

Constraints-Based Optimization Methods for Modeling Metabolism in Cancer Cells: The Warburg Effect Revisited*

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SIAM AN06 – 14 July 2006

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Cancer cells rely primarily on glycolysis instead of oxidative phosphorylation for their production of ATP. I will discuss how one of the leading methods used to model large-scale metabolic reaction networks in living cells, known as Stoichiometric Network Theory, can be used to study the differences between metabolism in normal cells and that in cancer cells. Results from this model illustrate the advantages that cancer cells gain by way of the Warburg effect and allow us to propose new diagnosis and treatment strategies.

* Joint work with Meredith D. Betterton.



Introduction

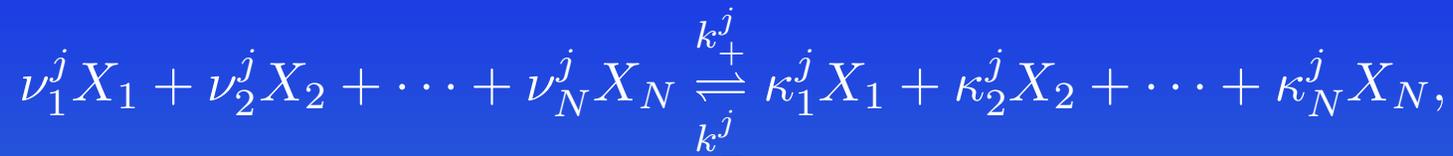
- ★ In 1924, the German biochemist, Otto Warburg, showed that many tumors relied on glycolysis instead of oxidative phosphorylation, even in the presence of oxygen (Warburg *et al.*, *Biochem. Z.*, 1924).
- ★ More recently, it has been shown that the Warburg effect is linked to mutations in signaling pathways that govern glucose uptake into cells, rather than mitochondrial defects (Garber, *J. Nat'l. Cancer Inst.*, 2004).
- ★ Activation of the Akt kinase signaling pathway has been shown to stimulate glucose consumption without affecting the rate of oxidative phosphorylation (Thompson *et al.*, *Cancer Res.*, 2004).
- ★ It has also been shown that the *myc* oncogene can turn on glycolysis (Dang, *Mol. Cell. Biol.*, 1997).
- ★ Hypoxia-inducible factor 1 (HIF-1) is well studied and has been shown to regulate a multiplicity of genes, including all of the glycolytic enzymes (Semenza *et al.*, *Mol. Cell. Biol.*, 1992).

Further Observations

- ★ Experiments have shown a 4 to 6-fold increase in glucose uptake in cancer cells (Thompson *et al.*, *Cancer Res.*, 2004; Ramanathan *et al.*, *PNAS*, 2005).
- ★ It has also been observed that cancer cells create a more acidic environment, perhaps to help them compete against normal cells so that they can proliferate.
- ★ Whereas most normal cells undergo apoptosis when the hypoxic stress is too intense or persists for too long, cancer cells appear to have a much higher resistance to hypoxia (Alarcón *et al.*, *J. Theor. Biol.*, 2004).
- ★ Some experiments have also shown increased levels of ribose-5-phosphate (R5P) and orotic acid, both of which are involved in nucleotide biosynthesis (Ramanathan *et al.*, *PNAS*, 2005).

The Law of Mass Action

For a system involving M reactions and N chemical species with j^{th} reaction



the law of mass action gives

$$\frac{dx_i(t)}{dt} = \sum_{j=1}^M (\kappa_i^j - \nu_i^j) (k_+^j x_1^{\nu_1^j} x_2^{\nu_2^j} \cdots x_N^{\nu_N^j} - k_-^j x_1^{\kappa_1^j} x_2^{\kappa_2^j} \cdots x_N^{\kappa_N^j}).$$

A closed system will go to equilibrium, whereas an open system will go to a nonequilibrium steady-state (NESS).

Open Systems

Starting with the original mass-action kinetics

$$\frac{dx_i(t)}{dt} = \sum_{j=1}^M (\kappa_i^j - \nu_i^j) (k_+^j x_1^{\nu_1^j} x_2^{\nu_2^j} \dots x_N^{\nu_N^j} - k_-^j x_1^{\kappa_1^j} x_2^{\kappa_2^j} \dots x_N^{\kappa_N^j}),$$

the detailed balance conditions can be broken by flux injection

$$\frac{dx_i(t)}{dt} = \sum_{j=1}^M (\kappa_i^j - \nu_i^j) (k_+^j x_1^{\nu_1^j} x_2^{\nu_2^j} \dots x_N^{\nu_N^j} - k_-^j x_1^{\kappa_1^j} x_2^{\kappa_2^j} \dots x_N^{\kappa_N^j}) + J_i^{ext},$$

or concentration clamping

$$\frac{dx_i(t)}{dt} = \sum_{j=1}^M (\kappa_i^j - \nu_i^j) (k_+^j c_0^{\nu_0^j} c_{N+1}^{\nu_{N+1}^j} x_1^{\nu_1^j} x_2^{\nu_2^j} \dots x_N^{\nu_N^j} - k_-^j c_0^{\kappa_0^j} c_{N+1}^{\kappa_{N+1}^j} x_1^{\kappa_1^j} x_2^{\kappa_2^j} \dots x_N^{\kappa_N^j}).$$

Nonequilibrium Thermodynamics

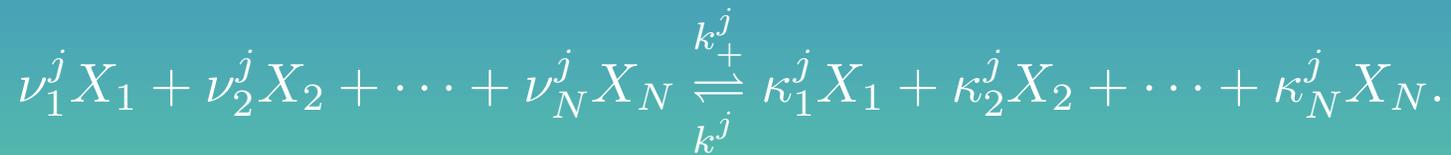
The chemical potential of a species is given by

$$\mu_i = \mu_i^o + RT \ln x_i,$$

from which we get the reaction potential, given by

$$\Delta\mu^j = RT \ln \left(\frac{k_-^j x_1^{\kappa_1^j} x_2^{\kappa_2^j} \cdots x_N^{\kappa_N^j}}{k_+^j x_1^{\nu_1^j} x_2^{\nu_2^j} \cdots x_N^{\nu_N^j}} \right),$$

for the j^{th} reaction



Turning to Stoichiometric Constraints-Based Approaches

- ★ Typically, it is not possible to solve for analytic solutions of the mass-action models because reaction networks are very large and complex.
- ★ There is a limit to the amount of information experimentalists can gather and, in most cases, it is not possible to obtain detailed kinetic-rate information.
- ★ For these reasons, approaches that rely only on the stoichiometry of a system, i.e., the static, algebraic structure of biochemical networks, within which chemical “motion” must take place, have been developed and do not require any kinetic-rate information.
- ★ This method of analysis has been successfully used to describe the functional states, or phenotypes, of many systems such as *E. coli* (Edwards and Palsson, *PNAS*, 2000), mitochondrial energy metabolism (Ramakrishna et al., *Am. J. Physiol. Reg. Int. Comp. Physiol.*, 2001), and metabolism in hepatocyte cells (Beard and Qian, *Am. J. Physiol. Endocrinol. Metab.*, 2005).

Stoichiometric Network Theory

Recall the general mass-action model with flux injection, where

$$\frac{dx_i(t)}{dt} = \sum_{j=1}^M (\kappa_i^j - \nu_i^j) (k_+^j x_1^{\nu_1^j} x_2^{\nu_2^j} \dots x_N^{\nu_N^j} - k_-^j x_1^{\kappa_1^j} x_2^{\kappa_2^j} \dots x_N^{\kappa_N^j}) + J_i^{ext}.$$

We can rewrite the system of equations in matrix form as

$$\frac{d\mathbf{x}}{dt} = \mathbf{S}\mathbf{J} + \mathbf{J}^{ext},$$

where $\mathbf{S} \in \mathbb{R}^{N \times M}$ is the stoichiometric matrix, $\mathbf{J} \in \mathbb{R}^M$ is the flux vector, and $\mathbf{J}^{ext} \in \mathbb{R}^N$ is the external flux vector.

Since this system is being driven by external fluxes, it will go to a NESS.

Flux Balance Constraints

In NESS, the concentrations of the chemical species are not changing and we have

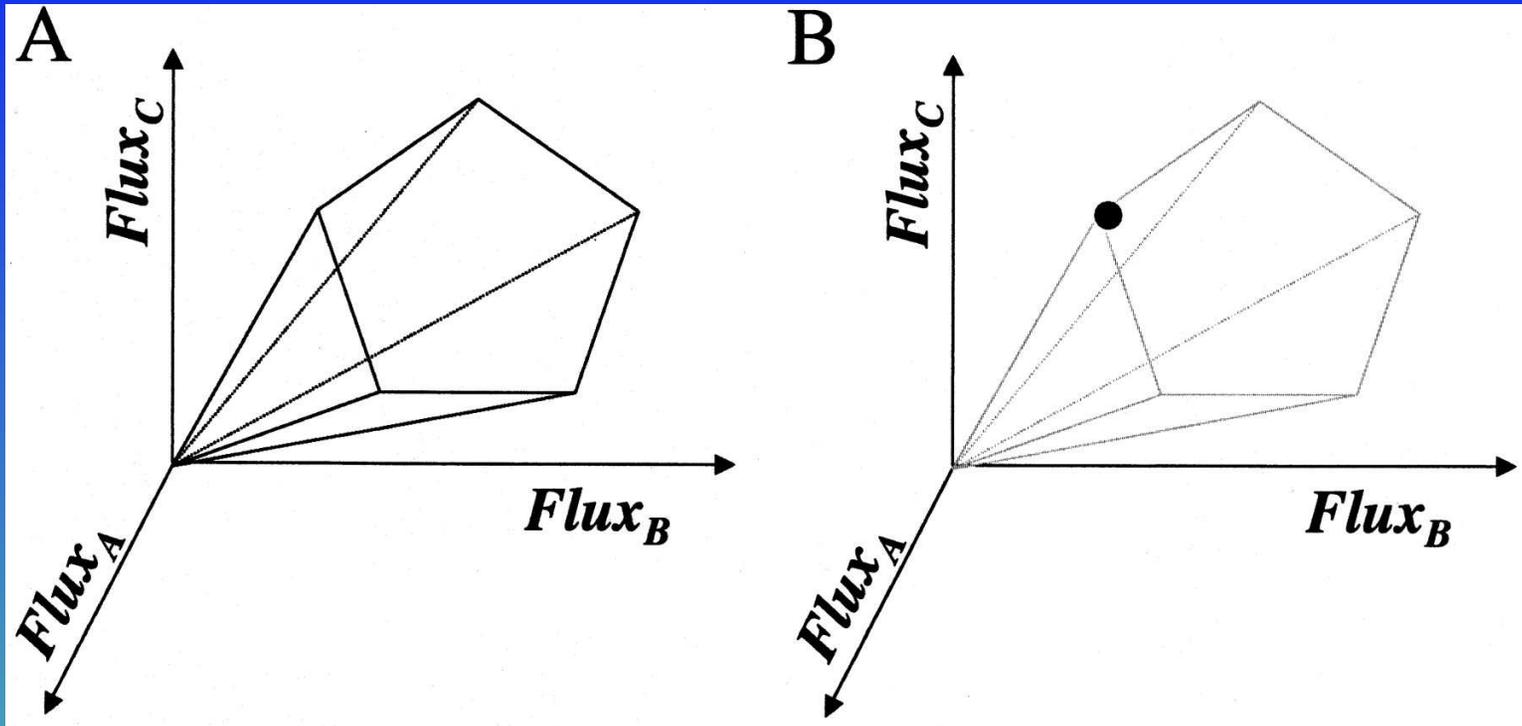
$$S\mathbf{J} = -\mathbf{J}^{ext},$$

which is known as the flux balance constraint of FBA. Note that this constraint is similar to Kirchoff's current law of electrical circuit theory.

Additional constraints can be applied to the NESS fluxes such as

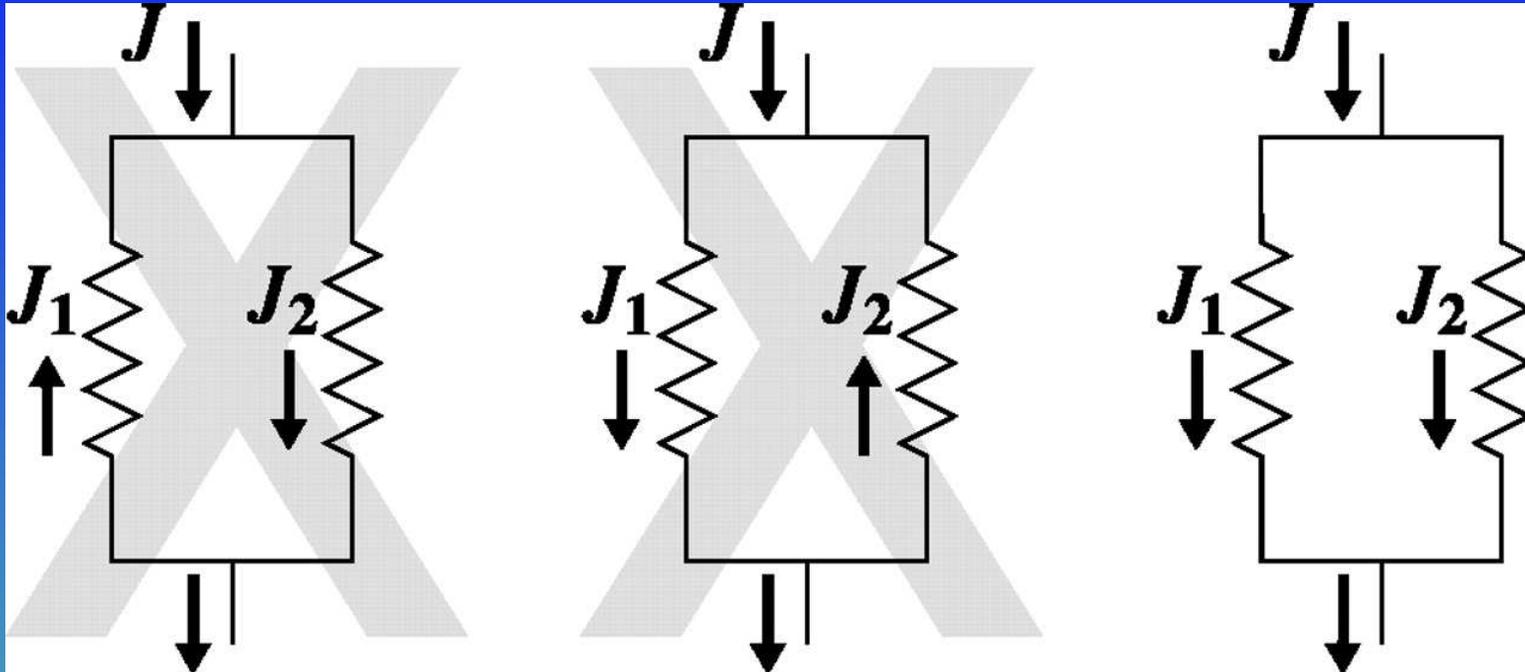
$$J_{lb}^j \leq J^j \leq J_{ub}^j \quad \forall j \in \{1, 2, \dots, M\}$$
$$(J^{ext})_{lb}^i \leq (J^{ext})^i \leq (J^{ext})_{ub}^i \quad \forall i \in \{1, 2, \dots, N\}.$$

Flux Balance Analysis



(Edwards and Palsson, *PNAS*, 2000)

Fluxes and Energy Gradients



(Beard *et al.*, *Biophys. J.*, 2002)

Energy Balance Analysis

Define $\mu \in \mathbb{R}^N$ as the vector of chemical potentials, then the vector of reaction potentials, $\Delta\mu \in \mathbb{R}^M$, is given by

$$S^T \mu = \Delta\mu.$$

We can define the nullspace matrix $K \in \mathbb{R}^{M \times (M-r)}$ with columns that form a basis for the nullspace of S , so that $SK = 0$. Then we have the constraint

$$K^T S^T \mu = K^T \Delta\mu = 0,$$

which is a statement of the conservation of energy and is similar to Kirchoff's loop or voltage law of electrical circuit theory.

Relationship Between Reaction Fluxes and Potentials

If we define the nonnegative forward and reverse reaction fluxes so that $\mathbf{J} = \mathbf{J}_+ - \mathbf{J}_-$, then the reaction potential is

$$\Delta\mu^j = RT \ln \left(\frac{J_-^j}{J_+^j} \right),$$

which leads us directly to the second law of thermodynamics, i.e.,

$$-J^j \Delta\mu^j = -RT \left(J_+^j - J_-^j \right) \ln \left(\frac{J_-^j}{J_+^j} \right) \geq 0.$$

Entropy must increase and the system must dissipate heat,

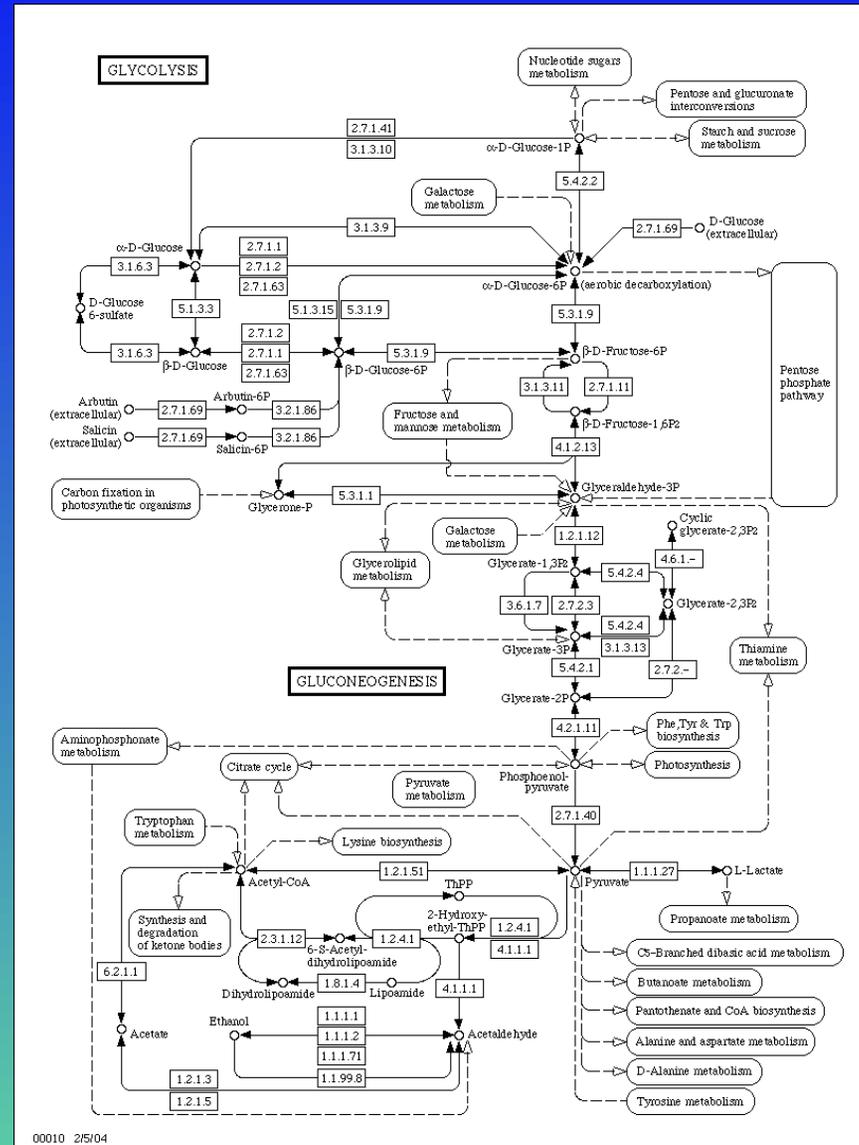
$$hdr = -\mathbf{J}^T \Delta\boldsymbol{\mu} > 0.$$

The Optimization Problem

$$\begin{aligned}
 & \min_{\mathbf{J}, \mathbf{J}_+, \mathbf{J}_-, \mathbf{J}^{ext}, \Delta\mu} && f(\mathbf{J}, \mathbf{J}_+, \mathbf{J}_-, \mathbf{J}^{ext}, \Delta\mu) \\
 & \text{s.t.} && \mathbf{S}\mathbf{J} + \mathbf{J}^{ext} = \mathbf{0} \\
 & && \mathbf{K}^T \Delta\mu = \mathbf{0} \\
 & && \text{diag} \left(e^{\Delta\mu/RT} \right) \mathbf{J}_+ - \mathbf{J}_- = \mathbf{0} \\
 & && \mathbf{J} - \mathbf{J}_+ + \mathbf{J}_- = \mathbf{0} \\
 & && \mathbf{J}_{lb} \leq \mathbf{J} \leq \mathbf{J}_{ub} \\
 & && \mathbf{0} \leq \mathbf{J}_+ < \infty \\
 & && \mathbf{0} \leq \mathbf{J}_- < \infty \\
 & && \mathbf{J}_{lb}^{ext} \leq \mathbf{J}^{ext} \leq \mathbf{J}_{ub}^{ext} \\
 & && \Delta\mu_{lb} \leq \Delta\mu \leq \Delta\mu_{ub}
 \end{aligned}$$

(Heuett and Qian, *J. Bioinf. Comp. Biol.*, 2006)

Glycolysis



Glycolysis and Lactate Dehydrogenase

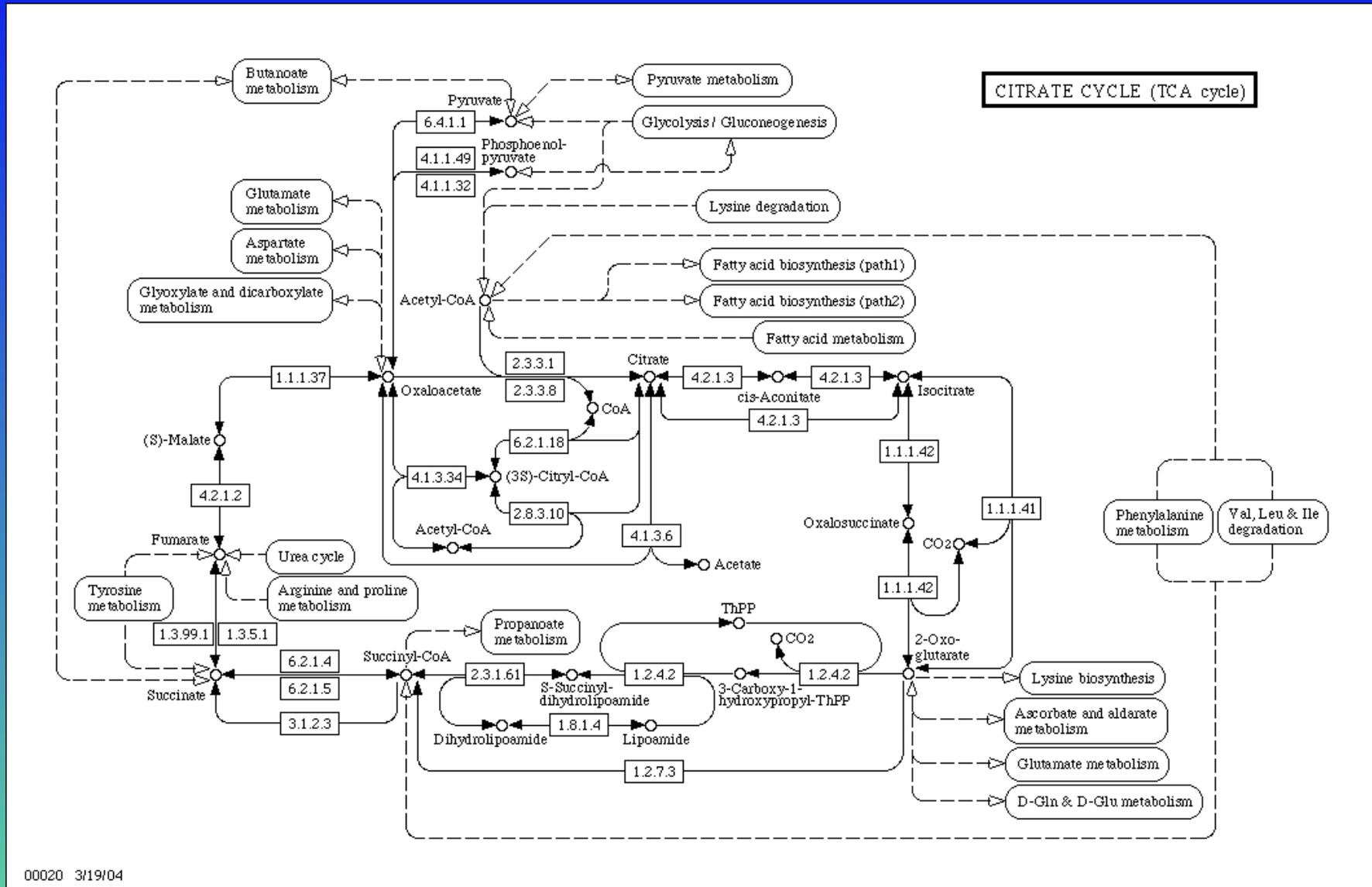
GLYCOLYSIS

| <u>RXN</u> | <u>REACTANTS</u> | <u>PRODUCTS</u> |
|------------|---|--|
| hk | GLC + ATP ⁴⁻ | G6P ²⁻ + ADP ³⁻ + H ⁺ |
| pgi | G6P ²⁻ | F6P ²⁻ |
| pfk | F6P ²⁻ + ATP ⁴⁻ | FBP ⁴⁻ + ADP ³⁻ + H ⁺ |
| pfp | F6P ²⁻ + H ⁺ + HPO ₄ ²⁻ | FBP ⁴⁻ + H ₂ O |
| ald | FBP ⁴⁻ | GAP ²⁻ + DHAP ²⁻ |
| tpi | DHAP ²⁻ | GAP ²⁻ |
| gapdh | GAP ²⁻ + NAD ⁺ + HPO ₄ ²⁻ | 1,3-BPG ⁴⁻ + NADH + H ⁺ |
| pgk | 1,3-BPG ⁴⁻ + ADP ³⁻ | 3-PGA ³⁻ + ATP ⁴⁻ |
| pgm | 3-PGA ³⁻ | 2-PGA ³⁻ |
| eno | 2-PGA ³⁻ | PEP ³⁻ + H ₂ O |
| pk | PEP ³⁻ + ADP ³⁻ + H ⁺ | PYR ⁻ + ATP ⁴⁻ |

LACTATE DEHYDROGENASE

| <u>RXN</u> | <u>REACTANTS</u> | <u>PRODUCTS</u> |
|------------|--|-------------------------------------|
| ldh | PYR ⁻ + NADH + H ⁺ | LAC ⁻ + NAD ⁺ |

TCA Cycle



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Transports and TCA Cycle

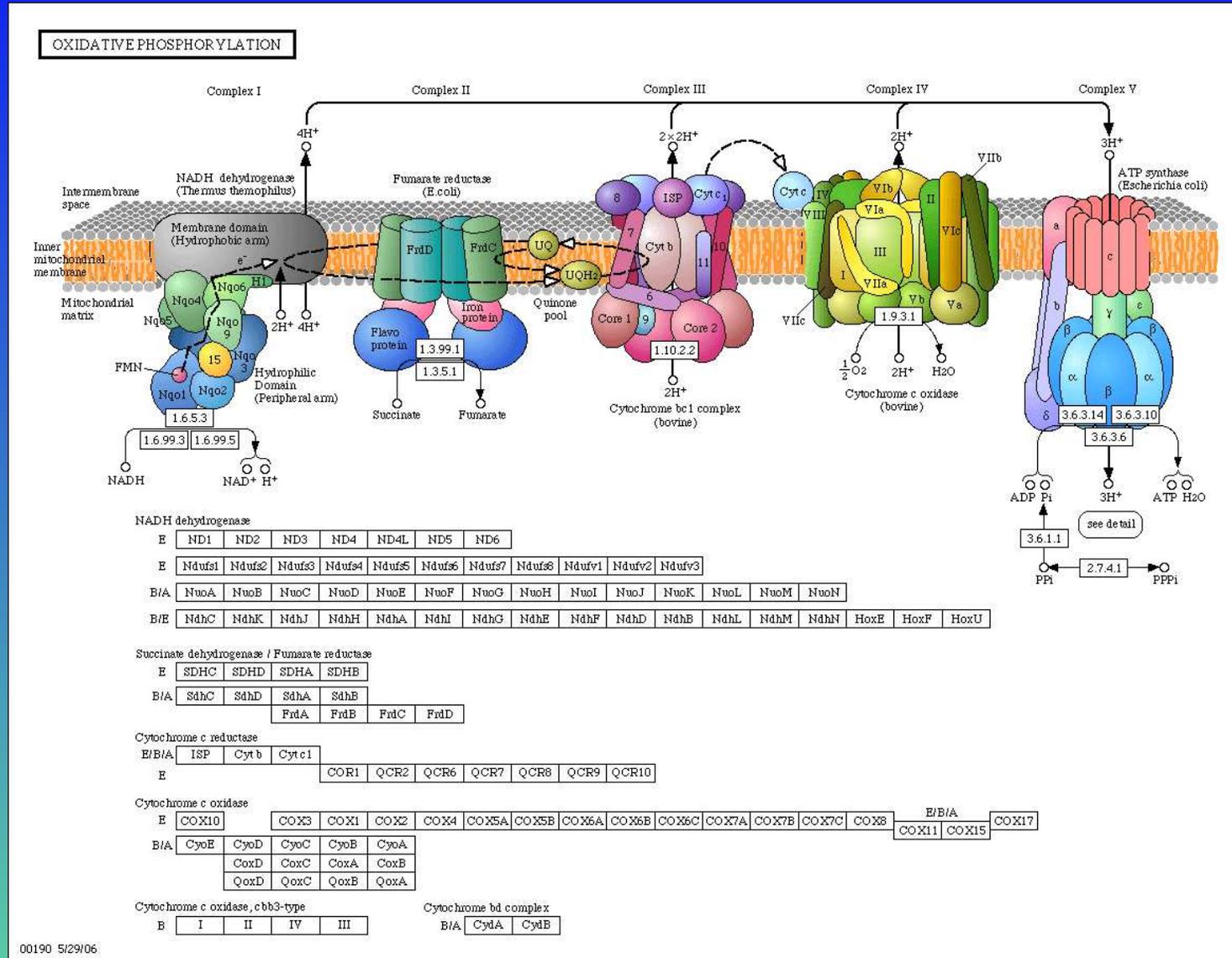
TRANSPORTS

| <u>RXN</u> | <u>REACTANTS</u> | <u>PRODUCTS</u> |
|------------|---|---|
| pmt | PYR^- | PYR_m^- |
| gps | $\text{NADH} + \text{H}^+ + \text{FAD}_m$ | $\text{NAD}^+ + \text{FADH}_{2m}$ |
| ant | $\text{ADP}_m^{3-} + \text{ATP}_m^{4-}$ | $\text{ADP}_m^{3-} + \text{ATP}_m^{4-}$ |
| pit | $\text{H}^+ + \text{HPO}_4^{2-}$ | $\text{H}_m^+ + \text{HPO}_{4m}^{2-}$ |

TCA CYCLE

| <u>RXN</u> | <u>REACTANTS</u> | <u>PRODUCTS</u> |
|------------|--|---|
| ace | $\text{PYR}_m^- + \text{CoA}_m + \text{NAD}_m^+ + \text{H}_2\text{O}$ | $\text{NADH}_m + \text{CO}_2 + \text{ACCoA}_m$ |
| glt | $\text{ACCoA}_m + \text{OA}_m^{2-} + \text{H}_2\text{O}$ | $\text{CoA}_m + \text{CIT}_m^{3-} + \text{H}_m^+$ |
| acn | CIT_m^{3-} | ICIT_m^{3-} |
| icd | $\text{ICIT}_m^{3-} + \text{NAD}_m^+ + \text{H}_2\text{O}$ | $\text{NADH}_m + \text{CO}_2 + \text{AKG}_m^{2-}$ |
| suc1 | $\text{AKG}_m^{2-} + \text{CoA}_m + \text{NAD}_m^+ + \text{H}_2\text{O}$ | $\text{NADH}_m + \text{CO}_2 + \text{SUCCoA}_m^-$ |
| suc2 | $\text{SUCCoA}_m^- + \text{GDP}_m^{3-} + \text{HPO}_{4m}^{2-}$ | $\text{GTP}_m^{4-} + \text{CoA}_m + \text{SUCC}_m^{2-}$ |
| sdh1 | $\text{SUCC}_m^{2-} + \text{FAD}_m$ | $\text{FADH}_{2m} + \text{FUM}_m^{2-}$ |
| fumA | $\text{FUM}_m^{2-} + \text{H}_2\text{O}$ | MAL_m^{2-} |
| mkh | $\text{MAL}_m^{2-} + \text{NAD}_m^+$ | $\text{NADH}_m + \text{OA}_m^{2-} + \text{H}_m^+$ |
| ndk2 | $\text{GTP}_m^{4-} + \text{ADP}_m^{3-}$ | $\text{GDP}_m^{3-} + \text{ATP}_m^{4-}$ |

Oxidative Phosphorylation



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Oxidative Phosphorylation

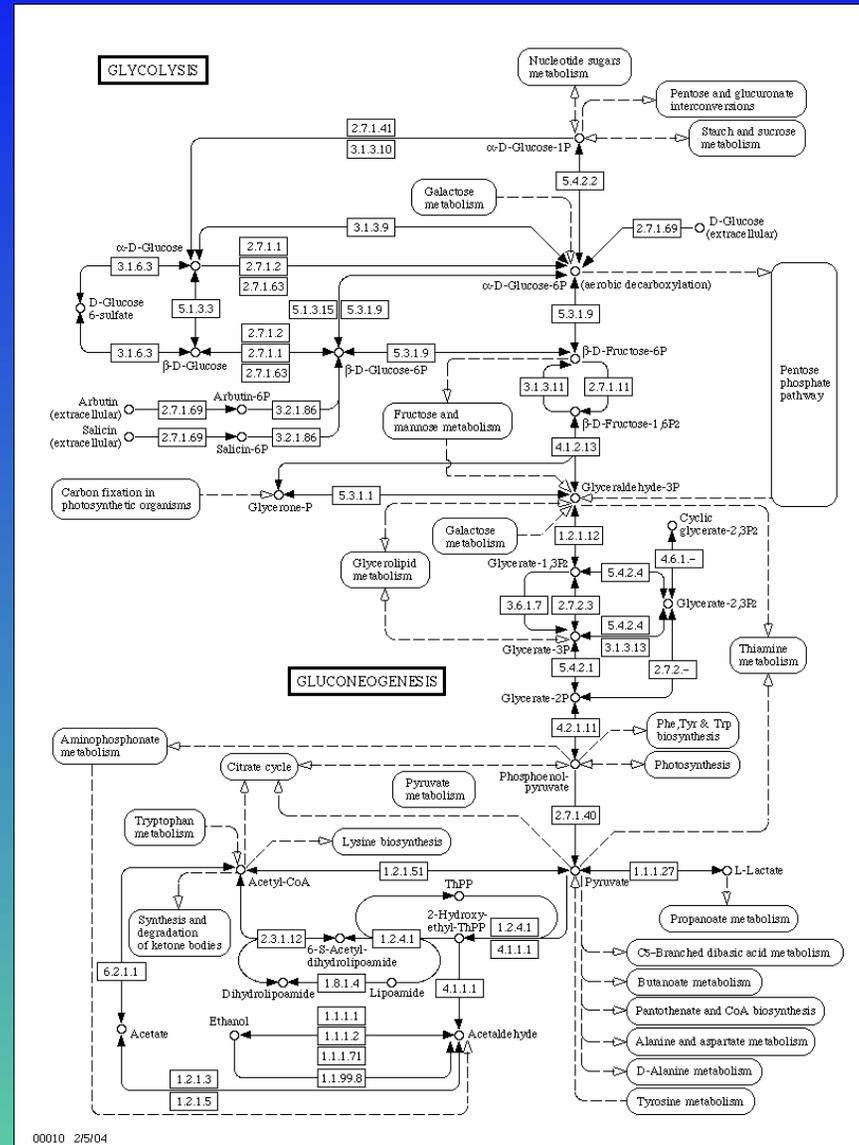
| <u>OXIDATIVE PHOSPHORYLATION</u> | | |
|----------------------------------|---|---|
| <u>RXN</u> | <u>REACTANTS</u> | <u>PRODUCTS</u> |
| nuo | $5 \text{H}_m^+ + \text{NADH}_m + \text{Q}$ | $\text{QH}_2 + \text{NAD}_m^+ + 4 \text{H}^+$ |
| sdh1 | $\text{FADH}_{2m} + \text{Q}$ | $\text{QH}_2 + \text{FAD}_m$ |
| cyo | $6 \text{H}_m^+ + \frac{1}{2} \text{O}_2 + \text{QH}_2$ | $\text{Q} + 6 \text{H}^+ + \text{H}_2\text{O}$ |
| f1atp | $3 \text{H}^+ + \text{ADP}_m^{3-} + \text{HPO}_4^{2-}$ | $2 \text{H}_m^+ + \text{H}_2\text{O} + \text{ATP}_m^{4-}$ |

Results

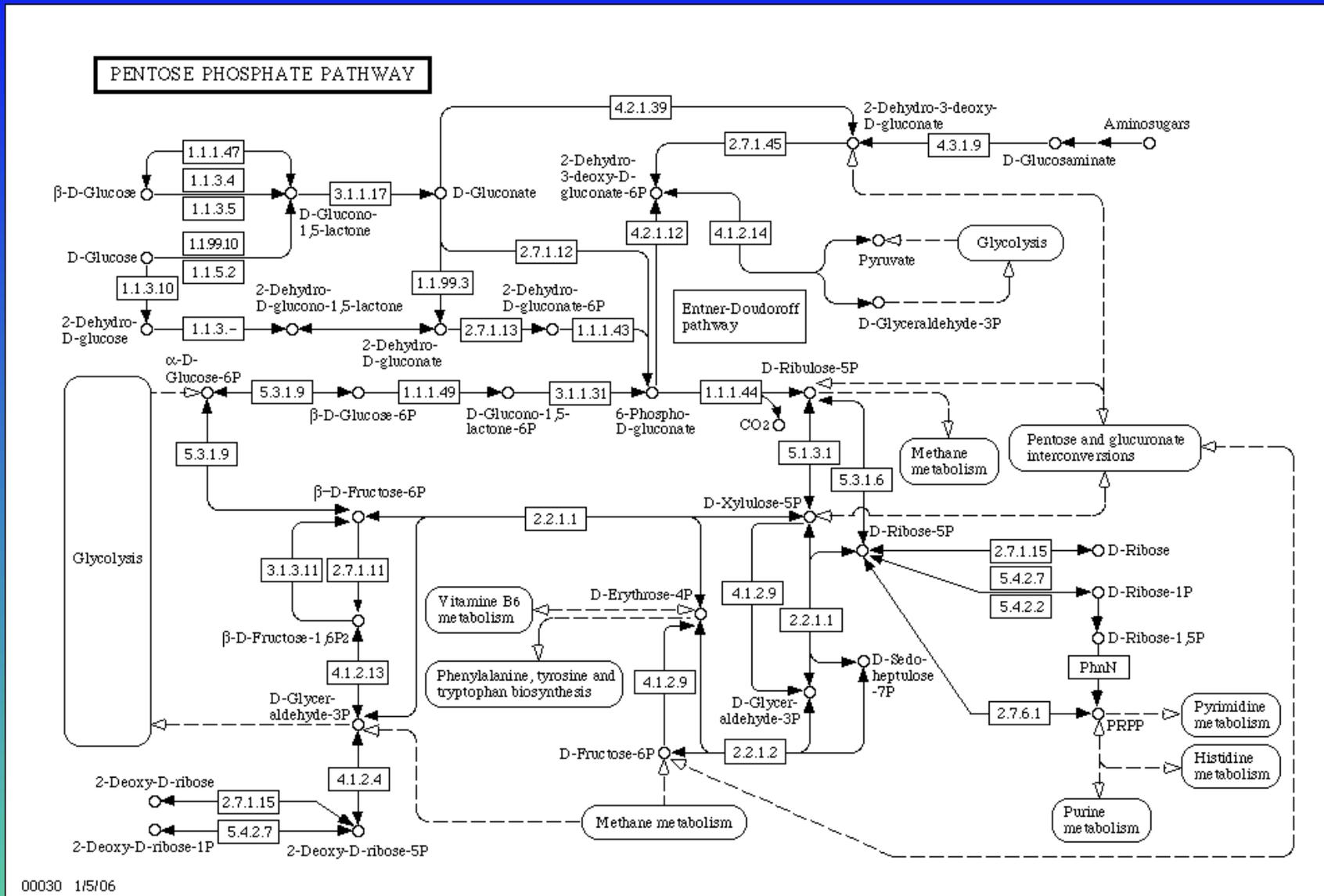
| | Reaction | Case 1 | Case 2 | Case 3 | Case 1 | Case 2 | Case 3 | Species |
|------------|----------|--------|--------|---------|--------|--------|----------|---------|
| GLYCOLYSIS | hk | 1 | 6 | 6 | 0 | 0 | 0 | H+ |
| | pgi | 1 | 6 | 6 | 38 | 38 | 6.3333 | H+_m |
| | pfk | 1 | 6 | 6 | -38 | -48 | -18 | H_20 |
| | pfp | 0 | 0 | 0 | 38 | 48 | 18 | HPO_4 |
| | ald | 1 | 6 | 6 | -38 | -48 | -18 | ATP |
| | tpi | 1 | 6 | 6 | 38 | 48 | 18 | ADP |
| | gapdh | 2 | 12 | 12 | 6 | 6 | 1 | O_2 |
| | pgk | 2 | 12 | 12 | -6 | -6 | -1 | CO_2 |
| | pgm | 2 | 12 | 12 | 1 | 6 | 6 | GLC |
| | eno | 2 | 12 | 12 | 0 | -10 | -11.6667 | LAC |
| | pk | 2 | 12 | 12 | | | | |
| | ldh | 0 | 10 | 11.6667 | | | | |
| TCA CYCLE | ace | 2 | 2 | 0.3333 | | | | |
| | glt | 2 | 2 | 0.3333 | | | | |
| | can | 2 | 2 | 0.3333 | | | | |
| | icd | 2 | 2 | 0.3333 | | | | |
| | suc1 | 2 | 2 | 0.3333 | | | | |
| | suc2 | 2 | 2 | 0.3333 | | | | |
| | sdh1 | 2 | 2 | 0.3333 | | | | |
| | fumA | 2 | 2 | 0.3333 | | | | |
| | mkh | 2 | 2 | 0.3333 | | | | |
| | ndk2 | 2 | 2 | 0.3333 | | | | |
| OX PHOS | nuo | 8 | 8 | 1.3333 | | | | |
| | sdh1 | 4 | 4 | 0.6667 | | | | |
| | cyo | 12 | 12 | 2 | | | | |
| | fl atp | 34 | 34 | 5.6667 | | | | |
| TRANS | Pit | 2 | 2 | 0.3333 | | | | |
| | pmt | 2 | 2 | 0.3333 | | | | |
| | gps | 2 | 2 | 0.3333 | | | | |
| | ant | 36 | 36 | 6 | | | | |

| UPPER BOUNDS | | |
|--------------|-----|-----|
| | O_2 | GLC |
| Case 1 | 6 | 1 |
| Case 2 | 6 | 6 |
| Case 3 | 1 | 6 |

Glycolysis



Pentose Phosphate Pathway



Pentose Phosphate Pathway

| <u>PENTOSE PHOSPHATE PATHWAY</u> | | |
|----------------------------------|------------------------|----------------------------|
| <u>RXN</u> | <u>REACTANTS</u> | <u>PRODUCTS</u> |
| g6pd | $G6P^{2-} + NADP^+$ | $6PdL^{2-} + NADPH + H^+$ |
| 6pgl | $6PdL^{2-} + H_2O$ | $6PGL^{2-} + H^+$ |
| 6pgd | $6PGL^{2-} + NADP^+$ | $Ru5P^{2-} + NADPH + CO_2$ |
| r5pi | $Ru5P^{2-}$ | $R5P^{2-}$ |
| r5pe | $Ru5P^{2-}$ | $Xu5P^{2-}$ |
| trk1 | $R5P^{2-} + Xu5P^{2-}$ | $S7P^{2-} + GAP^{2-}$ |
| tra | $S7P^{2-} + GAP^{2-}$ | $E4P^{2-} + F6P^{2-}$ |
| trk2 | $E4P^{2-} + Xu5P^{2-}$ | $GAP^{2-} + F6P^{2-}$ |

Results

| | Reaction | Case 1 | Case 2 | Case 3 | Case 1 | Case 2 | Case 3 | Species |
|------------|----------|--------|---------|---------|--------|----------|----------|---------|
| GLYCOLYSIS | hk | 1 | 6 | 6 | 0 | 0 | 0 | H+ |
| | pgi | 1 | -19.875 | 1.25 | 38 | 0 | 0 | H+_m |
| | pfk | 1 | 0 | 4.4167 | -38 | -29.875 | -14.8333 | H_20 |
| | pfp | 0 | -2.625 | 0 | 38 | 55.75 | 19.5833 | HPO_4 |
| | ald | 1 | -2.625 | 4.4167 | -38 | -55.75 | -19.5833 | ATP |
| | tpi | 1 | -2.625 | 4.4167 | 38 | 55.75 | 19.5833 | ADP |
| | gapdh | 2 | 3.375 | 10.4167 | 0 | 51.75 | 9.5 | NADP |
| | pgk | 2 | 3.375 | 10.4167 | 0 | -51.75 | -9.5 | NADPH |
| | pgm | 2 | 3.375 | 10.4167 | 6 | 6 | 1 | O_2 |
| | eno | 2 | 3.375 | 10.4167 | -6 | -31.875 | -5.75 | CO_2 |
| pk | 2 | 3.375 | 10.4167 | 1 | 6 | 6 | GLC | |
| ldh | 0 | 1.375 | 10.0833 | 0 | -1.375 | -10.0833 | LAC | |
| PPP | g6pd | 0 | 25.875 | 4.75 | | | | |
| | 6pgl | 0 | 25.875 | 4.75 | | | | |
| | 6pgd | 0 | 25.875 | 4.75 | | | | |
| | r5pi | 0 | 8.625 | 1.5833 | | | | |
| | r5pe | 0 | 17.25 | 3.1667 | Case 1 | 6 | 1 | |
| | trk1 | 0 | 8.625 | 1.5833 | Case 2 | 6 | 6 | |
| | tra | 0 | 8.625 | 1.5833 | Case 3 | 1 | 6 | |
| | trk2 | 0 | 8.625 | 1.5833 | | | | |
| TCA CYCLE | ace | 2 | 2 | 0.3333 | | | | |
| | glt | 2 | 2 | 0.3333 | | | | |
| | can | 2 | 2 | 0.3333 | | | | |
| | icd | 2 | 2 | 0.3333 | | | | |
| | suc1 | 2 | 2 | 0.3333 | | | | |
| | suc2 | 2 | 2 | 0.3333 | | | | |
| | sdh1 | 2 | 2 | 0.3333 | | | | |
| | fumA | 2 | 2 | 0.3333 | | | | |
| | mkh | 2 | 2 | 0.3333 | | | | |
| | ndk2 | 2 | 2 | 0.3333 | | | | |
| OX PHOS | nuo | 8 | 8 | 1.3333 | | | | |
| | sdh1 | 4 | 4 | 0.6667 | | | | |
| | cyo | 12 | 12 | 2 | | | | |
| | f1atp | 34 | 53 | 8.8333 | | | | |
| TRANS | Pit | 2 | 2 | 0.3333 | | | | |
| | pmt | 2 | 2 | 0.3333 | | | | |
| | gps | 2 | 2 | 0.3333 | | | | |
| | ant | 36 | 55 | 9.1667 | | | | |

Discussion

- ★ Ramanathan *et al.*, *PNAS*, 2005, suggested that cancer cells use the mitochondria and oxygen for pyrimidine synthesis rather than ATP production, i.e., there is a coupling between the nucleotide biosynthesis and the mitochondrial machinery to achieve the high rates of cell proliferation.
- ★ This suggestion was supported by their observations of higher R5P and orotic acid in cancer cells.
- ★ Our results also support this idea as we have seen a decoupling of ATP synthesis from oxidative phosphorylation. This change can come about simply by changing the glucose uptake, perhaps by way of the Akt kinase signaling pathway or the *myc* oncogene.

Conclusions

- ★ The classical methods for modeling biochemical networks are limited in their power. Using stoichiometric constraints-based approaches, we are able to quantitatively study the possible phenotypes of a system.
- ★ This method allows us to study a system on the whole genome scale and do *in silico* experiments instead of *in vitro* or *in vivo* experiments.
- ★ By combining FBA and EBA constraints, we are certain that the feasible solutions are mass balanced and thermodynamically realistic.
- ★ Using an SQP to solve the optimization problem allows us to combine the FBA and EBA constraints and consider many different objective functions.
- ★ SNT has been shown to be a very accurate and useful tool for studying mutant and disease affected organisms.

Conclusions

- ★ The glycolytic shift is one way to explain why positron emission tomography (PET), which uses a radiolabeled glucose analogue to track glucose uptake by tumors, is so good at picking out malignant and fast-growing tumors (Garber, *J. Nat'l. Cancer Inst.*, 2004).
- ★ New diagnosis strategies can be suggested. For example, one could look at the ratios of mitochondrial enzymes to glycolytic enzymes (Cuezva *et al.*, *Cancer Res.*, 2002; Cuezva *et al.*, *Carcinogenesis*, 2005).
- ★ Metabolic targeting may also provide new ways to treat cancer.
- ★ Whether the shift to aerobic glycolysis is an epiphenomenon or a requirement for transformation to cancer is still being debated, but it is evident that more work, focusing on metabolic phenotypes of cancer cells, is needed.

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- ★ my supervisor, Meredith Betterton,
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