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Development of high throughput miniaturized robotic platforms for metabolism investigations of microorganisms



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3 : RESTORE, Université Toulouse III - Paul Sabatier, EFS, Institut National de la Santé et de la Recherche Médicale, Centre National de la Recherche Scientifique

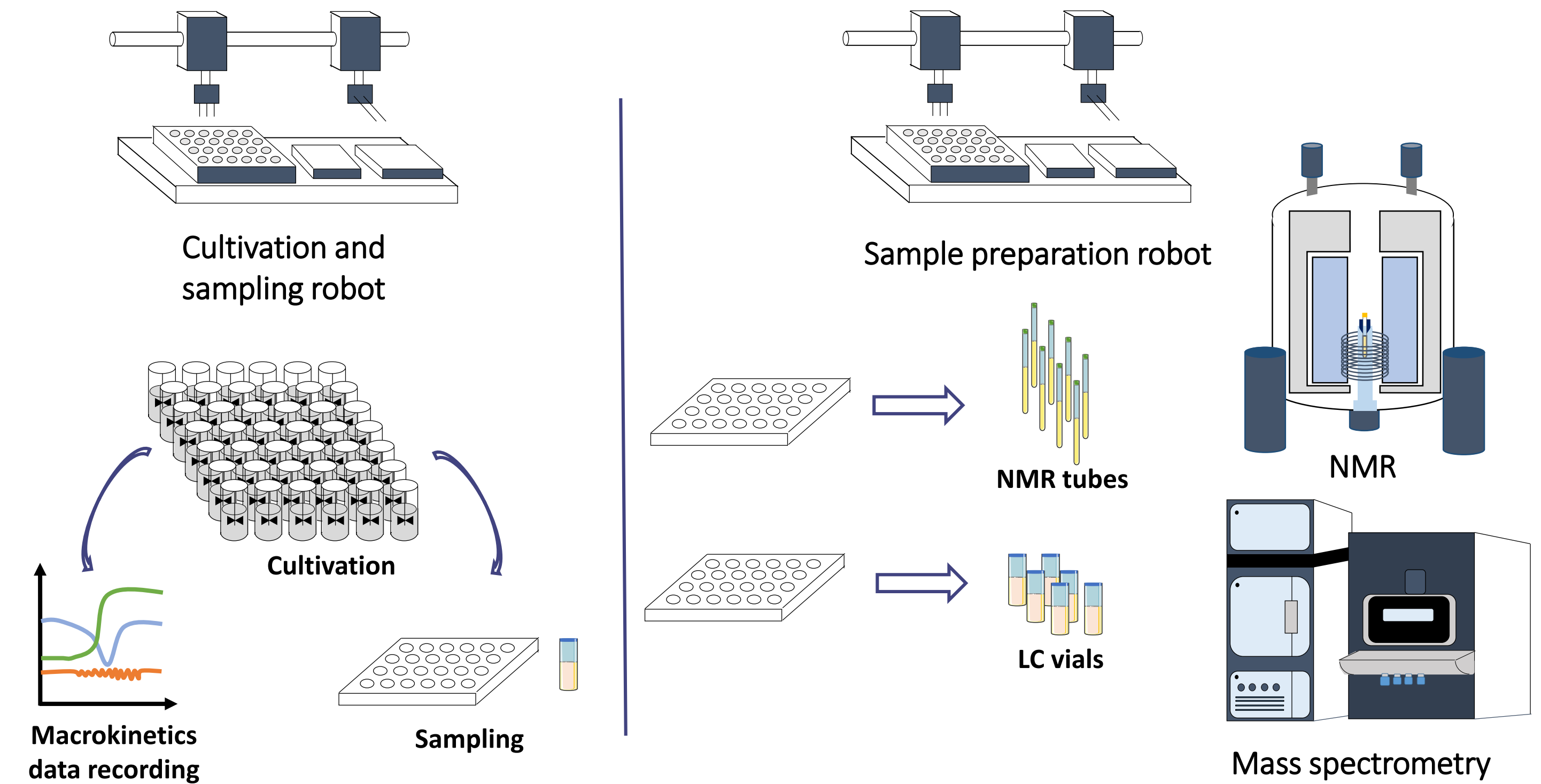
Context : MetaboHUB MetaToul platform : Robotic facilities

High-throughput (HT) omics approaches enable the large scale analysis of metabolism and its regulation. Among those, fluxomics (metabolic flux analysis) aims at measuring the actual rates of biochemical reactions in metabolic networks.

MetaToul develops and provides to the community a full methodological framework for high-throughput (HT) metabolism investigation applicable to a broad range of micro-organisms and applications. It includes experiment design and bioinformatical tools, analytical platforms (MS and NMR), and robotic platforms for cultivation and sample preparation.

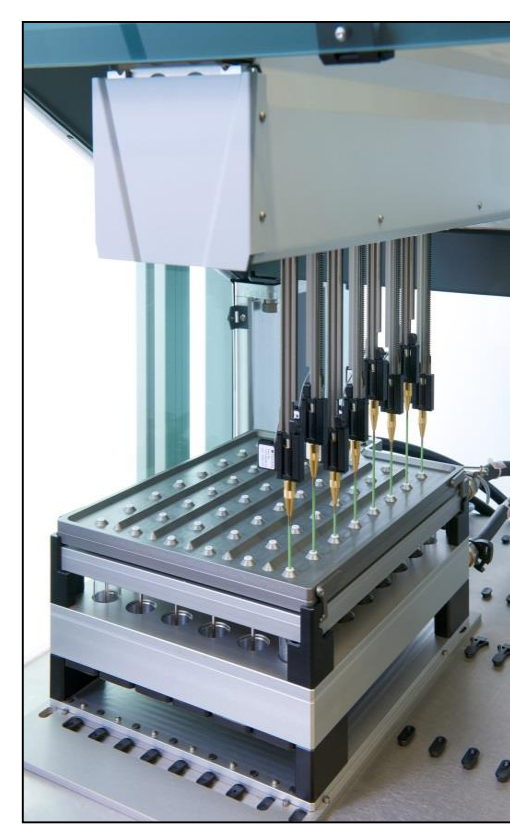
In this poster, you'll find a focus on the possibilities offered by our two robotic platforms :

- An automated, high-throughput cultivation and sampling workstation that allows to perform robust and HT metabolomics and fluxomics investigations in controlled physiological conditions.
- An sample preparation workstation for low-volume handling or small-quantity samples in order to analyze matrices for which we have few amounts available or to concentrate classical samples and have access to minority metabolites.



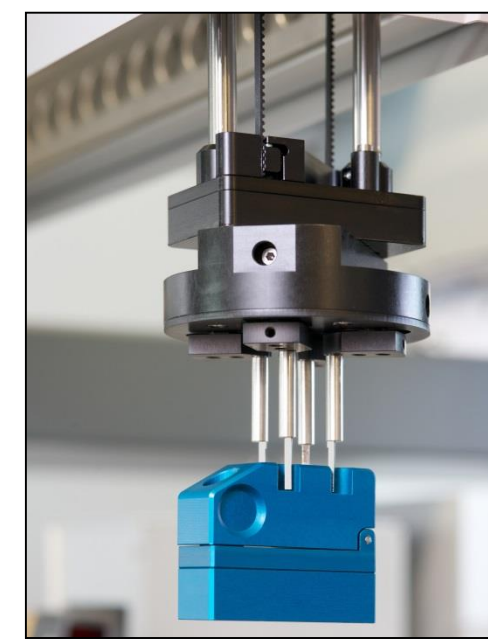
Robotic platform for cultivation and sampling

1 – Cell cultivations and tubes transfer

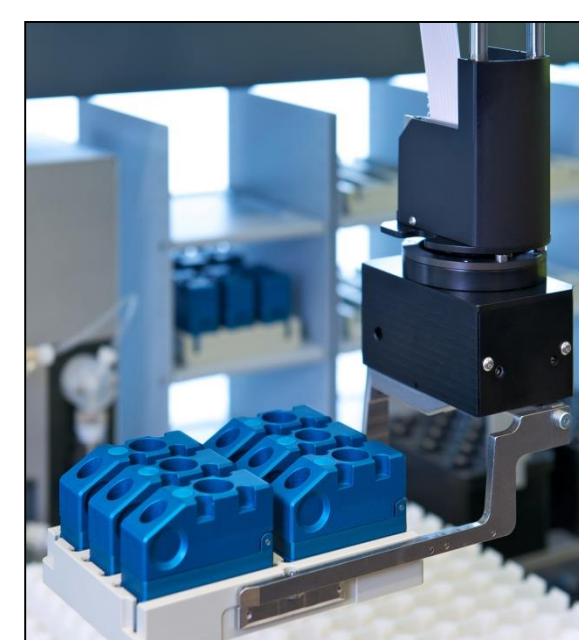


Eight-channel Liquid Handling Arm

48 monitored and controlled bioreactors



Pick and Place Arm

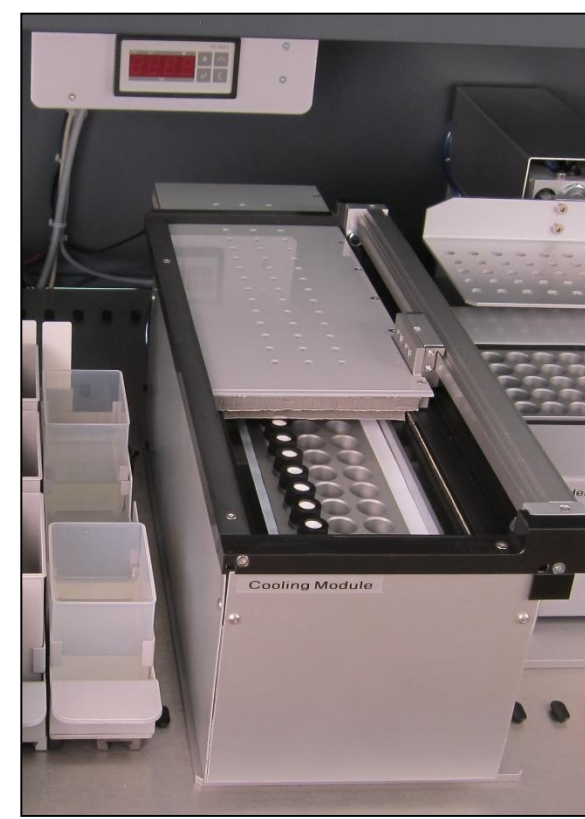


Robotic Manipulator arm

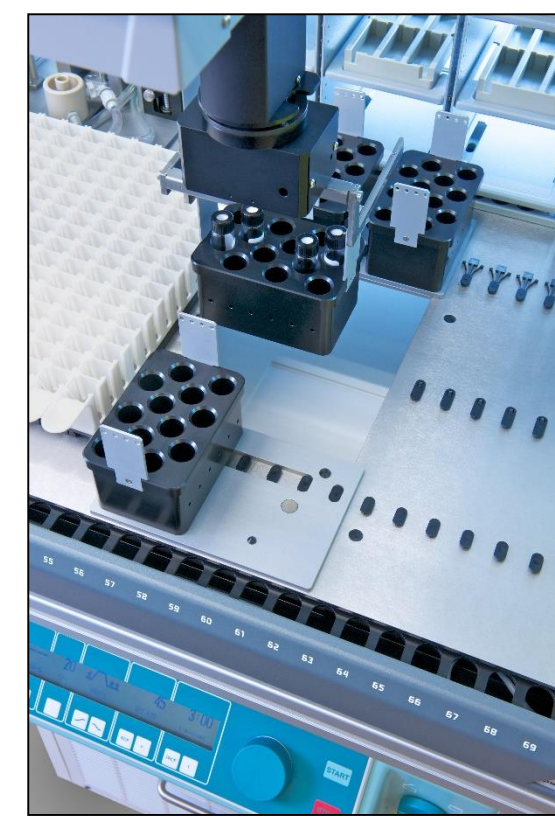
2 – Sampling devices



Heating module



Cooling module



Centrifuge



Filtration module

This robotic platform has the capability to run **48 cultivations in parallel** and **monitor pH, O₂ and OD₆₀₀** (optical density). This system also runs fully **automated metabolite harvesting and extraction** for downstream analyses.

Robotic platform for miniaturized sample preparation

1 – Pipetting system for small volumes (3 – 485 µL)

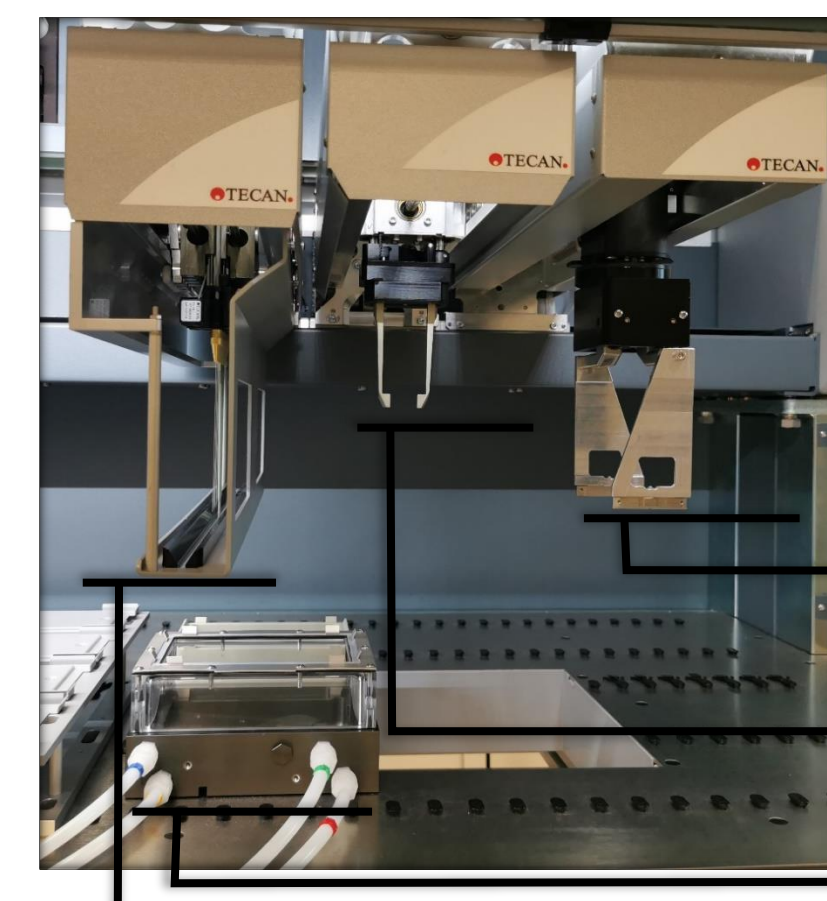


8 x 500 µL syringes

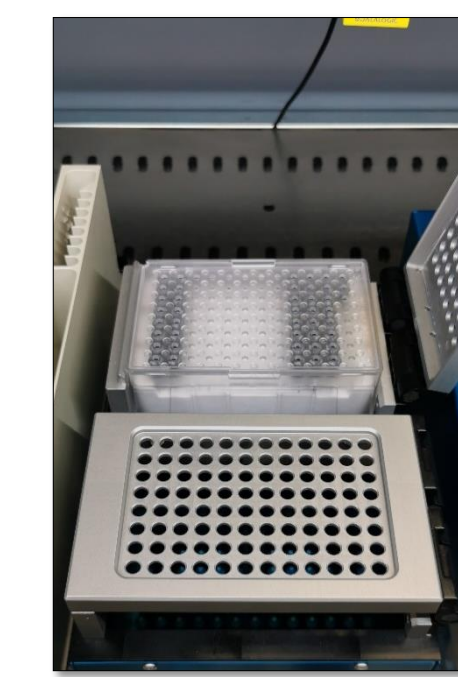


classical needles
1mm needles

2 – Miniaturized devices



RoMa arm with centric grippers
PnP arm
Module SPE SBS format
LiHa arm with downholder



NMR Tube Racks Cooling Carrier



Troughs cooling carrier
Customized Inserts for SBS format

This robotic platform is capable of **handling a complete range of sample racks** (NMR tubes, vials, multi-well plates, vials, cryotubes, etc.), as well as carrying out **various types of manipulation** such as dilution, aliquoting, addition of buffer and internal standard, all while **ensuring traceability** of all samples.

APPLICATIONS OF AUTOMATIC HIGH THROUGHPUT WORKFLOW

Monitoring physiology

- **48 cultivations** in parallel
- **Monitoring of :**
 - OD600 every 10 min
 - pH and pO₂ every 1 min
- **Collection of 48 culture medium** all along the cultivation kinetic
- **NMR analysis** to determine by-products production/consumption

HT Quantitative metabolomics

- **48 cultivations** in parallel
- **Monitoring of :**
 - OD600 every 10 min
 - pH and pO₂ every 1 min
- **Collection of 48 whole broth and filtrate** when OD target is achieved
- **Mass spectrometry analysis to determine intracellular pools :** amino acids, nucleotides, Krebs cycle, glycolysis and pentose phosphate pathway intermediates.

HT Fluxomic experiments

- **48 cultivations** in parallel
- **Monitoring of :**
 - OD600 every 10 min
 - pH and pO₂ every 1 min
- **Collection of 48 culture medium** all along the cultivation kinetic
- **Cells harvesting** at user defined OD600
- **Mass spectrometry analysis** to determine carbon isotopologues distributions
- **NMR analysis** to determine by-products production/consumption
- **Flux calculation**

Customized experiments

- **48 cultivations** in parallel
- **Monitoring of :**
 - OD600 every 10 min
 - pH and pO₂ every 1 min
- **Collection of supernatant or extract** all along the cultivation kinetic
- **Sample preparation of your samples**

Conclusions

⇒ This two systems allows to significantly accelerate the applications of metabolomics and fluxomics to investigate microbial metabolism, and to address challenges not only in **metabolic engineering, synthetic biology and biotechnology** but also in **health, toxicology and pharmacology, biomarker and drug development**.

⇒ **Customized protocols** can be designed on the two robotic platforms

⇒ Applications :

- **Large-scale analysis** of strains, mutants, species
- Large-scale analysis of the effects of environmental parameters, biological effectors, drugs, etc.
- **Design and metabolic optimization** of cell factories

Acknowledgments

MetaboHUB (MetaboHUB-ANR-11-INBS-0010)

