An Ockham Razor model of energy metabolism

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This chapter is dedicated to René Thomas who unfortunately left this world last January

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Abstract

We present an abstract model of energy metabolism that aims at understanding how activity level of biological functions and combination of nutrients influence metabolic shifts. One of the most frequently observed transition is between respiration and fermentation which is induced by high intake of glucose even in the presence of oxygen (Crabtree and Warburg effects). This glycolytic phenotype is observed in many micro-organisms including parasites and is also shared by all cancer cell lines which makes the Warburg glycolytic phenotype one of the most efficient target in oncology. Nutrients influence production yield of high added value compounds and the study of metabolic shifts is also of concern in bioproduction and fermentation processes. In order to help understanding how major metabolic actors influence these transitions, we developed an abstract and qualitative model of energy metabolism. To facilitate the interpretation of our results with respect to biological knowledge we restrict our variables to key metabolic or cellular components such as pathways, cellular functions, nutrients and important cofactors that play the role of regulators in this cellular system. Primary results on global dynamic phenotypes such as metabolic oscillations and Warburg/Crabtree effects are presented in this chapter. Our simulations have been done using a new software called DyMBioNet.

1 Introduction

Highly proliferative cells such as micro-organisms play a major role in biotechnology as their high turnover provides interesting yields for the industrial bio-

synthesis of high added value molecules such as food complement or biofuel. To adapt between cellular maintenance or cell growth (i.e. production of biomass) or between primary and secondary metabolism cells modify their metabolism with respect to environmental conditions. Nutrients, presence/absence of oxygen, carbon/nitrogen ratio are external regulators of cellular economy. They may induce metabolic shift such as the short term Crabtree effect that shows immediate shift from respiration to fermentation upon addition of excess sugar or the long term Crabtree effect that arise under steady-state conditions at high growth rates [1]. This effect which persists even in the presence of oxygen has also been observed in cancer cells by Otto Warburg in the early 20th century. The Warburg phenotype evolved towards a seemingly irreversible status due to the accumulation of mutations whereas the Crabtree effect is clearly reversible [2]. In the rest of this chapter, we do not make further distinctions between short and long term Crabtree effect or between reversible Crabtree and irreversible Warburg effect as this is not the scope of this chapter.

The Warburg glycolytic phenotype occurs in all tumor cell lines and therefore appears as a common anti-cancer target [3, 4, 5]. Other therapeutic areas such as infectious diseases (e.g. parasitic diseases), anti-aging or obesity also strongly depend on metabolism energetics. Controlling cellular fate is crucial not only to develop therapeutic strategy against infectious diseases or cancer but is also central to optimize yield in industrial bio-processes.

To study how metabolism can control such global cellular phenotypes, we developed an abstract model of energy metabolism. We made use of the Ockham's razor principle which asserts that between two equivalent models, the simpler, the better.

The next five sections describe: i) our qualitative modelling approach of energy metabolism, ii) a global view of energy metabolism and especially, the trade-off between efficient versus inefficient metabolism (respiration vs fermentation), iii) the Thomas modelling framework, iv) our qualitative model and the associated kinetic parameters, and finally v) four dynamics of energy metabolism under respiration, fermentation and Crabtree/Warburg initial conditions using DyMBioNet software.

2 Energy trade off in Cell Metabolism

Metabolism can be summarized as an oscillation between catabolism and anabolism. Catabolism degrades nutrients to extract electrons to be used during anabolism (synthesis of biological macro-molecules) or store in cofactors as a reservoir of energy. More specifically, electrons are stored in: i) cytoplasmic NAD(P)H which plays the role of electron container to synthesize

biomolecules or redox potential to release fermentation products, ii) mitochondrial NADH to create proton gradients and ATP through ATP-synthase in oxydative phosphorylation chain thanks to electron acceptor role of oxygen, iii) the third main reservoir concerns electron-pair bonds with primary metabolites (nucleotides, aromatic amino acids), plants alkaloids or flavor compounds issued from fermentation process. Most of secondary metabolites of industrial interest (i.e. food, biofuel) are produced during fermentation. The production yield of these metabolites are therefore sensitive to the metabolic modes of the cell especially to respiration and fermentation.

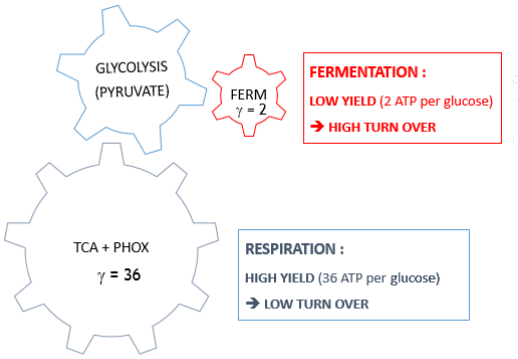


Figure 1: Energy metabolism: trade-off between fermentation and respiration. The Glycolysis pathway (upper wheel) is connected through pyruvate to two other pathways: fermentation with a fast turn over and Krebs cycle which has a high efficiency but lower turn over.

The shift from a highly efficient metabolism (respiration) to an inefficient metabolism (fermentation) at high glucose intake is one of the most important effects characterizing the Crabtree or Warburg effect mentioned above. The energy yield is decreasing from 36 to 2 molecules of ATP per molecule of glucose but the turnover of glucose (number of glucose molecule degraded per unit of time) is higher (Figure 1). These metabolic modes impact cell growth rate [6] and bio-production of secondary metabolites [7].

3 A coarse-grained qualitative approach of energy metabolism

In order to study the impact of external conditions (nutrient, drugs) on the global phenotypes of energy metabolism, especially the Crabtree or Warburg effect, we focus on the biological actors that are directly related to these phenotypes. The variables of our model correspond therefore to coarse-grained

metabolic descriptors such as metabolic pathways, biological functions, key cofactors or cellular nutrients (Figure 2).

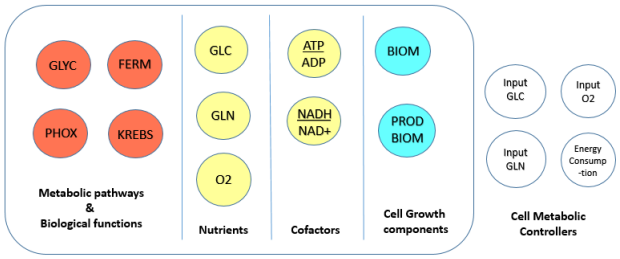


Figure 2: Four main classes of actors for the energy metabolism: biological functions or pathways (red), nutrients and cofactor (yellow), cell growth components (blue) and controls of cellular inputs (white).

The advantage of this qualitative modelling is to get closer connection with experimental facts as global effect of cellular functions or pathways are usually well known. The parametrization of the model depends only on biological knowledge and not on molecular interactions for which kinetics constants are more difficult to estimate *in vivo*. The model includes four classes of cellular variables and one class of external cursors controlling nutrients and consumption of energy. The four internal cell variables are: i) biological functions or metabolic pathways such as Glycolysis, Fermentation, Krebs cycle and Oxidative Phosphorylation (red circles in figure 2), ii) nutrients, which concern carbon source symbolized by glucose (GLC), nitrogen source symbolized by glutamine (GLN) and oxygen (O2), iii) energy and redox cellular level symbolized by the ratio of cofactors ATP/ADP and NADH/NAD+ and finally iv) the biomass-related components that correspond to anabolism.

The ATP/ADP ratio captures the energetic balance of the cell. It encapsulates all the other related molecules such as GTP, AMP and inorganic phosphate (Pi). Other important nutrients necessary for cell growth such as vitamins are supposed to be present implicitly. This abstraction level is not as high for the NADH/NAD+ ratio which corresponds explicitly to the redox molecular components directly involved in the Glycolysis pathway and Krebs cycle. The NADPH/NADP+ cofactors are the redox energy cofactors for anabolism which are not explicitly involved in the Crabtree or Warburg transitions. For that reason there were only implicitly included into the ”Biomass Production” variables (blue cycle in Figure 2).

4 The Thomas Modelling Framework

René Thomas has developed in the 70's a discrete modelling approach for gene networks [8, 9] where a gene never consumes its activators or inhibitors contrarily to metabolism where a product consumes its substrate. In our qualitative model of energy metabolism, variables corresponding to biological functions or metabolic pathways do not consume their resources. For example, Glycolysis "activates" the Krebs cycle but it is nevertheless not "consumed" by Krebs. We consequently decided to use the Thomas formalism instead of a formalism dedicated to metabolic reactions such as BIOCHAM [10, 11]. Exceptionally, for cofactors and nutrients, there are consumptions which are then modelled by negative retro-actions (see full model in Figure 8).

4.1 Interaction network with multiplexes

The static representation of a biological network can be drawn using a directed graph, in which directed arrows (activation/inhibition) represent the action of one variable on its target variable. An example is given for the interaction between Glycolysis and Krebs in Figure 3.

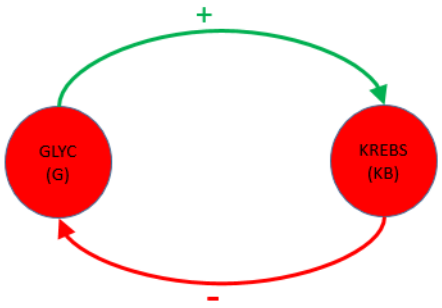


Figure 3: An oversimplified metabolic relation between Glycolysis and Krebs (only for pedagogical purposes). The end product of Glycolysis is a nutrient for Krebs (green positive arrow). Moreover a high level of activity of Krebs can produce citrate, which may inhibit Glycolysis (red negative arrow). Note that Glycolysis is not *consummed* by Krebs.

Moreover, in formal modelling, actors are not always independent variables. It is often necessary to group together several concomitant molecular conditions into a single statement for an activation or an inhibition to be present. This is the role of a *multiplex*, that encodes the basic important

logical conditions for the action to be present [12]. If the multiplex is FALSE, then its action (activation or inhibition) is ignored (see Figure 4). More than one multiplex can act on a given variable. Each multiplex represents only one resource for its target node, i.e. one well-defined but complex biological regulation event. The naive representation of Figure 3 can be replaced by 2 multiplexes (Figure 4) that encapsulate more detailed information than just + and - signs.

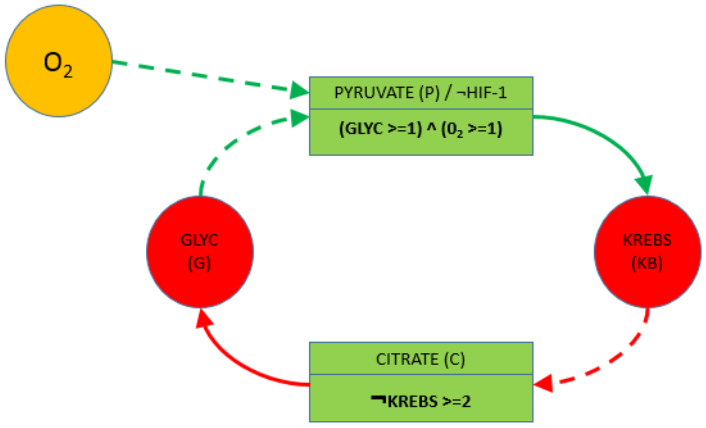


Figure 4: Simplified interactions between Glycolysis and Krebs with multiplexes (green boxes). Top multiplex is called PYRUVATE to mention the metabolite connecting the two pathways without making it an explicit variable of the model, note that oxygen is also needed to act on Krebs ($\wedge$ stands for the conjunction). Similarly, CITRATE refers to the inhibitory effect of high level of citrate on phosphofructokinase. More precisely, it reduces glycolysis by enhancing the inhibitory effect of ATP. So, a high level of Krebs may finally inhibit Glycolysis ($\neg$ stands for the negation of being a resource for Glycolysis).

The top multiplex of Figure 4 informs that pyruvate, the end-product of GLYCOLYSIS will aliment KREBS. The information that pyruvate is a resource of KREBS is encoded into a multiplex called "Pyruvate" (denoted by P) which says symbolically that GLYCOLYSIS is functional ("GLYC $\geq$ 1") and can activate KREBS (Figure 4). Note that there is no retroactive loop on this top part to say that KREBS consumes pyruvate and therefore GLYCOLYSIS. Note also that there is an additional condition in the Pyruvate multiplex which concerns oxygen. This is a rough illustration of our specific question, i.e. the shift between aerobic or anaerobic metabolism in cancer cells or microorganisms. In this particular case, the assertion that oxygen is present stipulates (in cancer cell) the absence of Hypoxia-Induced Factor 1 (HIF1) which inhibits

mitochondria in anoxic condition. The statement on the presence of oxygen in the “P” multiplex ensures therefore that HIF-1 is “off”.

The second multiplex at the bottom of Figure 4 mentions that citrate (produced by KREBS) can inhibit GLYCOLYSIS (through PhosphoFructoKinase) if accumulated in the cytoplasm. The minus inhibition sign is then logically encoded by a negative logical statement (the negation ¬) in the second multiplex concerning KREBS.

4.2 Activity levels and thresholds

To each variable is assigned a number of qualitative activity states. Certain variables like O2 are boolean describing only the absence or presence of this nutrient. Other variables are multivalued in order to capture finer level of biological activities. These qualitative states are defined according to a given question of interest.

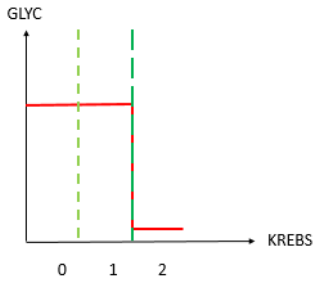


Figure 5: Three activity states for KREBS. The inhibition of GLYCOLYSIS by citrate multiplex is effective only if CITRATE is in excess, which occurs when KREBS is above the threshold of 2 (over-expressed)

For sake of simplicity, GLYCOLYSIS and KREBS variables as well as PYRUVATE and CITRATE multiplexes are represented by G, KB, P and C respectively. In addition, the assignment of a name to a multiplex is important as it denotes a particular molecular mechanism in the energy metabolism. The name is given to help biologists to define the kinetic parameters of the model (see Table 1). The variable KREBS is multivalued (0,1,2) in multiplex C. Il this multiplex, Krebs is prefixed by a ¬ symbol, which means that it inhibits glycolysis (represented also by a red line) only when it is over-expressed (0=low, 1=high, 2=over-expressed) as depicted in Figure 5.

4.3 Kinetic parameters

A particular combination of resources acting on a variable determines its fate if we leave the variable evolve without limit in time, the rest of the system being supposedly frozen. The long time activity level of the variable of interest corresponds to what is often referred to as kinetic parameters, which can be represented using $K_{\nu,\omega}$, where ν is the variable and ω represents the set of resources (identified by solid arrows in Figure 4).

A variable that possesses n multiplexes as potential resources, has 2^n different potential sets of resources and consequently 2^n kinetic parameters. In Figure 4, KREBS (respectively GLYC) has multiplex P (respectively multiplex C) as the sole input and therefore 2 possible sets of resources each. Table 1 lists randomly chosen values for the parameters K.

Kinetic Parameters	Values
K_{KB}	0
$K_{KB,P}$	1
K_G	0
$K_{G,C}$	2

Table 1: An arbitrary set of kinetic parameters. The first and third parameters indicate that in absence of resources, respectively the Krebs cycle (KB) and the glycolysis (G) are attracted toward a state (0) that is too low to allow them having an action on the rest of the system. If the multiplex PYRUVATE becomes a resource for Krebs (second parameter) then Krebs is attracted toward a "normal" activity level (1). If the multiplex CITRATE becomes a resource of glycolysis (that is an *absence* of citrate, fourth parameter) then, according to this toy pedagogical example, glycolysis is attracted to a its highest level (2).

These kinetic parameters are not "dynamical" parameters as they remain fixed during the simulations but they give all the information that is necessary to deduce the complete dynamics of the system.

Determining the values for the set of K parameters is based purely on the biological knowledge. Using formulas from the multiplex, we can quickly deduce the set of multiplexes which are resources. In Figure 4, multiplex P is a resource of Krebs only when glycolysis is equal or above 1. In a similar vein, multiplex C is a resource of G when Krebs is less than 2. This method gives us a total of 9 parameters, as shown in Table 2.

Current state		Resources ($K_{\nu,\omega}$)		Attracting State	
G	KB	K_G	K_{KB}		
0	0	C	-	2	0
0	1	C	-	2	0
0	2	-	-	0	0
1	0	C	P	2	1
1	1	C	P	2	1
1	2	-	P	0	1
2	0	C	P	2	1
2	1	C	P	2	1
2	2	-	P	0	1

Table 2: States, applicable parameters and their values. These are only toy values according to Table 1, chosen to obtain Figure 6.

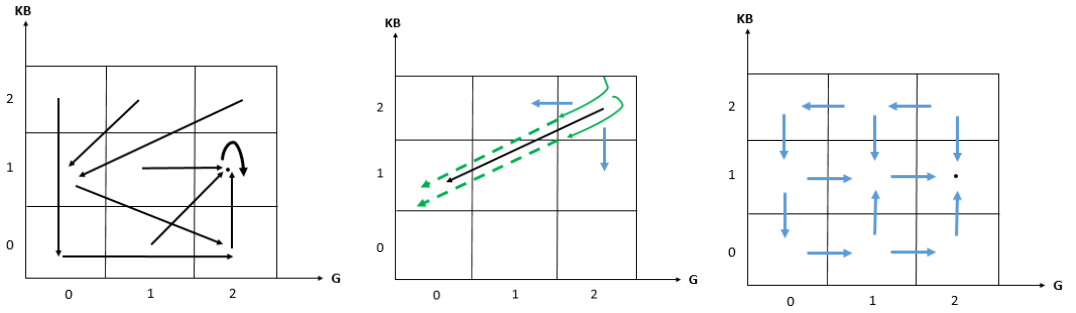
4.4 Transition graphs

In a dynamical system, a transition from a current state (denoted by η) to a next state (denoted by η'), can be represented by $\eta \rightarrow \eta'$, as seen in Figure 6. As demonstrated in [15], the probability that all variables pass through their respective thresholds at the same time is negligible *in vivo*. This means that the system is asynchronous and therefore only one variable is likely to evolve (increase or decrease) over a unit of time while other variables remain unchanged. As demonstrated by Houssine Snoussi, this way of discretizing the state space is compatible with continuous approaches such as stepwise linear differential equations [13]. From Table 2, assuming normoxic condition in which case Oxygen is always present ($O2$=1) in the cells, we would obtain the synchronous state graph of Figure 6a.

Focusing on state (2,2) in Figure 6b, we obviously see that imaginary continuous trajectories (green) would quit the state (2,2) by going either in state (1,2) or in state (2,1). According to [13], the René Thomas approach retains the transitions (blue) : (2,2) $\rightarrow$ (1,2) and (2,2) $\rightarrow$ (2,1). By doing so in each state,we get the transition graph of Figure 6c.

More formally,

- ν can change from state $\eta(\nu)$ to state $\eta'(\nu) = \eta(\nu) + 1$ only if $K_{\nu,\omega} > \eta$ and it is then required that $\eta'(x) = \eta(x)$ for all $x \neq \nu$.
- ν can change from state $\eta(v)$ to state $\eta'(\nu) = \eta(\nu) - 1$ only if $K_{\nu,\omega} < \eta$ and it is then required that $\eta'(x) = \eta(x)$ for all $x \neq \nu$.
- Accordingly, a state η is *stable* when for all variables x, $K_{x,\omega} = \eta(x)$ (i.e. there is no variable ν that gives rise to a possible transition).



(a) Synchronous State graph (b) Asynchronous Transitions (c) Asynchronous Transition graph

Figure 6: State transitions. The asynchronous dynamic (a) directly obtained from Table 2 would be biologically incorrect because it contains "jumps" from 0 to 2 that do not reflect a continuous increase of activity and because it contains simultaneous variable changes (diagonal arrows). Asynchronous variable changes are required for compatibility with models based on differential equations (b) where the probability to cross two frontiers at the same time is null. So, Table 2 results finaly to the asynchronous transition graph (c).

5 Our qualitative model

5.1 Introduction

Based on the Thomas framework, we applied a method [14] in 5 steps as follows:

1. What are the actors? (variables of the system, see section 3)
2. Which variables or combination of variables act on a given variable? (interaction graph)
3. How many qualitative levels for each variable and in which order are the targets influenced by a given variable? (link between targets and qualitative levels that determines the elementary comparisons in the multiplexes)
4. Identify the Kinetic parameters
5. Validate the global behavior of the model

5.2 Threshold order

For each main actor of section 3, we have firstly looked at the actors on which it acts. For example, Krebs acts on Glycolysis, on the production of biomass

(PROD-BIOM), as well as GLN and NADH/NAD+. After a first round of discussions based on the literature, it appears that Krebs acts on Glyc and Prod. BIOM mainly via the excess production of Citrate and at the same level of Citrate. So, this gives rise to three targets for Krebs as in Figure 7 (for the moment ignoring the thresholds):

We then assume that for a reason or another, Krebs goes progressively from a state where it is completely off to a state where it is running at its maximum (over-expressed).

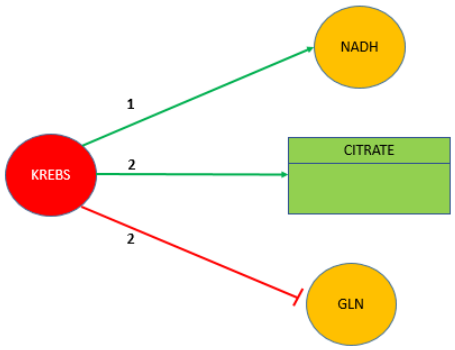


Figure 7: Threshold order for Krebs. Krebs acts on three actors including one multiplex. NADH is the first actor to be activated as Krebs's level rises up and, according to the purpose of our model, one sees no reason in the literature to distinguish the abstract levels of Krebs where CITRATE and GLN are produced by Krebs (else Krebs would get an additional level 3, which seems useless according to the questions under consideration).

As soon as Krebs is active, it produces NADH. So, this is the first actor activated by Krebs (threshold of 1). Krebs may increase its activity by consuming alphaKeto Glutarate (αKG), one end-product of glutaminolysis (cancer phenotype) which has the effect to produce citrate that exits mitochondria to be transformed into malate. This citrate-malate shuttle has also the effect of replenishing NAPDH for the biomass production. Both actions (consumption of alpha-ketoglutarate, end-product of glutaminolysis and over-production of citrate is a result of high level of krebs (level 2) without further distinction.

This reasoning allowed us to put the thresholds 1 and 2 in Figure 7. We did the same work for all the variables and finally we obtain the interaction graph of Figure 8.

Our abstract model of energy metabolism is made of 10 variables, 11 multiplexes (i.e. regulation mechanisms) and 4 metabolic controllers to setup the external nutrient conditions.

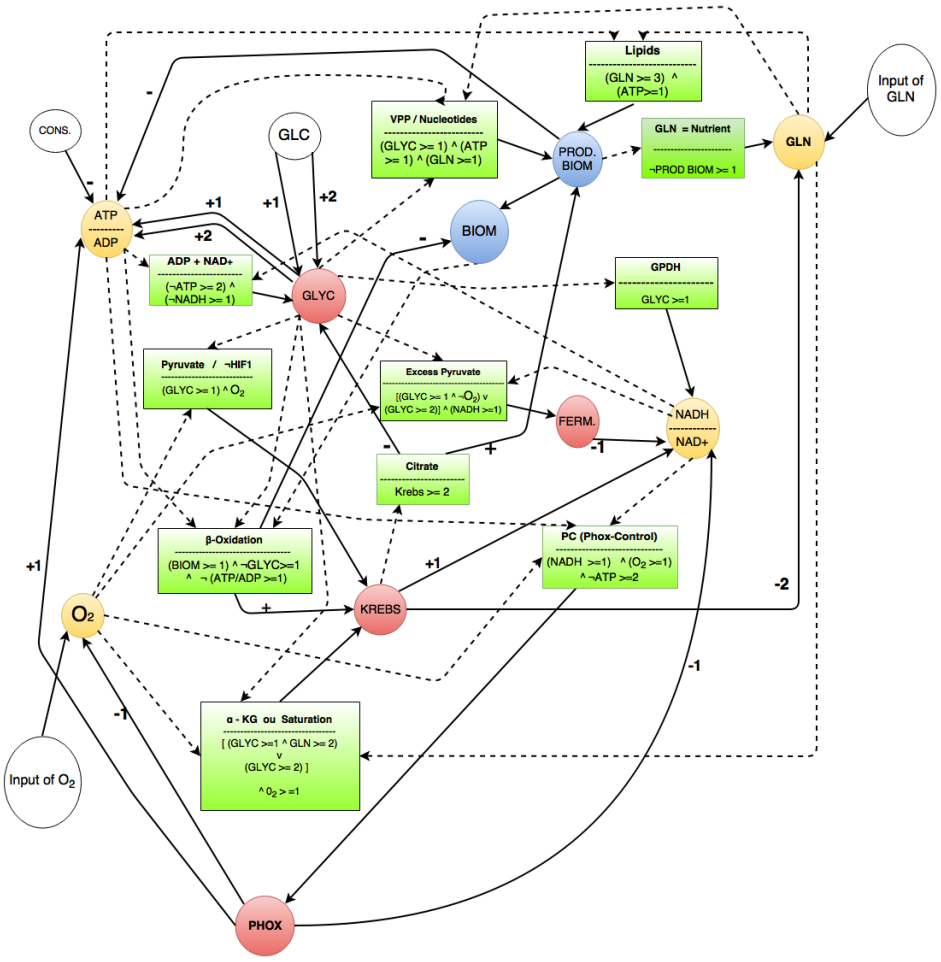


Figure 8: Model for energy metabolism resulting from answering three questions:i) what are the actors? ii) what interacts with what? and iii) which priority? The last component (kinetic parameters) is given in Table 4.

5.3 Identification of the K parameters

For each actor ν of Figure 8, we have considered the 2^n possible sets of resources and for each of them, we have determined the level towards which ν is attracted. To do so, we consider a virtual experiment where the considered resources of ω of ν are "frozen" in the system and we inventory which ones of the targets of ν would be directly affected by ν.

If the previous stage of the method (threshold order) has been properly done, there is a level l of ν such that all targets with a threshold $t \leq l$ are

affected, then $K_{\nu,\omega} = l$. For example, consider the 3 possible resources of Krebs(Kb) in Figure 8. There are 8 configurations to treat. For example, when $\omega = \{P\}$ (only Pyruvate), there is normal pyruvate (from glycolysis) and Krebs is attracted towards a level where it is strong enough to produce NADH, but not enough to inhibit GLN or produce excess of Citrate. So, $K_{Kb,P} = 1$. In table 3, a $-$ (respectively x) means absence (respectively presence) of a resource. The column $values$ corresponds to the threshold levels of the variable (here the variable Krebs can take only 0,1 and 2 as possible values). If a C appears as value, this means this condition cannot occur.

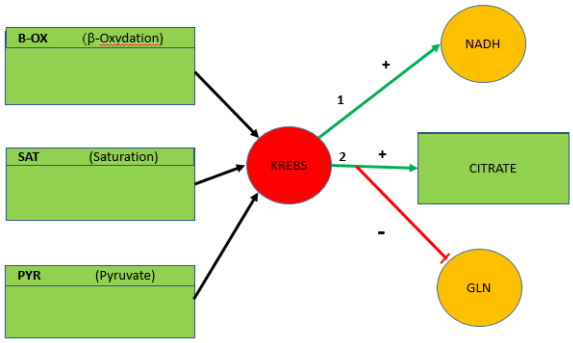


Figure 9: Identification method for the kinetic parameters of Krebs: we consider successively each of the 8 possible subsets of $\{\beta$-OX,SAT,PYR$\}$, assume that Krebs benefits from that subset (say ω) of resources for an infinite period of time, and finally deduce from the literature on which targets Krebs will finally act. Then $K_{KREBS,\omega}$ equals 0 of no target activated, 1 if only NADH is activated, and else 2, see Table 3.

We did the same work for the 7 other K parameters. Some sets of resources are inconsistent. For example Pyruvate and β-Oxidation are contradictory. Inconsistent sets of resources are identified by C in Table 3, where only 4 sets of resources are consistent. The same work is applied for all variables and we got the whole set of 100 parameters of the model, owing to the large panel of convergent literature (see Table 4).

6 Results

6.1 The DyMBioNet Software

We have developed an extension of SMBioNet [15], DyMBioNet (short for Dynamic Modelling of Biological Networks), which we use to simulate the

Resources for Krebs, $K_{KREBS,\omega}$			
PYR	β-OX	α-KG	Values
-	-	-	0
-	-	x	C
-	x	-	1
-	x	x	C
x	-	-	1
x	-	x	2
x	x	-	C
x	x	x	C

Table 3: Set of resources for Krebs. "-" and "x" respectively stand for the absence or the presence of a resource. A qualitative level for the KREBS variable is assigned (under the column "values") for each of the $2^3 = 8$ possible combinations of resources for this central variable. Some combinations are contradictory (C).

dynamics of the energy metabolism. DyMBioNet also includes proof checking techniques with Temporal Logic (CTL) to confirm the existence of certain metabolic states under specific nutrient or drug conditions. It has a built-in user-friendly interface and a suitable chart for demonstrating how the system evolves over time. This formal logic framework will also help in the future to propose most pertinent experiments to validate or refute certain hypotheses concerning the Warburg effect.

In the following subsections, we illustrate four of the key behaviours that participate to validate our model.

6.1.1 Respiration under normal condition

The nutrients were set up for normal respiration conditions, i.e. presence of oxygen, normal level of glucose intake and low level of nitrogen source (GLN). Simulation shows that under these nutrient conditions, the system is maintained in respiration mode or return to respiration if it starts in fermentation mode (FERM = 1 at the initialisation of the simulation).

In this respiration condition, glutamine is not an important ingredient to fuel this machinery. The default value of 0 for the variable `Input_GLN` corresponds to a basic level of input nitrogen source. The GLN nutrient variable was used to model the excess of glutamine or the glytaminolysis phenotype which is often activated in cancer cells [16]. A representative simulation under normal respiration mode is shown in Figure 10.

Metabolic oscillations can be observed from all the simulations we performed. The three basic metabolites which are often used to observed metabolic

Variable	K Parameters & values
ATP/ADP	$K_{ATP/ADP,\{cons.,phox\}} = 1$, $K_{ATP/ADP,\{cons.,phox,glyc_2\}} = 1$, $K_{ATP/ADP,\{cons.,phox,pbm\}} = 2$, $K_{ATP/ADP,\{cons.,phox,pbm,glyc2\}} = 2$, $K_{ATP/ADP,\{glyc1,pbm,glyc2\}} = 1$, $K_{ATP/ADP,\{glyc1,phox,pbm,glyc2\}} = 1$, $K_{ATP/ADP,\{glyc1,cons.,glyc2\}} = 1$, $K_{ATP/ADP,\{glyc1,cons.,pbm\}} = 2$, $K_{ATP/ADP,\{glyc1,cons.,pbm,glyc2\}} = 2$, $K_{ATP/ADP,\{glyc1,cons.,glyc2\}} = 1$, $K_{ATP/ADP,\{glyc1,cons.,phox\}} = 1$, $K_{ATP/ADP,\{glyc1,cons.,pbm\}} = 2$, $K_{ATP/ADP,\{glyc2,cons.,pbm,glyc2\}} = 2$
BIOM (BM)	$K_{BM,\{\beta\_ox\}} = 1, K_{BM,\{\beta\_ox,pbm\}} = 1$
FERM	$K_{FERM,\{ex\_pyr\}} = 1$
GLYC	$K_{GLYC,\{NAD+,GLU1\}} = 1$, $K_{GLYC,\{NAD+,GLU1,\ GLU2\}} = 1$, $K_{GLYC,\{NAD+,CIT,GLU1\}} = 1$, $K_{GLYC,\{NAD+,CIT,GLU1,GLU2\}} = 2$
GLN	$K_{GLN,\{in\_gln\}} = 3, K_{GLN,\{in\_gln,krebs\}} = 3$, $K_{GLN,\{in\_gln,pbm\}} = 3, K_{GLN,\{in\_gln,krebs,pbm\}} = 3$
KREBS	$K_{KREBS,\{\beta-OX\}} = 1, K_{KREBS,\{PYR\}} = 1$, $K_{KREBS,\{PYR,\alpha-KG\}} = 2$
NADH	$K_{NADH,\{Krebs,Phox\}} = 1$, $K_{NADH,\{Krebs,glyc,phox\}} = 1$, $K_{NADH,\{glyc,krebs,ferm\}} = 1$, $K_{NADH,\{glyc,phox,ferm\}} = 1$, $K_{NADH,\{krebs,phox,ferm\}} = 1$, $K_{NADH,\{glyc,krebs,phox,ferm\}} = 1$
OXYG	$K_{O_2,\{in\_O_2\}} = 1, K_{O_2,\{in\_O_2,phox\}} = 1$
PHOX	$K_{PHOX,\{PC\}} = 1$
PROB_BIOM (PBM)	All K parameters are equal to 1, except $K_{PBM,\{\}}$
Control Variables for c in GLC,CONS., GLN or IN_O_2)	K_c=value assigned to c

Table 4: List of K parameters for the whole model (K parameters that do not appear in the table have values 0)

oscillations O2, NADH and ATP are oscillating between low and medium state. Glycolysis (GLYC) is also oscillating, which is due to the NADH/NAD+ and ATP/ADP oscillators. The biomarkers of respiration and metabolic oscil-

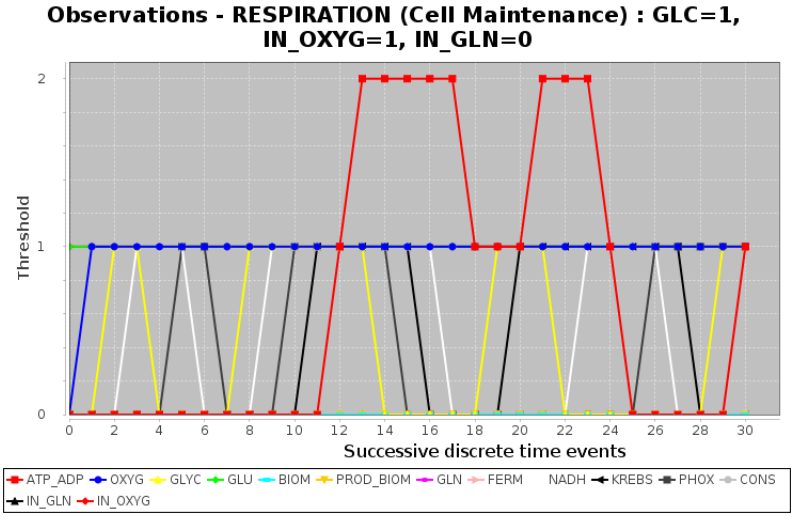


Figure 10: Respiration (Cell Maintenance). The initial state expresses normal cell maintenance conditions with medium level for glucose (GLC=1), presence of oxygen input and no excess of glutaminolysis or anaplerotic reactions. Time goes from left to right, with a unique variable change at each time step. So, one sees when a variable increases due to a line of its color that rises, and a decreasing line indicates a decrease. Increases and decreases are always from one unit at each time step.

lations (NADH via Krebs) and PHOX oscillate as well. The model therefore reproduces the basic metabolic oscillating behaviours.

Let us point out that discrete abstract frameworks are, by construction, not quantitative. Within quantitative frameworks one could try to identify parameters with a sufficient precision in order to determine whether the oscillations are damped. Here the price to pay for abstraction is to be unable to address this question.

6.1.2 Biomass production conditions

Glutamine, which circulates with the highest concentration among amino acids, serves as a major bioenergy substrate and nitrogen donor for proliferating cells [16]. The amount of ATP in highly proliferative cell is not dramatically different from a quiescent cell in respiratory mode. The addition of glutamine triggers the accumulation of biomass. In this context, glucose and glutamine are considered essential nutrients providing ATP and carbon skeletons for building blocks of macromolecules respectively. This justifies the following set of initial values $[GLU = 1, \texttt{Input\_OXYG} = 1$ and $\texttt{Input\_GLN} = 1]$ for this

simulation that are critical nature of proliferative phenotypes (see Figure 11).

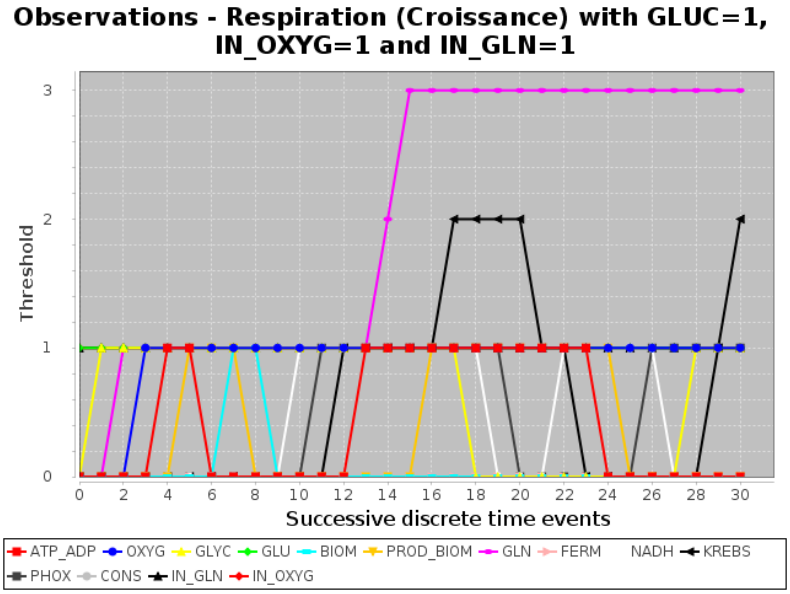


Figure 11: Respiration during cell division. The initial state expresses normal cell growth conditions with medium level for glucose, presence of oxygen input and presence of anaplerotic reactions.

All the biomarkers for oscillating metabolism are clearly visible during this simulation in which other nodes in the graph keep their same initial state. The availability of glutamine (by turning $[GLU = 1, \texttt{Input\_OXYG} = 1]$) has an effect on the production of biomass and later on the biomass. The nitrogen source for building blocks like nucleotides as well as DNA coincides with the presence of glutamine in the cell. We can also observe that glutamine at a given moment becomes an active nutrient for Krebs cycle through alpha Ketoglutarate. This is marked by the shift of Krebs from level 1 to 2 in the simulated chart.

6.2 Fermentation Condition

In normal fermentation, the minimal required conditions are firstly O2=0 (a very low level or no more oxygen available) and the presence of either a low or high glucose concentration in cells. We investigate the effects of consumption of ATP in the metabolic system. It means that PHOX will stay at level 0 after a certain amount of time. Whether we start with an initial value of 0 or 1 for fermentation, there will be a tendency for fermentation to go towards 1 and

stay at 1. In all fermentation processes, the principal purpose is to regenerate NAD+ so that glycolysis can continue.

Most of the biomarkers of fermentation (GLYC, NADH, FERM and ATP/ADP) have the tendency to oscillate: Glycolysis is high when NAD+ and ATP are high, and Glycolysis is low when NADH and ADP is high! We can also witness that Krebs and Phox as well, stay at level=0 during fermentation which is in accordance with biological observations.

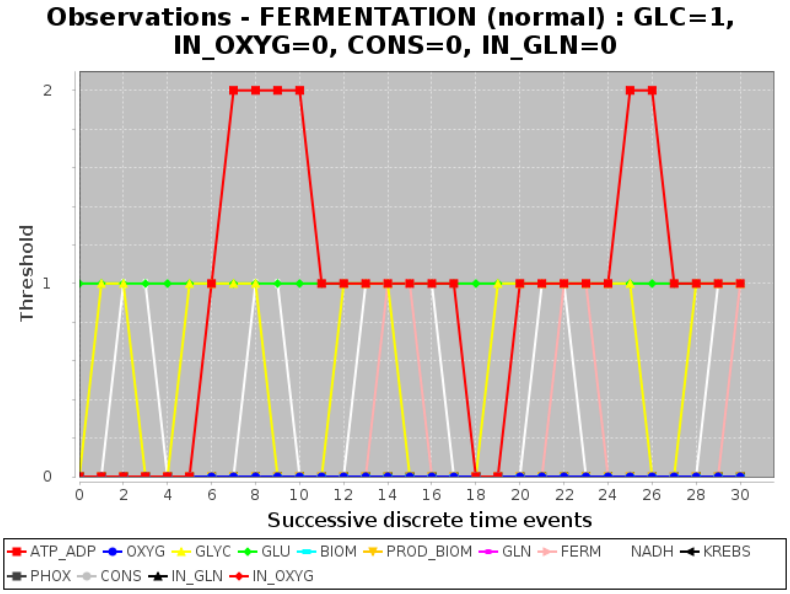


Figure 12: Initial state leading to fermentation, characterized by an absence of oxygen input and normal nutrient level (GLC=1).

6.3 Crabtree/Warburg effect

The capability to ferment sugars into ethanol is a key metabolic trait of yeasts. Crabtree-positive yeasts use fermentation even in the presence of oxygen, where they could, in principle, rely on the respiration pathway. This biologically observed phenomenon is surprising because fermentation has a much lower ATP yield than respiration (2 ATP vs. approximately 18 ATP per glucose) [2]. This normally occurs at high glucose level (GLU=2) and `Input_Oxygen`=1.

In accordance with these biological observations: Initially, the model state is in the Krebs phase and shortly afterwards it ends in the fermentation phase. Consequently, our model is able to reproduce the Crabtree effect.

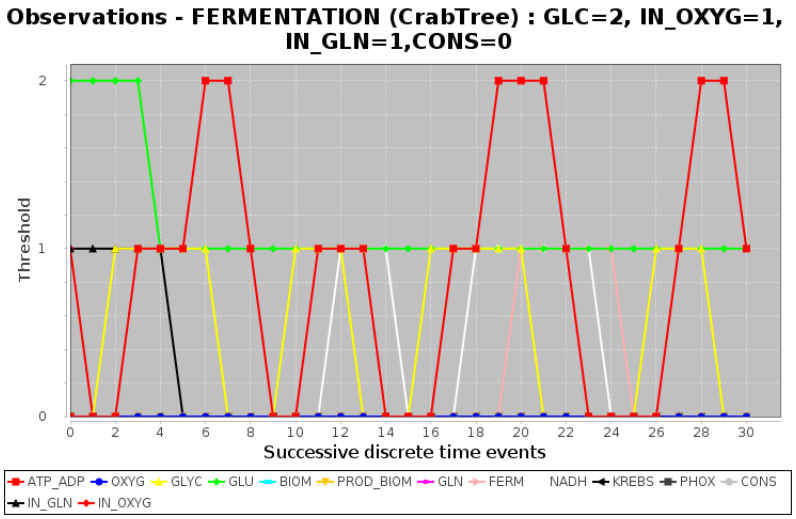


Figure 13: Fermentation showing the Crabtree effect under high glucose input, in the presence of oxygen

7 Conclusion

Our coarse-grained modelling of energy metabolism allows us to study how main actors of metabolism (including nutrients) can influence or affect global metabolic phenotypes such as metabolic oscillations or metabolic transitions between fermentation and respiration. The high level of abstraction has the advantage to directly relate the variables of the model to the biological knowledge or readouts from cellular phenotypic screens.

This abstract model of energy metabolism reproduces the basic aspects of energy metabolism dynamics such as metabolic oscillations and the Warburg/Crabtree effect. This metabolic transition from respiration to fermentation is confirmed with the DyMBioNet simulation when the glucose intake variable is modified from medium (GLC=1) to high (GLC=2).

A long term goal of this study is to propose pertinent experiments using formal logic to confirm or refute certain hypotheses concerning energy metabolism or to study the consistency of anti-Warburg strategies. For other problematics such as optimizing trade off between biomass and storage for bioproduction, new variables need to be incorporated to make the model more suitable for these new problems.

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