Análise de Fluxos Metabólicos Empregando Carbono Marcado (^{13}C-MFA)

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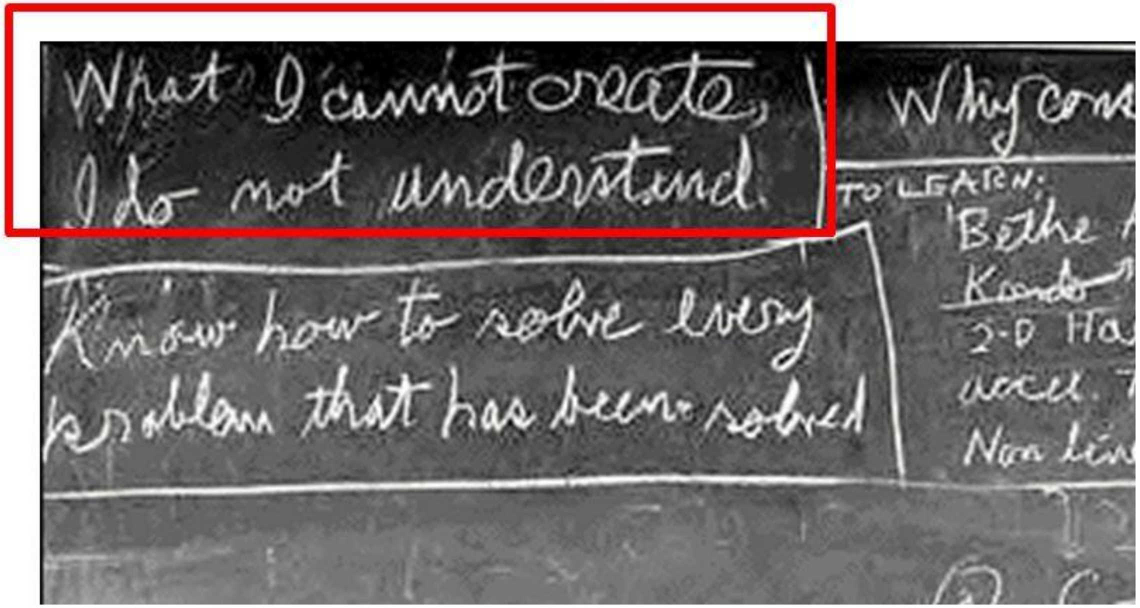
BMM5729-Análise Sistêmica e Engenharia do Metabolismo Microbiano

Por que construímos modelos?

"What I cannot create,
I do not understand"

Richard Feynman



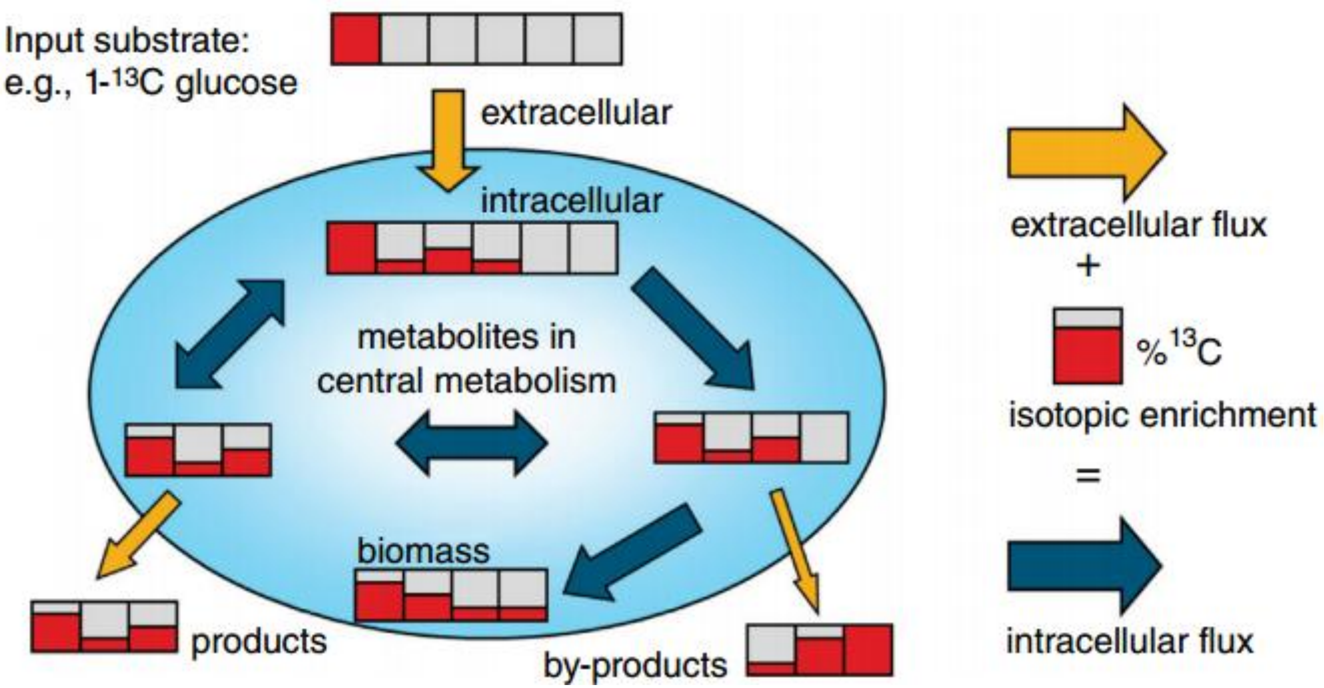
Por que construímos modelos?

“If you can’t model your Process, you don’t understand it. If you don’t understand it, you can’t improve it. And, if you can’t improve it, you won’t be competitive in the 21st century.”

Jim Trainham, DuPont, 1994

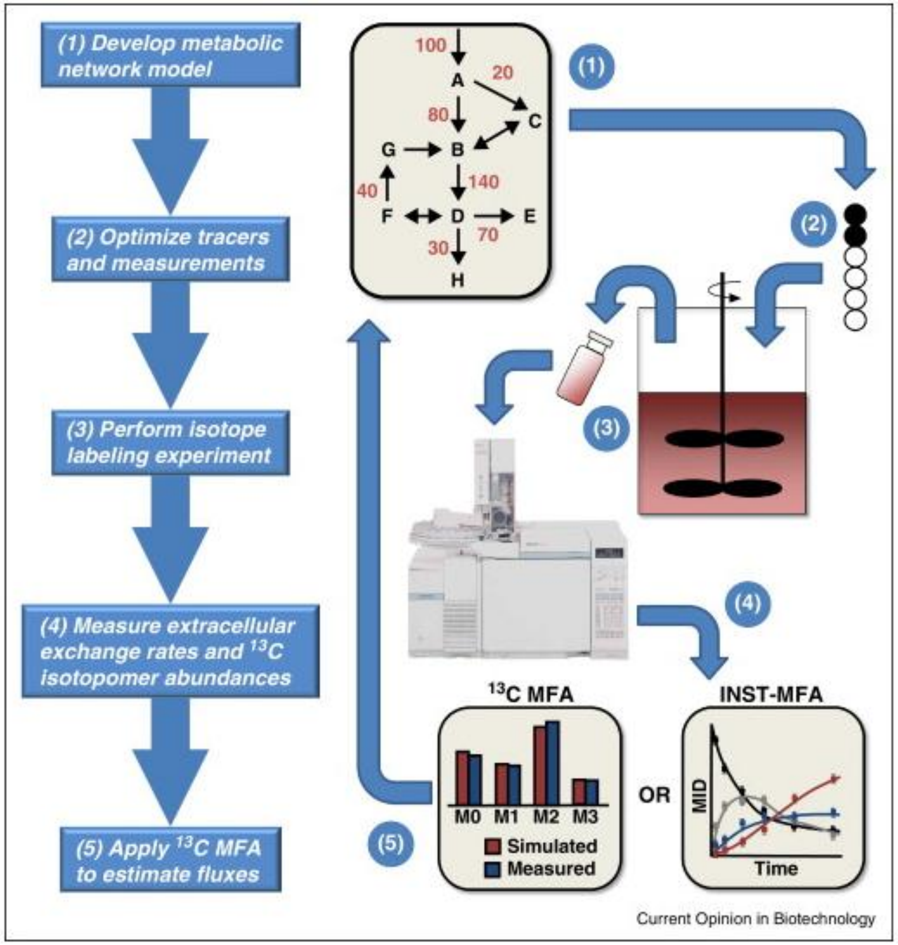
Por que construímos modelos?

Visão Geral



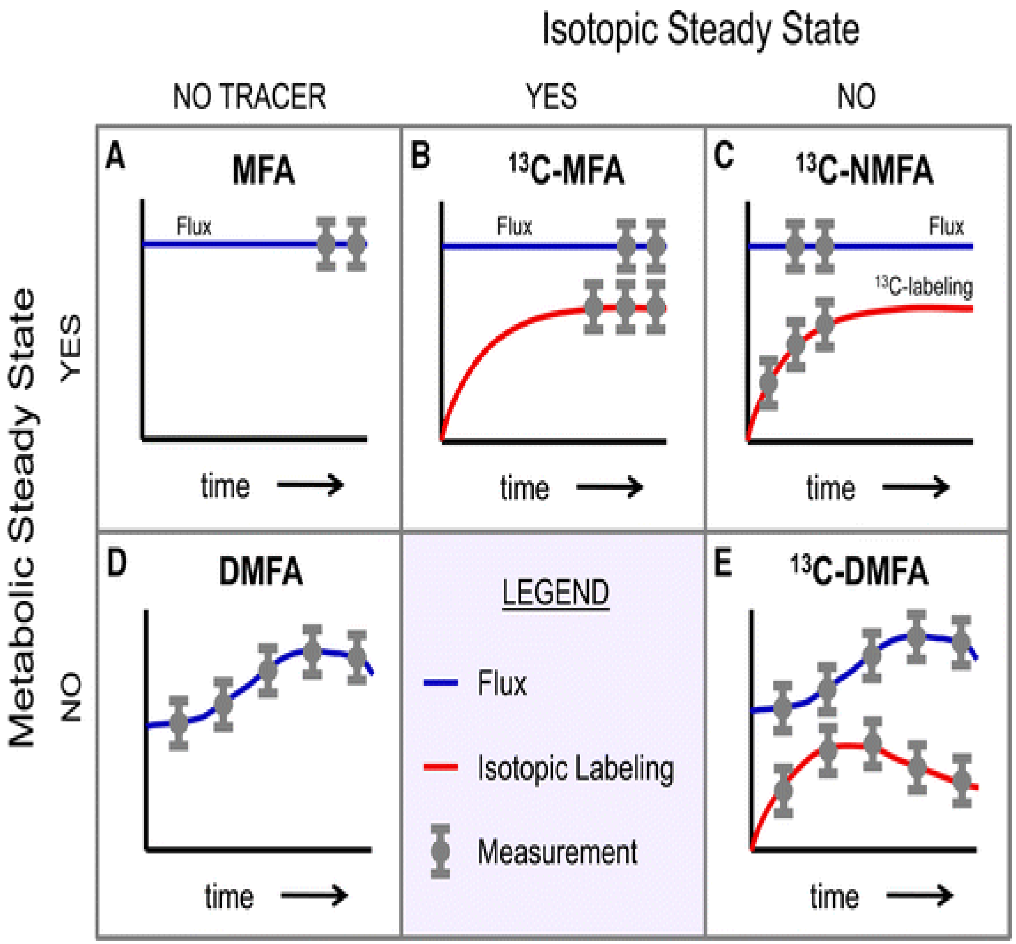
Fonte: WIECHERT, W. ^{13}C-Metabolic Flux Analysis. Metabolic Engineering, mar. 2001.

Etapas da ^{13}C-MFA



Current Opinion in Biotechnology

Classificação dos métodos de MFA



Fonte: Antoniewicz, Maciek R. "Methods and Advances in Metabolic Flux Analysis: A Mini-Review." Journal of Industrial Microbiology & Biotechnology 42, no. 3 (March 2015)

^{13}C-NMFA e ^{13}C-DMFA

- **Vantagens**

➢ Estimação de uma quantidade maior de fluxos metabólicos

➢ Estudos de estados transientes
Redução de custos experimentais

➢ Crescimento em CO_2

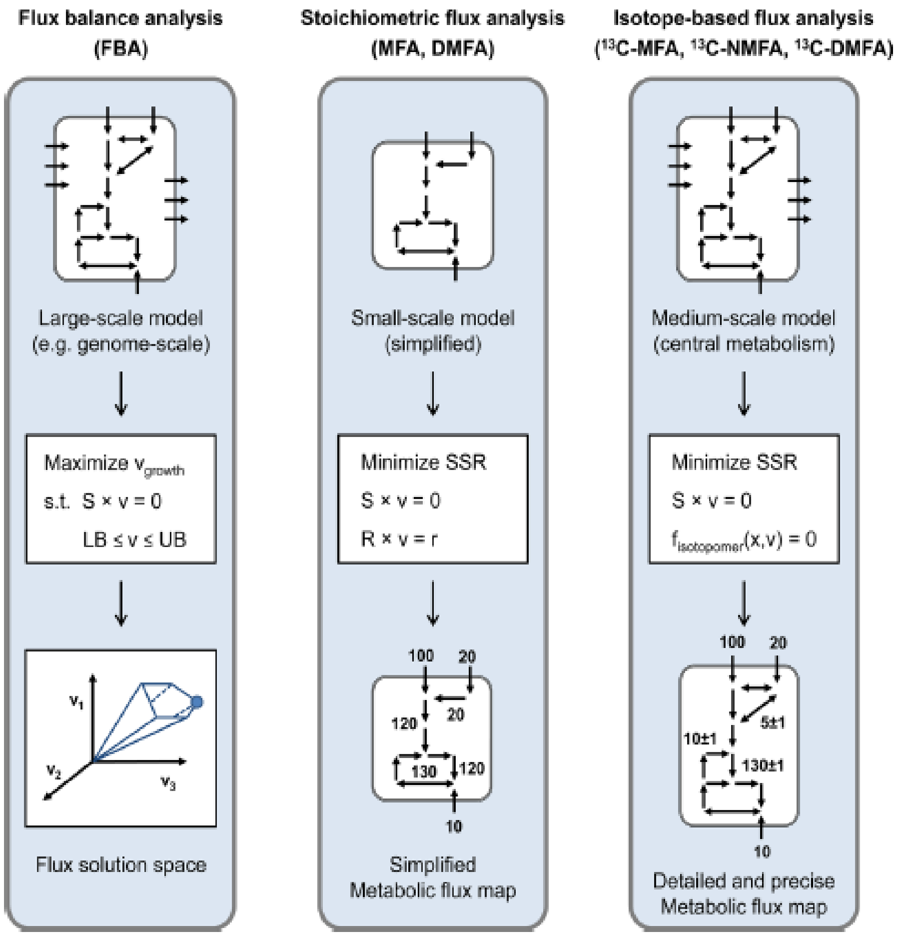
- **Desafios**

➢ Número maior de medidas

➢ Amostragem rápida

➢ Métodos computacionais

Abordagem de cada método



Flux balance analysis (FBA)

Large-scale model (e.g. genome-scale)

Maximize v_{growth}

s.t. $S \times v = 0$

$LB \leq v \leq UB$

Flux solution space

Stoichiometric flux analysis (MFA, DMFA)

Small-scale model (simplified)

Minimize SSR

$S \times v = 0$

$R \times v = r$

Simplified Metabolic flux map

Isotope-based flux analysis (^{13}C-MFA, ^{13}C-NMFA, ^{13}C-DMFA)

Medium-scale model (central metabolism)

Minimize SSR

$S \times v = 0$

$f_{isotopomer}(x,v) = 0$

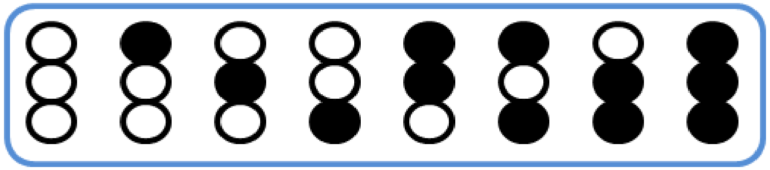
Detailed and precise Metabolic flux map

Fonte: Antoniewicz, Maciek R**.** "Methods and Advances in Metabolic Flux Analysis: A Mini-Review." Journal of Industrial Microbiology & Biotechnology 42, no. 3 (March 2015)

Conceito chave: Isotopômeros

Isotopômeros : Moléculas isômeras com átomos isótopos

Isotopômeros de posição



Conceito chave: Isotopômeros

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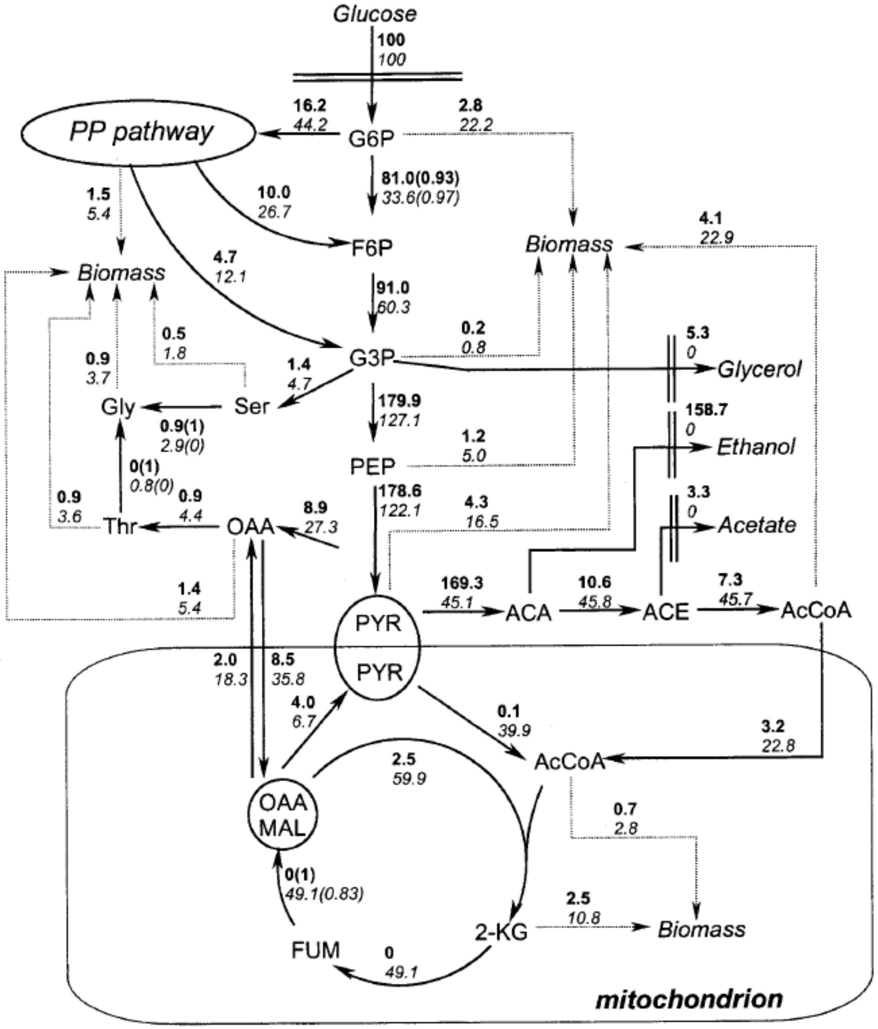
Marcação nos átomos

átomo 1

átomo 2

átomo 3

Conceito chave: transições atômicas nas reações



Conceito chave: transições atômicas nas reações

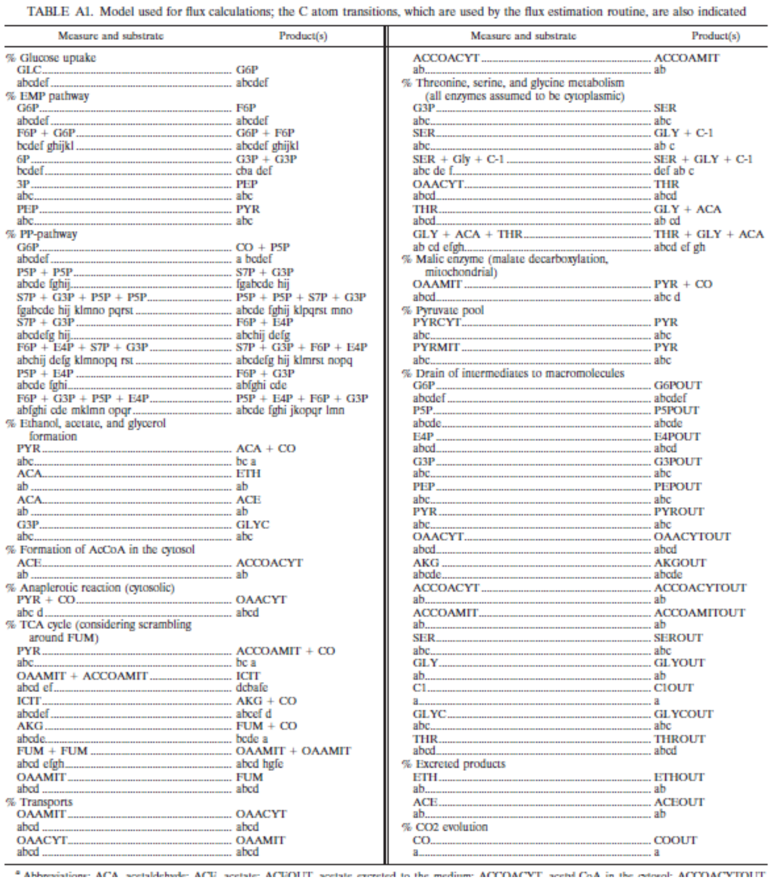
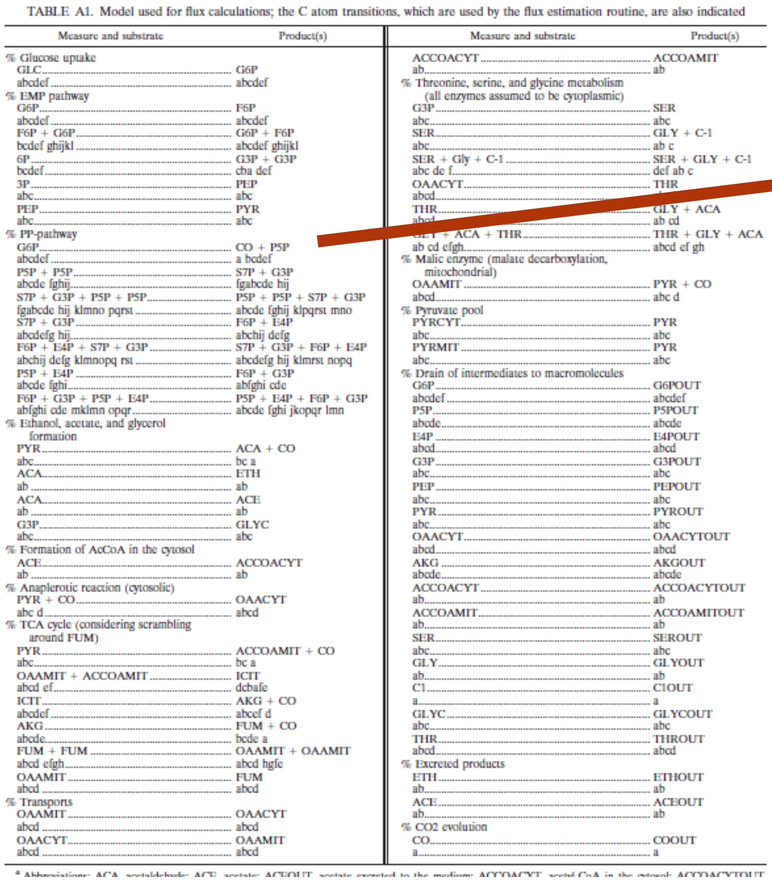


TABLE A1. Model used for flux calculations; the C atom transitions, which are used by the flux estimation routine, are also indicated

Measure and substrate	Product(s)
% Glucose uptake	
GLC	G6P
abcdef	abcdef
% EMP pathway	
G6P	F6P
abcdef	abcdef
F6P + G6P	G6P + F6P
bcdef ghijkl	abcdef ghijkl
6P	G3P + G3P
bcdef	cba def
3P	PEP
abc	abc
PEP	PYR
abc	abc
% PP-pathway	
G6P	CO + P5P
abcdef	a bcdef
P5P + P5P	S7P + G3P
abcde fghij	fgabcde hij
S7P + G3P + P5P + P5P	P5P + P5P + S7P + G3P
fgabcde hij klmno pqrst	abcde fghij klpqrst mno
S7P + G3P	F6P + E4P
abcdefg hij	abchij defg
F6P + E4P + S7P + G3P	S7P + G3P + F6P + E4P
abchij defg klmnopq rst	abcdefg hij klmrst nopq
P5P + E4P	F6P + G3P
abcde fghi	abfghi cde
F6P + G3P + P5P + E4P	P5P + E4P + F6P + G3P
abfghi cde mklmn opqr	abcde fghi jkopqr lmn
% Ethanol, acetate, and glycerol formation	
PYR	ACA + CO
abc	bc a
ACA	ETH
ab	ab
ACA	ACE
ab	ab
G3P	GLYC
abc	abc
% Formation of AcCoA in the cytosol	
ACE	ACCOACYT
ab	ab
% Anaplerotic reaction (cytosolic)	
PYR + CO	OAACYT
abc d	abcd
% TCA cycle (considering scrambling around FUM)	
PYR	ACCOAMIT + CO
abc	bc a
OAAMIT + ACCOAMIT	ICIT
abcd ef	dcbafe
ICIT	AKG + CO
abcdef	abcef d
AKG	FUM + CO
abcde	bcde a
FUM + FUM	OAAMIT + OAAMIT
abcd efgh	abcd hgfe
OAAMIT	FUM
abcd	abcd
% Transports	
OAAMIT	OAACYT
abcd	abcd
OAACYT	OAAMIT
abcd	abcd
ACCOACYT	ACCOAMIT
ab	ab
% Threonine, serine, and glycine metabolism (all enzymes assumed to be cytoplasmic)	
G3P	SER
abc	abc
SER	GLY + C-1
abc	ab c
SER + Gly + C-1	SER + GLY + C-1
abc de f	def ab c
OAACYT	THR
abcd	abcd
THR	GLY + ACA
abcd	ab cd
GLY + ACA + THR	THR + GLY + ACA
ab cd efgh	abcd ef gh
% Malic enzyme (malate decarboxylation, mitochondrial)	
OAAMIT	PYR + CO
abcd	abc d
% Pyruvate pool	
PYRCYT	PYR
abc	abc
PYRMIT	PYR
abc	abc
% Drain of intermediates to macromolecules	
G6P	G6POUT
abcdef	abcdef
P5P	P5POUT
abcde	abcde
E4P	E4POUT
abcd	abcd
G3P	G3POUT
abc	abc
PEP	PEPOUT
abc	abc
PYR	PYROUT
abc	abc
OAACYT	OAACYTOUT
abcd	abcd
AKG	AKGOUT
abcde	abcde
ACCOACYT	ACCOACYTOUT
ab	ab
ACCOAMIT	ACCOAMITOUT
ab	ab
SER	SEROUT
abc	abc
GLY	GLYOUT
ab	ab
C1	C1OUT
a	a
GLYC	GLYCOUT
abc	abc
THR	THROUT
abcd	abcd
% Excreted products	
ETH	ETHOUT
ab	ab
ACE	ACEOUT
ab	ab
% CO2 evolution	
CO	COOUT
a	a

[a] Abbreviations: ACA, acetaldehyde; ACE, acetate; ACEOUT, acetate excreted to the medium; ACCOACYT, acetyl CoA in the cytosol; ACCOACYTOUT

Conceito chave: transições atômicas nas reações



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abchij defg klmnopq rst	abcdefg hij klmrst nopq
P5P + E4P	F6P + G3P
abcde fghi	abfghi cde
F6P + G3P + P5P + E4P	P5P + E4P + F6P + G3P
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abcd	[illegible]
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PEP	PEPOUT
abc	abc
PYR	PYROUT
abc	abc
OAACYT	OAACYTOUT
abcd	abcd
AKG	AKGOUT
abcde	abcde
ACCOACYT	ACCOACYTOUT
ab	ab
ACCOAMIT	ACCOAMITOUT
ab	ab
SER	SEROUT
abc	abc
GLY	GLYOUT
ab	ab
C1	C1OUT
a	a
GLYC	GLYCOUT
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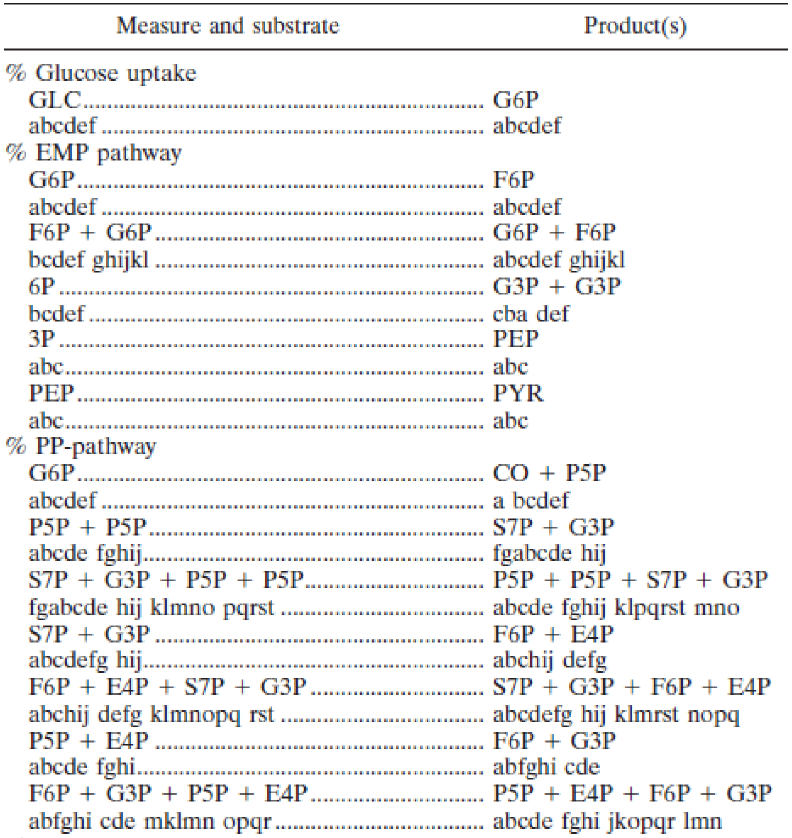

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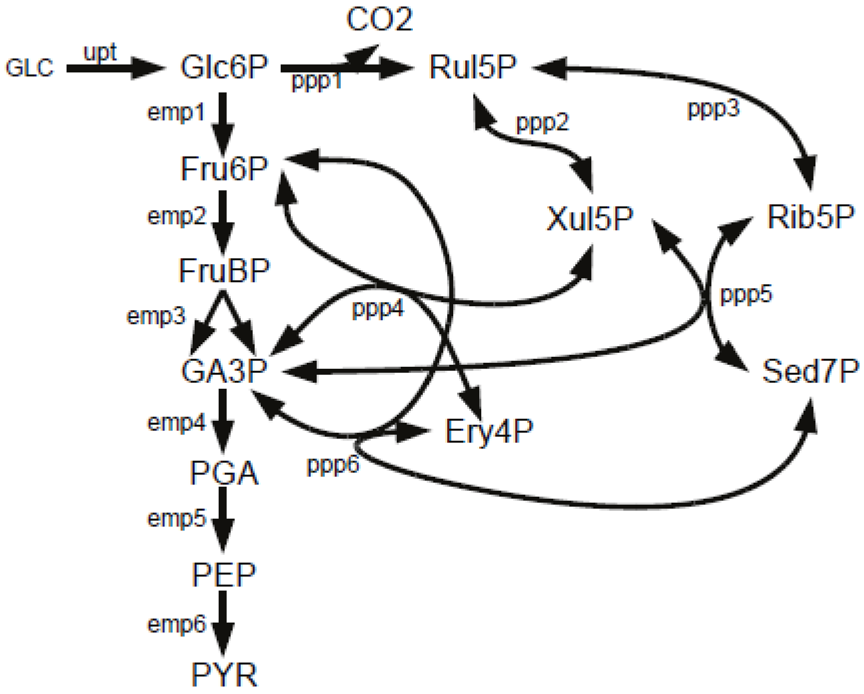
fgabcde hij klmno pqrst	abcde fghij klpqrst mno
S7P + G3P	F6P + E4P
abcdefg hij	abchij defg
F6P + E4P + S7P + G3P	S7P + G3P + F6P + E4P
abchij defg klmnopq rst	abcdefg hij klmrst nopq
P5P + E4P	F6P + G3P
abcde fghi	abfghi cde
F6P + G3P + P5P + E4P	P5P + E4P + F6P + G3P
abfghi cde mklmn opqr	abcde fghi jkopqr lmn

Conceito chave: Fluxos bidirecionais

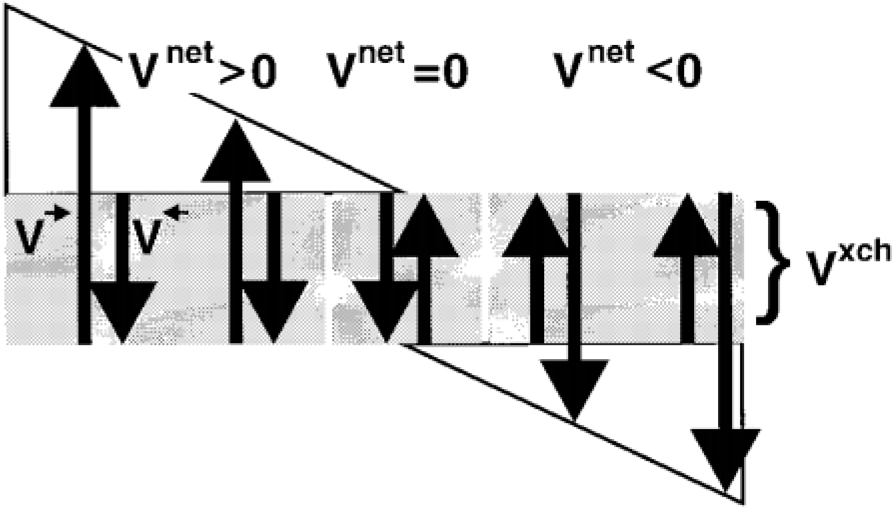


PPP3 $\vec{v}$: Rul5P $\rightarrow$ Rib5P $\overleftarrow{v}$: Rul5P $\leftarrow$ Rib5P

Conceito chave: Fluxos bidirecionais

$$v^{net} = \overrightarrow{v} - \overleftarrow{v}$$

$$v^{xch} = min(\overrightarrow{v}, \overleftarrow{v})$$

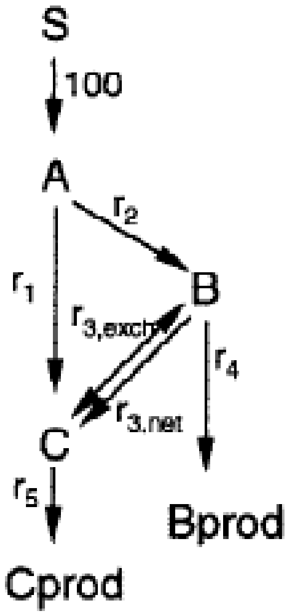


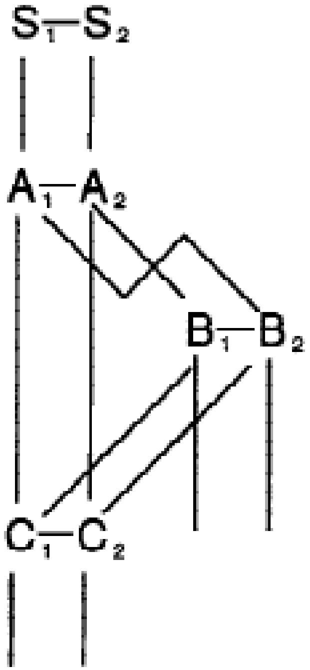
Conceito chave: Fluxos livres

Balanços dos metabólitos:

$$
\begin{aligned}
&A\colon 100 - r_1 - r_2 = 0\\
&B\colon r_2 - r_{3,\mathrm{net}} - r_4 = 0\\
&C\colon r_1 + r_{3,\mathrm{net}} - r_5 = 0
\end{aligned}
$$

Rede metabólica:



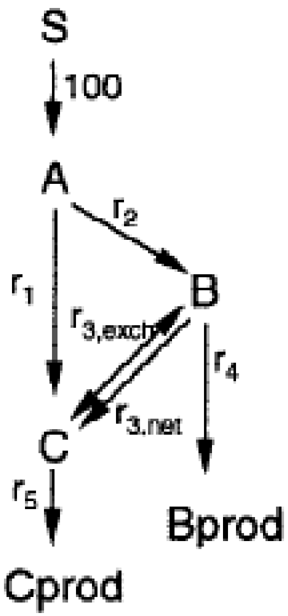


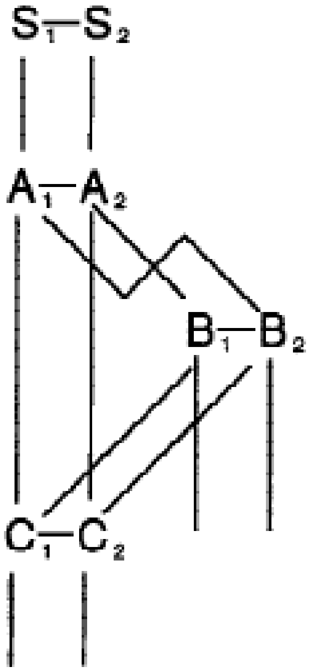
Conceito chave: Fluxos livres

Balanços dos metabólitos:

$$A: 100 - r_1 - r_2 = 0$$
$$B: r_2 - r_{3,\text{net}} - r_4 = 0$$
$$C: r_1 + r_{3,\text{net}} - r_5 = 0$$

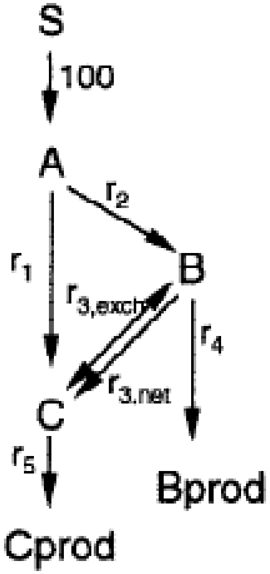
Rede metabólica:

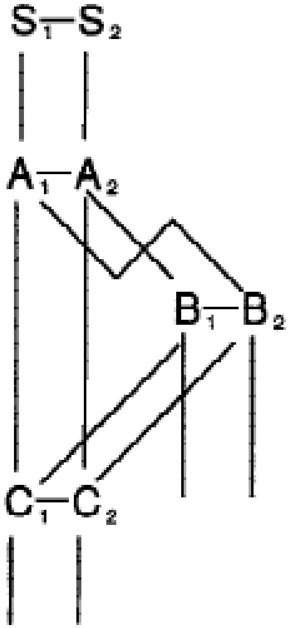




Escolho (fluxos livres)		Calculo pelos balanços		
r_1	r_3	r_2	r_4	r_5
50	0	50	50	50
0	100	100	0	100

Modelo Matemático

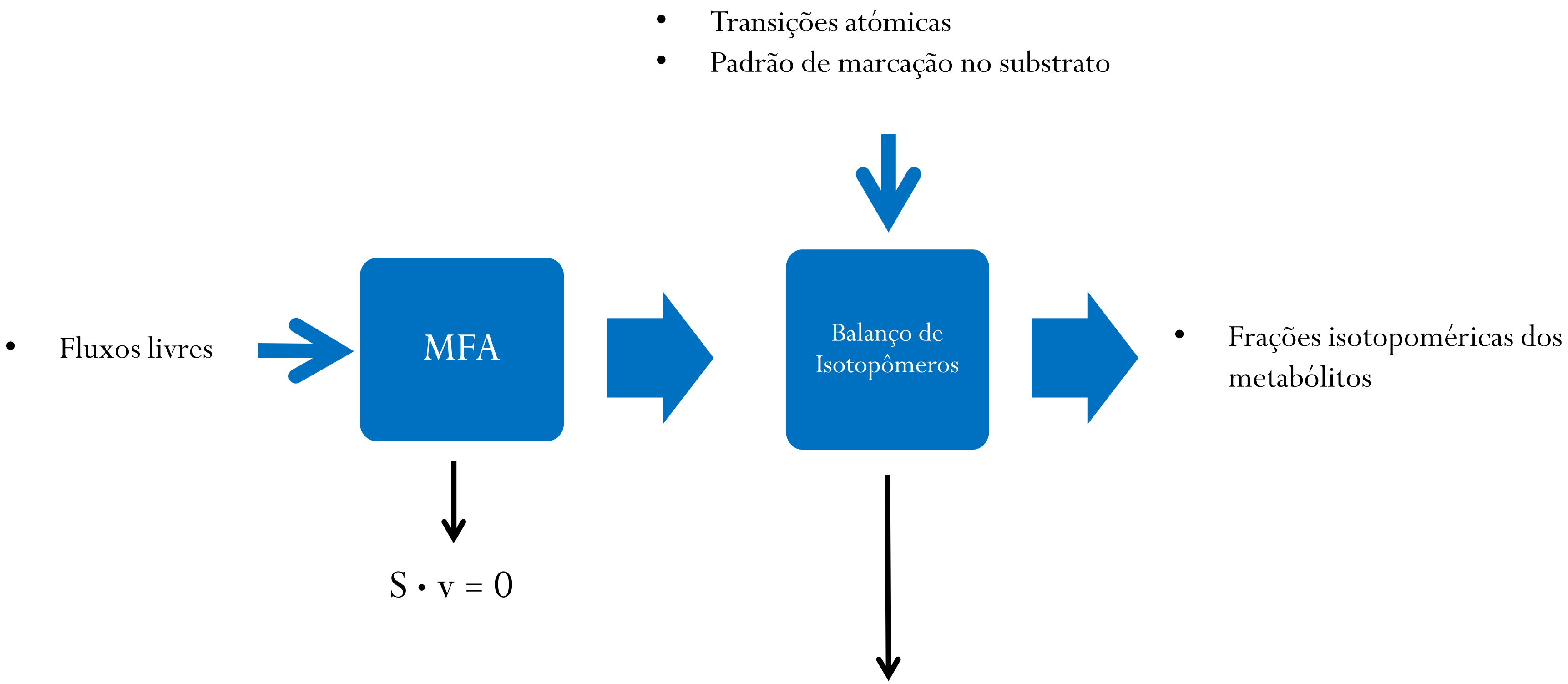




Balanços de marcação nos átomos dos metabólitos:

$$
\begin{array}{cccccccc}
-(r_1+r_2)A_1 & & & & & & = & -100S_1 \\
 & -(r_1+r_2)A_2 & & & & & = & -100S_2 \\
 & r_2A_2 & -(r_{3,\text{exch}}+r_{3,\text{net}}+r_4)B_1 & & r_{3,\text{exch}}C_1 & & = & 0 \\
r_2A_1 & & & -(r_{3,\text{exch}}+r_{3,\text{net}}+r_4)B_2 & & r_{3,\text{exch}}C_2 & = & 0 \\
r_1A_1 & & (r_{3,\text{exch}}+r_{3,\text{net}})B_1 & & -(r_{3,\text{exch}}+r_5)C_1 & & = & 0 \\
 & r_1A_2 & & (r_{3,\text{exch}}+r_{3,\text{net}})B_2 & & -(r_{3,\text{exch}}+r_5)C_2 & = & 0
\end{array}
$$

Modelo Matemático



- Transições atómicas
- Padrão de marcação no substrato

- Fluxos livres

MFA

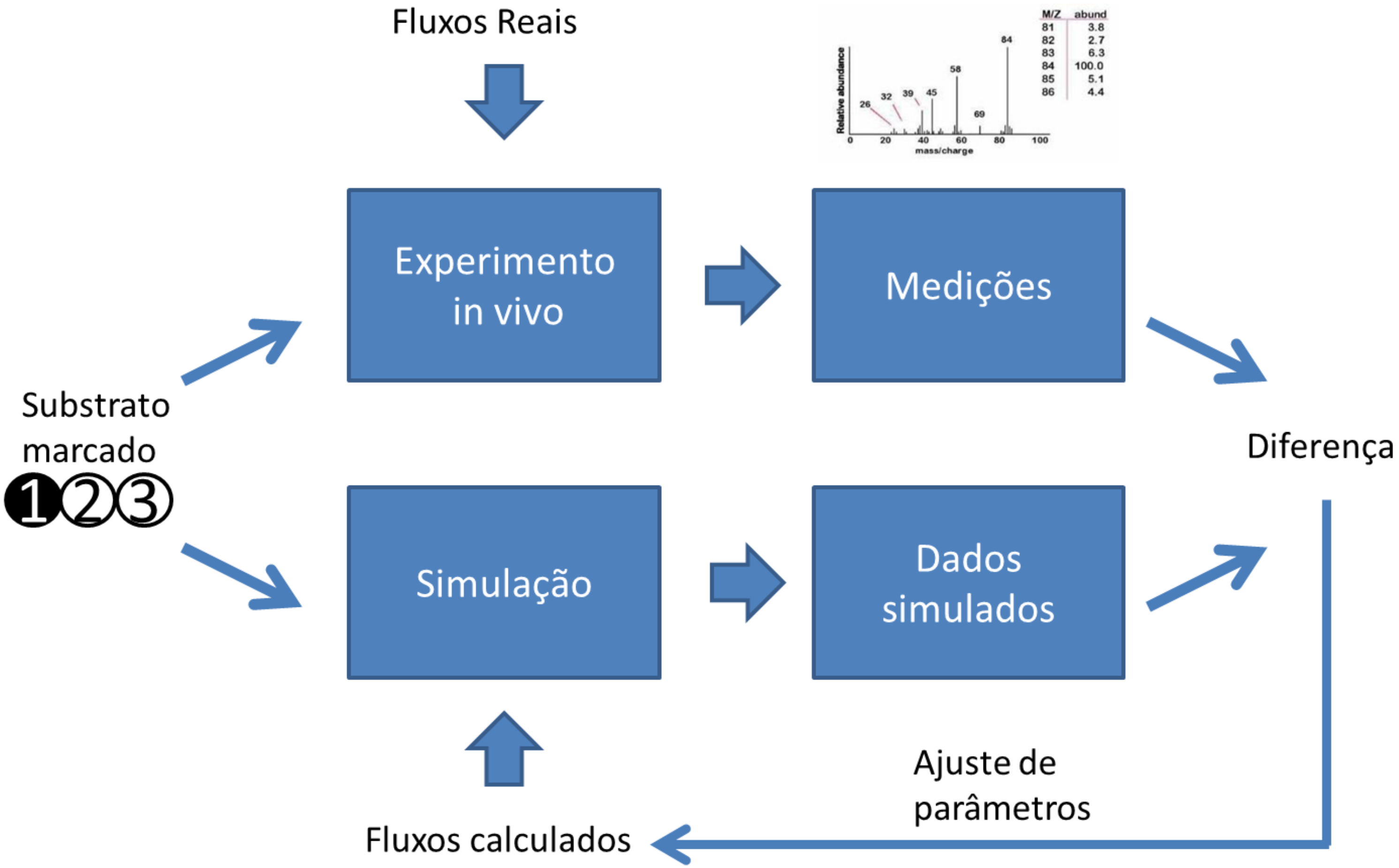
$S \cdot v = 0$

Balanço de Isotopômeros

- Frações isotopoméricas dos metabólitos

$$\bar{f}(v,\bar{x},\bar{x}^{inp}) = \frac{1}{2}\bar{x}^{T}\cdot\Big(\sum_{i}\overrightarrow{v}\cdot\overrightarrow{\bar{Q}}_{i}+\overleftarrow{v}\cdot\overleftarrow{\bar{Q}}_{i}\Big)\cdot\bar{x}+\Big(\sum_{i}\overrightarrow{v}_{i}\cdot\overrightarrow{\bar{P}}_{i}+\sum_{i}\overleftarrow{v}_{i}\cdot\overleftarrow{\bar{P}}_{i}\Big)\cdot\bar{x}+\Big(\sum_{i}\overrightarrow{v}_{i}\cdot\bar{P}_{i}^{inp}\Big)\cdot\bar{x}^{inp}=0$$

Estimação de fluxos



M/Z	abund
81	3.8
82	2.7
83	6.3
84	100.0
85	5.1
86	4.4

Fluxos Reais

Experimento in vivo

Medições

Substrato marcado ❶②③

Simulação

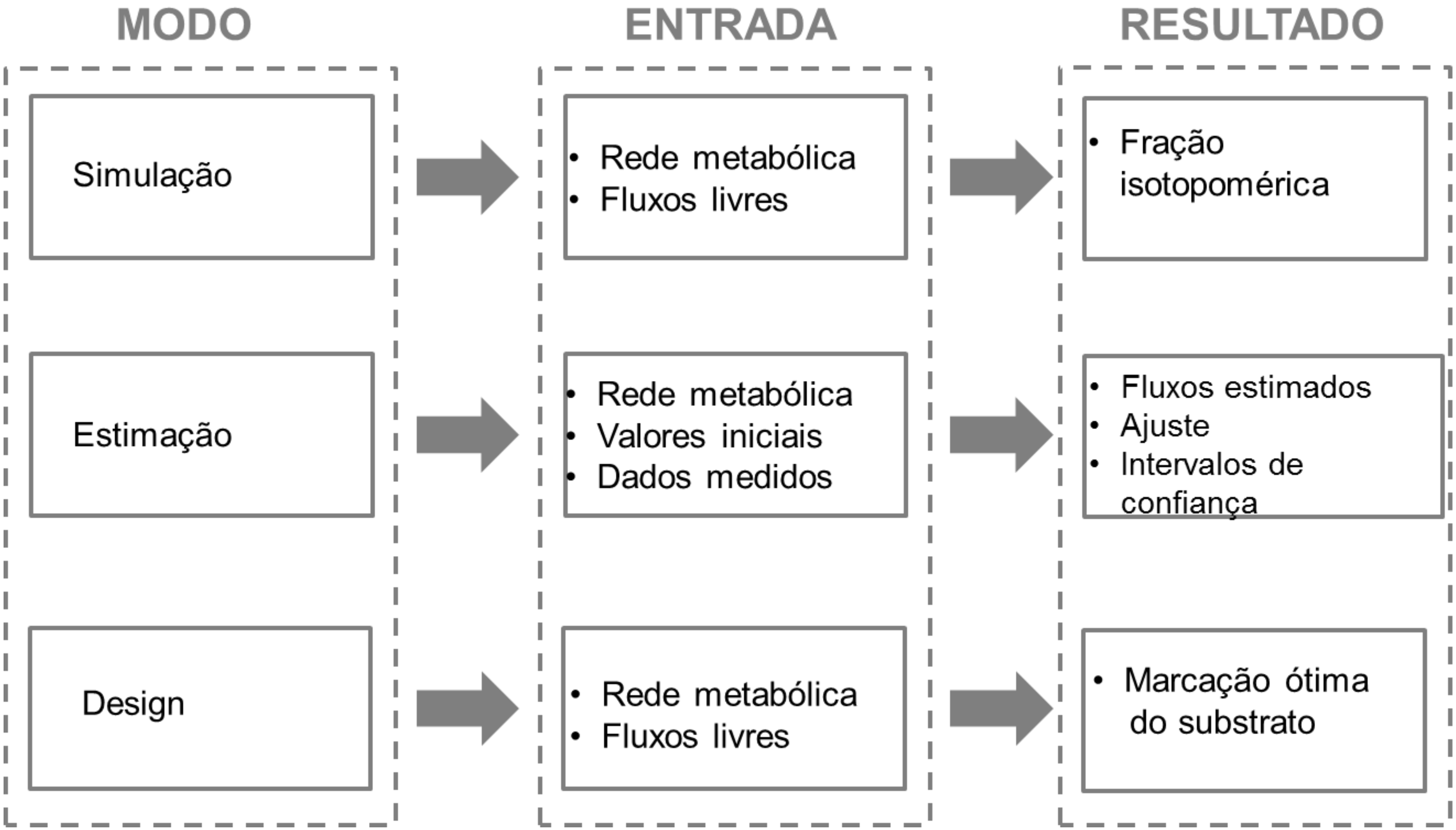
Dados simulados

Diferença

Ajuste de parâmetros

Fluxos calculados

Ferramenta computacional



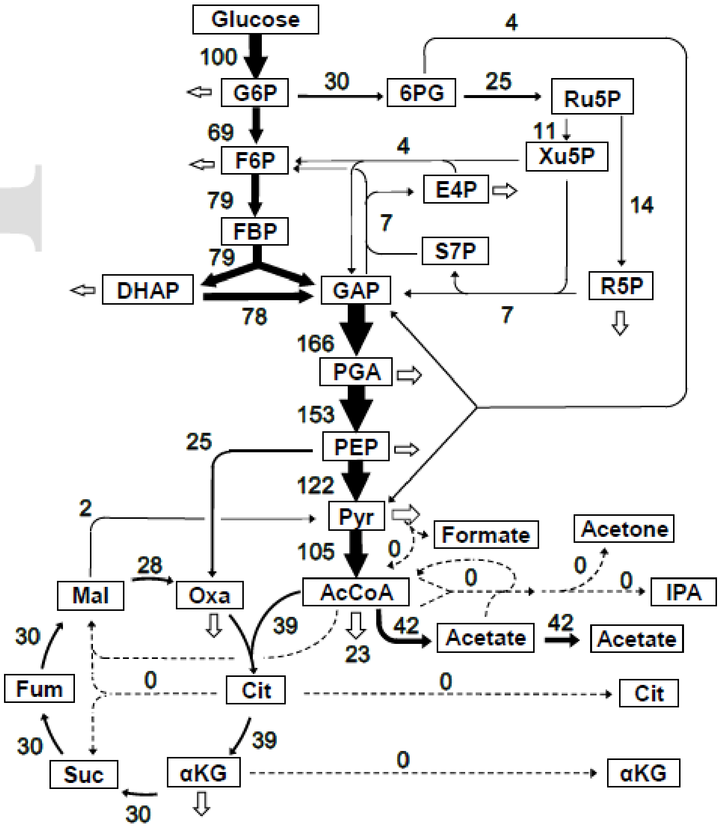
MODO	ENTRADA	RESULTADO
Simulação	• Rede metabólica • Fluxos livres	• Fração isotopomérica
Estimação	• Rede metabólica • Valores iniciais • Dados medidos	• Fluxos estimados • Ajuste • Intervalos de confiança
Design	• Rede metabólica • Fluxos livres	• Marcação ótima do substrato

Aplicações:

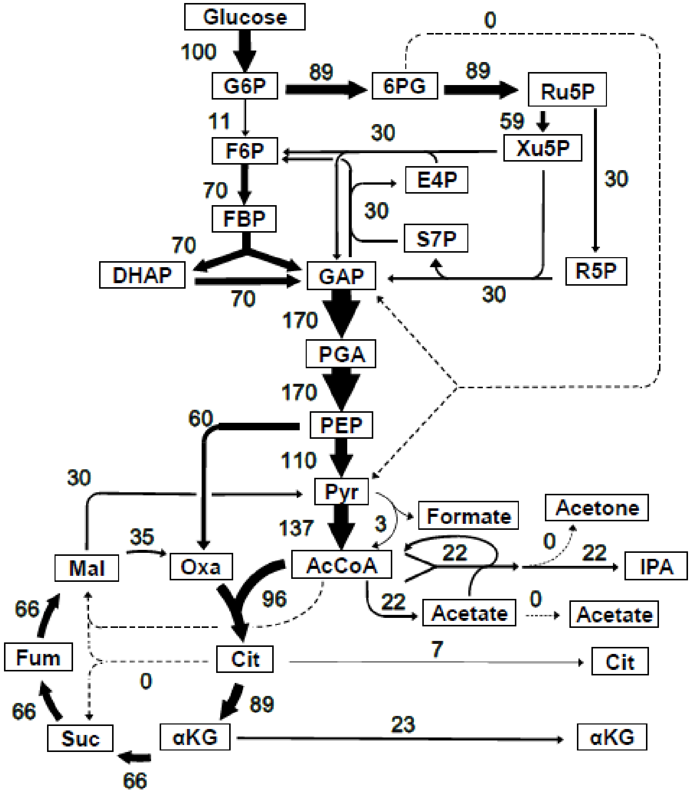
Metabolic engineering of isopropyl alcohol-producing *Escherichia coli* strains with ^{13}C-metabolic flux analysis

Nobuyuki Okahashi, Fumio Matsuda, Katsunori Yoshikawa, Tomokazu Shirai, Yoshiko Matsumoto, Mitsufumi Wada, Hiroshi Shimizu ✉

(a)



(b)



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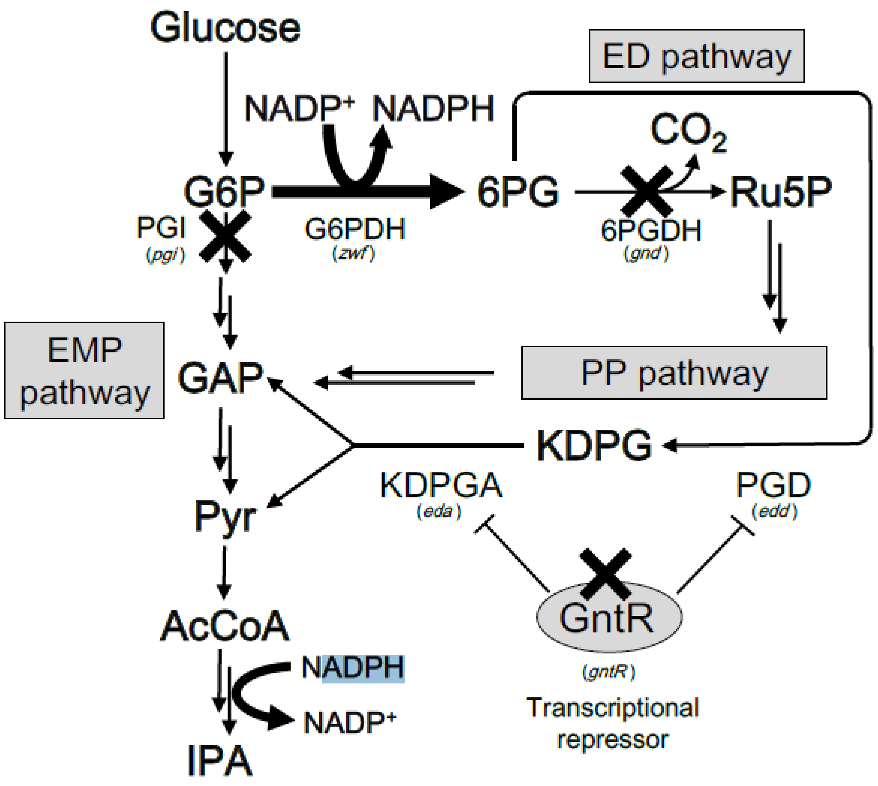

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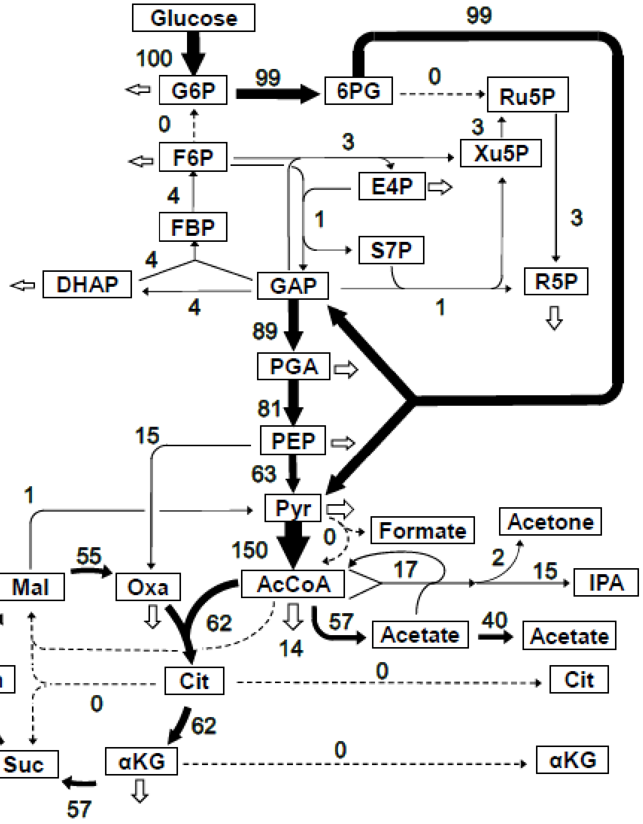

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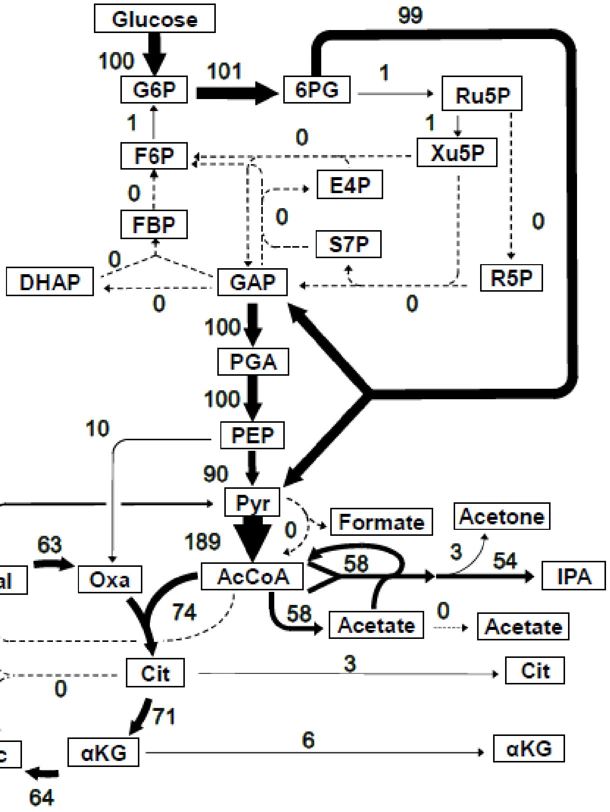


Aplicações:

Application of ^{13}C flux analysis to identify high-productivity CHO metabolic phenotypes

Neil Templeton[a,1], Kevin D. Smith[b], Allison G. McAtee-Pereira[a], Haimanti Dorai[b], Michael J. Betenbaugh[c], Steven E. Lang[b,2], Jamey D. Young[a,d,*]

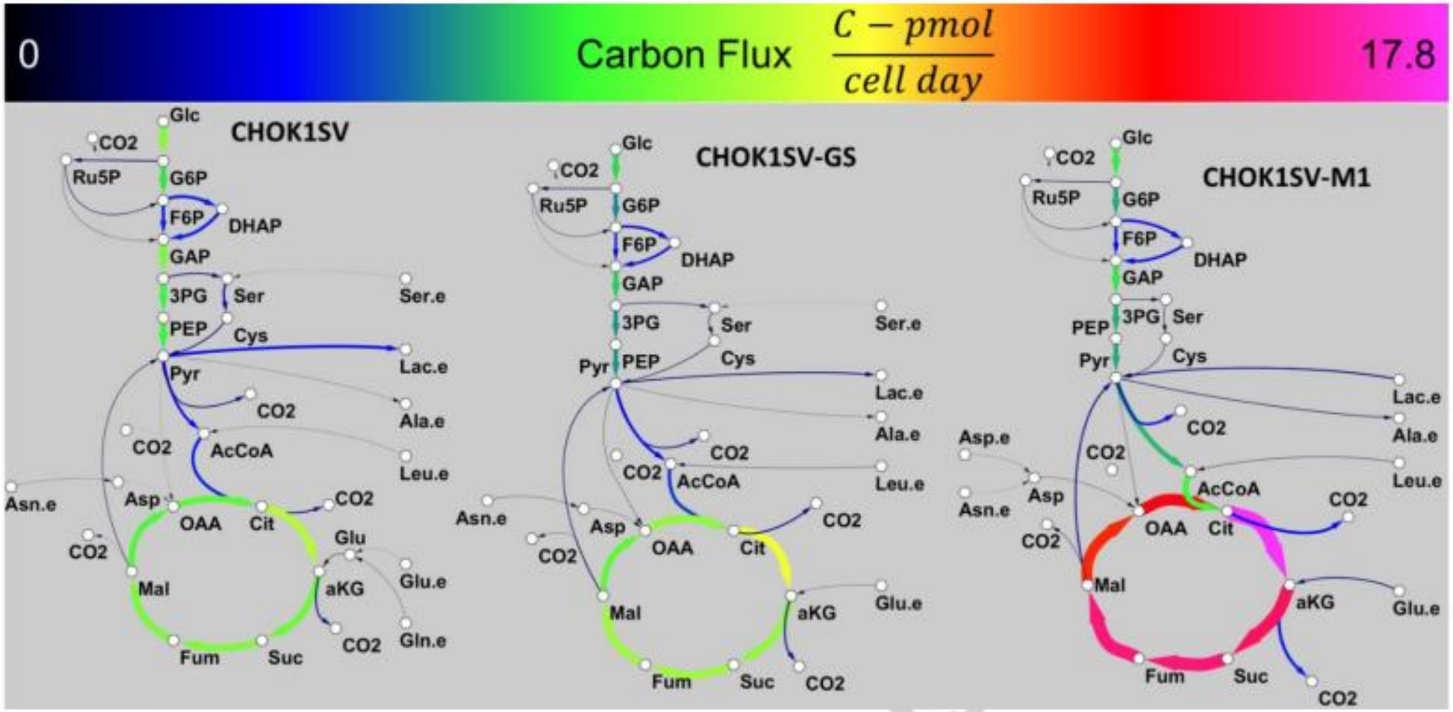
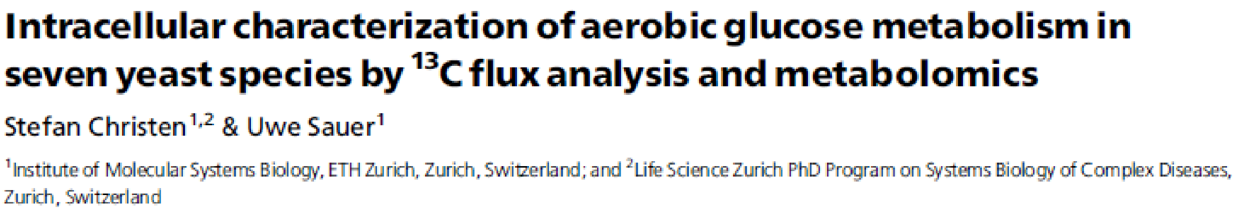


Fig 6. Comparison of flux maps for CHOK1SV parent, CHOK1SV-GS vector control, and CHOK1SV-M1 IgG-producing clone. Major carbon fluxes are shown as C-pmol/cell/day. Both width and color of directional arrows are scaled to the magnitude of carbon flux.

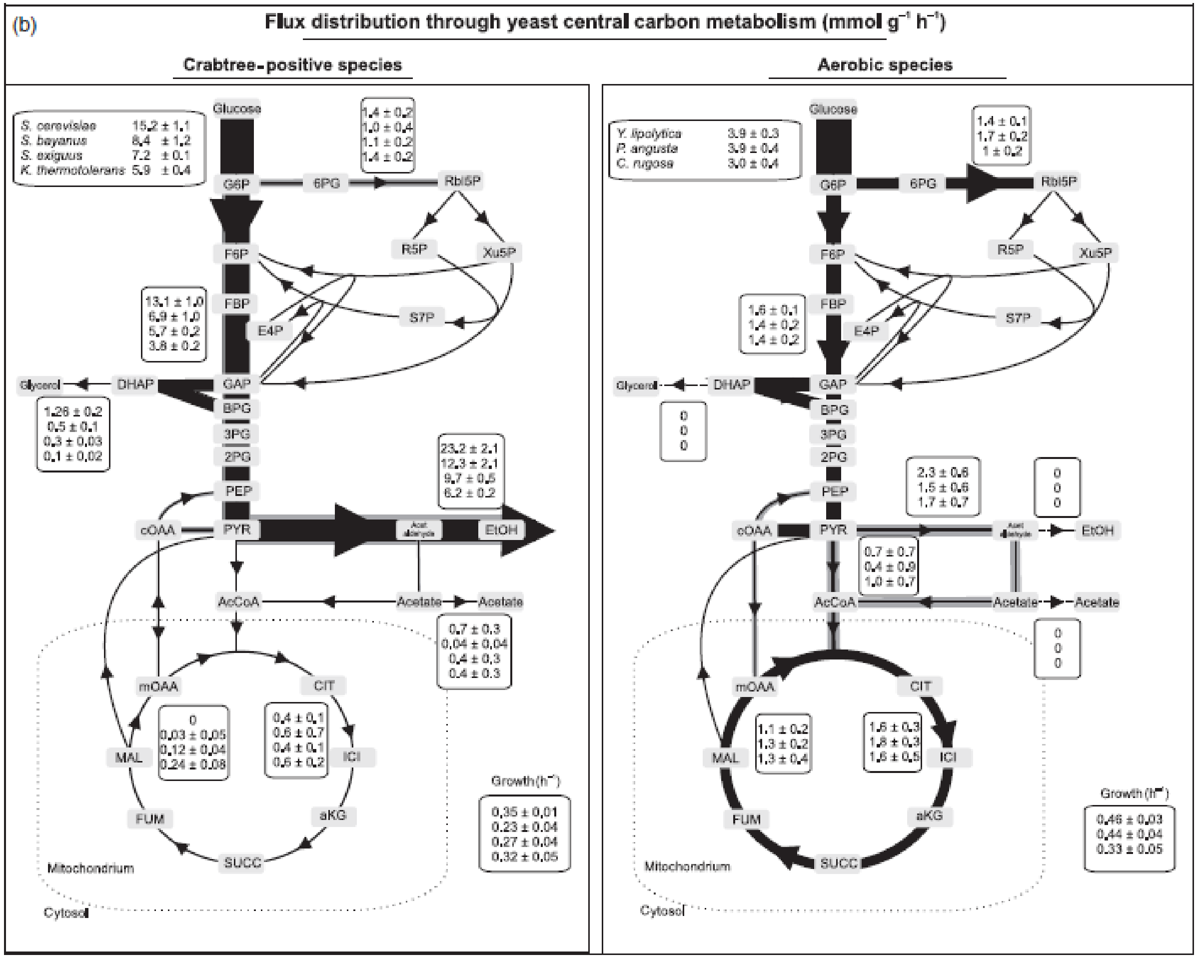
Aplicações:



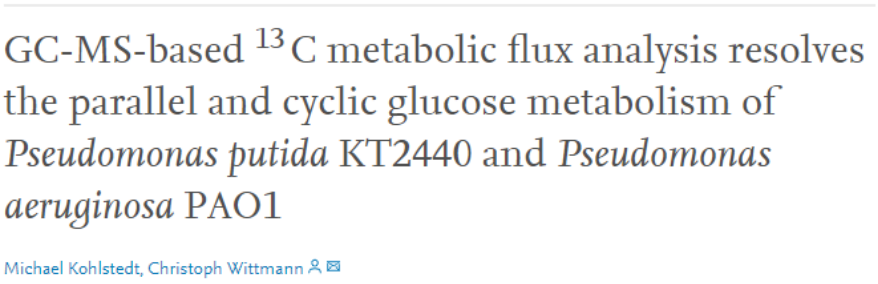
Intracellular characterization of aerobic glucose metabolism in seven yeast species by ^{13}C flux analysis and metabolomics

Stefan Christen[1,2] & Uwe Sauer[1]

[1]Institute of Molecular Systems Biology, ETH Zurich, Zurich, Switzerland; and [2]Life Science Zurich PhD Program on Systems Biology of Complex Diseases, Zurich, Switzerland

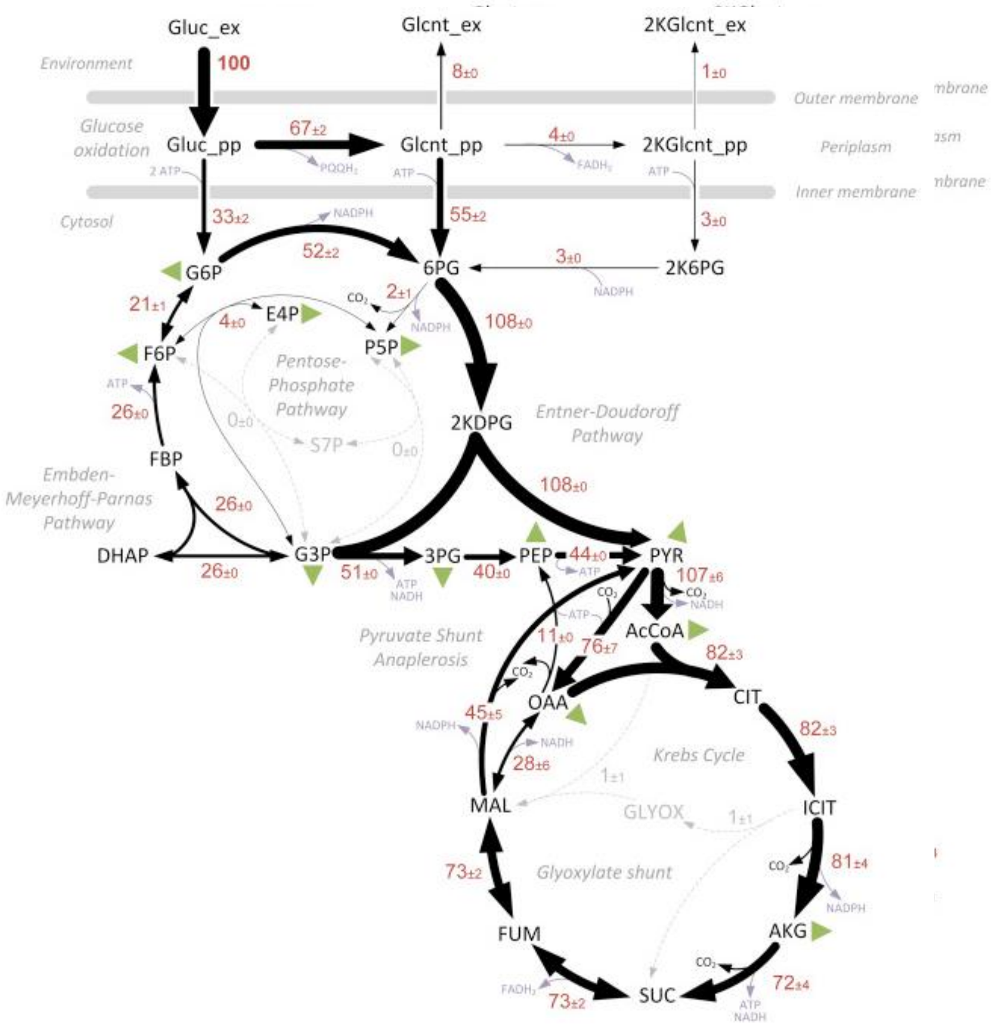


Aplicações:



GC-MS-based ^{13}C metabolic flux analysis resolves the parallel and cyclic glucose metabolism of *Pseudomonas putida* KT2440 and *Pseudomonas aeruginosa* PAO1

Michael Kohlstedt, Christoph Wittmann

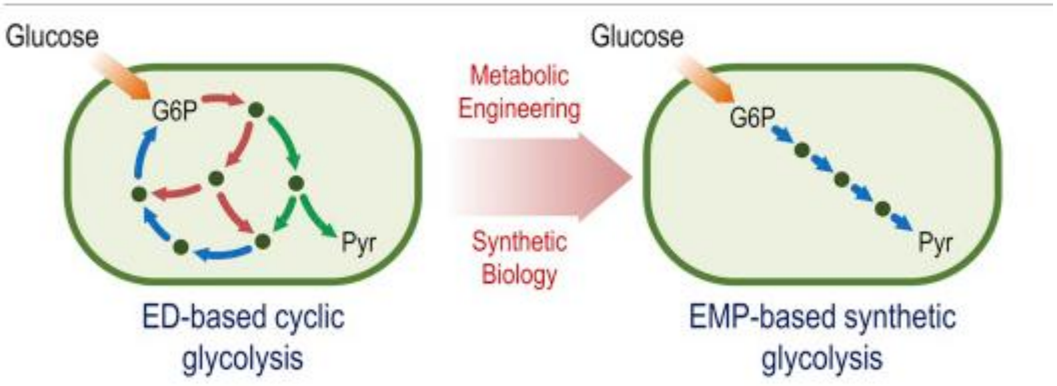


Aplicações:

Functional implementation of a linear glycolysis for sugar catabolism in *Pseudomonas putida*

Alberto Sánchez-Pascuala[a,1], Lorena Fernández-Cabezón[b], Víctor de Lorenzo[a,**], Pablo I. Nikel[b,*]

[a] Systems and Synthetic Biology Program, Centro Nacional de Biotecnología (CNB-CSIC), Campus de Cantoblanco, 28049 Madrid, Spain
[b] The Novo Nordisk Foundation Center for Biosustainability, Technical University of Denmark, 2800 Kongens Lyngby, Denmark



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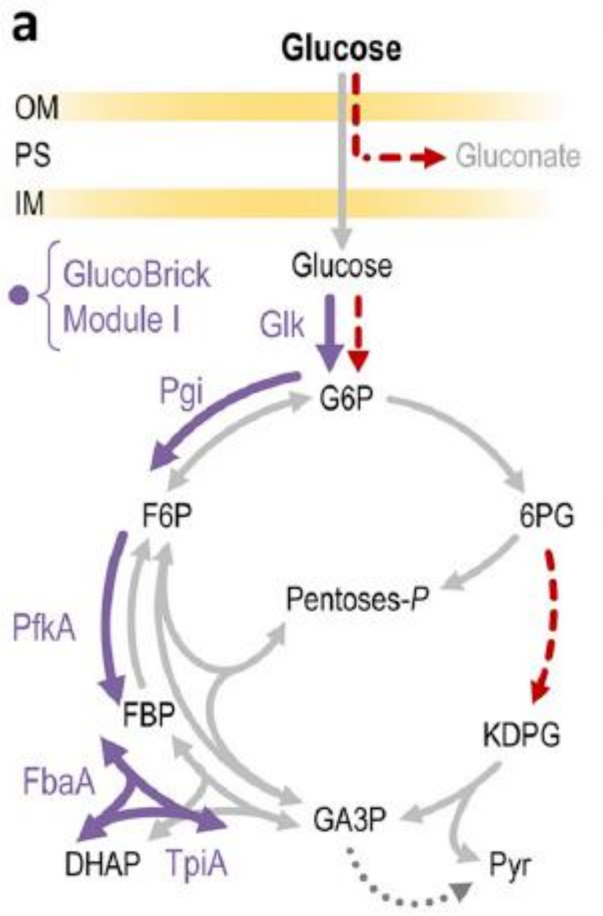

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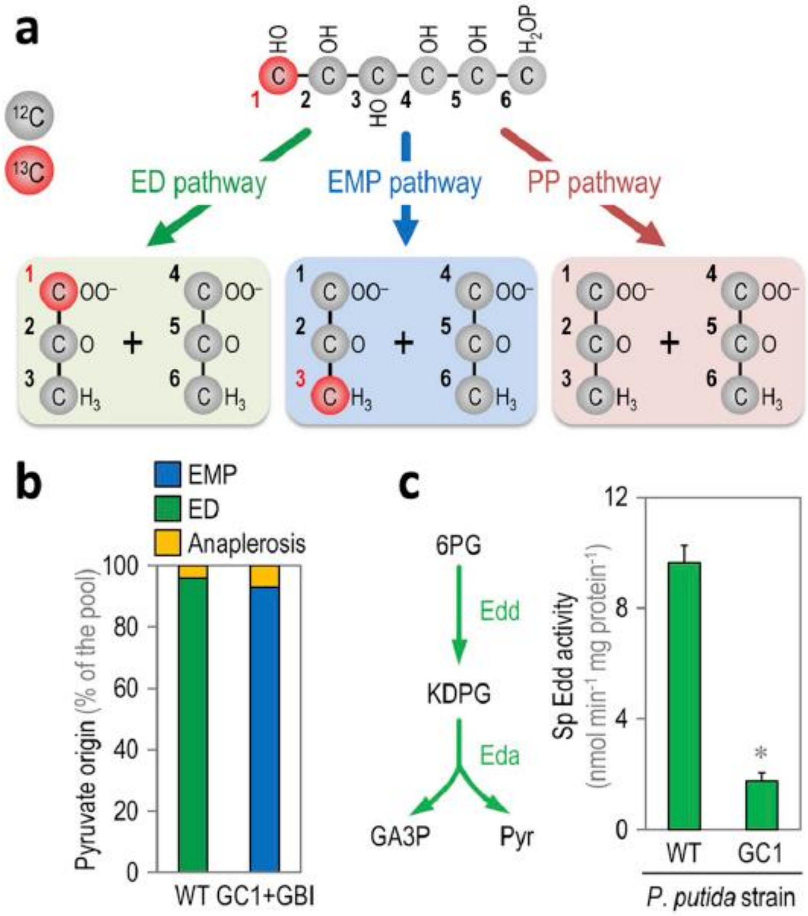

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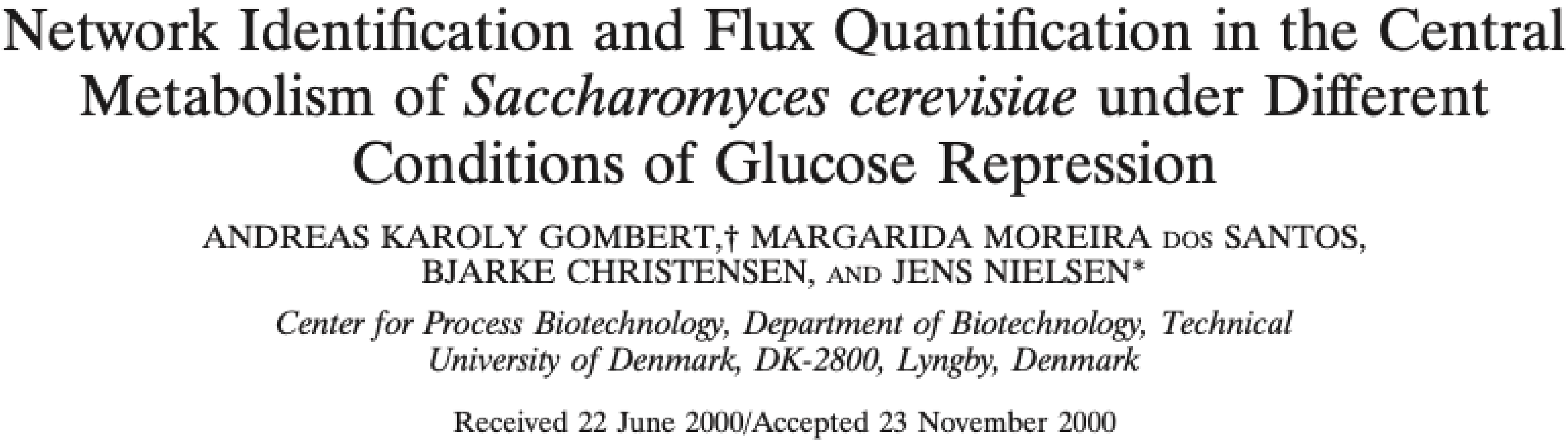
Exercício: *Saccharomyces cerevisiae*

Obejtivos:

-Comparar FBA x ^{13}C-MFA

-Esclarecer limitações de ^{13}C-MFA



Network Identification and Flux Quantification in the Central Metabolism of *Saccharomyces cerevisiae* under Different Conditions of Glucose Repression

ANDREAS KAROLY GOMBERT,† MARGARIDA MOREIRA DOS SANTOS,
BJARKE CHRISTENSEN, AND JENS NIELSEN*

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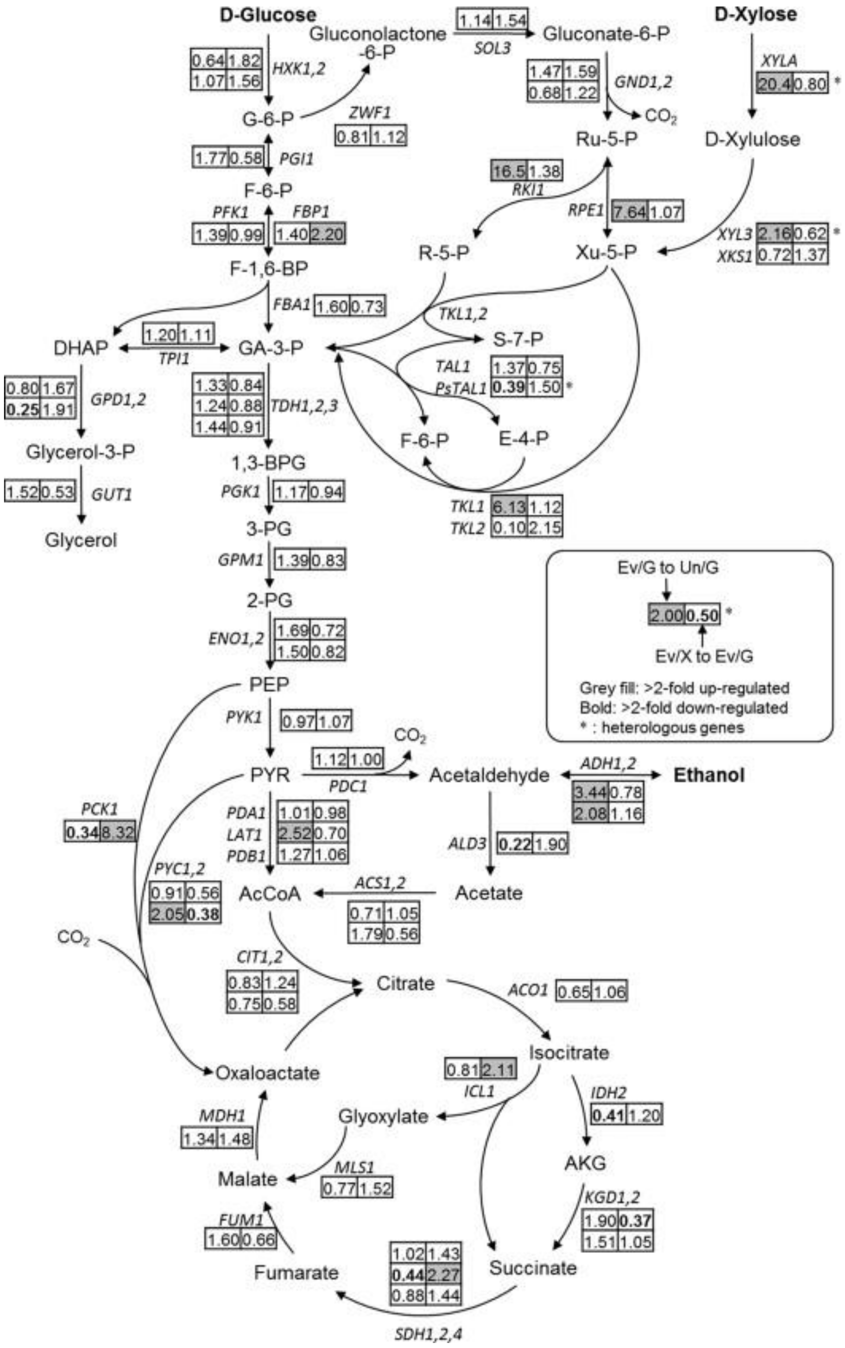
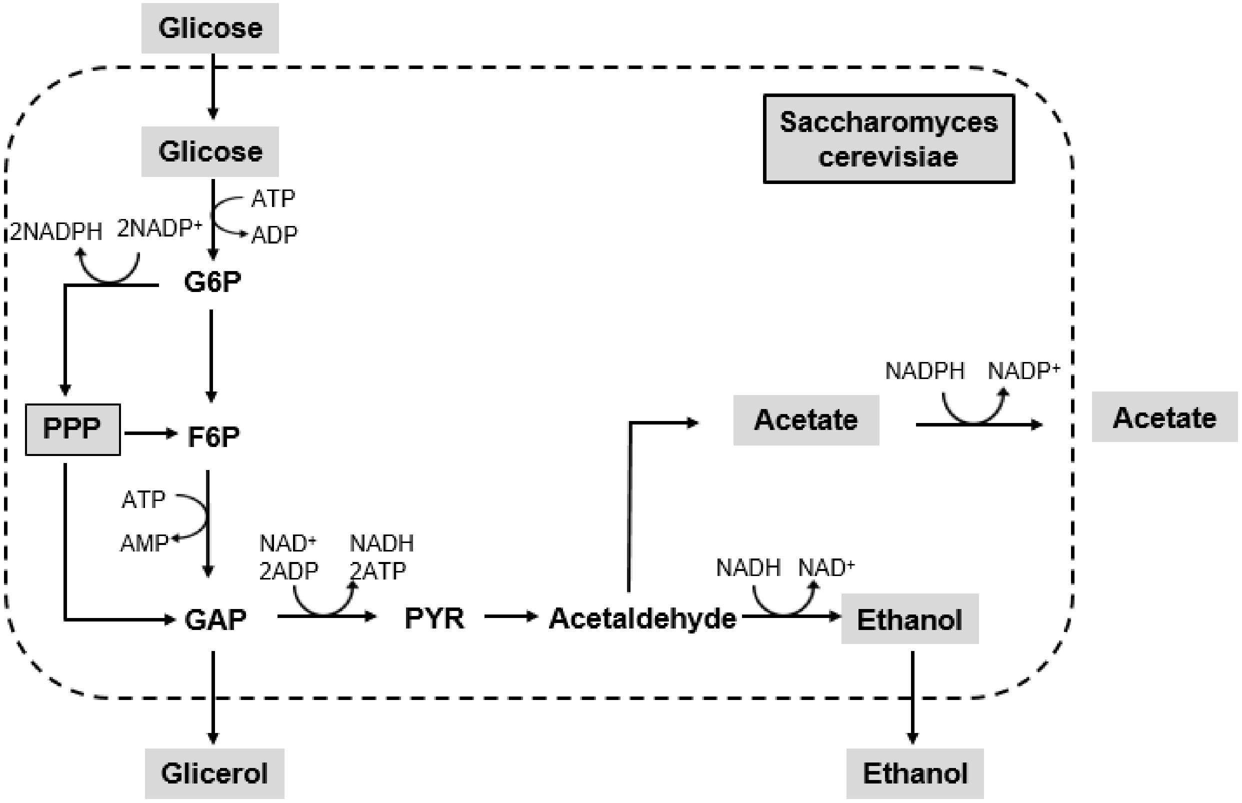

D-Glucose
Gluconolactone -6-P
1.14 1.54
SOL3
Gluconate-6-P
D-Xylose
0.64 1.82
1.07 1.56
HXK1,2
G-6-P
ZWF1
0.81 1.12
1.47 1.59
0.68 1.22
GND1,2
CO2
Ru-5-P
XYLA
20.4 0.80 *
D-Xylulose
1.77 0.58
PGI1
F-6-P
16.5 1.38
RKI1
RPE1
7.64 1.07
PFK1
FBP1
1.39 0.99
1.40 2.20
R-5-P
Xu-5-P
XYL3 2.16 0.62 *
XKS1 0.72 1.37
F-1,6-BP
FBA1 1.60 0.73
TKL1,2
1.20 1.11
DHAP
TPI1
GA-3-P
S-7-P
TAL1
PsTAL1
1.37 0.75
0.39 1.50 *
0.80 1.67
0.25 1.91
GPD1,2
1.33 0.84
1.24 0.88
1.44 0.91
TDH1,2,3
F-6-P
E-4-P
Glycerol-3-P
1,3-BPG
1.52 0.53
GUT1
PGK1 1.17 0.94
TKL1 6.13 1.12
TKL2 0.10 2.15
Glycerol
3-PG
GPM1 1.39 0.83
Ev/G to Un/G
2.00 0.50 *
Ev/X to Ev/G
Grey fill: >2-fold up-regulated
Bold: >2-fold down-regulated
* : heterologous genes
2-PG
1.69 0.72
1.50 0.82
ENO1,2
PEP
PYK1 0.97 1.07
CO2
1.12 1.00
PYR
PDC1
Acetaldehyde
ADH1,2
Ethanol
3.44 0.78
2.08 1.16
PCK1
0.34 8.32
PDA1 1.01 0.98
LAT1 2.52 0.70
PDB1 1.27 1.06
ALD3 0.22 1.90
PYC1,2
0.91 0.56
2.05 0.38
AcCoA
ACS1,2
Acetate
0.71 1.05
1.79 0.56
CO2
CIT1,2
0.83 1.24
0.75 0.58
Citrate
ACO1 0.65 1.06
Isocitrate
0.81 2.11
ICL1
Oxaloactate
IDH2
0.41 1.20
Glyoxylate
MDH1
1.34 1.48
AKG
Malate
MLS1
0.77 1.52
KGD1,2
1.90 0.37
1.51 1.05
FUM1
1.60 0.66
1.02 1.43
0.44 2.27
0.88 1.44
Fumarate
Succinate
SDH1,2,4

Simplificações na rede

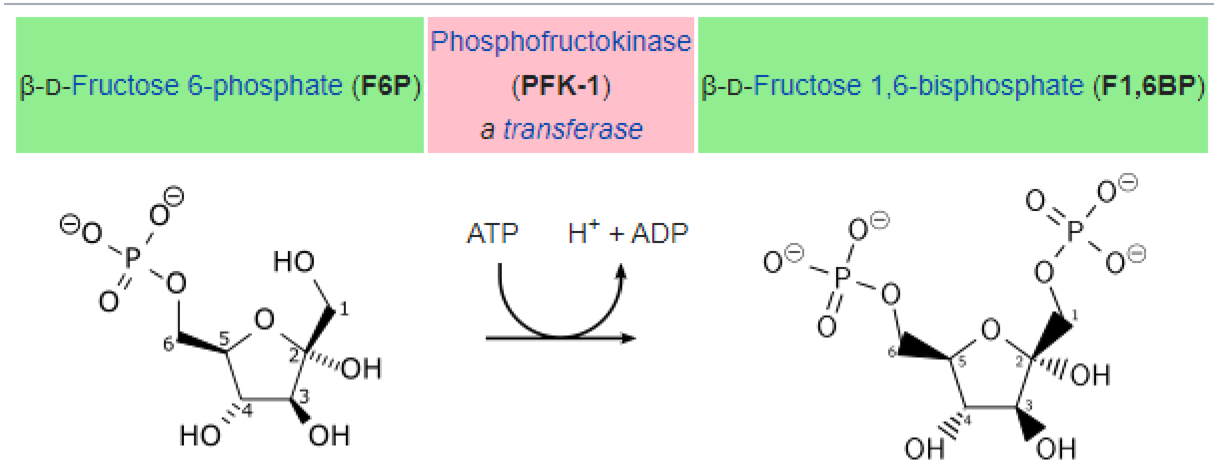
- Reações lineares
- Equilíbrio rápido



FBA: hipóteses

- Cultivo em anaerobiose
- Baixo crescimento celular
- Alta produção de etanol
- Composição da biomassa desconhecida

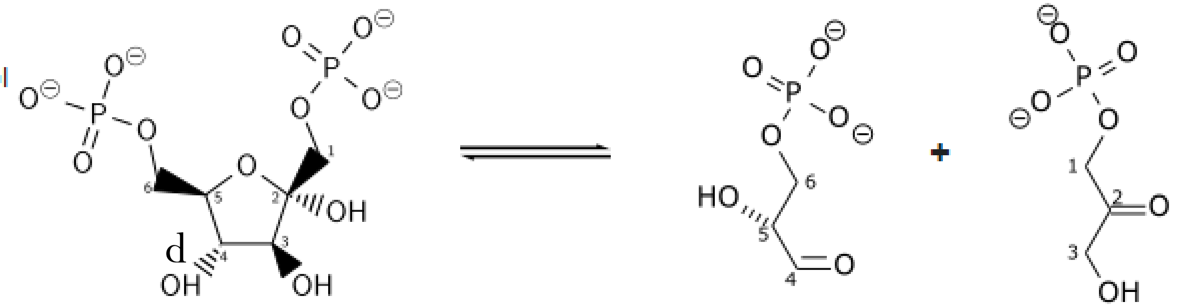
^{13}C-MFA: Transições



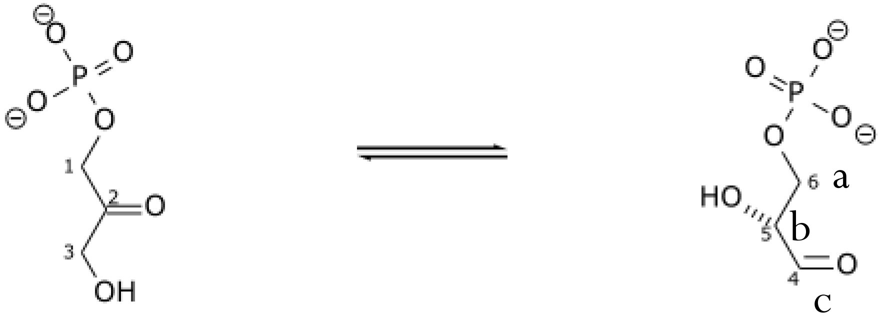
β-D-Fructose 6-phosphate (**F6P**)	Phosphofructokinase (**PFK-1**) a *transferase*	β-D-Fructose 1,6-bisphosphate (**F1,6BP**)

^{13}C-MFA: Transições

β-D-Fructose 1,6-bisphosphate (**F1,6BP**)	Fructose-bisphosphate aldolase (**ALDO**) *a lyase*	D-Glyceraldehyde 3-phosphate (**GADP**)	Dihydroxyacetone phosphate (**DHAP**)

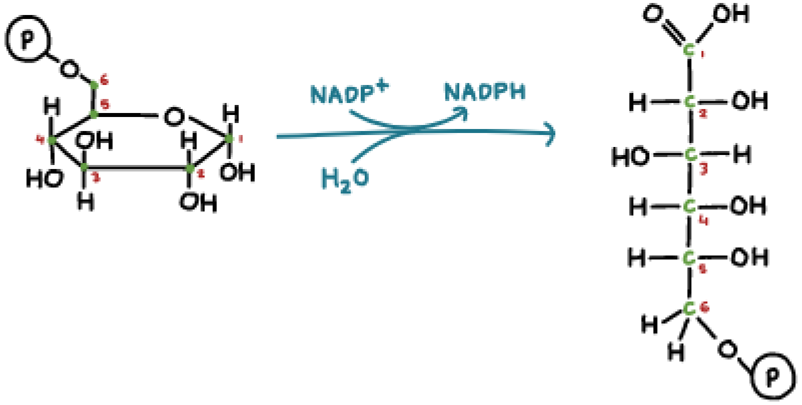


Dihydroxyacetone phosphate (**DHAP**)	Triosephosphate isomerase (**TPI**) *an isomerase*	D-Glyceraldehyde 3-phosphate (**GADP**)

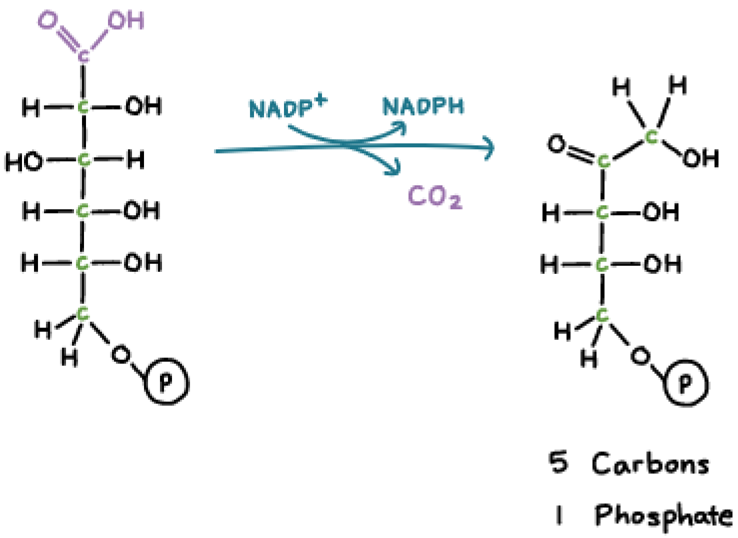


^{13}C-MFA: Transições

Oxidative Phase : Step 1



Oxidative Phase : Step 2



^{13}C-MFA: Dados medidos

- 75%[1-^{13}C]-Glicose + 25%[4-^{13}C]-Glicose

Isotopômeros de posição GAP	
1	0.1548
2	0.0000
3	0.0269

Isotopômeros de posição E4P	
1	0.0509
2	0.2326
3	0.0000
4	0.0490

Mínimos quadrados

$$\sum_{1}^{n} (predição\ do\ modelo - dado\ experimental)^{2}$$