

Exploration of metabolism in microbial ecosystems

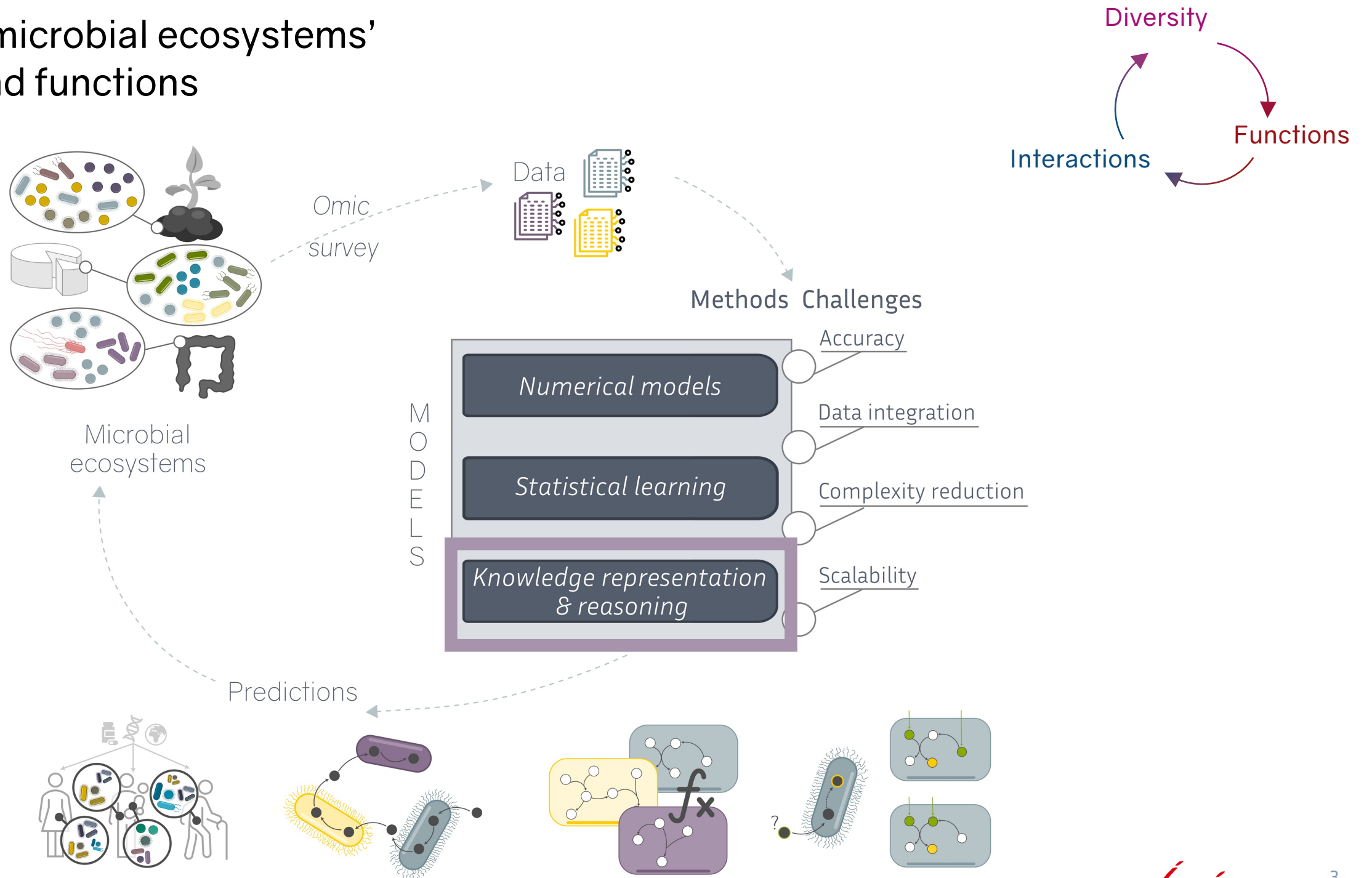
From the metabolic network to the (discrete) model



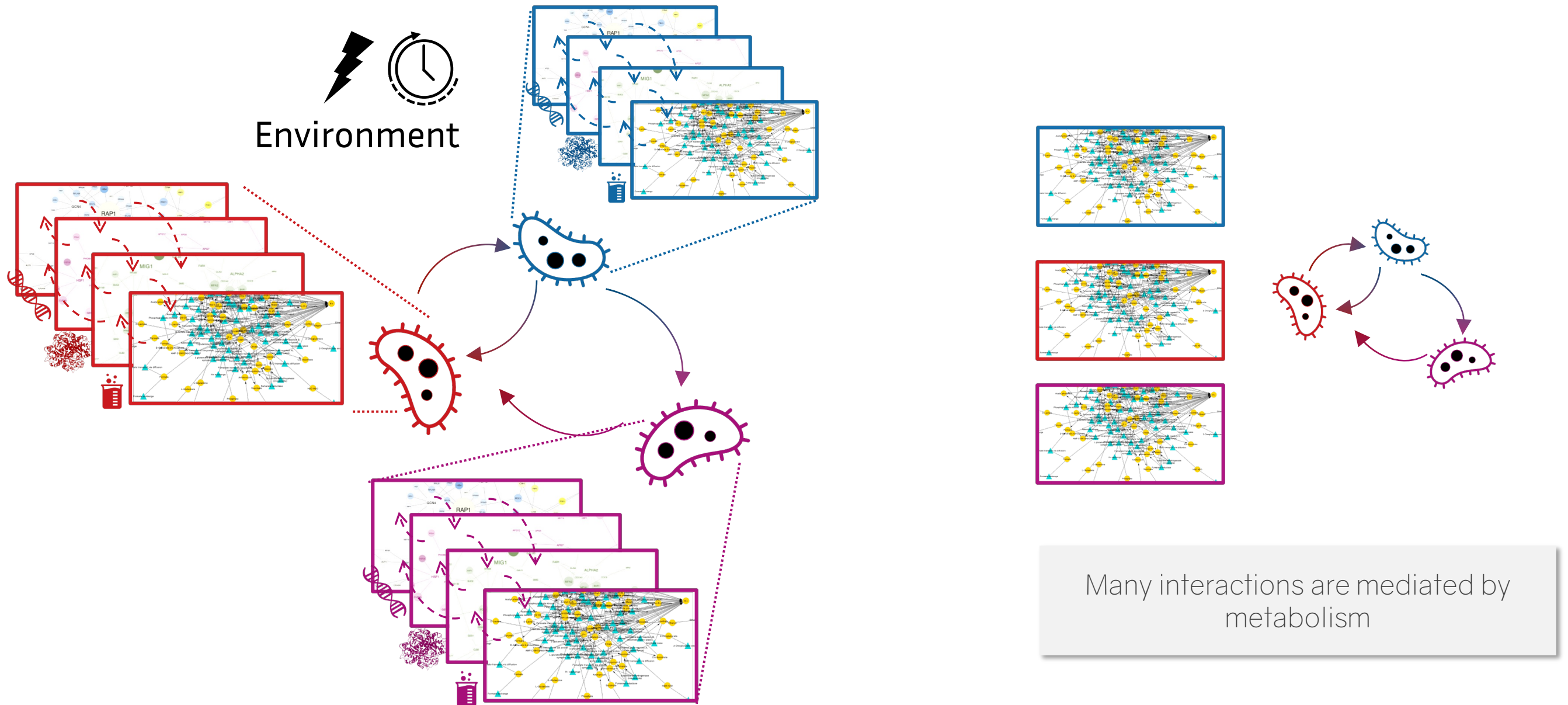
AI for Microbiome Workshop

October 2nd, 2025

Modelling microbial ecosystems' diversity and functions



From (microbial) systems biology to systems ecology



Predicting gastrointestinal drug effects using contextualized metabolic models

Marouen Ben Guebila¹, 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

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

Received: 9 November 2021

Accepted: 30 November 2022

Published online: 19 January 2023

Almut Heinken^{1,2,3}, Johannes Hertel^{1,4}, Geeta Acharya⁵, Dmitry A. Ravcheev^{1,2}, Malgorzata Nyga⁶, Onyedika Emmanuel Okpala⁷, Marcus Hogan^{1,2}, Stefania Magnúsdóttir⁸, Filippo Martinelli^{1,2}, Bram Nap^{1,2}, German Preciat⁹, Janaka N. Edirisinghe^{10,11}, Christopher S. Henry¹¹, Ronan M. T. Fleming^{1,9} & Ines Thiele^{1,2,12,13}

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

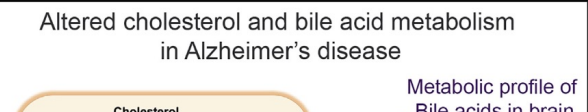
Ines Thiele^{1,2,3,4,*}, Swagatika Sahoo^{4,†}, Almut Heinken¹, Johannes Hertel^{1,5}, Laurent Heirendt⁴, Maïke K Aurich⁴ & Ronan MT Fleming^{1,6,**}

Systematic assessment of secondary bile acid metabolism in gut microbes reveals distinct metabolic capabilities in inflammatory bowel disease

Almut Heinken¹, Dmitry A. Ravcheev¹, Federico Baldini², Laurent Heirendt², Ronan M. T. Fleming³ and Ines Thiele^{1,2,4*}

Metabolic Network Analysis Reveals Altered Bile Acid Synthesis and Metabolism in Alzheimer’s Disease

Graphical Abstract



Authors

Priyanka Baloni, Cory C. Funk, Jingwen Yan, ..., The Alzheimer’s Disease Metabolomics Consortium, Rima Kaddurah-Daouk, Nathan D. Price

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 Seshadri², Natalia Ivanova², Panagiotis G. Kougias^{1,5*}, Nikos Kyrpides² and Irini Angelidaki¹

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

Emrah Özcan^{1,2}, Merve Seven³, Burcu Şirin³, Tunahan Çakır⁴, Emrah Nikerel³, Bas Teusink¹, Ebru Toksoy Öner²

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

Arianna Basile^a, Stefano Campanaro^{a,b,*}, Adam Kovalovszki^c, Guido Zampieri^{a,d}, Alessandro Rossi^a, Irini Angelidaki^c, Giorgio Valle^{a,1}, Laura Treu^{a,1}

Longitudinal flux balance analyses of a patient with episodic colonic inflammation reveals microbiome metabolic dynamics

Arianna Basile^{a,b}, Almut Heinken^{b,c}, Johannes Hertel^d, Larry Smarr^e, Weizhong Li^f, Laura Treu^a, Giorgio Valle^a, Stefano Campanaro^{a,g}, and Ines Thiele^{b,c,g,h,i}

Integrating metagenomic binning with flux balance analysis to unravel syntrophies in anaerobic CO₂ methanation

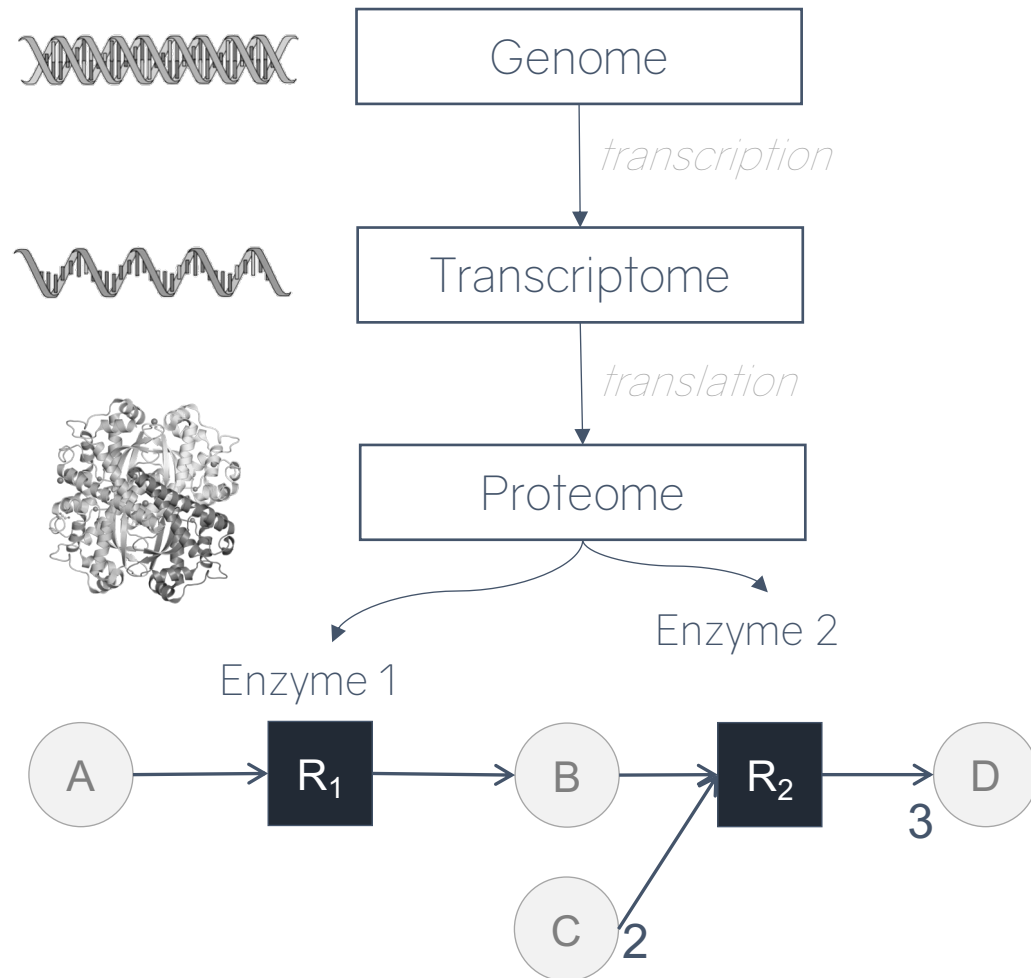
Nicola De Bernardini¹, Arianna Basile¹, Guido Zampieri¹, Adam Kovalovszki², Beatrix De Diego Diaz³, Elisabetta Offer¹, Nantharat Wongfaed⁴, Irini Angelidaki⁵, Panagiotis G. Kougias^{6*}, Stefano Campanaro^{1,7,*†} and Laura Treu^{1†}

1. Metabolic networks





Connecting genomes to functions

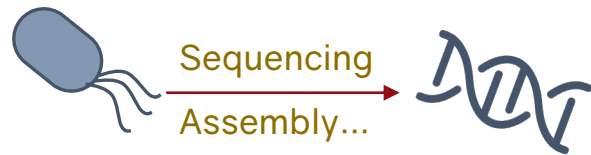


1 genome $\rightarrow$ 1,000 to 5,000 reactions

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

Functional repertoire of the cell

Metabolic networks



> protein_1
ATGGAATCGCATAGCA...
> protein_2
AATGCCTGATCGAATA...

Functional annotation

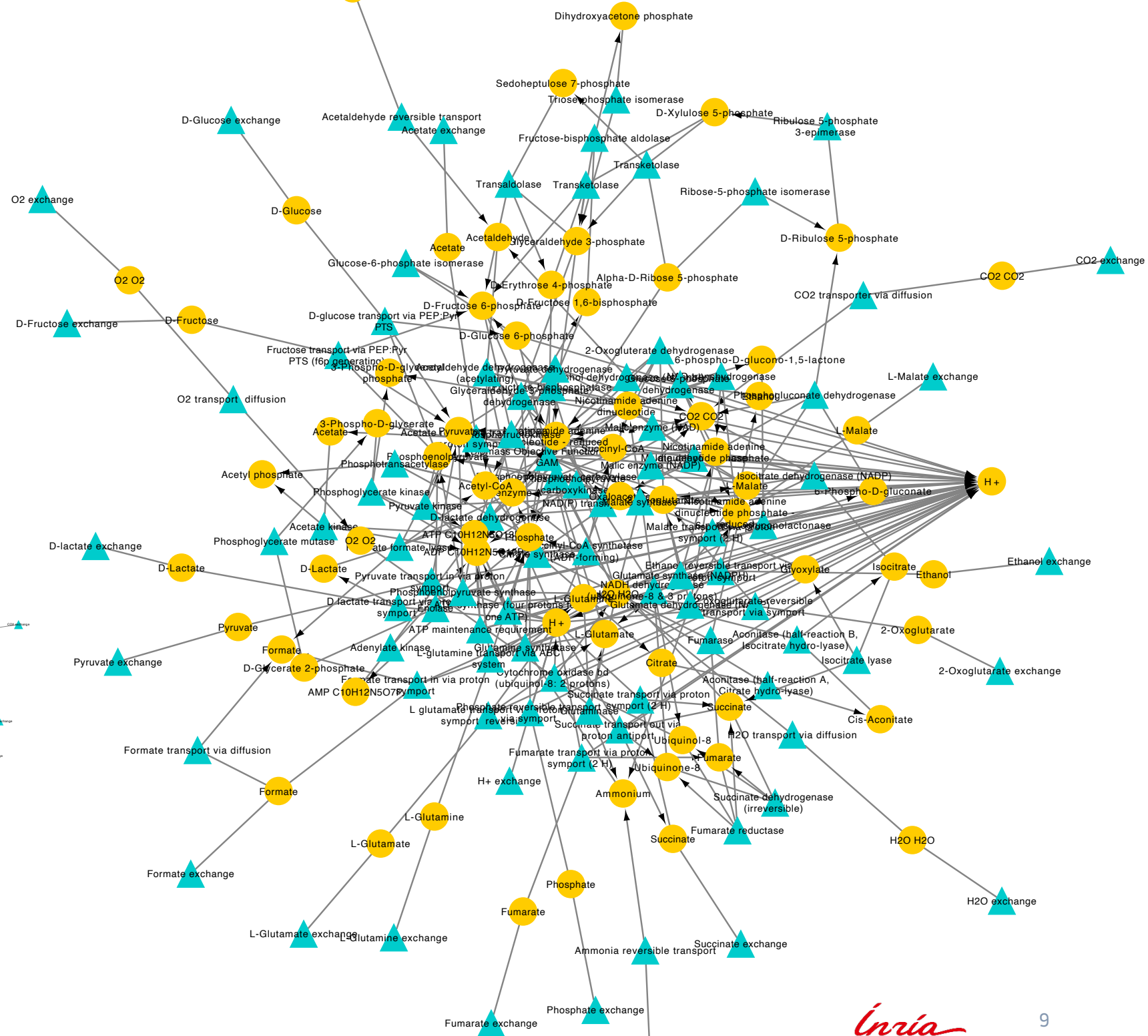
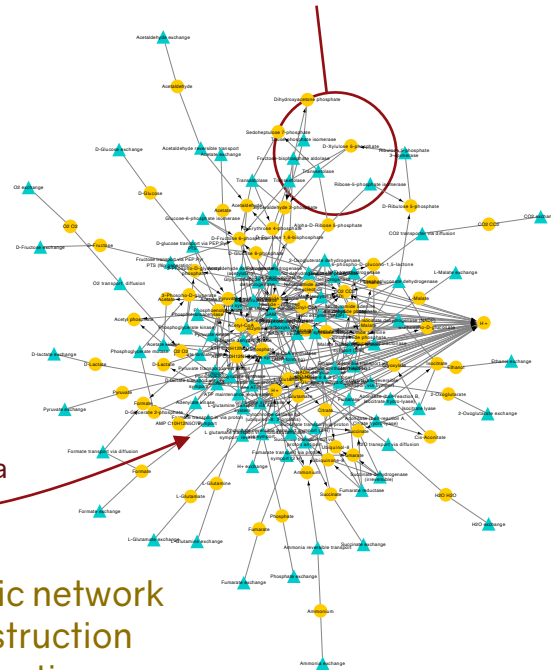
protein	name	EC-number	GO	KEGG	...
protein_1	pfkA	2.7.1.11	-	K00850	...
protein_2	eno	4.2.1.11	-	K01689	...
...	...	...	...	...	...

± other omics data

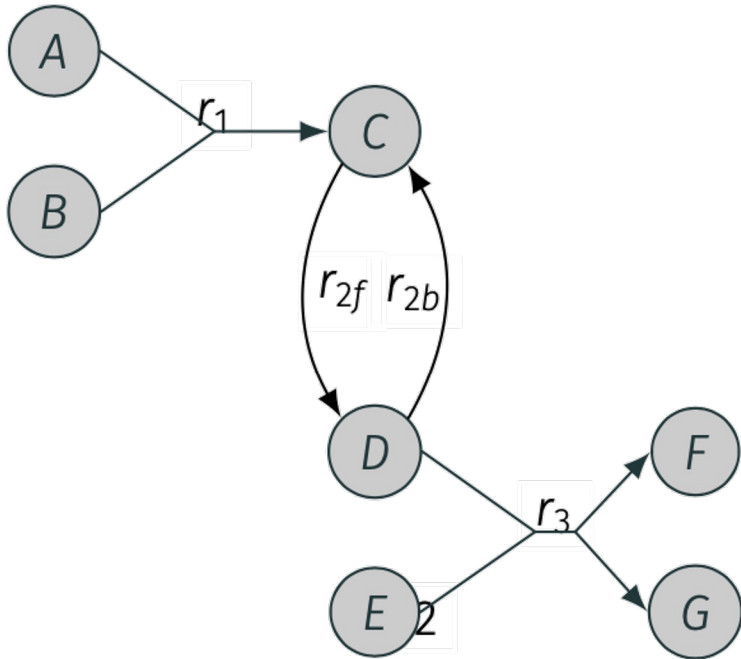
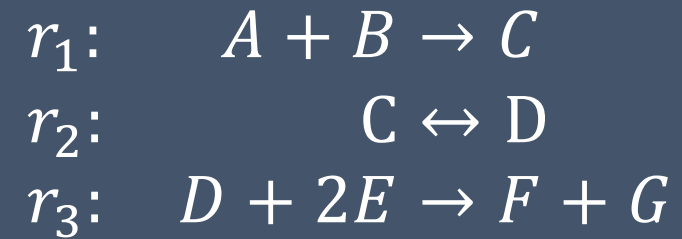
Function	
Carbohydrate degradation	
Lipid synthesis	
Vitamin biosynthesis	
Antibiotic resistance	
...	...

Metabolic network
reconstruction
& curation

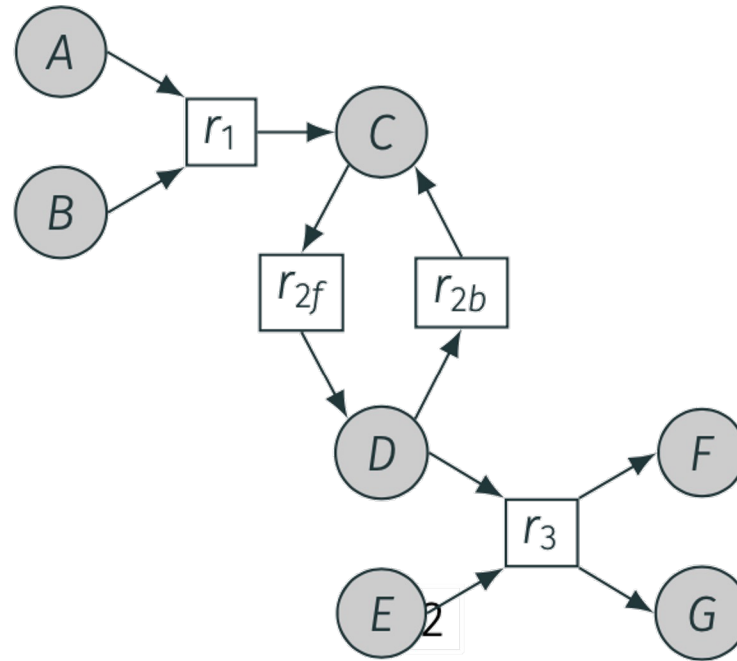
1000-5000
reactions
 $A + 2B \rightarrow 2C + D$



Representations of metabolic networks



Hypergraph



Bipartite graph

$$\begin{matrix} & r_1 & r_{2f} & r_{2b} & r_3 \\ \begin{matrix} A \\ B \\ C \\ D \\ E \\ F \\ G \end{matrix} & \begin{pmatrix} -1 & 0 & 0 & 0 \\ -1 & 0 & 0 & 0 \\ +1 & -1 & +1 & 0 \\ 0 & +1 & -1 & -1 \\ 0 & 0 & 0 & -2 \\ 0 & 0 & 0 & +1 \\ 0 & 0 & 0 & +1 \end{pmatrix} & = S \end{matrix}$$

Stoichiometric matrix

Tutorial content

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

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

2. Reconstruction of metabolic networks





Input data

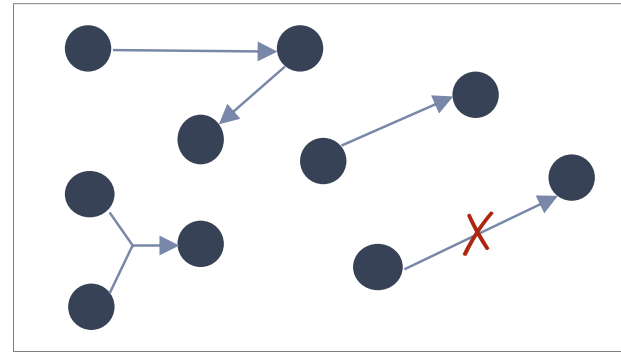
- minimal input: a genome or a proteome
 - a reference genome
 - a Metagenomic-Assembled Genome (MAG)
- Genomes must be structurally annotated
 - e.g. with Prodigal [Hyatt et al 2010] for prokaryotes
- Some tools require also a functional annotation
 - e.g. with EggNOG mapper [Cantalapiedra et al 2021]
 - Prokka [Seeman 2014]
 - Bakta [Schwengers 2021]

⚠️ MAG completeness
(Eisenhofer et al 2023)



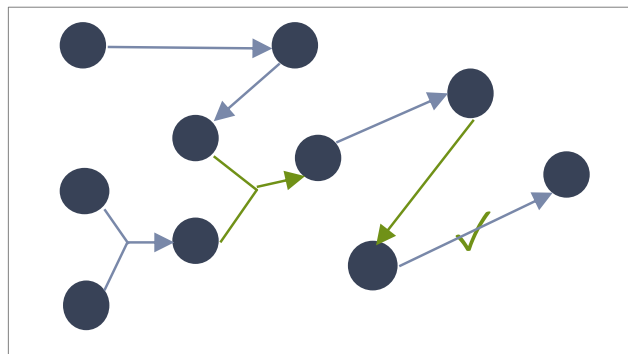
Metabolic network inference: bottom-up procedure

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



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

2. Semi-manual / automatic curation: *guided*



f_x



Metabolic objective
e.g. biomass / growth

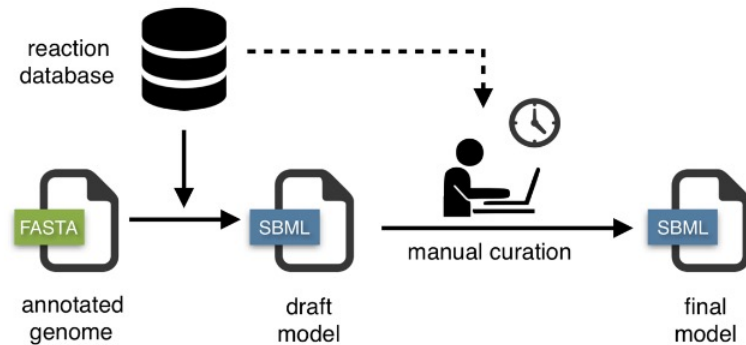
Automated in gapseq [Zimmermann et al 2021]
Meneco [Prigent et al 2017],
BioISO [Cruz et al 2021]...

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

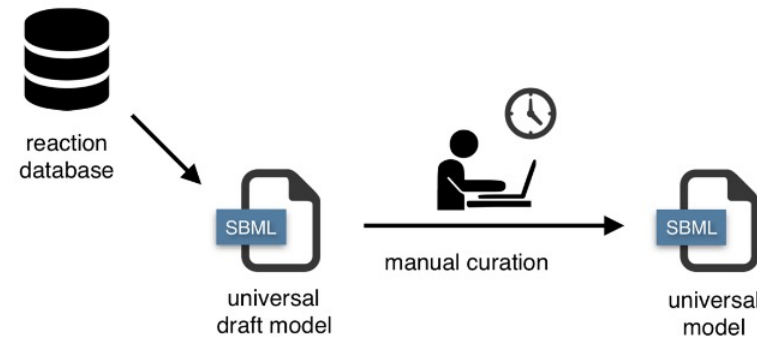


Alternative: top-down reconstruction

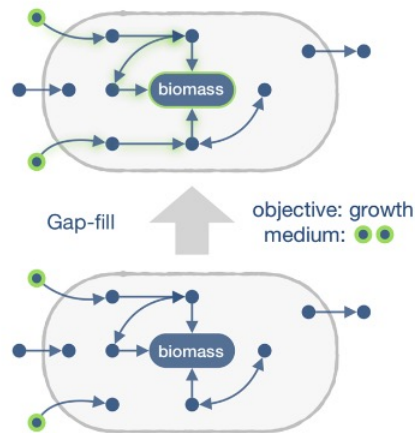
A Classic reconstruction workflow



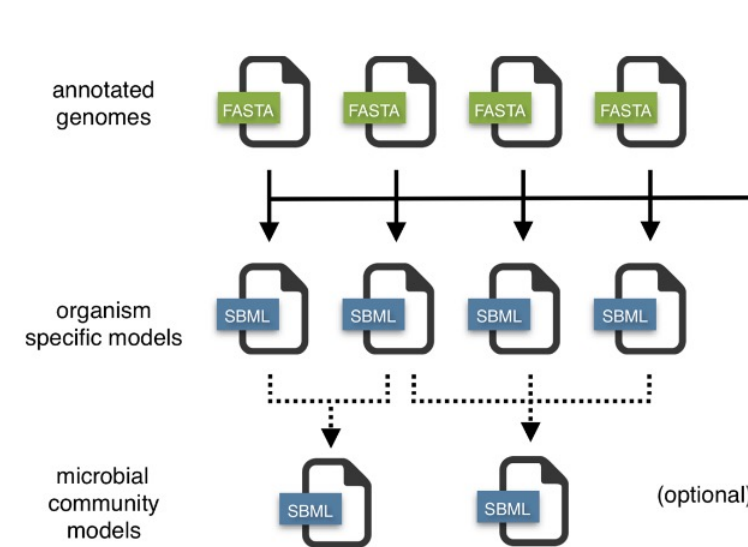
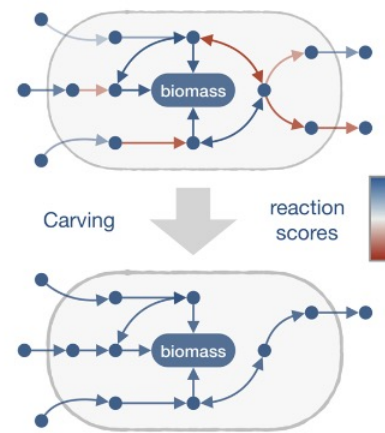
B CarveMe reconstruction workflow



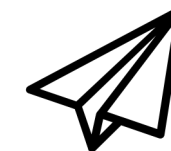
C Bottom-up reconstruction



D Top-down reconstruction



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]
- Bakta [Schwengers 2021]

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

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3. Discrete metabolic models

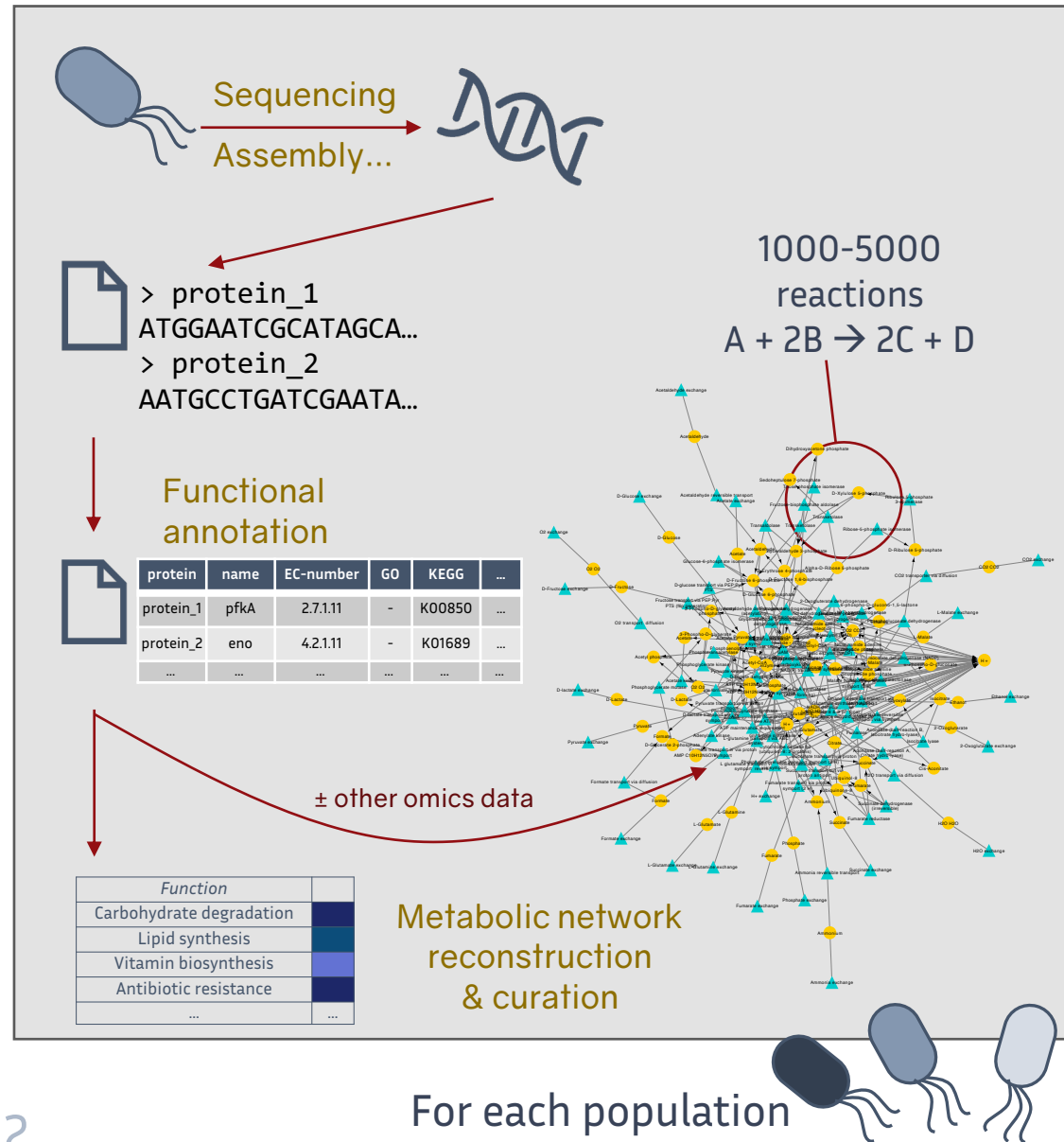


“We were promised AI...”



Yes, but old-fashioned AI! Buckle up to enter the fascinating
world of machine... reasoning

Metabolic modelling

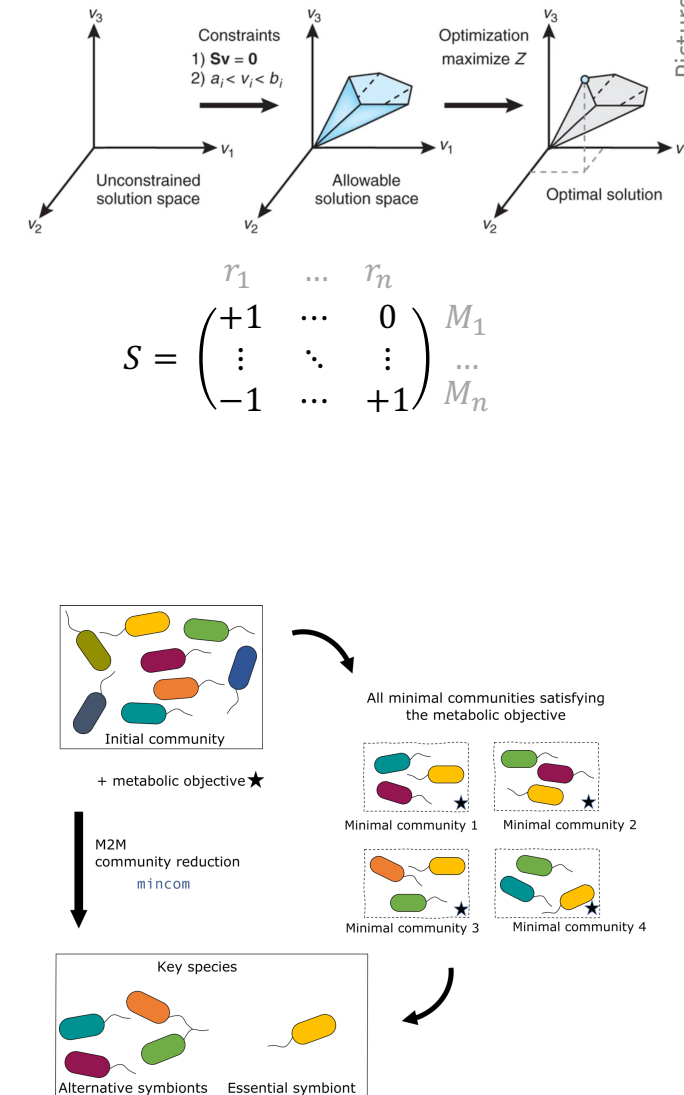


Environmental conditions

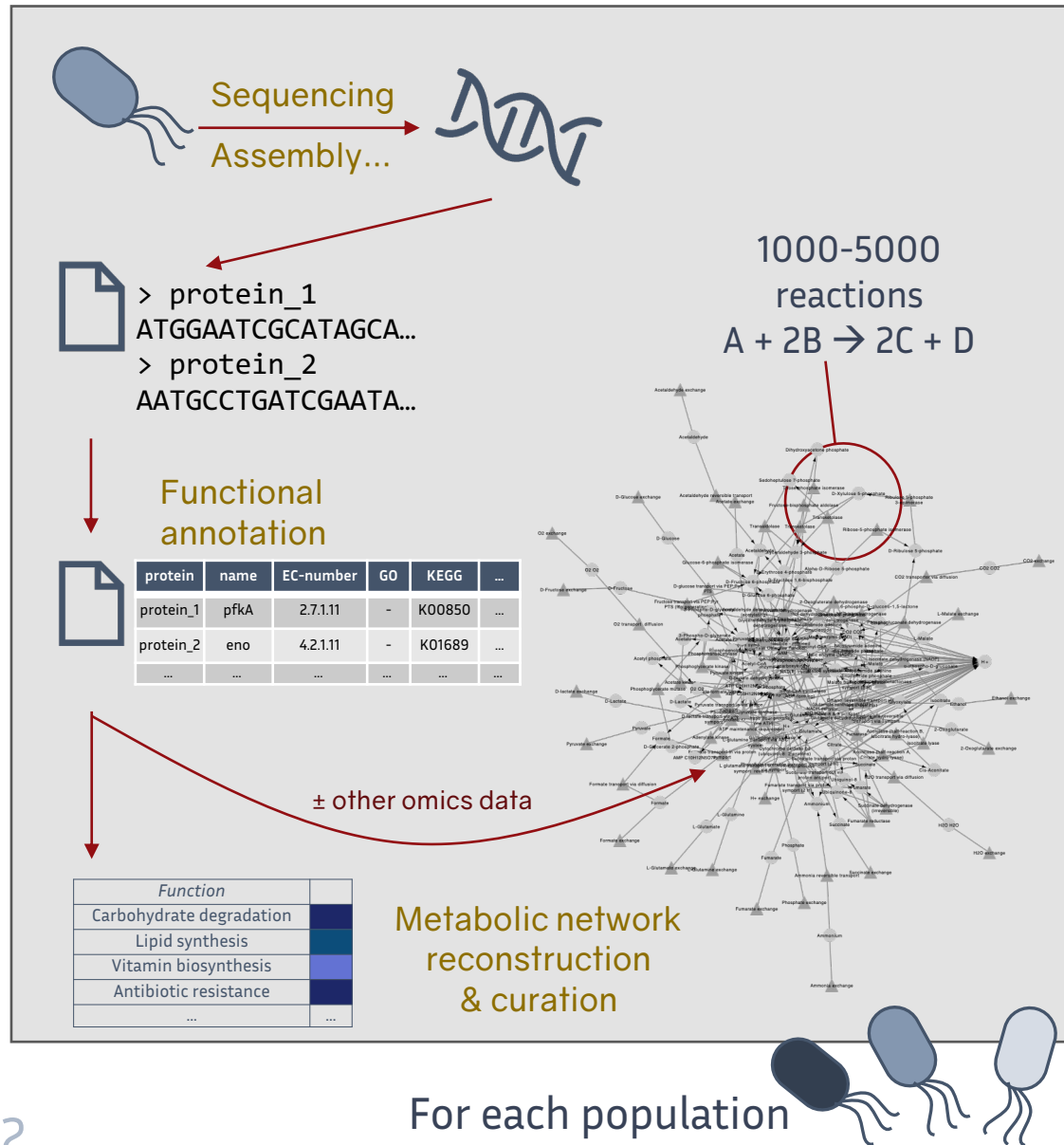
± metabolic objective

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

Automated reasoning using logic paradigms and combinatorial optimisation problems



Metabolic modelling



Environmental conditions

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

± metabolic objective

Automated reasoning using logic paradigms and combinatorial optimisation problems

Not what we'll do today!

Constraints
1) $Sv = 0$

Optimization
maximize Z

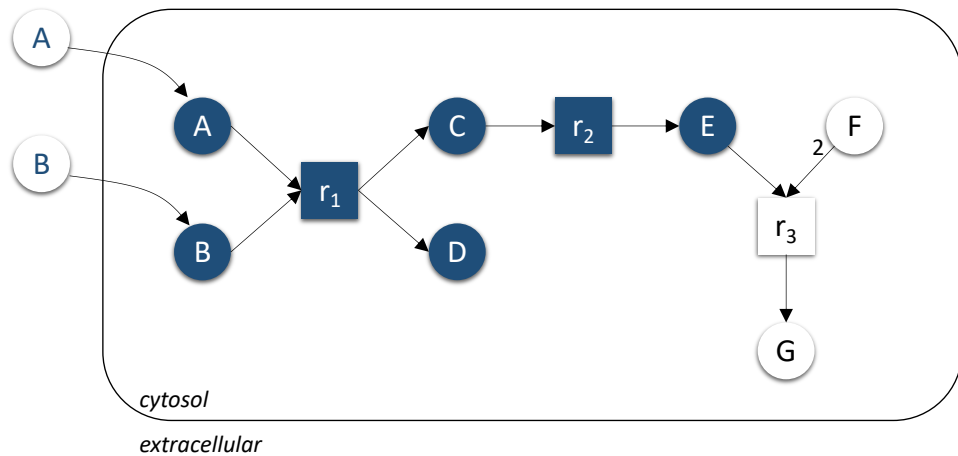
$\begin{pmatrix} -1 & \dots & +1 \end{pmatrix} M_n$

Today's topic!

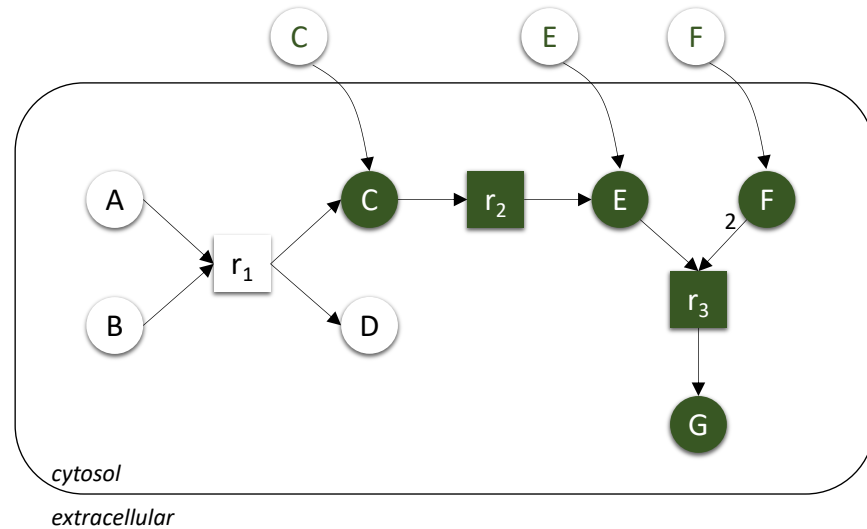
$\begin{pmatrix} +1 & \dots & 0 \end{pmatrix}$

$\begin{pmatrix} -1 & \dots & +1 \end{pmatrix}$

Boolean abstraction of metabolic producibility



Network expansion [Ebenhöh et al 2004]
 → Scope of seeds
 → Discrete modelling



$$\text{scope}(G, S) = \bigcup_i M_i,$$

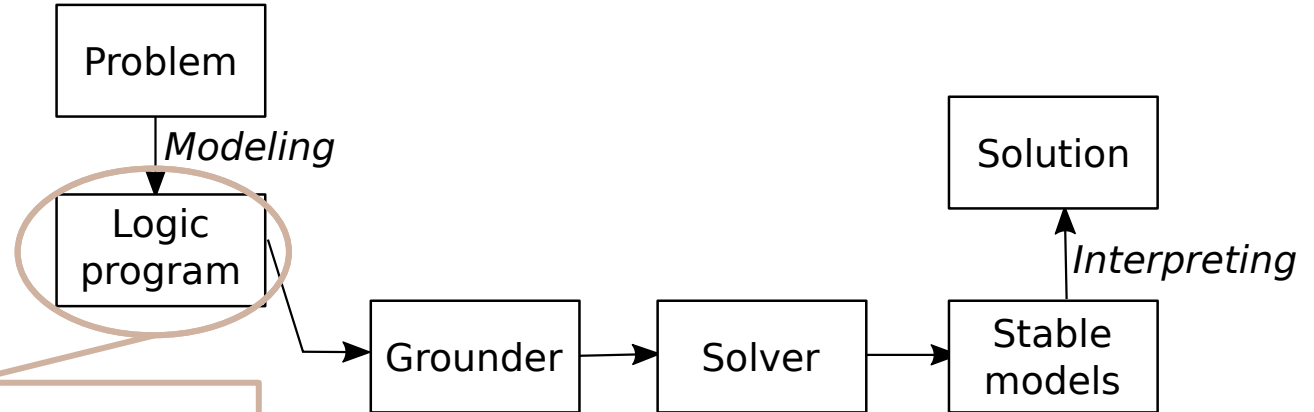
where $M_0 = S$ and

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

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

A flexible implementation system

- Answer Set Programming (ASP)
- Logic paradigm
- Knowledge representation & reasoning
- Optimisation + combination to linear prog.

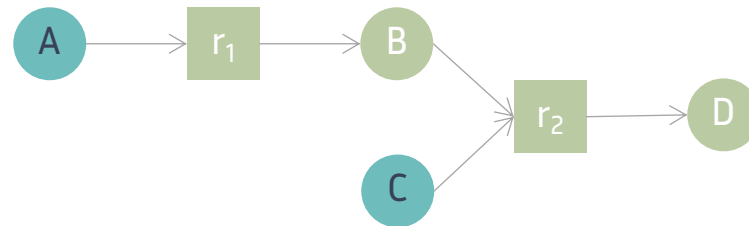


```
reaction("r1").  
reactant("A", "r1").  
product("B", "r1").
```

```
reaction("r2").  
reactant("B", "r2").  
reactant("C", "r2").  
product("D", "r2").
```

```
seed("A").  
seed("C").
```

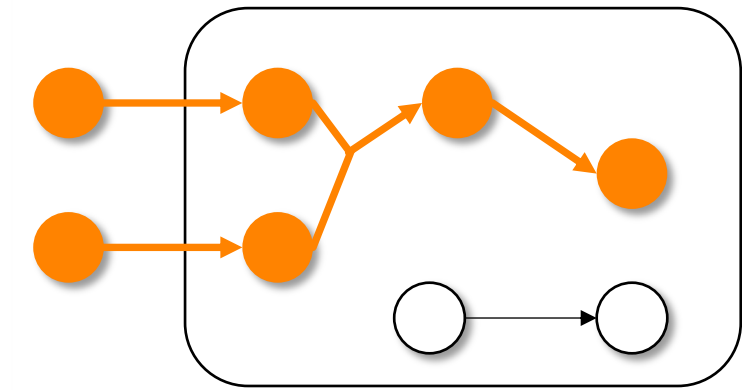
```
scope(M) :- seed(M).  
scope(M) :- product(M,R); reaction(R); scope(N) : reactant(N,R).
```



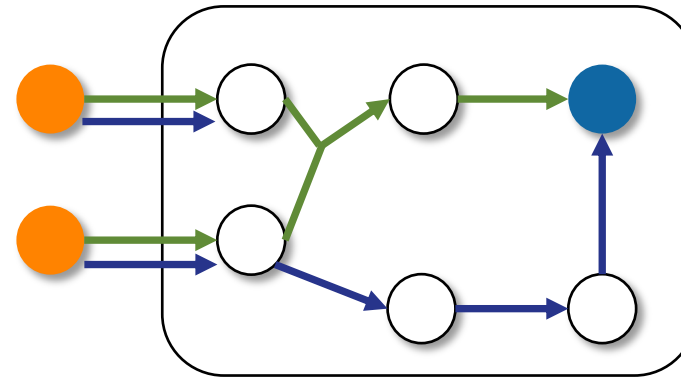
Machine reasoning

Applications of the Boolean abstraction of producibility

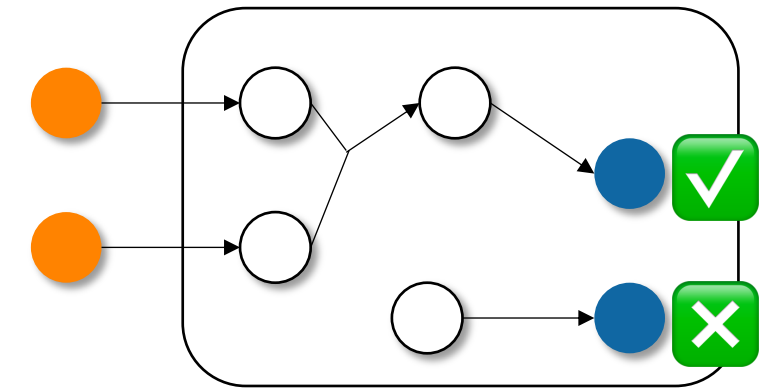
Prigent et al 2017, Aite et al 2017, Frioux et al 2018,
Frioux et al 2019, Ghassemi Nedjad et al 20224



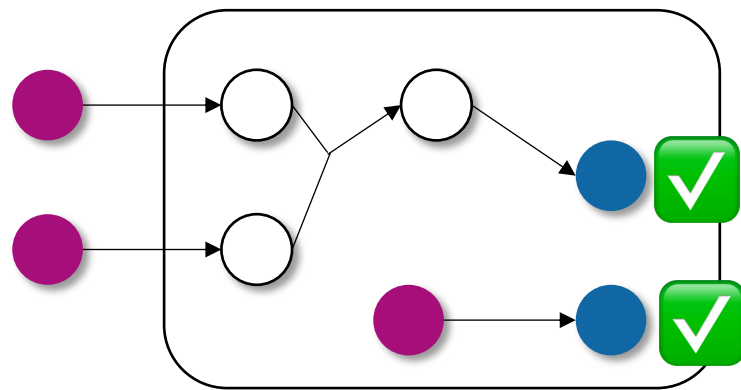
Scope calculation / Activated reactions



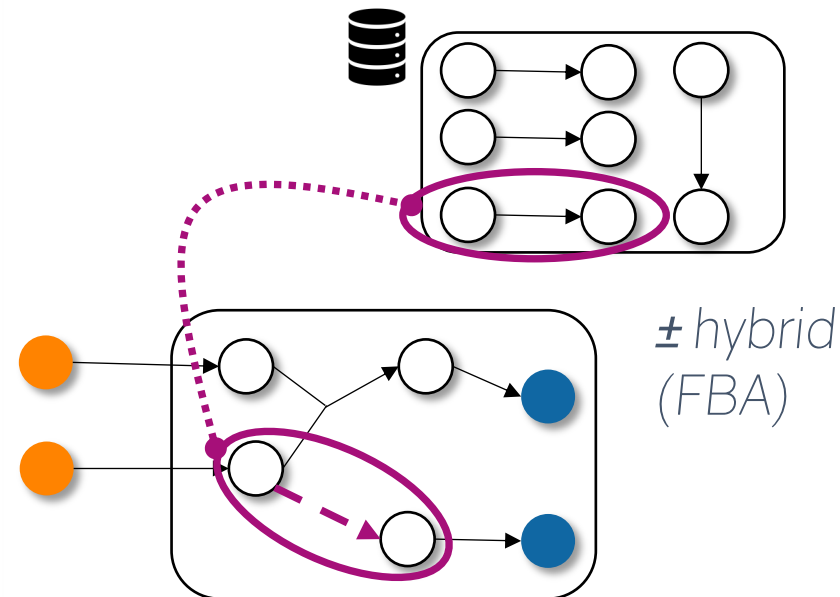
Pathway detection



Producibility checking

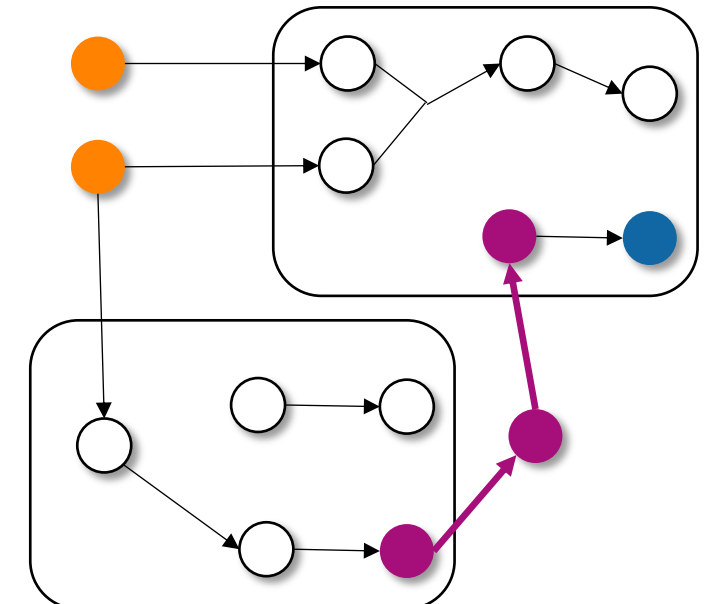


Medium prediction



Gap-filling

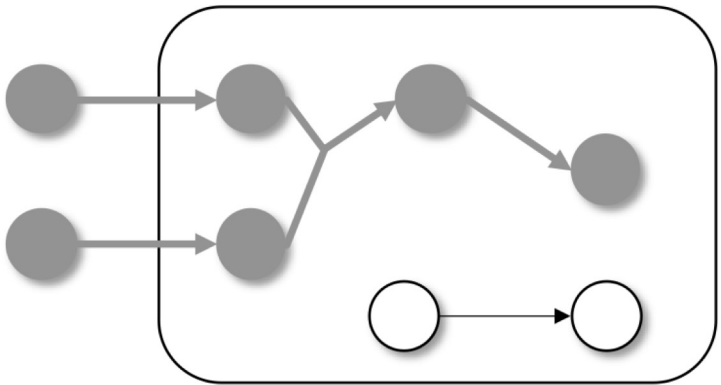
$\pm$ hybrid
(FBA)



Community analysis

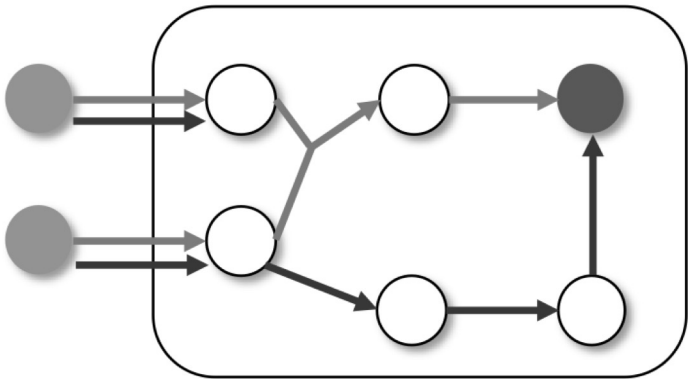
Applications of the Boolean abstraction of producibility

MeneTools



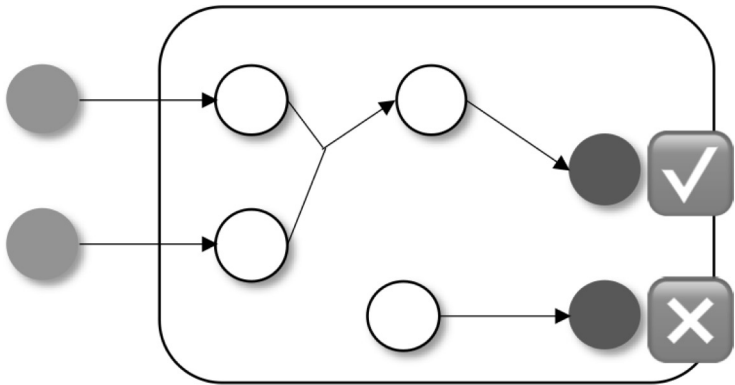
Scope calculation / Activated reactions

MeneTools



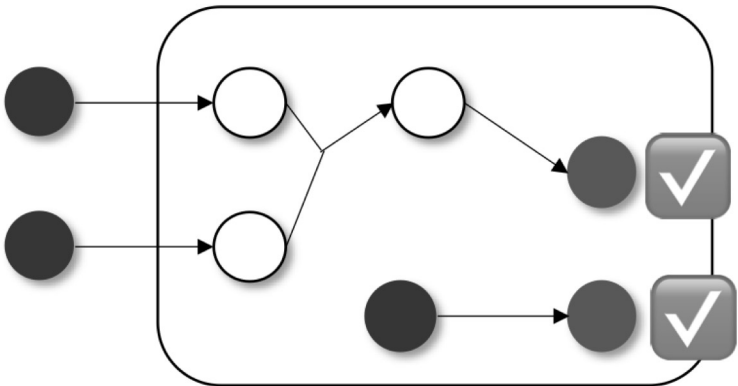
Pathway detection

MeneTools



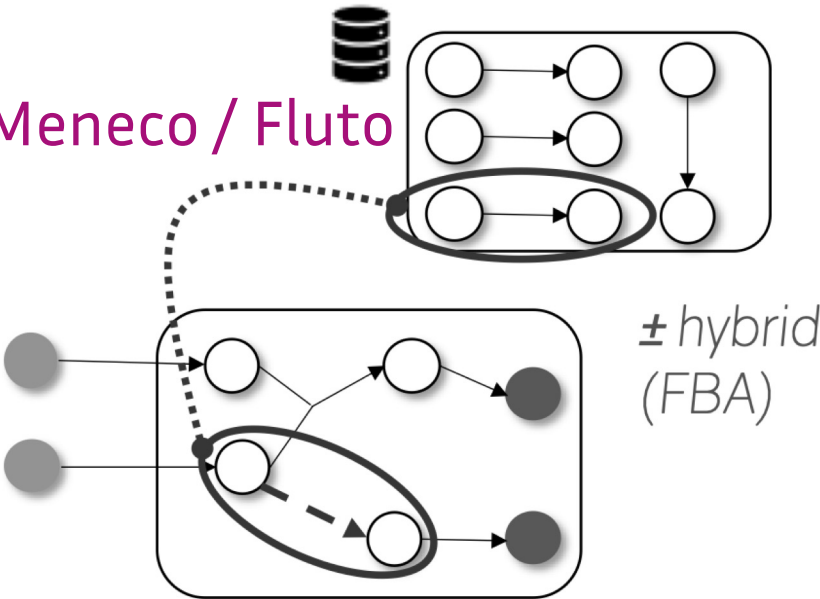
Producibility checking

Seed2LP



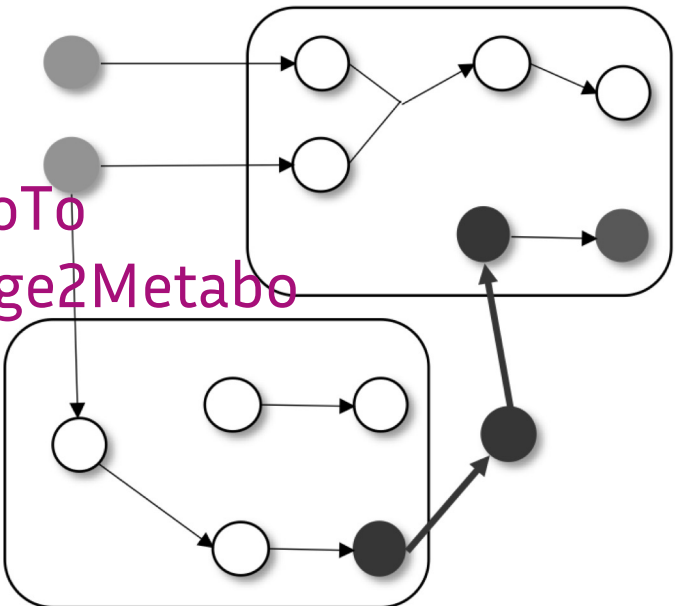
Medium prediction

Meneco / Fluto



Gap-filling

MiSCoTo
Metage2Metabo



Community analysis

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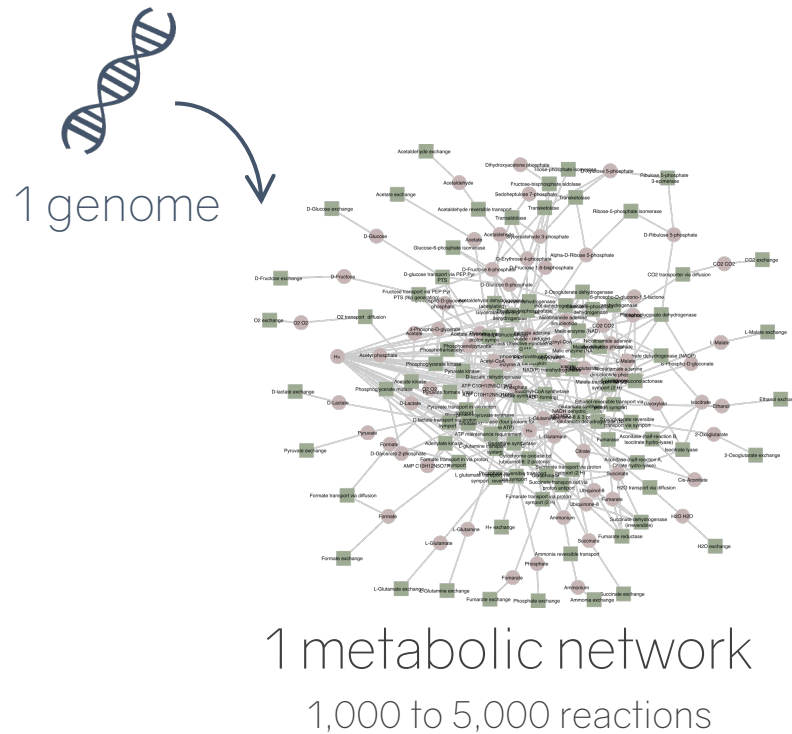
- a. Using toy data
- b. Using real data

4. Mining metabolic models of microbial communities



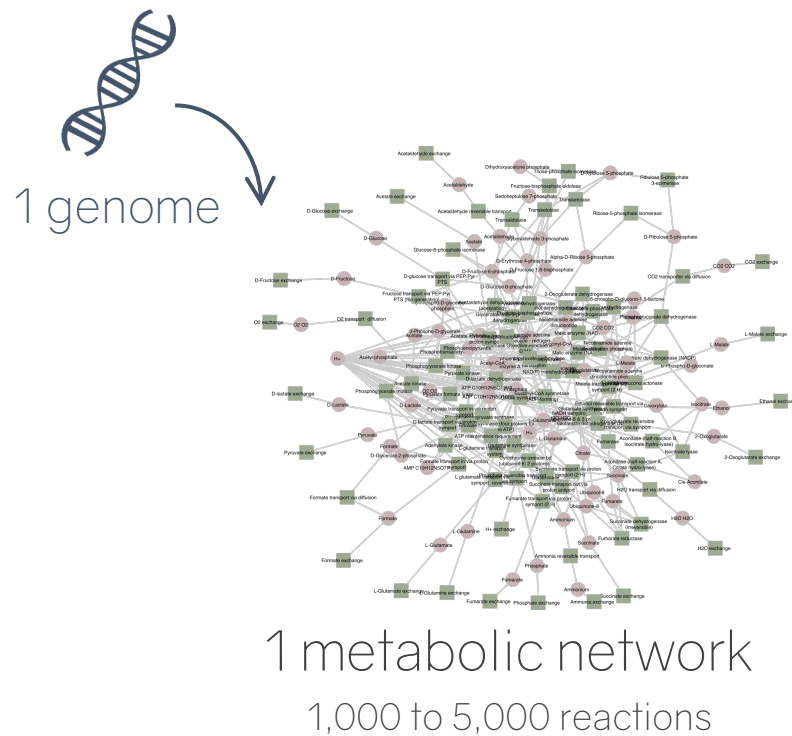
Scale, screen, simplify

Metabolic networks, models, and communities

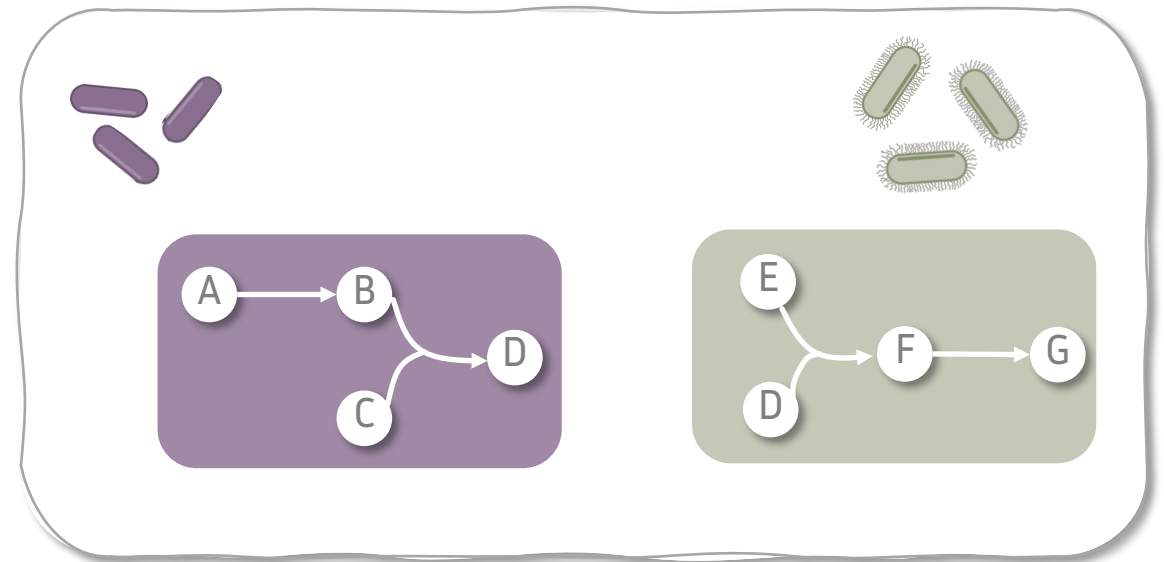


Functional repertoire of the cell.
Can be obtained for all community members
through automatic inference

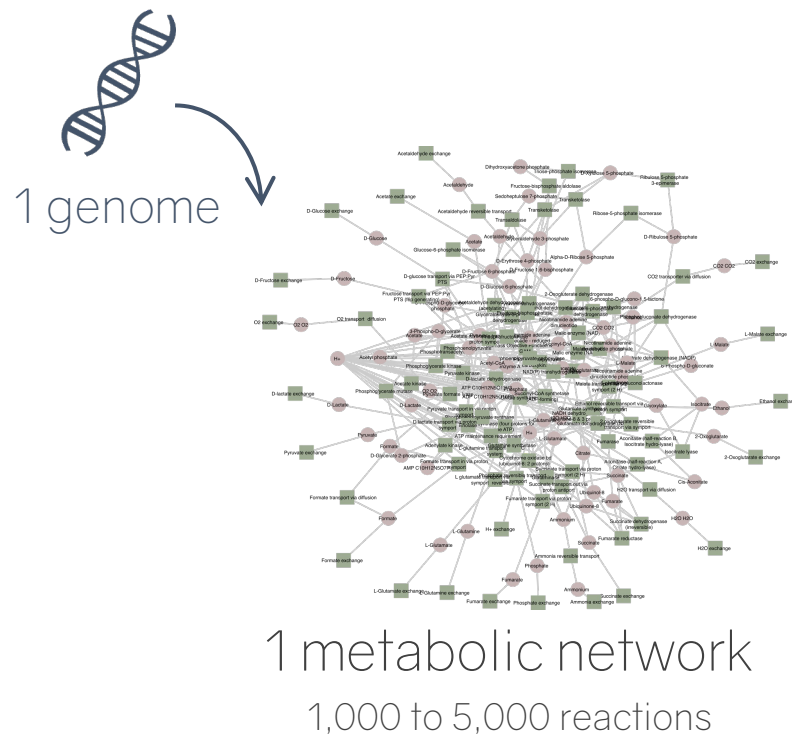
Metabolic networks, models, and communities



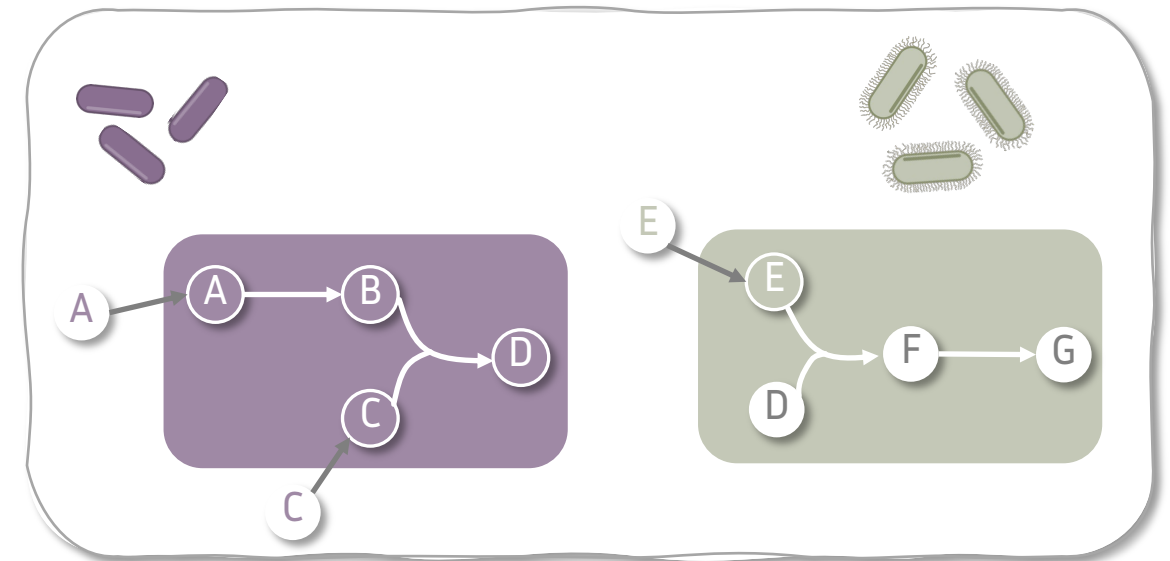
Functional repertoire of the cell.
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Metabolic networks, models, and communities

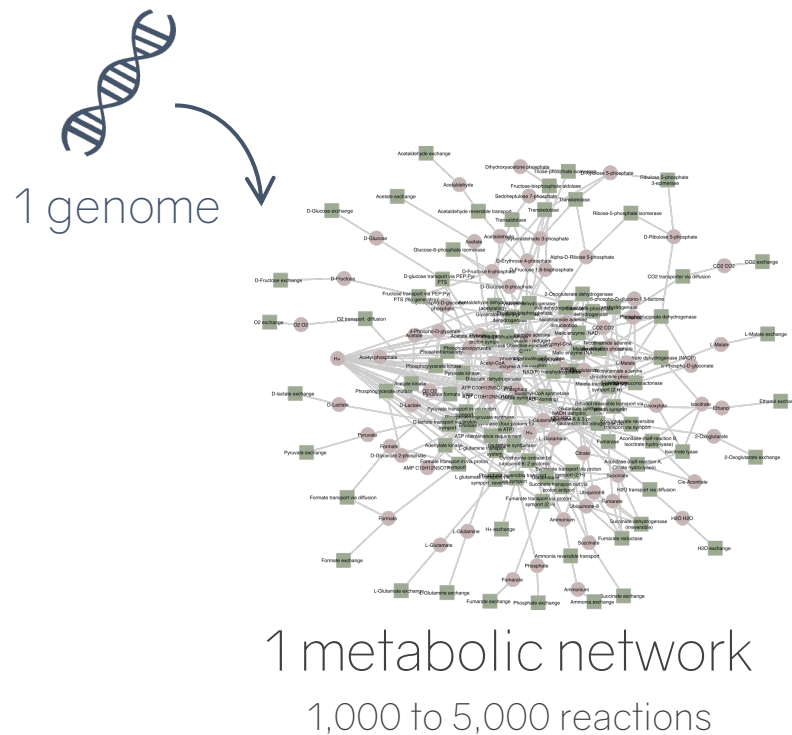


Functional repertoire of the cell.
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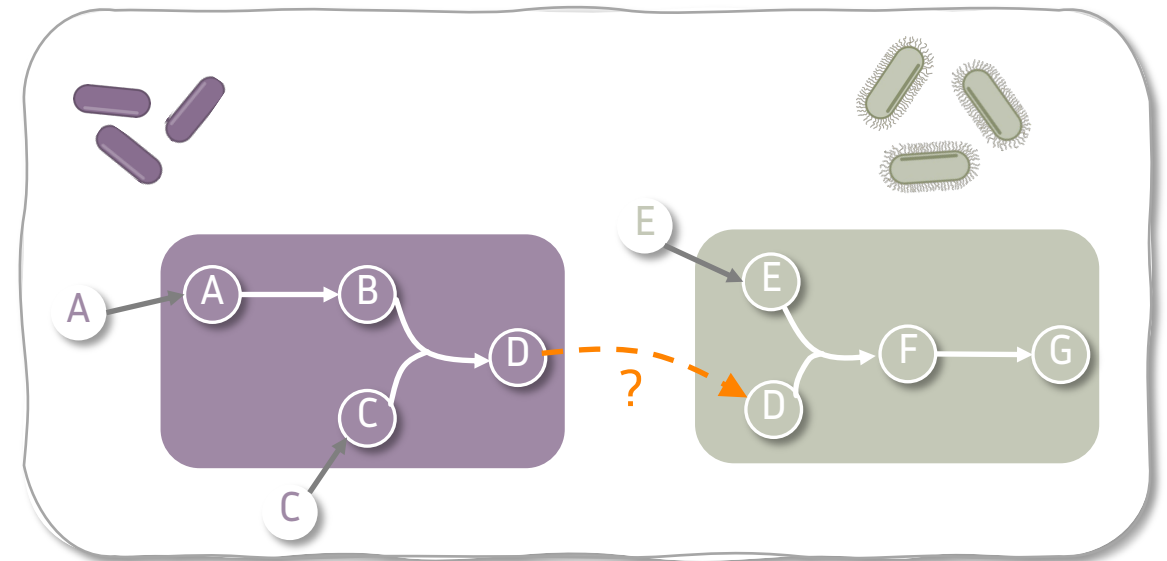


Populations have their individual metabolic potential

Metabolic networks, models, and communities



Functional repertoire of the cell.
Can be obtained for all community members
through automatic inference



Populations have their individual metabolic potential
Metabolic potential expands through cross feeding in
communities, unlocking new metabolic pathways

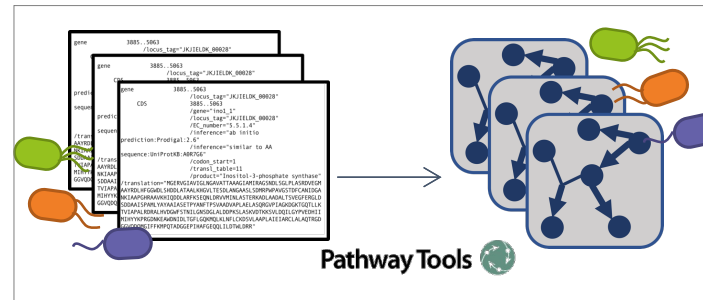
Metage2Metabo:

A pipeline for metabolic screening of communities

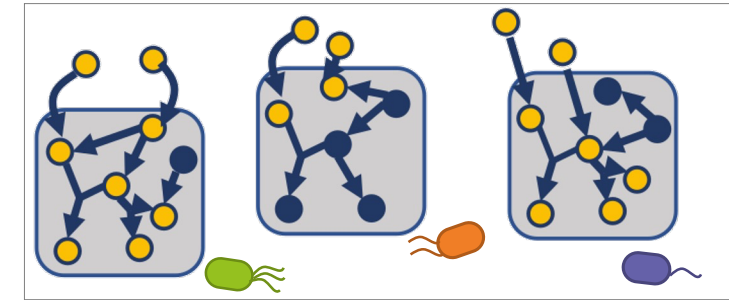
Microbiota

- MAGs
- Reference genomes

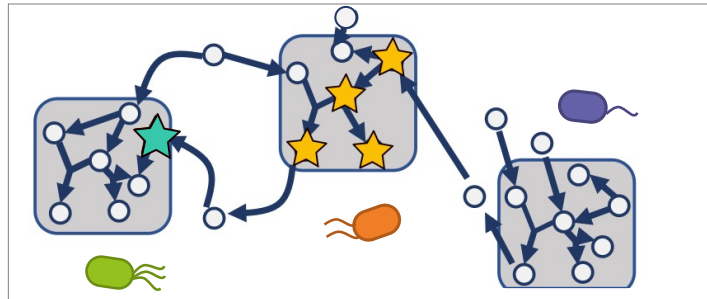
Automatic GSMN reconstruction



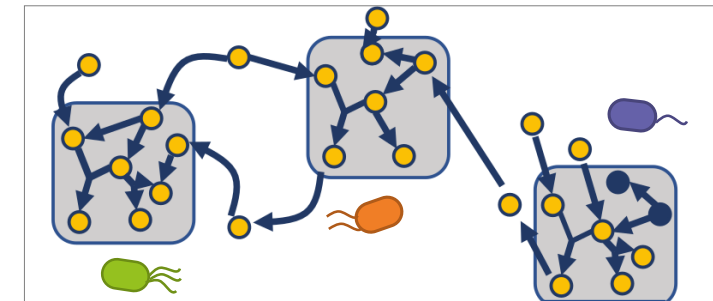
Individual metabolic capabilities



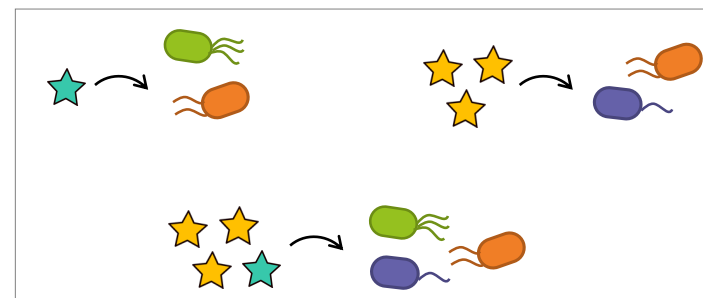
Added value of synergistic interactions



Collective metabolic capabilities



Minimal community selection



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

Github

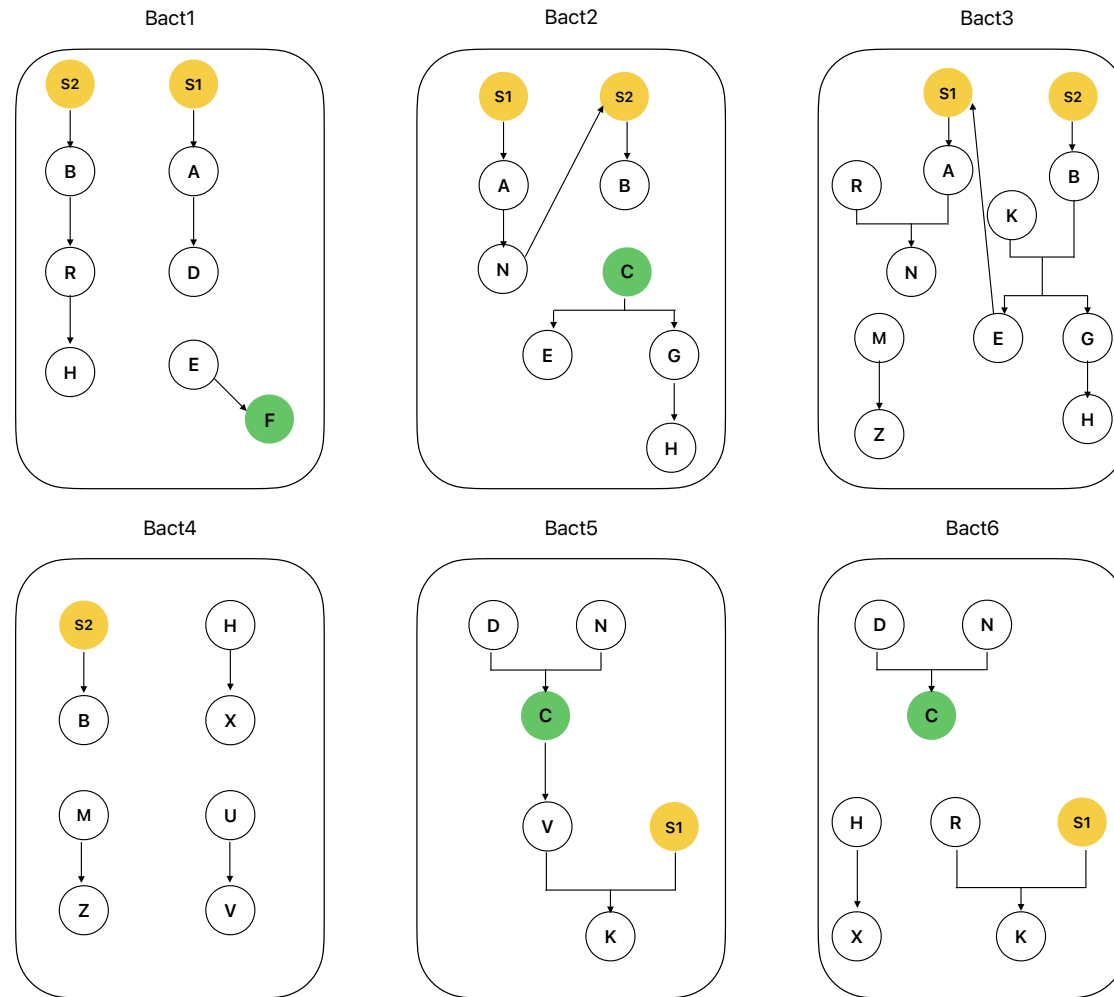
Aureme/Metage2Metabo

[Belcour*, Frioux* et al. eLife 2020]

[Karp et al. Bioinformatics 2002]

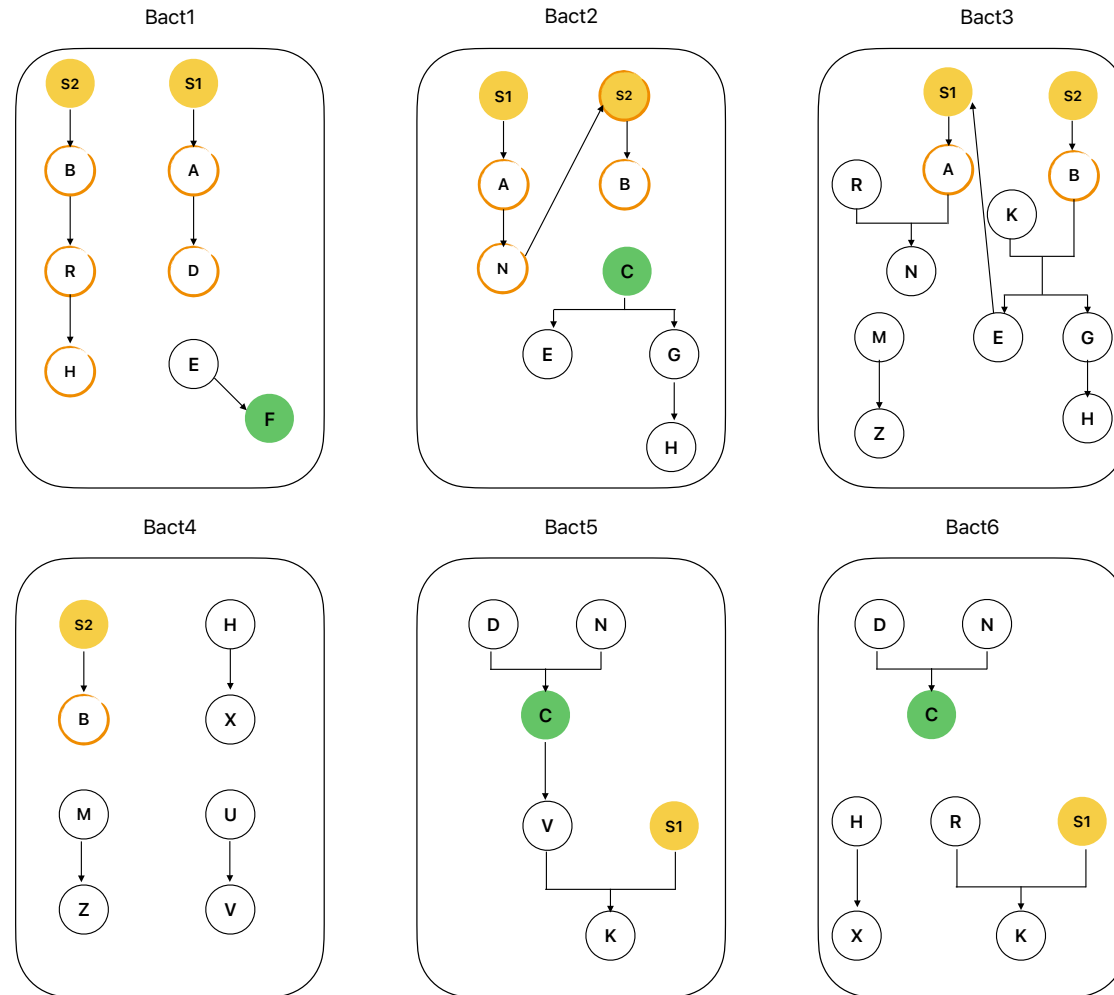
[Frioux et al. Bioinformatics 2018]

Metage2Metabo: application to an example



A community of 6 species, in an environment (yellow),
with compounds of interest (green)

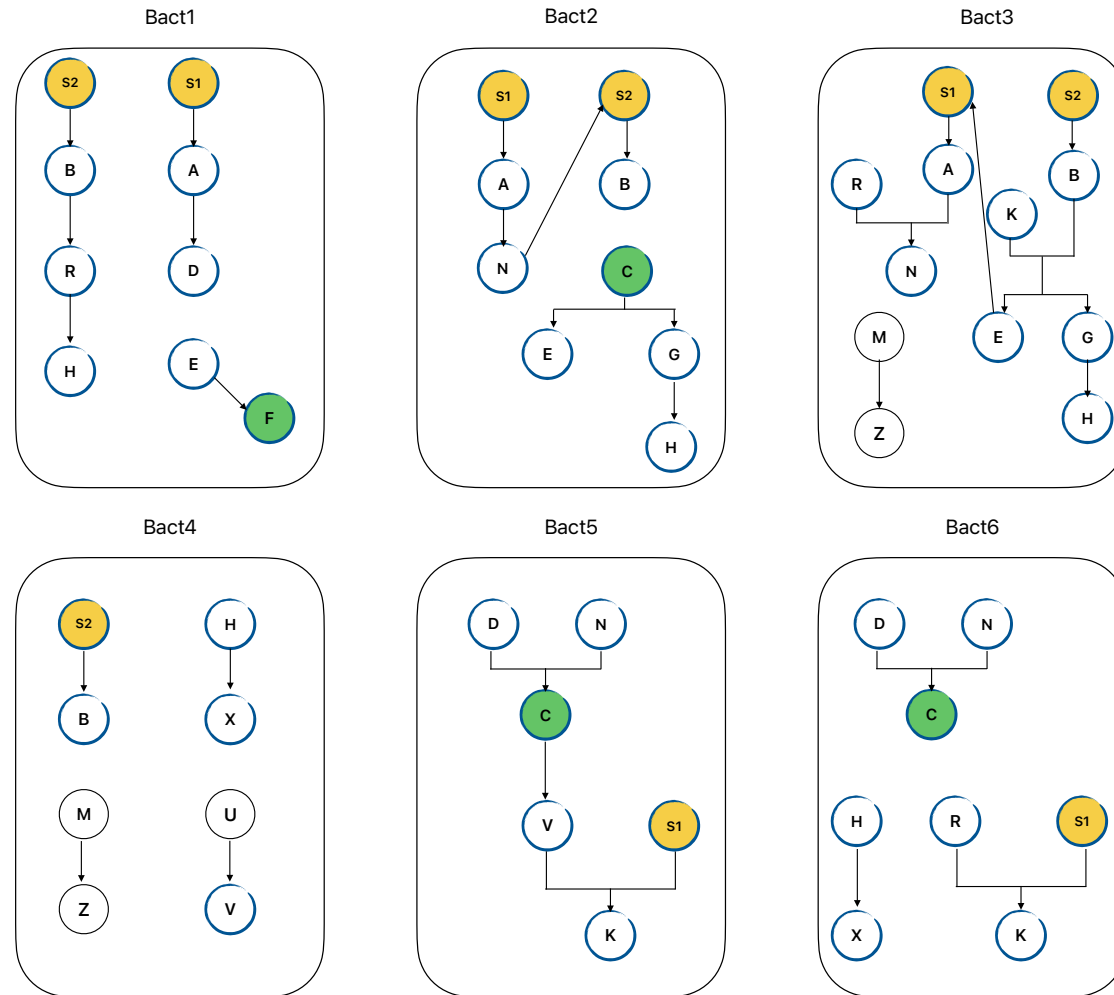
Metage2Metabo: application to an example



Symbiont	Individual Scope
bact1	A, B, D, R, H
bact2	A, B, N, S2
bact3	A, B
bact4	B
bact5	
bact6	

Individual metabolic potential of each symbiont
(orange nodes)

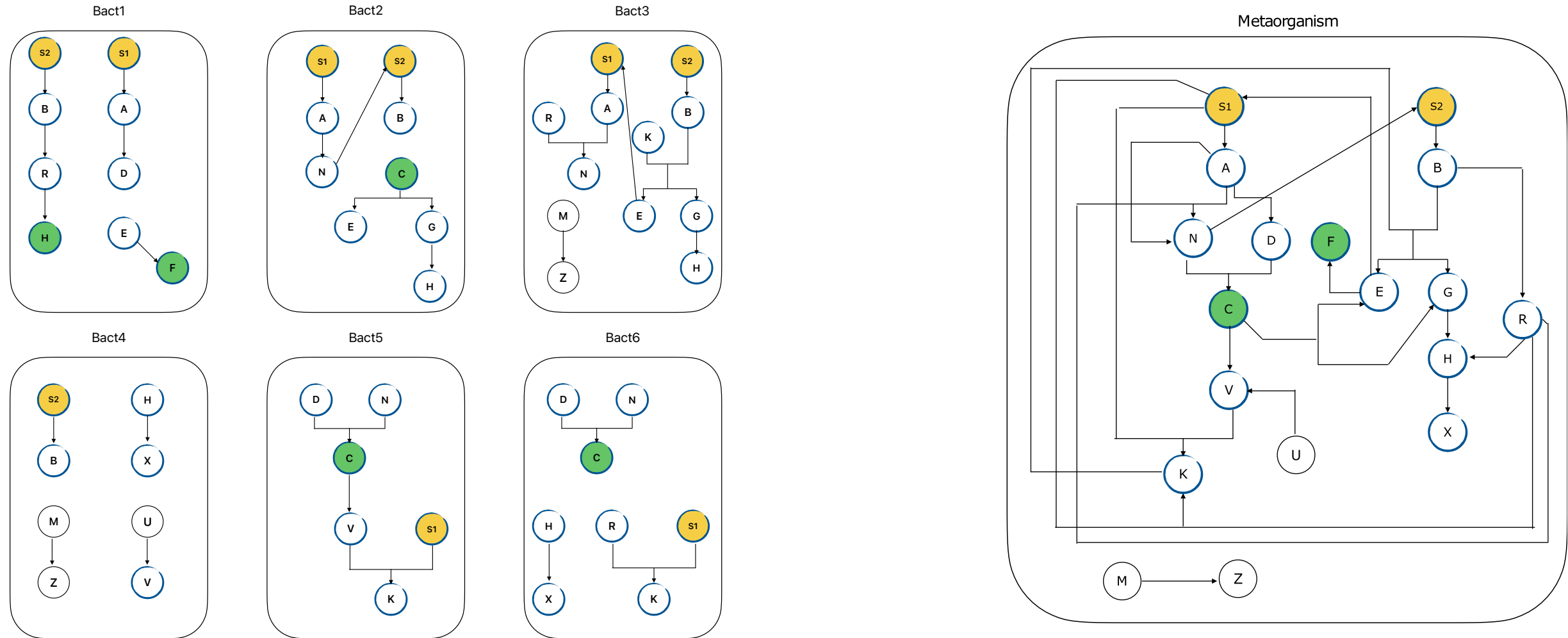
Metage2Metabo: application to an example



Symbiont	Individual Scope	+ community prod
bact1	A, B, D, R, H	F
bact2	A, B, N, S2	E, G, H
bact3	A, B	E, G, N, H, S1
bact4	B	X
bact5		C, K, V
bact6		C, K, X

Synergistic interactions increase the potential for the community (blue nodes)...

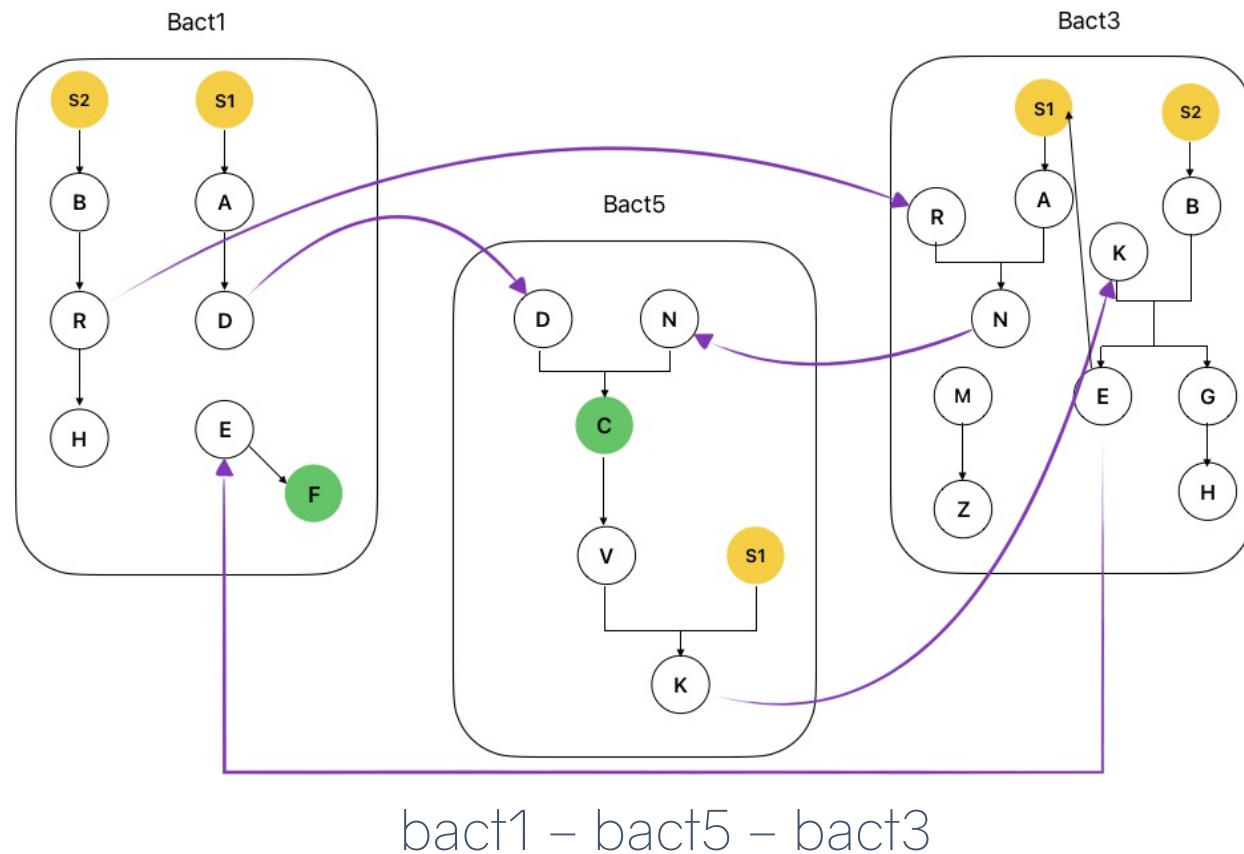
Metage2Metabo: application to an example



Synergistic interactions increase the potential for the community (blue nodes)...

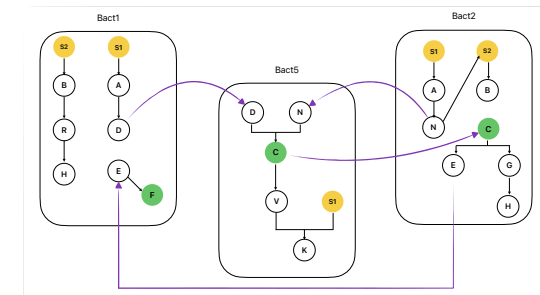
... similarly to the way a meta-organism of the community would behave

Metage2Metabo: application to an example

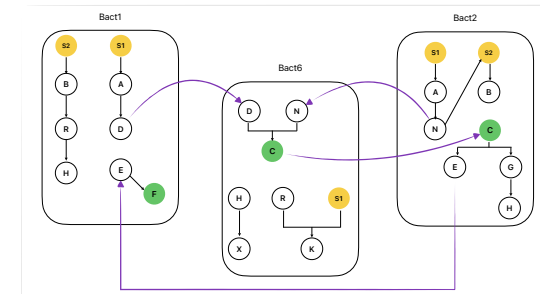


Selecting a minimal community producing the compounds of interest

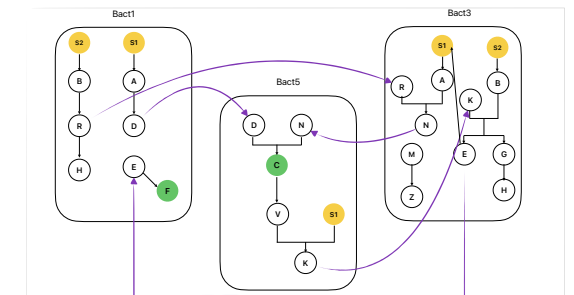
bact1 – bact5 – bact2



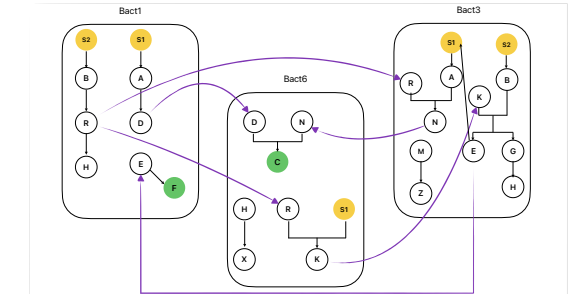
bact1 – bact6 – bact2



bact1 – bact5 – bact3



bact1 – bact6 – bact3



4 equivalent solutions,
4 to 5 metabolic interactions

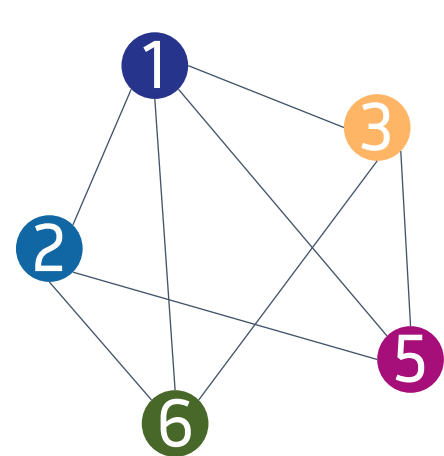
Possibility to enumerate all solutions or to get the players occurring in minimal communities

Metage2Metabo: application to an example

Minimal communities:

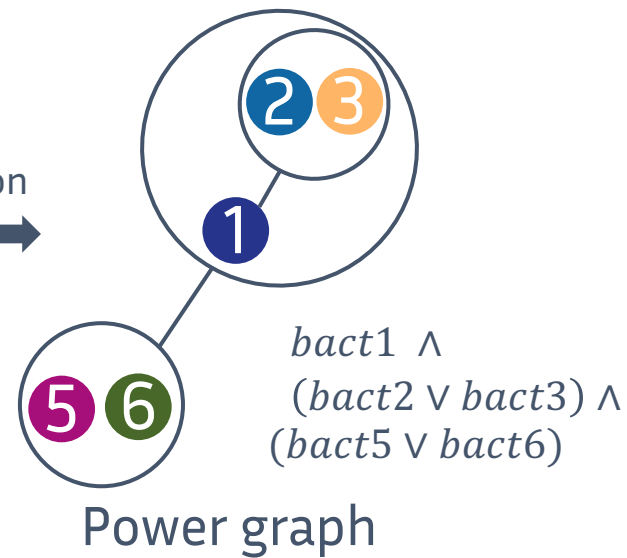
- bact1 - bact2 - bact5
- bact1 - bact2 - bact6
- bact1 - bact3 - bact5
- bact1 - bact3 - bact6

KEY SPECIES



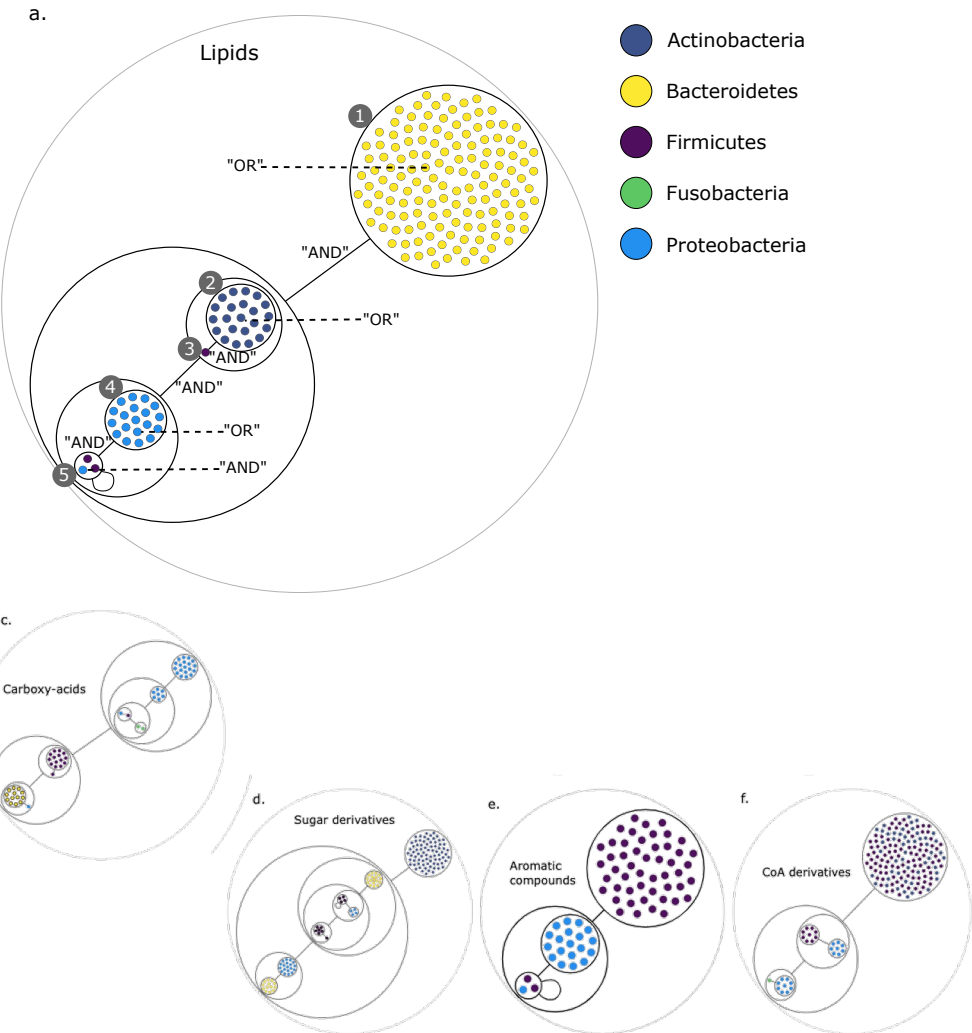
Association graph

compression



Power graph

Highlighting symbionts with equivalent roles



Reveals interesting patterns on real data

Tutorial content

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

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

5. Tutorial



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Acknowledgements

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