

# **Análise sistêmica e engenharia do metabolismo microbiano Biologia Sintética e Biologia de Sistemas.**

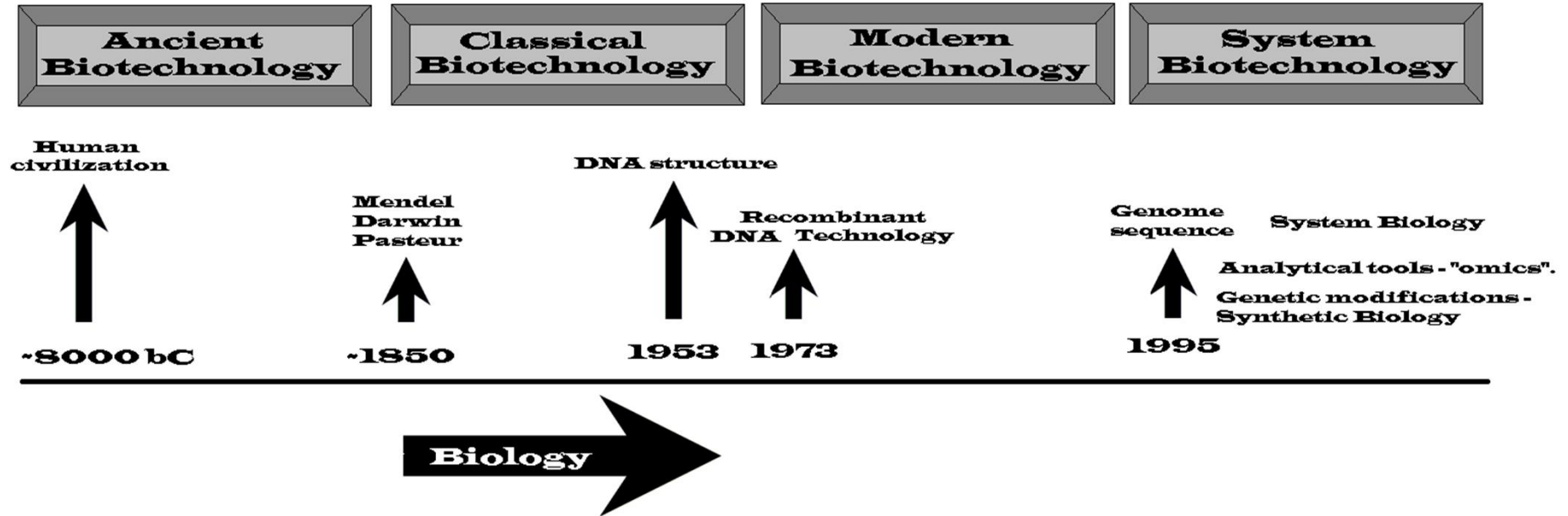
---

José Gregório Cabrera Gomez

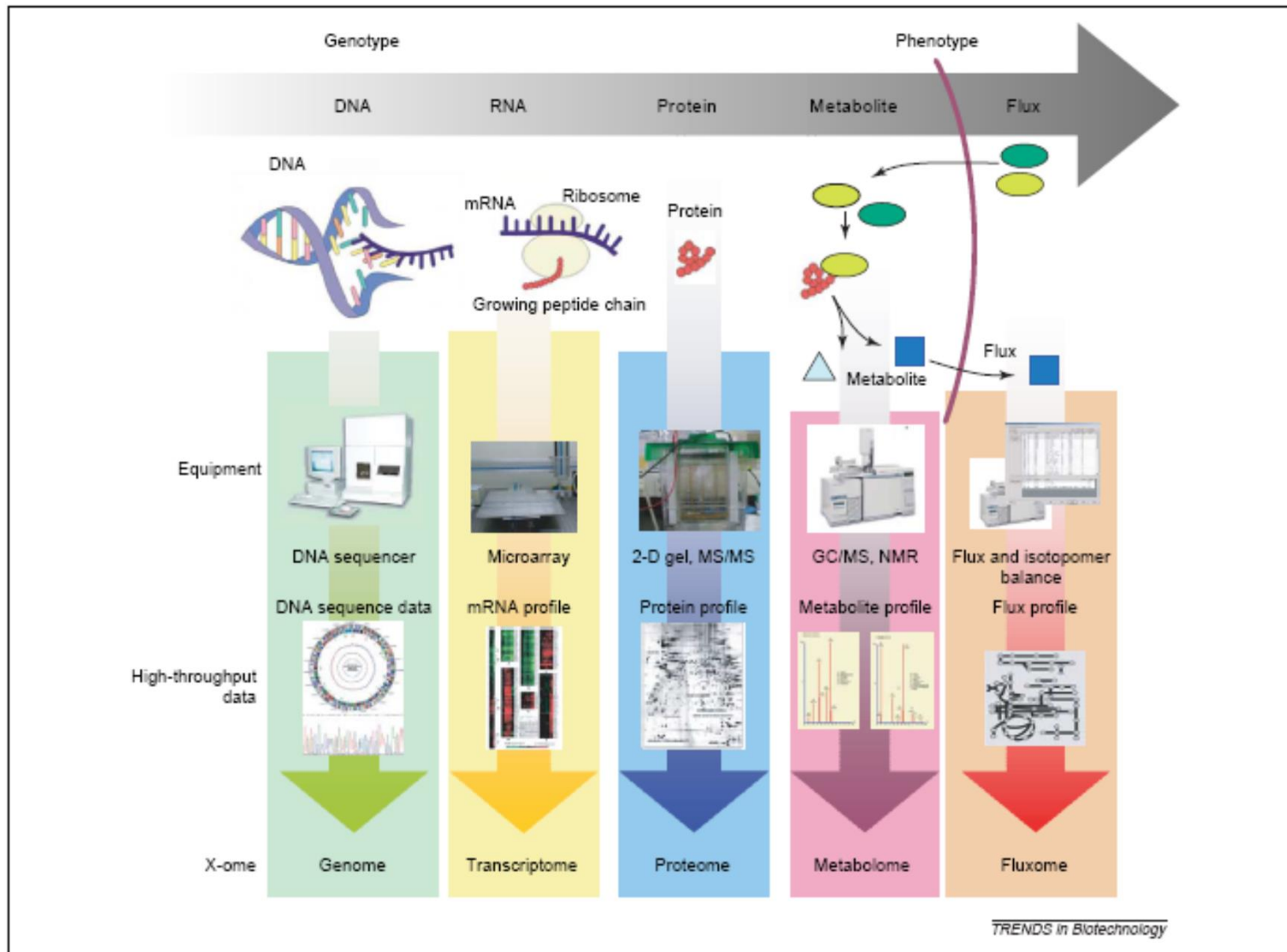


DEPARTAMENTO DE  
**MI**Cro**B**iologia  
UNIVERSIDADE DE SÃO PAULO

# Biology and Biotechnology



# ômicas



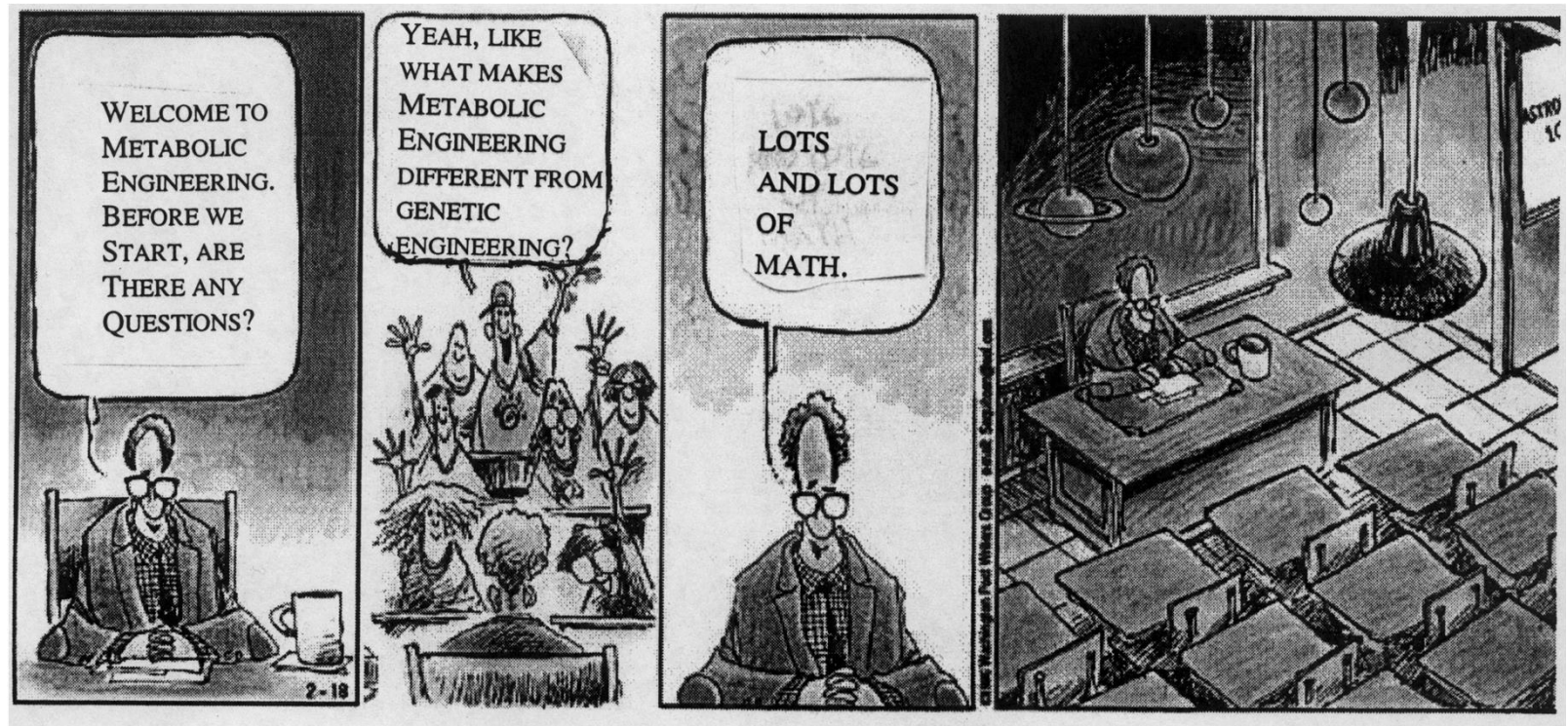
**Figure 1.** High-throughput omics research. Genomics advanced by the development of high-speed DNA sequencing is now accompanied by transcriptome profiling using DNA microarrays. Proteome profiling is joining the high-throughput race as 2D-gel electrophoresis combined with mass spectrography is advancing. Metabolome profiling is also rapidly advancing with the development of better GC/MS, LC/MS and NMR technologies. Isotopomer profiling followed by challenging with isotopically labeled substrate allows determination of flux profiles in the cell (fluxome).

Metabolic engineering is the improvement of cellular activities by manipulation of enzymatic, transport, and regulatory functions of the cell with the use of recombinant DNA technology. The opportunity to introduce heterologous genes and regulatory elements distinguishes metabolic engineering from traditional genetic approaches to improve strains.

... An interactive cycle of a genetic change, an analysis of the consequences, and the design of a further change...

Toward a Science of Metabolic Engineering.

James E. Bailey Science, 252: 1668-1675.





# Metabolic Engineering

The knockout or overexpression of genes, usually used in Genetic Engineering, frequently does not result in product yield improvements due to resistance in the metabolism. Therefore, a better knowledge of the metabolism is needed to promote metabolism engineering as a whole to improve biotechnological processes.

**Vallino & Stephanopoulos, 1992**

Metabolic engineering is an enabling science, and distinguishes itself from applied genetic engineering by the use of advanced analytical tools for identification of appropriate targets for genetic modifications and possibly even the use of mathematical models to perform *in silico* design of optimized cell factories.

**Nielsen & Jewett, 2008 FEMS Yeast Res.**

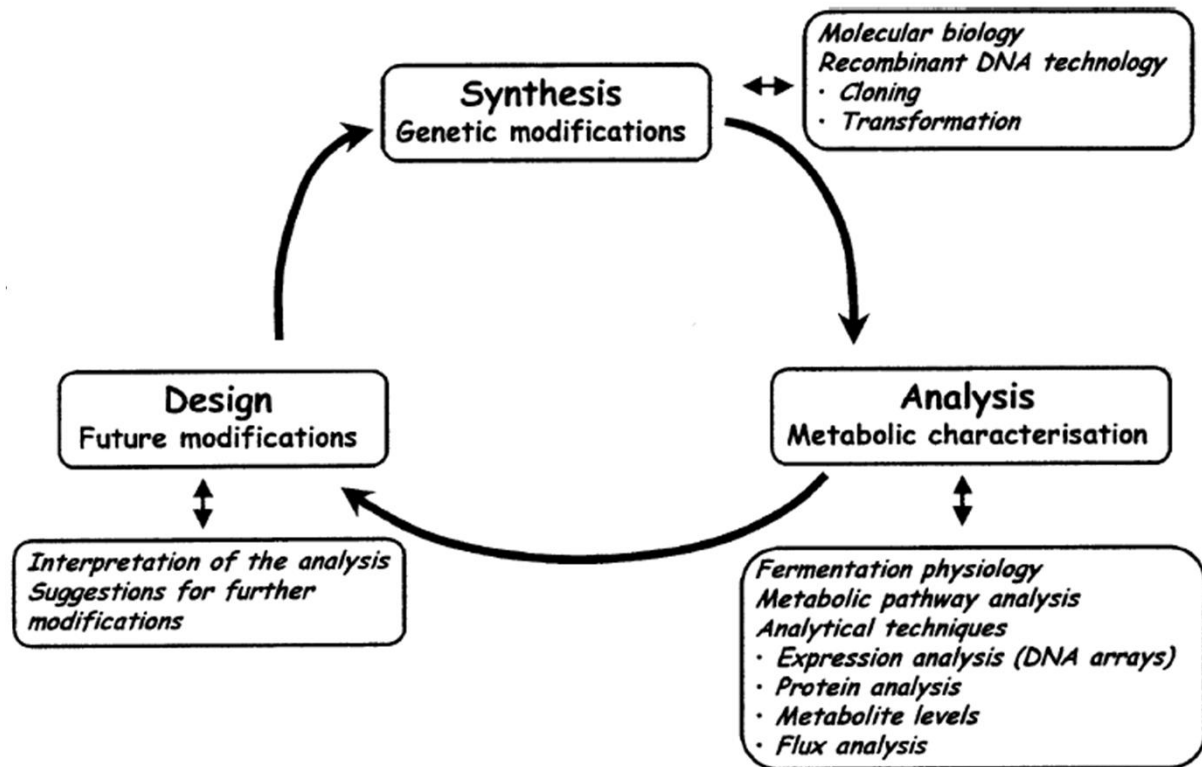


TABLE 1. Overview of reactions, metabolites, and ORFs in reconstructed metabolic networks<sup>a</sup>

Organism	No. of reactions	No. of metabolites	No. of metabolic ORFs	Total no. of ORFs	% of ORFs involved in metabolism
<i>H. pylori</i>	444	340	268	1,638	16
<i>H. influenzae</i>	477	343	362	1,880	19
<i>E. coli</i>	720	436	695	4,485	15
<i>S. cerevisiae</i>	1,175	584	708	5,773	12 <sup>b</sup>

<sup>a</sup> The reconstructed networks are described in references 6, 8, 17, and 18.

<sup>b</sup> The value is based on a recent gene count (3).

Table 3. Frequency of precursor metabolites and cofactors in a *Saccharomyces cerevisiae* genome scale model\*

Precursor metabolite	No of reactions	Cofactor	No of reactions
Glucose-6P	16	ATP	188
Fructose-6P	18	ADP	146
Ribose-5P	20	NADH	65
Erythrose-4P	6	NAD <sup>+</sup>	78
Glyceraldehyde-3P	13	NADPH	78
3-Phosphoglycerate	6	NADP <sup>+</sup>	86
Phosphoenolpyruvate	12		
Pyruvate	27		
Acetyl-CoA	32		
2-Oxoglutarate	38		
Succinyl-CoA	3		
Oxaloacetate	12		

\*The data are taken from the metabolic model developed by Forster et al. (2003).

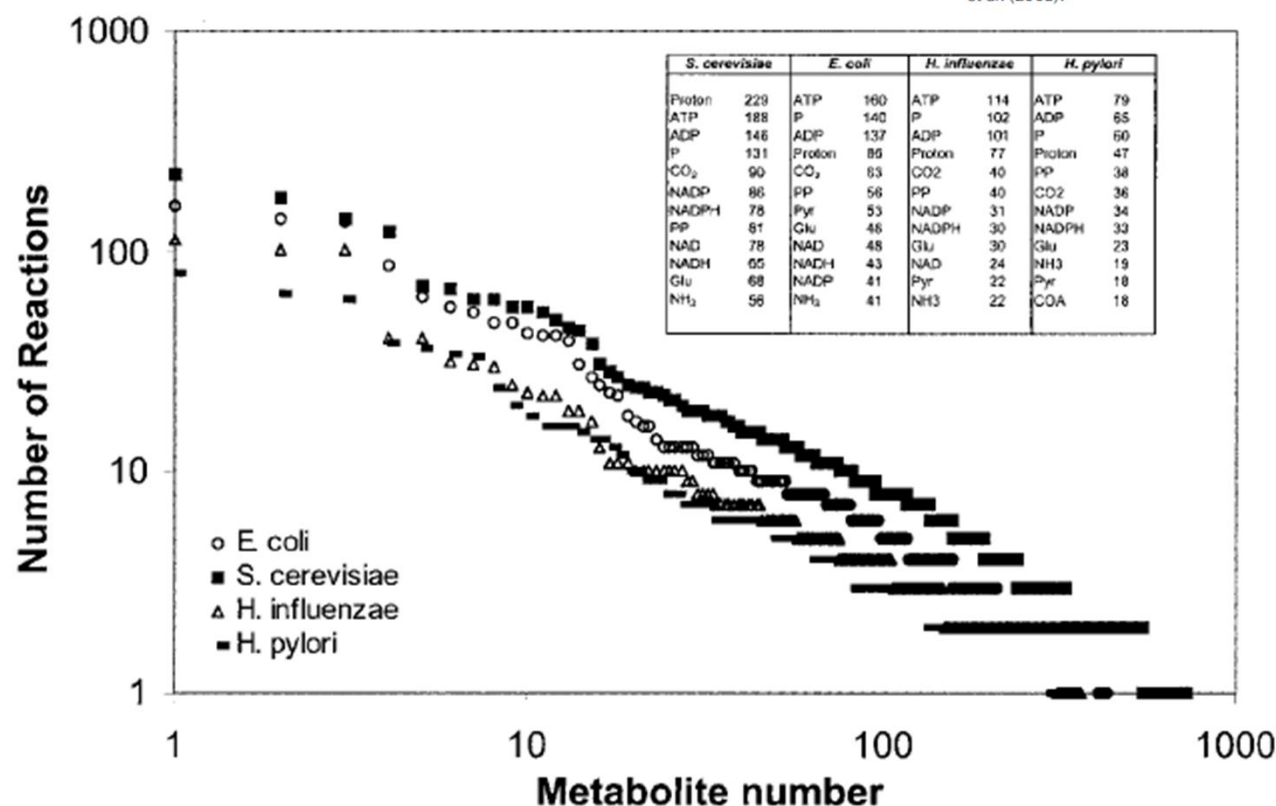
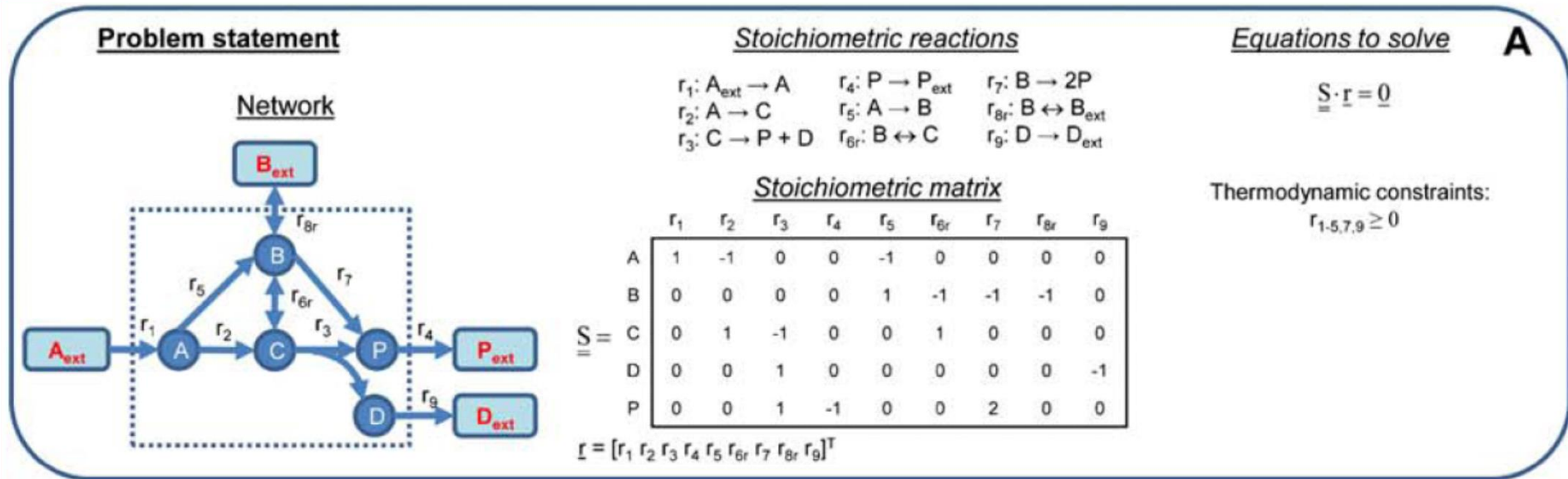


FIG. 1. Frequency plot of the number of reactions that each metabolite appears in for four different reconstructed metabolic networks. For each metabolic network the 10 metabolites that appear in the most reactions are listed. PP, pyrophosphate; COA, coenzyme A. The numbers in the box specify the numbers of reactions the 10 most frequently used metabolites participate in for the four different microorganisms.

# analysis of cellular metabolism



$$\frac{d}{dt} \underline{C} = \underline{S} \times \underline{r} - \mu \times \underline{C}, \quad \mu \cdot C \text{ (negligible)} \quad S \cdot r = 0 \text{ (Eq 2)}$$

$$dC/dt = 0 \text{ (steady state)} \quad r_i \geq 0 \text{ (Eq 3)}$$

Tools for analysis of cellular metabolism can be grouped into three categories, all of them developed from the same mathematical model:

- (1) Metabolic flux analysis,
- (2) Flux balance analysis and
- (3) Metabolic pathway analysis (Elementary mode analysis).

# Metabolic Flux Analysis

$$S_{=u} = \begin{matrix} & r_3 & r_4 & r_5 & r_{6r} & r_7 \\ \begin{matrix} A \\ B \\ C \\ D \\ P \end{matrix} & \begin{bmatrix} 0 & 0 & -1 & 0 & 0 \\ 0 & 0 & 1 & -1 & -1 \\ -1 & 0 & 0 & 1 & 0 \\ 1 & 0 & 0 & 0 & 0 \\ 1 & -1 & 0 & 0 & 2 \end{bmatrix} \end{matrix}$$

$$S_{=m} = \begin{matrix} & r_1 & r_2 & r_{8r} & r_9 \\ \begin{matrix} A \\ B \\ C \\ D \\ P \end{matrix} & \begin{bmatrix} 1 & -1 & 0 & 0 \\ 0 & 0 & -1 & 0 \\ 0 & 1 & 0 & 0 \\ 0 & 0 & 0 & -1 \\ 0 & 0 & 0 & 0 \end{bmatrix} \end{matrix}$$

## Measured fluxes

$$\underline{r}_m = \begin{bmatrix} r_1 \\ r_2 \\ r_{8r} \\ r_9 \end{bmatrix} = \begin{bmatrix} 1 \\ 0.3 \\ 0 \\ 0.75 \end{bmatrix}$$

## Equations to solve

$$\underline{S} \cdot \underline{r} = \underline{0}$$

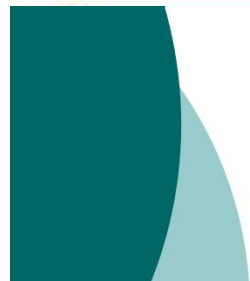
$$\begin{bmatrix} S_{=u} \\ S_{=m} \end{bmatrix} \begin{bmatrix} \underline{r}_u \\ \underline{r}_m \end{bmatrix} = \underline{0}$$

$$\underline{r}_u = -S_{=u}^{-1} \cdot S_{=m} \cdot \underline{r}_m$$

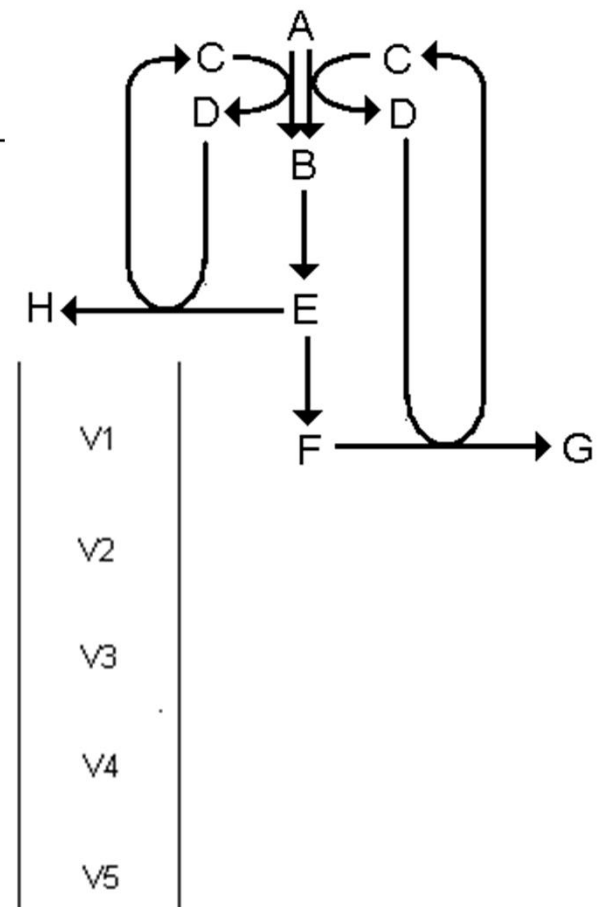
## Solution

$$\underline{r}_u = \begin{bmatrix} r_3 \\ r_4 \\ r_5 \\ r_{6r} \\ r_7 \end{bmatrix} = \begin{bmatrix} 0.75 \\ 1.25 \\ 0.7 \\ 0.45 \\ 0.25 \end{bmatrix}$$

**B**



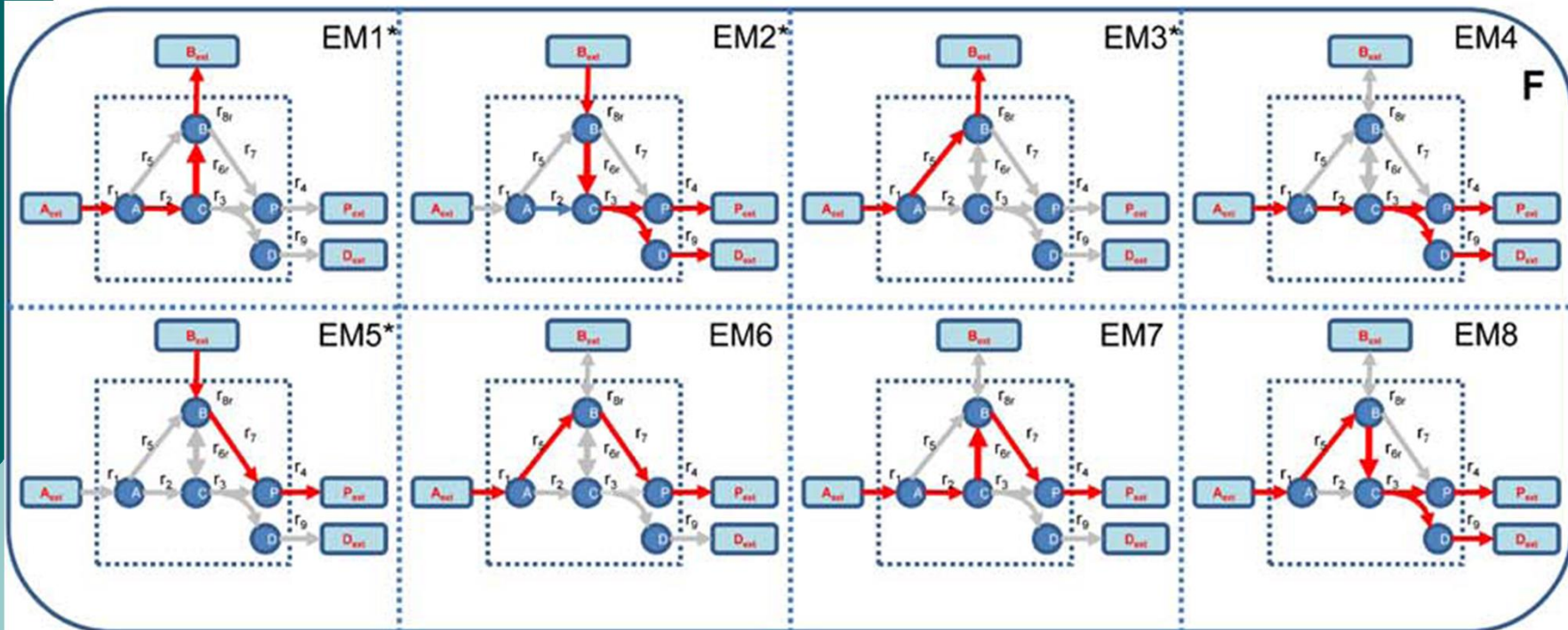
A	B	E	F	C	D	G	H
-1	1	0	0	-1	1	0	0
0	-1	1	0	0	0	0	0
0	0	-1	1	0	0	0	0
0	0	0	-1	1	-1	1	0
0	0	-1	0	1	-1	0	1



$$F = J - K$$



# Metabolic pathway analysis (Elementary (flux) analysis)



$$S \cdot r = 0 \text{ (Eq 2)}$$

$$r_i \geq 0 \text{ (Eq 3)}$$

Elementary mode analysis calculates all solutions in the admissible flux space by solving Eq 2 in conjunction with the thermodynamic constraint (3) and additional non-decomposability and systematic independence constraints. Each solution (re)presents an elementary (flux) mode.

### Flux Balance Analysis

Obj:  $\max r_4$

s.t.:  $\underline{S} \cdot \underline{r} = \underline{0}$

$$r_1 = 1$$

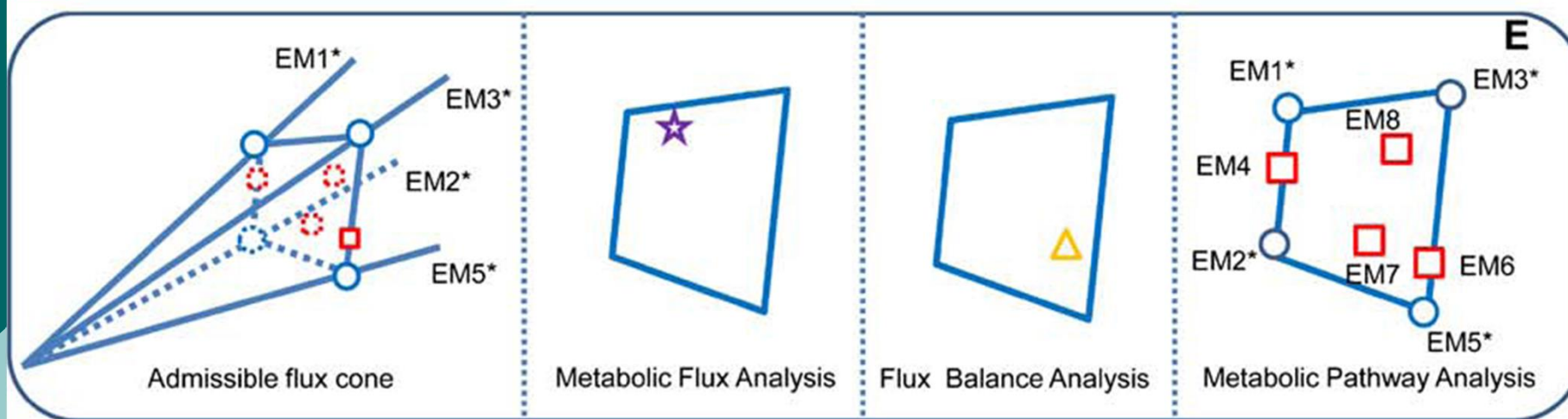
$$r_{8r} = 0$$

$$r_{2-5,7,9} \geq 0$$

$$\underline{r} = \begin{bmatrix} r_1 \\ r_2 \\ r_3 \\ r_4 \\ r_5 \\ r_{6r} \\ r_7 \\ r_{8r} \\ r_9 \end{bmatrix} = \begin{bmatrix} 1 \\ 0.35 \\ 0 \\ 2 \\ 0.65 \\ -0.35 \\ 1 \\ 0 \\ 0 \end{bmatrix}$$

**C**

# Interpretação Geométrica



- ✓ O cone de fluxos admissíveis representa todas as possíveis vias que podem existir.
- ✓ Alguns modos elementares ficam na face ou na base do cone.
- ✓ AFM identifica somente uma via que se localiza em qualquer local do cone. ABF representa somente uma via em qualquer local do cone e satisfaz a função objetivo definida.
- ✓ AVM identifica todas as vias geneticamente independentes, com vias extremas em azul e modos elementares em vermelho.





# Metabolic Engineering

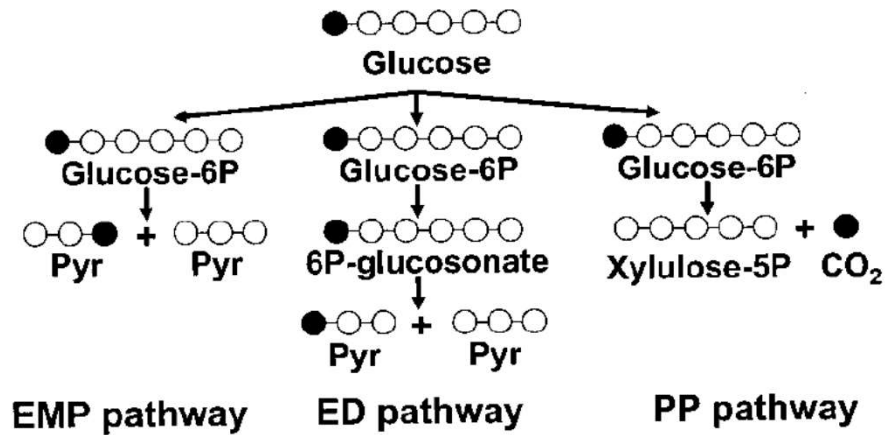


FIG. 2. Illustration of how measurement of the  $^{13}\text{C}$  enrichment patterns can be used to identify active pathways. EMP, Embden-Meyerhof-Parnas; ED, Entner-Doudoroff; PP, pentose phosphate.

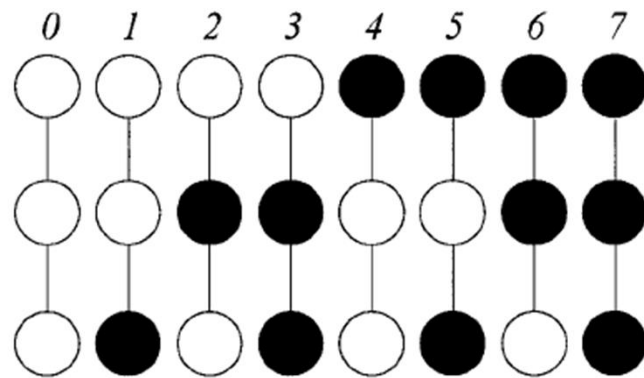
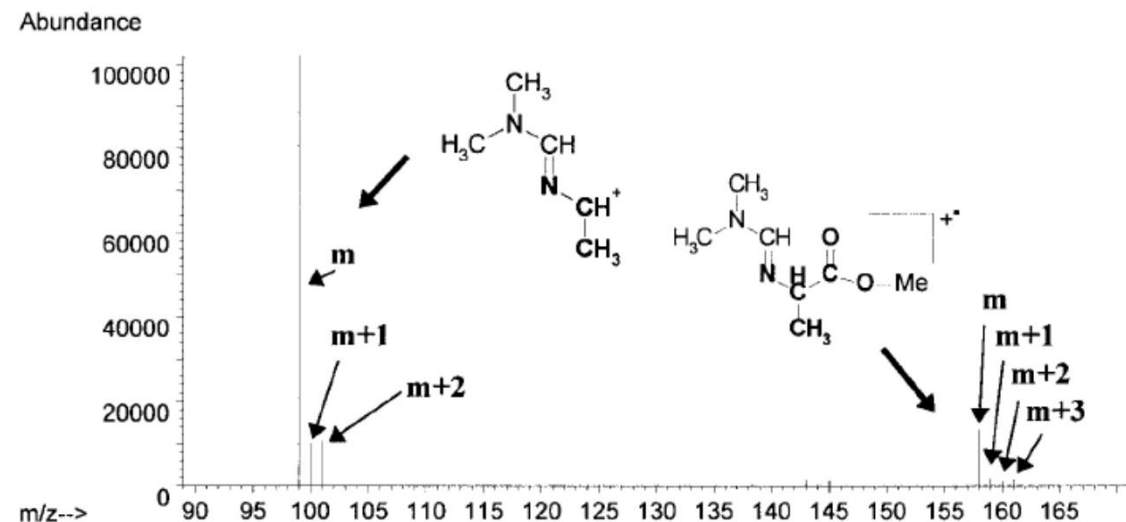
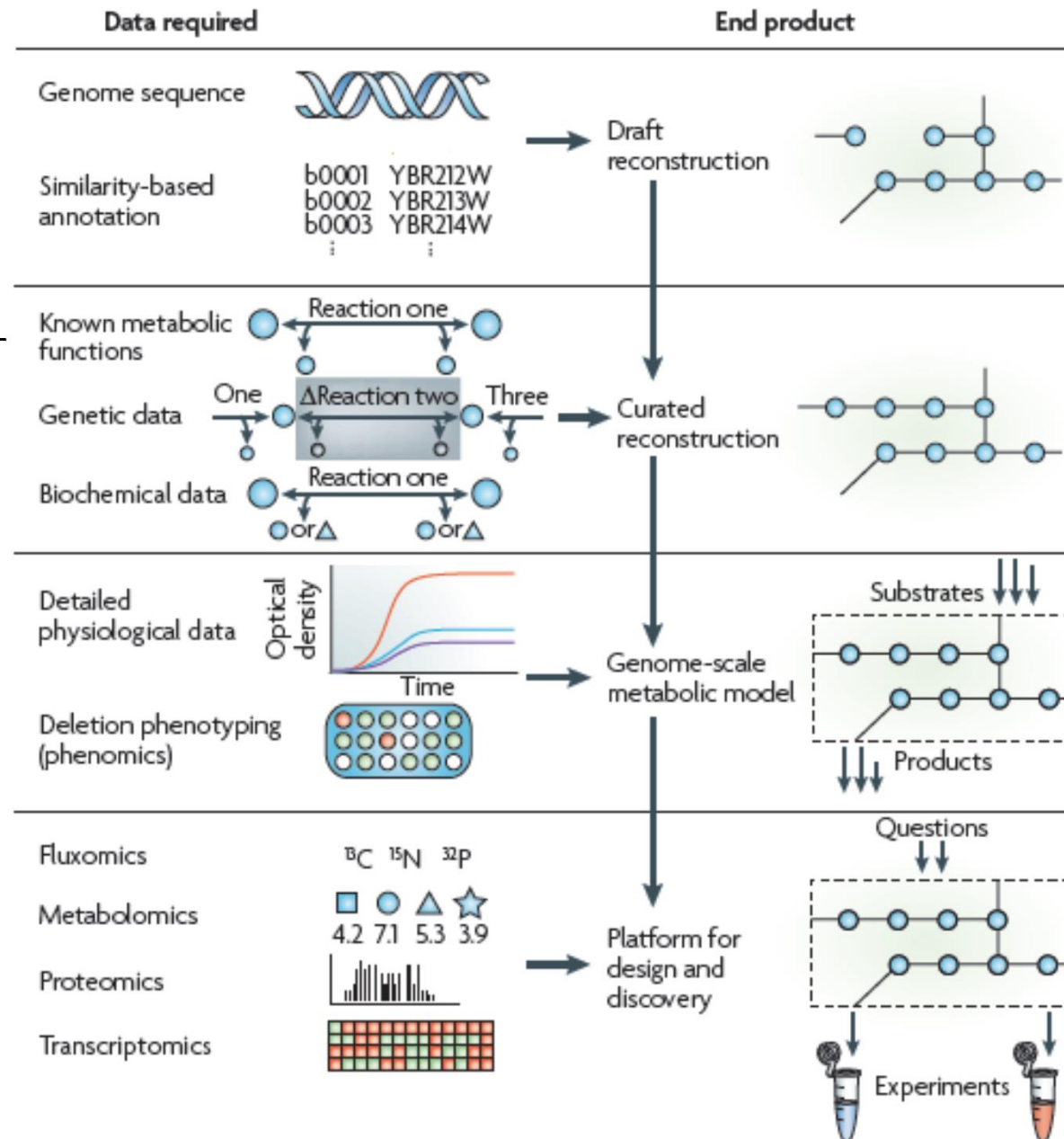


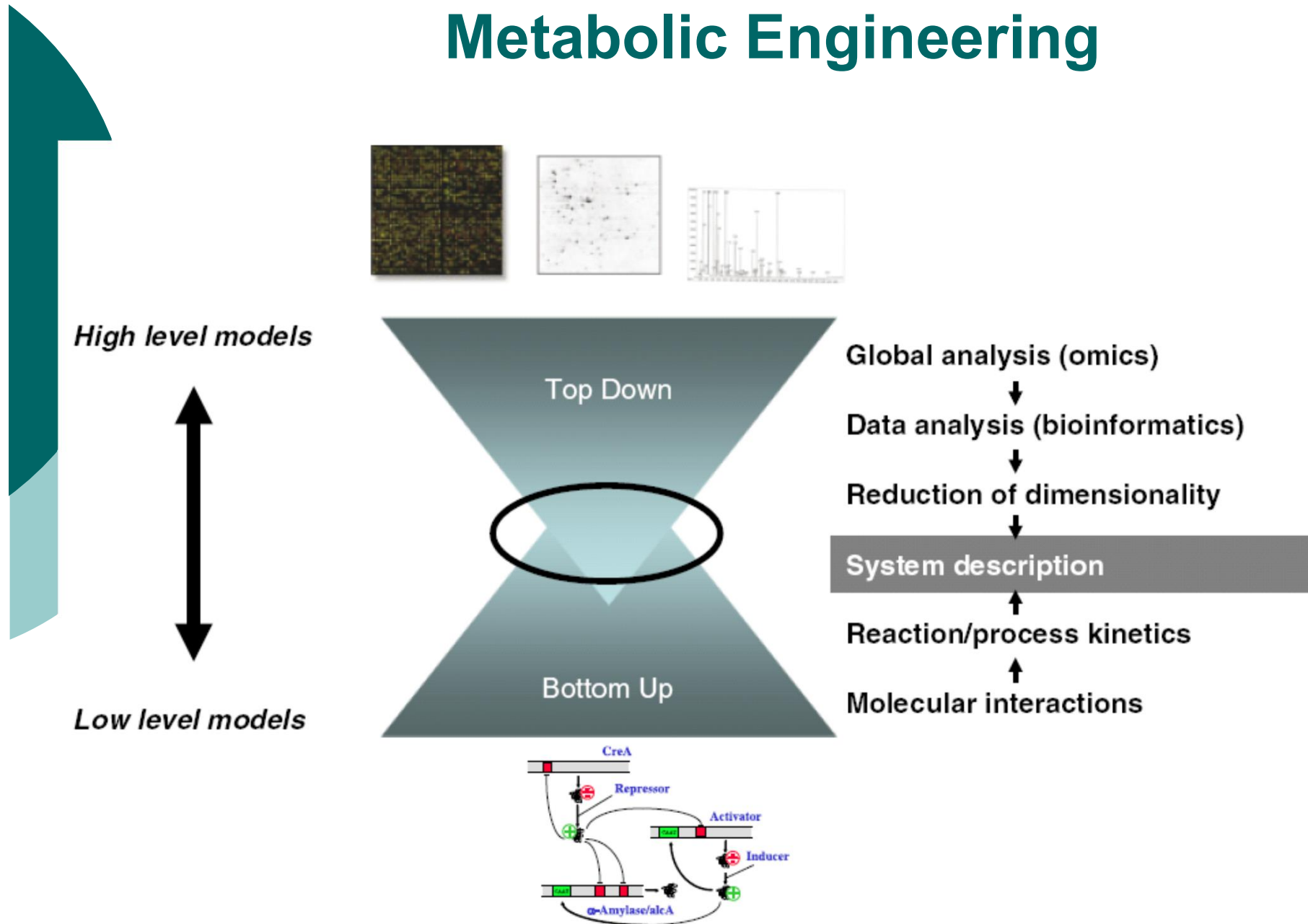
FIG. 1. The eight different positional isotopomers of a  $\text{C}_3$  molecule. The isotopomers are enumerated from zero to seven, corresponding to binary number formed using the white circles ( $^{12}\text{C}$ ) as zeros and the black circles ( $^{13}\text{C}$ ) as ones.



# Redes metabólicas em escala genômica.



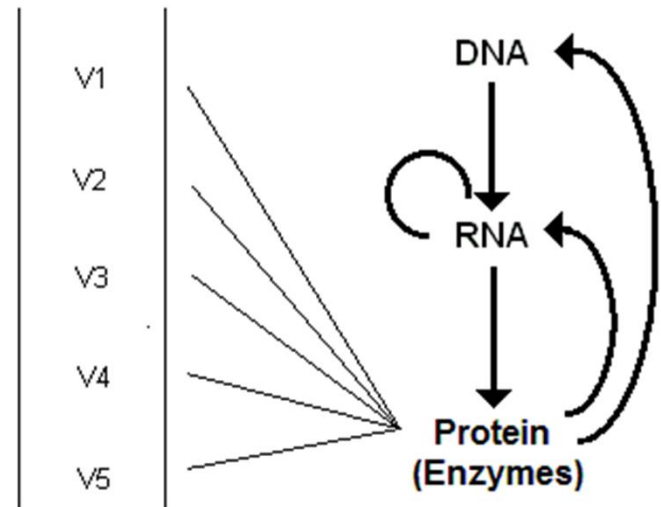
# Metabolic Engineering



# Metabolic Engineering

-1	1	0	0	-1	1	0	0
0	-1	1	0	0	0	0	0
0	0	-1	1	0	0	0	0
0	0	0	-1	1	-1	1	0
0	0	-1	0	1	-1	0	1

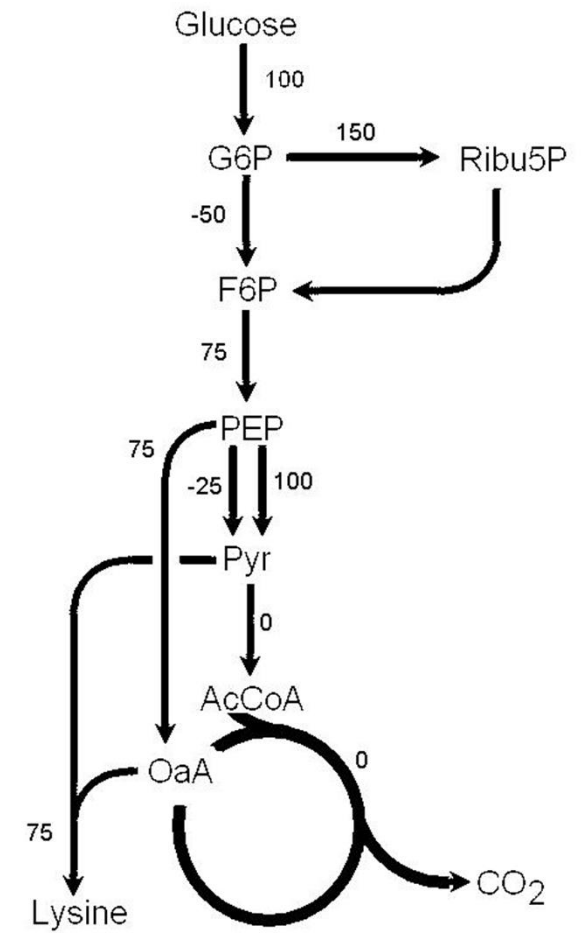
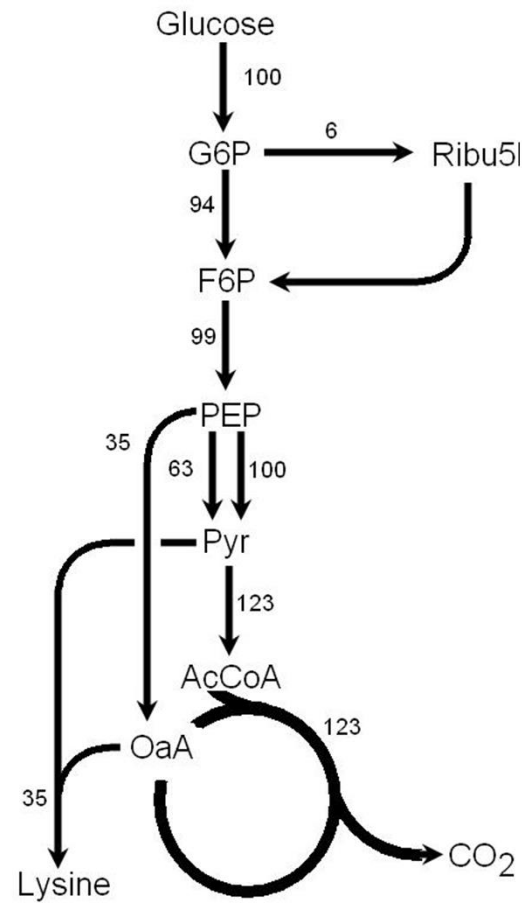
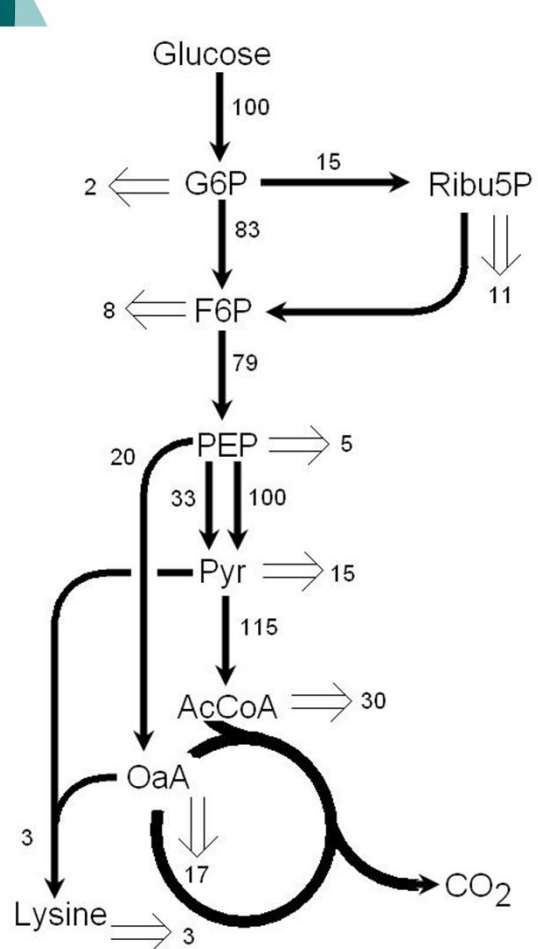
Metabolic level  
regulation

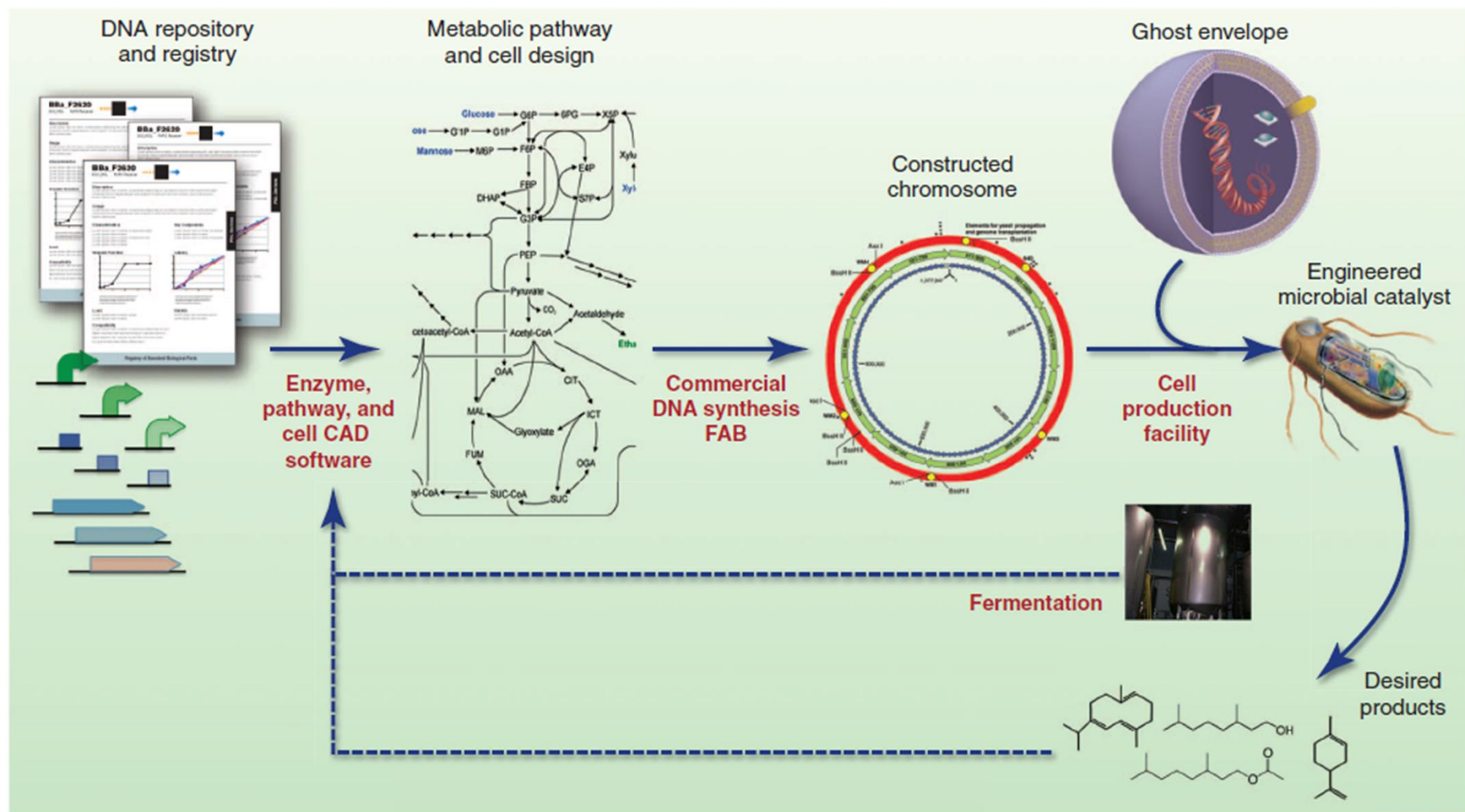


Hierarchical level  
regulation



# Fluxos metabólicos






**Fig. 3.** The future of engineered biocatalysts. Pathways, enzymes, and genetic controls are designed from characteristics of parts (enzymes, promoters, etc.) by means of pathway and enzyme CAD software. The chromosomes encoding

those elements are synthesized at a FAB and incorporated into a ghost envelope to obtain the new catalyst. The design of the engineered catalyst is influenced by the desired product and the production process.

# Referências

- 
1. Nielsen, J. 2003. It is all about metabolic fluxes. *Journal of Bacteriology*, 185: 7031-7035.
  2. Nielsen, J. & Jewett, M.C. 2008. Impact of system biology on metabolic engineering of *Saccharomyces cerevisiae*. *FEMS Yeast Research*, XX: XXXX-XXXX.
  3. Tyo, K.E.; Alper, H.S. & Stephanopoulos, G.N. 2007. — Expanding the metabolic engineering toolbox: more options to engineer cells. *Trends in Biotechnology*, 25: 132-137.
  4. Keasling, J. 2010. Manufacturing molecules through metabolic engineering. *Science*, 330: 1355-1358.
  5. Bailey, J.E. 1991. Toward a science of metabolic engineering. **Science**, 252: 1668-1674.
  6. Stephanopoulos, G. & Vallino, J.J. 1991. Network rigidity and metabolic engineering in metabolite overproduction. **Science**, 252: 1675-1681.
  7. Gomez, J.G.C. 2016. Engenharia metabólica, Biologia de Sistemas e o desenvolvimento de processos biotecnológicos microbianos. *Revista Brasileira de Engenharia Química*, 32 (1): 6-13.