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## Exploring the fluxotype of the *E. coli* Y-ome using high-resolution fluxomics

Cécilia Bergès, Edern Cahoreau, Pierre Millard, Brice Enjalbert, Mickael Dinclaux, Maud Heuillet, Hanna Kulyk, Lara Gales, Noémie Butin, Maxime Chazalviel, et al.

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# Exploring the fluxotype of the *E. coli* Y-ome using high-resolution fluxomics

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A robust and integrated workflow to measure high-resolution fluxotypes

*Cécilia Bergès & Edern Cahoreau, Pierre Millard, Brice Enjalbert, Mickael Dinclaux, Maud Heuillet, Hanna Kulyk, Lara Gales, Noémie Butin, Maxime Chazalviel, Tony Palama, Matthieu Guionnet, Sergueï Sokol, Lindsay Peyriga, Floriant Bellvert, Stéphanie Heux and Jean-Charles Portais.*

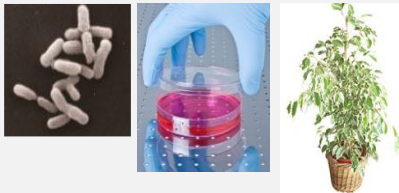
# INTRODUCTION : Fluxomics and fluxotypes

## Fluxomics :

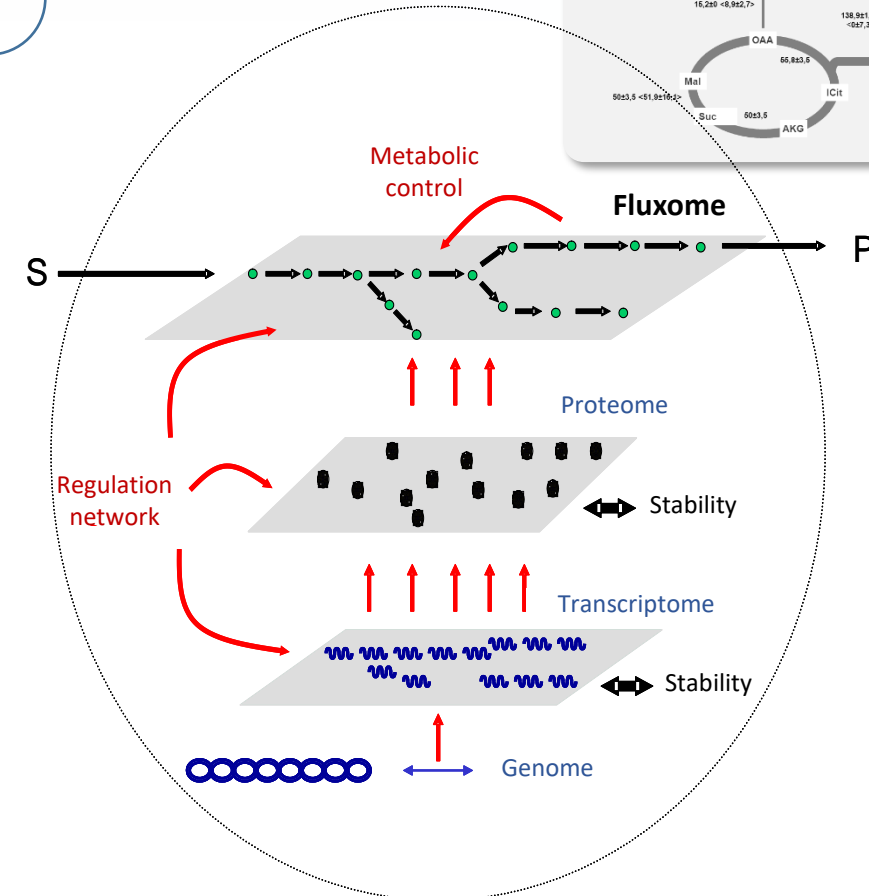
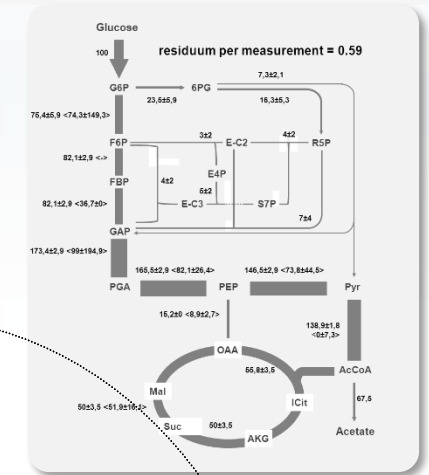
Measures the **actual rates of biochemical reactions** in metabolic networks (i.e. the **Fluxome**). It measures the actual output of the **integrated response of the gene-protein-metabolite interaction network** and provides direct access to the cellular phenotype in a **quantitative manner**.

## A number of applications :

- Microorganisms, plant cells & tissues, animal cells
- Systems biology
- Biotechnology
- Health, drug targeting

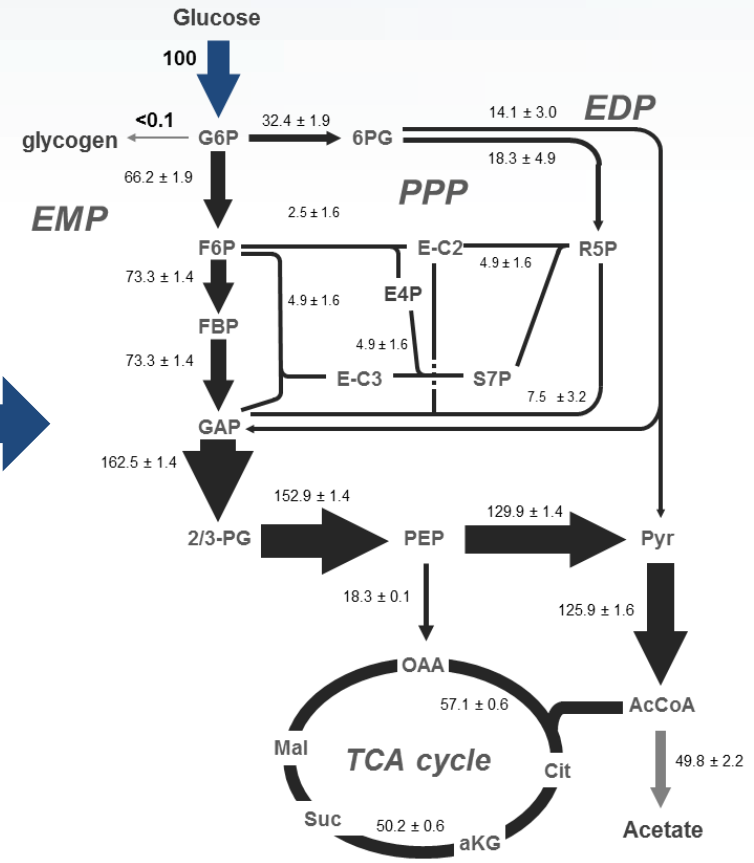
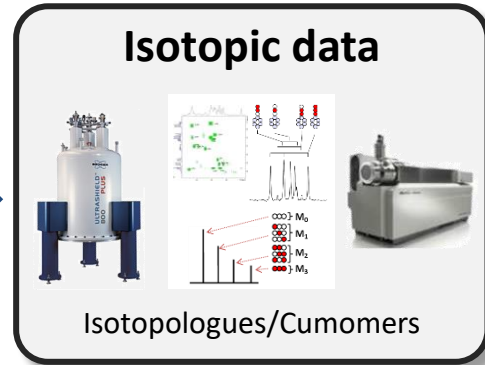
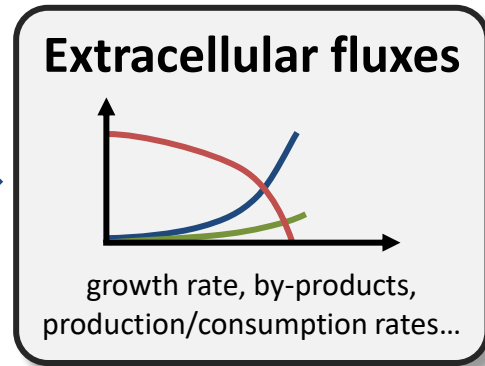
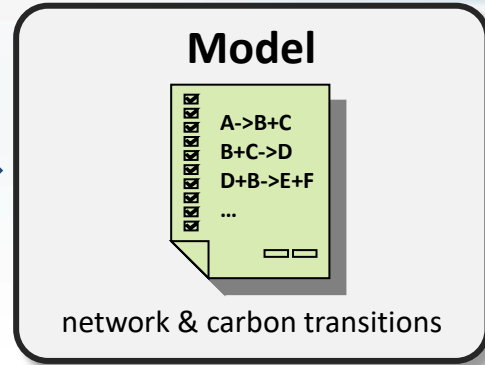


**Fluxotype = Metabolic flux phenotype** : the particular distribution of metabolic fluxes measured for a given strain under given physiological conditions

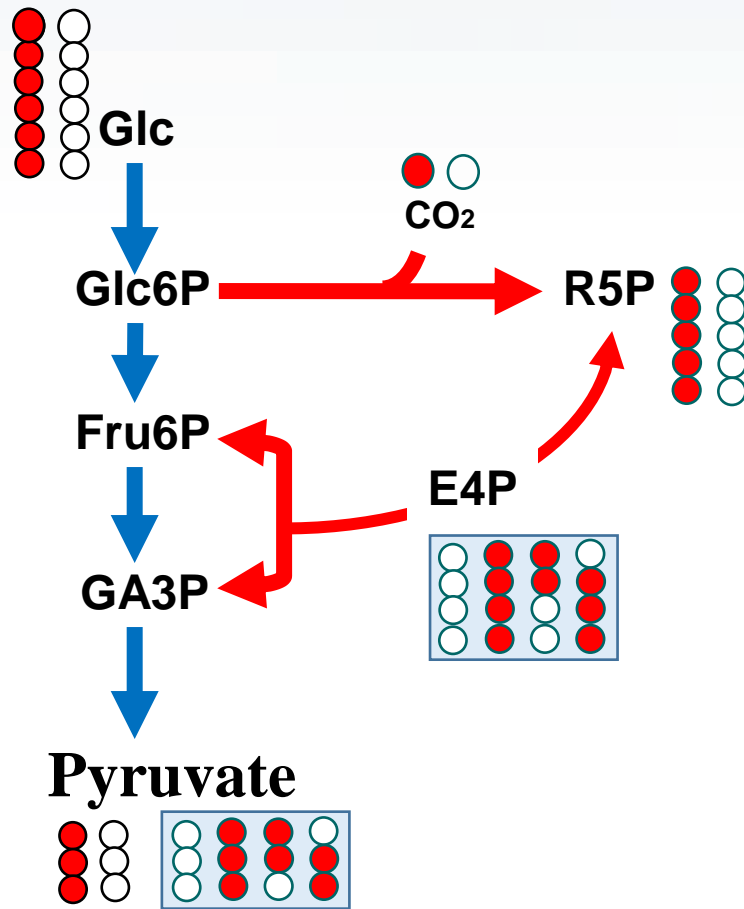


# INTRODUCTION : $^{13}\text{C}$ fluxomic analyses

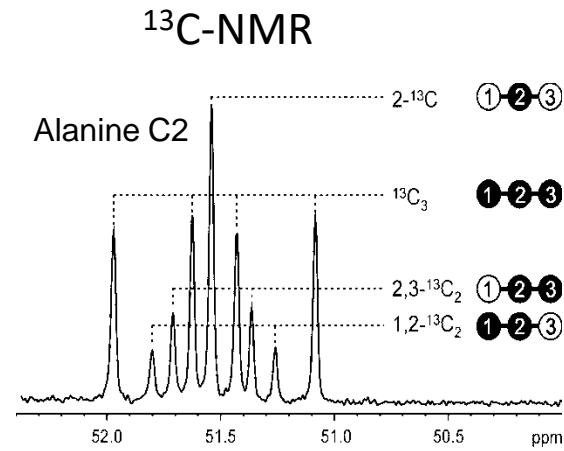
$^{13}\text{C}$ -labeled carbon source



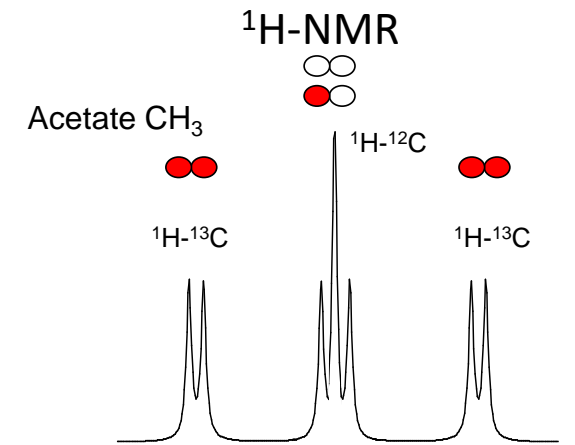
# <sup>13</sup>C targeted fluxomics example



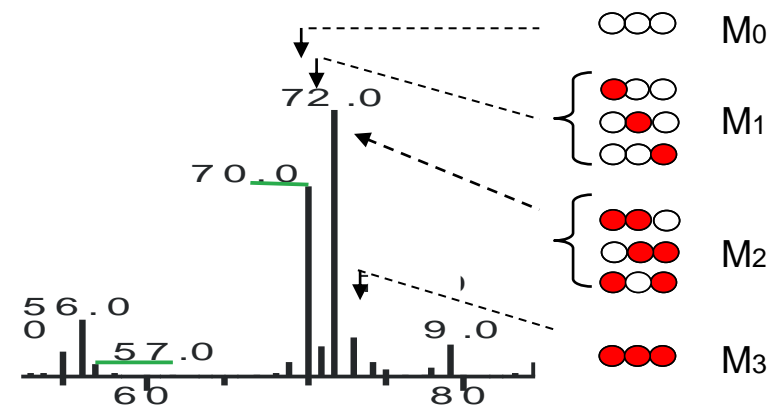
## NMR: positional isotopomers



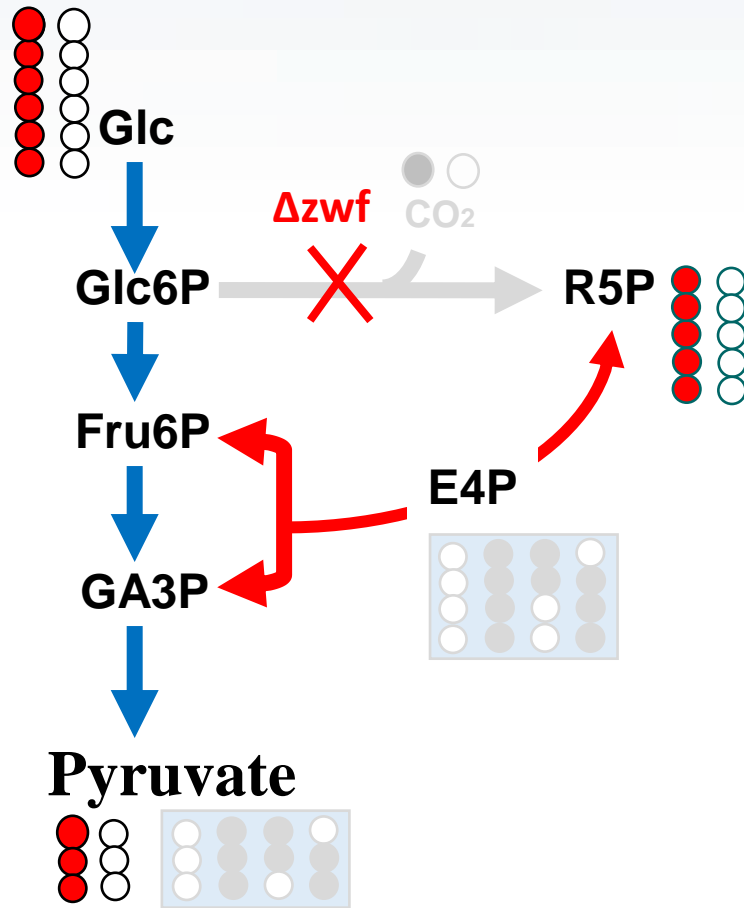
## NMR: <sup>13</sup>C enrichment



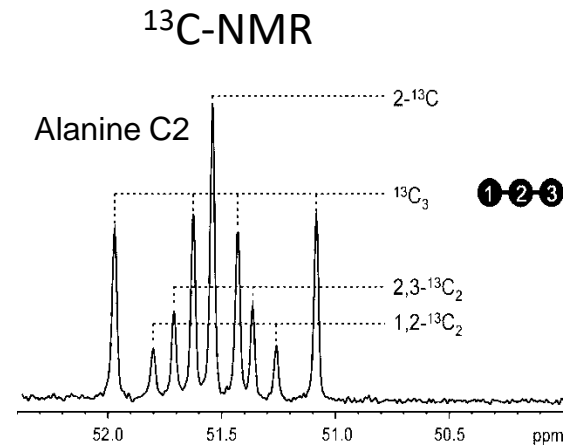
## MS: isotopologues



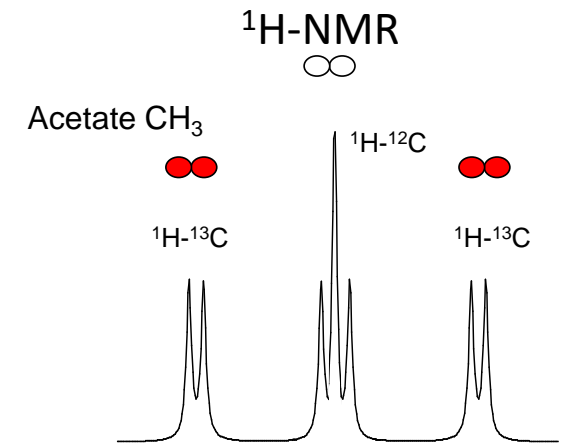
# <sup>13</sup>C targeted fluxomics example



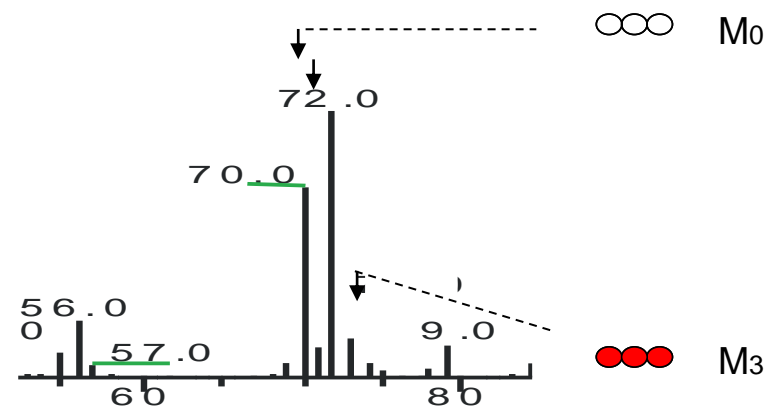
## NMR: positional isotopomers



## NMR: <sup>13</sup>C enrichment

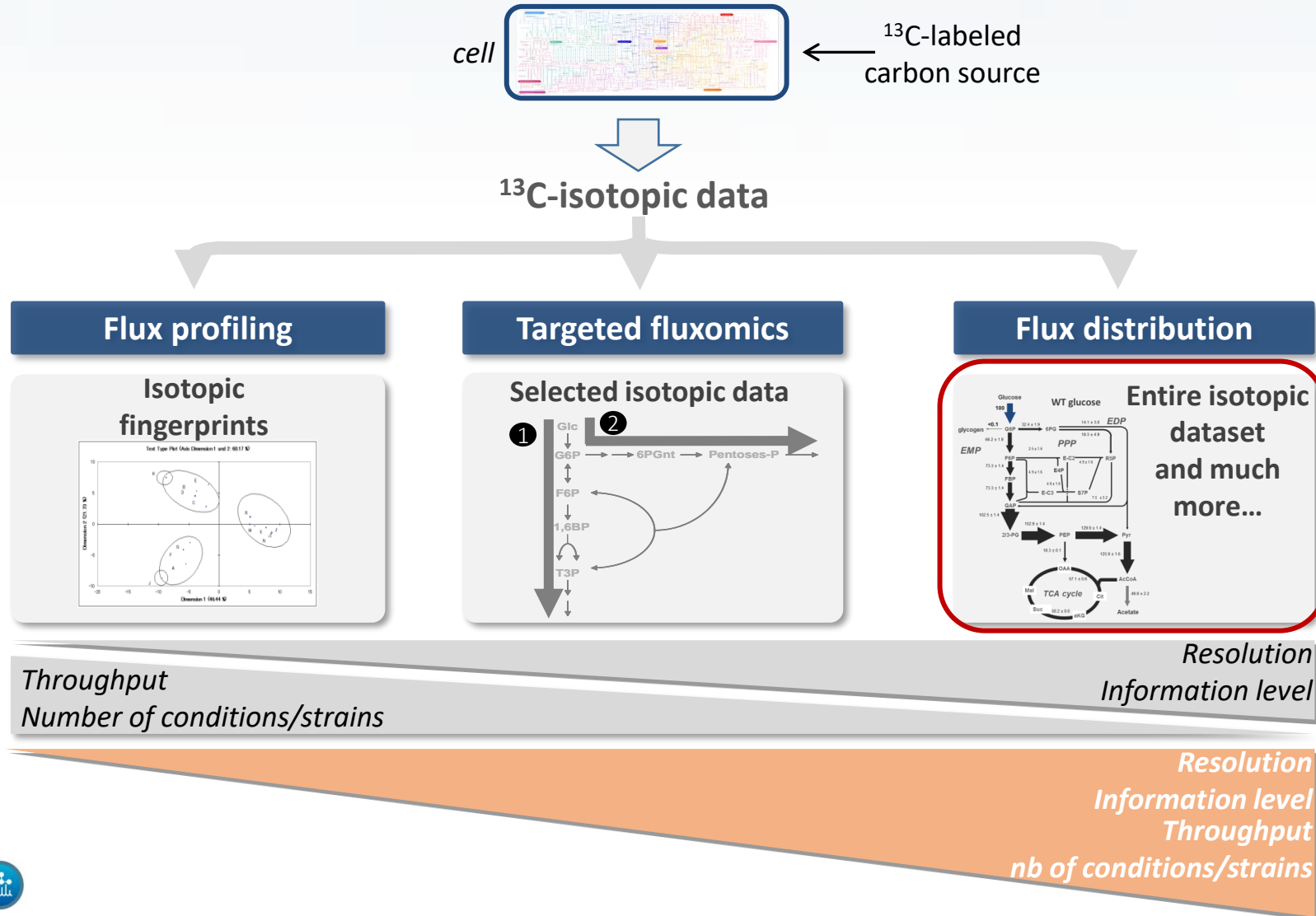


## MS: isotopologues



# INTRODUCTION : $^{13}\text{C}$ fluxomic analyses

Different  $^{13}\text{C}$  exploitation approaches : Different levels of information and throughput :



Highly manual and tedious work



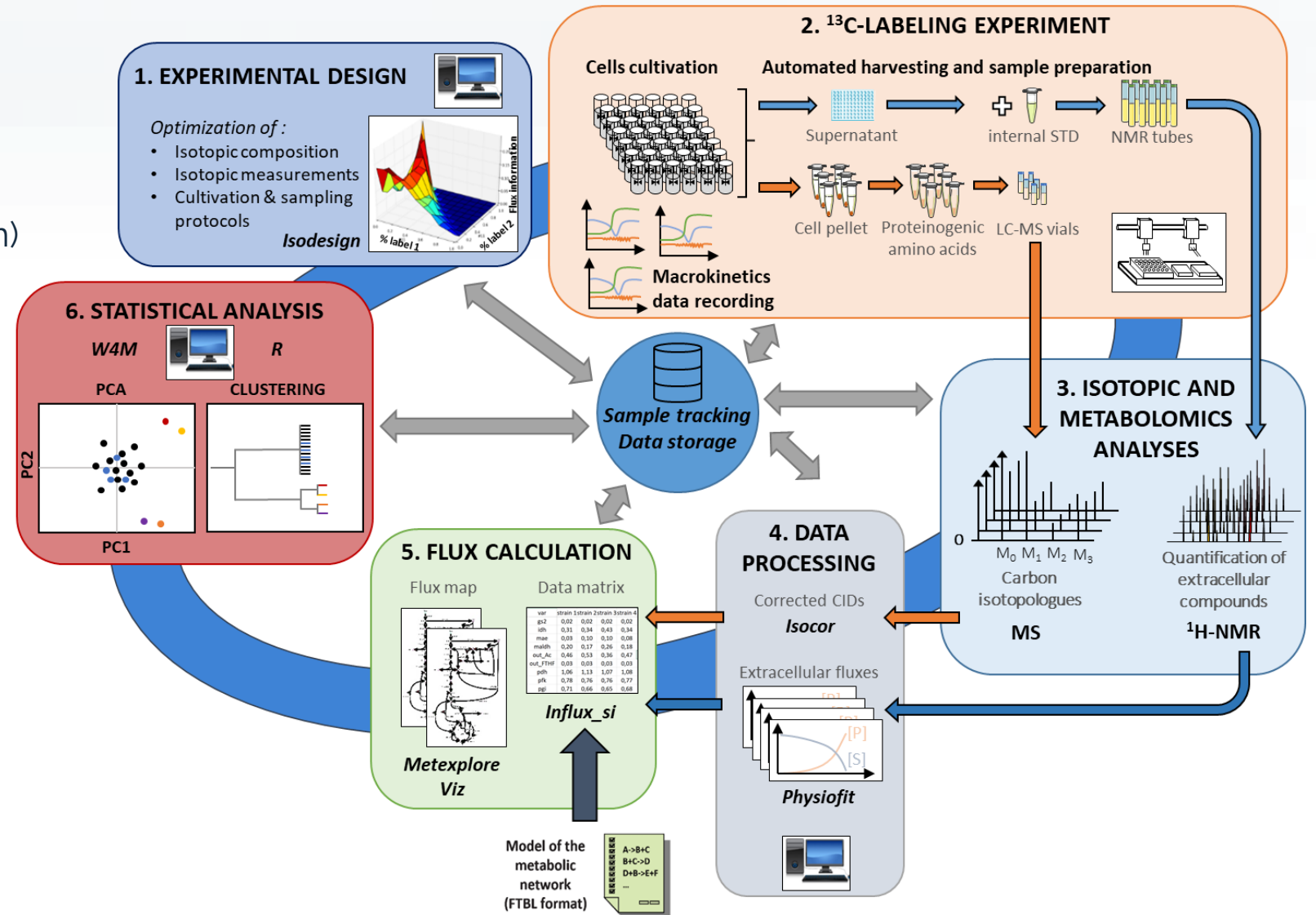
Many steps, long process

# How to go faster, deeper, stronger...

## What do we need to improve :

- Throughput
- Robustness
- Interactivity (data  $\leftrightarrow$  results  $\leftrightarrow$  brain)

## Needs for a development of an integrated fluxomics workflow :





# Application to the Fluxotyping of *E.coli* Y-ome

**E. coli BW25113**



- 4 624 857 pdb
- 4 487 genes
- 4 234 protein-encoding genes
- 122 RNA genes
- 131 pseudogenes
- **Unknown function protein encoding genes ?**  
-> **The Y-ome**

- Does Y-ome genes have a role in metabolism activity ?
- Does they affect flux phenotype ?
- If yes, can we affect a potential role to these Y-genes ?

Molecular Systems Biology (2006) doi:10.1038/msb4100050  
© 2006 EMBO and Nature Publishing Group All rights reserved 1744-4292/06  
www.molecular-systemsbiology.com  
Article number: 2006.0008

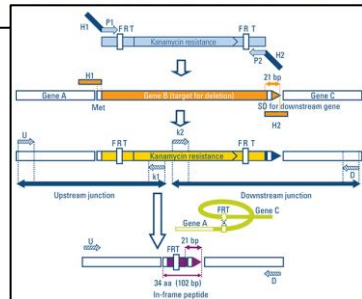
molecular systems biology

## Construction of *Escherichia coli* K-12 in-frame, single-gene knockout mutants: the Keio collection

Tomoya Baba<sup>1,2</sup>, Takeshi Ara<sup>1</sup>, Miki Hasegawa<sup>1,3</sup>, Yuki Takai<sup>1,3</sup>, Yoshiko Okumura<sup>1</sup>, Miki Baba<sup>1</sup>, Kirill A Datsenko<sup>4</sup>, Masaru Tomita<sup>1</sup>, Barry L Wanner<sup>4,\*</sup> and Hirotsada Mori<sup>1,2,\*</sup>

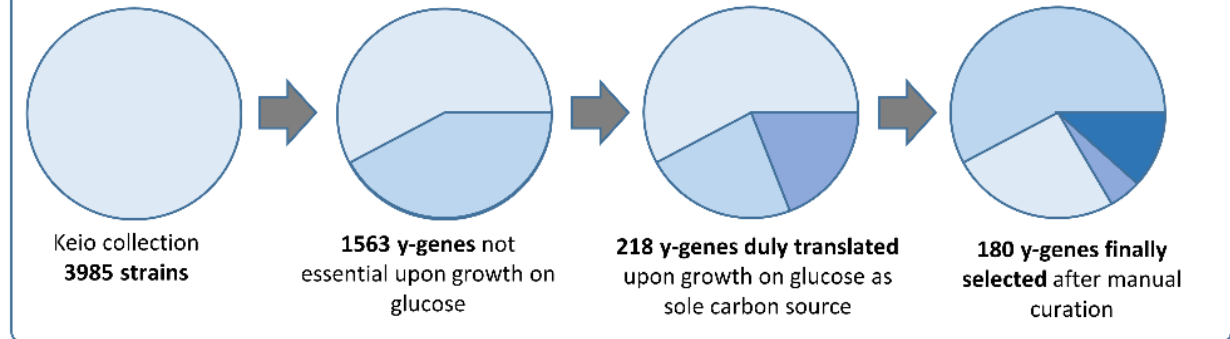
### The Keio collection

The most complete Gene deletion strain collection of *E. coli*



Baba *et al.*, Mol Syst Biol. 2006

## 1) Y-ome *E. coli* : strain selection

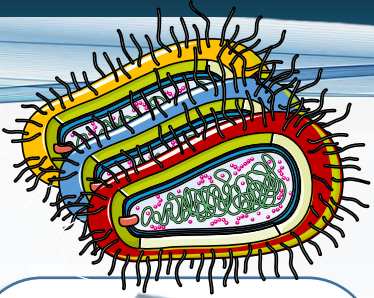


Proteomic data from Schmidt *et al.*, Nat Biotechnol, 2016

Databases used for selection and curation of *y*-genes : *Ecocyc*, *Uniprot*



# High-resolution fluxotyping of the *E. coli* Y-ome



## Robotic platforms

Cultivation, monitoring, sampling and preparation

## IsoCor

MS isotopic data correction

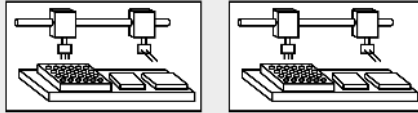
## Physiofit

Calculation of in/output fluxes from NMR data



## 2) HR Fluxotyping of the 180 selected strains

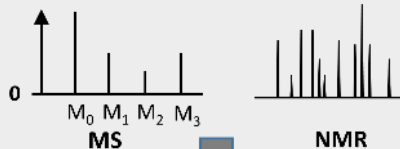
### <sup>13</sup>C labelling experiment



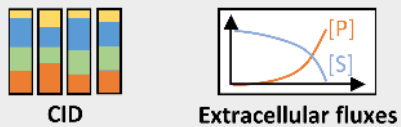
Cells cultivation & sampling

Sample preparation

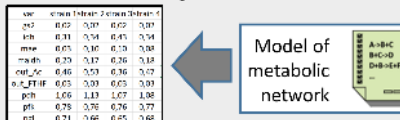
### Data analysis



### Data processing



### Intracellular fluxes calculation



## A huge amount of data to process

- 198 fully controlled cultivations (pH, O<sub>2</sub>, growth)
- 1074 growth measurements points(OD)
- 1248 samples collected
- more than 20000 isotopic data measured
- **18612 fluxes were calculated for 198 flux maps**



## Targeted LC-MS/MS

Thermo LTQ Orbitrap

Targeted analysis on 15 Proteinogenic Amino acids  
<sup>13</sup>C isotopic profiling



## NMR

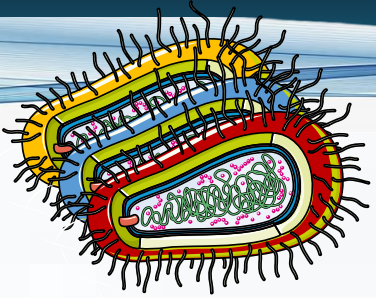
Bruker Ascend 800MHz – 5mm QPCI cryoprobe

Analysis of **exometabolome**  
**Quantification of substrates and products** and <sup>13</sup>C enrichments

## Influx<sub>si</sub>

Serial flux calculation from a unique starting metabolic network model

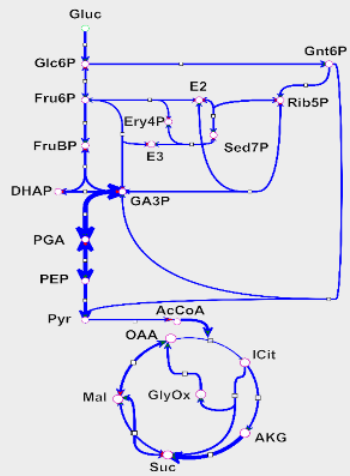
# How to evaluate fluxotype results of the *E. coli* Y-ome ?



## How to evaluate this big data set ?

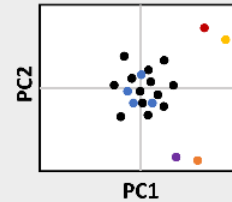
### 3) Fluxotype evaluation

High-resolution fluxotypes: metabolic maps with flux values and standard deviations

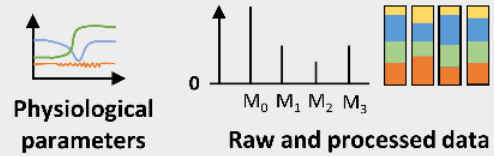


PCA on intracellular fluxes

Outlier Strains



Checking of data outliers



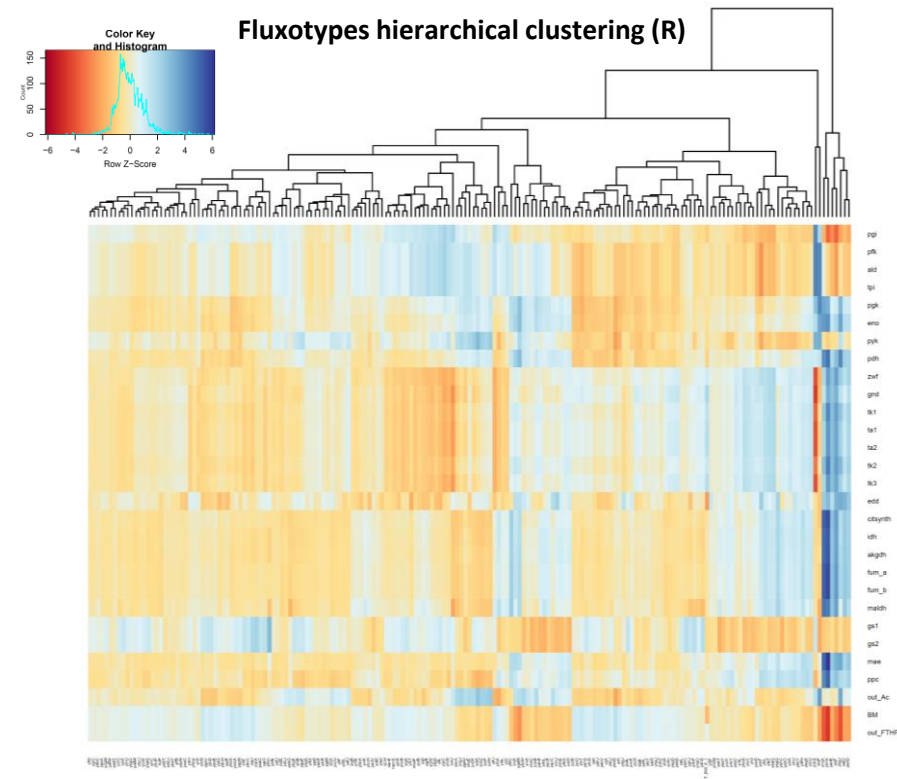
### Data curation

- Statistical analyses (HC, PCA)
- Removal of outliers

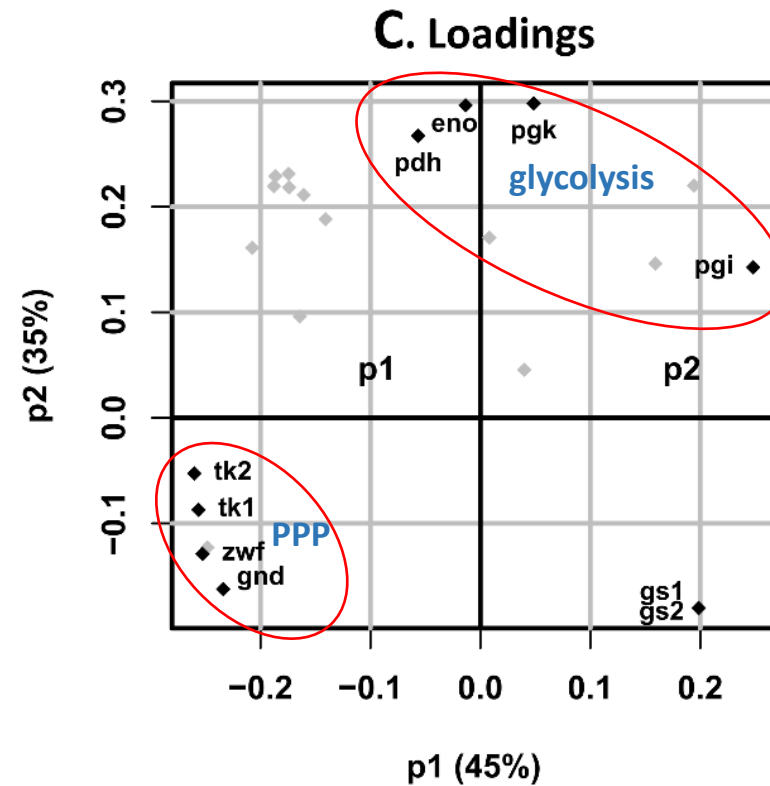
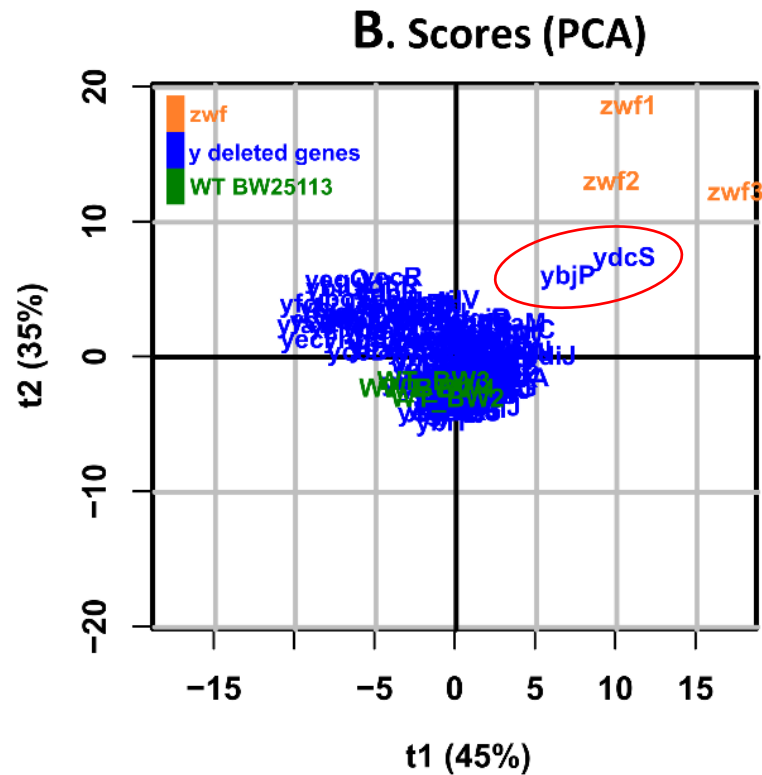
-> Replication of strains of interest and control strains

Comparison of growth parameters between the *E. coli* Y-ome strains and the WT strain

	$\mu$ (hr <sup>-1</sup> )	<i>qglc</i> (mmol.gDW <sup>-1</sup> .hr <sup>-1</sup> )	Biomass yield (gDW <sup>-1</sup> .mmol <sup>-1</sup> )	<i>qace</i> (mmol.gDW <sup>-1</sup> .hr <sup>-1</sup> )
BW25113 (n = 4)	0.66 ± 0.02	-6.41 ± 0.30	0.10 ± 0.01	2.29 ± 0.24
<i>E. coli</i> Y_ome (average of 180 strains)	0.57 ± 0.06	-5.81 ± 1.02	0.10 ± 0.02	2.79 ± 0.65



# Comparison of fluxotypes of $\gamma$ -ome strains with those of their parental strain

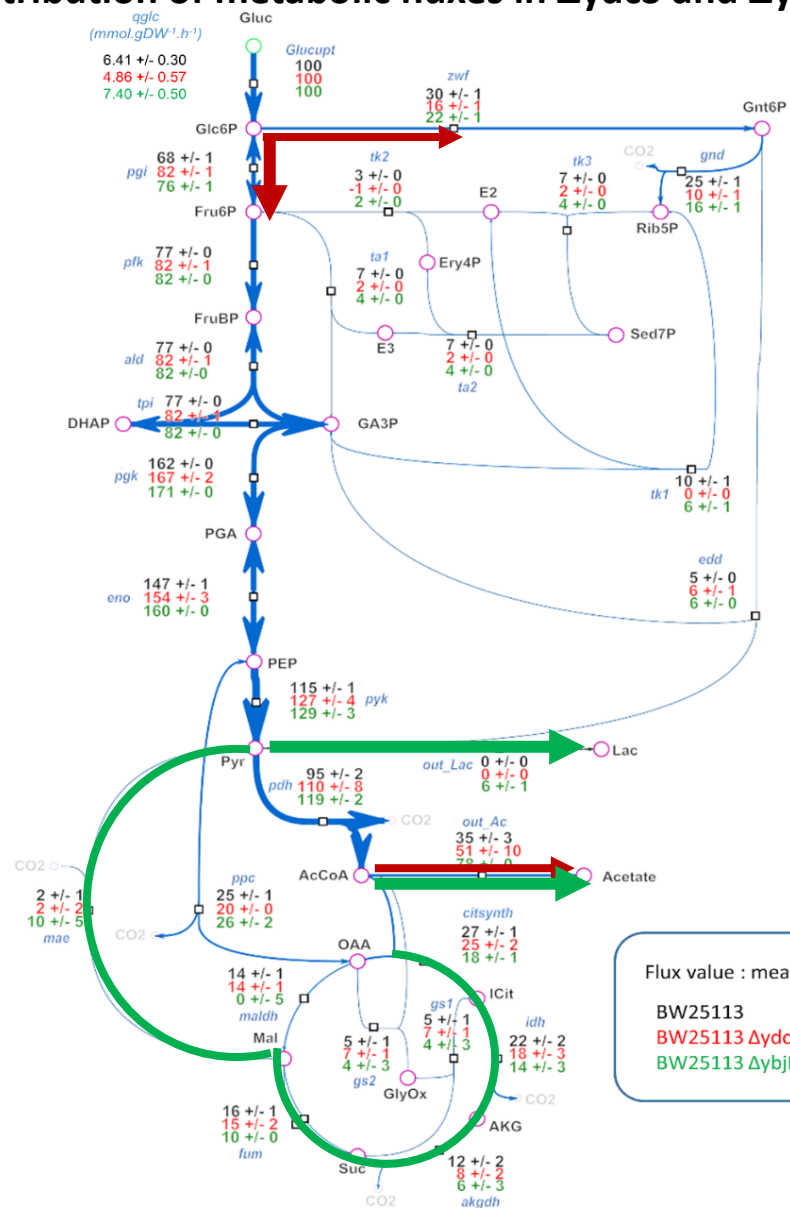


- For a large majority of mutant strains the flux data clustered with those of the WT strains
- Most of the deleted  $\gamma$ -genes has a limited impact on the distribution of intracellular metabolic fluxes

**Two mutant strains did not cluster with the WT strains :  $\Delta ybjP$  &  $\Delta ydcS$**

# Biological conclusion : Metabolic fluxes of $\Delta ydcS$ and $\Delta ybjP$ strains

## Distribution of metabolic fluxes in $\Delta ydcS$ and $\Delta ybjP$ strains



- Modification of glycolysis/PPP partition
- Alteration in TCA cycle and related pathways (pdh, acetate / lactate excretion)

- **ydcS : decrease in PPP utilization**

- **ybjP : rerouting around TCA/ malic enzyme, lactate production**

- Overall increase in acetate production metabolism

Specific pathways?

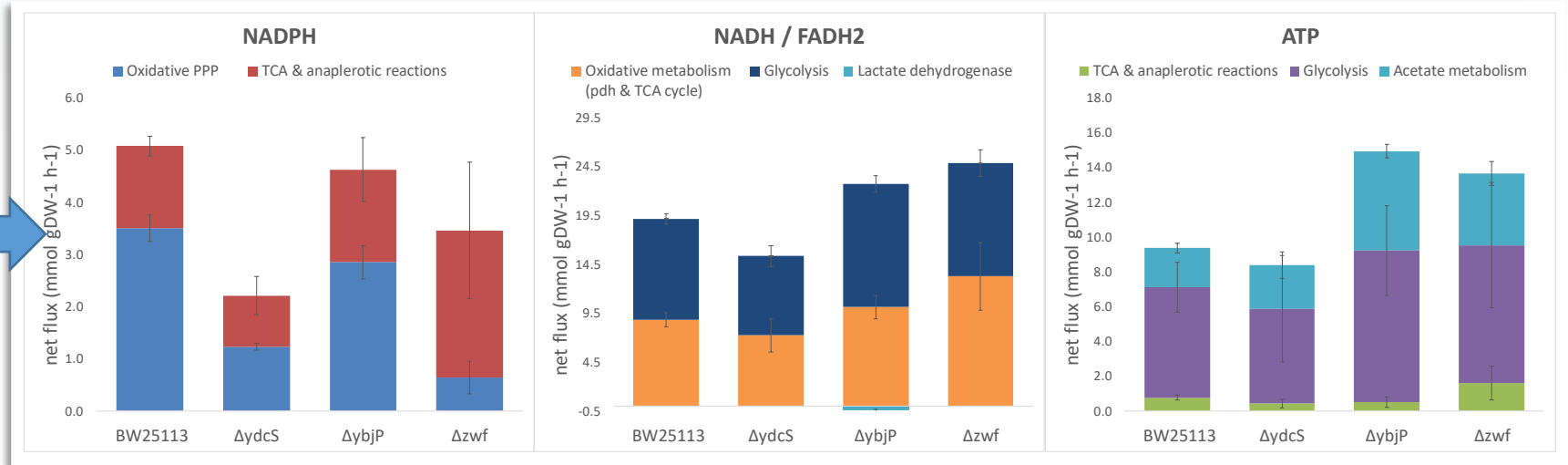
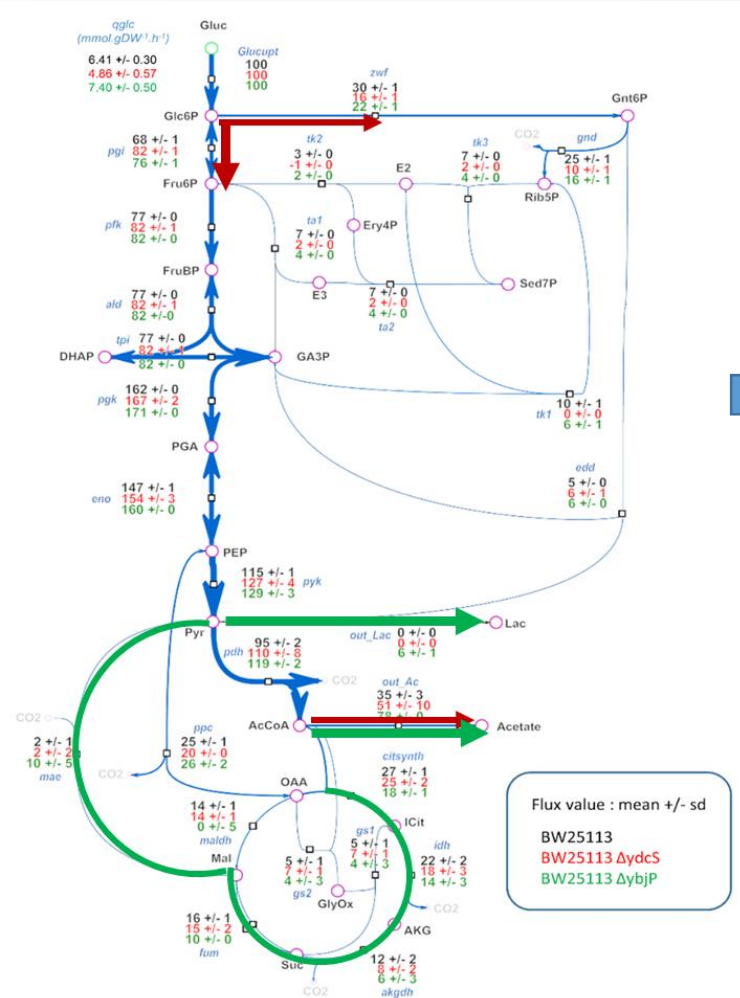
more a global rearrangement of flux distribution?

No direct link to a potential function... yet



# Biological conclusion : Metabolic fluxes of $\Delta ydcS$ and $\Delta ybjP$ strains

## Production of NADPH, NADH/FADH<sub>2</sub> and ATP from the central carbon metabolism



**$ydcS$  :** ↓ NADPH production rate  
↓ ATP production rate

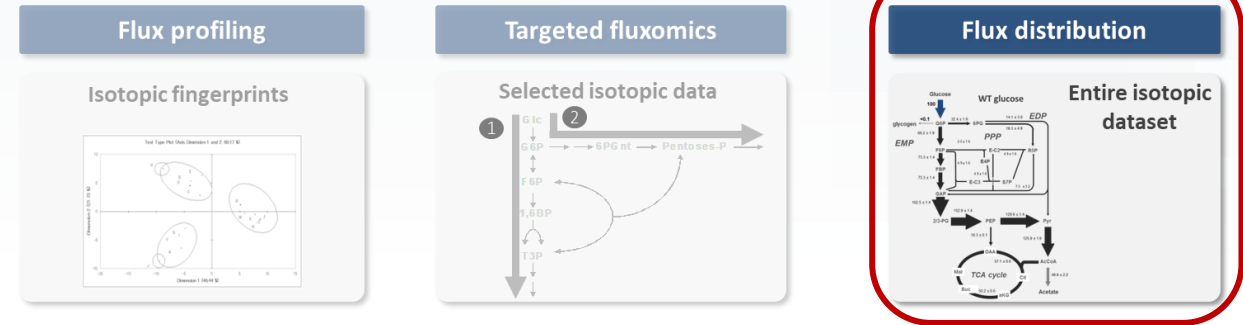
**$ybjP$  :** ↑ production rate of ATP  
▬ NADPH production



# Evaluation of data size and quality in HT fluxomics investigations

## HR fluxotyping workflow comparison :

	Leighty et al., 2013 [37]	Crown et al., 2015 [38]	Long & Antoniewicz 2019 [35]	Millard et al., 2014 [30]	Long et al., 2016 [39]	Heux et al., 2014 [22]	Haverkorn van Rijsewijk et al., 2011 [31]	This Paper	
<i>E. coli</i> strains	MG1655	MG1655	BW25113 $\Delta$ tpiA mutant	MG1655	BW25113	MG1655	BW25113 + Keio mutants	MG1655 + BW25113 + keio mutants	
Number of measured fluxotypes	1	1	1	1	1	20	190	198	
Number of different label input(s) by strain or condition	6	14	2	1	1	2	2	1	
Fluxome resolution = number of (net) fluxes by fluxotype	71	71	75	84	71	23	23	94	
Isotopic resolution power = number of isotopic data/ number of calculated fluxes	7.52	17.55	5.07	1.4	1.94	2.57	1.61	1.13	
Total flux dimension = number of fluxes per fluxotype $\times$ number of fluxotypes	71	71	75	84	142	460	4370	18,612	
Global flux precision = median RSD over the global dataset	12%	15%	7.80%	19%	23%	285%	14%	32%	
Global pathway precision = median RSD within specific pathways	S.A. (n = 1)	S.A. (n = 1)	S.A. (n = 1)	S.A. (n = 1)	S.A. (n = 1)	S.A. (n = 23)	S.A. (n = 23)	S.A. (n = 198)	biological replicates (n = 20)
Glycolysis	1%	2%	4.20%	3%	3.50%	553%	7%	3%	1%
ppp + edp	14%	11%	17%	131%	10%	41%	25%	24%	7%
tca + gs	11%	19%	4.90%	20%	31%	795%	19%	40%	21%
anaplerosis	122%	144%	87%	13%	1822%	7%	15%	42%	20%
output fluxes	12%	25%	4.7%	15%	42.4%	426%	0%	10%	14%



**198 isotopically-resolved flux maps generated in 33 days**  
**194 hrs of human time ~ 1hr / flux map**

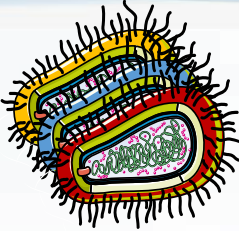
Resolution  
 Information level  
 Throughput  
 Nb of conditions/strains

## New metrics introduced :

- **Isotopic resolution power = 1.13**
  - **Total flux dimension = 18612**
  - **Global flux precision = 32 % (from sensitivity analysis)**
- HT and HR character of this workflow
- Compromise resolution / throughput



# Conclusions - Perspectives



## HT & HR fluxotyping workflow :

- Significant **improvements** in the parallelization, automation and implementation of fluxomics in **large-scale investigations**
- Good level of **flux precision / number of isotopic data collected**

## Y-ome investigation :

- The **vast majority** of the 180 mutations investigated has a fluxotype **similar to the WT strain**
- **2 interesting fluxotypes identified : Ydcs & Ybjp**
- **help annotation of gene functions -> EcoCyc referenced**

## Perspectives of developpements and applications

- **Expanding capacities** of fluxotyping on **other environment / carbon sources**
- Opening to other organisms: **Yeasts, other microorganisms, mammalian cells**
- Opening to **untargeted fluxomics** and **unknown organisms**
- Integration of fluxomics workflow in an improved and **integrated and <sup>13</sup>C fluxomics workflow**

**EcoCyc** Change Current Database  
Search in Current Database:

gene polypeptide  
**ybjP** **DUF3828 domain-containing lipoprotein YbjP**  
*Escherichia coli* K-12 substr. MG1655

Accession IDs: G6450 (EcoCyc), b0905, ECO0956, P75818 (UniProt)  
Length: 516 bp / 171 aa  
Map Position: [903,952 - 904,467] (19.4)  
Location: inner membrane

Summary GO Terms (2) Essentiality Protein Features Operons References Show All

Summary of Regulatory Influences on ybjP

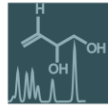
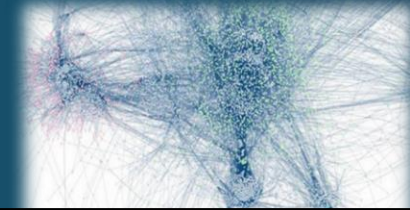
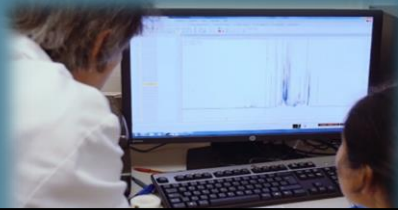
Diagram showing regulatory influences on ybjP: TdcC and GcsFp regulate ybjP, which in turn regulates YbjP.

Summary  
YbjP is a verified lipoprotein according to unpublished data by S. Matsuyama et al. (cited in [Juncker03]).  
Deletion of ybjP is associated with significant changes in the distribution of carbon fluxes in central metabolism [Berges21].  
Additional Citations: [Rao20]





# Thanks for your attention













*metabolites*



Article

## Exploring the Glucose Fluxotype of the *E. coli* $\gamma$ -ome Using High-Resolution Fluxomics

Cécilia Bergès <sup>1,2,+</sup> , Edern Cahoreau <sup>1,2,+</sup> , Pierre Millard <sup>1</sup> , Brice Enjalbert <sup>1</sup> , Mickael Dinclaux <sup>1</sup> , Maud Heuillet <sup>1,2</sup>, Hanna Kulyk <sup>1,2</sup>, Lara Gales <sup>1,2</sup>, Noémie Butin <sup>1,2,3</sup>, Maxime Chazalviel <sup>4</sup> , Tony Palama <sup>1,2</sup> , Matthieu Guionnet <sup>1,2</sup> , Sergueï Sokol <sup>1</sup> , Lindsay Peyriga <sup>1,2</sup> , Floriant Bellvert <sup>1,2</sup>, Stéphanie Heux <sup>1</sup> and Jean-Charles Portais <sup>1,2,3,\*</sup> 