text{m}/\text{h}$) and run our simulation for one hour (3600pt). In accordance with the model we have constructed, we can get the simulation results as: [Methionine]= $22.61\mu\text{m}$, [AdoMet]= $102.23\mu\text{m}$, [AdoHcy]= $10.06\mu\text{m}$, [Homocysteine]= $0.64\mu\text{m}$, [Cystathionine]= $35.06\mu\text{m}$. (Figure 6)

Excess methionine intakes result in serious diseases

We simulate this situation by increasing the amount of metin. Finkelstein and Martin [5] indicated that the fraction of Hcy, which is the source for cystathionine synthesis, is a function of the concentration of AdoMet. The higher intake of dietary methionine, the higher AdoMet concentration is detected. Increasing the concentration of [AdoMet] will elevate more

transulfuration fraction, and resulting in more cystathionine synthesis. We can observe a drastically increasing in [AdoMet] and [Cystathionine] in Figure 7. High level of cystathionine (cystathinuria) usually leads to some disease such as Down Syndrome [14], hepatic cancer [8], and liver disease. [6]

Effect of folate deficiency

After taking off the folate supply in our model, we can see that the concentration of both [Methionine] and [AdoMet] decreases slightly, at the same time, the concentration of [Homocysteine] and [Cystathionine] increases (Figure 8). This explains the phenomena described in some literatures. In our model, we have successfully proven that lack of folate will cause colorectal cancer [3, 15] and neural tube defects (NTDs) [4].

Effect of high and low expression of CBS activity

We modified the concentration of CBS in the model and found some interest results. Figure 9 showed that the cycle with broken CBS activity. When the cycle with low CBS activity, transulfuration function was lost and cystathionine synthesis is stopped which causes cardiovascular disease [17]. On the other hand, in Figure 10, when CBS activity is too high, cystathionine increase and result in Down Syndrome [14].

Methionine accumulation may cause a serious outcome

It is also interesting to understand the effect of MATI and MATIII at very low level of concentration. In our model, we assume that the initial concentration of both MATI and MATIII will be consumed within a very short time (~ 1 minute), in this situation, we can see that methionine accumulates quickly after running out of MATI and MATIII. All the subsequent intermediates syntheses are therefore completely blocked (Figure 11). This means that the whole cycle is damaged and severe results are expected.

Effect of betaine and methionine synthase (MS) deficiency

Betaine is a chemical that activate enzyme BHMT. In order to simulate the effect of betaine and MS deficiency, we set [BHMT] and [MS] equal to zero. The result is that [Cystathionine] arise moderately (Figure 12 and 13) and will result in diseases like higher CBS activity does.

4. Conclusion

Metabolic pathway is not a close and independent system. Other amino acids and intermediates may also interact with the reaction in the methionine cycle. In





this paper, we do not take them into account. A complete simulation of amino acids metabolic pathway is hard to simulate due to the complexity in this cycle. There are still many relations between amino acids and intermediates are still unknown. A partial metabolic pathway may have significant biological meanings; nevertheless, some factors that are ignored may cause an unexpected compact on the outcome. In our next step, we will take more factors into account and hopefully to explore more meaning knowledge good to human beings, especially in the cancers and diseases. KEGG [19] provides a lot of metabolic pathways, which indicate those metabolites interacting within the methionine cycle. The full-size simulation is a very challenge job and we want to improve our model to live up to this goal.

5. Acknowledges

We appreciate the financial supports of Tzu-Chi Research Grant TCMRC94012 and NSC 95-2221-E-320-001.

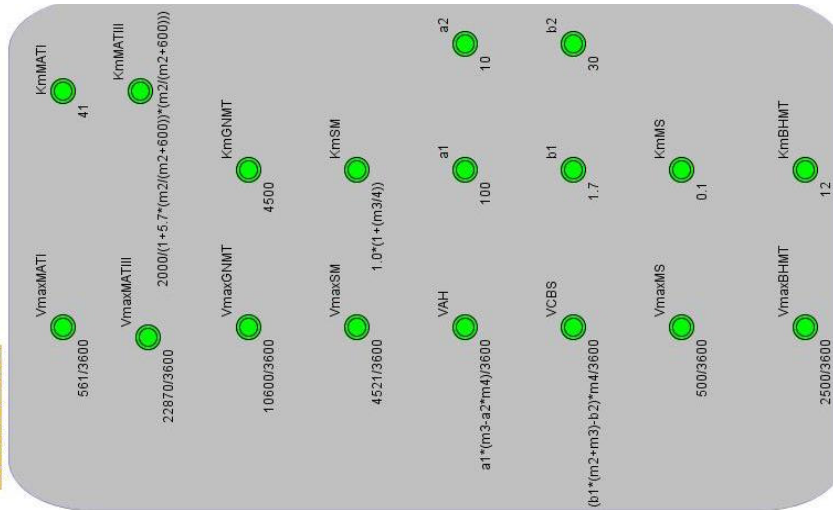
6. References

- [1] Brattström, Lars, David EL Wilcken, 2000. Homocysteine and cardiovascular disease: cause or effect? *Am J Clin Nutr.* 72, 315–23.
- [2] Doi, A., Nagasaki, M., Fujita, S., Matsuno, H., Miyano, S., 2004. Genomic Object Net:II. Modeling biopathways by hybrid functional Petri net with extension. *Applied Bioinformatics.* 2, 185– 188.
- [3] Duthie, S.J., 1999. Folic acid deficiency and cancer: mechanisms of DNA instability. *Brit. Med. Bull.* 55, 578–592.
- [4] Eskes, T.K.A.B., 1998, Neural tube defects, vitamins and homocysteine. *Eur J Pediatr.* 157, 139–141
- [5] Finkelstein, J., 1990. Methionine metabolism in mammals. *J. Nutr. Biochem.* 1, 228–237.
- [6] Finkelstein, J., 2003. Methionine metabolism in liver disease. *Am. J. Clin. Nutr.* 77, 1094–1095.
- [7] L, Miller A., 2003. The methionine-homocysteine cycle and its effects on cognitive diseases. *Altern Med Rev.* 8, 7-19.
- [8] L, Helson, Peterson R.H., Schwartz M.K., 1973. Cystathionine excess in children with hepatic cancer. *Cancer Res.* 33, 1570-3.
- [9] M, Tomita, Hashimoto, K., Takahashi, K., Shimizu, T., Matsuzaki, Y., Miyoshi, F., Saito, K., Tanida, S., Yugi, K., Venter, J.C. and Hutchison, C., 1999. E-Cell: software environment for whole cell simulation. *Bioinformatics.* 15 (1), 72–84.
- [10] Matsuno, H., Tanaka, Y., Aoshima, H., Doi, A., Matsui, M., Miyano, S., 2003. Biopathways Representation and Simulation on Hybrid Functional Petri Net. *In Silico Biology.* 3(3), 389– 404.
- [11] Martinov, M., Vitvitsky, V., Mosharov, E., Banerjee, R., Ataullakhanov, F., 2000. A substrate switch: a new mode of regulation in the methionine metabolic pathway. *J. Theor. Biol.* 204, 521–532.
- [12] Nagasaki, M., Doi, A., Matsuno, H., Miyano, S., 2004. A Versatile Petri Net Based Architecture for Modeling and Simulation of Complex Biological Processes. *Genome Informatics.* 15(1), in press.
- [13] Nagasaki, M., Doi, A., Matsuno, H., Miyano, S., 2004. Genomic Object Net: I A platform for modeling and simulating biopathways. *Applied Bioinformatics.* 2, 181– 184.
- [14] Pogribna, M., Melnyk, S., Pogrybny, I., Chango, A., Yi, P., James, J., 2001. Homocysteine metabolism in children with Down Syndrome: in vitro modulation. *Am. J. Hum. Genet.* 69, 88–95.
- [15] Potter, J.D., 1999. Colorectal cancer: molecules and populations. *J. Nat. Cancer Inst.* 91, 916 – 932.
- [16] Reed, Michael C., H. Frederik Nijhout, Rachel Sparks, Cornelia M. Ulrich, 2004. A mathematical model of the methionine cycle. *Journal of Theoretical Biology.* 226, 33– 43
- [17] Refsum, H., Ueland, P.M., Nygard, O., Vollset, S.E., 1998. Homocysteine and cardiovascular disease. *Ann. Rev. Med.* 49, 31 – 62.
- [18] <https://biospice.org/index.php>
- [19] <http://www.genome.jp/kegg/>
- [20] <http://www.e-cell.org/>





VARIABLE



Methionine Cycle Simulation

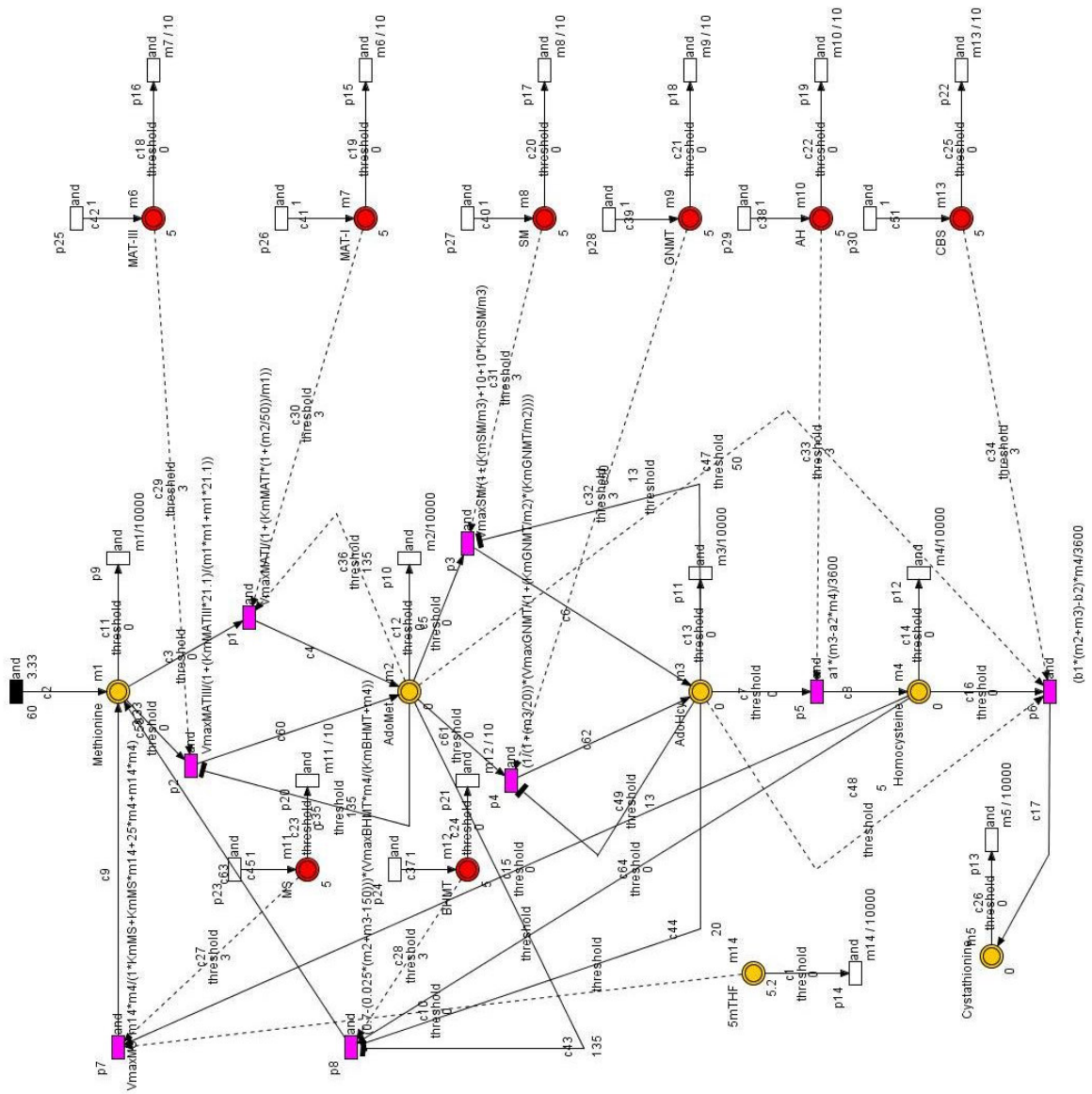


Figure 5. Simulation of methionine cycle and the variable are shown with all properties in each element.



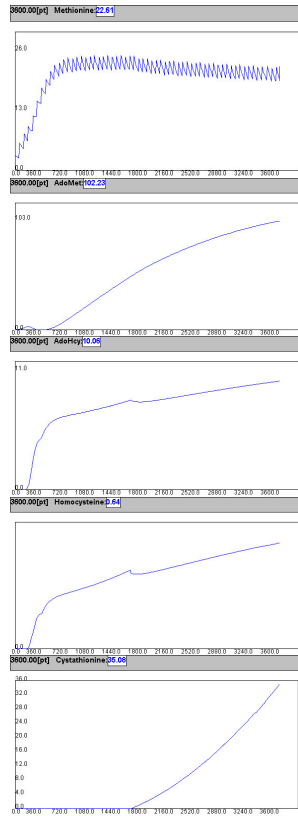


Figure 6. Simulation of normal methionine cycle.

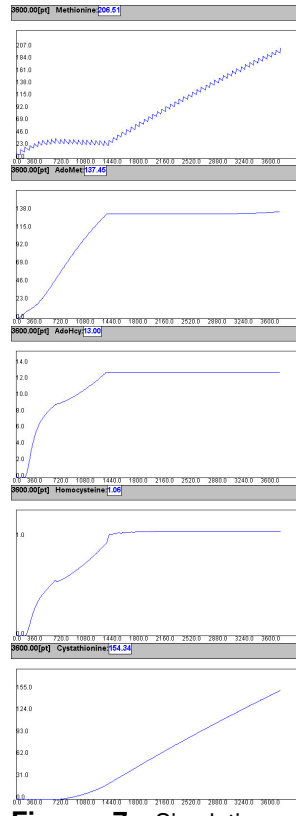


Figure 7. Simulation of excess methionine intake.

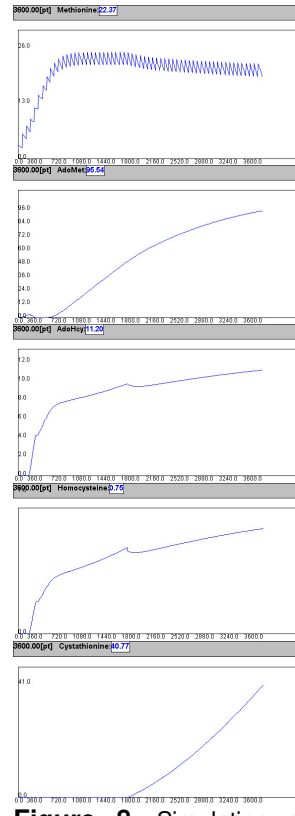


Figure 8. Simulation of folate deficiency.

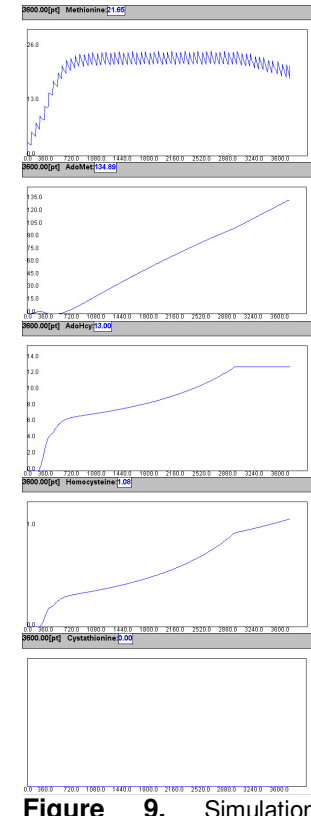


Figure 9. Simulation of cystathionine beta-synthase deficiency.

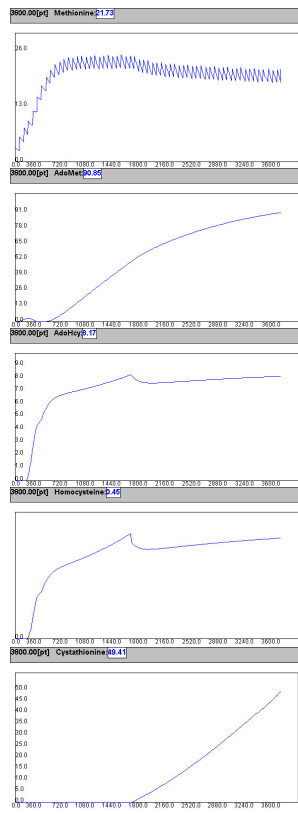


Figure 10. Simulation of methionine cycle in cystathionine beta-synthase deficiency.

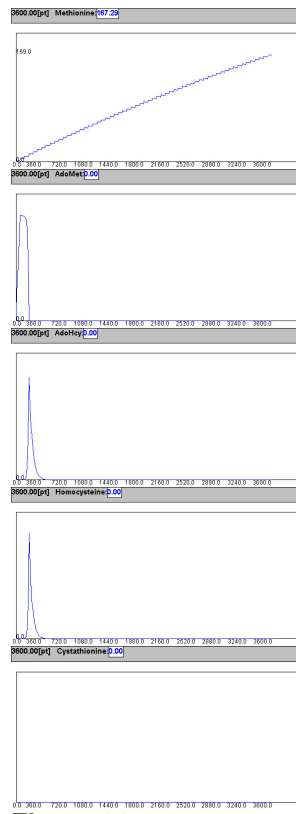


Figure 11. Simulation of methionine cycle in MAT1/MATIII deficiency.

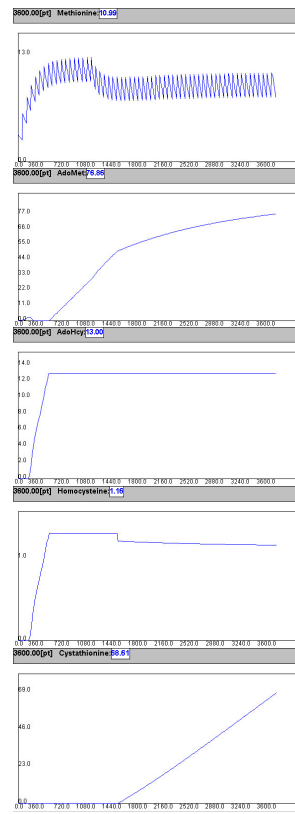


Figure 12. Simulation of methionine cycle in betaine deficiency.

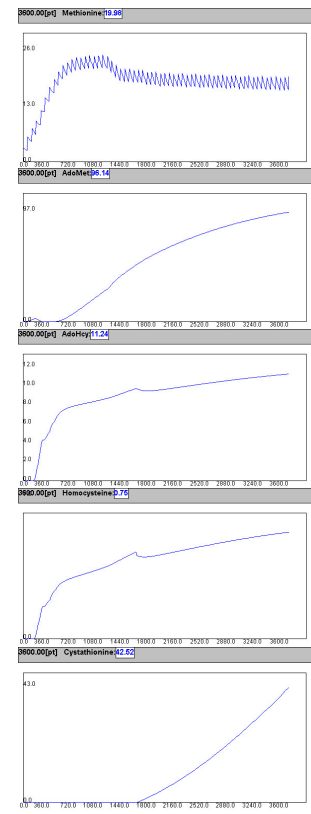


Figure 13. Simulation of methionine cycle in methionine synthase deficiency..

