LECTURE 2: METABOLIC ENGINEERING

Introduction to Biocybernetics

Daniel Georgiev

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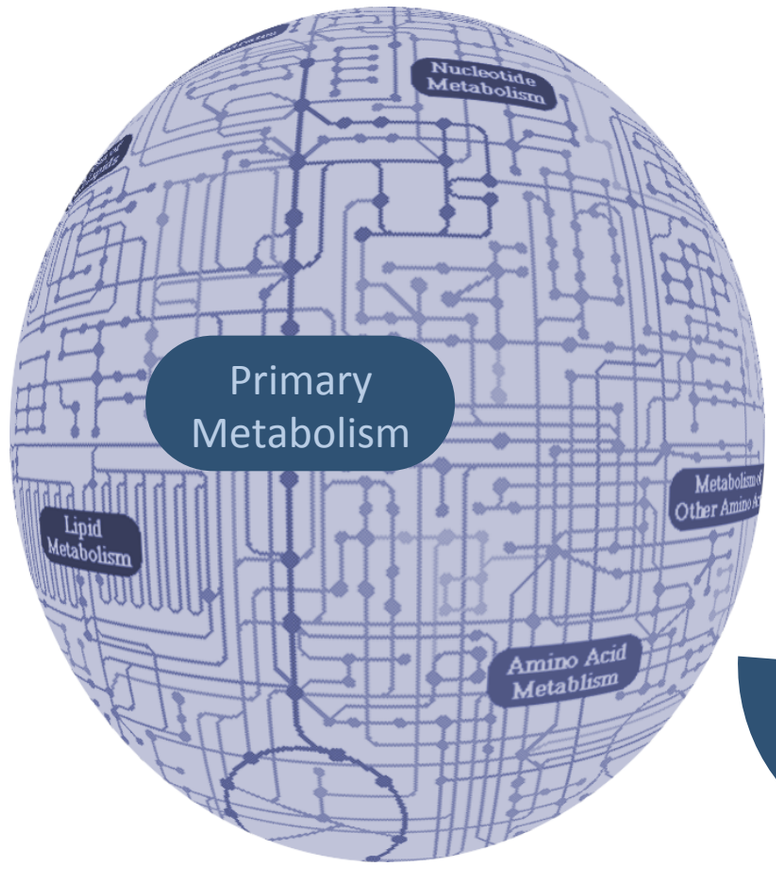
OSNOVA

- Biosynthesis
- Anabolism/Catabolism
- Central metabolism
- Amino acid synthesis
- Metabolic engineering
- Regulation
- Mass action kinetics
- Michaelis-menten kinetics
- Stead state approximation
- Flux balance analysis

Catabolism

Bioremediation
Biomass Decomposition

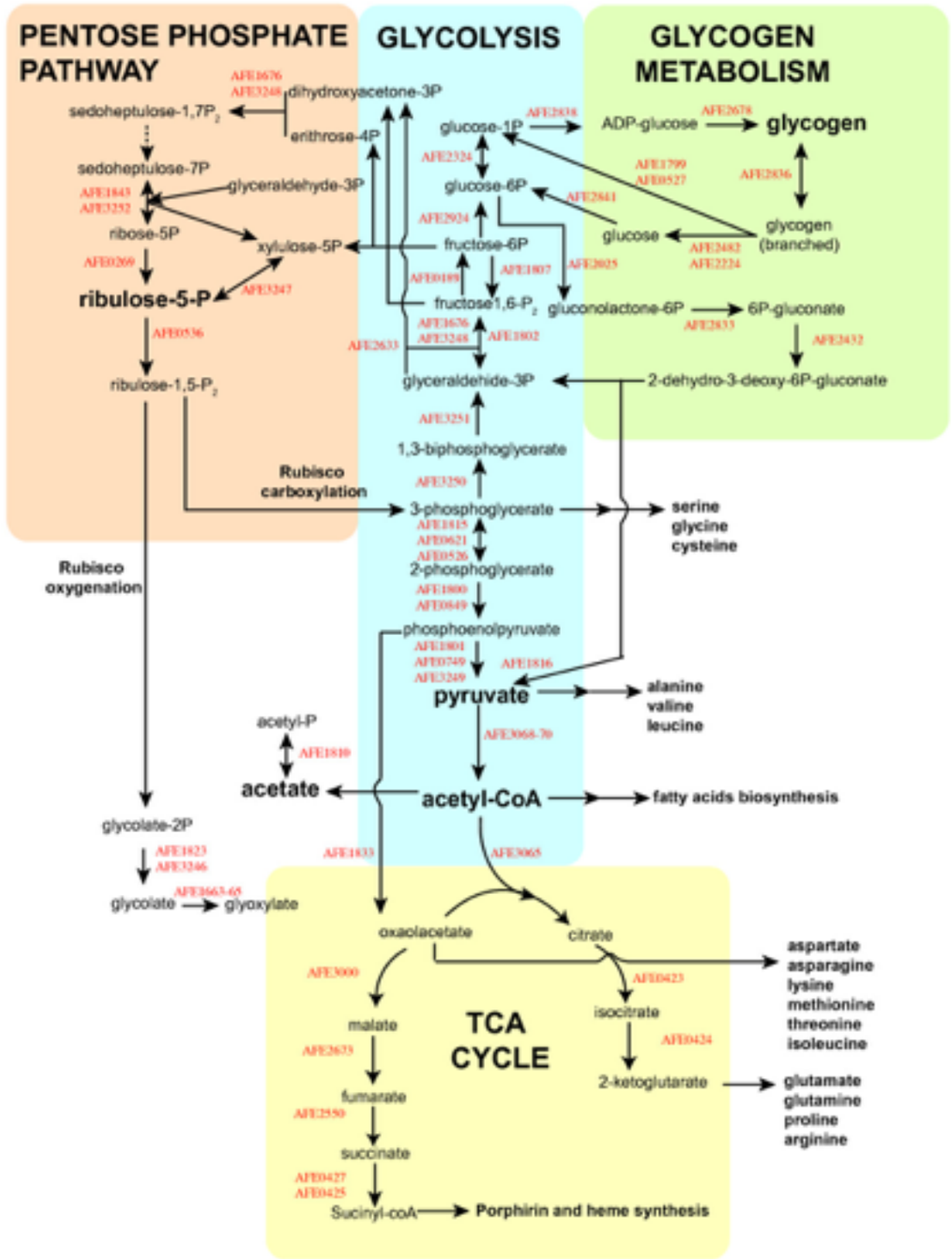


Anabolism

Natural Product Biosynthesis
Biofuels

ANABOLISM/CATABOLISM

Existing biotechnologies are based on the merging of inherent and synthetic metabolic pathways for the breaking down and building up of compounds.



CENTRAL METABOLISM

The cells central metabolism is associated with carbohydrate breakdown and ATP production. Intermediate metabolites also serve as precursors for all other molecules.

Step 1: Augment the organism with a sufficient set of enzymes to convert metabolites already available to the cell into the desired product (or vice versa for catabolism) until activity is detectable

Step 2: Optimize the organism by:

- Improving the expression of bottleneck enzymes using directed evolution, truncations, or fusion proteins
- Trying homologs of the enzymes used in step 1
- Finding the expression level of the enzymes that gives rise to optimal product formation
- Knocking-up, down, or out native enzymes to increase flux through the desired pathway
- Doing system-wide transposon or chemical mutagenesis and screen for improved yield

METABOLIC ENGINEERING

The above steps outline the metabolic engineering process.

- Traditionally meant to try and make an organism overproduce a compound present on the primary metabolism map
- Also involves adding enzymes to create compounds that aren't part of primary metabolism (secondary metabolites)
- Even when producing secondary metabolites, production yields are often limited by flux through primary metabolic pathways
- So, much effort goes into increasing the production of key branching-off points for major secondary metabolite classes:

METABOLIC ENGINEERING

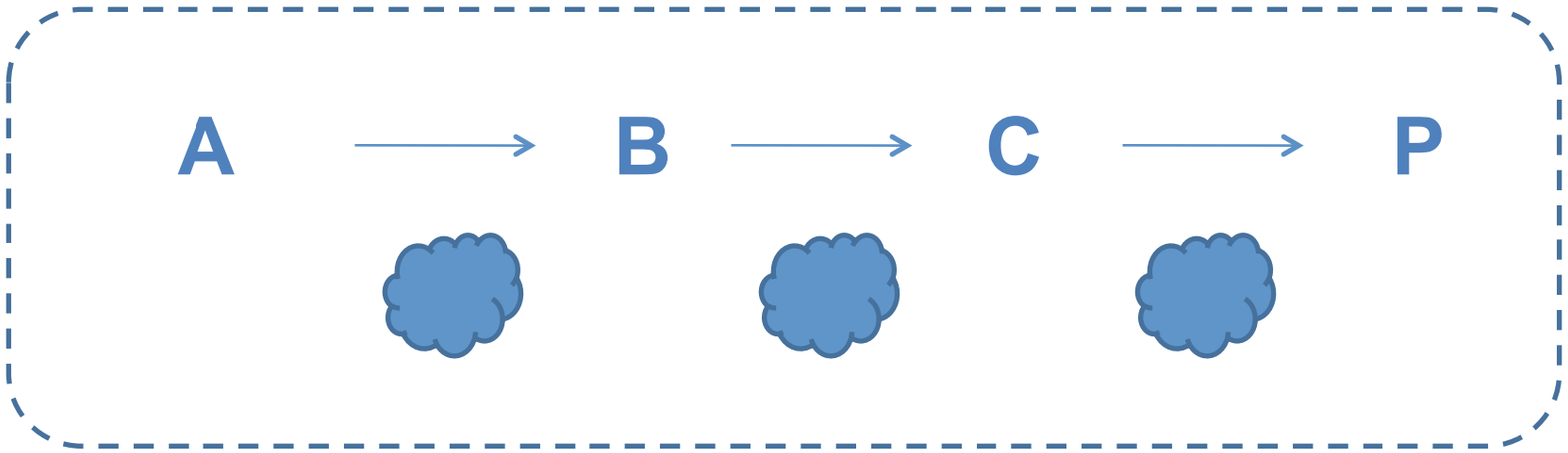
The above steps outline the metabolic engineering process.

- Bacteria are neat and tidy—they tend to keep (most) of the enzymes related to a particular secondary metabolite in operons
- Eukaryotes aren't
- Many natural products are produced along with many other related compounds through competing reactions with various enzymes
- Sometimes these enzymes aren't specific to a particular pathway

- If the organism is amenable to it, can use genetics to find genes that when removed result in a truncation of the pathway
- Can find the enzymes based on homology to similar enzymes (degenerate oligonucleotides, northern blot of cDNA libraries, synthetic metagenomics)
- Various genetic selections and screens can be used to sift through cDNA libraries
- Can use protein engineering to modify known enzymes to the new activity

DETERMINING THE ENZYME

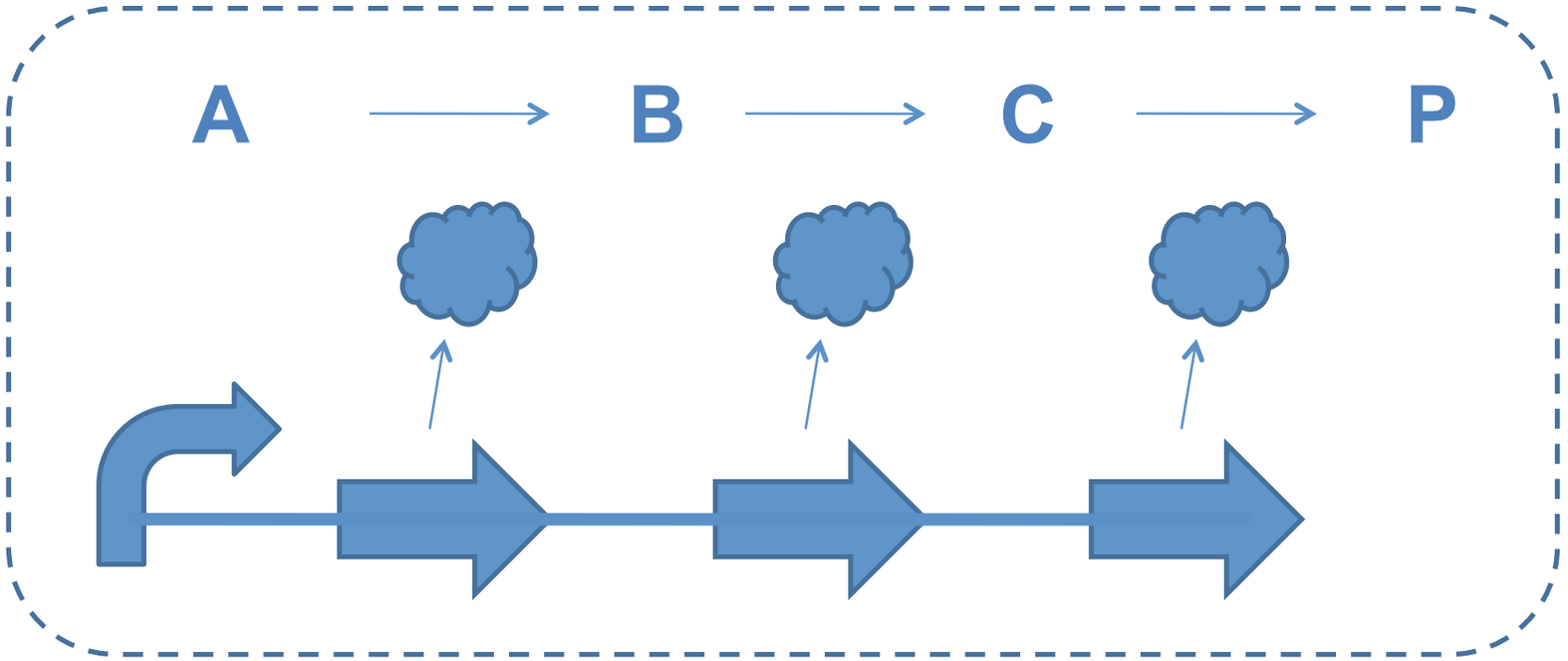
The above steps illustrate how one may find an enzyme for performing the desired steps.



A liposomal system

METABOLIC PATHWAY SCHEMA

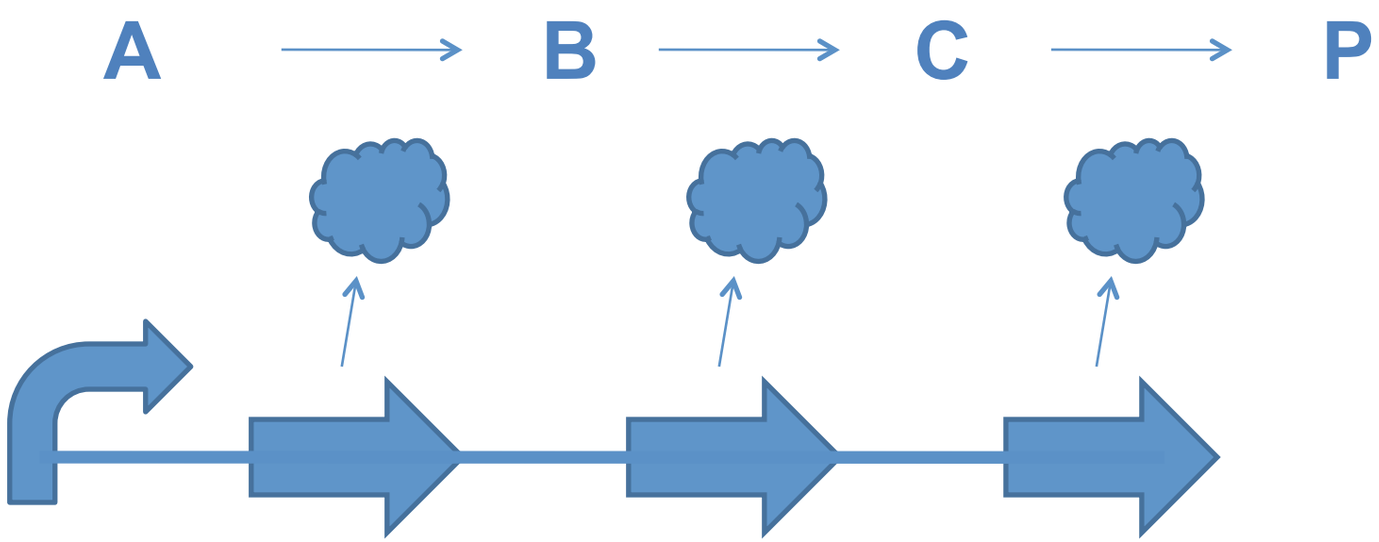
Organisation of metabolic pathway.



A cellular system

METABOLIC PATHWAY SCHEMA

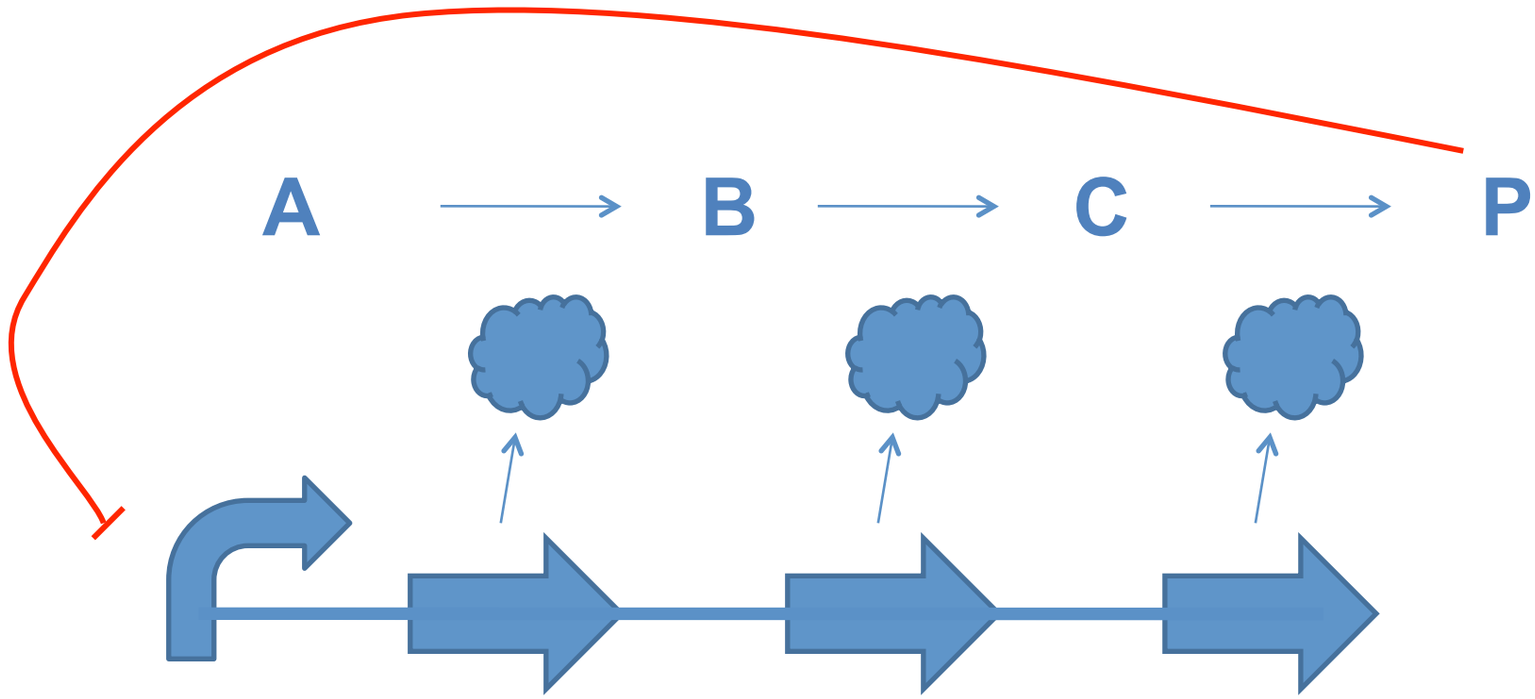
Organisation of metabolic pathway.



Introducing regulation into biosynthetic pathways is a very current topic in synthetic biology

METABOLIC PATHWAY SCHEMA

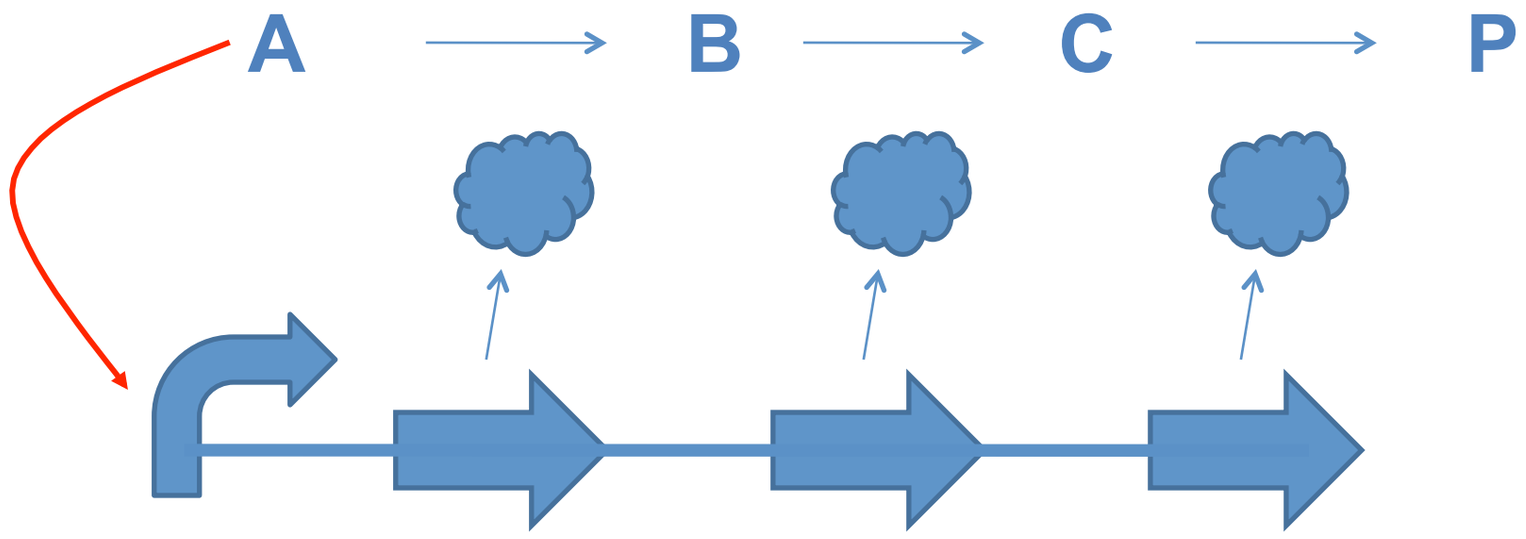
Organisation of metabolic pathway.



Negative Feedback

METABOLIC PATHWAY SCHEMA

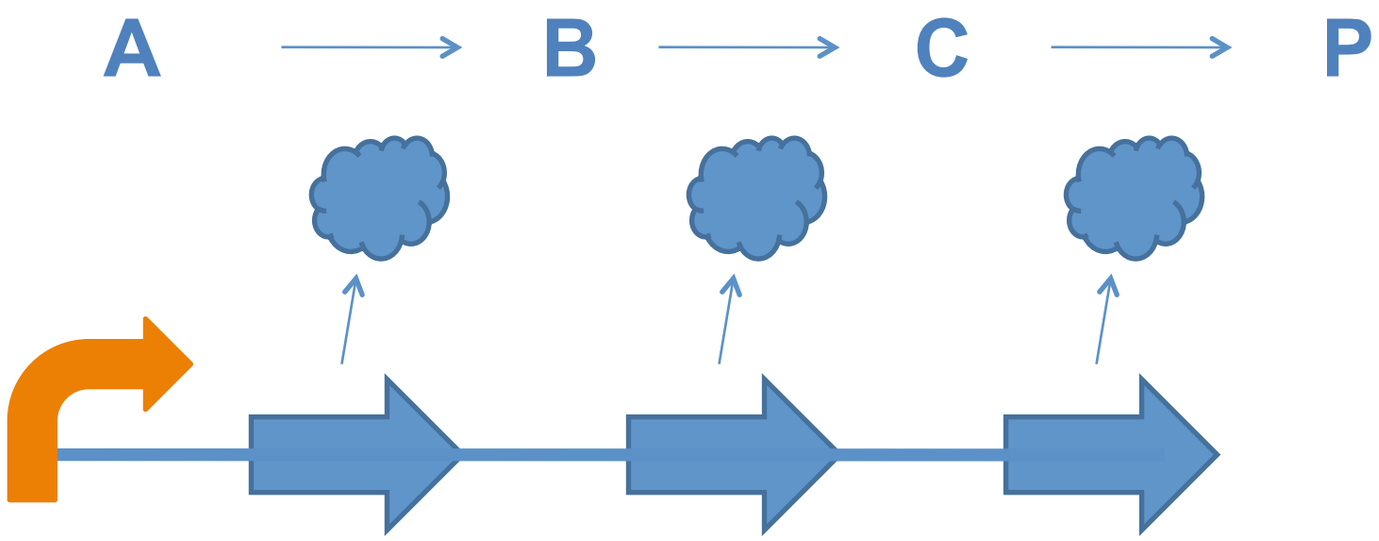
Organisation of metabolic pathway.



Substrate Activation
Ex: arabinose catabolism

METABOLIC PATHWAY SCHEMA

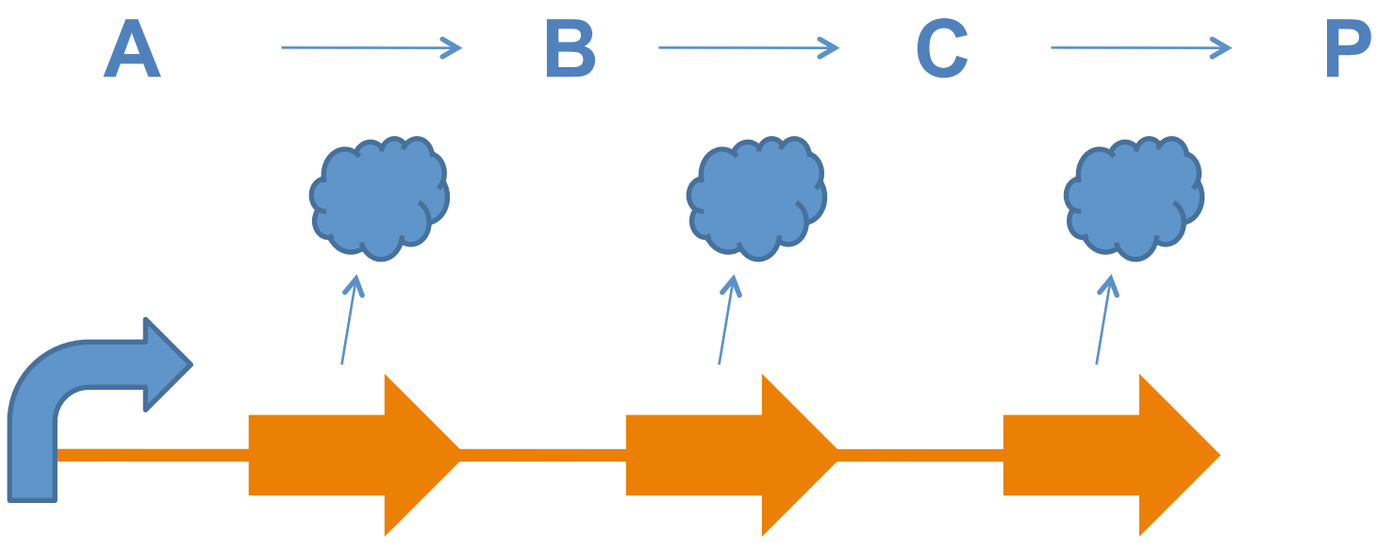
Organisation of metabolic pathway.



Changing promoters eliminates transcriptional control

METABOLIC PATHWAY SCHEMA

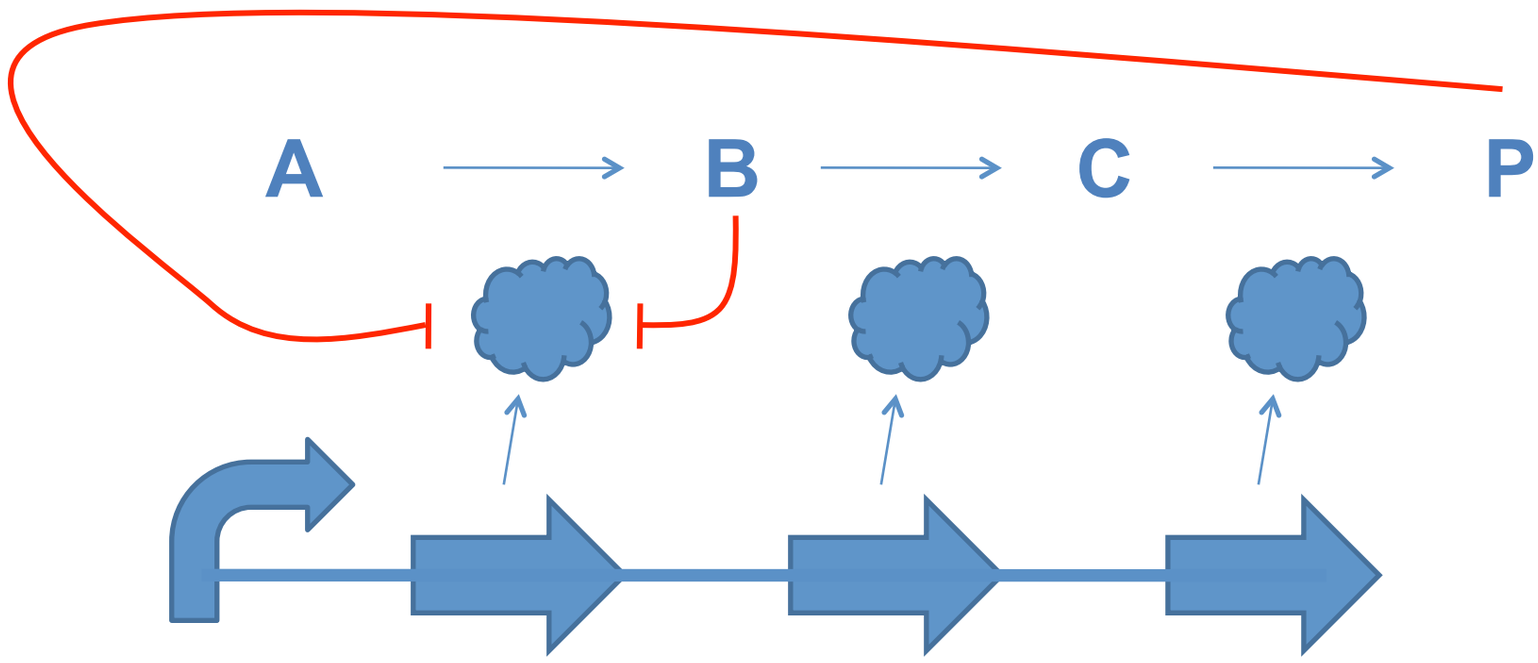
Organisation of metabolic pathway.



Shuffled codon usage and changing 5’ UTRs eliminates translational control

METABOLIC PATHWAY SCHEMA

Organisation of metabolic pathway.

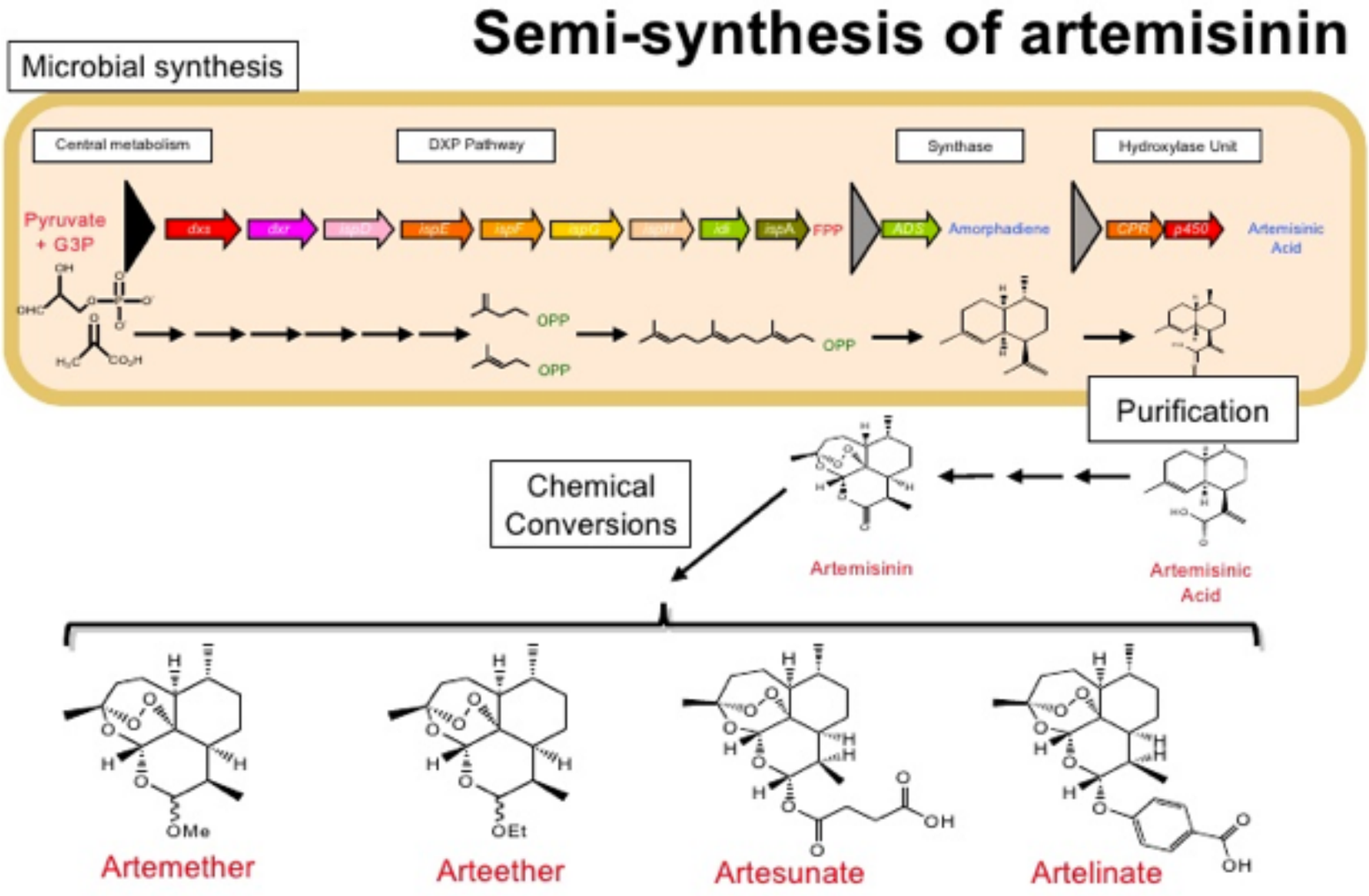


Allostery ex: phosphoenolpyruvate inhibition of phosphofructokinase, PMID 2952886

Product inhibition ex: hexokinase, PMID 5460798

METABOLIC PATHWAY SCHEMA

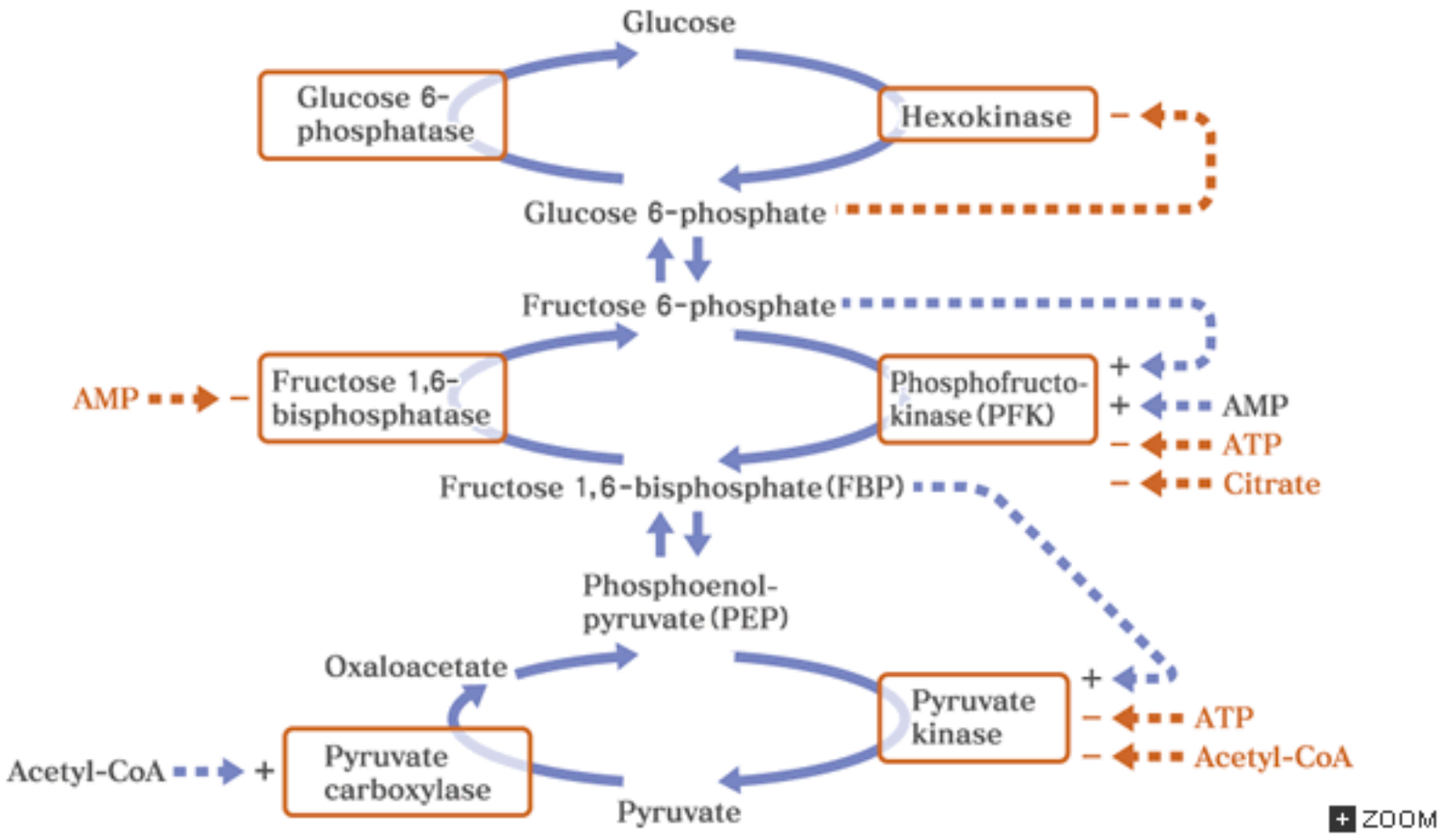
Organisation of metabolic pathway.



MALARIA FACTS:
mosquito carrying parasites
247mil cases in 2008
treatement with artemisinin
$2.25 cost/dose x 10 doses
expensive ($4/person/year)
700 tons needed

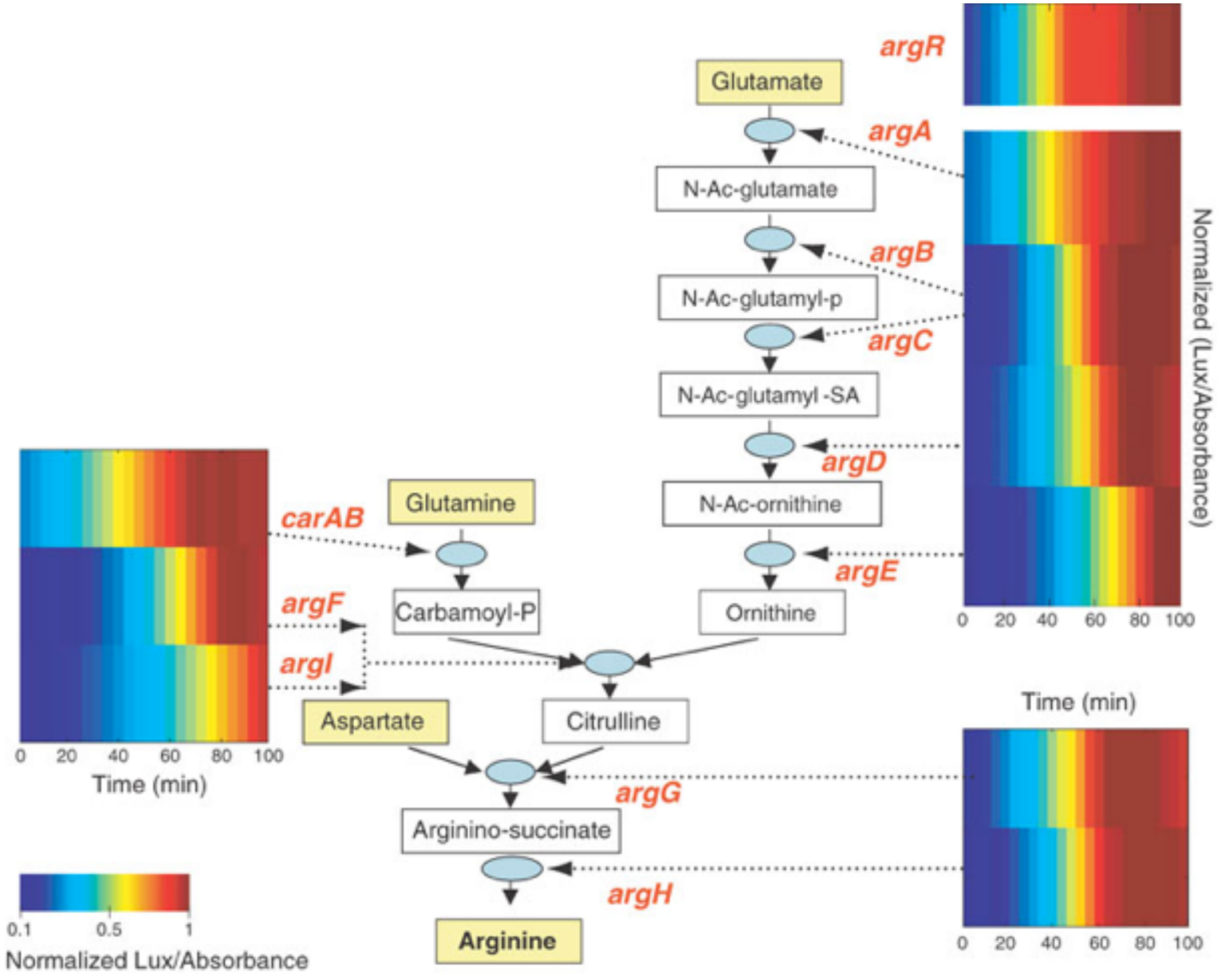
ARTEMISINIC ACID IN YEAST

Artemisinin is the active compound in an efficient anti-maleria drug.



GLYCOLYSIS

Glycolysis is common to nearly all organisms and is the basic pathway for generating ATP from glucose.



ARGENINE METABOLISM

Amino acid argenine is metabolised from other amino acids through the above pictured metabolic pathways. Production of biosynthetic enzymes is synchronised in FIFO order.

MASS ACTION KINETICS

$$n_1 X_{r1} + n_2 X_{r2} \xrightarrow{k_1} X_p$$

BINDING REACTIONS

Mass action kinetics is also called the first order approximation of chemical reactions.

MICHAELIS-MENTEN KINETICS

$$E + S \underset{k_3}{\overset{k_1}{\rightleftharpoons}} ES \overset{k_2}{\rightleftharpoons} E + P$$

ENZYMATIC REACTIONS

Enzymatic reactions include many intermediate steps. Modelling them is inefficient. Time scale separation is used to reduce to one step with Michaelis-Menten kinetics.

STEADY STATE FORMULATION

$$\frac{dX}{dt} = Sr\left(X\right)$$

$$\text{at equilibrium}$$

$$0 = Sr\left(x\right)$$

STEADY STATE ANALYSIS OF FLUXES

Enzymatic reactions involve many parameters and regulation. Stoichiometry is better known (still not always exact!!)

FLUX BALANCE ANALYSIS

$$\max_r \left(c^T r\right)$$

$$\text{subject to}$$

$$0 = Sr\left(x\right)$$

$$r \leq r^+$$

$$r \geq r^-$$

OPTIMIZATION OF FLUXES

It is conjectured that the real fluxes maximise some utility. This utility is unknown but we can make some good guesses.

Flux variability analysis

$$r_i^+ = \max_r (r_i)$$

subject to

$$c^T r = c^T r^*$$
$$0 = Sr\,(x)$$
$$r \leq r^+$$
$$r \geq r^-$$

$$r_i^- = \min_r (r_i)$$

subject to

$$c^T r = c^T r^*$$
$$0 = Sr\,(x)$$
$$r \leq r^+$$
$$r \geq r^-$$

ROBUSTNESS OF FLUXES

Usually, the optimal solution r^* is not unique. One can ask how much a given flux is allowed to vary without violating the metabolic optimum.

Flux variability analysis

$$r_i^+ = \max_r (r_i)$$

subject to

$$c^T r = c^T r^*$$
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