"Mathematical and Computational Analysis of Central Carbon Pathways for Efficient Metabolic Engineering"

"Metabolic Engineering with No Parameters"

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Systems Engineering:

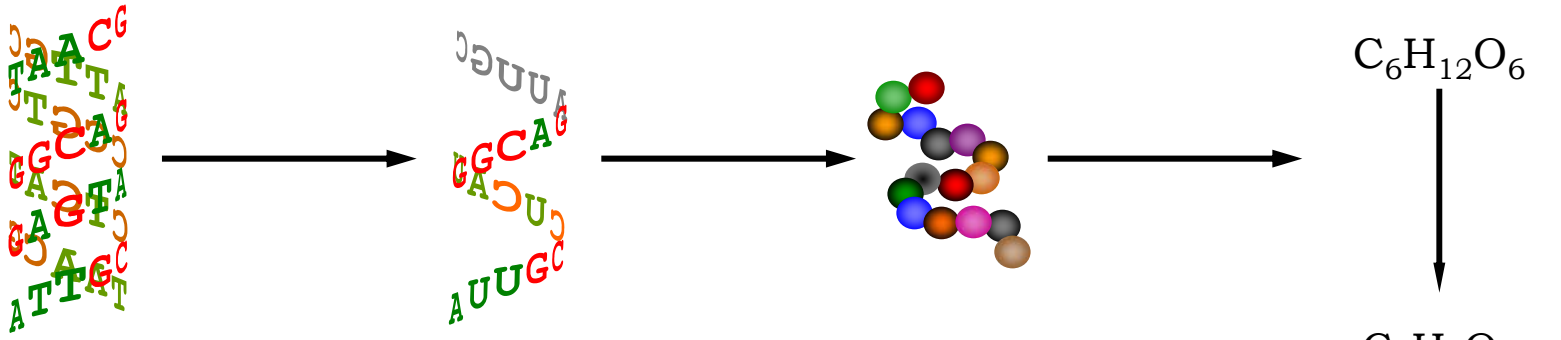
"Retrofitting (and troubleshooting) chemical plants"

Metabolic Systems Engineering:

"Retrofitting (and troubleshooting) ancient chemical plants"

Complexity:

"Absence of a flowchart"

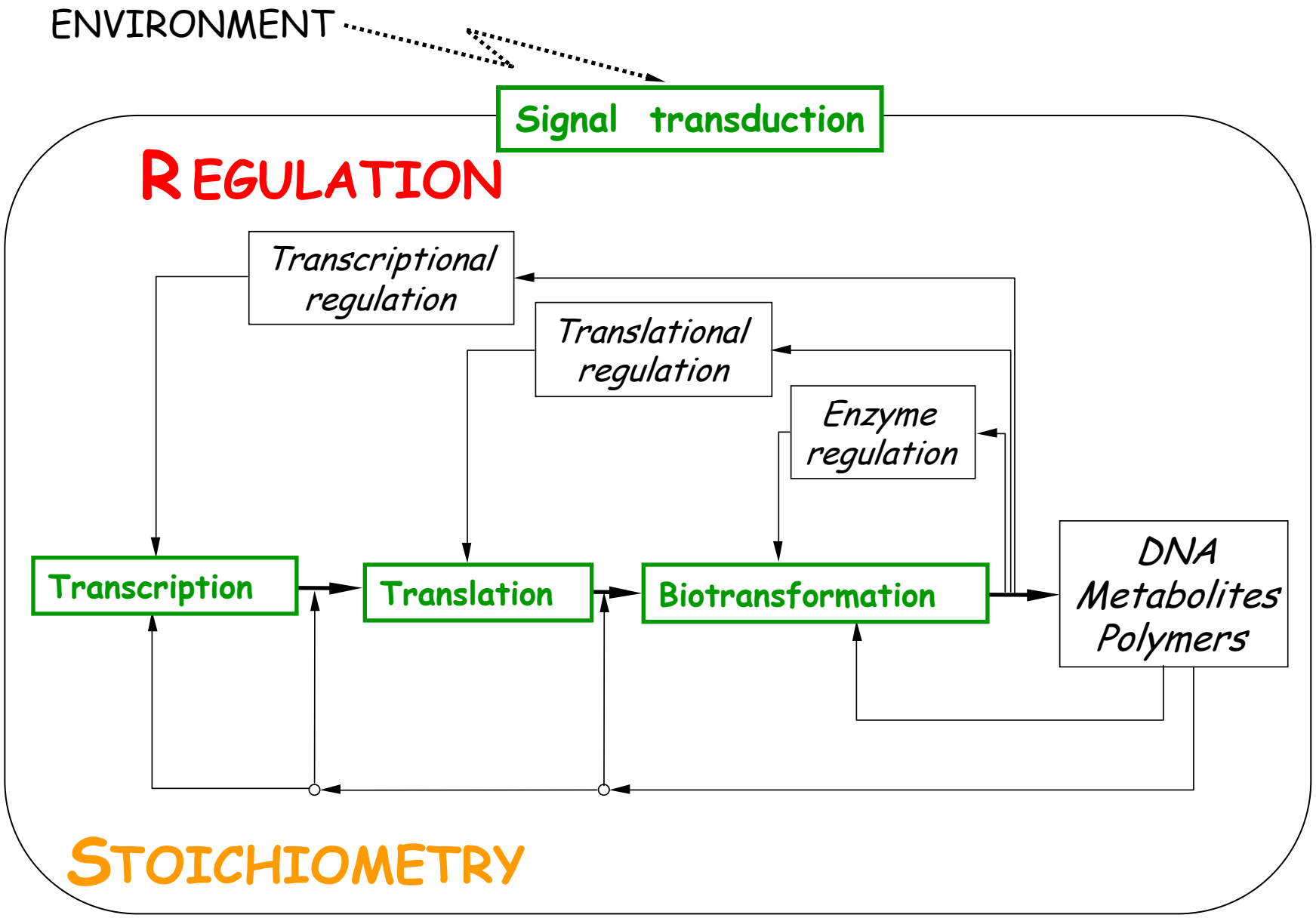


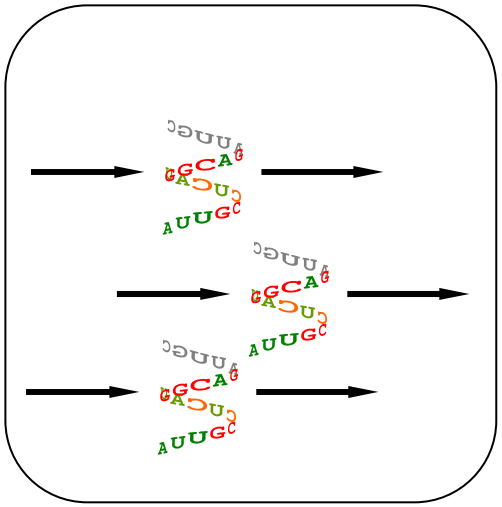
Transcription

Translation

Biotransformation

THE ENGINEERING VIEW OF A LIVING CELL





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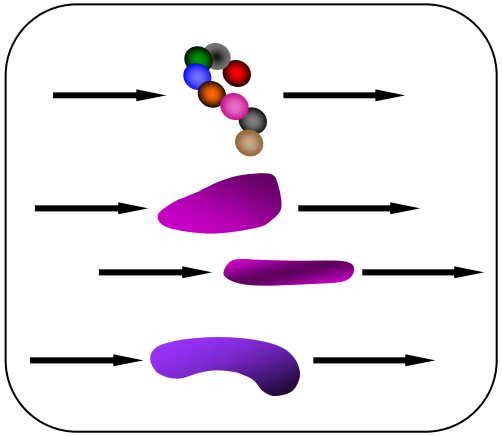
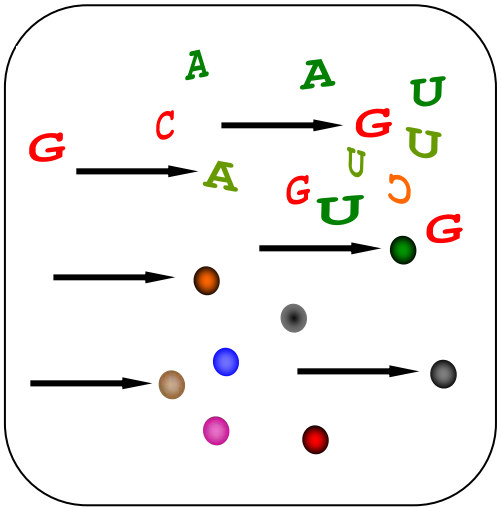
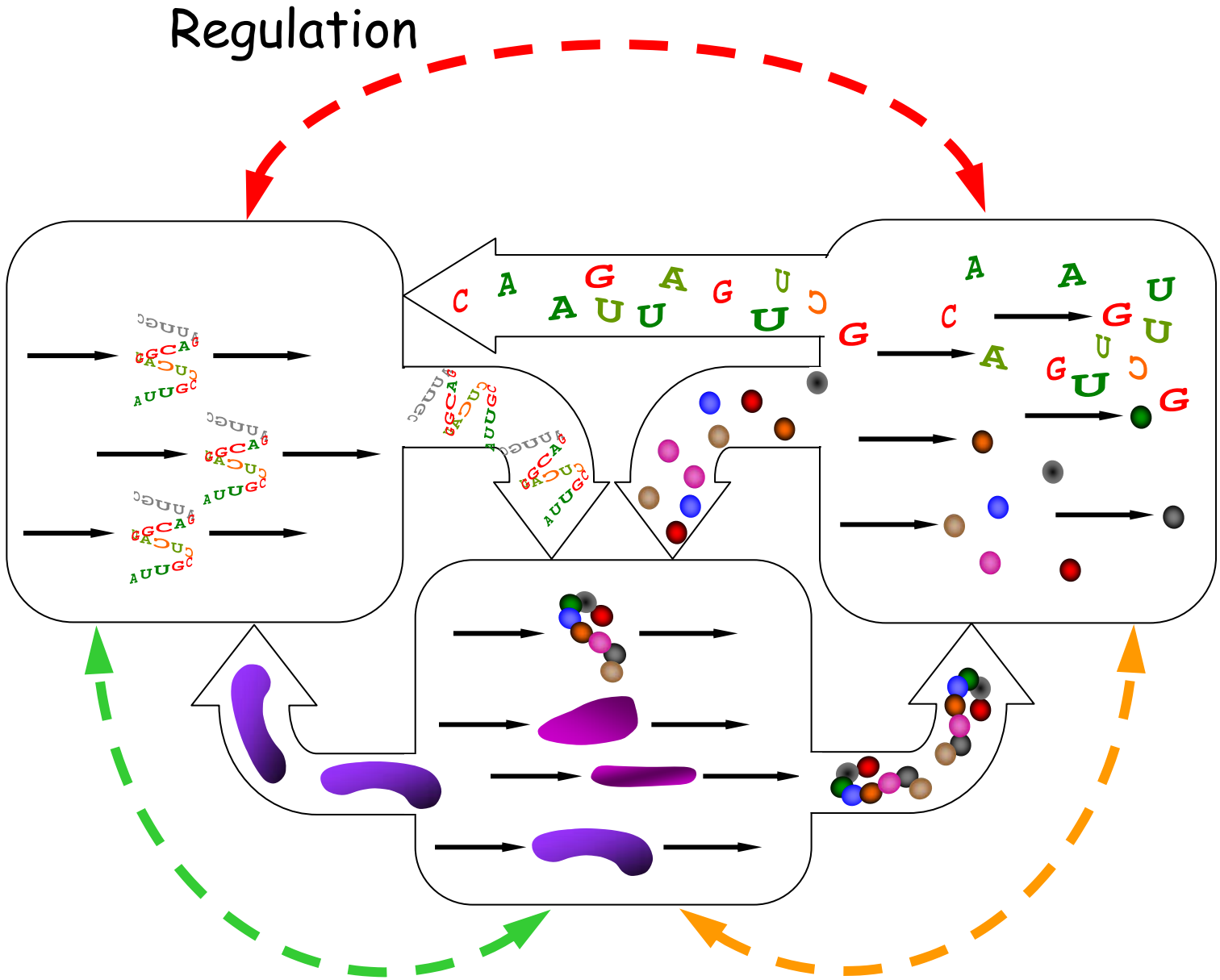
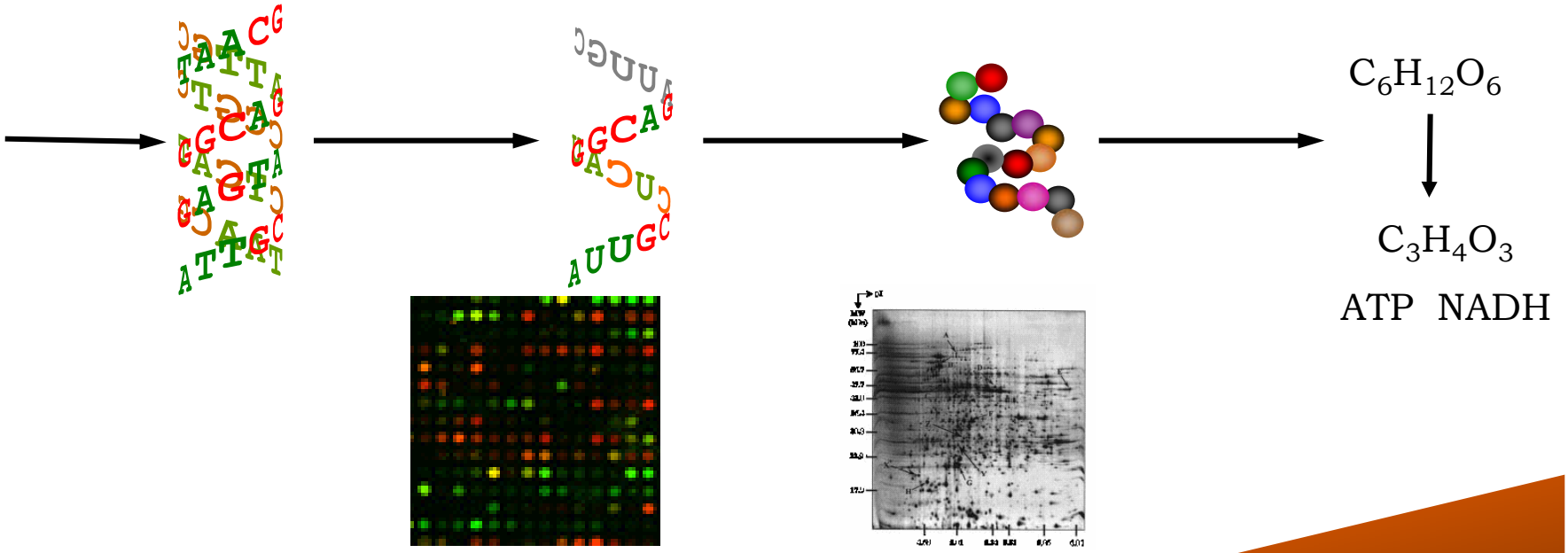


Translation



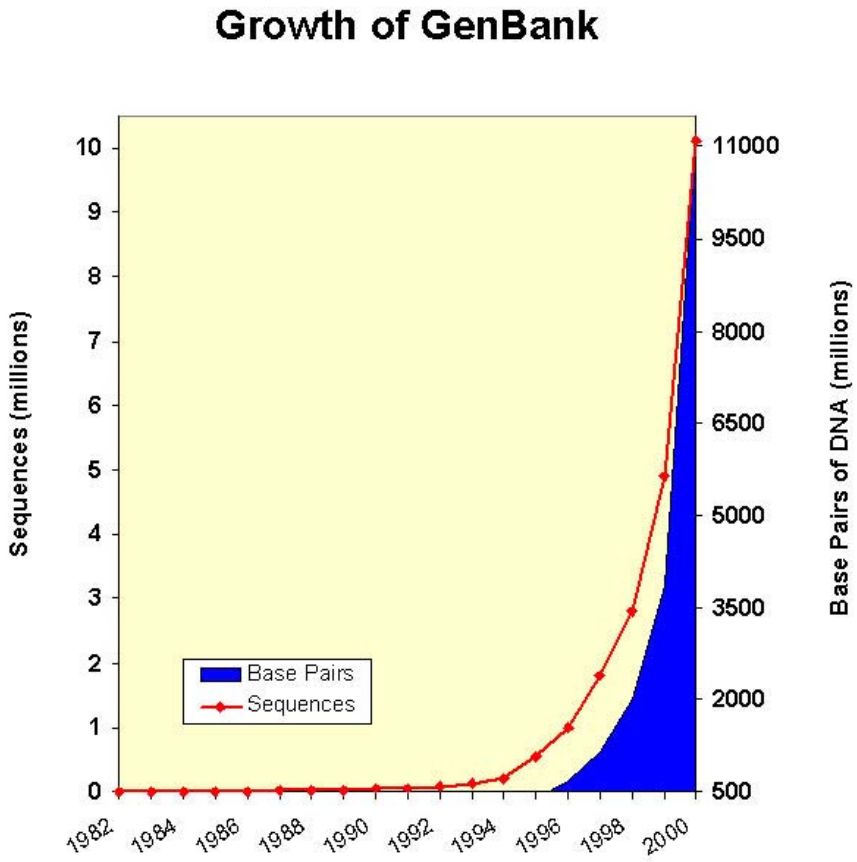
Biotransformation


Regulation

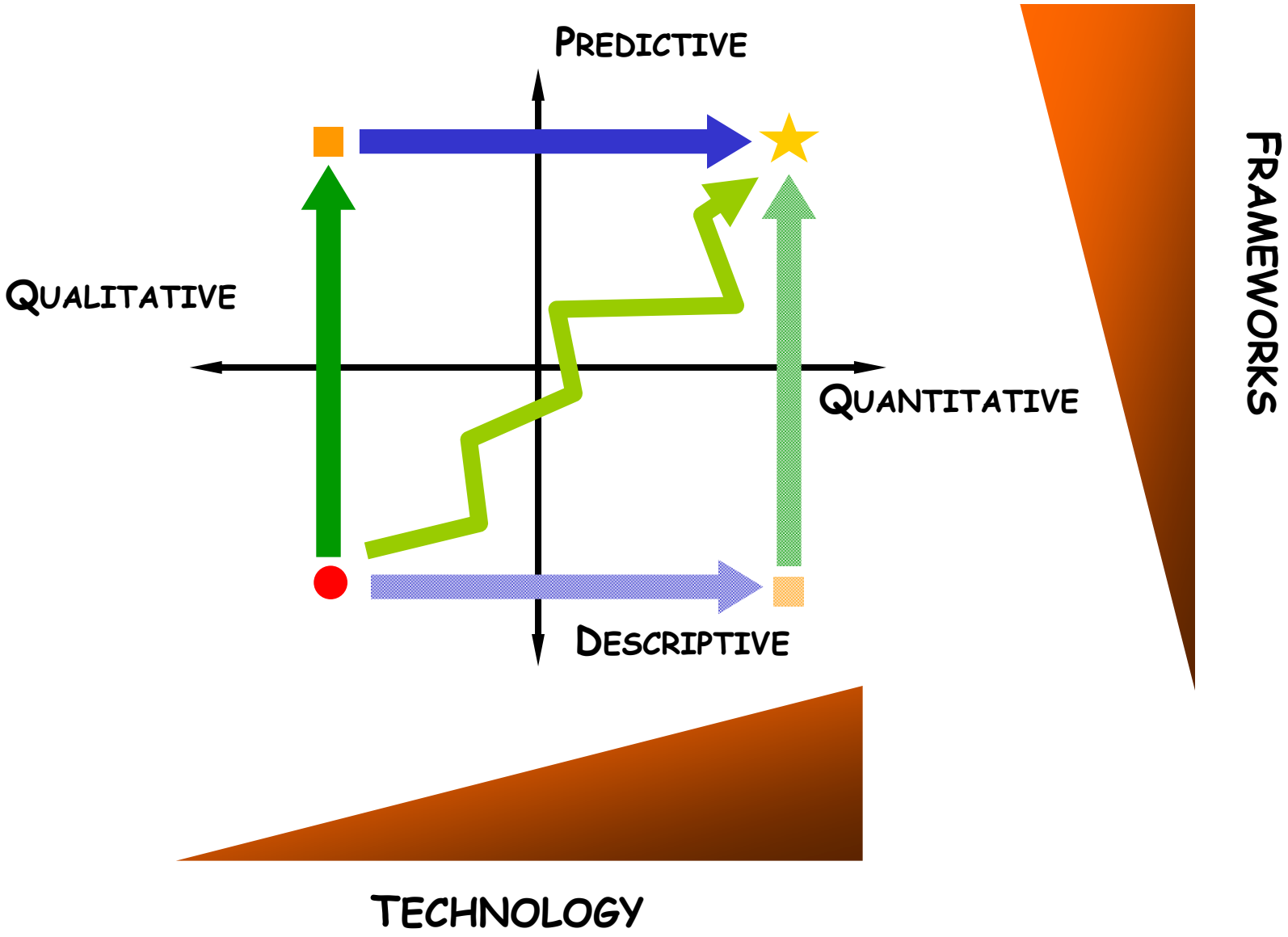


TECHNOLOGY LIMITATIONS

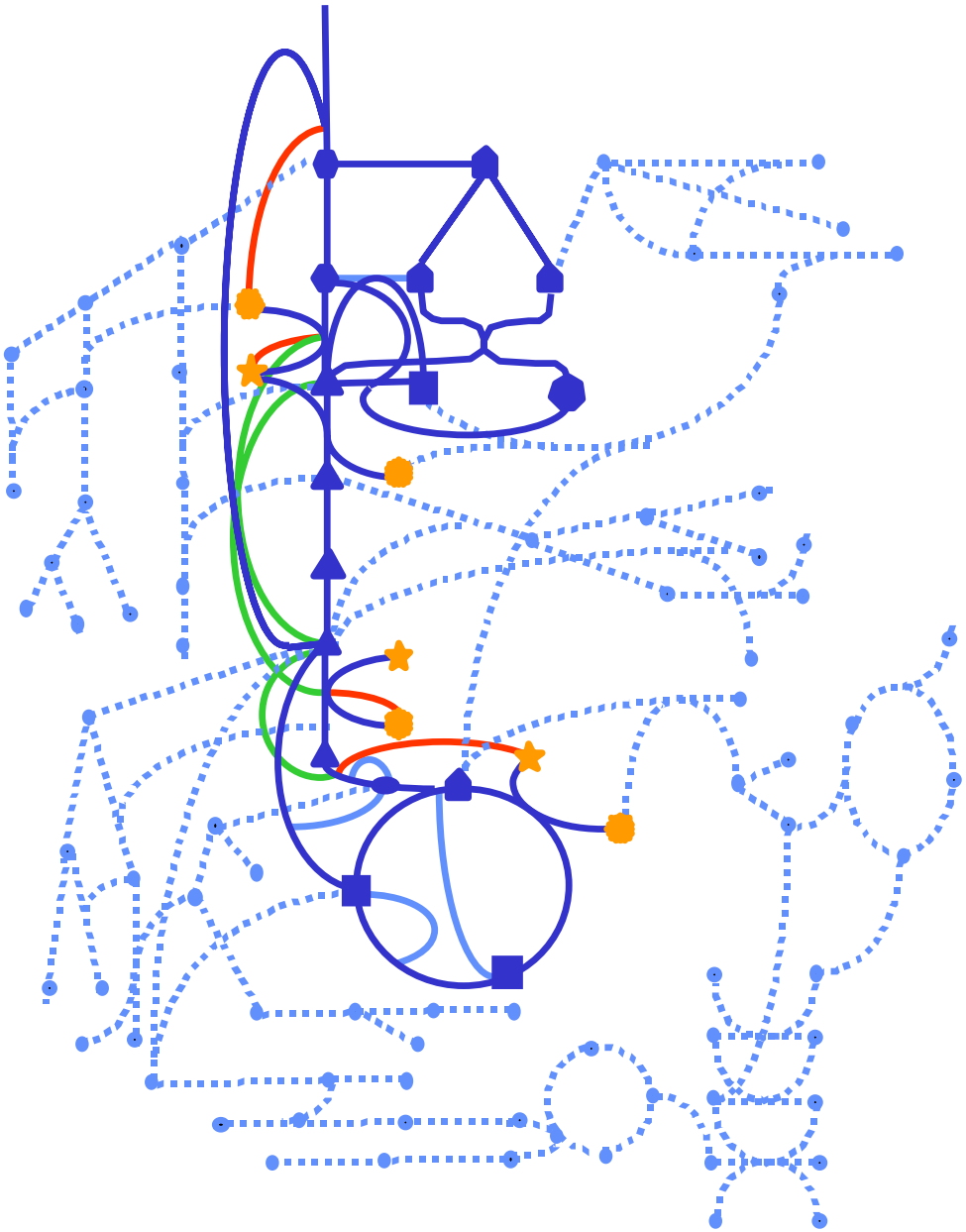
UNDERSTANDING vs INFORMATION



$$\frac{d(\text{understanding})}{dt} \propto \frac{1}{\frac{d(\text{information})}{dt}}$$


PREDICTIVE
QUALITATIVE
QUANTITATIVE
DESCRIPTIVE
FRAMEWORKS
TECHNOLOGY

The Metabolic Maze



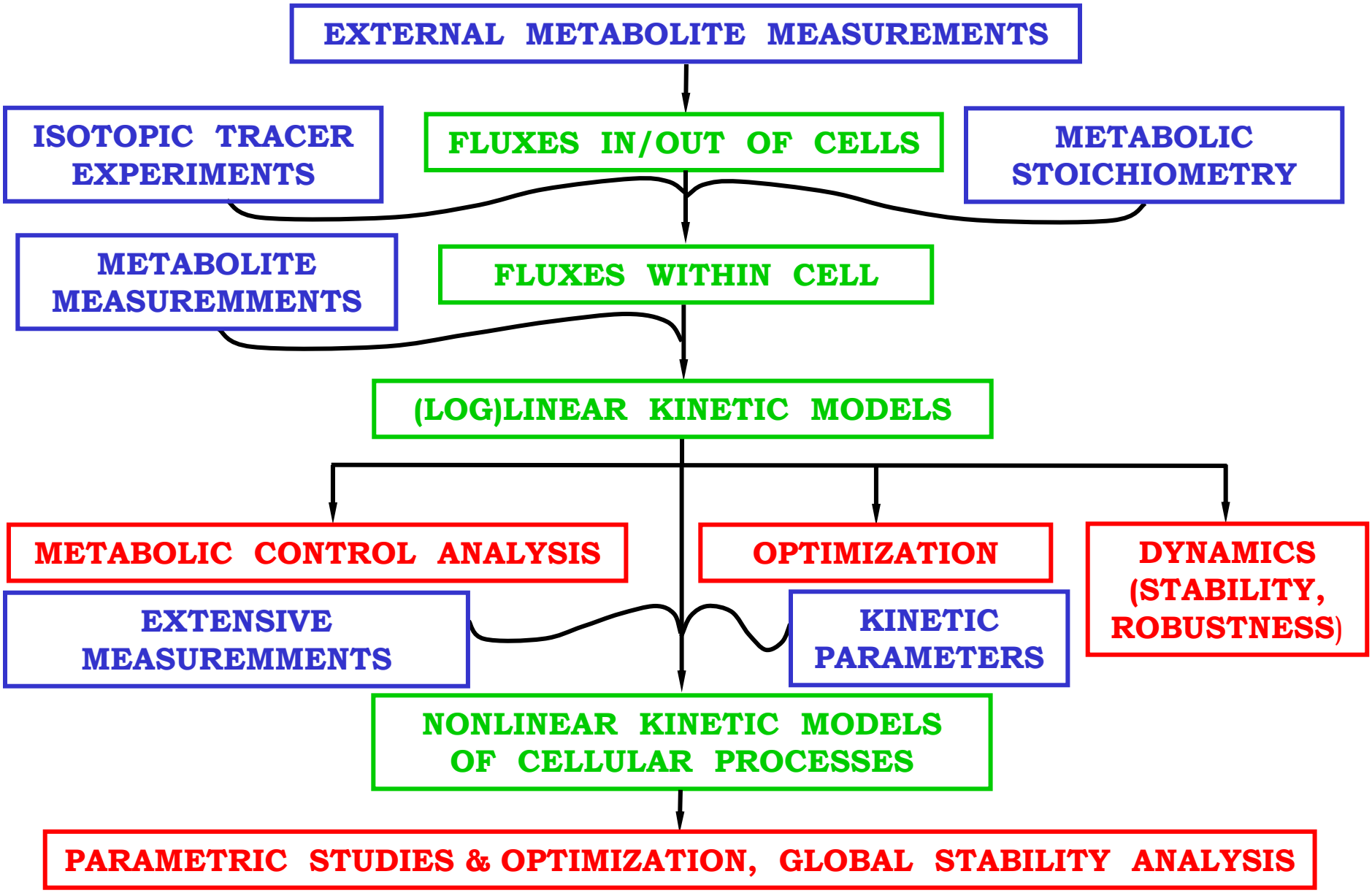
Complex biology with no parameters

James E. Bailey

Recent publication of analyses of the human that each one accepts and all of the products ce, it is possible to for- l reaction network that e repertoire of possible ystems within that cell. this state of enlighten- or the future; it was not in the network formulated by Palsson and coworkers[3]. Starting from a master reaction network containing all of the metabolic reactions possible in a bacterium (available—in principle—from its genome sequence), this approach then derives equations that constrain the feasible reaction rates (typically

It is incorrect to say that these shortcomings represent flaws or errors in the models outlined above or in their analysis. Such limitations will arise in any mathematical model, which is written from the beginning to describe and understand a particular set of phenomena and interactions. It is expected that models developed for one type of question may not be useful to describe other variations. However, because of the importance of understanding the effects on phenotype of the types of genetic modifications just listed, expanded models should be formulated that encompass more aspects of regulation. Then, although computer solu-

HIERARCHY OF MATHEMATICAL STRUCTURES FOR UTILIZING EXPERIMENTAL INFORMATION : *METABOLISM*



Adapted from J.E. Bailey, *Biotechnol. Prog.*, **14**(1), 8-20 (1998)

METABOLIC FLUX ANALYSIS

"*Knowledge of the flux distribution can provide guidance for metabolic engineering*"

- There exist many methods for the *estimation* of metabolic fluxes in metabolic pathways:
  - ^{13}C-NMR
  - GS/MS
  - ^{1}H-NMR
  - Metabolic optimization criteria
  - Constraints-based framework

Metabolic Flux Analysis and Metabolic Engineering

- Can we use metabolic flux measurements to obtain guidance for metabolic engineering ?

Guidance :

- which is the best candidate enzyme/reaction step for engineering in order to achieve a specific objective ?

- which metabolic fluxes and metabolites respond in a *similar* manner to genetic and environmental changes ?

METABOLIC FLUX ANALYSIS

"Knowledge of the flux distribution can provide guidance for metabolic engineering"

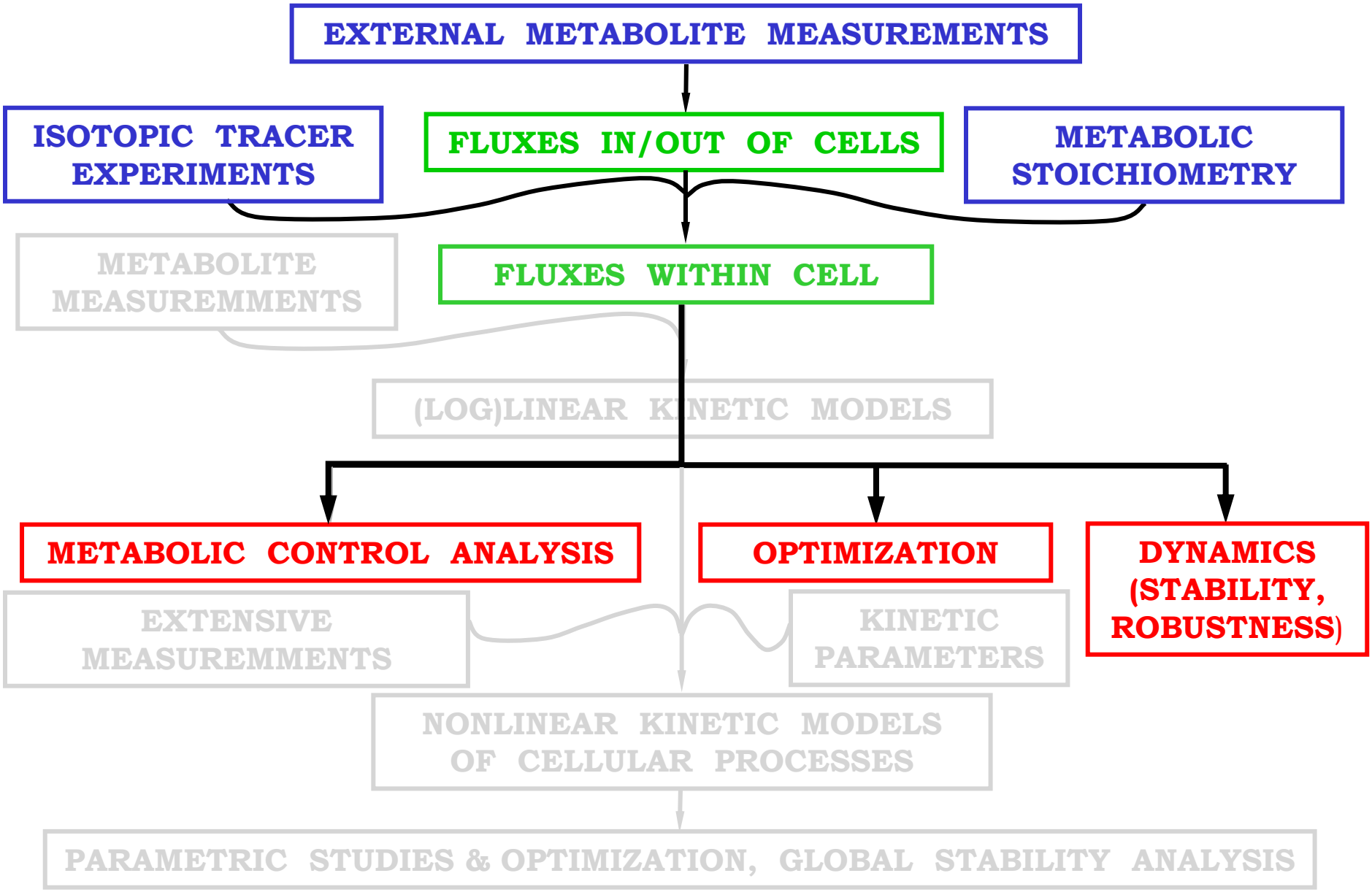
- **Metabolic flux analysis does not consider kinetic constraints, whereas metabolic engineering deals with manipulation of pathway kinetics**

estimation

of metabolic fluxes in metabolic pathways

- *There exist many methods for the*
- *^{13}C-NMR*
- *GS/MS*
- *^{1}H-NMR*
- *Metabolic optimization criteria*
- *Constraints-based framework*

HIERARCHY OF MATHEMATICAL STRUCTURES FOR UTILIZING EXPERIMENTAL INFORMATION : *METABOLISM*



Bioinformatics and Metabolic Engineering

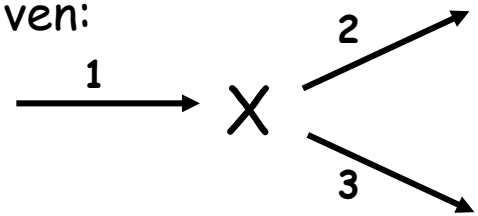
the subject of the emerging field of bioinformatics. In general, we define bioinformatics as the methods and framework aiming at the extraction of biological knowledge from sequence, expression, proteomic, and isotopic tracer distribution data. The *upgrade of information content* is the main theme of bioinformatics research.

Metabolic Engineering **2**, 157–158 (2000)

Gregory Stephanopoulos
Massachusetts Institute of Technology

Metabolic Engineering

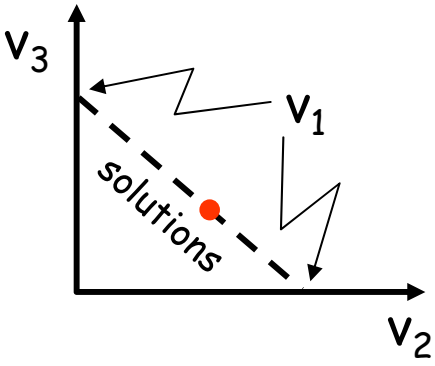
Given:



How can we increase flux through enzyme 2 ?

Metabolic Flux Analysis

$$v_1 - v_2 - v_3 = 0 \Rightarrow v_3 = v_1 - v_2$$



Metabolic Engineering

$$v_1 - v_{max,2}\frac{X}{K_1 + X} - v_{max,3}\frac{X}{K_1 + X} = 0$$

Which kinetic parameter we should change and how in order to increase flux through enzyme 2 ?

Complex biology with no parameters

James E. Bailey

Recent publication of analyses of the human genome sequence dramatically signals a turn in biology research from reductionist dissection to systems integration. Mathematical and computational tools, clearly indispensable for genome assembly and sequence comparisons, have otherwise become almost

that each one accepts and all of the products that each one can make, it is possible to formulate a master global reaction network that represents the complete repertoire of possible biochemical reaction systems within that cell.

However, reaching this state of enlightenment remains a goal for the future; it was not

in the network formulated by Palsson and coworkers[3]. Starting from a master reaction network containing all of the metabolic reactions possible in a bacterium (available—in principle—from its genome sequence), this approach then derives equations that constrain the feasible reaction rates (typically

is not usually, if ever, necessary. Qualitative and quantitative understanding and corresponding methodologies for designing desired properties of many complex systems have been successfully achieved in the fields of chemistry, physics, and the associated engineering disciplines without knowing all aspects of systems structure and certainly without knowing all parameter values involved. The same must be possible for biology.

METABOLIC ENGINEERING

$$v_1 - v_{max,2}\frac{X}{K_1 + X} - v_{max,3}\frac{X}{K_1 + X} = 0$$

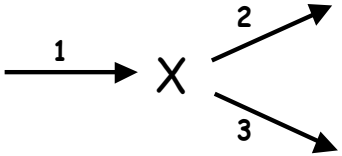
- Even if we know the kinetic mechanism
  we still face uncertainties in:
  $V_{max,2}$, $V_{max,3}$, K_2 , K_3 , and X

- Quantitative estimation of the kinetic parameters and the metabolite concentrations are:
  - Expensive
  - Time consuming
  - Infeasible for every metabolite within a cell

- Proposed approach:

  - **Monte Carlo Metabolic Control Analysis**

Metabolic Engineering: Quantitative guidance through Metabolic Control Analysis (MCA)



$C_{e_2}^{v_2} = \frac{d\ln v_2}{d\ln e_2}$: % change in the metabolic flux 2 for a unit % change in activity of enzyme 2 (flux control coefficient)

$$\ln\left(\frac{v_2}{v_{2,o}}\right) = C_{v_1}^{v_2} \cdot \ln\left(\frac{e_1}{e_{1,o}}\right)$$

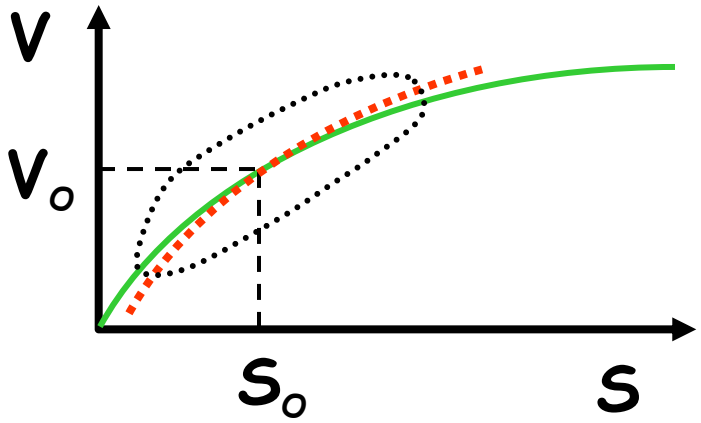
$$C_{e_1}^{v_2} = \frac{V_1 \cdot \varepsilon_2}{V_2 \cdot \varepsilon_2 + V_3 \cdot \varepsilon_3} = \frac{\varepsilon_2}{\alpha \cdot \varepsilon_2 + (1-\alpha) \cdot \varepsilon_3}$$

where

$\alpha = (v_2 / v_1)$ and $\varepsilon_i = \frac{\partial \ln v_i}{\partial \ln x} = \frac{x}{k_i + x} \Rightarrow 0 \le \varepsilon_i \le 1$

A SIMPLIFIED MATHEMATICAL FORMALISM

$$S + E \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} SE \xrightarrow{k_2} P + E$$



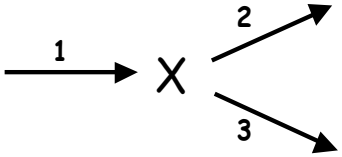
$$V = k_2 \cdot [E] \frac{S}{K + S}$$

$$V = V_o \left(1 + \varepsilon \cdot \ln\left(\frac{S}{S_o} \right) + \pi \cdot \ln\left(\frac{[E]}{[E]_o} \right) \right)$$

$$0 \le \varepsilon = \frac{K}{K + S_o} \le 1$$

$$\pi = 1$$

Metabolic Engineering: Quantitative guidance through Metabolic Control Analysis (MCA)



$$C_{e_2}^{v_2} = \frac{V_1 \cdot \varepsilon_2}{V_2 \cdot \varepsilon_2 + V_3 \cdot \varepsilon_3} = \frac{\varepsilon_2}{\alpha \cdot \varepsilon_2 + (1-\alpha) \cdot \varepsilon_3}$$

Depends only on:

- steady state fluxes (accessible through Metabolic Flux Analysis)
- ε_i (well-defined bounds)

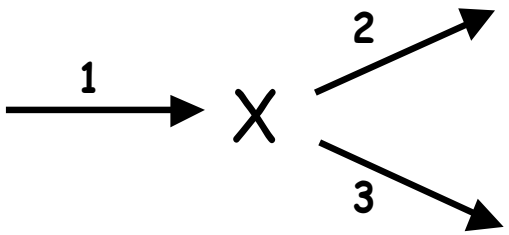
Our computational and statistical approach:

Monte Carlo Metabolic Control Analysis

- generate random values for ε_i
- calculate populations of control coefficients
- perform statistical analysis

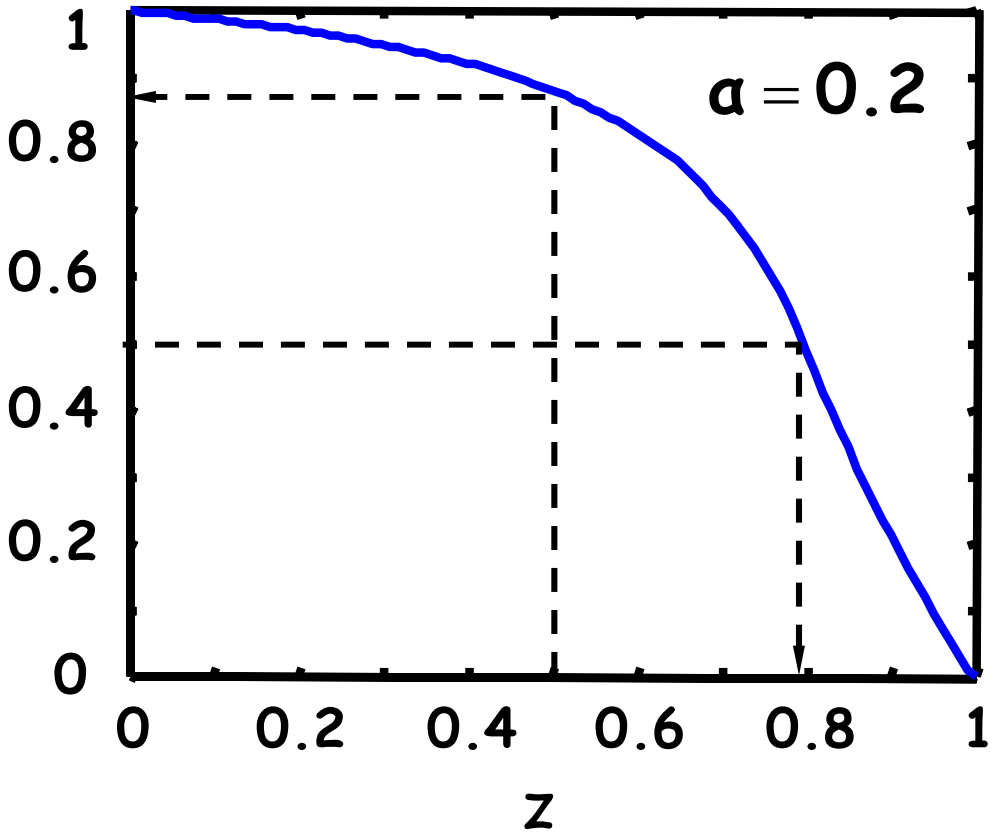
Monte Carlo Metabolic Control Analysis:

Branched Pathway (w/o regulation)



$$\alpha = \frac{v_2}{v_1}$$

$P\left(C_2^2 \geq z\right)$



Monte Carlo Metabolic Control Analysis:

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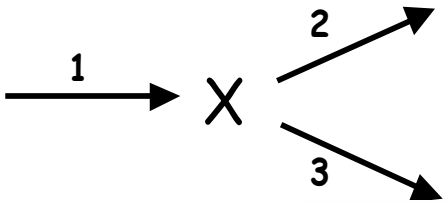

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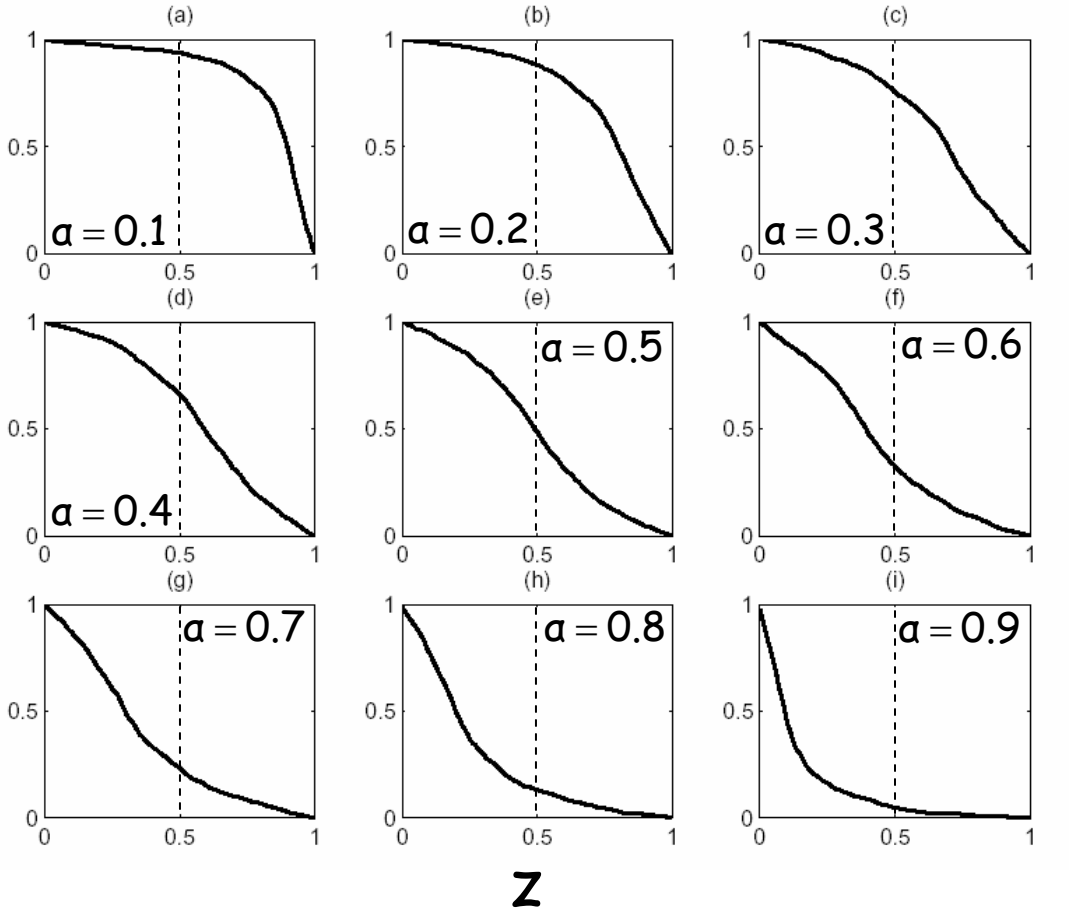
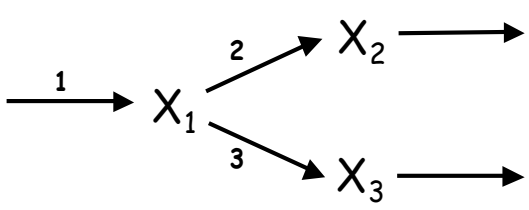


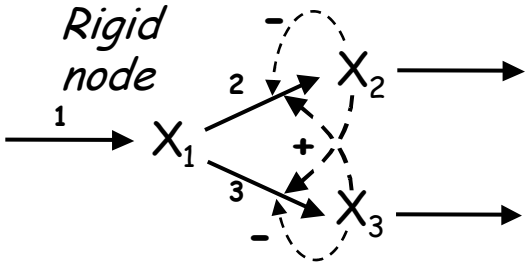
Monte Carlo Metabolic Control Analysis:

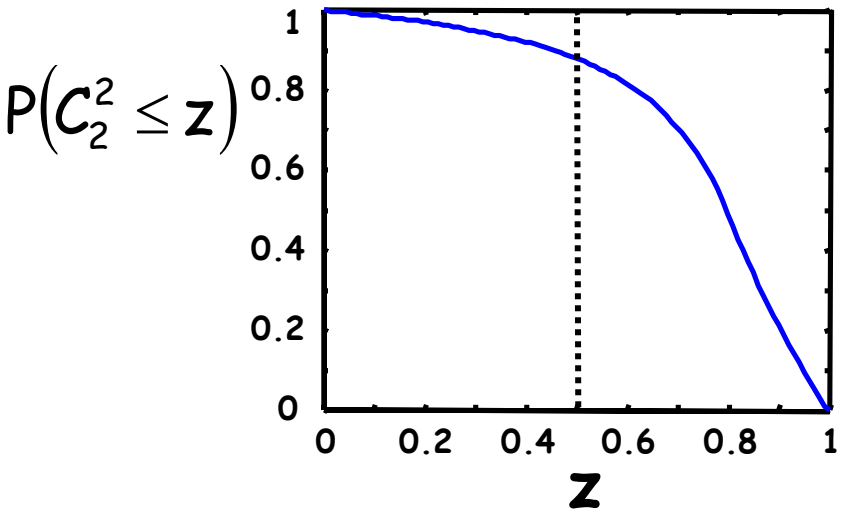
Branched Pathway: Effects of regulation

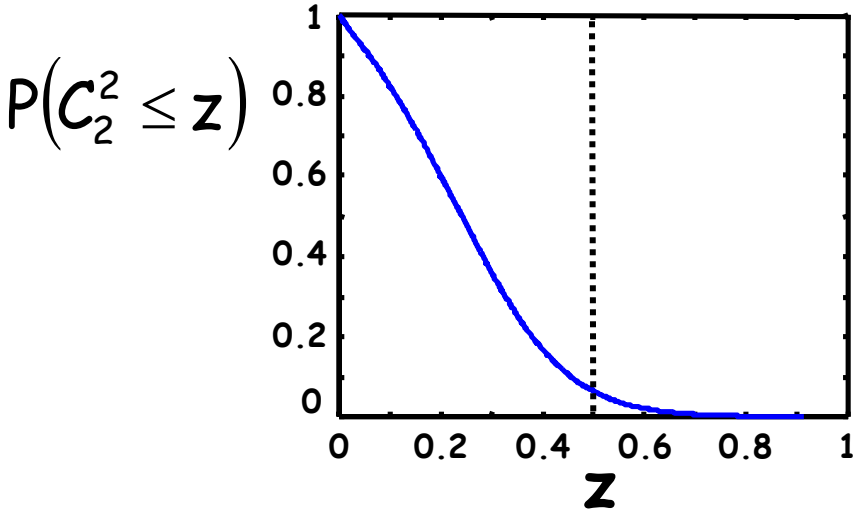
Regulation in the branched pathway reduces the sensitivity of the pathway to perturbations



$$\alpha = \frac{v_2}{v_1} = 0.2$$







Monte Carlo Metabolic Control Analysis

- Requires knowledge of stoichiometry and metabolic fluxes alone
- Relies on large-scale computation
- Employs non-parametric statistics
- Provides quantitative insight and guidance for metabolic engineering

Bioinformatics approaches to Metabolic Engineering in the post-genomic era

Post-genomic era: large-scale information from multiple levels increases uncertainty.

- Exploit computational power
- Employ statistical analysis
- Methods for decision under uncertainty

Acknowledgements

- National Science Foundation
- National Aeronautics & Space Administration
- Department of Energy