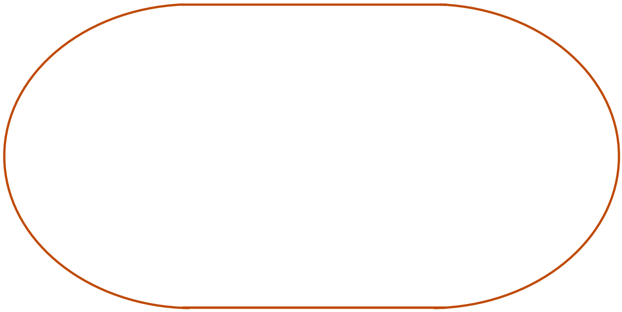
Null Space, Subsets, Elementary Modes and Conserved Cycles
Nepal 2018

Mark Poolman

June 29, 2018

Friday L3

The Problem



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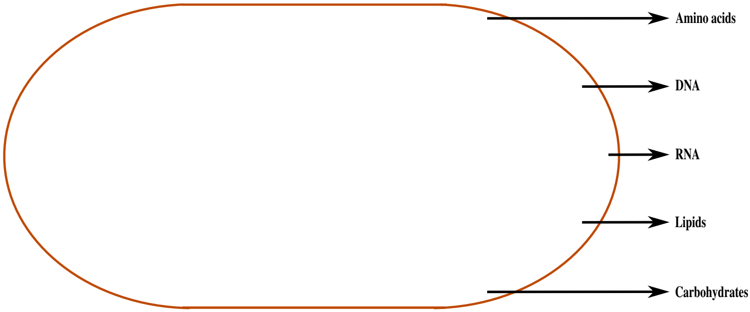

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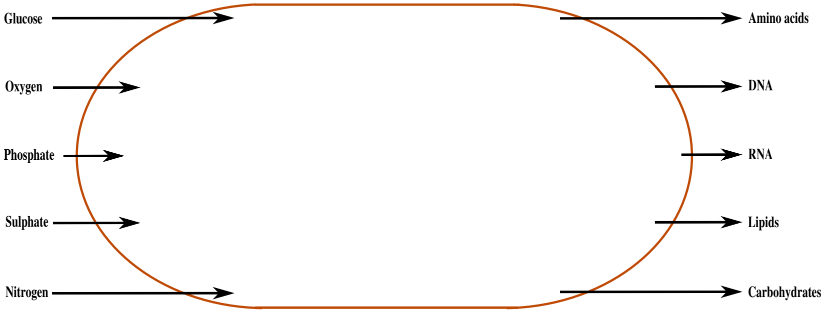
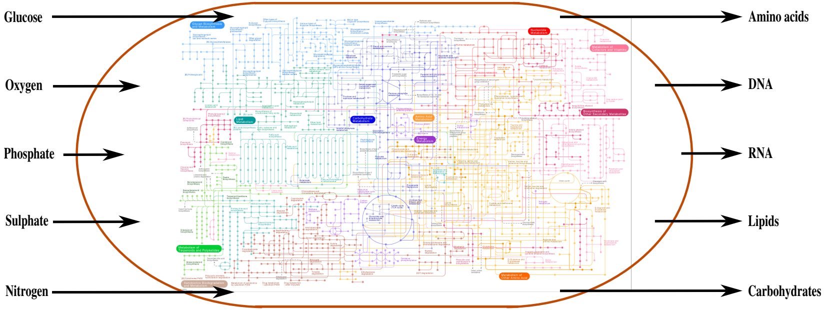

Glucose
Oxygen
Phosphate
Sulphate
Nitrogen
Amino acids
DNA
RNA
Lipids
Carbohydrates

The Problem



How to connect input(s) to output(s) ??

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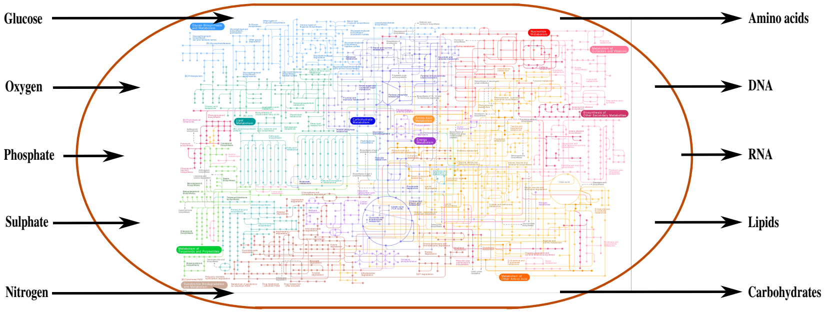


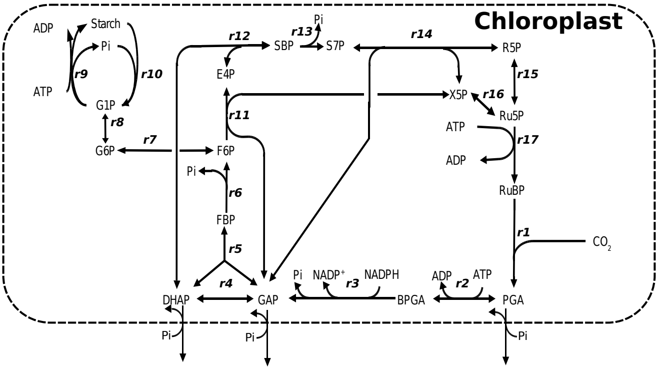
How to connect input(s) to output(s) ??

Motivation

What do we want to know - can we:

- Predict network behaviour (assign fluxes to reactions)?
- Predict the effect of network modification?
- Predict the modification needed to achieve a specific effect?

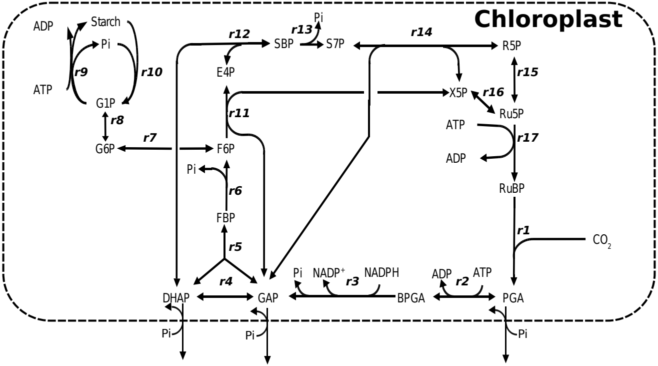
Example - The Calvin Cycle



Questions:

- Which reactions are essential?
- What does knowledge of flux in one reaction tell us about flux in another?
- What does knowledge of one metabolite concentration tell us about the concentration of another?

Example - The Calvin Cycle



Questions:

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Definition of a metabolic model

1. A set of *External* metabolites - inputs and outputs.
2. A set of *Internal* metabolites - no net production or consumption.
3. A set of reactions that inter-convert them defined by:
   - Stoichiometry.
   - Directionality.
   - Reversibility.

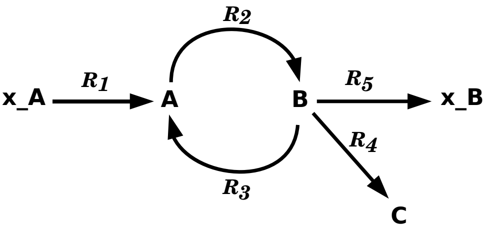
Fundamental assumptions

- Reactions interconvert substrates and products whilst conserving mass.
- Transporters are a special case of reaction (interconvert internal with external metabolites)
- Rate of change concentration is sum of reaction rates.
- This is assumed to tend to zero in the long term (steady state)

Note

- Reactions are not enzymes.
- Enzymes are not genes.

Modelling networks of reactions



$$\frac{dA}{dt} = R_1 + R_3 - R_2$$
$$\frac{dB}{dt} = R_2 - R_3 - R_4 - R_5$$
$$\frac{dC}{dt} = R_4$$

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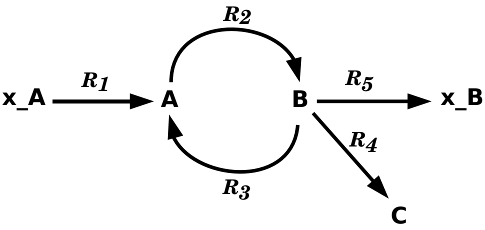

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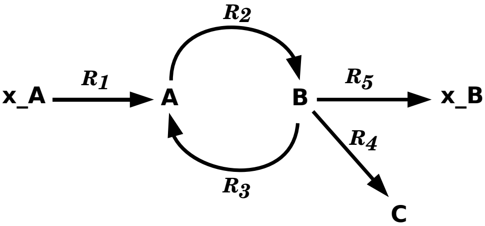

$$\begin{bmatrix} \frac{dA}{dt} \\ \frac{dB}{dt} \\ \frac{dC}{dt} \end{bmatrix} = \begin{bmatrix} 1 & -1 & 1 & 0 & 0 \\ 0 & 1 & -1 & -1 & -1 \\ 0 & 0 & 0 & 1 & 0 \end{bmatrix} \begin{bmatrix} R_1 \\ R_2 \\ R_3 \\ R_4 \\ R_5 \end{bmatrix} = \begin{bmatrix} 0 \\ 0 \\ 0 \end{bmatrix}$$

Or more succinctly:

$$\mathbf{Nv} = \mathbf{0}$$

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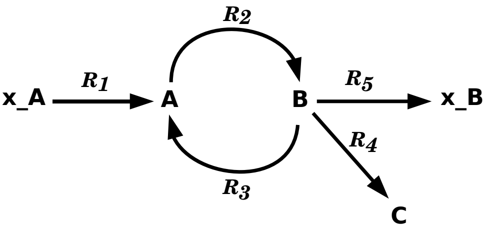

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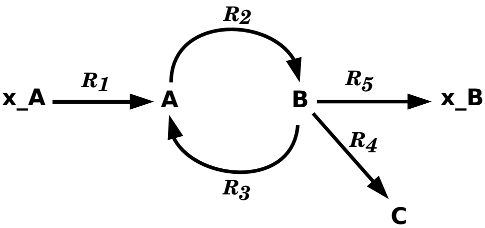

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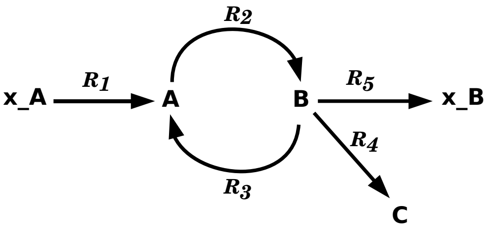

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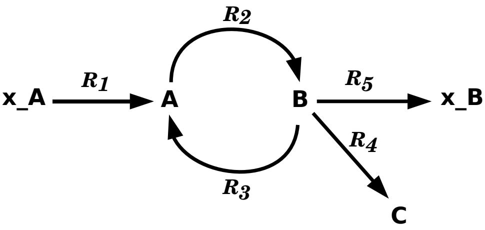

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So What ?!

v is not unique

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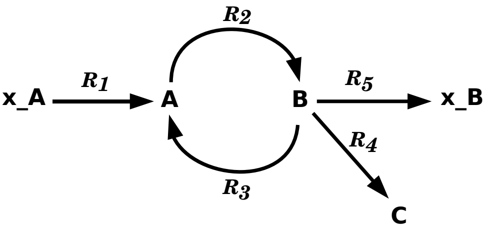

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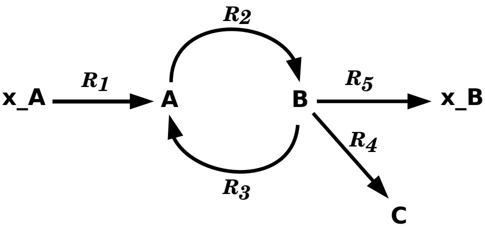

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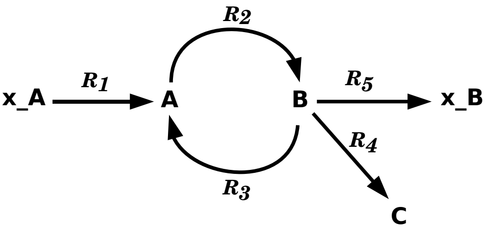
Kernels



$$\mathbf{K} = \begin{bmatrix} 1 & 0 \\ 1 & 1 \\ 0 & 1 \\ 0 & 0 \\ 1 & 0 \end{bmatrix} \begin{bmatrix} w_1 \\ w_2 \end{bmatrix} = \begin{bmatrix} R_1 \\ R_2 \\ R_3 \\ R_4 \\ R_5 \end{bmatrix} = \begin{bmatrix} 1w_1 + 0w_2 \\ 1w_1 + 1w_2 \\ 0w_1 + 1w_2 \\ 0w_1 + 0w_2 \\ 1w_1 + 0w_2 \end{bmatrix} \begin{matrix} \longleftarrow \text{subset} \\ \\ \\ \longleftarrow \text{dead} \\ \longleftarrow \text{subset} \end{matrix}$$

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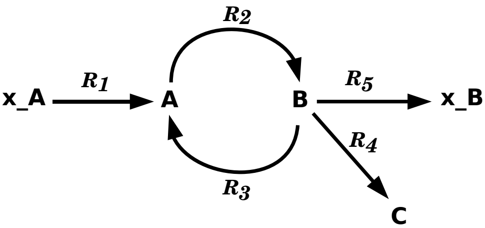

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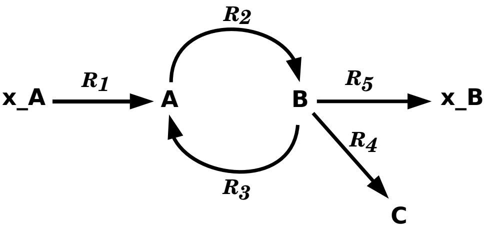

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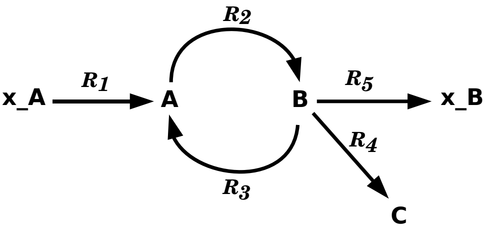

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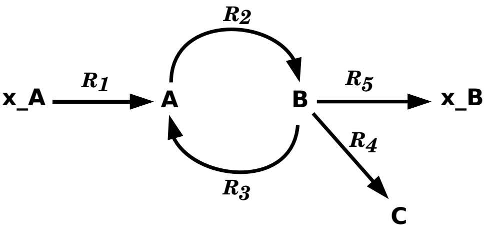

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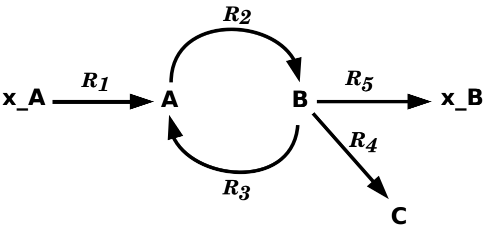

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More about subsets

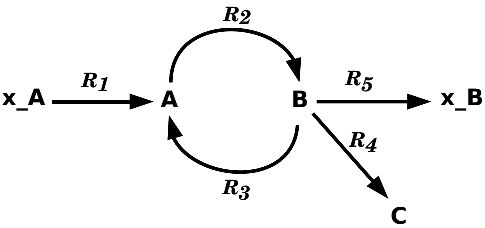
1. All reactions in a subset *must* carry flux in a fixed ratio.
2. Subsets have a single *net* stoichiometry.
3. If any single reaction is removed from a subset, the remaining reactions will be dead.
4. If one or more reactions in a subset are irreversible, the whole subset is irreversible.

See: Pfieffer *et al* (1999) **15**, 251–257.

Significance of the kernel

- The kernel captures steady-state invariants of a network that are independent of environment, metabolite levels etc.
- Any and all steady state flux distributions can be represented as a linear combination of columns of the null space.
- A dead reaction will *always* be dead regardless of kinetic parameters.
- Reactions in subsets carry steady-state flux in fixed ratio regardless of kinetic parameters.
- Unexpected behaviour in other results can often be explained by consideration of the kernel.

Kernels are not unique



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$$\mathbf{E} = \begin{bmatrix} 1 & 1 & 0 \\ 1 & 0 & 1 \\ 0 & -1 & 1 \\ 0 & 0 & 0 \\ 1 & 1 & 0 \end{bmatrix}$$

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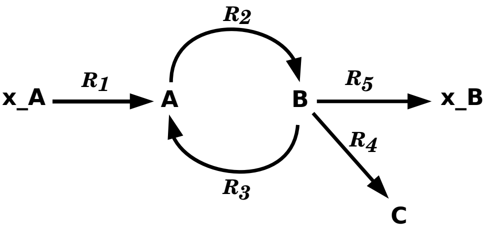

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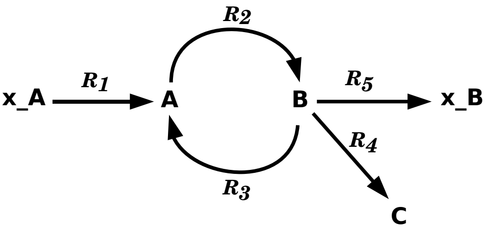

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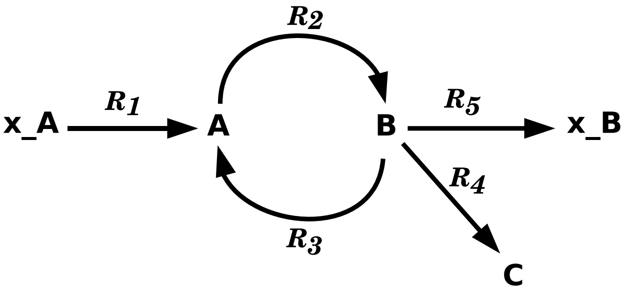
Elementary modes (1)

Definition:
A set of reactions in a system that:

- Balance all internal metabolites.
- Respect reversibility.
- Cannot be decomposed. (ie a *minimal* set of reactions)
- Are associated with a single net stoichiometry involving only external metabolites (or none).

Elementary modes (2)

Non Elementary modes



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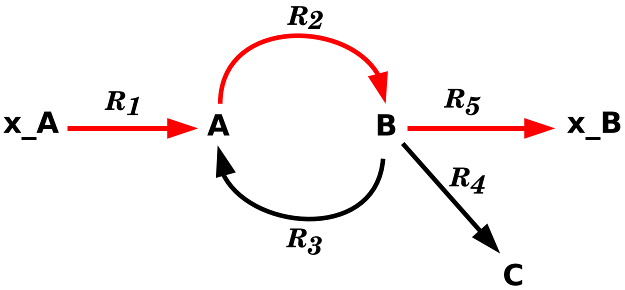

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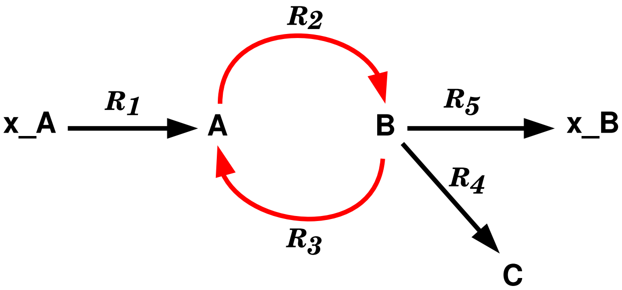

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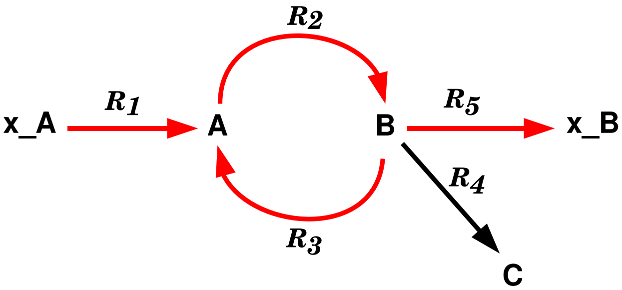

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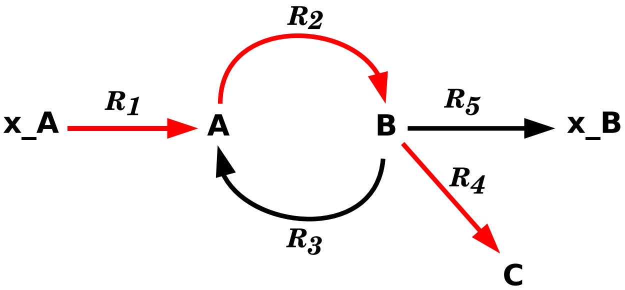


Elementary modes - Summary

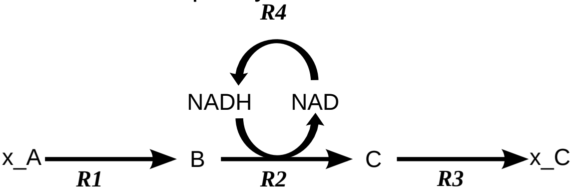
- Elementary modes represent independent paths in a system.
- They provide an *objective* definition of pathways.
- The set of reactions in an EM is unique.
- Every EM is associated with a net stoichiometry which may or may not be unique.
- The net metabolic behaviour of a system can always be expressed as a linear combination of its EMs.

So far we have considered relationships between steady-state reaction fluxes.

Can we say anything about metabolite concentrations?

Conserved cycles

Consider a simple cycle:



From inspection NAD + NADH are constant.

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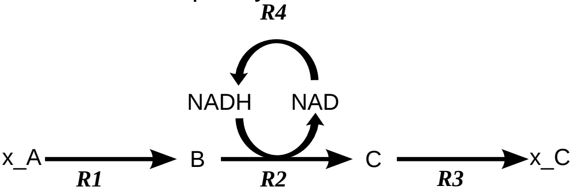

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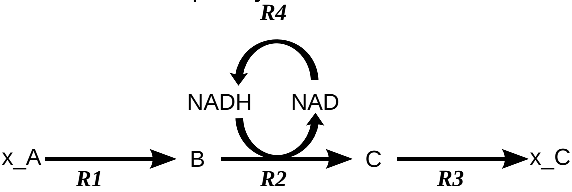

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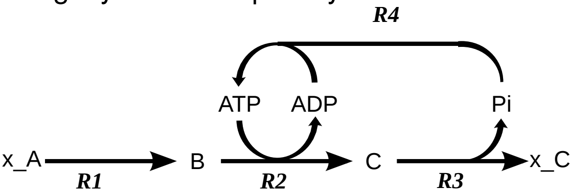
A slightly more complex cycle:



From inspection ADP + ATP and ATP + Pi are constant (?).

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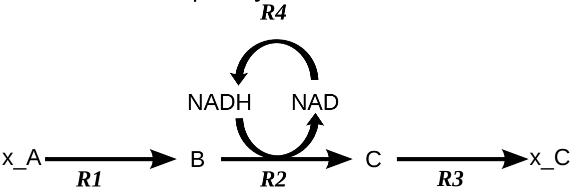

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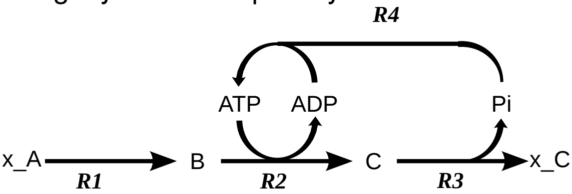

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Conserved cycles

These relationships are called

Moiety Conservation relationships.

They can be determined by analysis of the stoichiometry matrix.

Conserved cycles

$$
\mathbf{N} = \begin{array}{r|cccc}
 & R1 & R2 & R3 & R4 \\
B & 1 & -1 & 0 & 0 \\
C & 0 & 1 & -1 & 0 \\
NAD & 0 & 1 & 0 & -1 \\
NADH & 0 & -1 & 0 & 1
\end{array}
$$

dB/dt = R1 - R2
dC/dt = R2 - R3
dNAD/dt = R2 - R4
dNADH/dt = R4 - R2

$$dNAD/dt = -dNADH/dt$$

Integrating:

$$NAD = -NADH + k$$

$$NAD + NADH = k$$

k is the conserved total.

Conserved cycles

$$\mathbf{N} = $$

	R1	R2	R3	R4
B	1	-1	0	0
C	0	1	-1	0
NAD	0	1	0	-1
NADH	0	-1	0	1

dB/dt = R1 - R2
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Conserved cycles

		R1	R2	R3	R4	
	B	1	-1	0	0	dB/dt = R1 - R2
$\mathbf{N} =$	C	0	1	-1	0	dC/dt = R2 - R3
	NAD	0	1	0	-1	dNAD/dt = R2 - R4
	NADH	0	-1	0	1	dNADH/dt = R4 - R2

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Conserved cycles

$$\mathbf{N} =$$

	R1	R2	R3	R4	
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C	0	1	-1	0	dC/dt = R2 - R3
NAD	0	1	0	-1	dNAD/dt = R2 - R4
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Conserved cycles

		R1	R2	R3	R4	
$\mathbf{N} =$	B	1	-1	0	0	dB/dt = R1 - R2
	C	0	1	-1	0	dC/dt = R2 - R3
	NAD	0	1	0	-1	dNAD/dt = R2 - R4
	NADH	0	-1	0	1	dNADH/dt = R4 - R2

$$dNAD/dt = -dNADH/dt$$

Integrating:

$$NAD = -NADH + k$$

$$NAD + NADH = k$$

k is the conserved total.

Conserved cycles

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	R1	R2	R3	R4	
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Structural analysis - The left Null-space

Such relationships can be identified from the *left* null space $\mathbf{K_l}$ which has the property:

$$\mathbf{K_l N} = \mathbf{0}$$

the dimension of which is equal to the number of conservation relationships in the system:

for the first example

$$\mathbf{K_l} = \begin{matrix} B & C & NAD & NADH \\ 0 & 0 & 1 & 1 \end{matrix} \quad \text{(one conservation relationship)}$$

and for the second example

$$\mathbf{K}_l = \begin{matrix} B & C & ADP & ATP & Pi \\ 0 & 0 & 1 & 1 & 0 \\ 0 & 1 & 0 & 1 & 1 \end{matrix} \quad \text{two conservation relationships}$$

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Structural analysis - The left Null-space

Notes:

- $\mathbf{K_l}$ is not always unique - there may not be a single way to represent the conservation relationships in a system.
- Negative elements in $\mathbf{K_l}$ do not imply negative concentrations.
- It is not possible to guarantee that an all positive $\mathbf{K_l}$ can be found.

Significance of $\mathbf{K_l}$

- Very important consideration in design of kinetic modelling software.
- Introduces “hidden” parameters in kinetic models.
- Changing concentrations in a model can lead to unexpected results.
- Need to identify the *dependent* metabolite in each relationship.
- The left null-space has received relatively scant attention and represents a potentially fruitful area for further theoretical research.

Conclusions

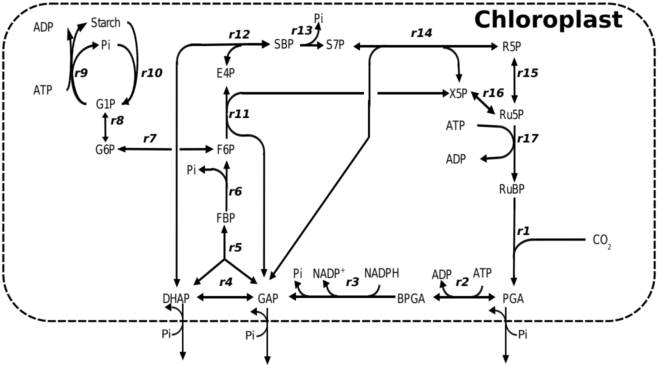
From consideration of the stoichiometry matrix, along with assumptions about reaction reversibility, we can:

- Identify independent routes through metabolic networks.
- Identify sets of reactions that carry flux in fixed ratios.
- Identify groups of metabolites with interdependent concentration values.

So Now ...

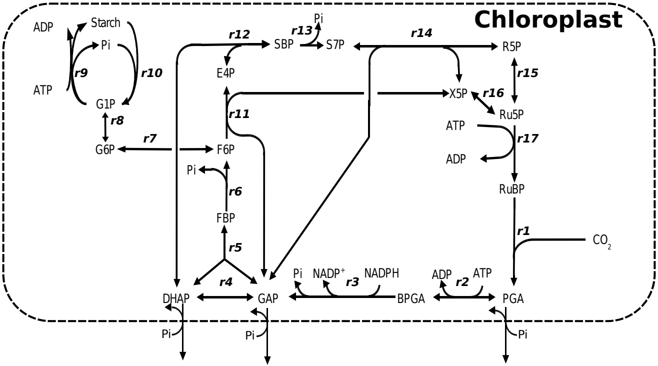
We have the *theoretical* tools to answer the questions posed earlier .

Example - The Calvin Cycle (Practical 4)



Questions:

- What are the routes from starch to triose phosphate. Will they work in the dark?
- Which reactions will show correlated flux.
- Why do plants need the triose-phosphate - phosphate anti-porter?



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