

Metabolic Networks : Visual Analysis of Elementary Flux Modes.



Beurton-Aimar M.¹, Sansen J.¹ and Nguyen Vu Ngoc T.²

¹LaBRI - UMR 5800, Univ. Bordeaux, France

²EMBL - EBI - Cambridge, UK



Working with networks and pathways in molecular biology

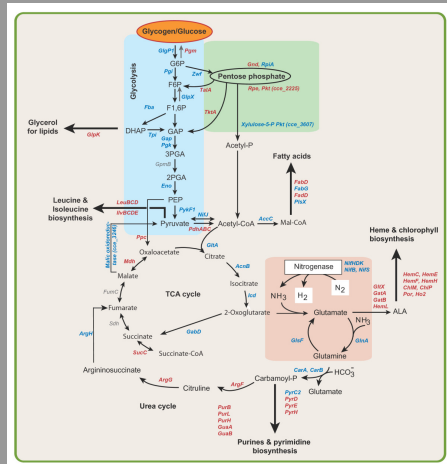
Context

- ▶ Metabolic Networks Specificities : graph model and granularity of the studying.
- ▶ Finding Feasible Pathways : searching for equilibrium and steady state.
- ▶ How to Explore Results : visual tool to dynamically find patterns in the network.

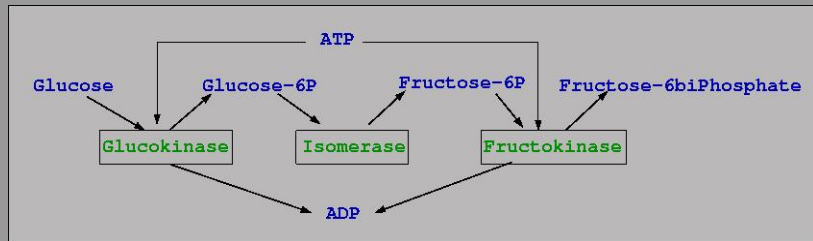
Metabolic Network Design - Granularity of the studying

Building Metabolic Network

- ▶ Association of several pathways.
- ▶ **Graph model** : nodes and edges.
- ▶ **Different models**: reaction graph, metabolite graph or both.



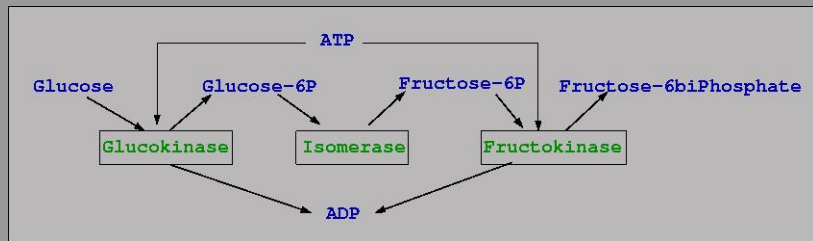
Metabolic Pathway Example



A piece of the Pentose Phosphate Pathway

<i>Enzyme</i>	<i>Substract</i>	<i>Produce</i>
Glucokinase :	Glucose + ATP	= Glucose-6P + ADP
Isomerase :	Glucose-6P	= Fructose-6P
Fructokinase :	Fructose-6P + ATP	= Fructose-6biPhosphate + ADP

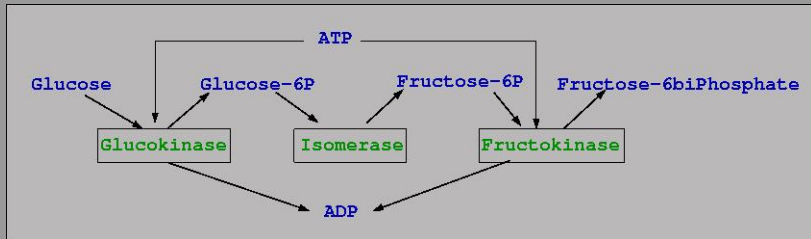
Metabolic Pathway Example



Interaction Matrix

Met./Réact.	Glucokinase	Isomerase	Fructokinase
Glucose	-1	0	0
ATP	-1	0	-1
Gluc-6P	1	-1	0
ADP	1	0	1
Fruct-6P	0	1	-1
Fruct-6biPhosphate	0	0	1

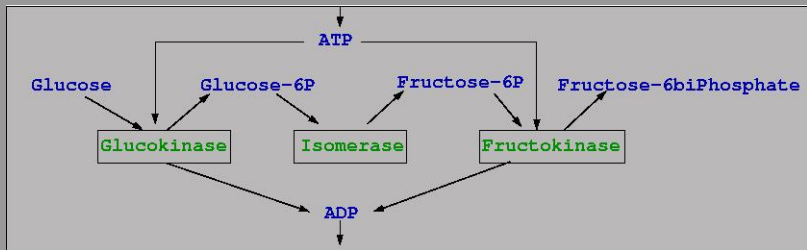
Metabolic Pathway Example



Interaction Matrix

Met./React.	Glucokinase	Isomerase	Fructokinase
Glucose	-1	0	0
ATP	-1	0	-1
Gluc-6P	1	-1	0
ADP	1	0	1
Fruct-6P	0	1	-1
Fruct-6biPhosphate	0	0	1

Metabolic Pathway Example



Interaction Matrix

Met/React	Glucokinase	Isomerase	Fructokinase	T-atp	T-adp
ATP	-1	0	-1	1	0
Gluc-6P	1	-1	0	0	0
ADP	1	0	1	0	-1
Fruct-6P	0	1	-1	0	0

Metabolic Network Design

Flux analysis

- ▶ Dynamic analysis :
 - ▶ Tools : Michaelis Menten equation, flux balance analysis ...
 - ▶ Requirements : quantitative information as kinetics of reactions
- ▶ Static analysis :
 - ▶ Tools : graph theory, linear algebra, logic operators ...
 - ▶ Requirements : qualitative and structural information as definition of the set of reactions, frontiers of the model ...

Metabolic Network Design

Flux analysis

- ▶ Dynamic analysis :
 - ▶ Tools : Michaelis Menten equation, flux balance analysis ...
 - ▶ Requirements : quantitative information as kinetics of reactions
- ▶ Static analysis :
 - ▶ Tools : graph theory, linear algebra, logic operators ...
 - ▶ Requirements : qualitative and structural information as definition of the set of reactions, frontiers of the model ...

Questions to the model.

Questions

Enzyme / Pathway Activity

- ▶ **Functional relationships** between two enzymes.
- ▶ **Substitutes** to produce a metabolite .
- ▶ Plasticity, **robustness** of a metabolic pathway..

Finding the way through a graph

- ▶ Identification of **routes** to realize an objective function.
- ▶ Identification of control points, reactions or metabolites **hubs**.

Analysis of the Graph Structure

Quantification of properties

- ▶ Diameter, arity of nodes, clustering coefficient .
⇒ few relevant informations.

Metabolic Graph course

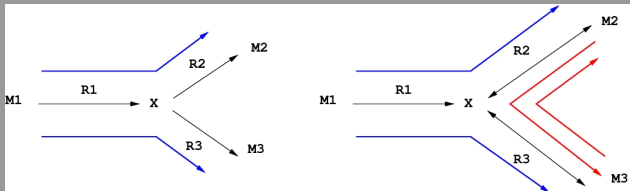
- ▶ Elementary Flux Modes ¹, EFMs : set of unique and minimal set of reactions interacting together at the steady-state.
- ▶ Taking into account reversibility and stoichiometry of reactions.

¹Schuster et al Journal of Biological Systems - 1995

Elementary Flux Modes

Method

- ▶ Using linear algebra to find all possible combinations of reactions leading to steady-state.
- ▶ Using stoichiometric matrix.
- ▶ Allowing to validate the network description.
- ▶ Not need of kinetic values.



Elementary Flux Modes

Computation

▶ Tools:

- ▶ **CellNetAnalyser**(previously Metatool):
www2.mpi-magdeburg.mpg.de/projects/cna/cna.html,
- ▶ **EfmTools**: www.csb.ethz.ch/tools/software/efmtool.html,
- ▶ **RegEfmtools**: tiny www.biotec.boku.ac.at.

▶ Example of EFMs with their reaction names.

(11) Tg6p (2 Vpgi_p) (-2 Vpgi) (-3 Vfbp) (3 Vepi_p) (-3 Vac_g) (3 tr3) (-3 tr5) (3 node1) Gly_p1 (3 t9PPPC)
irreversible

(12) Tg6y Vpgi_p -Vpgi Vepi_p Vinv -Vac_g (-3 tr5) Vgdh node1 Vasp (3 t9PPPC) (2 VCO2) irreversible

▶ Matrix format: all lines have the same size.

```
1 0 2 -2 0 0 0 -3 0 3 0 0 0 -3 0 0 0 0 3 0 -3 0 3 0 0 0 0 0 0 1 0 0 0 0 0 0 0 0 0 3 0 ....
1 0 1 -1 0 0 0 0 0 1 0 0 1 -1 0 0 0 0 0 0 -3 1 1 0 0 0 0 0 0 0 0 0 1 0 0 0 0 0 0 3 2 ....
```

Result Analysis

Elementary Flux Modes

- ▶ Huge number of EFMs - several thousands.
- ▶ Classification:
 - ▶ Classical clustering: difficulties due to EFM properties.
 - ▶ Specific tools: dual computing of list of reactions leading feasible pathways.

Result Analysis

Elementary Flux Modes

- ▶ Huge number of EFMs - several thousands.
- ▶ Classification:
 - ▶ Classical clustering: difficulties due to EFM properties.
 - ▶ Specific tools: dual computing of list of reactions leading feasible pathways.

Minimal Cut Sets

- ▶ **Idea:** find the reaction/set of reactions unable pathways.
- ▶ Taking into account reversibility of reactions and stoichiometry.

Minimal Cut Sets

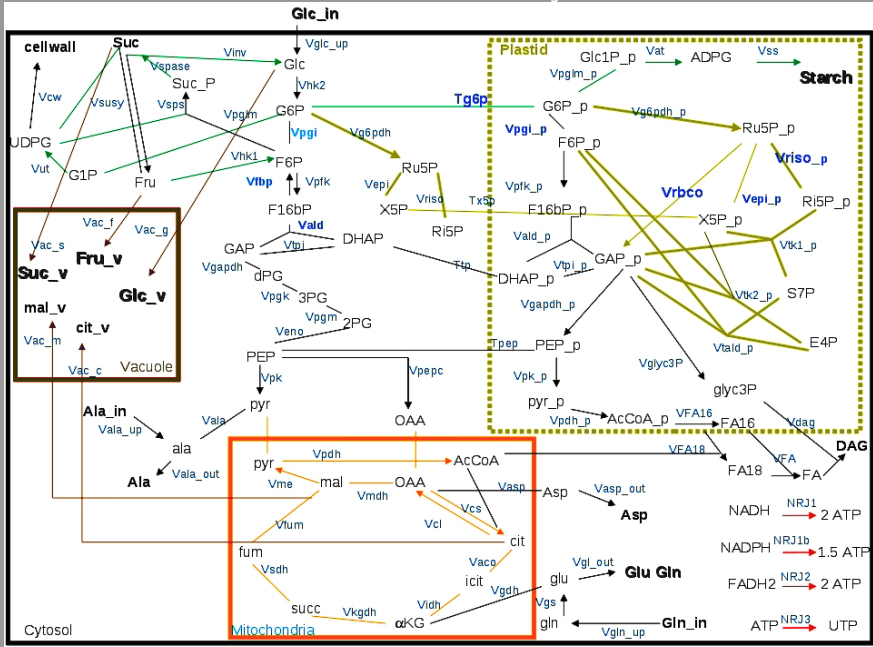
Dual Problem: cutting flux

- ▶ **Graph theory:** searching the set of nodes building a minimal cut.
- ▶ **Biology:** consequences of stopping/missing the activity of an enzyme?

Definition

- ▶ A MCS², is a unique and smallest set of reactions whose removal from the network would stop a given metabolic function.
- ▶ Hope to obtain **less MCS than EFMs** for a given network.
- ▶ **93,009** MCS have been obtained by computation of the plant cell network vs **114,614** EFMs.
- ▶ Result too large to be analyzed like it.

Central Metabolism of Plant Cell Analysis



Central Metabolism of Plant Cell Analysis

Identification of network structure

- ▶ **Concrete example:** finding rules/constraints to produce 5 output metabolites if no entry of Glucose.
- ▶ Analysis of MCS with small size.

Results

- ▶ MCS of size 2: Identification of a set of mandatory reactions.
- ▶ MCS of size 3, 4 ...: Identification of branches/switches through the network.

Central Metabolism of Plant Cell Analysis

Identification of network structure

- ▶ **Concrete example:** finding rules/constraints to produce 5 output metabolites if no entry of Glucose.
- ▶ Analysis of MCS with small size.

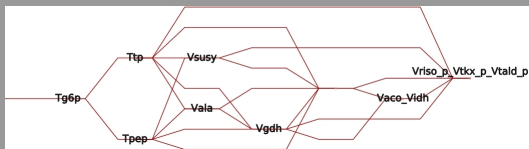
Results

- ▶ MCS of size 2: Identification of a set of mandatory reactions.
- ▶ MCS of size 3, 4 ...: Identification of branches/switches through the network.

How to visualize this information?

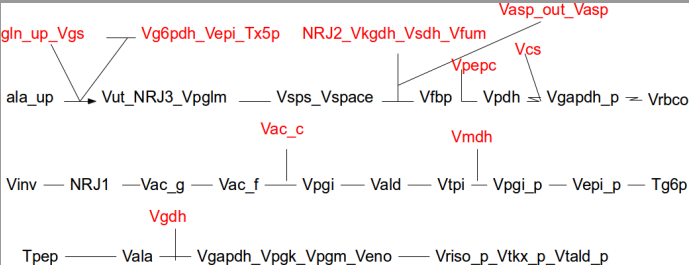
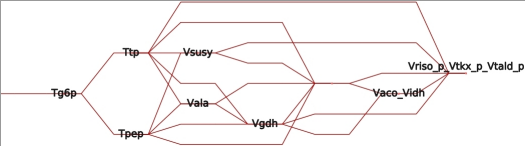
Graph display?

Complex to understand!



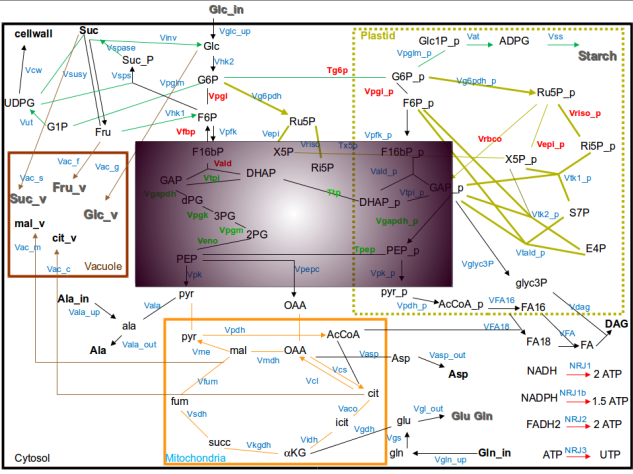
Graph display?

Complex to understand!



Graph display?

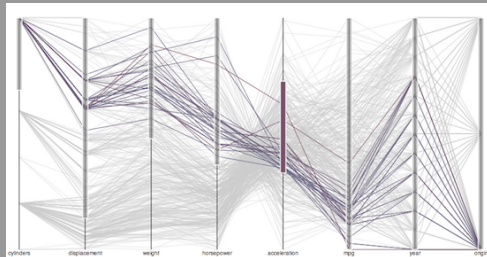
Complex to understand!



Parallel Coordinates

Methods

- ▶ Display a multivariate matrix: columns are data attributes, lines/polylines are elements³.
- ▶ Attributes are vertical axes.
- ▶ For big data set, polylines can be aggregated in groups.



from <http://http://mbostock.github.io/protovis/ex/cars.html>.

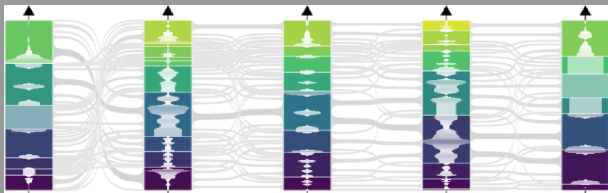
Parallel Coordinates

Visualization of EFMs

- ▶ Vertical axes are reactions, lines are EFMs.
- ▶ Values on axes present information about **absence** (0) of a reaction or about its **forward** (1) or **backward** activity (2).

CoPHI: Parallel Coordinates Highly Interactive

- ▶ Web application - javascript environment.
- ▶ Developed by Joris Sansen and the MaBioVis team at LaBRI (<http://www.labri.fr/perso/jsansen/CoPHI.html>).



www.labri.fr/perso/gricher/

IMPORT

Vac_f-newOrdering-avecnomReaction.mat

Delimiter: space

Row labels:

Column labels:

Clustering type: None

Load

RENDERING

Color scheme: Viridis

Histogram/Distortion:

Weight/Range size:

Per axis/Global:

DATA TABLE OPTIONS

Highlighted only:

Include deleted:

Download

Issue "tracker"

Vac_f	Tgfp	Vpgj	Vpgj_p	Vtbp	Vald	Vibco	VuL_NRJ3_Vpgim	Vvps_Vspace	Vvni	Vvpi_p	Vviso_p_Vbxx_p_Vtald_p	Ttp	Ttpep	Vtpi	Vg6poh_Vvpi_TxSp	Vvp_k_p_Vpdp_h_p_VFAX_Vdag_Vgycj3P	NRJ2_Vkqdh_Vsdh_Vfum	Vl
1	1	2	1	1	2	1	1	1	1	2	2	2	2	0	0	1	0	1
1	1	2	1	1	2	1	1	1	1	1	2	2	2	0	0	1	1	1
1	1	2	1	1	2	1	1	1	1	1	2	2	2	0	0	1	1	0
1	1	2	1	1	2	1	1	1	1	1	2	2	2	0	0	1	0	1
1	1	2	1	1	2	1	1	1	1	1	2	2	2	0	0	1	0	1
1	1	2	1	1	2	1	1	1	1	1	2	2	2	0	0	1	0	0
1	1	2	1	1	2	1	1	1	1	1	2	2	2	0	1	1	0	0
1	1	2	1	1	2	1	1	1	1	1	2	2	2	0	1	1	1	0

15:32 02/09/2015

IMPORT

Vac_f-newOrdering-avecnomReaction.mat

Delimiter: space

Row labels: None

Column labels: None

Clustering type: None

Load

RENDERING

Color scheme: Viridis

Histogram/Distortion: None

Weight/Range size: None

Per axis/Global: Global

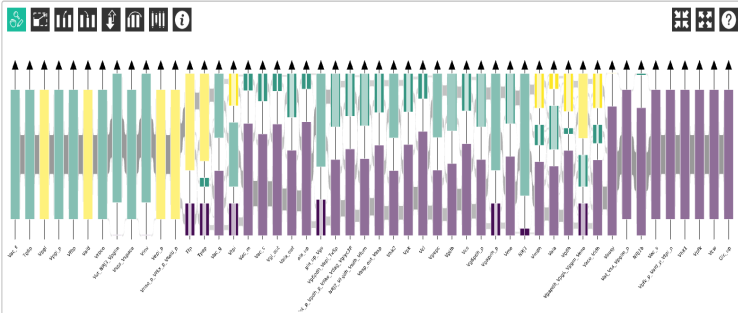
DATA TABLE OPTIONS

Highlighted only: None

Include deleted: None

Download

Issue "tracker"



Vac_f	Tg6p	Vp6j	Vp6j_p	Vb6p	Vald	Vbco	Vut_NR33_Vp6jm	Vps_Vspace	Vmv	Vepi_p	Vriso_p_Vtkx_p_Vtald_p	Ttp	Tpep	Vac_g	Vtpi	Vac_m	Vac_c	Vgl_out	Vals_out	ala_up	gln_up_Vgs	Vg6pdh_Vepi_T5p	Vp		
1	1	2	1	1	2	1	1	1	1	1	2	2	2	2	0	0	0	0	0	0	0	1	0	1	
1	1	2	1	1	2	1	1	1	1	1	2	2	2	2	0	0	0	0	0	0	0	1	0	1	
1	1	2	1	1	2	1	1	1	1	1	2	2	2	2	1	0	0	0	0	0	0	0	1	0	1
1	1	2	1	1	2	1	1	1	1	1	2	2	2	2	0	0	0	0	0	0	0	1	0	0	1
1	1	2	1	1	2	1	1	1	1	1	2	2	2	2	0	0	0	0	0	0	0	0	0	0	1
1	1	2	1	1	2	1	1	1	1	1	2	2	2	2	1	0	0	0	0	0	0	0	0	0	1
1	1	2	1	1	2	1	1	1	1	1	2	2	2	2	0	0	0	0	0	0	0	1	0	0	1
1	1	2	1	1	2	1	1	1	1	1	2	2	2	2	1	0	0	0	0	0	0	1	1	1	1
1	1	2	1	1	2	1	1	1	1	1	2	2	2	2	0	0	0	0	0	0	0	1	1	1	1

Conclusion

Another way to display connections through the network

- ▶ Global view of selection.
- ▶ Rate of EFMs concerns by response to the question.

Dynamic interface driven by pre-treatments

- ▶ Pre-treatments lead by the biological questions.
- ▶ Dynamic interface to explore possibilities.
- ▶ Changing the way to consider displaying of metabolic graphs.