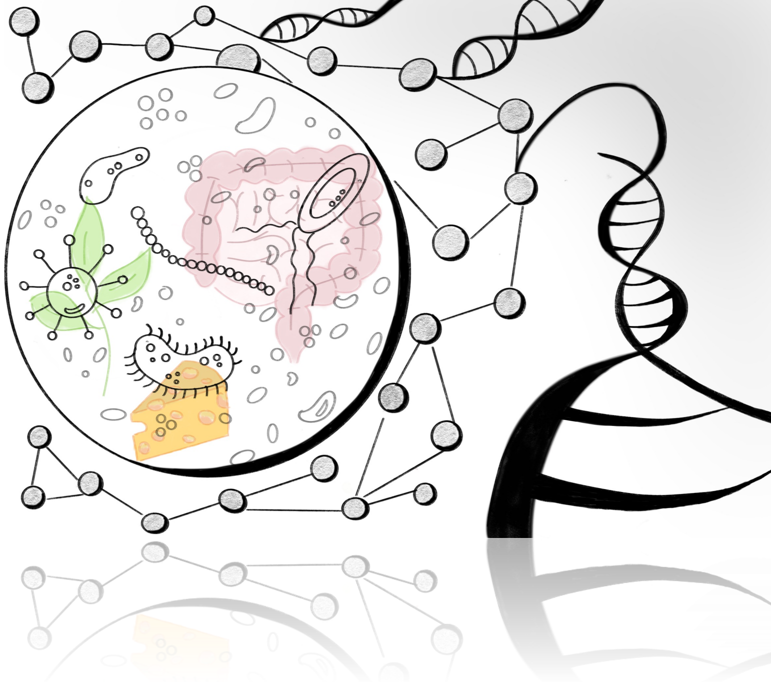
In silico exploration of metabolism in microbial ecosystems:

from the metabolic network to the model



Clémence Frioux

8th EBAME Workshop

28/10/2023

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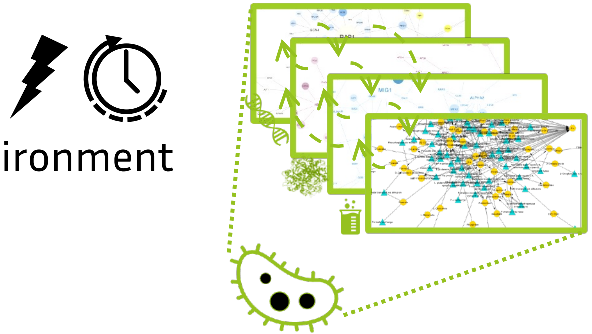
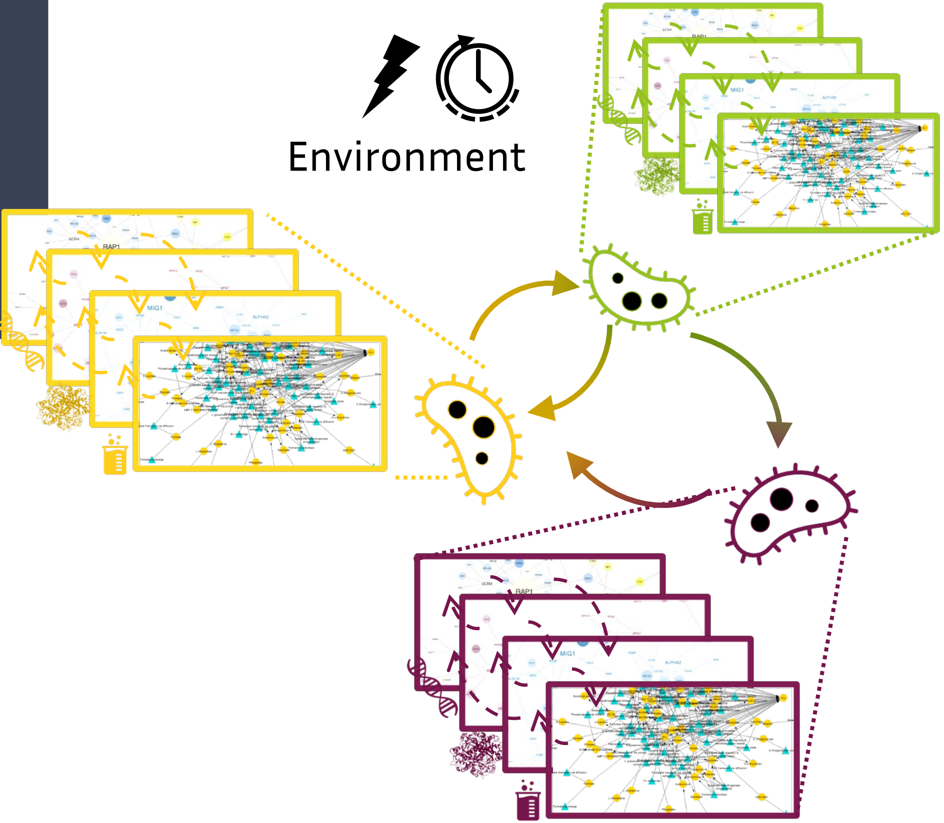
Inria centre at the University of Bordeaux
clemencefrioux@mamot.fr
@c_frioux

From (microbial) systems biology to systems ecology



Environment

From (microbial) systems biology to systems ecology



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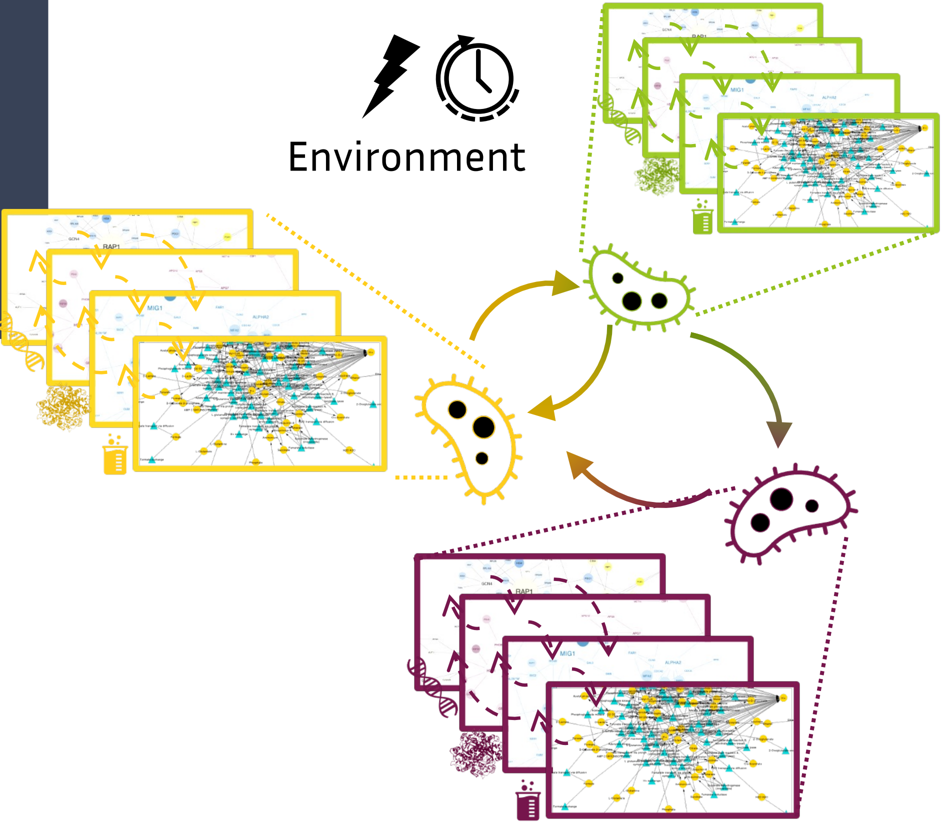
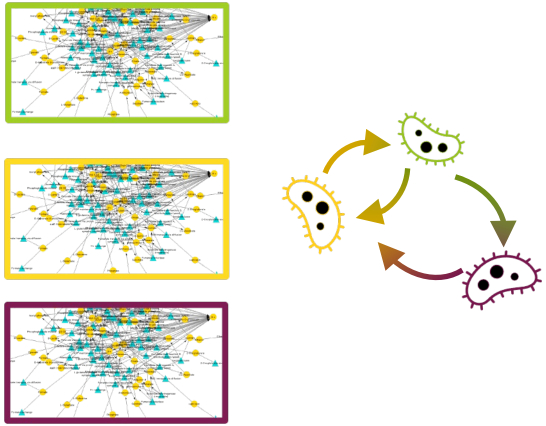
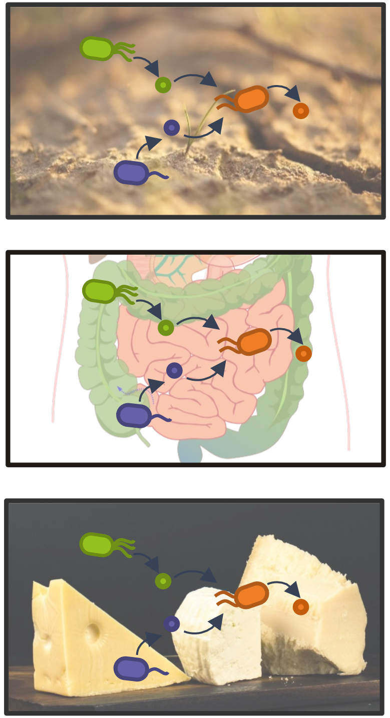




Most interactions are
mediated by metabolism

Integrating the biological data flood into models

Ecosystems



“Omics” data

“Who” is there?
What can they do?
What do they actually do?
What molecules are present?

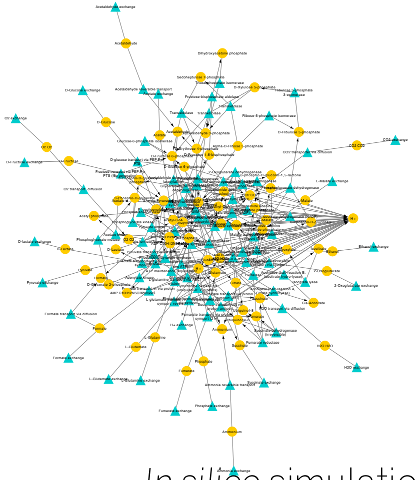
(Meta)genomics
Metataxonomics

Model generation

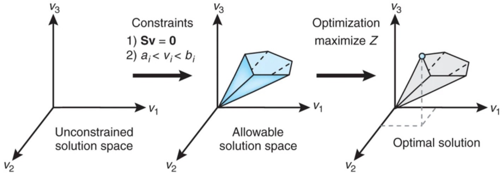
Metatranscriptomics
Metabolomics...

Model refinement

Models

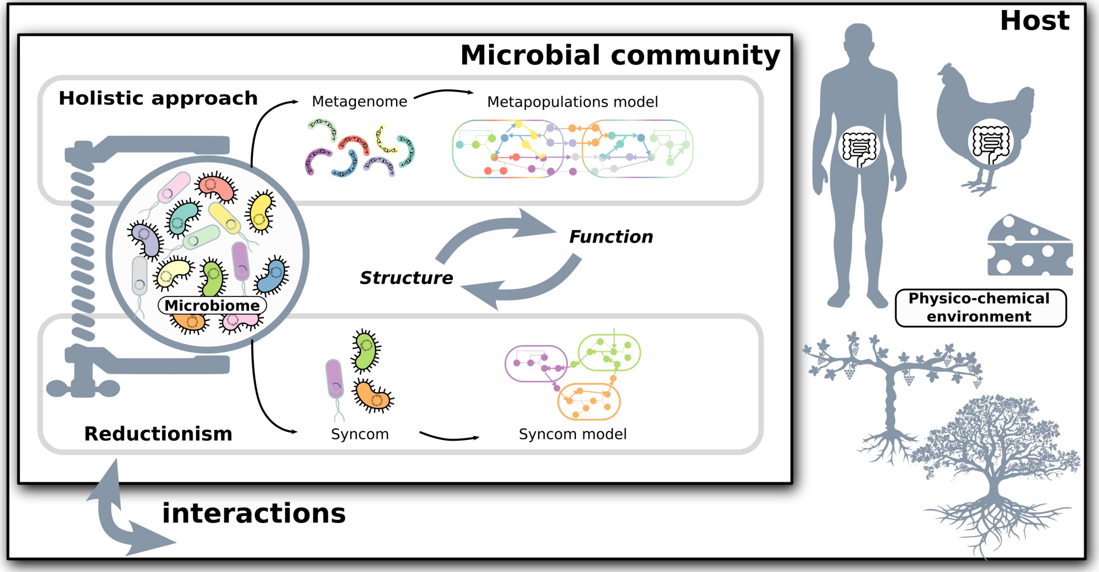


In silico simulations
Digital twins?



Picture from Orth et al, Nat. Biot. 2010

Outline



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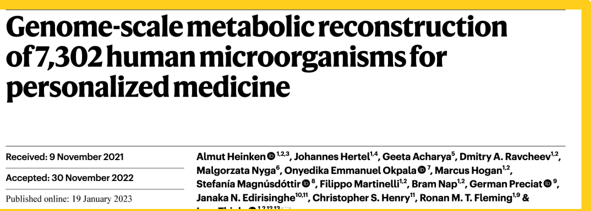
1. Metabolic networks
2. Reconstruction of metabolic networks
3. A discrete model of metabolism
4. Mining metabolic models of large communities
5. Tutorial – hands on

1. Metabolic networks



Genome-scale metabolic reconstruction of 7,302 human microorganisms for personalized medicine

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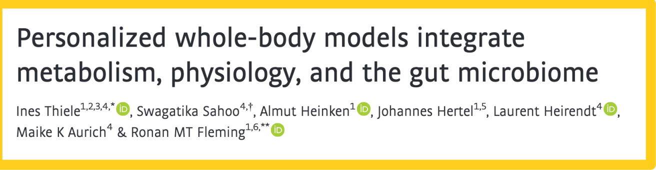

Personalized whole-body models integrate metabolism, physiology, and the gut microbiome

Ines Thiele[1,2,3,4,*], Swagatika Sahoo[4,†], Almut Heinken[1], Johannes Hertel[1,5], Laurent Heirendt[4], Maike K Aurich[4] & Ronan MT Fleming[1,6,**]


RESEARCH ARTICLE

Predicting gastrointestinal drug effects using contextualized metabolic models

Marouen Ben Guebila[1], Ines Thiele[1,2,3]*

1 Luxembourg Centre for Systems Biomedicine, University of Luxembourg, Esch-sur-Alzette, Luxembourg, **2** School of Medicine, National University of Ireland, Galway, University Road, Galway, Ireland, **3** Discipline of Microbiology, School of Natural Sciences, National University of Ireland, Galway, University Road, Galway, Ireland


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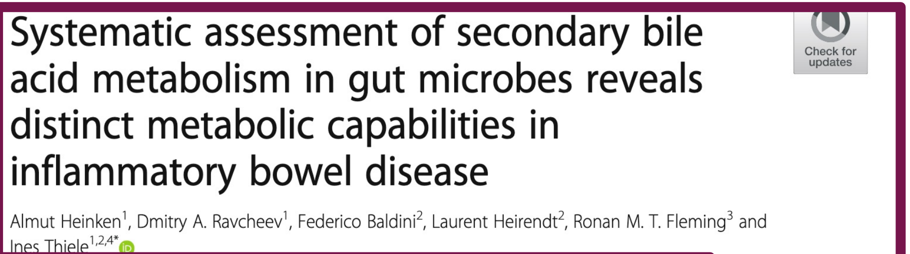
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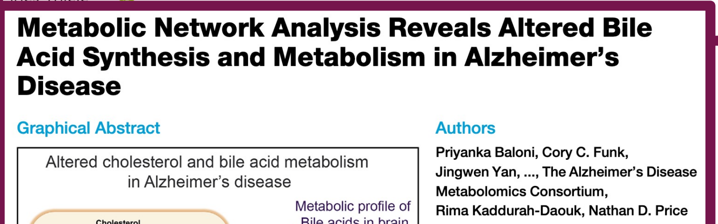
Check for updates

Almut Heinken[1], Dmitry A. Ravcheev[1], Federico Baldini[2], Laurent Heirendt[2], Ronan M. T. Fleming[3] and Ines Thiele[1,2,4*]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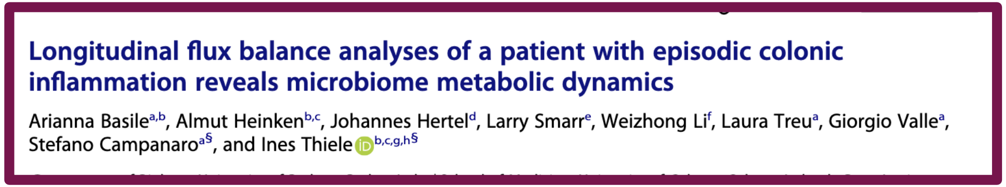
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Metabolic dependencies govern microbial syntrophies during methanogenesis in an anaerobic digestion ecosystem

Xinyu Zhu[1,2], Stefano Campanaro[3,4], Laura Treu[1,3]*, Rekha Seshadri[2], Natalia Ivanova[2], Panagiotis G. Kougias[1,5]*, Nikos Kyrpides[2] and Irini Angelidaki[1]


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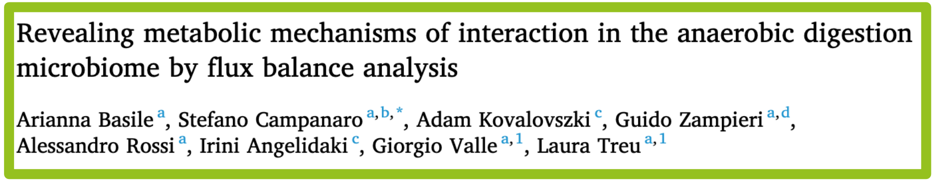
Revealing metabolic mechanisms of interaction in the anaerobic digestion microbiome by flux balance analysis

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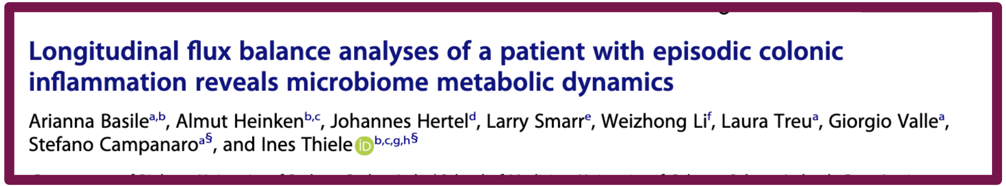
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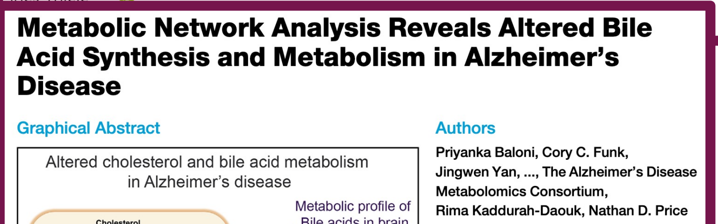
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Graphical Abstract

Altered cholesterol and bile acid metabolism in Alzheimer's disease

Metabolic profile of Bile acids in brain

Cholesterol

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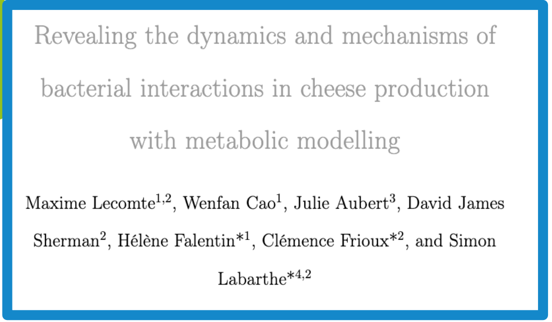

Revealing the dynamics and mechanisms of bacterial interactions in cheese production with metabolic modelling

Maxime Lecomte[1,2], Wenfan Cao[1], Julie Aubert[3], David James Sherman[2], Hélène Falentin*[1], Clémence Frioux*[2], and Simon Labarthe*[4,2]

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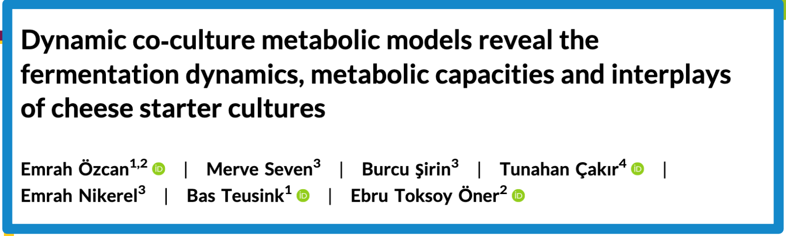

Dynamic co-culture metabolic models reveal the fermentation dynamics, metabolic capacities and interplays of cheese starter cultures

Emrah Özcan[1,2] | Merve Seven[3] | Burcu Şirin[3] | Tunahan Çakır[4] | Emrah Nikerel[3] | Bas Teusink[1] | Ebru Toksoy Öner[2]

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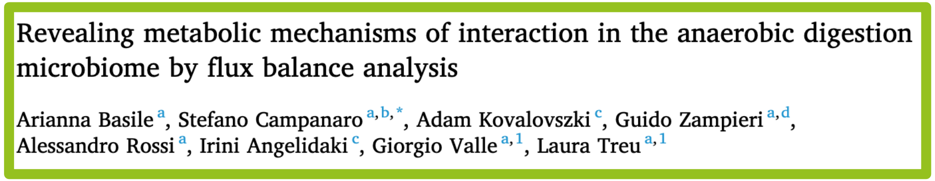

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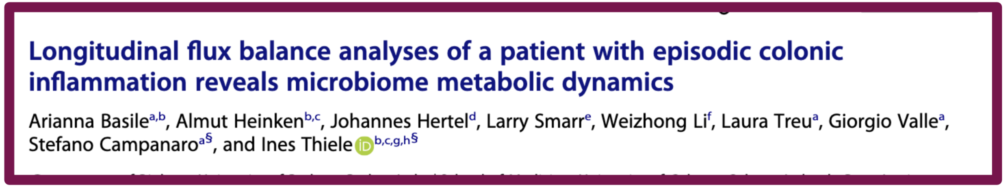

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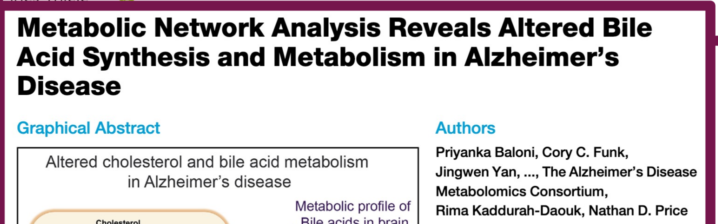

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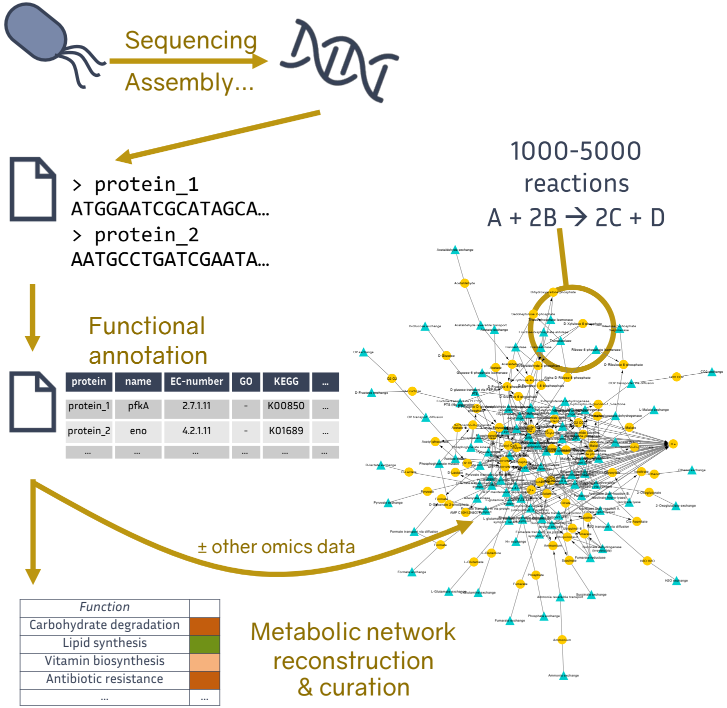
Metabolic modelling



Sequencing

Assembly…

```
> protein_1
ATGGAATCGCATAGCA…
> protein_2
AATGCCTGATCGAATA…
```

Functional annotation

protein	name	EC-number	GO	KEGG	…
protein_1	pfkA	2.7.1.11	-	K00850	…
protein_2	eno	4.2.1.11	-	K01689	…
…	…	…	…	…	…

± other omics data

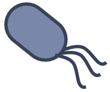
Function	
Carbohydrate degradation	🟧
Lipid synthesis	🟩
Vitamin biosynthesis	🟧
Antibiotic resistance	🟧
…	…

Metabolic network reconstruction & curation

1000-5000 reactions

A + 2B → 2C + D

Metabolic modelling



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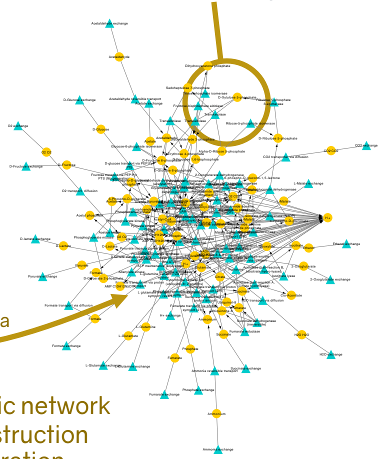

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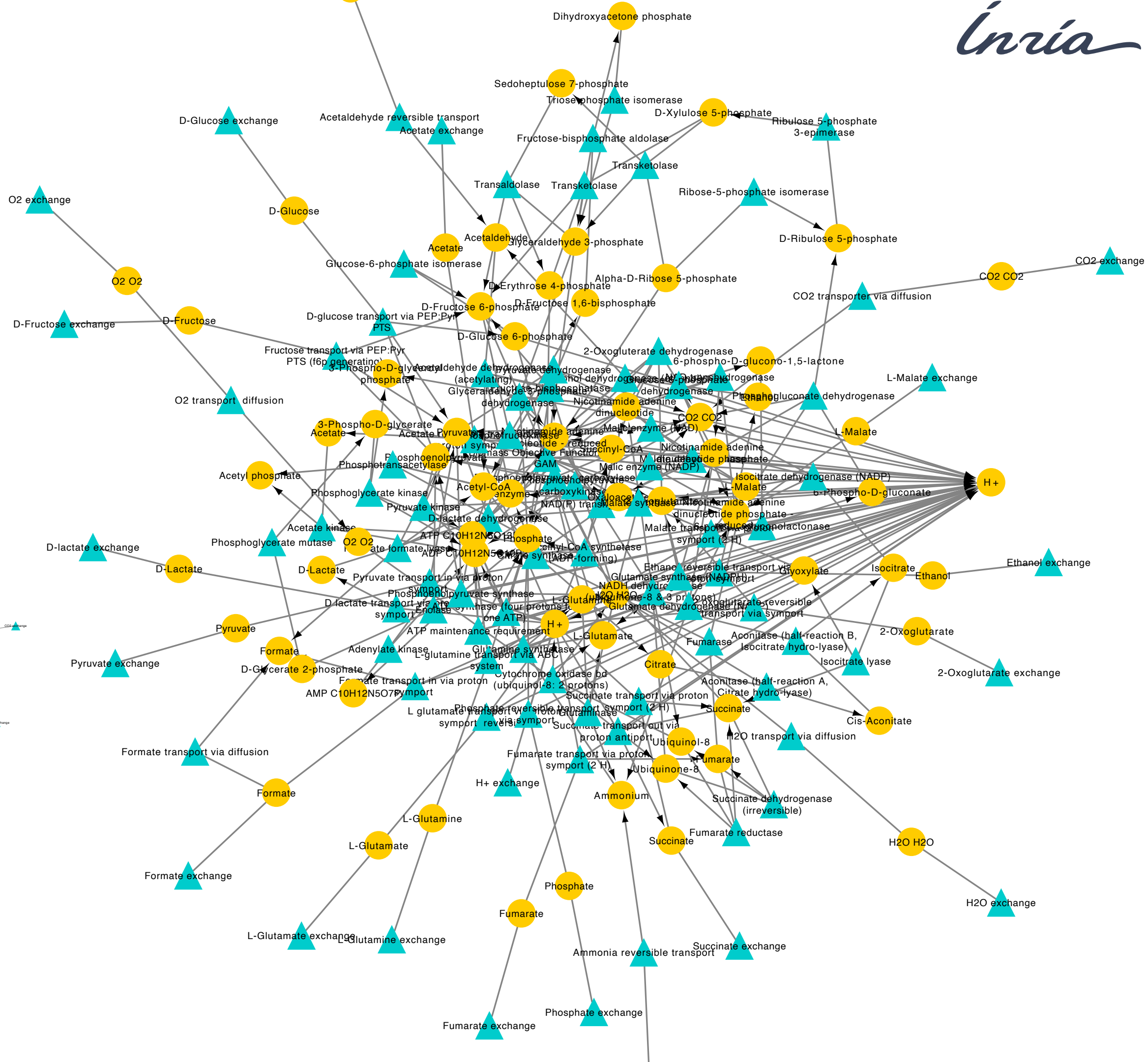
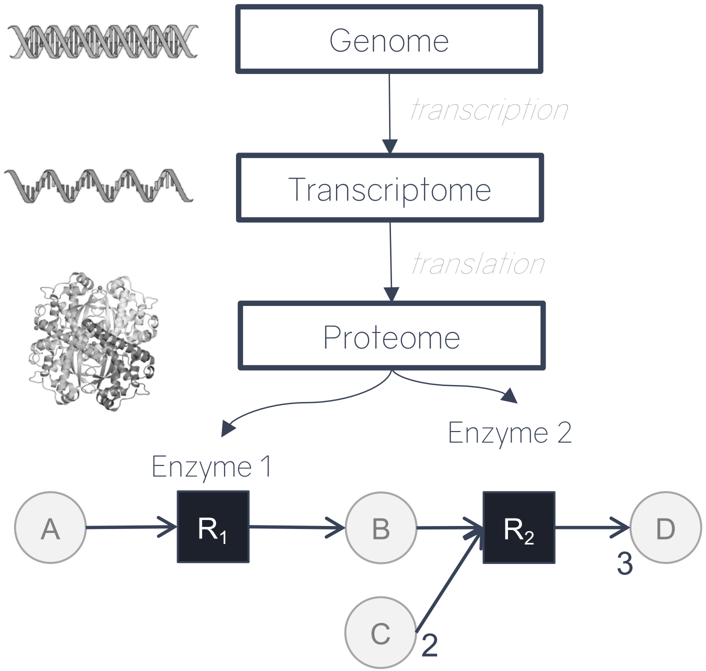


Connecting genomes to functions

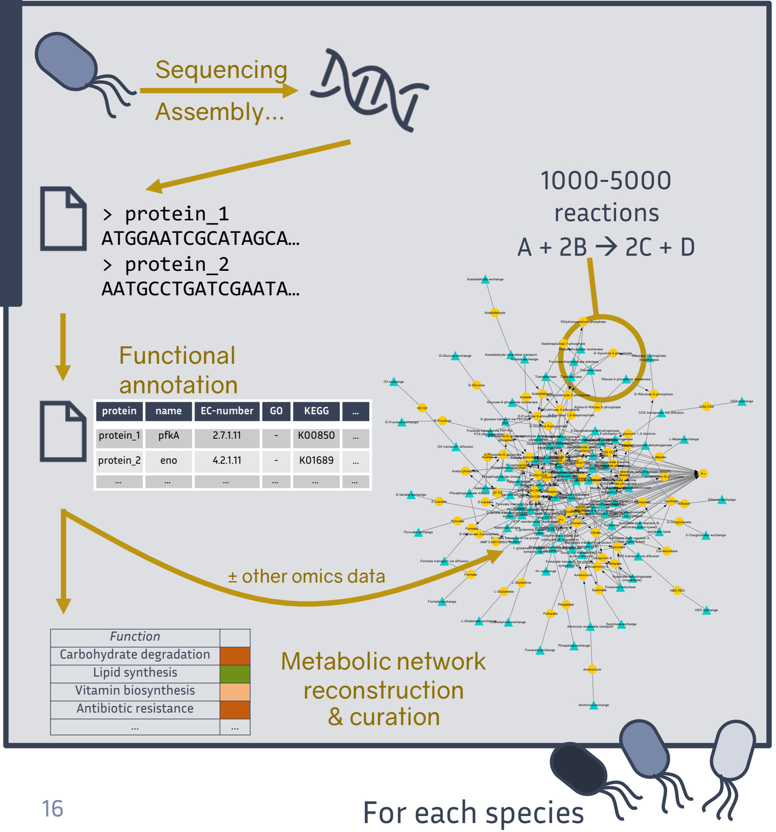


1 genome → 1,000 to 5,000 reactions

Abstraction of genomic information in genome-scale metabolic networks (GSMNs)

Functional repertoire of the cell

Metabolic modelling



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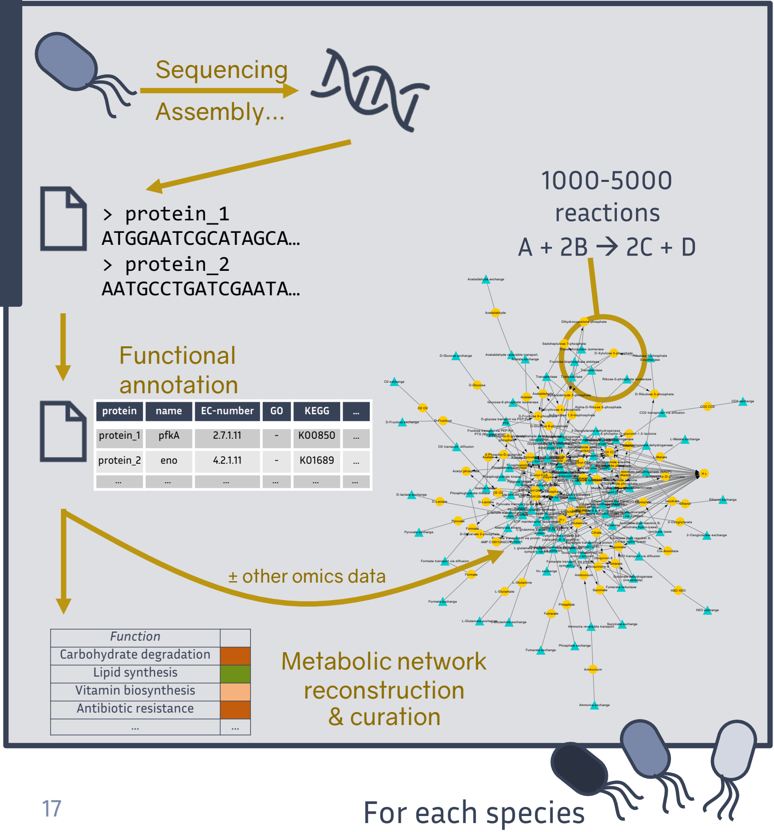
…	…

For each species

]

Metabolic modelling



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± other omics data

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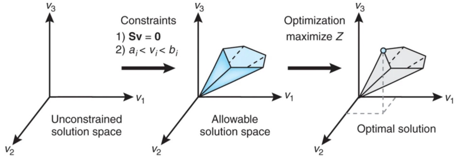
Metabolic network reconstruction & curation

For each species

Environmental conditions

± metabolic objective

Flux analysis (FBA, FVA...)
Constraint-based modelling

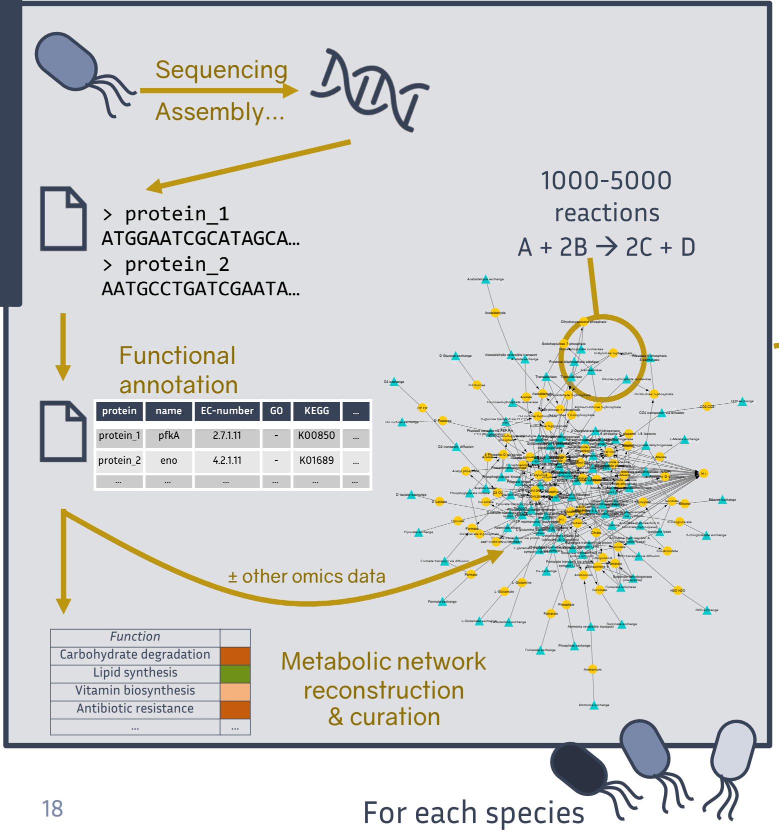


$$S = \begin{array}{ccc|c} r_1 & \cdots & r_n & \\ \hline +1 & \cdots & 0 & M_1 \\ \vdots & \ddots & \vdots & \cdots \\ -1 & \cdots & +1 & M_n \end{array}$$

Damien Éveillard's tutorial

from Orth et al, Nat. Biot. 2010

Metabolic modelling



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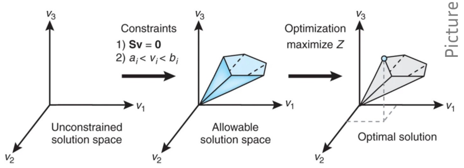

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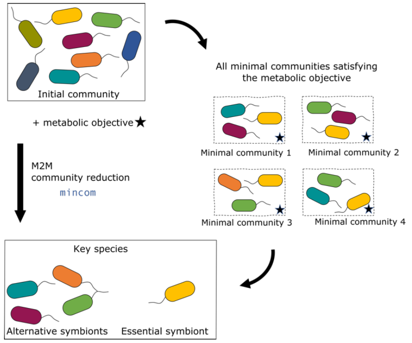
Damien Éveillard's tutorial

Automated reasoning using logic paradigms

This tutorial



Picture from Orth et al, Nat. Biot. 2010

Representations of metabolic networks

$$\begin{aligned}
r_1&: \quad A + B \rightarrow C \\
r_2&: \quad \mathrm{C} \leftrightarrow \mathrm{D} \\
r_3&: \quad D + 2E \rightarrow F + G
\end{aligned}$$

Representations of metabolic networks

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r_2: $\mathrm{C} \leftrightarrow \mathrm{D}$
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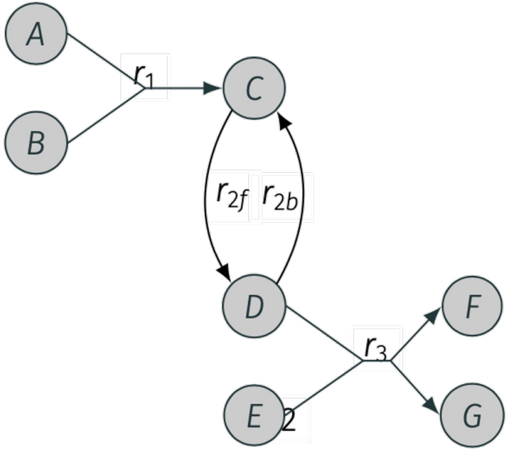

Hypergraph

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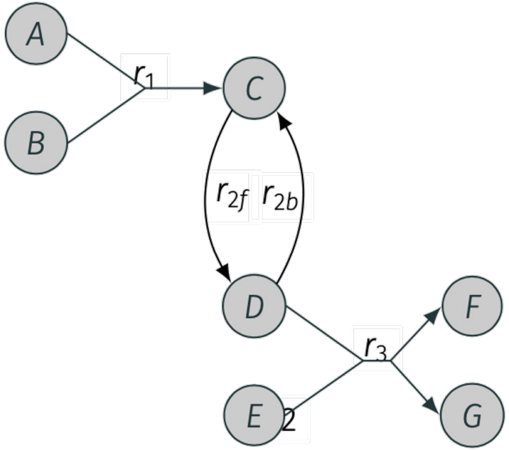

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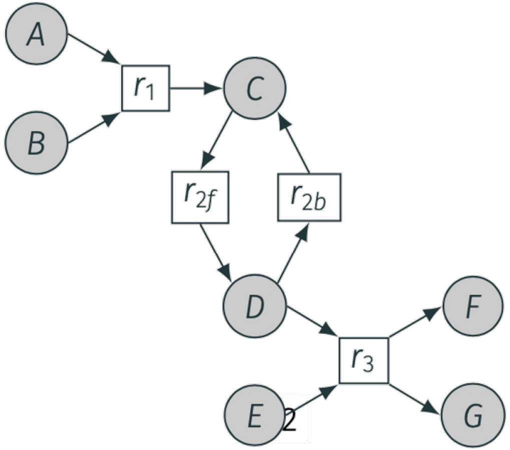

Bipartite graph

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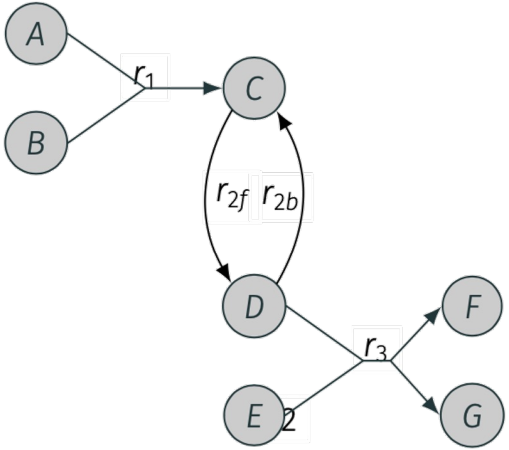

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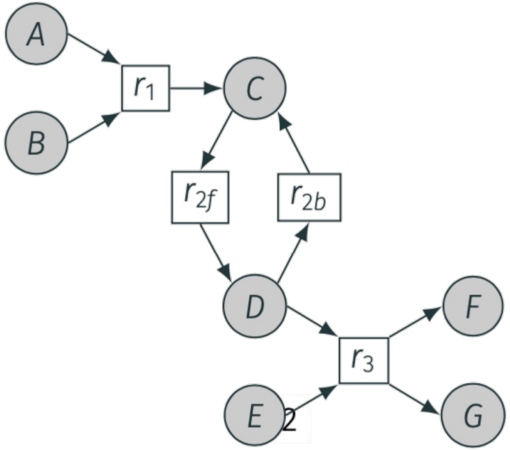


Bipartite graph

$$\begin{array}{c} \\ A \\ B \\ C \\ D \\ E \\ F \\ G \end{array} \begin{array}{c} \begin{array}{cccc} r_1 & r_{2f} & r_{2b} & r_3 \end{array} \\ \left(\begin{array}{cccc} -1 & 0 & 0 & 0 \\ -1 & 0 & 0 & 0 \\ +1 & -1 & +1 & 0 \\ 0 & +1 & -1 & -1 \\ 0 & 0 & 0 & -2 \\ 0 & 0 & 0 & +1 \\ 0 & 0 & 0 & +1 \end{array}\right) \end{array} = S$$

Stoichiometric matrix

1. **Metabolic network exploration:**
    a. Visualisation of metabolic networks
    b. Exploration of the exchange format: SBML
2. **Metabolic network reconstruction**
    a. Structural annotation with Prodigal
    b. Reconstruction with gapseq
    c. Reconstruction from Kegg knowledge base
    d. Exploration of metabolic networks reconstructed with Pathway Tools
3. **Metabolic network modelling of a unique species**
    a. Using toy data
    b. Using real data
4. **Metabolic network modelling of a community**
    a. Using toy data
    b. Using real data

https://gitlab.inria.fr/cfrioux/ebame

2. Reconstruction of metabolic networks

Input data

- **minimal input: a genome or a proteome**
  - a reference genome
  - a Metagenomic-Assembled Genome (MAG)

⚠ MAG completeness (Eisenhofer et al 2023)

- **Genomes must be structurally annotated**

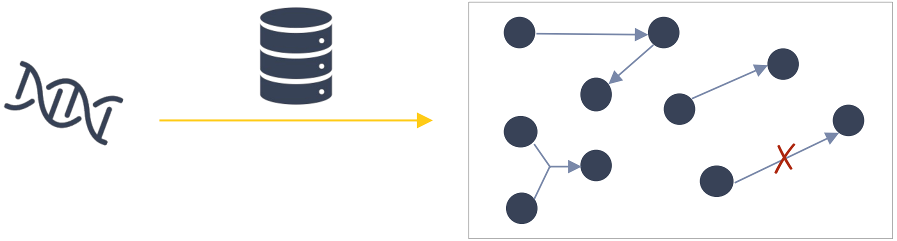
  e.g. with Prodigal [Hyatt et al 2010] for prokaryotes

- **Some tools require also a functional annotation**

  e.g.with EggNOG mapper [Cantalapiedra et al 2021] or Prokka [Seeman 2014]

Metabolic network inference: bottom-up procedure

1. Automatic inference from sequences: *non-guided*



Gapseq [Zimmermann et al 2021]
Pathway Tools [Karp et al 2018]…

Metabolic network inference: bottom-up procedure

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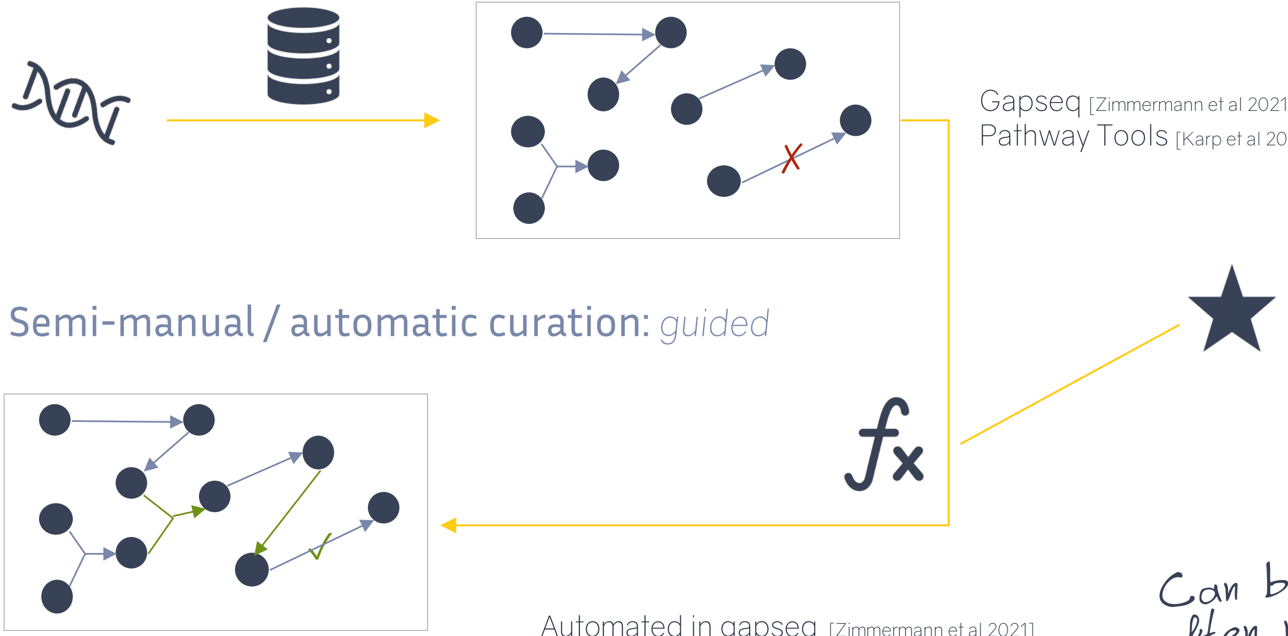

Gapseq [Zimmermann et al 2021]
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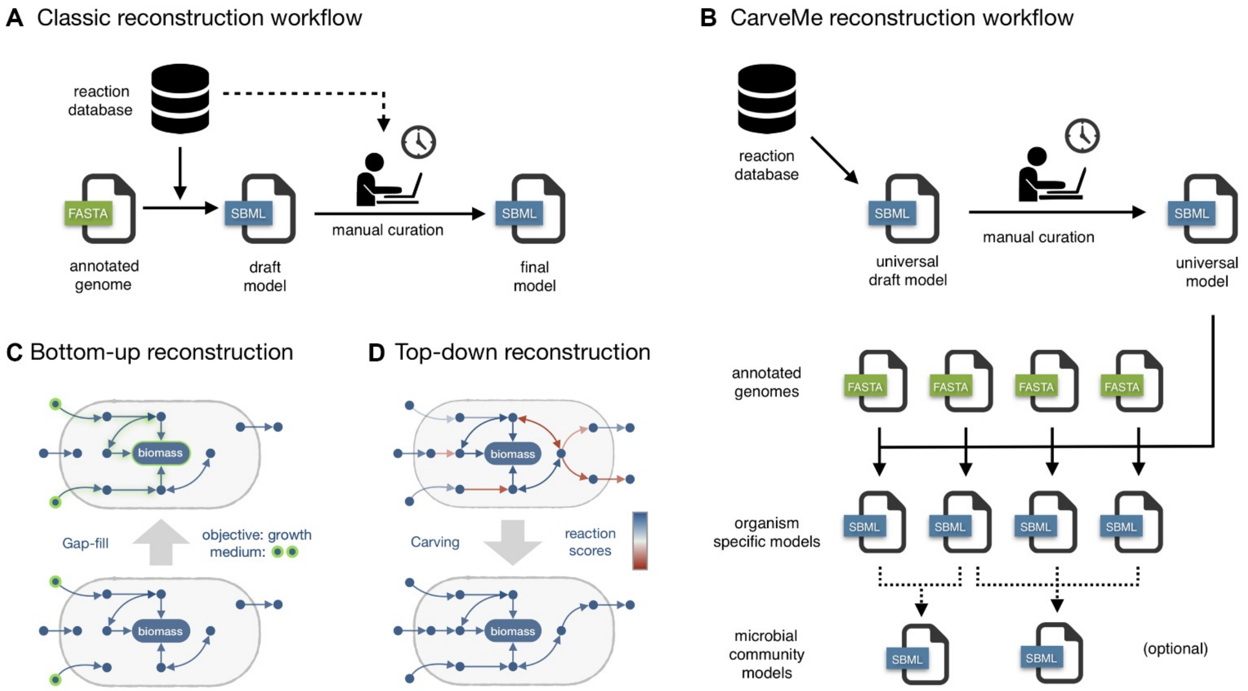
2. Semi-manual / automatic curation: *guided*

Metabolic objective
e.g. biomass / growth

Automated in gapseq [Zimmermann et al 2021]
Meneco [Prigent et al 2017],
BioISO [Cruz et al 2021]…

Can be fastidious but often necessary for high-quality GSMNs…

Alternative: top-down reconstruction



[figure from Machado et al 2018]

Metabolic models ready for constraint-based simulation

A rise in non-optimal use-cases

Cheaper sequencing
(Meta)genomics

Non-model organisms

- Limited experimentations
- Unknown biomass composition (metabolic objective)

Large-scale community modelling

- Impossible manual curation
- Uncertainty on/missing functions
- Scalability of optimisations-based simulation

→ The choice of the mathematical/computational model for simulation must account for the data and the question to be answered

Resources for (draft) reconstruction

Functional annotation

- eggNOG mapper [Cantalapiedra et al 2021]
- Prokka [Seeman 2014]

Tools scalable to microbial communities

- Pathway Tools [Karp et al 2019]
- Carveme [Machado et al 2018]
- gapseq [Zimmermann et al 2021]
- AutoKEGGRec [Karlsen et al 2018]

Collections of reconstructions

- KEGG [Kanehisa et al 2021]
- BioCyc [Karp et al 2019]
- Bigg [King et al 2016]
- Biomodels [Malik-Sheriff et al 2019]
- Virtual metabolic human [Noronha et al 2018]
- AGORA 2 [Heinken et al 2023]
- EMBL GEMs [Machado et al 2018]

A network, then what?

Exploration

- reactions, metabolic pathways, association of genes to reactions
- visualisation (usually complicated)

Modelling

- predicting the behaviour of the microbe/community in given conditions
- finding members of interest within the community
- screen the metabolic potential

Tutorial content

https://gitlab.inria.fr/cfrioux/ebame

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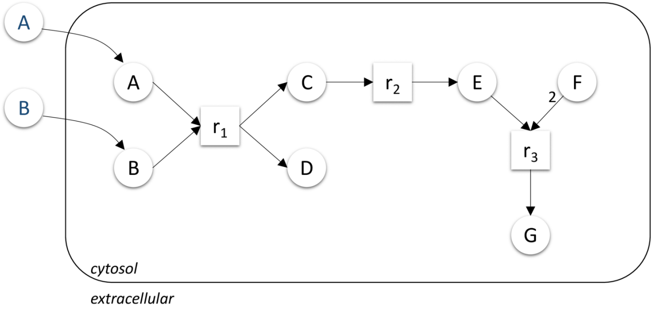
3. A discrete model of metabolism

Boolean abstraction of metabolic producibility



Network expansion [Ebenhöh et al 2004]

→ Scope of seeds

→ Discrete modelling

$$scope(G,S) = \bigcup_i M_i\,,$$

where $M_0 = S$ and

$$M_{i+1} = M_i \cup products(\{r \in R \mid reactants(r) \subseteq M_i\})$$

Qualitative simulation of metabolic producibility ignoring stoichiometric coefficients and w/o objective function

[Kruse et al 2008] [Handorf et al 2005]

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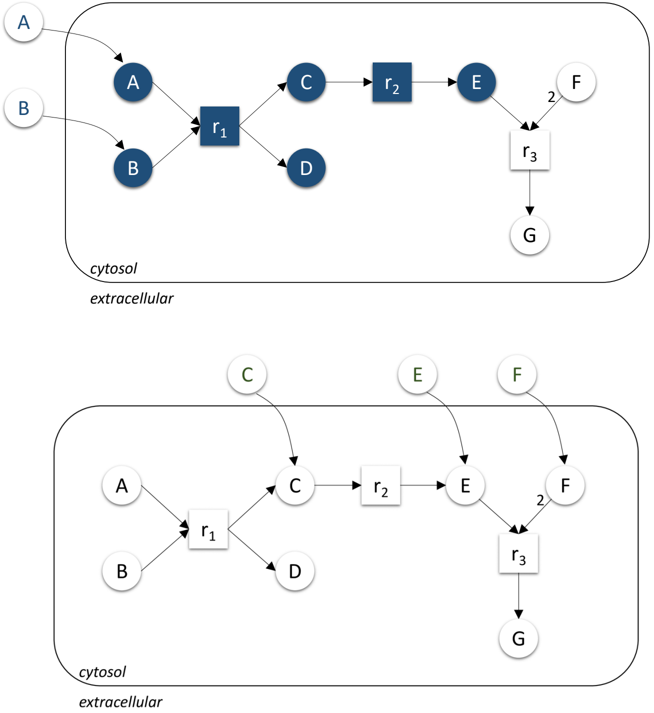

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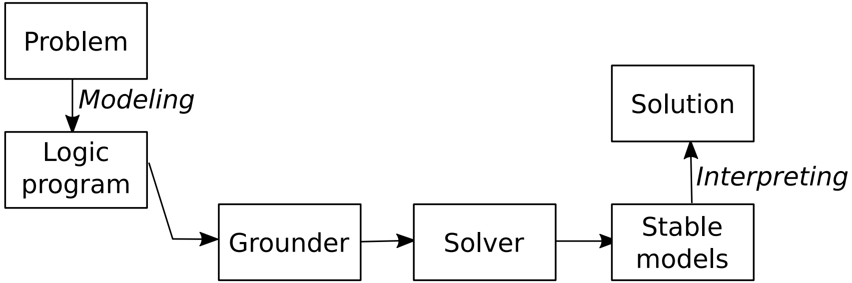
A flexible implementation system

[Gebser et al 2012]

- Answer Set Programming
- Logic paradigm
- Knowledge representation & reasoning
- Optimisation



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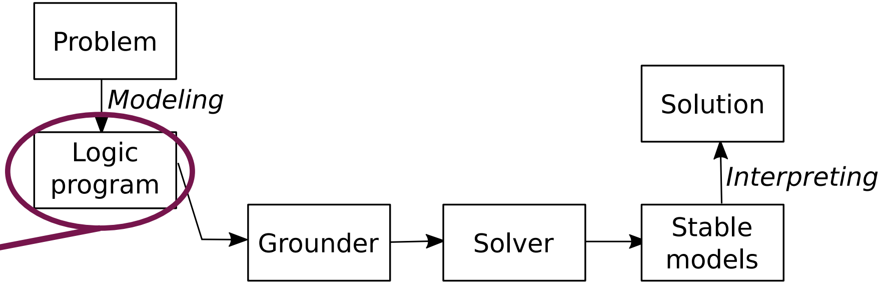


Problem

Modeling

Logic program

Grounder

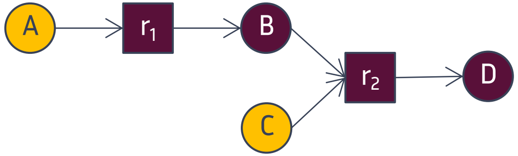
Solver

Stable models

Interpreting

Solution

```
reaction(“r1”).
reactant(“A”, “r1”).
product(“B”, “r1”).
%
reaction(“r2”).
reactant(“B”, “r2”).
reactant(“C”, “r2”).
product(“D”, “r2”).
%
seed(“A”).
seed(“C”).
%
scope(M) :- seed(M).
scope(M) :- product(M,R); reaction(R); scope(N) : reactant(N,R).
```



Prigent et al 2017, Aite et al 2017,
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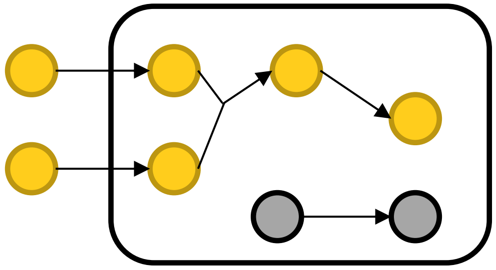

Scope calculation

Applications of the Boolean abstraction of producibility

Prigent et al 2017, Aite et al 2017,
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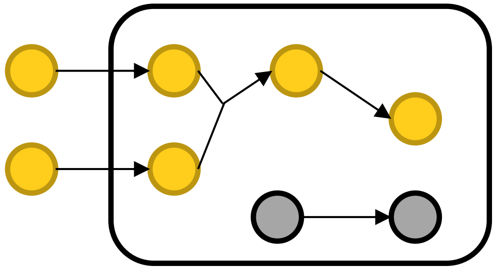

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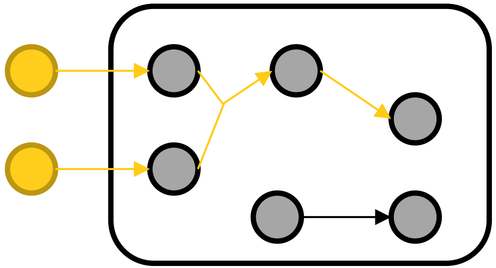

Activated reactions

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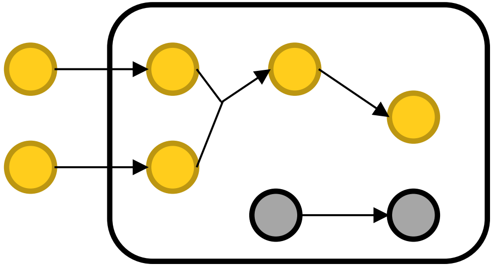

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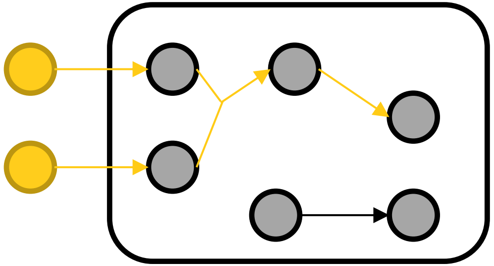

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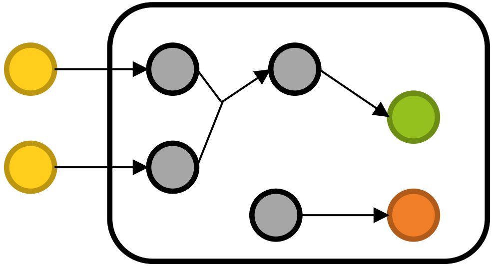

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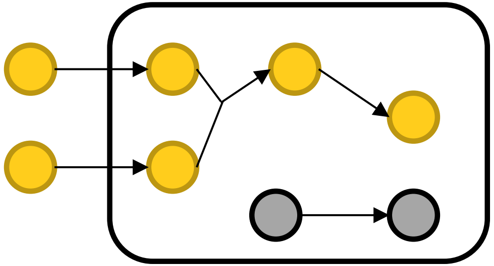

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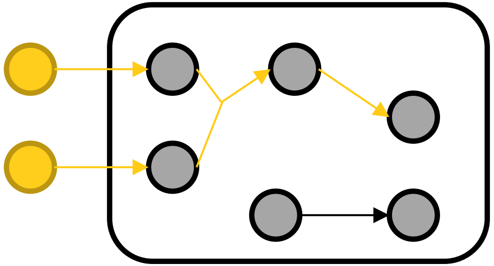

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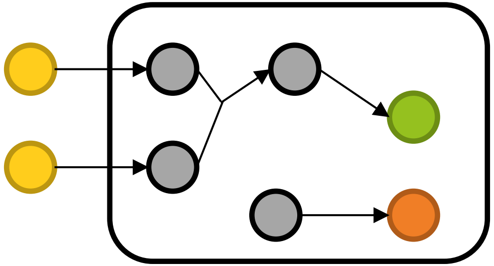

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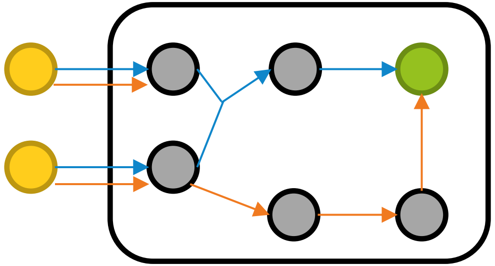

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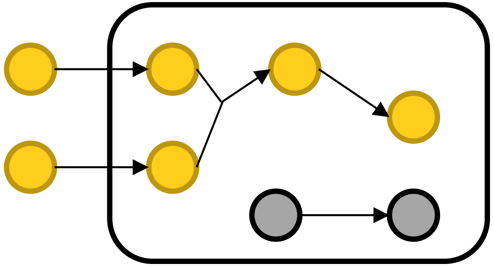

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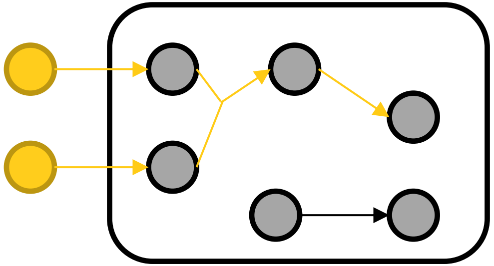

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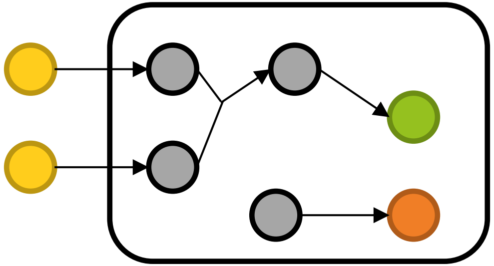

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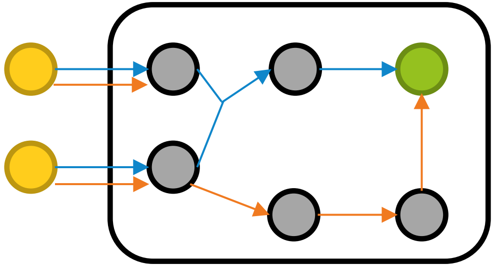

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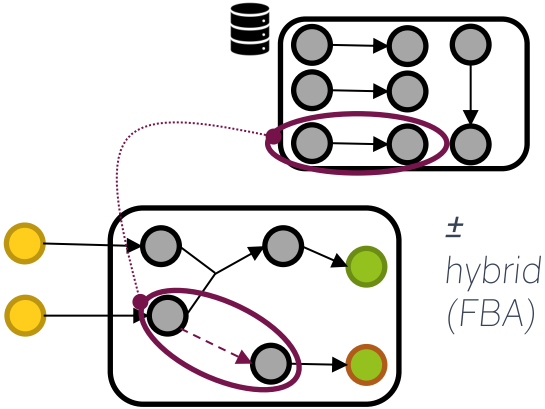

± hybrid (FBA)

Gap-filling

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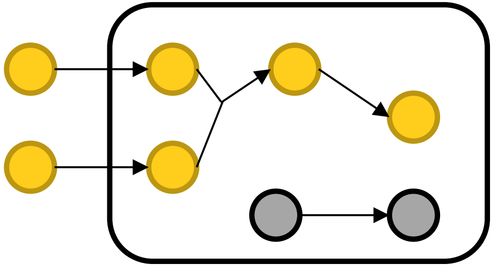

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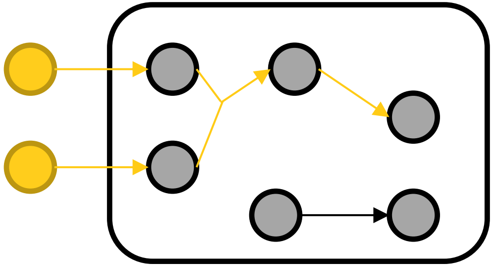

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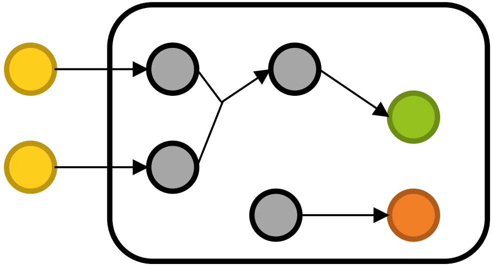

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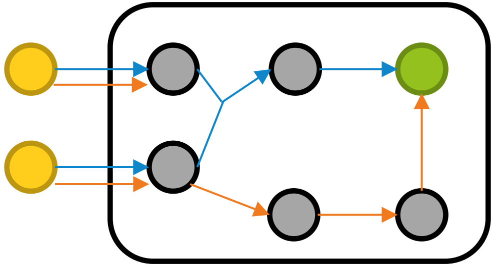

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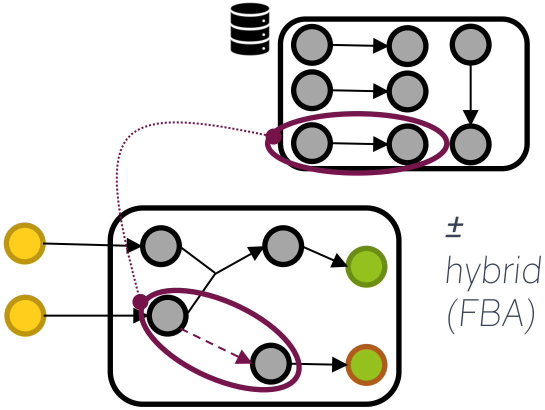

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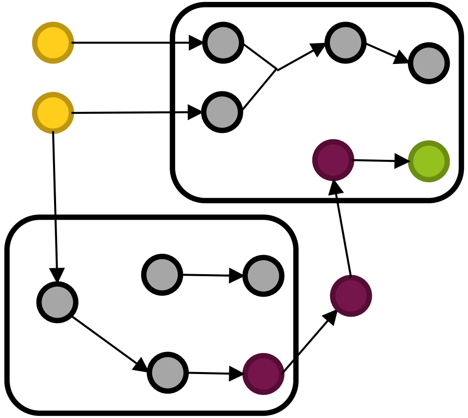
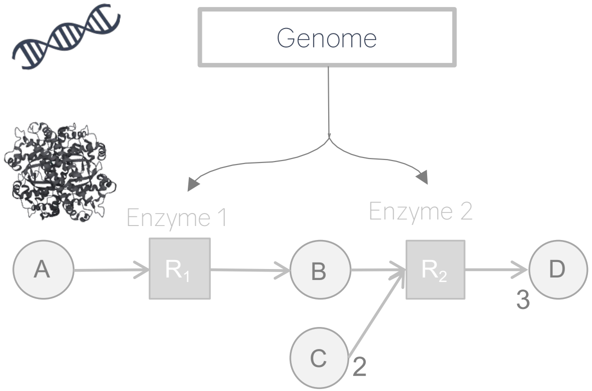


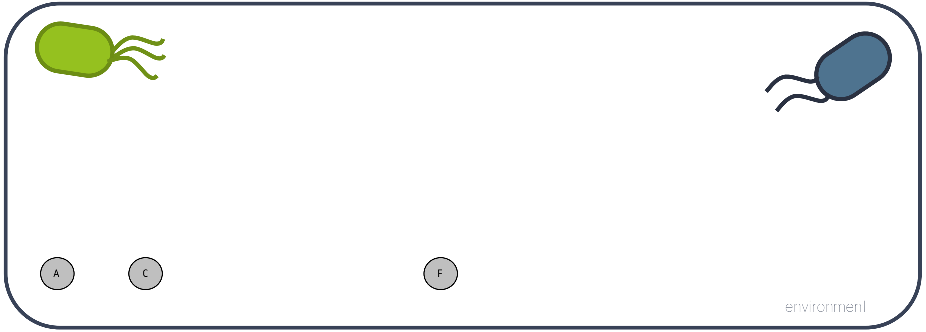
Community analysis

4. Mining metabolic models of large communities

Scale, Screen, Simplify

Metabolic networks and communities



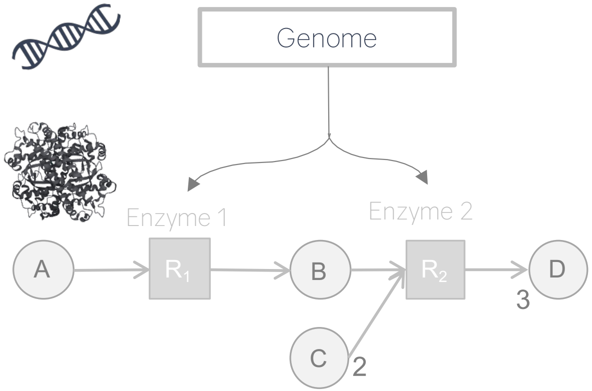


1 genome → 1,000 to 5,000 reactions

Genome-scale metabolic network (GSMN)

Functional repertoire of the cell

Metabolic networks and communities



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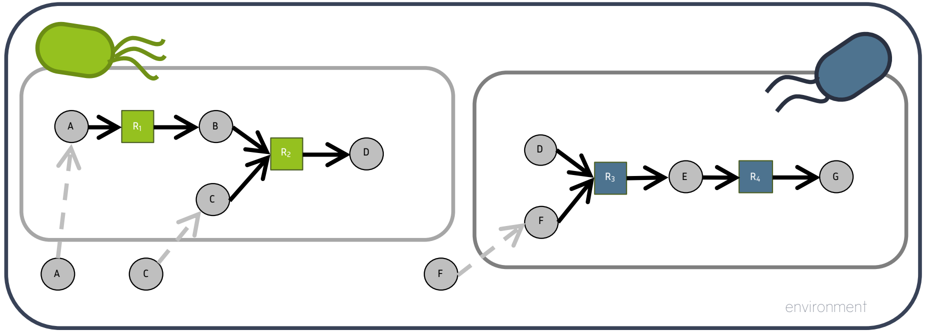
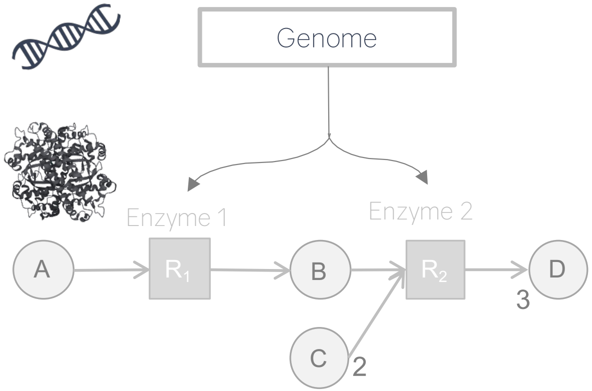


1. Reconstruct GSMNs for all genomes

Metabolic networks and communities



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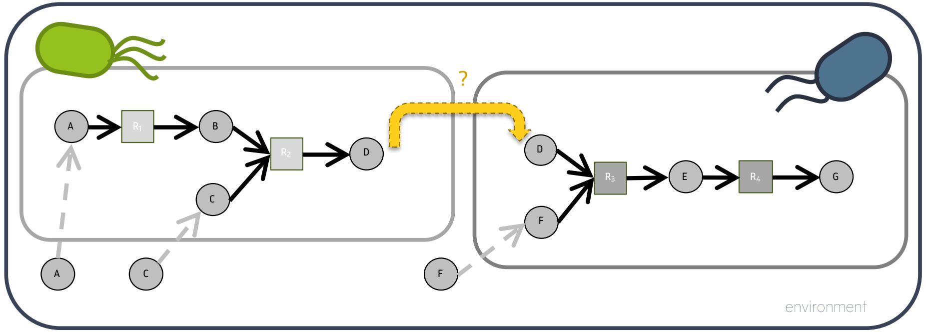

1. Reconstruct GSMNs for all genomes
2. Assess putative complementarity between them

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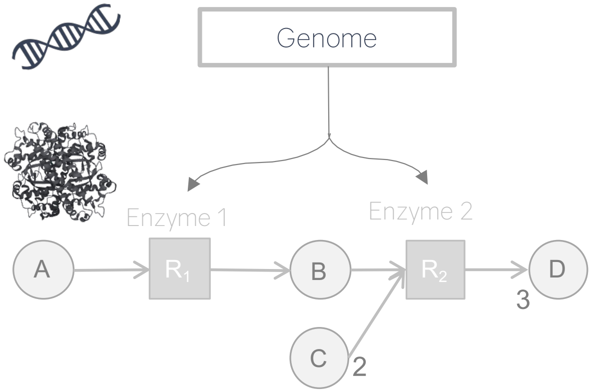

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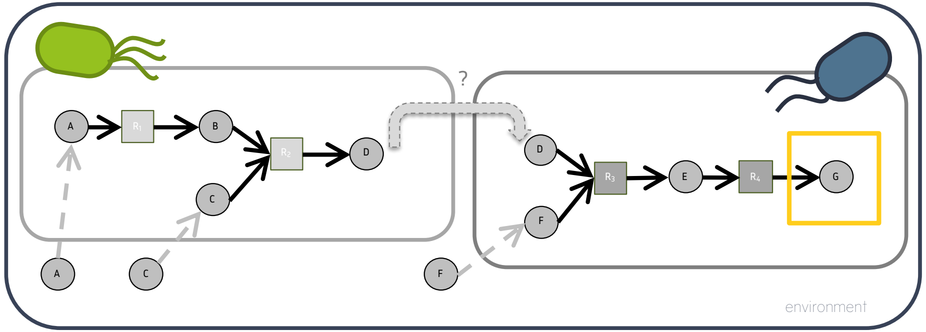

1. Reconstruct GSMNs for all genomes
2. Assess putative complementarity between them
3. Identify key species associated to a function

A pipeline for metabolic screening of communities

Microbiota
- MAGs
- Reference genomes

Systematic screening of **metabolic potential** and **mutualistic potential** in a microbiota

`Github`
`Aureme/Metage2Metabo`

[Belcour*, Frioux* et al. eLife 2020]
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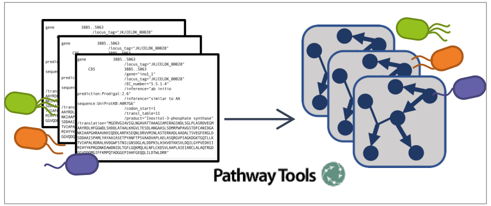
Automatic GSMN reconstruction



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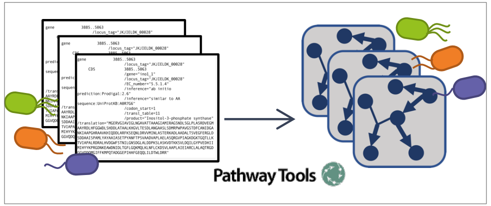
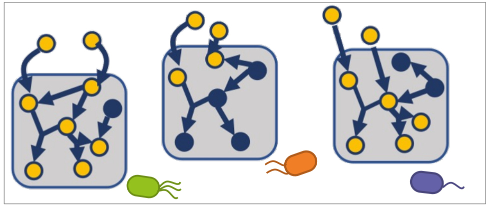


Individual metabolic capabilities



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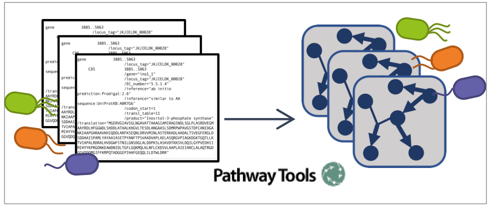

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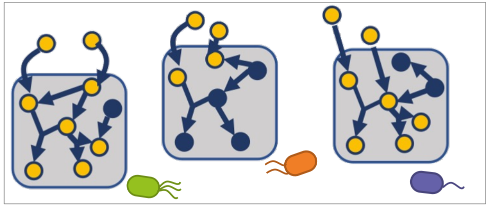
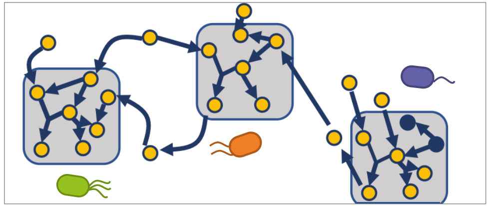


Collective metabolic capabilities



Systematic screening of **metabolic potential** and **mutualistic** potential in a microbiota

Github
Aureme/Metage2Metabo

[Belcour*, Frioux* et al. eLife 2020]
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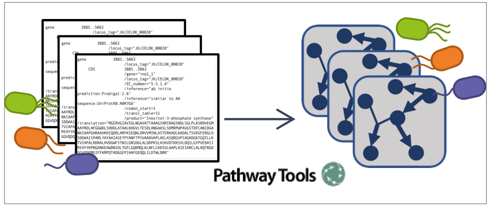
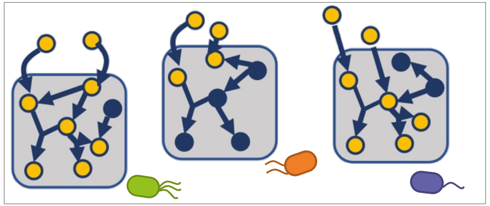
A pipeline for metabolic screening of communities

Microbiota
- MAGs
- Reference genomes

Automatic GSMN reconstruction



Individual metabolic capabilities



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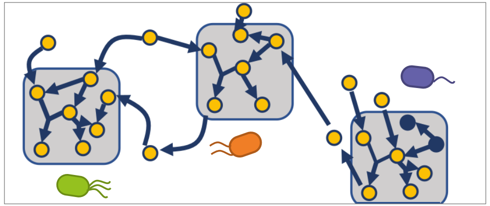
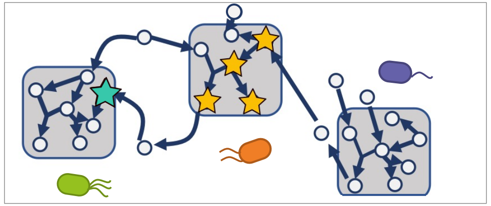


Added value of cross feeding



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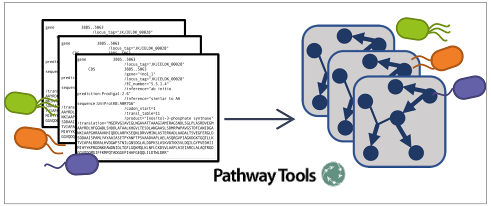

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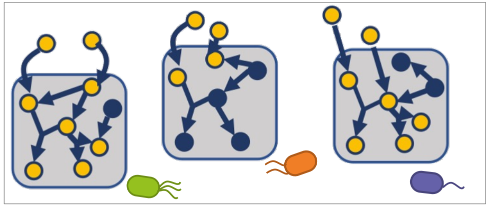

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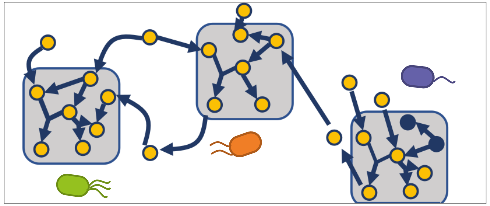

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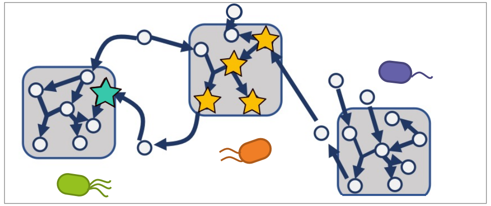
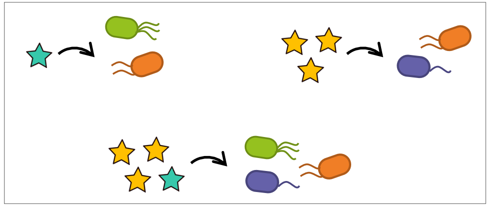


Minimal community selection



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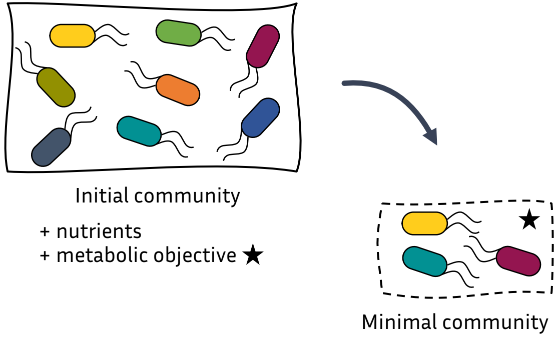
Key species

- Minimal community selection:
  combinatorial problem solving
- However… 1 solution (= 1 minimal community) among millions? **Huge combinatorics**
- *Key species* = species found in at least one of the predicted minimal communities (MC)
  > *Essential symbionts* : species predicted in **every** MC
  > *Alternative symbionts* : species predicted is some but not all MC



The concept of key species addresses the combinatorial challenge brought by functional redundancy in microbiomes

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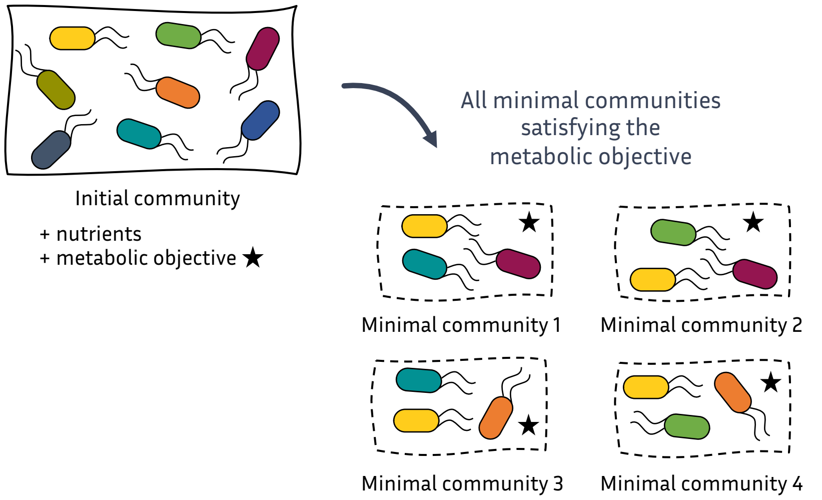

Initial community

+ nutrients
+ metabolic objective ★

All minimal communities satisfying the metabolic objective

Minimal community 1

Minimal community 2

Minimal community 3

Minimal community 4

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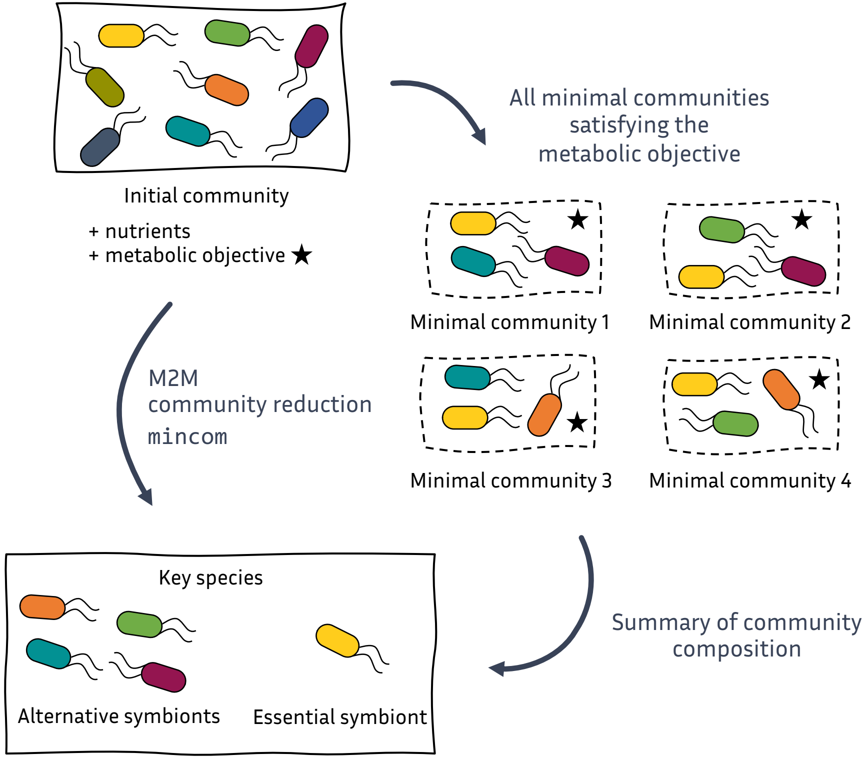

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M2M
community reduction
`mincom`

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Summary of community composition

Key species

Alternative symbionts

Essential symbiont

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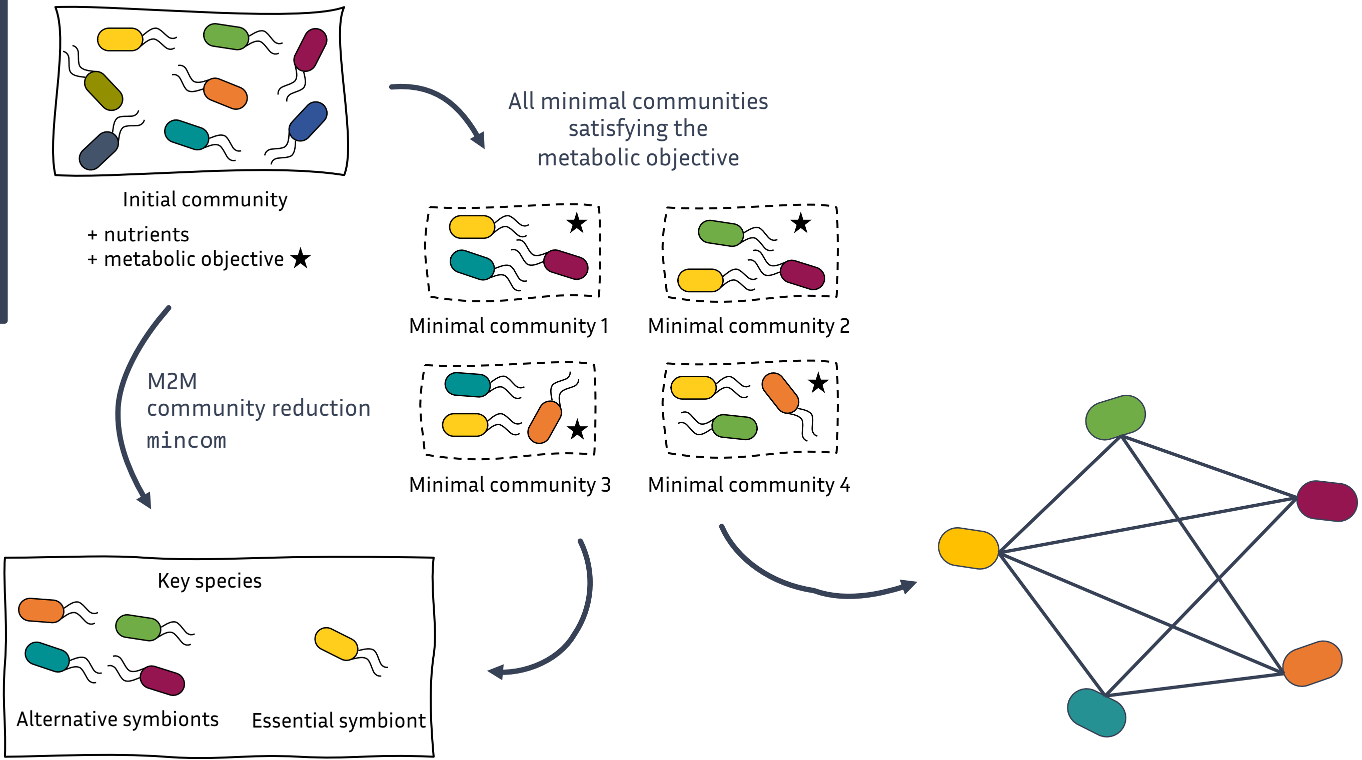
From key species to association analyses



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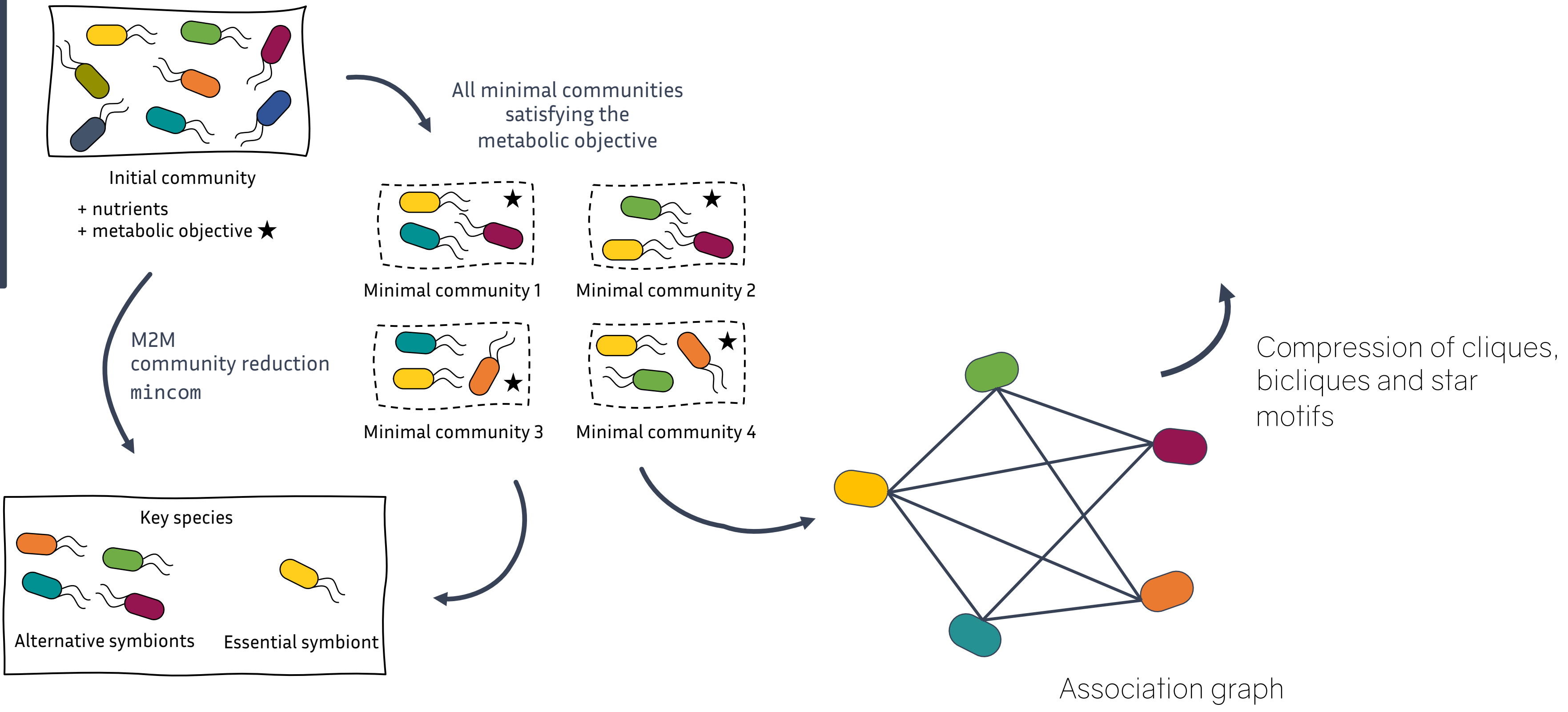

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Key species

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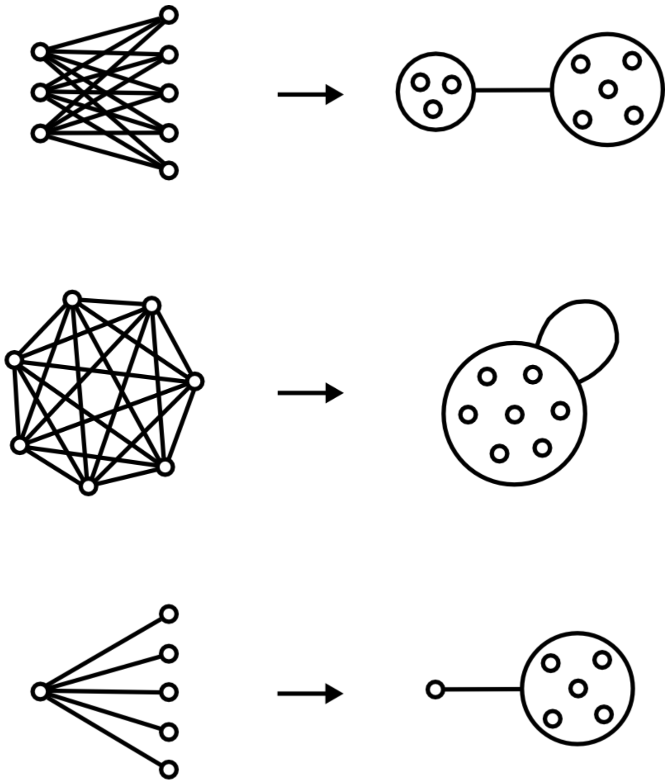
Association graph

Compression of cliques,
bicliques and star
motifs

Power graph compression

[Royer et al. PLoS CB 2008]



Biclique

Clique

Star

[Belcour*, Frioux et al. eLife 2020]
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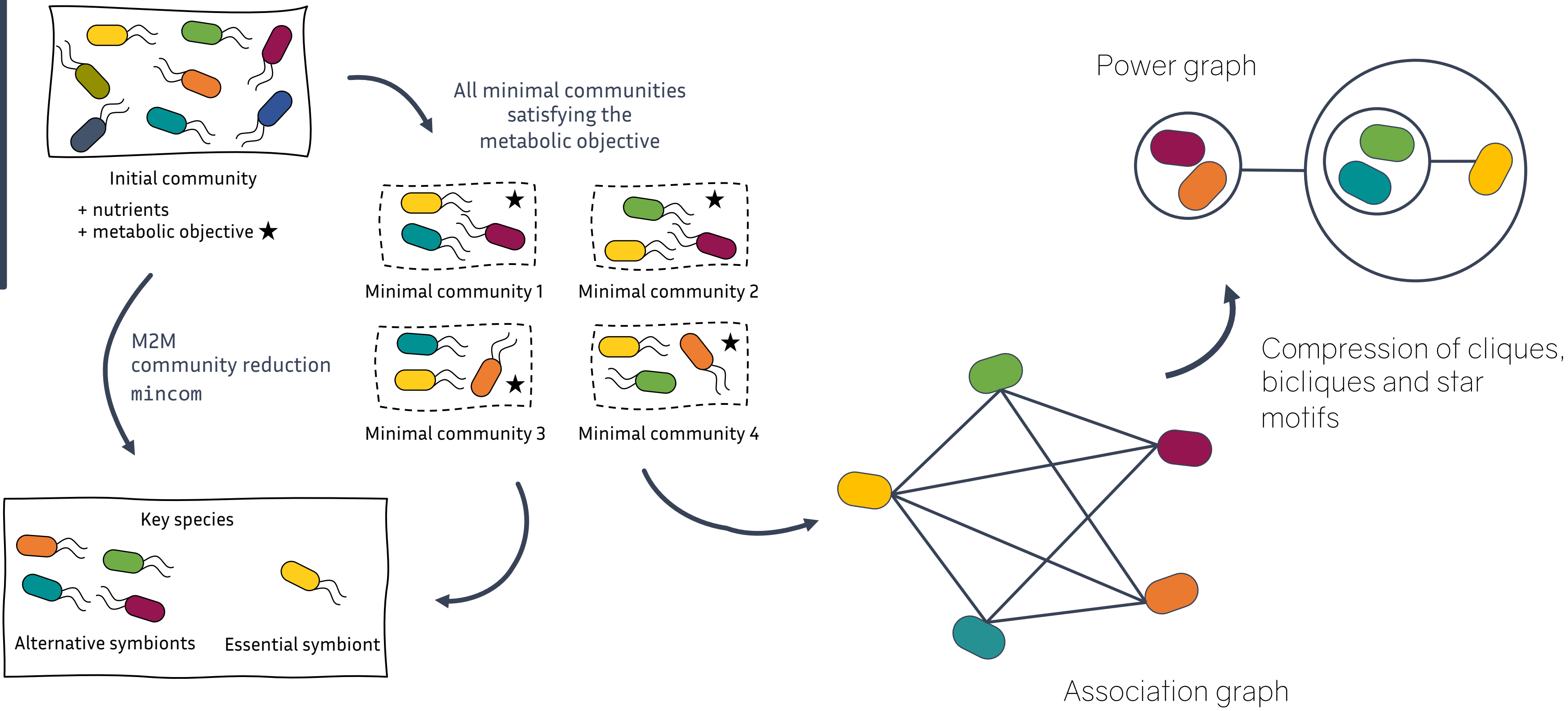
From key species to association analyses



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Association graph

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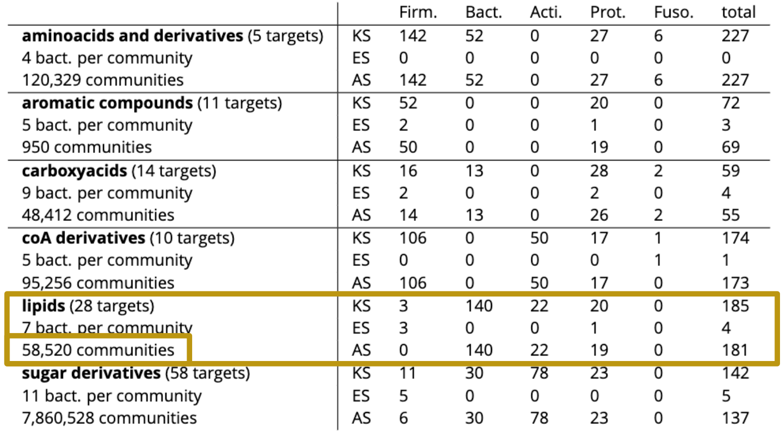
Key species by groups of metabolic end-products



		Firm.	Bact.	Acti.	Prot.	Fuso.	total
aminoacids and derivatives (5 targets)	KS	142	52	0	27	6	227
4 bact. per community	ES	0	0	0	0	0	0
120,329 communities	AS	142	52	0	27	6	227
aromatic compounds (11 targets)	KS	52	0	0	20	0	72
5 bact. per community	ES	2	0	0	1	0	3
950 communities	AS	50	0	0	19	0	69
carboxyacids (14 targets)	KS	16	13	0	28	2	59
9 bact. per community	ES	2	0	0	2	0	4
48,412 communities	AS	14	13	0	26	2	55
coA derivatives (10 targets)	KS	106	0	50	17	1	174
5 bact. per community	ES	0	0	0	0	1	1
95,256 communities	AS	106	0	50	17	0	173
lipids (28 targets)	KS	3	140	22	20	0	185
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58,520 communities	AS	0	140	22	19	0	181
sugar derivatives (58 targets)	KS	11	30	78	23	0	142
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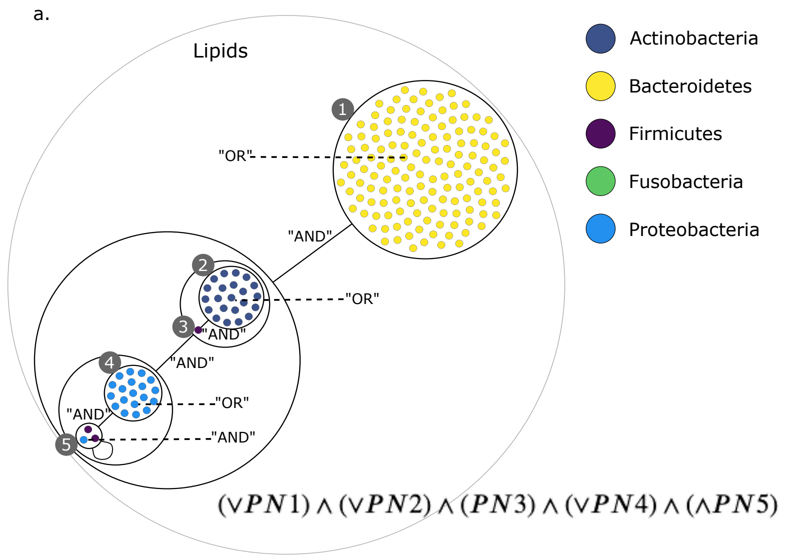

$$(\vee PN1) \wedge (\vee PN2) \wedge (PN3) \wedge (\vee PN4) \wedge (\wedge PN5)$$

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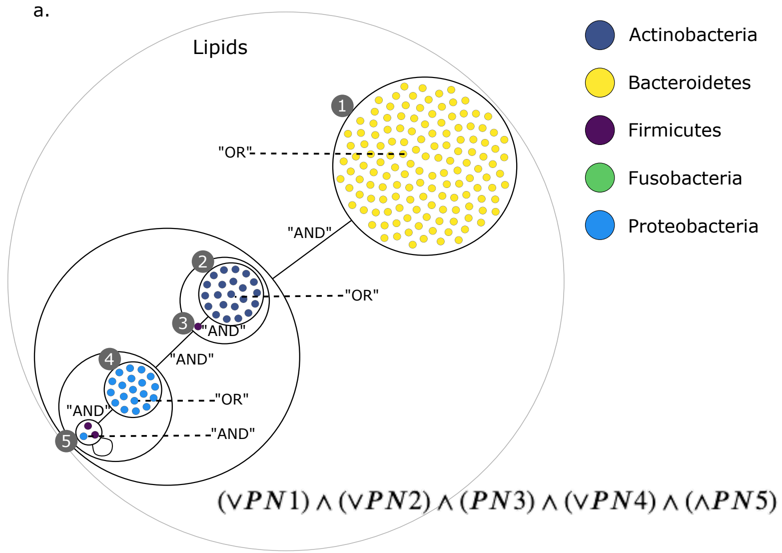

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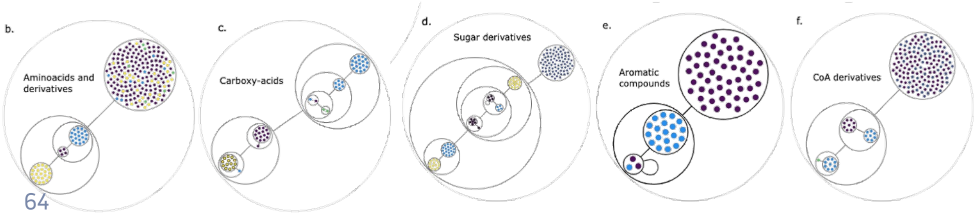

Visualisation of key species and their association in minimal communities highlights groups of equivalent roles in the community

5. Tutorial

https://gitlab.inria.fr/cfrioux/ebame

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1. **Metabolic network exploration:**
    a. Visualisation of metabolic networks
    b. Exploration of the exchange format: SBML
2. **Metabolic network reconstruction**
    a. Structural annotation with Prodigal
    b. Reconstruction with gapseq
    c. Reconstruction from Kegg knowledge base
    d. Exploration of metabolic networks reconstructed with Pathway Tools
3. **Metabolic network modelling of a unique species**
    a. Using toy data
    b. Using real data
4. **Metabolic network modelling of a community**
    a. Using toy data
    b. Using real data

Acknowledgements

Discrete modelling of metabolism

- Arnaud Belcour
- Méziane Aite
- Anne Siegel
- Chabname Ghassemi Nedjad
- Maxime Lecomte
- Coralie Muller
- Falk Hildebrand

EBAME organisation team

- Lois Maignien
- Stéphane Renard
- Christophe Blanchet

HPC: