

Automated workflows for ^{13}C -Fluxomics

Increasing throughput and accessibility of ^{13}C -Fluxomics

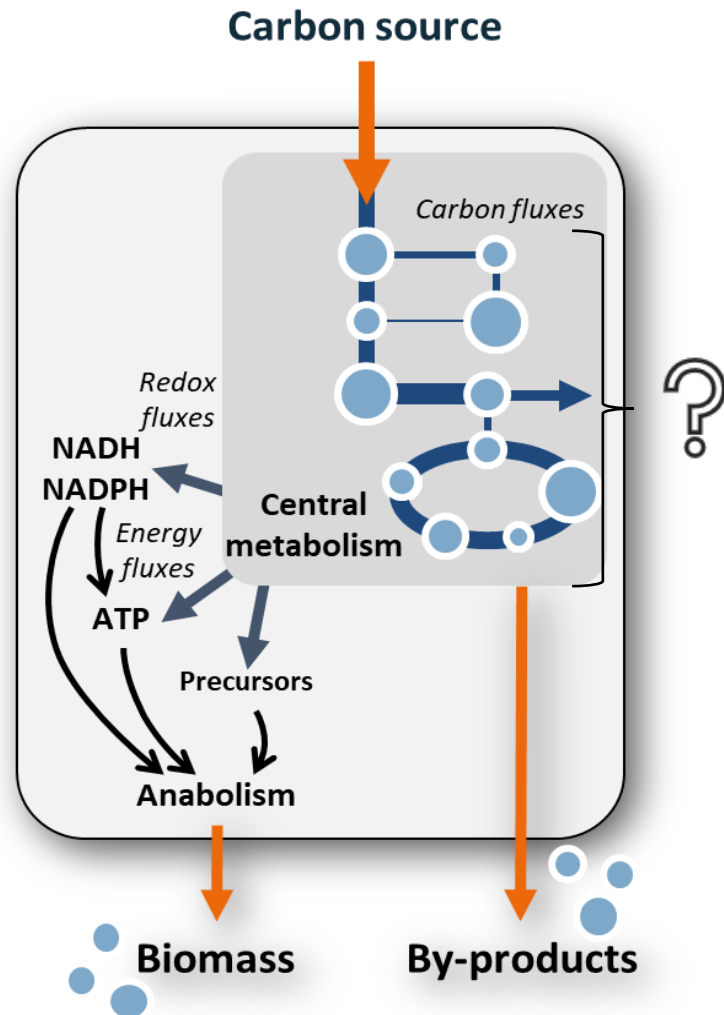
Loïc Le Grégam, Floriant Bellevert, Yann Guitton, Fabien Jourdan, Jean-Charles Portais, Pierre Millard

^{13}C -fluxomics

⇒ Quantification of the **actual rates** of (bio)chemical reactions within metabolic networks

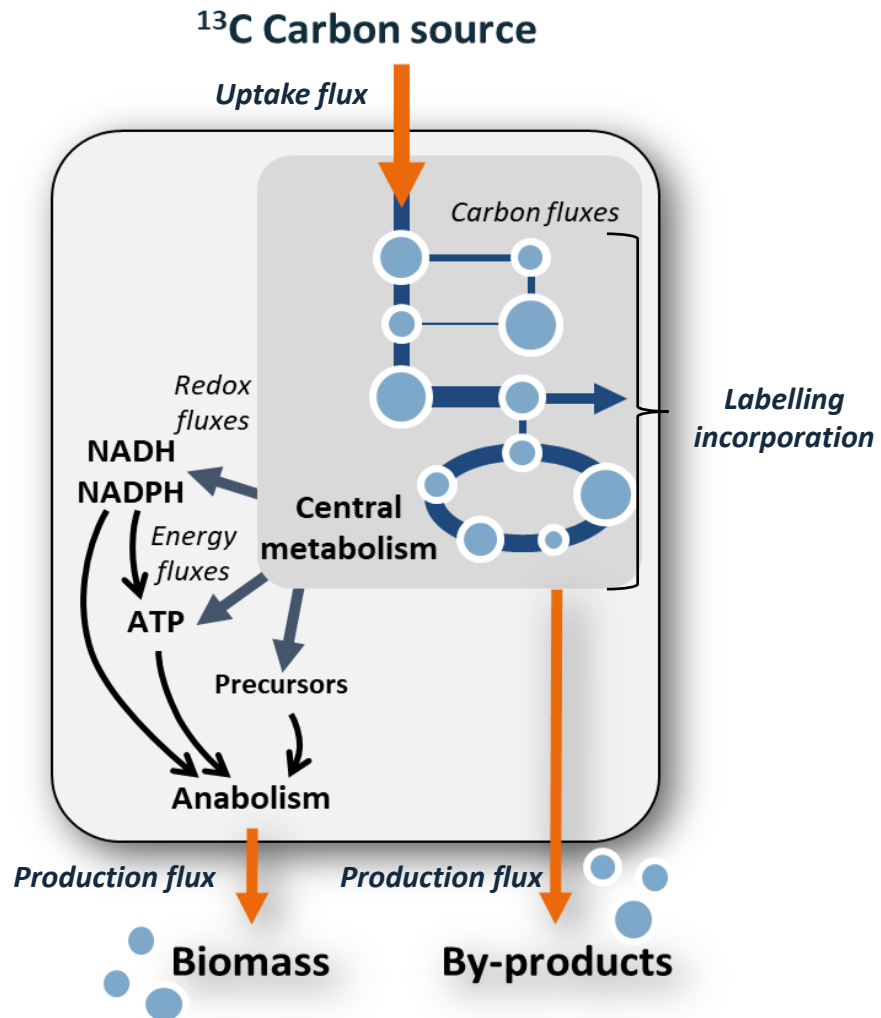
^{13}C -fluxomics

➔ Quantification of the **actual rates** of (bio)chemical reactions within metabolic networks



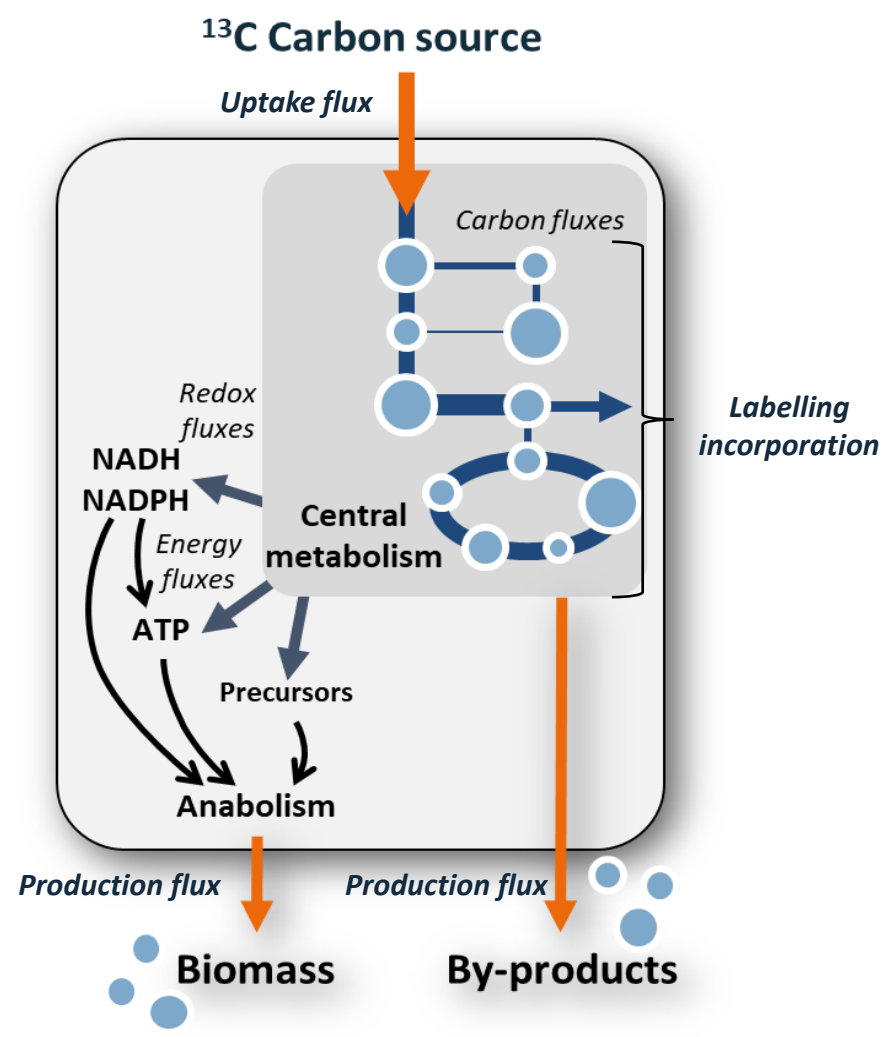
^{13}C -fluxomics

➔ Quantification of the **actual rates** of (bio)chemical reactions within metabolic networks

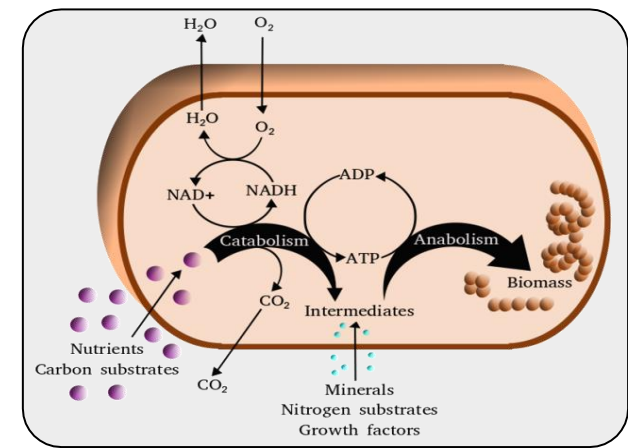


¹³C-fluxomics

➔ Quantification of the **actual rates** of (bio)chemical reactions within metabolic networks



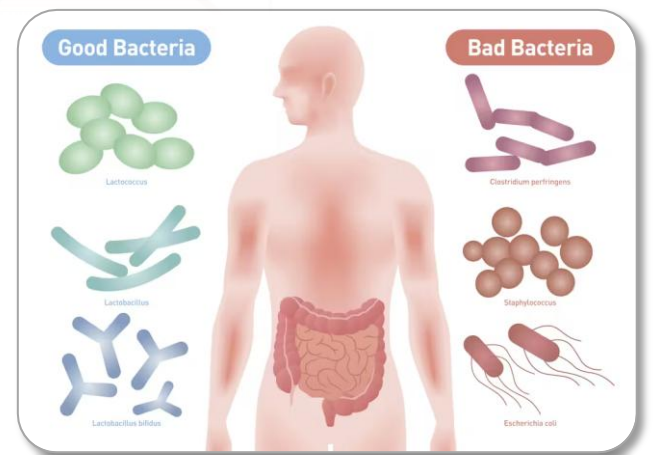
Fundamental knowledge



Biotechnology applications

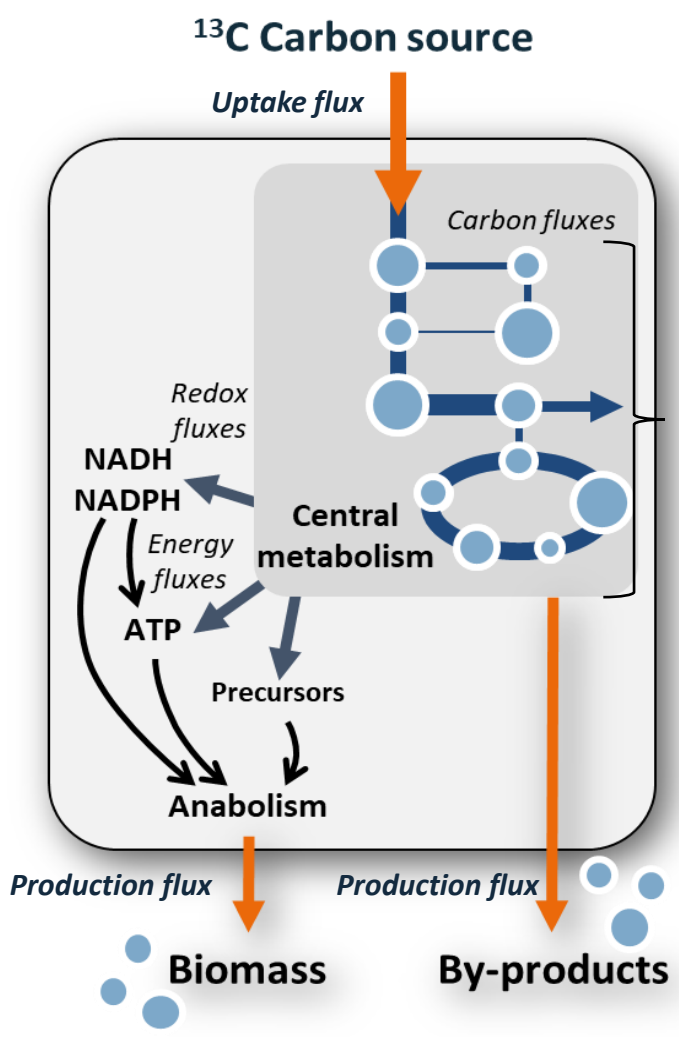


Health applications

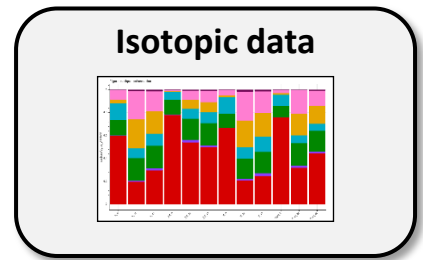


¹³C-fluxomics

➔ Quantification of the **actual rates** of (bio)chemical reactions within metabolic networks

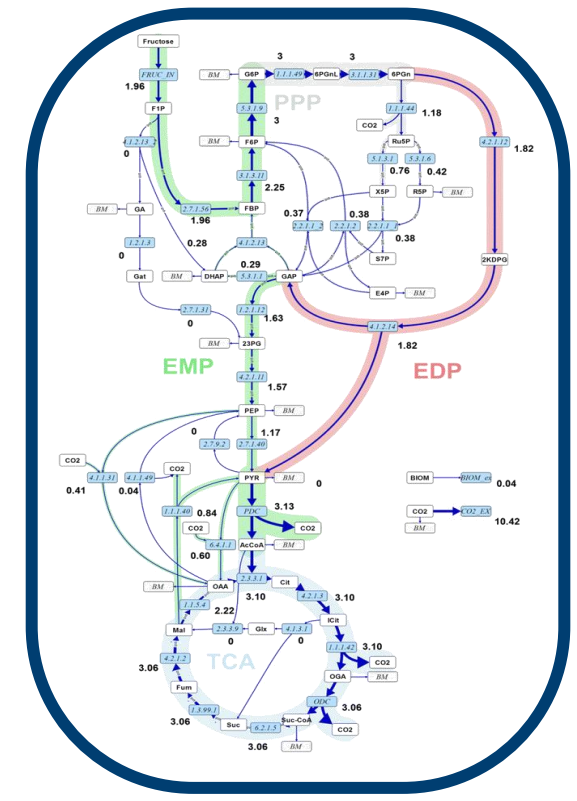
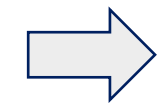
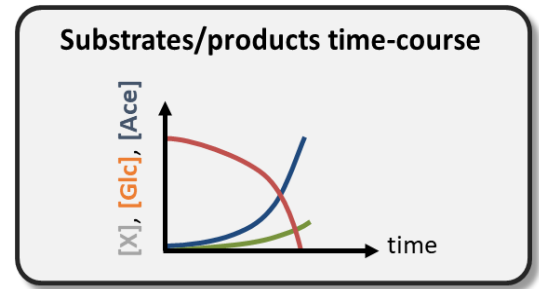


Labelling incorporation

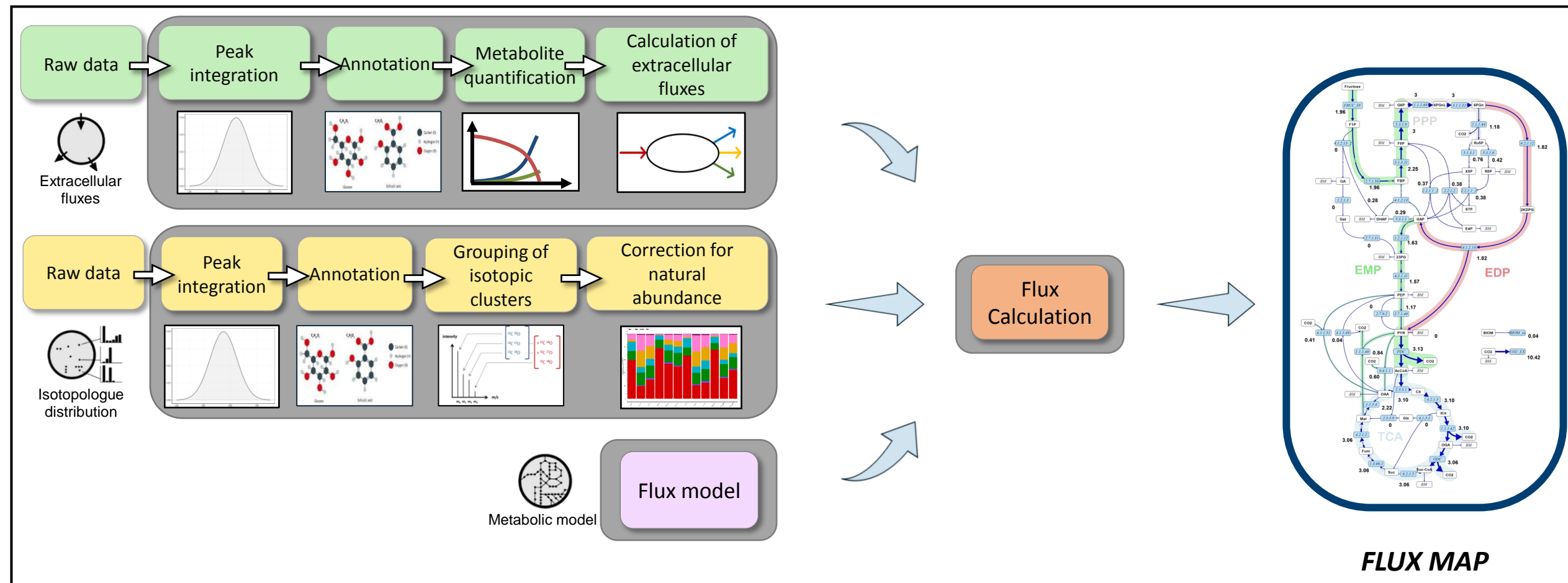


Network topology

dA/dt	1	-1	-1	0	0	0	$v1$
dB/dt	0	1	0	-1	0	0	$v2$
dC/dt	0	0	1	0	-1	0	$v3$
dD/dt	0	0	0	1	1	-1	$v4$
							$v5$
							$v6$



Data processing & flux calculation: a functional overview



Objectives and strategy



Throughput
Precision
Reproducibility
Coverage



Reduce complexity
Streamline the
process



Increase **flexibility** to
integrate **multiple**
approaches



Objectives and strategy

Throughput

Precision

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Coverage

Reduce complexity

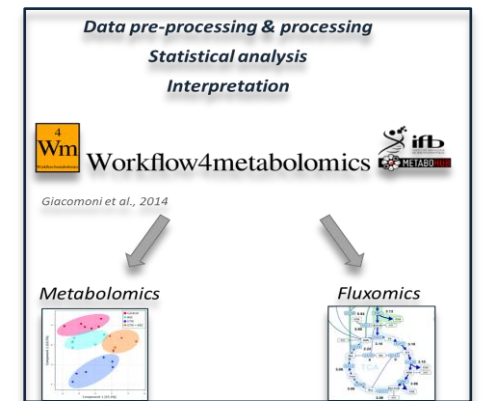
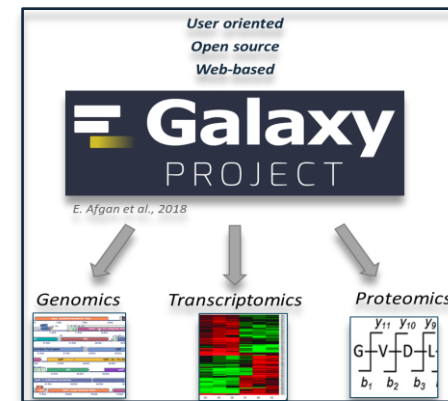
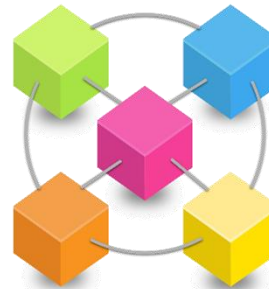
Streamline the process

Increase flexibility to integrate multiple approaches

→ Creation of automated computational workflows

Strategy:

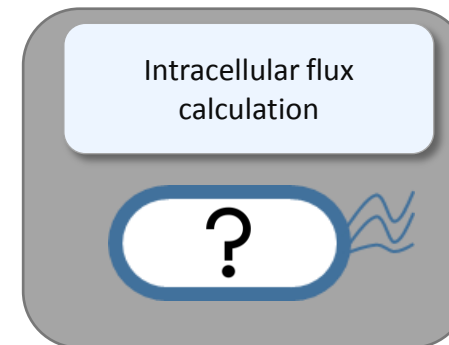
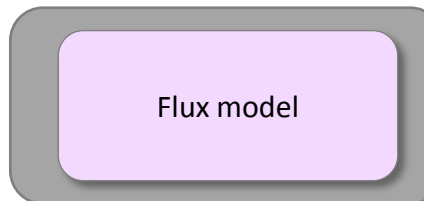
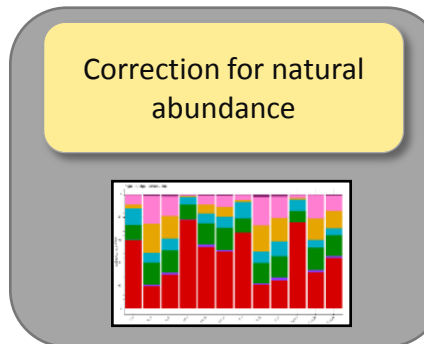
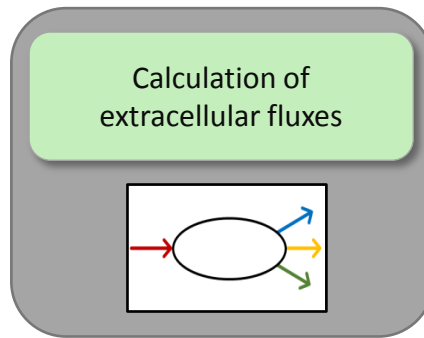
Microservices



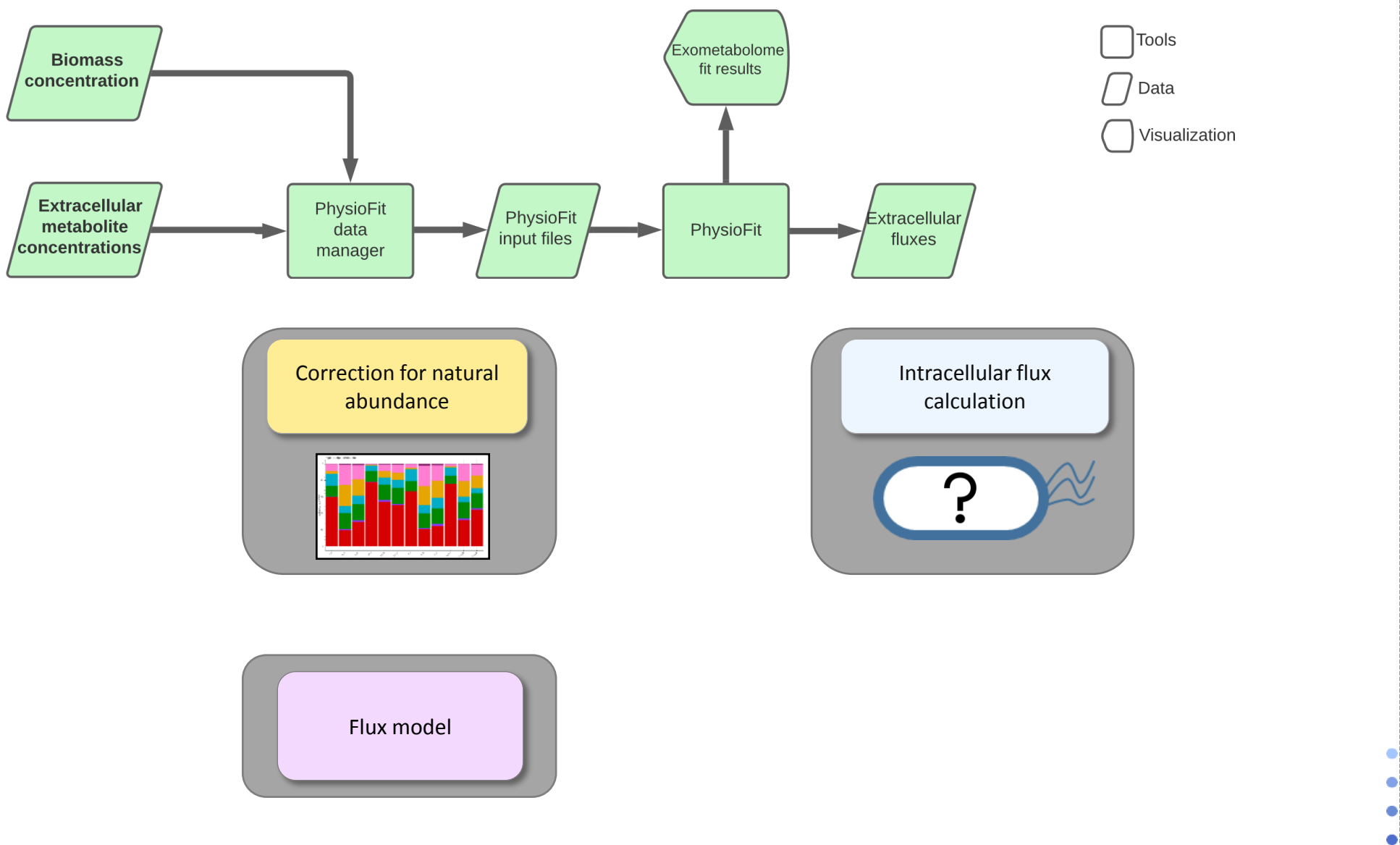
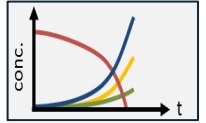
- Integrate the **minimal set of tools for stationary ¹³C-fluxomics**
- Extend the workflow to cover **upstream computational steps** and **additional approaches**



Workflow implementation



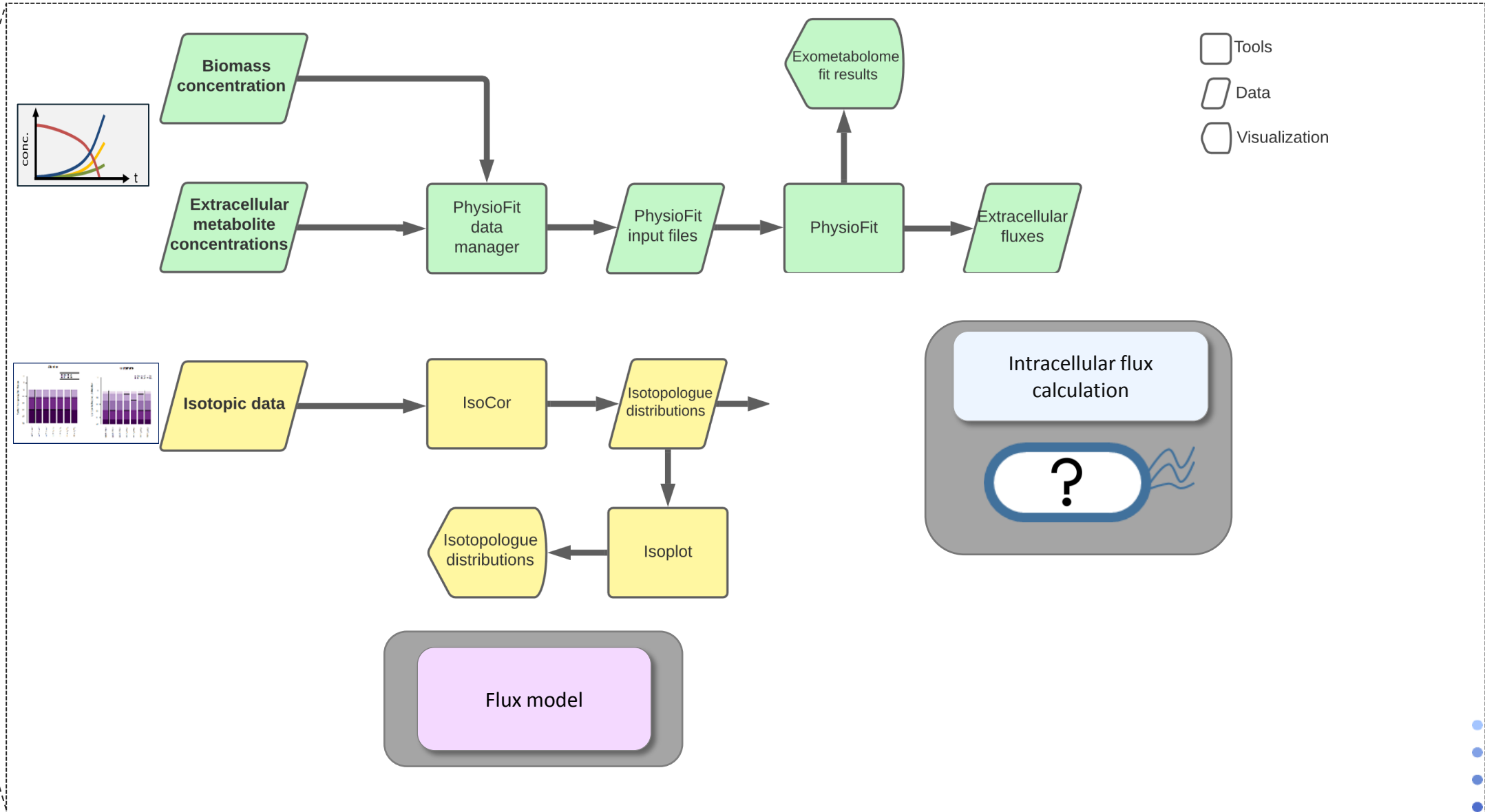
The minimal workflow



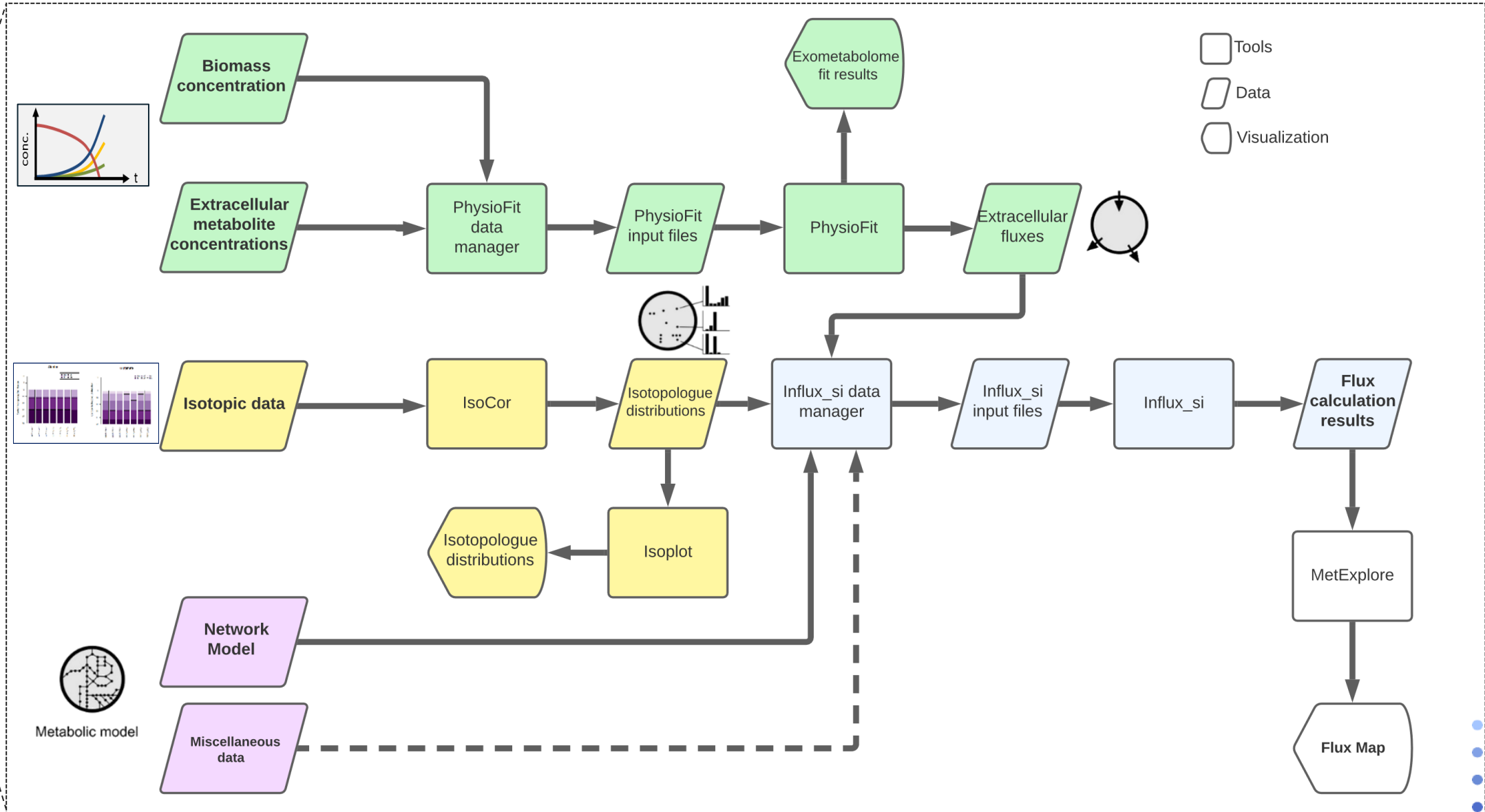
- Tools
- Data
- Visualization



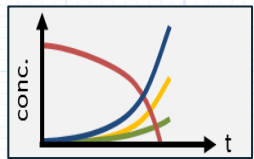
The minimal workflow



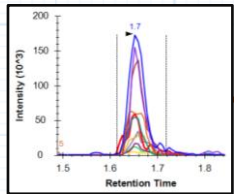
The minimal workflow



Results: a first functional workflow



Exometabolome dynamics



MS Isotopic data

Network topology

dA/dt	1	-1	-1	0	0	0	v1
dB/dt	0	1	0	-1	0	0	v3
dC/dt	0	0	1	0	-1	0	v4
dD/dt	0	0	0	1	1	-1	v5
							v6

Flux model

8: PhysioFit Manager

- File containing the time-course measurements of biomass concentrations *
- File containing the time-course measurements of the different metabolite concentrations *
- physiofit_input (tabular)

10: PhysioFit

- Upload data file containing biomass and concentration values
- plots (pdf)
- fluxes (tabular)
- stats (tabular)
- config (json)
- log (txt)

9: Isotope Correction for mass spectrometry labeling experiments

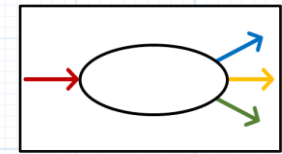
- measurements Data file *
- metabolites db *
- derivatives db *
- isotopes db *
- output1 (tabular)
- output2 (txt)

11: Isoplot: Generate plots from isocor output

- Upload data that has been corrected by Isocor (.tsv format)
- Upload template file containing plot metadata (.xlsx format) *
- data_output (csv)
- plots_output (zip)
- log_output (txt)

12: Influx Data Connector: Handling influx_si data inputs in Galaxy workflows

- File containing network for experiments (.netw file) *
- Output of isocor which corresponds to the .miso input (.tsv file)
- File containing types of variables for experiments (.tvar file) *
- Output of physiofit which corresponds to the .mflux input (.csv file)
- File containing label inputs for experiments (.linp file)
- File containing specie concentrations for experiments (.mnet file)
- File containing constraints on fluxes and specie concentrations for experiments (.constr file)
- File containing options for influx_si (.opt file)
- list_output (input)
- stdout (txt)
- stderr (txt)



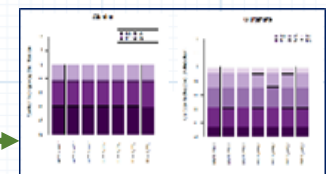
Extracellular fluxes

Intracellular fluxes

13: influx_si

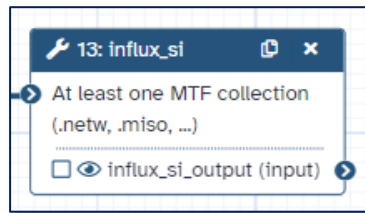
- At least one MTF collection (.netw, .miso, ...)
- influx_si_output (input)

SBML model

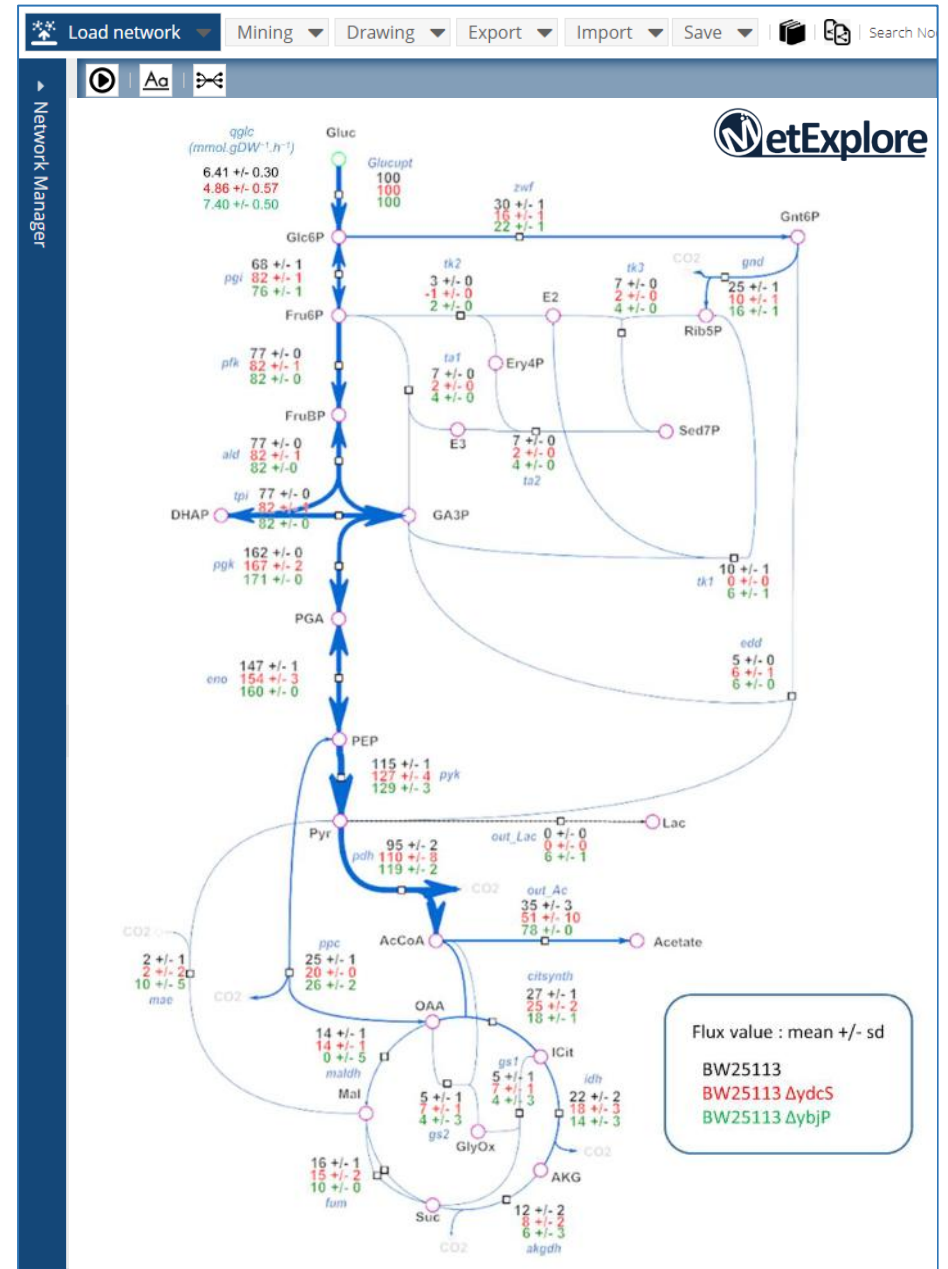


Carbon Isotopologue Distribution

Results: a first (minimal) workflow



SBML model



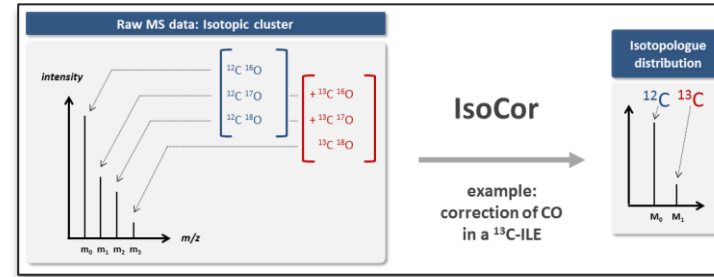
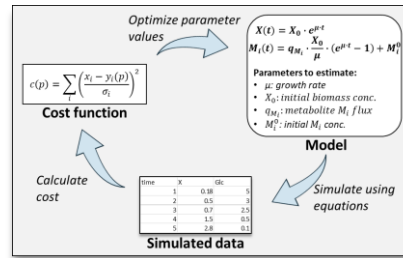
Data from Berges et al., 2021

metexplore.toulouse.inrae.fr



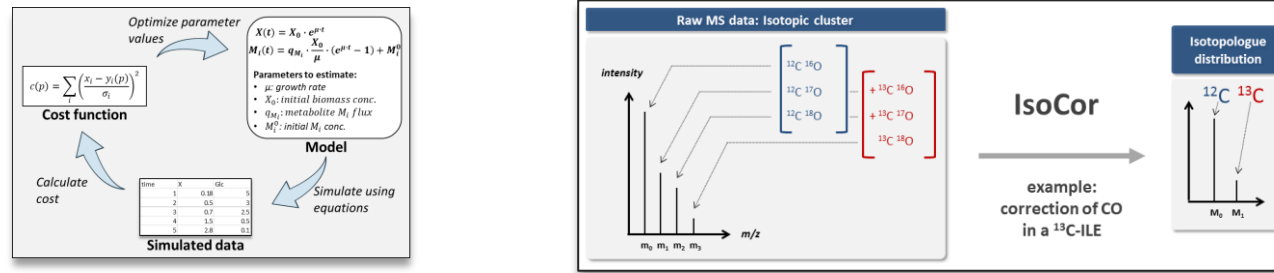
Concluding remarks

- Development and implementation of standalone tools for metabolic studies

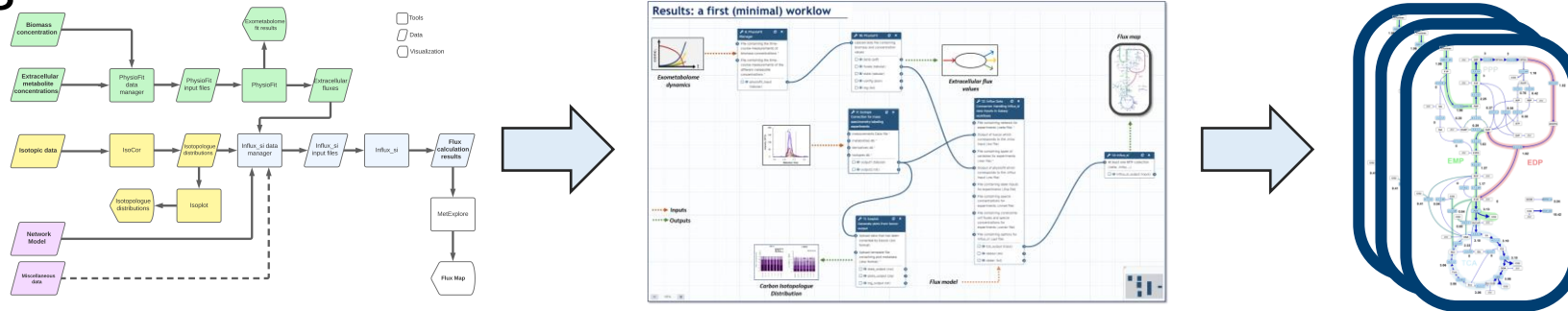


Concluding remarks

- Development and implementation of standalone tools for metabolic studies

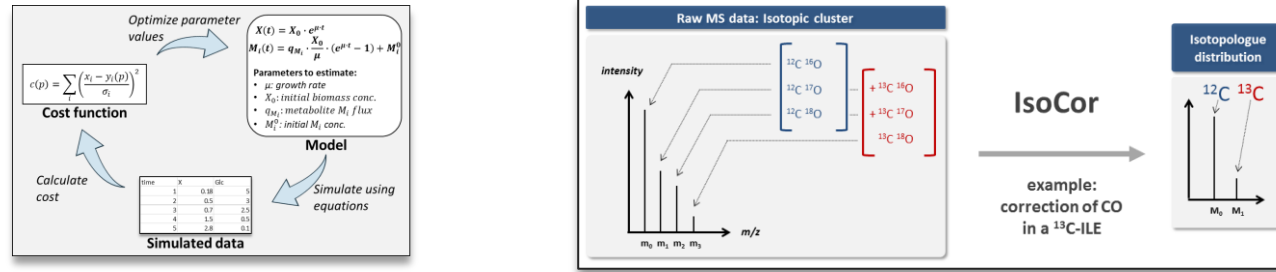


- Implemented workflow enables **high throughput & reproducible** ^{13}C -flux studies

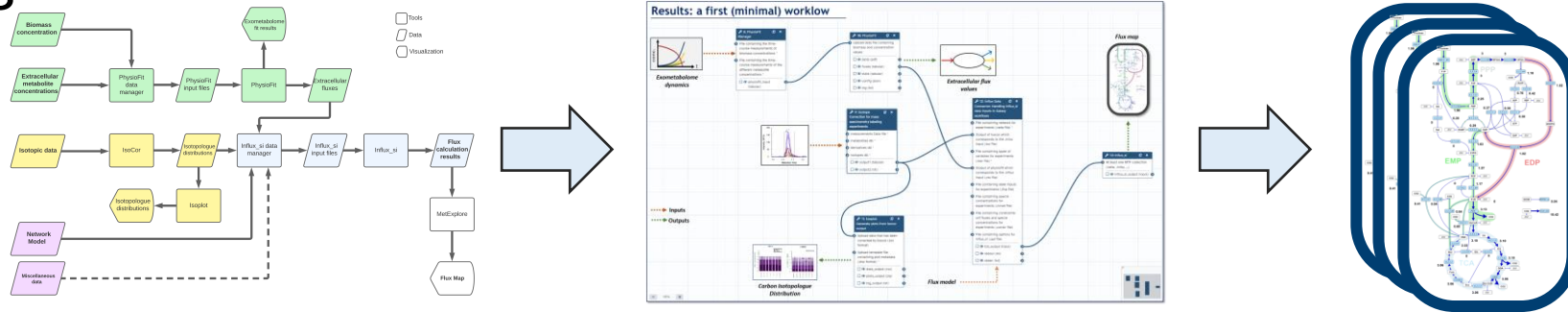


Concluding remarks

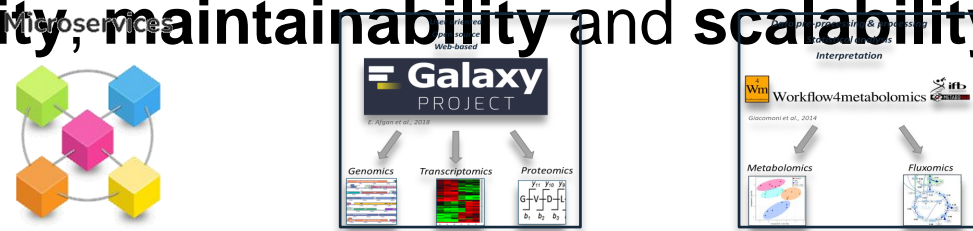
- Development and implementation of standalone tools for metabolic studies



- Implemented workflow enables **high throughput & reproducible** ¹³C-flux studies



- Enhanced **flexibility, maintainability** and **scalability** through microservice architecture



An automated workflow for high-throughput ^{13}C -fluxomics



Loïc Le Grégam^{1,2}, **Sergueï Sokol**^{1,2}, **Yann Guitton**^{3,4}, **Fabien Jourdan**^{2,5}, **Florian Bellvert**^{1,2}, **Jean-Charles Portais**^{2,6}, **Pierre Millard**^{1,2}



Introduction

The goal of isotope-based fluxomics approaches is to quantify metabolic fluxes in living organisms. ^{13}C -fluxomics provides a detailed phenotypic description of the actual metabolic state of studied organisms. To achieve this, a combination of several experimental and computational steps is required. However, the complexity of the computational steps, coupled with the need to select the right software while manually ensuring data interoperability, makes the process error-prone, time-consuming, and hard to reproduce effectively.

The objective of this project is to overcome these limitations by providing a **flexible, reproducible, user-friendly, and high-throughput ^{13}C -fluxomics workflow** that integrates the tools needed for flux calculation.

Material and Methods

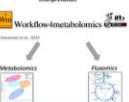
Microservices



Integration of a minimal set of tools for stationary ^{13}C -fluxomics on Workflow4Metabolomics.

Flexible architecture eases implementation of upstream computational steps and thus the integration of additional approaches.

Data pre-processing & processing



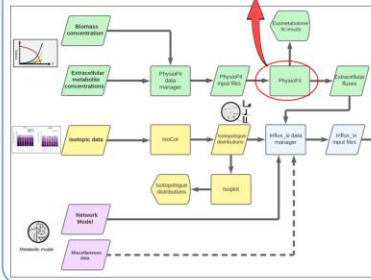
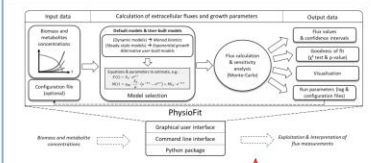
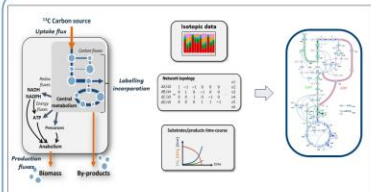
Results and discussion

The data needed for flux calculation:

- Flux Model:** Model containing information on the reactions within the network (stoichiometry and carbon transitions) as well as information on the labelled substrate(s), constraints on the model, etc
- Isotopic data:** Carbon Isotope/Distribution measurements from different metabolites of the metabolic network
- Extracellular flux measurements:** Uptake and production fluxes calculated from biomass and extracellular metabolite dynamics

Interoperability of tools is key for building the workflow. Each tool can be used alone, or in tandem with other software. Logs and configuration files ensure **reproducibility**.

Implementation of **PhysioFit**, a software to quantify extracellular fluxes and growth parameters. **LINKS** --- >>>

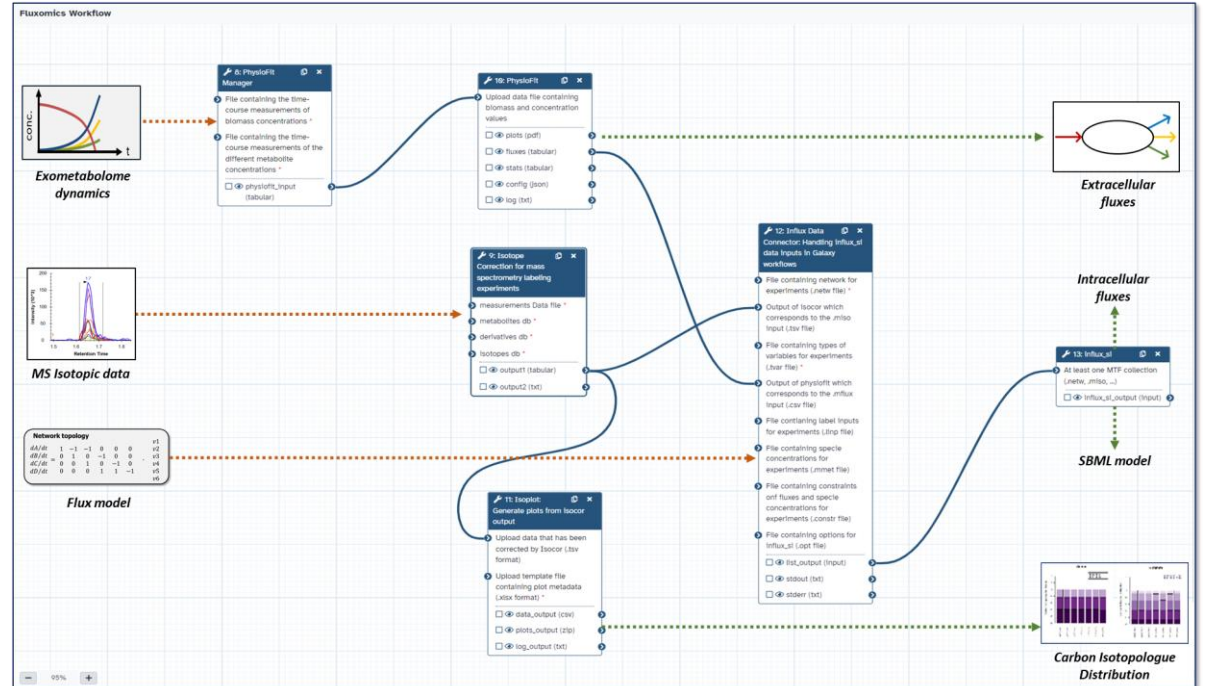


Conclusions

Reproducible and high-quality ^{13}C -fluxomics experiments are critical for **functional studies of metabolic systems**, as well as for **identifying metabolic levers to optimize biotechnological processes** for drug development, industrial applications and fundamental understanding of metabolism. The development of an automated workflow for data processing and flux calculation enables **reproducible, high-throughput experimental metabolic flux analysis**. The flexible microservice architecture allows for future integration of new approaches and customization, helping scientists apply it to a **wide range of biological questions** and keeping this work relevant for years to come.

References

- [1] *mflux_s: increasing numerical stability and precision for metabolic flux analysis in isotope labelling experiments*, Sokol et al., 2012
- [2] *Workflow4Metabolomics: a collaborative research infrastructure for computational metabolomics*, Giacomoni et al., 2015
- [3] *Recent advances in high-throughput ^{13}C -fluxomics*, Heux et al., 2017
- [4] *Isocor: isotope correction for high-resolution MS labeling experiments*, Millard et al., 2018
- [5] *Exploring the Glucose Fluxotype of the E. coli y-ome using high-resolution fluxomics*, Berges et al., 2021
- [6] *PhysioFit: a software to quantify cell growth parameters and extracellular fluxes*, Le Grégam et al., 2024



Session 5 – Poster 38
Thank you for listening!

