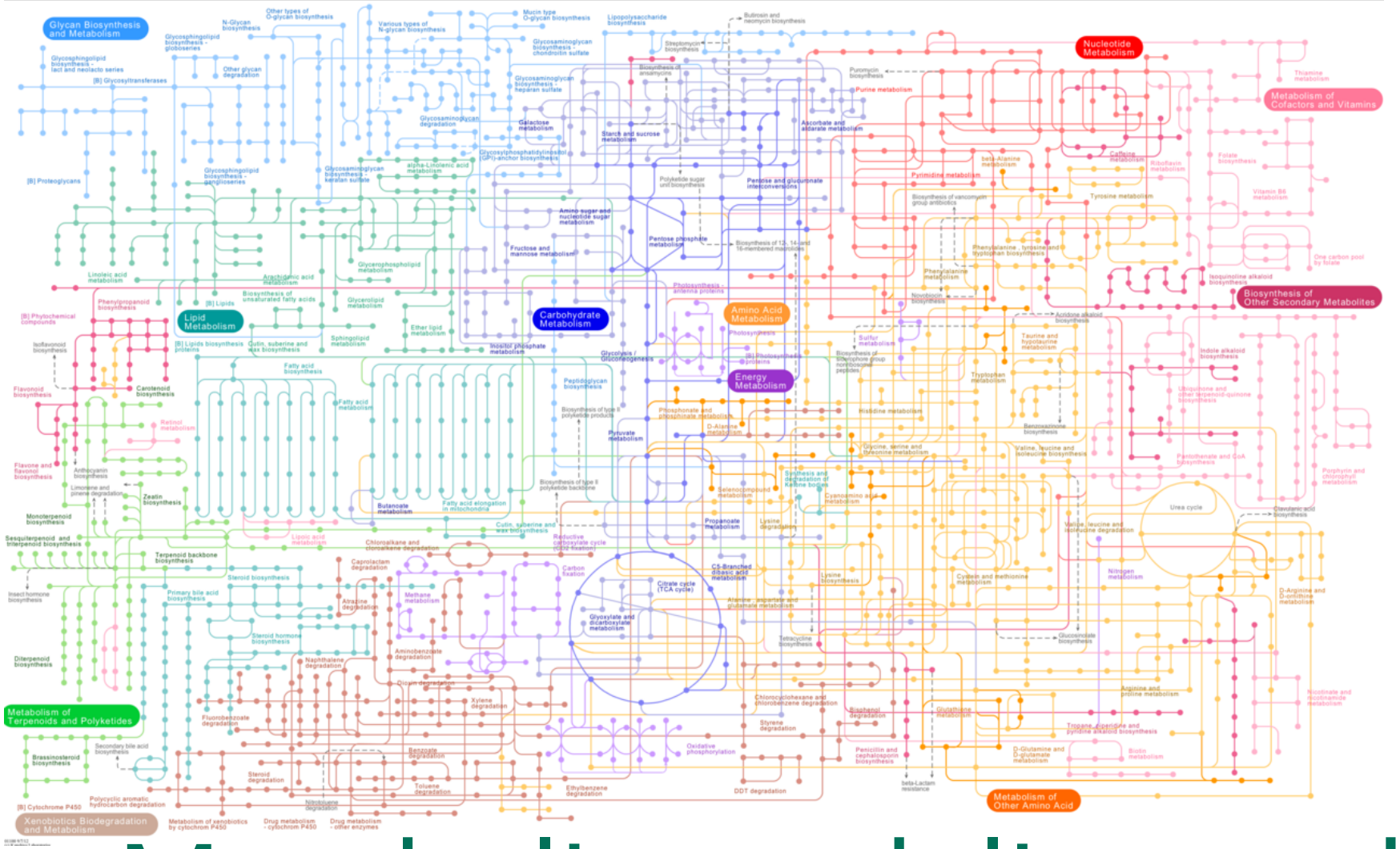


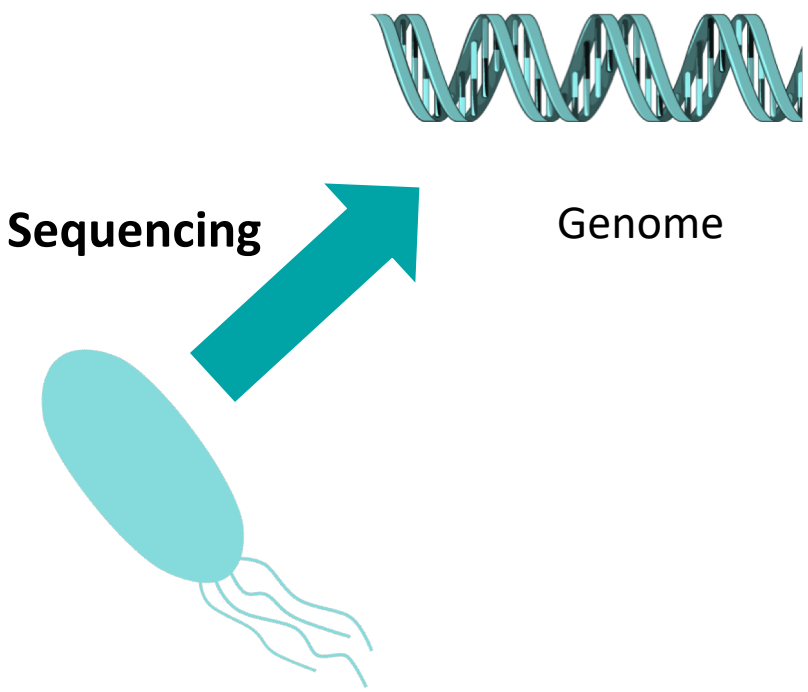
cnrs INRAE

lipme

C. Baroukh

Metabolic modeling at the scale of an organism/ecosystem

Metabolic modeling in a nutshell



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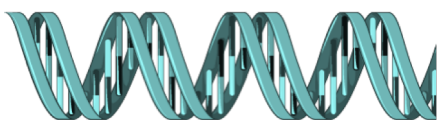
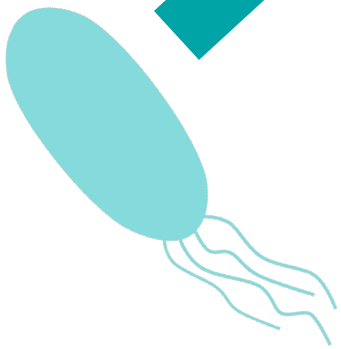


Reconstruction

Sequencing



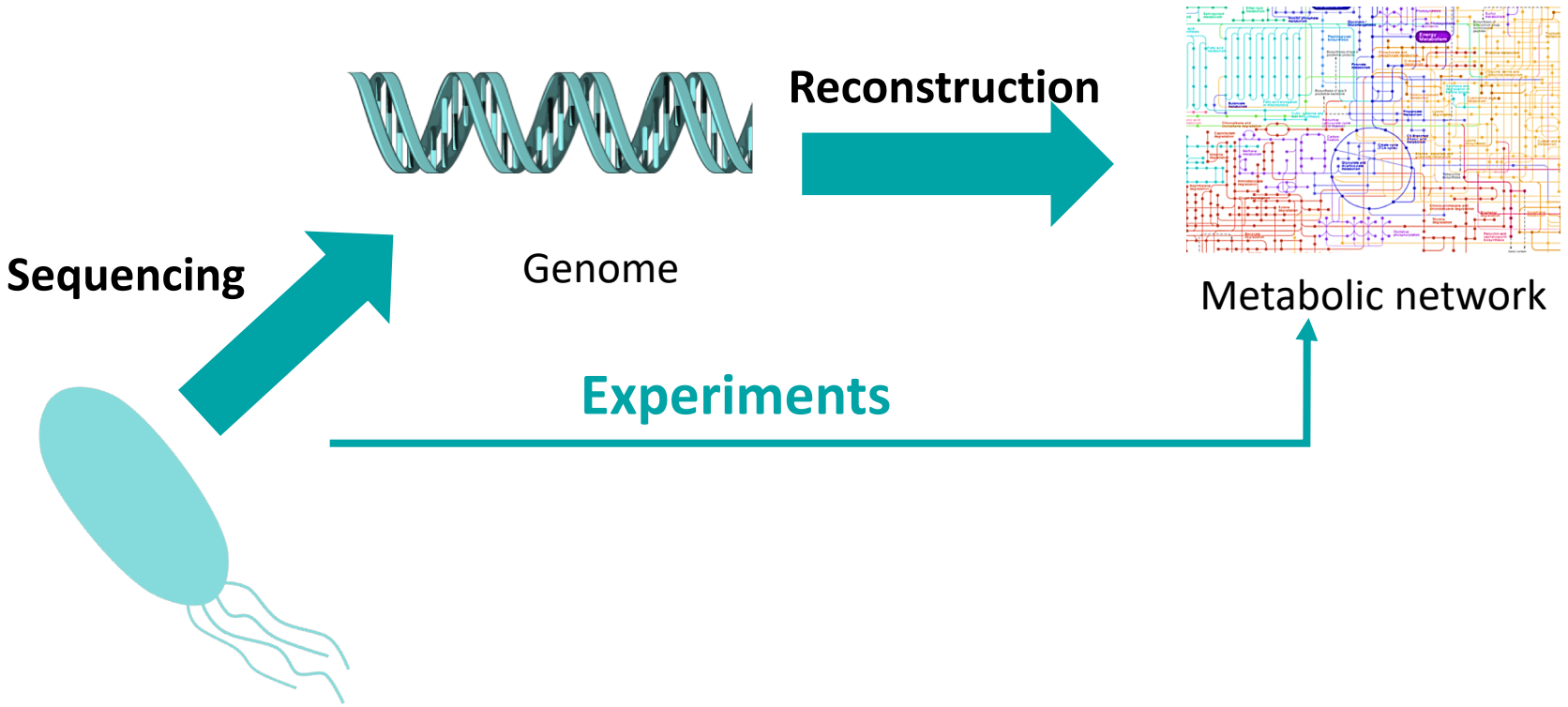
Genome

Using orthologies to species with known metabolic network

A 2 step process:

1. Automatic reconstruction
2. Manual curation to fill gaps and remove false positives

Metabolic modeling in a nutshell



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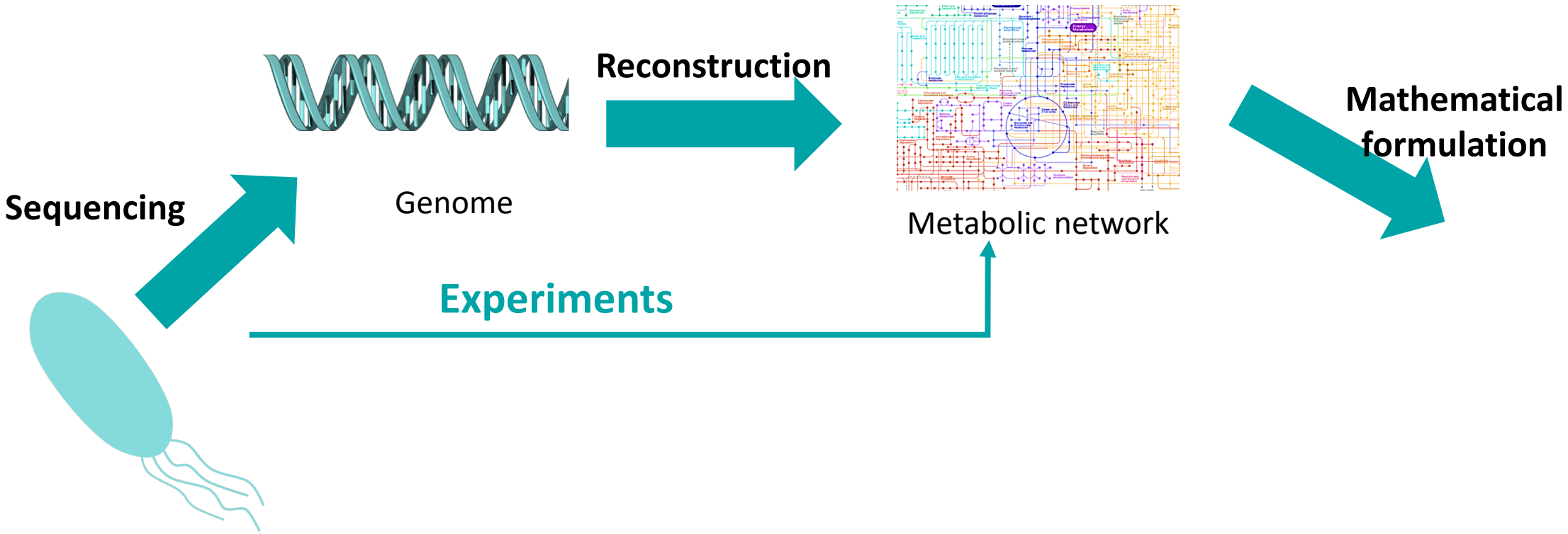
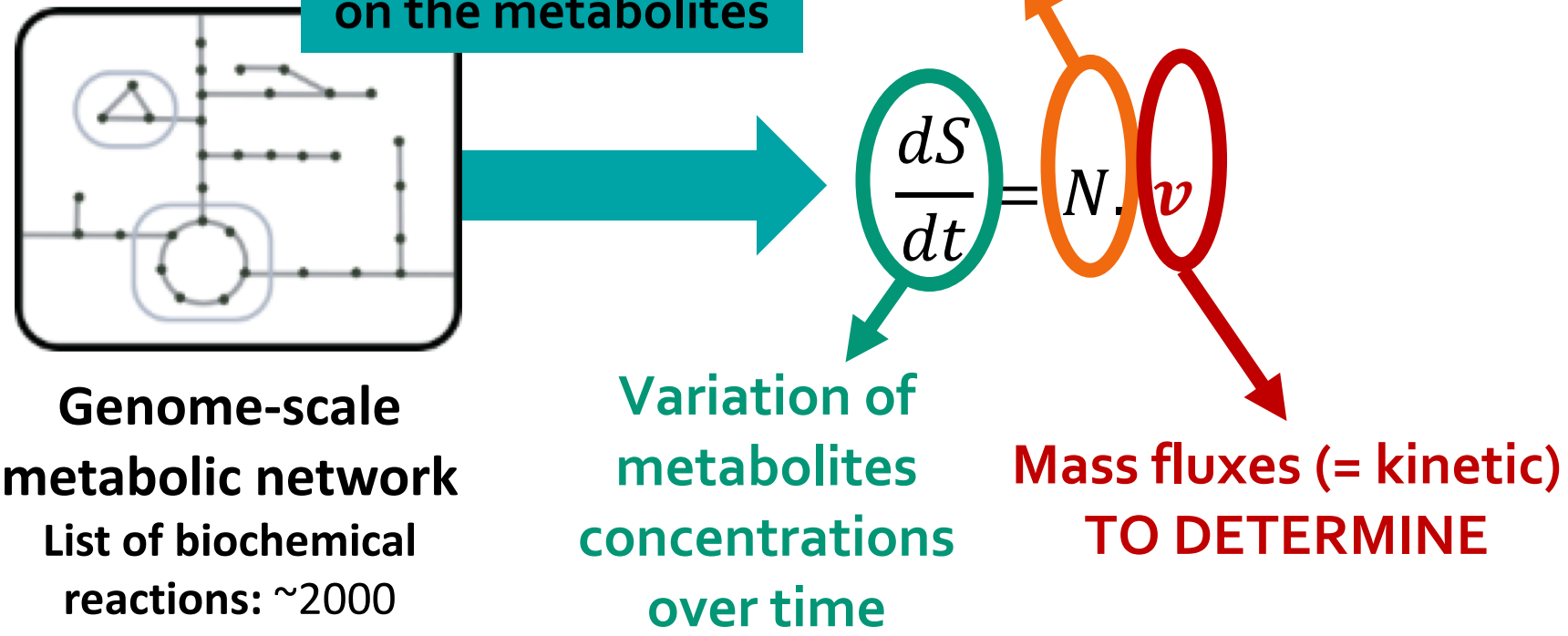


Mass balance on a metabolic system



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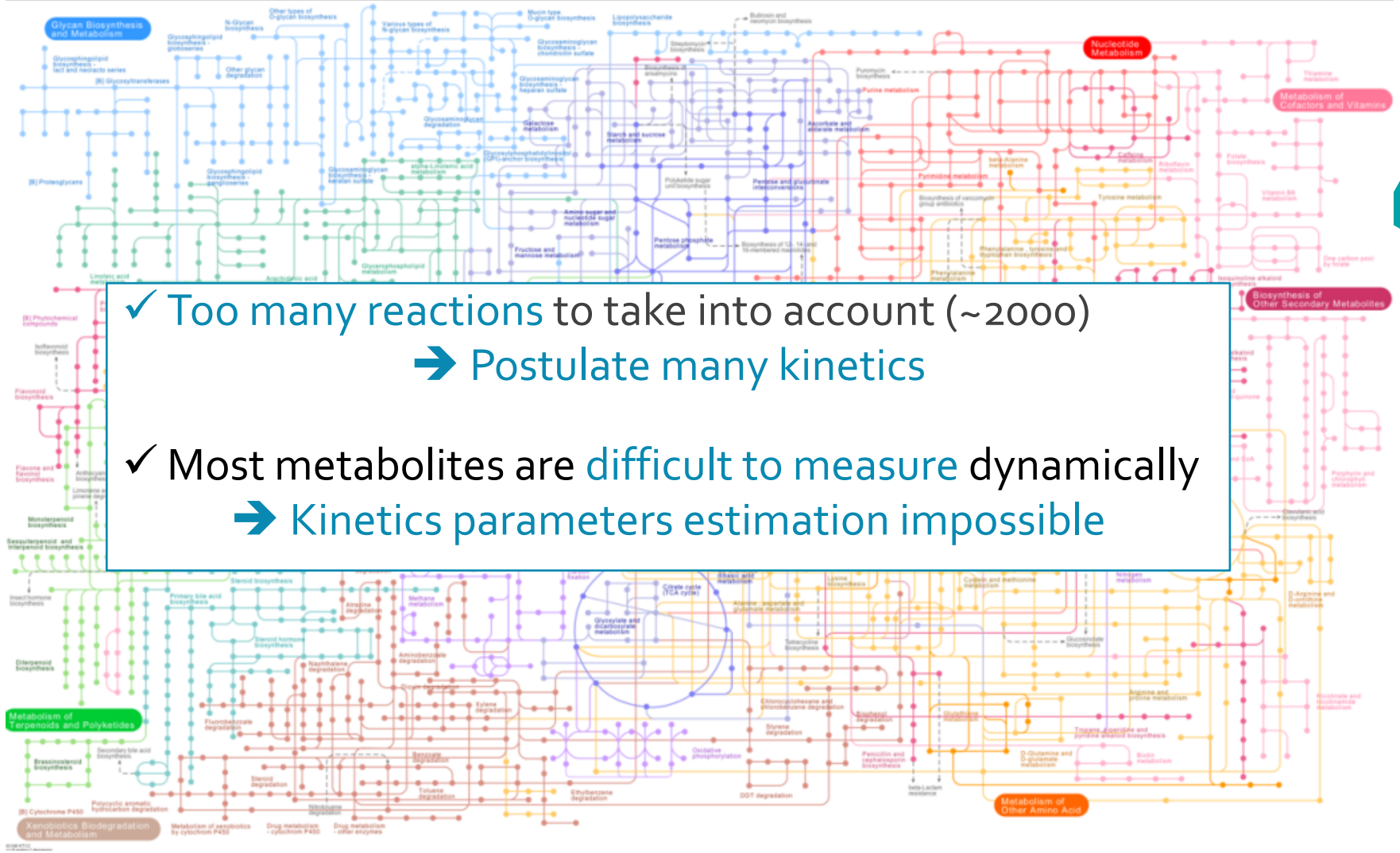


✓ Too many reactions to take into account (~2000)

➔ Postulate many kinetics

✓ Most metabolites are difficult to measure dynamically

➔ Kinetics parameters estimation impossible

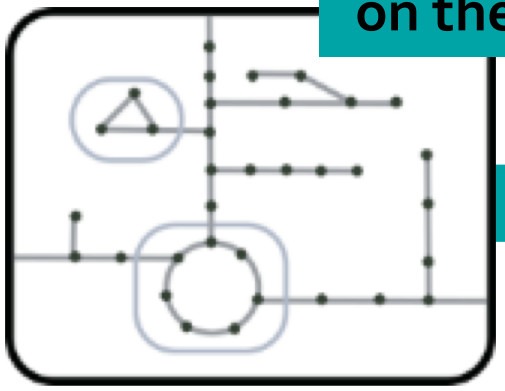
Mathematical formulation

$$\frac{dS}{dt} = N.v$$

Large size ODE
Dynamical system

Quasi-steady state assumption

Mass balance on the metabolites



Genome-scale metabolic network

List of biochemical reactions: ~2000

Stoichiometric matrix

$$\frac{dS}{dt} = N . v$$

Variation of metabolites concentrations over time

Mass fluxes of reactions TO DETERMINE

First hypothesis: Quasi-steady-state assumption

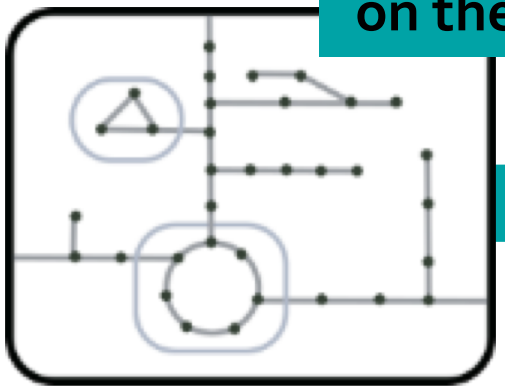
Internal metabolites consumed once they are produced

$$\begin{cases} \frac{dS_{ext}}{dt} = N_{S_{ext}} . v \\ N_{S_{int}} . v = 0 \end{cases}$$

TO DETERMINE

Quasi-steady state assumption

Mass balance on the metabolites



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TO DETERMINE

BUT indeterminate system !!!!

Flux Balance Analysis

First hypothesis: internal metabolism are assumed at quasi-steady-state

Second hypothesis: metabolism is assumed optimal by evolution

Flux Balance Analysis

First hypothesis: internal metabolism are assumed at quasi-steady-state

Second hypothesis: metabolism is assumed optimal by evolution

➔ Solve an optimization problem

Flux Balance Analysis

First hypothesis: internal metabolism are assumed at quasi-steady-state

Second hypothesis: metabolism is assumed optimal by evolution

Optimization problem

Objective (maximization or minimization)

e.g. Biomass synthesis maximization

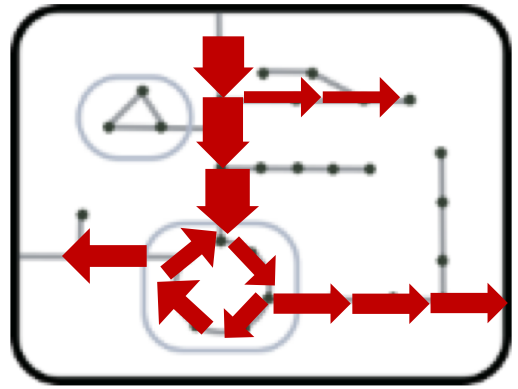
Constraints

$N_{S_{int}} . \boldsymbol{v} = 0$ **and**

e.g Limitation of substrate assimilation

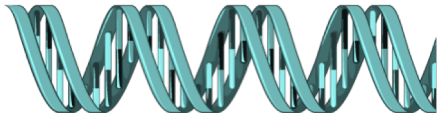
e.g. Irreversible reactions fluxes ≥ 0

e.g. Energetic cost for maintenance processes



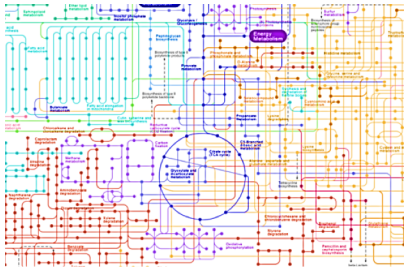
Fluxes of matter ($\boldsymbol{v}$)

Metabolic modeling in a nutshell



Genome

Reconstruction



Metabolic network

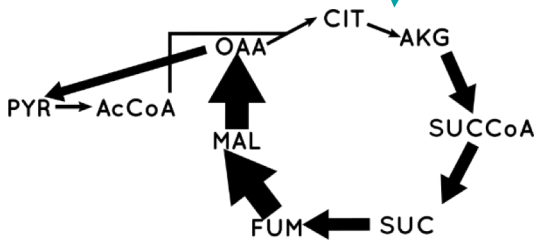
Mathematical formulation

$$\frac{dM}{dt} = K.v.B$$

Large size ODE
Dynamical system

Simplification and simulation

Small ODE system
Semi-dynamic system

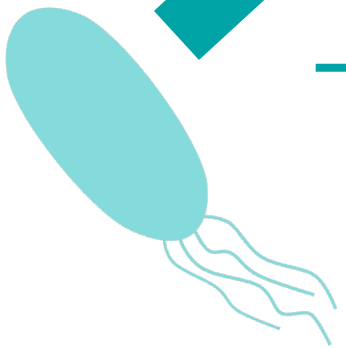


Matter fluxes

Tests *in silico*

Experiments

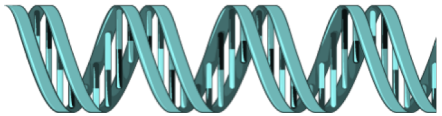
Sequencing



Flux Balance Analysis- uses

- Prediction of intracellular fluxes
- Comparison of fluxes distribution between
  - Several environmental conditions
  - Several strains
- Study the impact of
  - The deletion of one or genes
  - The inhibition or catalysis of a metabolic reaction
- Study the metabolic/genetic modifications to perform so as to, e.g., optimize a bioprocess

Metabolic modeling in a nutshell



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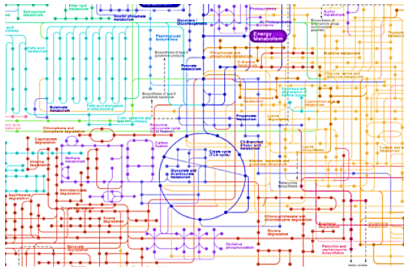

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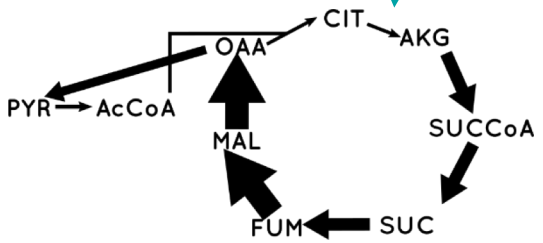


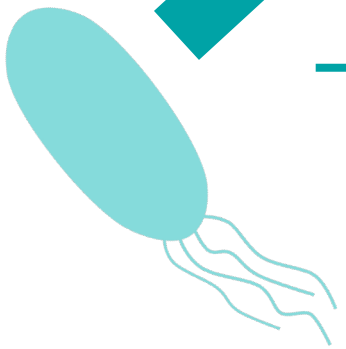
Matter fluxes

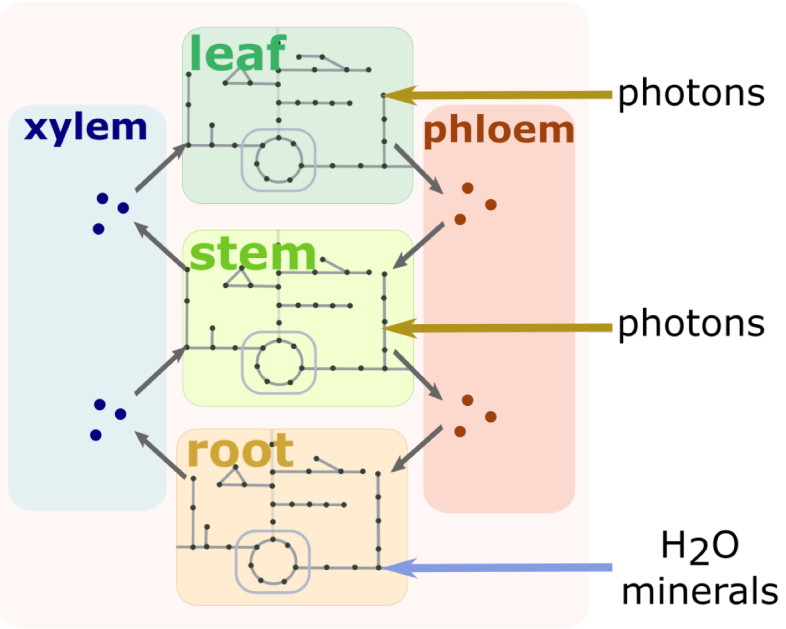
Tests *in silico*

A better understanding of metabolic behaviours

Experiments

Sequencing





$$\frac{dS}{dt} = N.v$$

An example of result

Xylella fastidiosa

Infection of over a 100 plant species

Grapevine, Olive tree, …

Leaf necrosis, leaf drying

Fastidious bacteria :
isolation and *in vitro* cultures difficult

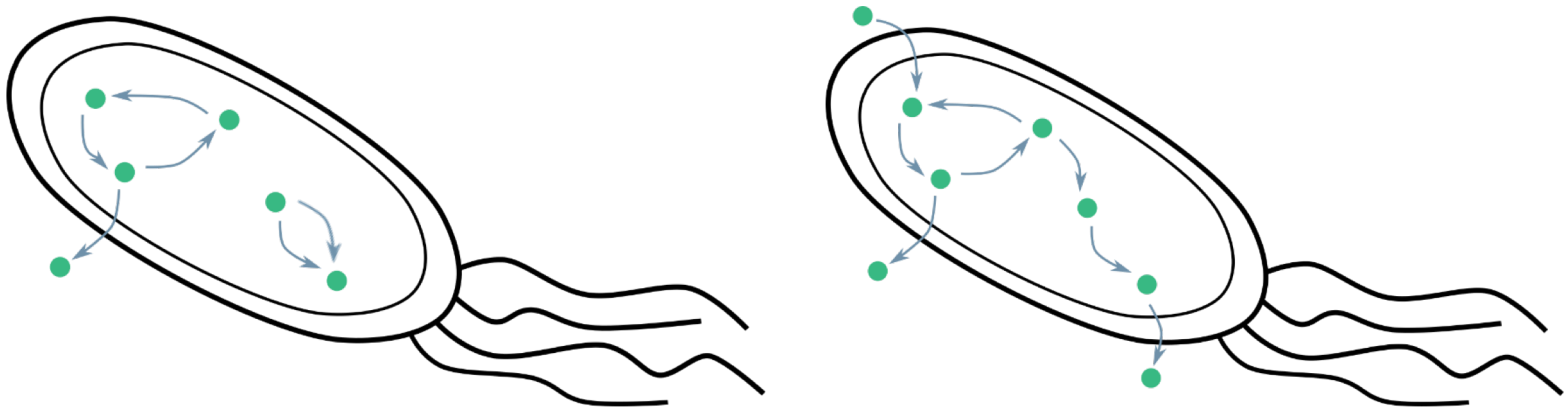
➔ Objective : **a better understanding of its metabolism and its fastidious growth**

Metabolic reconstruction process


```
>Q2V9N9
MRNTLVSHYQHIIEYRRKSLMGVVFVNYARSFLAGTMALSAVCLN
LNPSYSRVQNFKLKDAGNDPAGILAYHSGIGTVPERFDWNTLDAN
LGIGREAFPVKTDEAGSSRTFLLIRNLARAVAQHDSDTIESELFQ
SVEQRFVDIMQSIQKDPKAEFPRSAVKHLVAGYGAEKYKYSPKLV
ATVARISGITSTSVTLNVCRDVPTYIPNARLIPYACAGVGWGFIG
HLIAGAFLQGVFGSVKHQDIDTDVVAGNISKAASHISSTFRLKNL
>O83055
MGACISVYARFALGCGVFFLHGAVLDGVSRAFSSSAAFSGSAELS
KSSWKLAFPLLPKKGATYTSFSGEDPIWVELSLKGLKVDFESALG
ATLHLYDVSFSVGKDPVFPSNFAQLWTPFITTSYESRSVKYAPGF
SFSSNGIWKSAPSVTSKVKGKGTNSRRMPADPHSKYGLGTEFTLV
GPDQTHHQNKDTVLWNVGARLTLSPGAGFKIVCAFDAGTPYKKGA
```

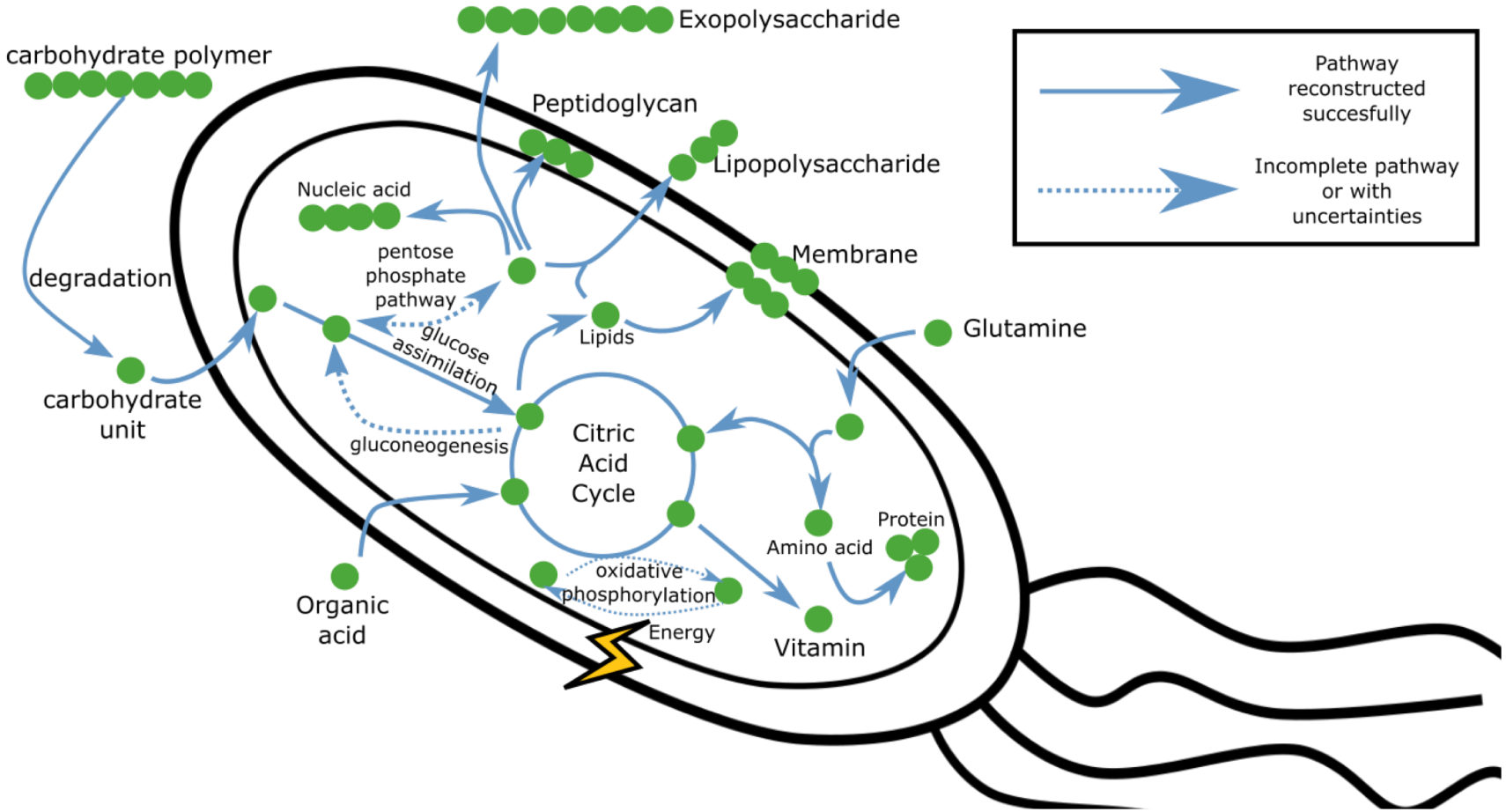
Reference metabolic networks used

1. ***E. coli***
2. ***R. solanacearum***
3. ***P. aeruginosa***
4. ***R. eutropha***
5. ***B. subtilis***



Gerlin L.

A complete network, but minimal

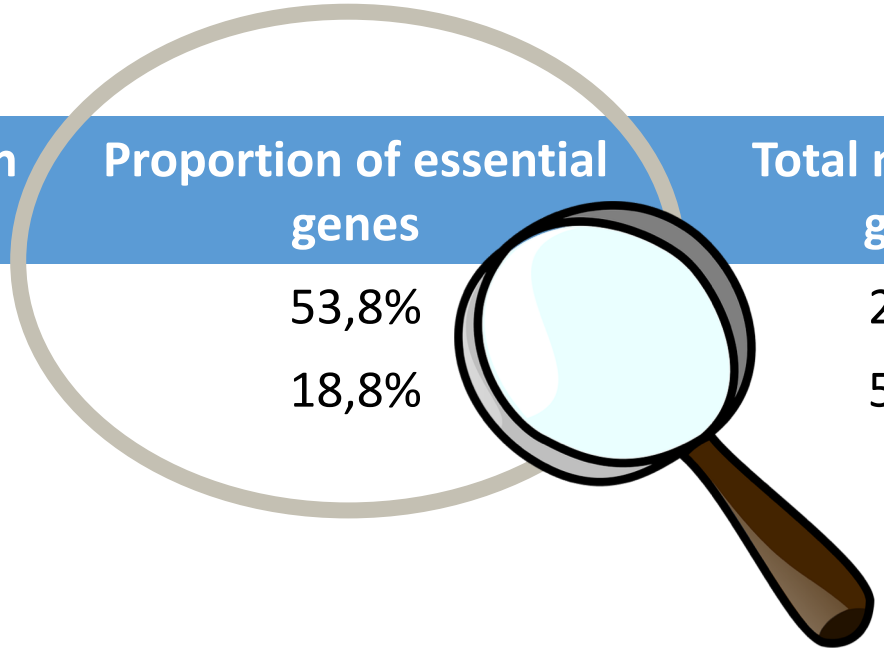


Gerlin L.

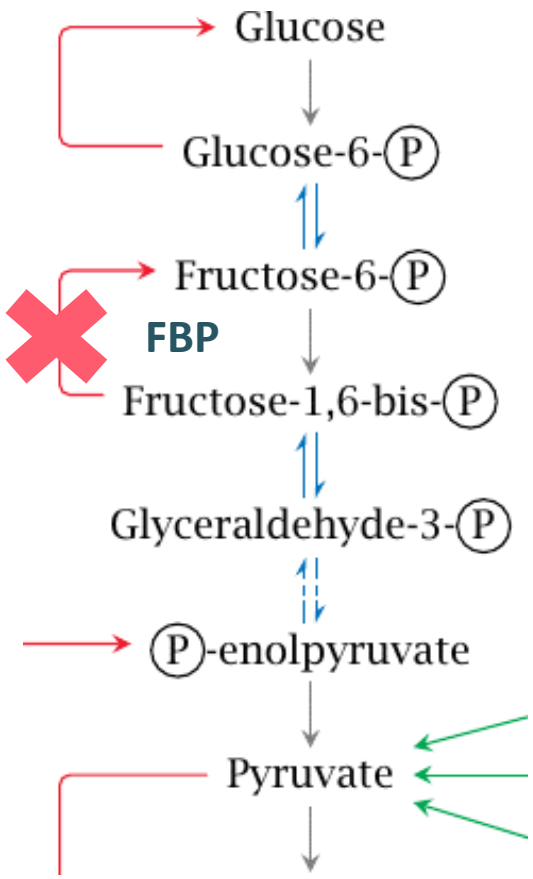
A network lacking robustness

Gene deletion study (*in silico*) = numerous FBA

Organism	Total number of genes in the network	Proportion of essential genes	Total number of genes
Xylella fastidiosa	537	53,8%	2 782
Ralstonia solanacearum	1474	18,8%	5 194



Where is the fructose-1,6-bisphosphatase?



Glycolysis / Gluconeogenesis

FBP

Fructose-1,6-bisphosphatase
EC 3.1.3.11

- A key enzyme of the gluconeogenesis pathway
- An enzyme conserved in « all » living organisms:

« Same » enzyme for animals/plants/bacteria/etc

- Loss in all *Xylella* strains sequenced

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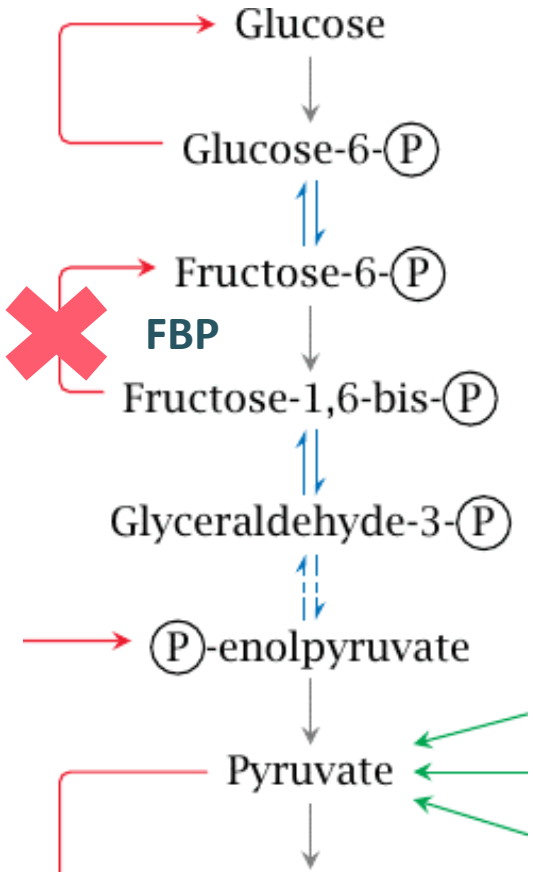

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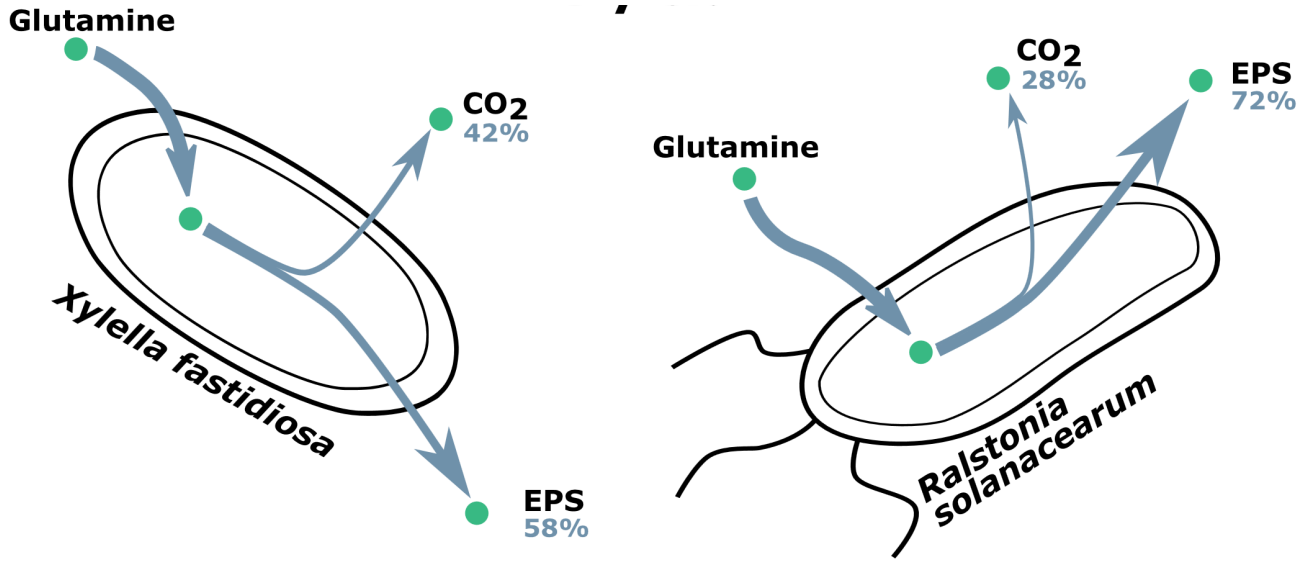
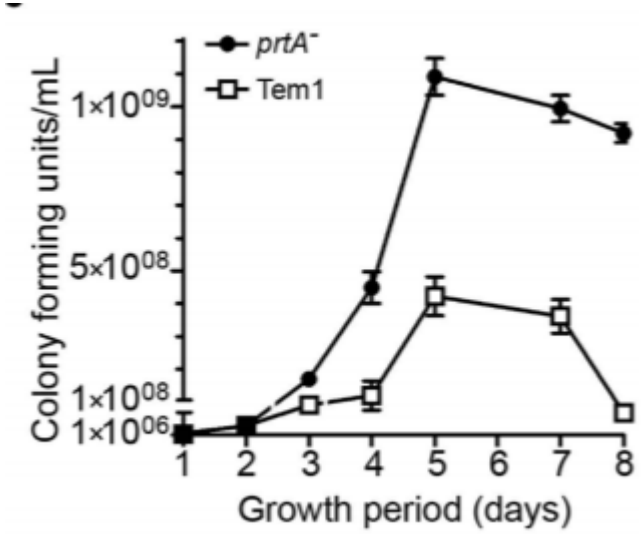
FBA prediction: FBP activity needs to be 3.37 times lower to make the strain fastidious (generation time from 1.45h^{-1} to 103h^{-1})

Efficiency study: exopolysaccharides (EPS) production



(Gouran et al., 2016)



➔ **Inefficient EPS production in *Xylella***

➔ **Evolution drove *Xylella* to slow growth: strategy of virulence ? (low population level to remain undetected by the host)**

prtA⁻: reduced biofilm (EPS)/protein secretion

➔ Enhanced growth

In conclusion

- We can perform metabolic modeling at the scale of the organism

BUT with additional assumptions : quasi-steady-state and optimality

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➔A complementary approach to conventional biological approaches, which gives a different view of the biological system

Extra slides

Optimisation problems

1. Formulation

Optimization problem

Objective (maximization or minimization)

$$\min_{\boldsymbol{v}} f(\boldsymbol{v})$$

Constraints

$$A.\boldsymbol{v} = b$$
$$A_{ineq}.\boldsymbol{v} \leq b_{ineq}$$
$$lb \leq \boldsymbol{v} \leq ub$$

2. Solve using solver

3. Explore result

$$f(v) = f_{opt}$$
$$\boldsymbol{v} = v_{opt}$$