LECTURE 10: METABOLISM AND FLUX BALANCE ANALASIS

Introduction to Cellular System Modelling

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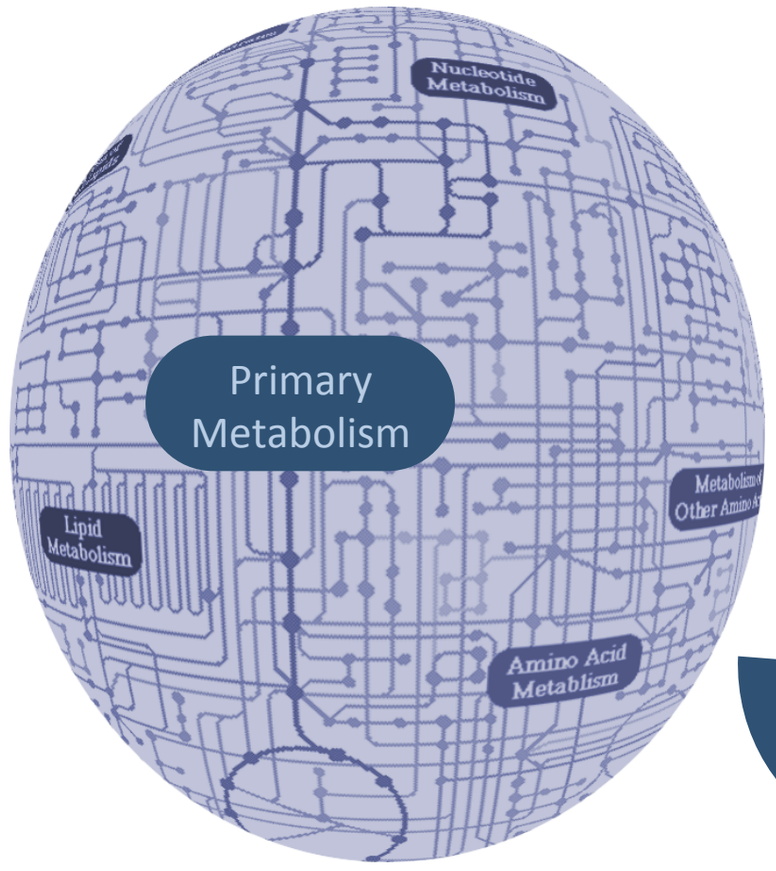
OSNOVA

- 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

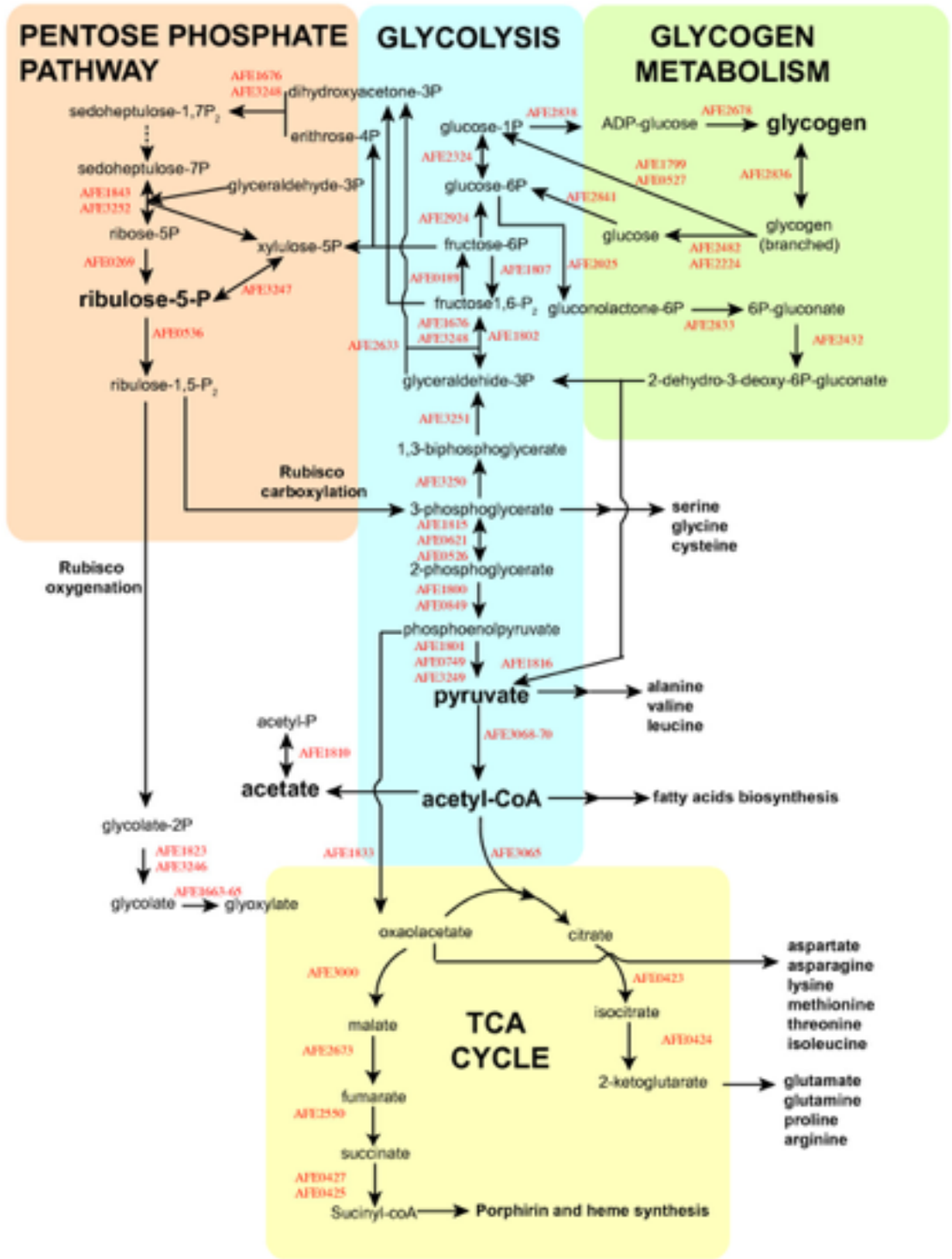


Anabolism

Natural Product Biosynthesis
Biofuels

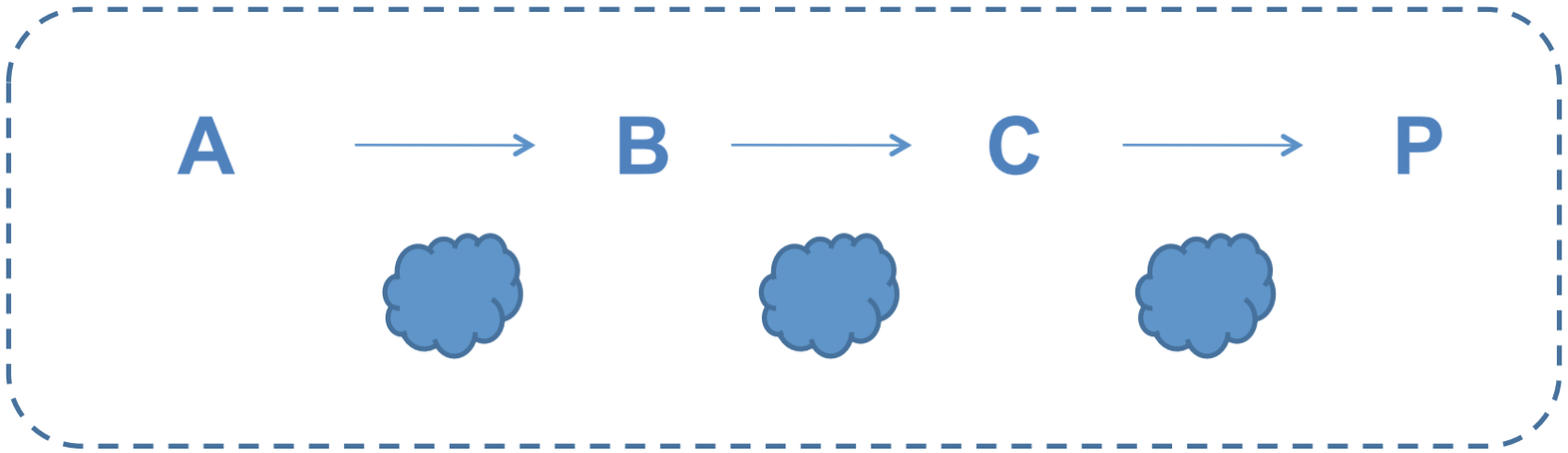
ANABOLISM/CATABOLISM

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



CENTRAL METABOLISM

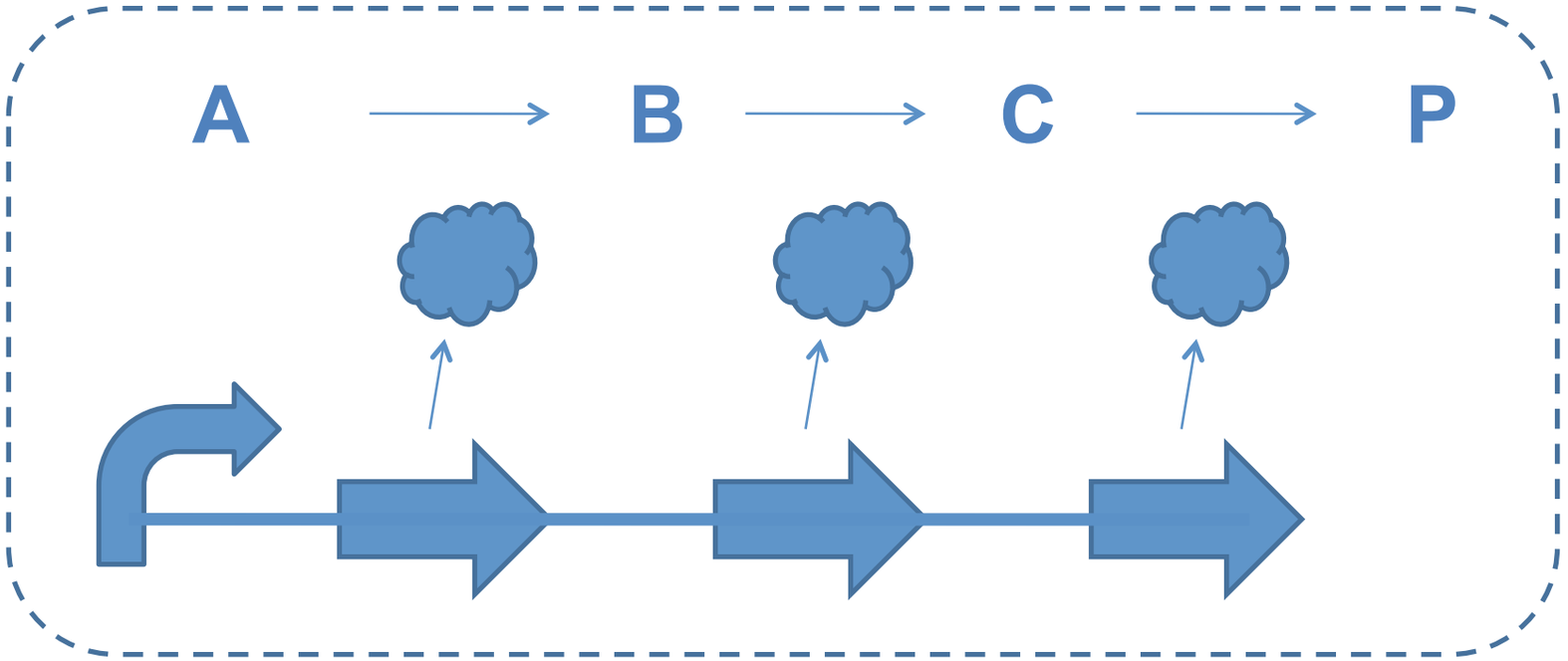
The cells central metabolism is associated with carbohydrate breakdown and ATP production. Intermediate metabolites also serve as precursors for all other molecules.



A liposomal system

METABOLIC PATHWAY SCHEMA

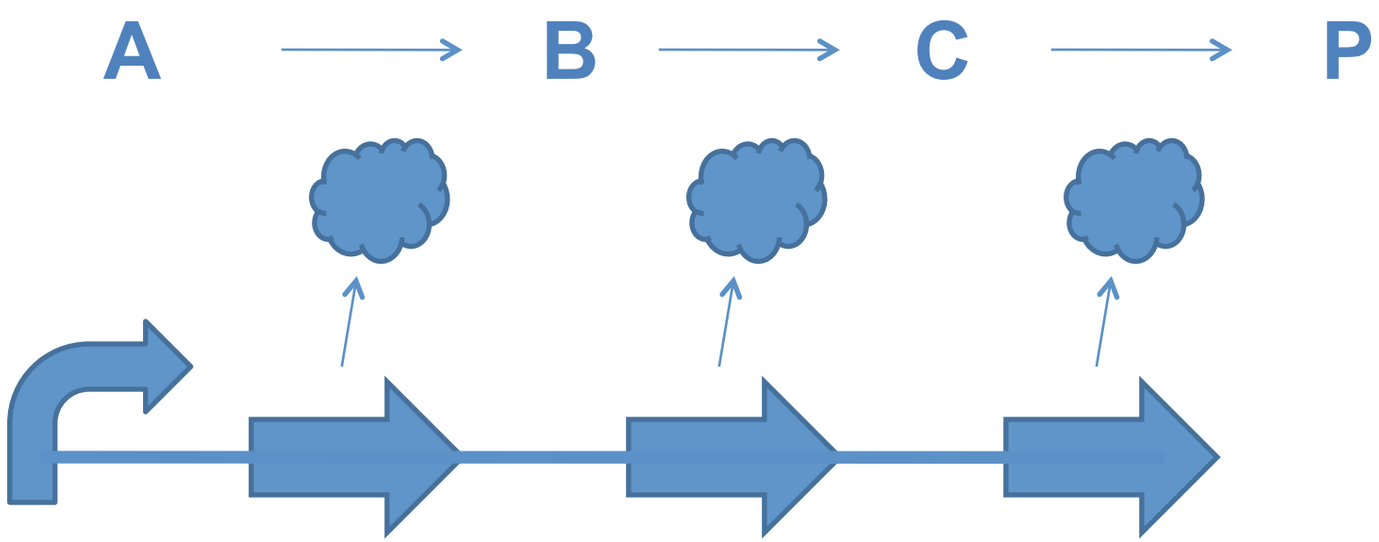
Organisation of metabolic pathway.



A cellular system

METABOLIC PATHWAY SCHEMA

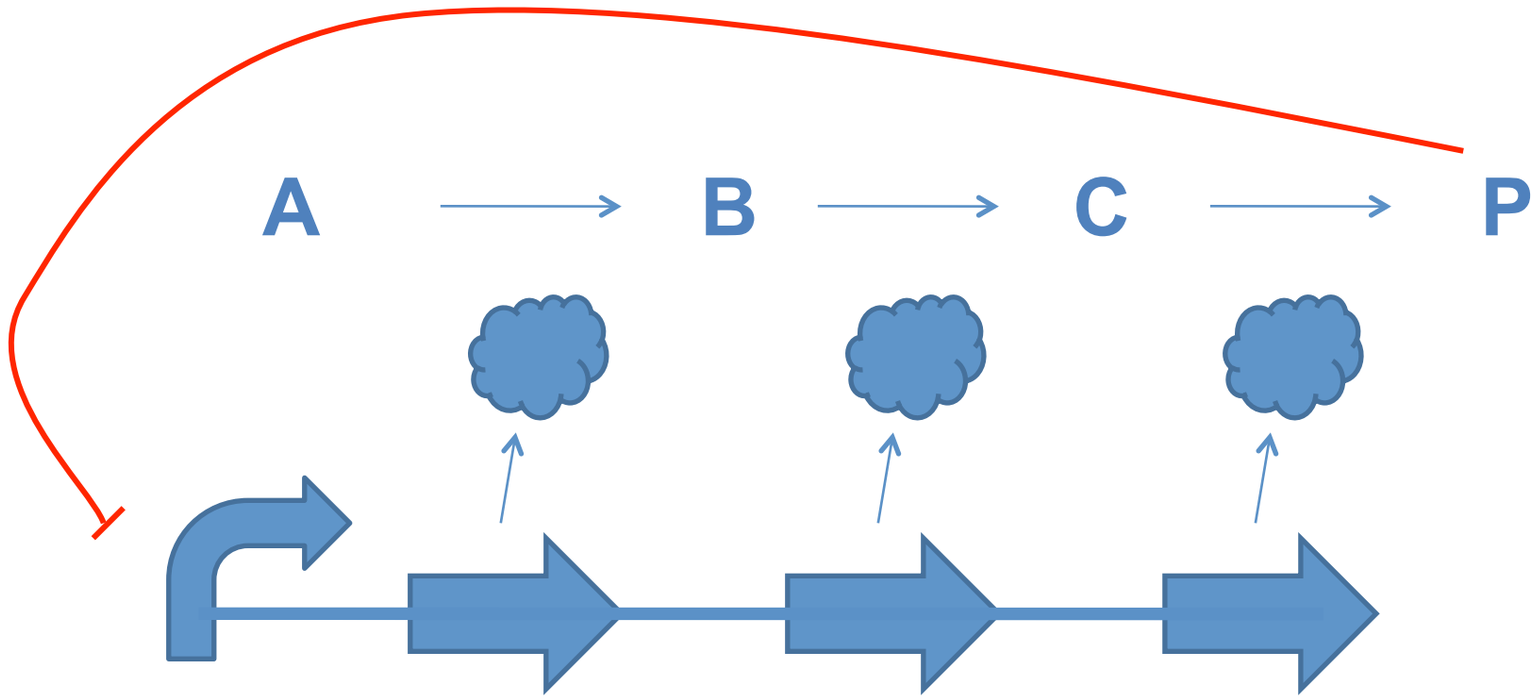
Organisation of metabolic pathway.



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

METABOLIC PATHWAY SCHEMA

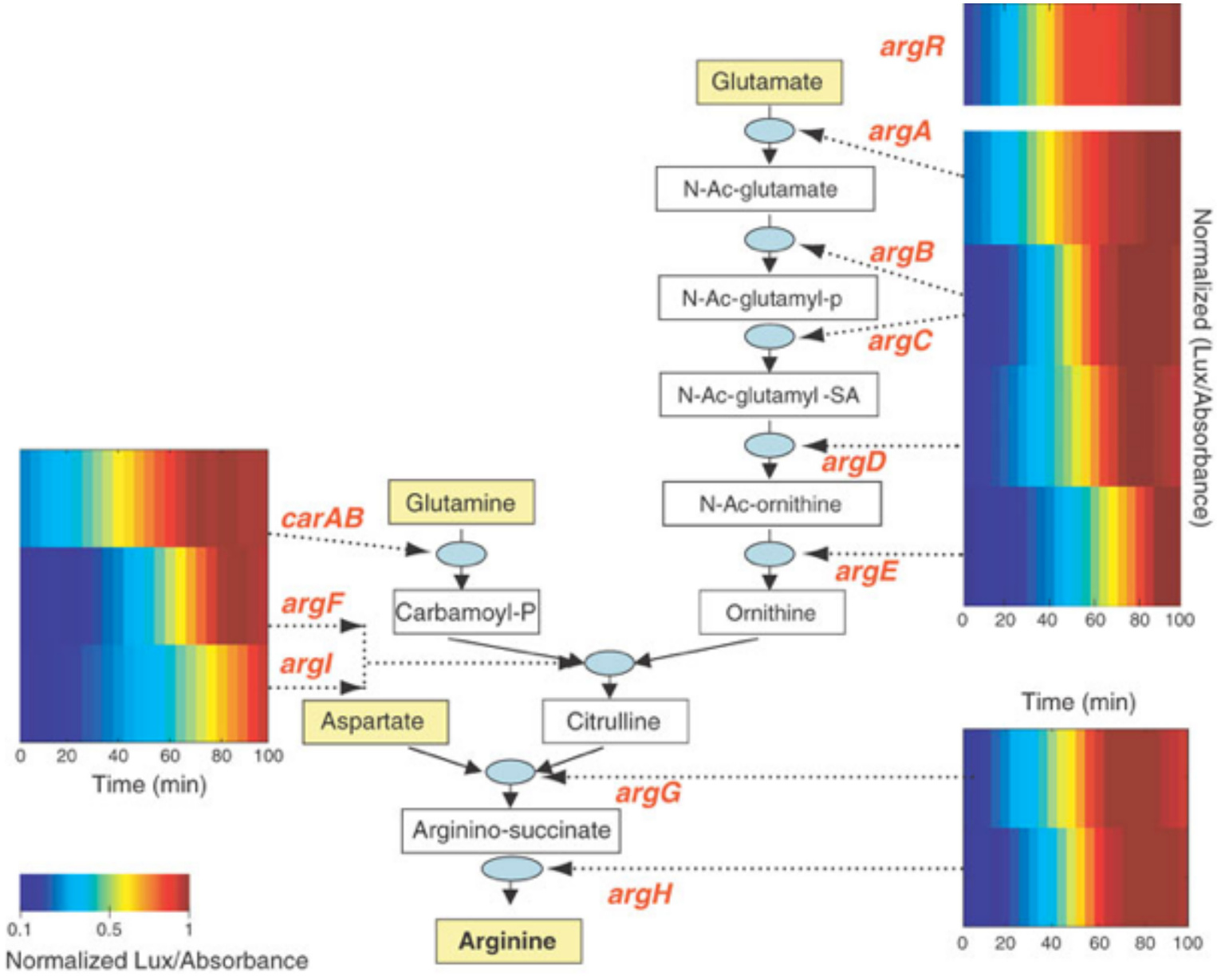
Organisation of metabolic pathway.



Negative Feedback

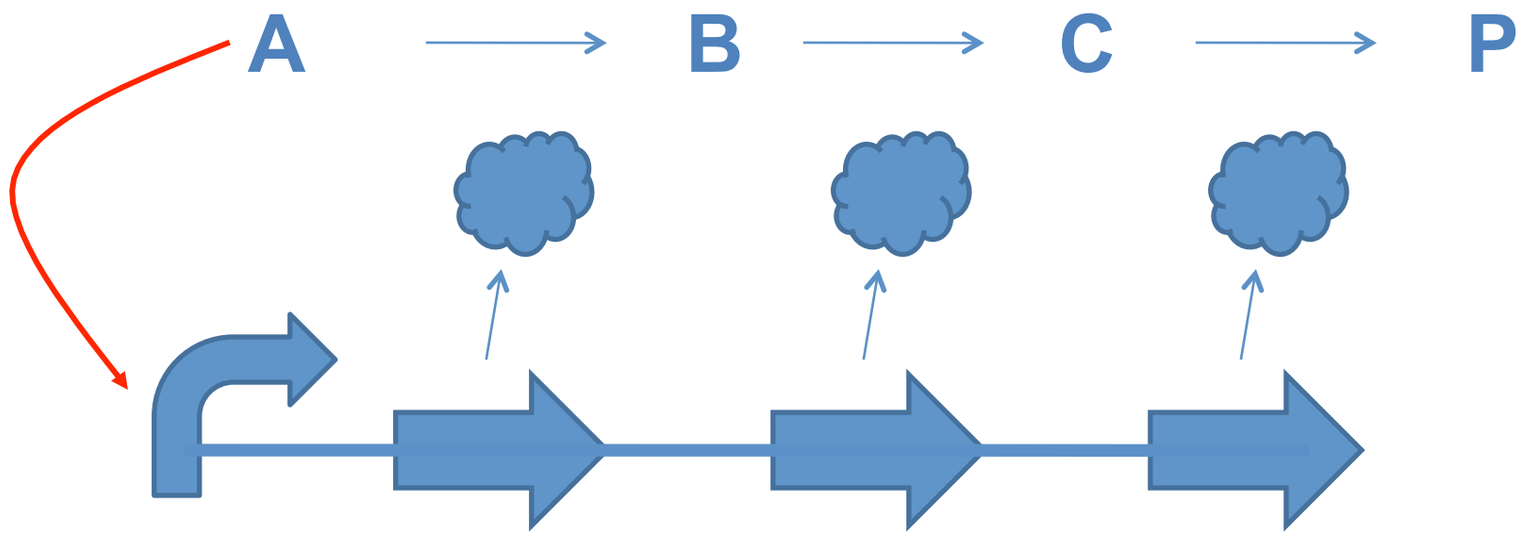
METABOLIC PATHWAY SCHEMA

Organisation of metabolic pathway.



ARGENINE METABOLISM

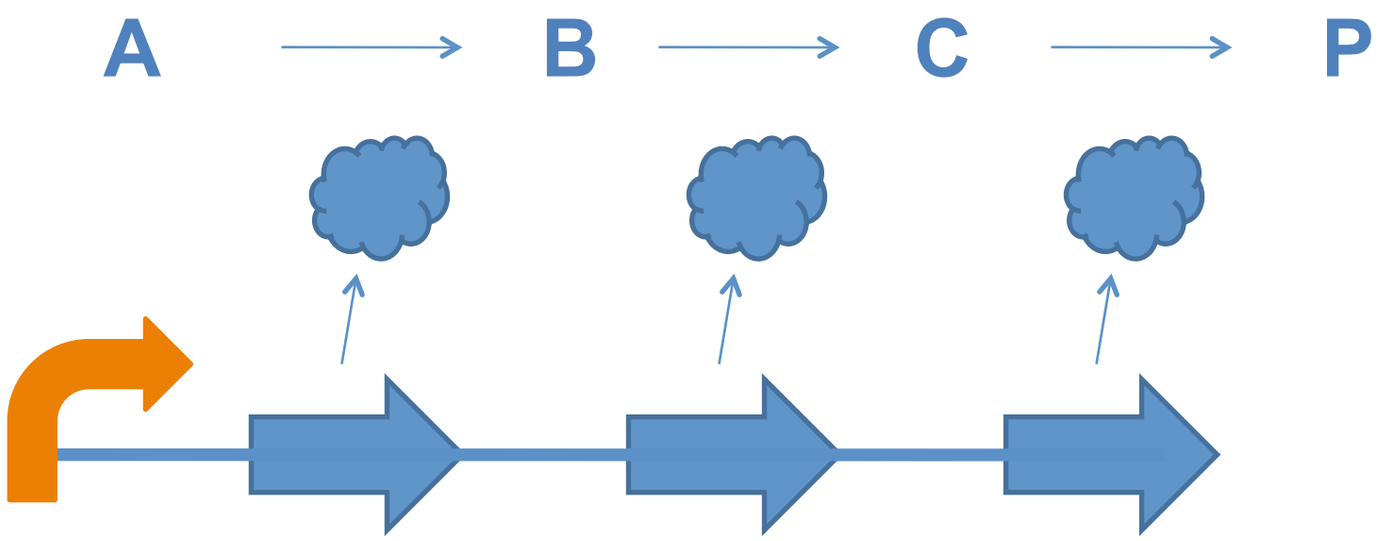
Amino acid argenine is metabolised from other amino acids through the above pictured metabolic pathways. Production of biosynthetic enzymes is synchronised in FIFO order.



Substrate Activation
Ex: arabinose catabolism

METABOLIC PATHWAY SCHEMA

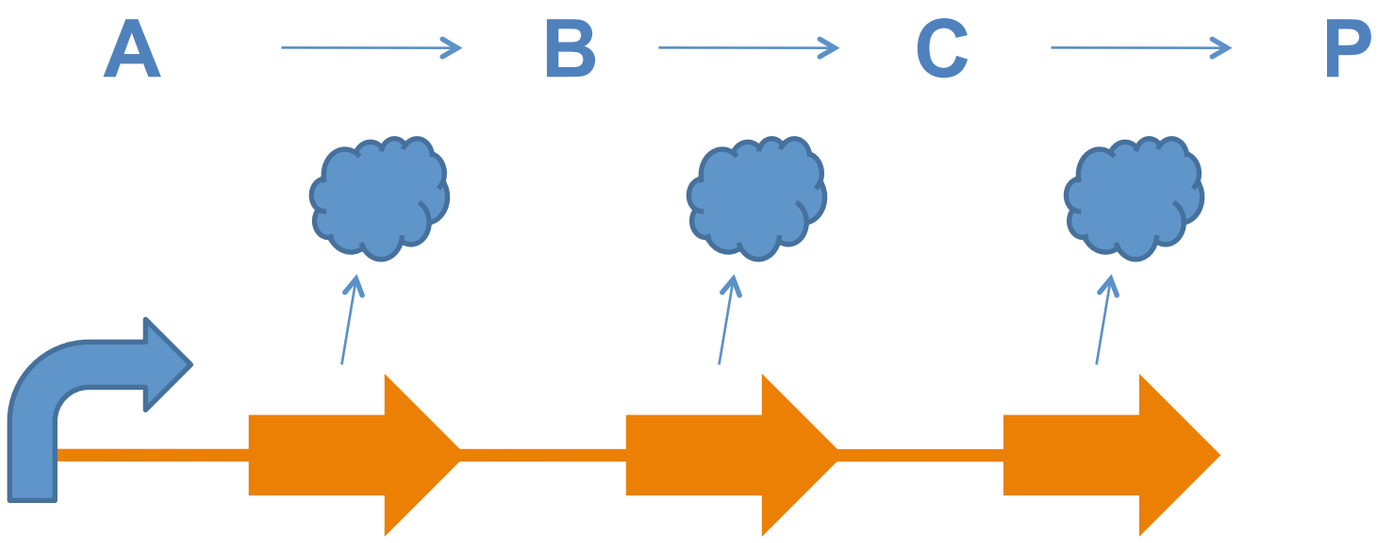
Organisation of metabolic pathway.



Changing promoters eliminates transcriptional control

METABOLIC PATHWAY SCHEMA

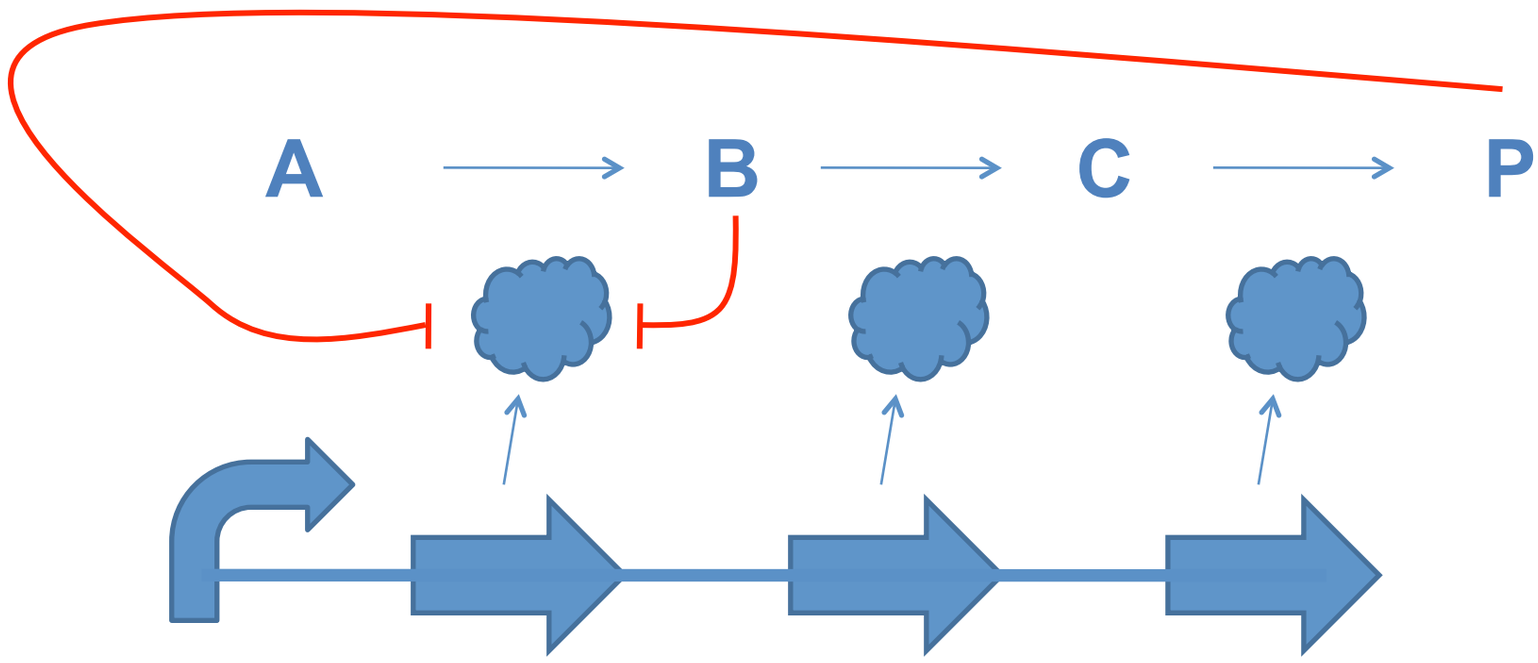
Organisation of metabolic pathway.



Shuffled codon usage and changing 5’ UTRs eliminates translational control

METABOLIC PATHWAY SCHEMA

Organisation of metabolic pathway.

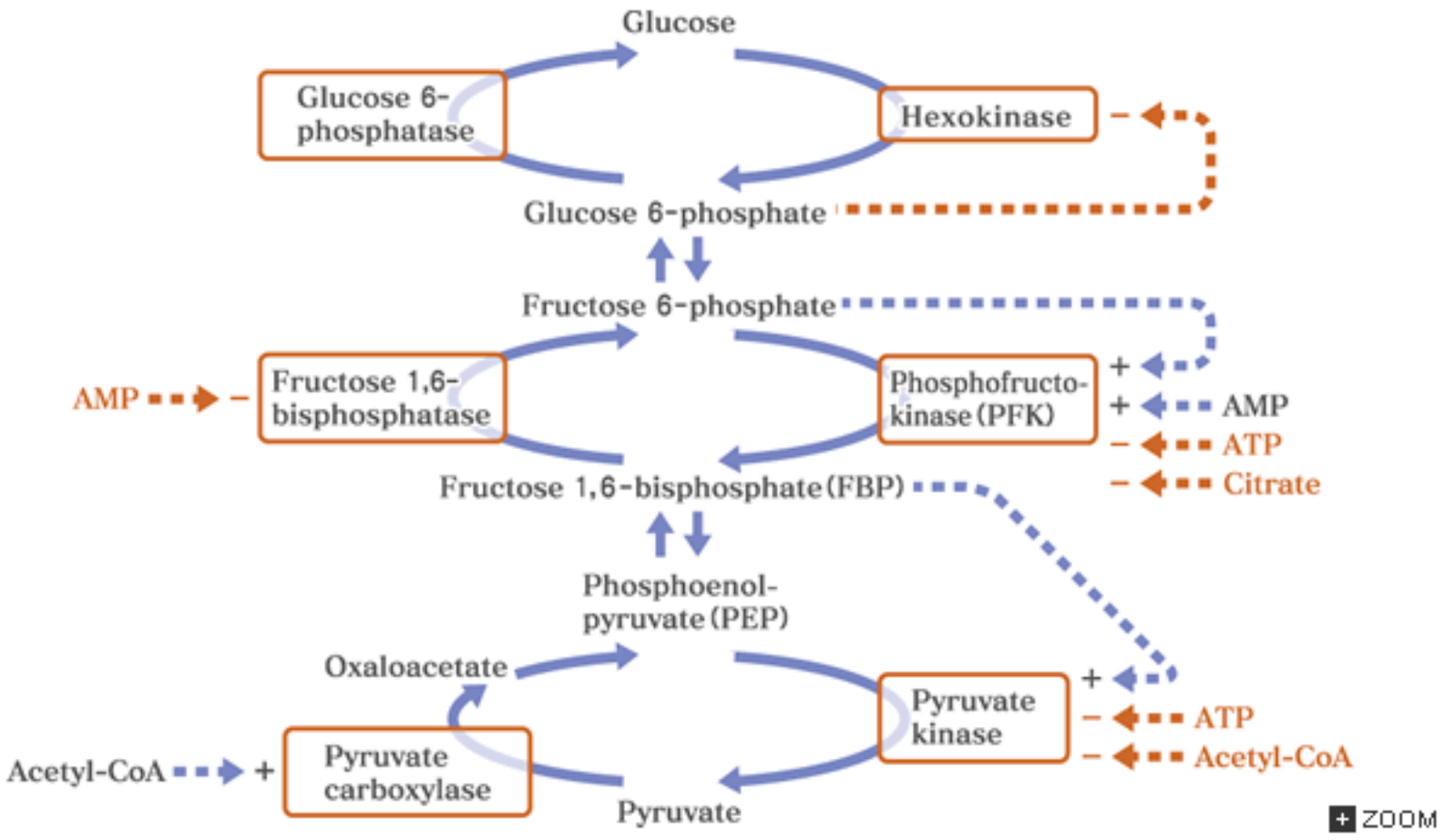


Allostery ex: phosphoenolpyruvate inhibition of phosphofructokinase, PMID 2952886

Product inhibition ex: hexokinase, PMID 5460798

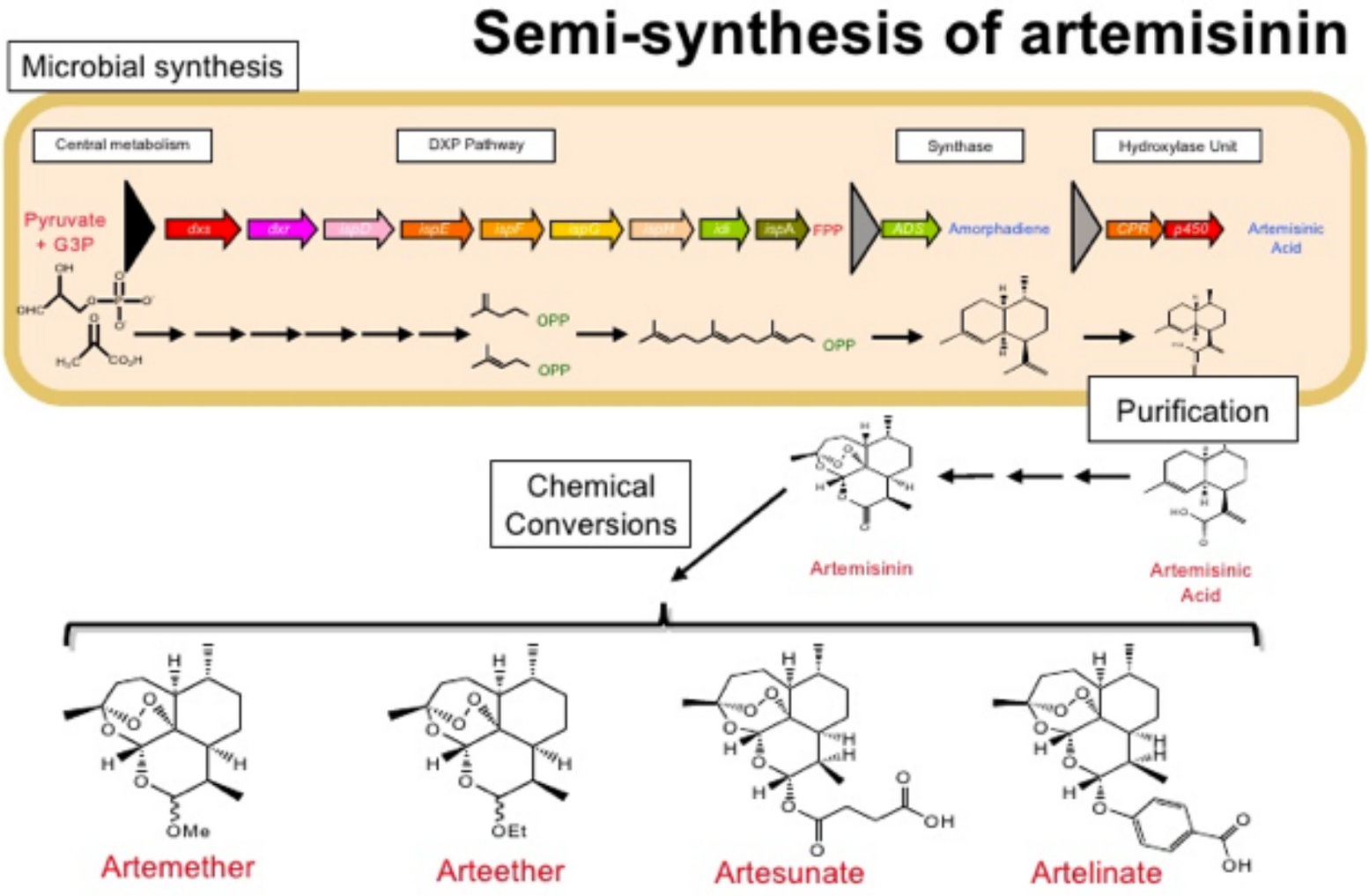
METABOLIC PATHWAY SCHEMA

Organisation of metabolic pathway.



GLYCOLYSIS

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



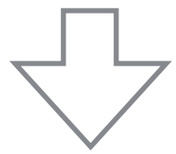
MALARIA FACTS:
mosquito carrying parasites
247mil cases in 2008
treatement with artemisinin
$2.25 cost/dose x 10 doses
expensive ($4/person/year)
700 tons needed

ARTEMISINIC ACID IN YEAST

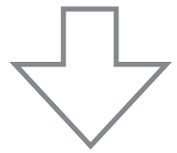
Artemisinin is the active compound in an efficient anti-maleria drug.

MICHAELIS-MENTEN KINETICS

$$E + X_1 + X_2 \underset{k_1^-}{\overset{k_1^+}{\rightleftharpoons}} C \xrightarrow{k_p} E + X_3 + X_4$$



$$X_1 + X_2 \xrightarrow{f(X, E_T)} X_3 + X_4$$



$$X_1 X_2 f\left(X, E_T\right) = \frac{k_P E_T X_1 X_2}{K_M + X_1 X_2} + \text{Regulation}\left(X\right)$$

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, which are still drastically overparametrized in real world networks.

MICHAELIS-MENTEN KINETICS

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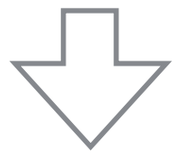
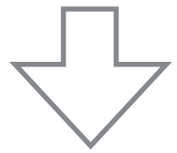


$$X_1 + X_2 \xrightarrow{f(X, E_T)} X_3 + X_4$$



$$r_1(X) = X_1 X_2 f(X, E_T) = \frac{k_P E_T X_1 X_2}{K_M + X_1 X_2} + \text{Regulation}(X)$$

ENZYMATIC REACTIONS

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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

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

$$\text{subject to}$$

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

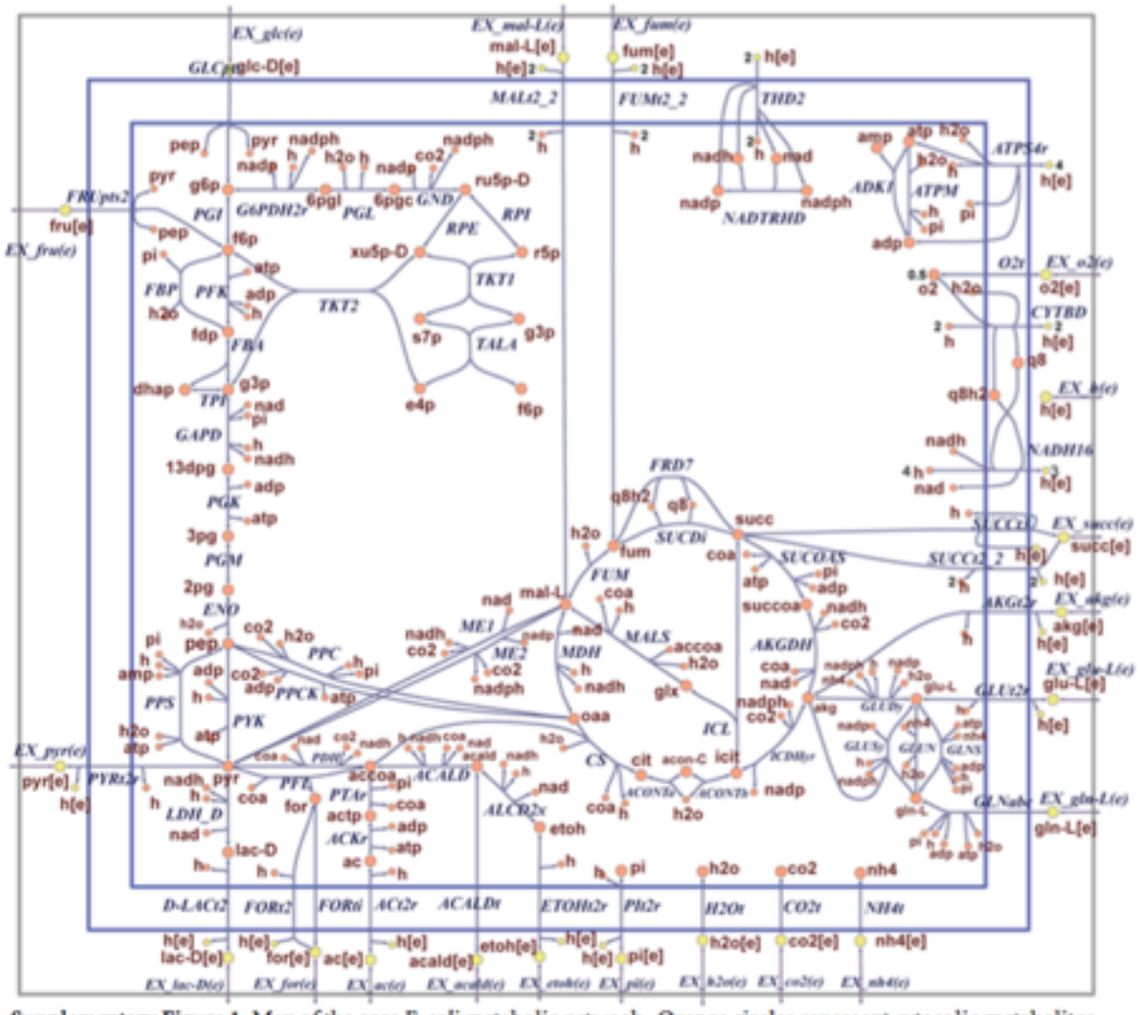
$$r \leq r^+$$

$$r \geq r^-$$

OPTIMIZATION OF FLUXES

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

METABOLIC NETWORK

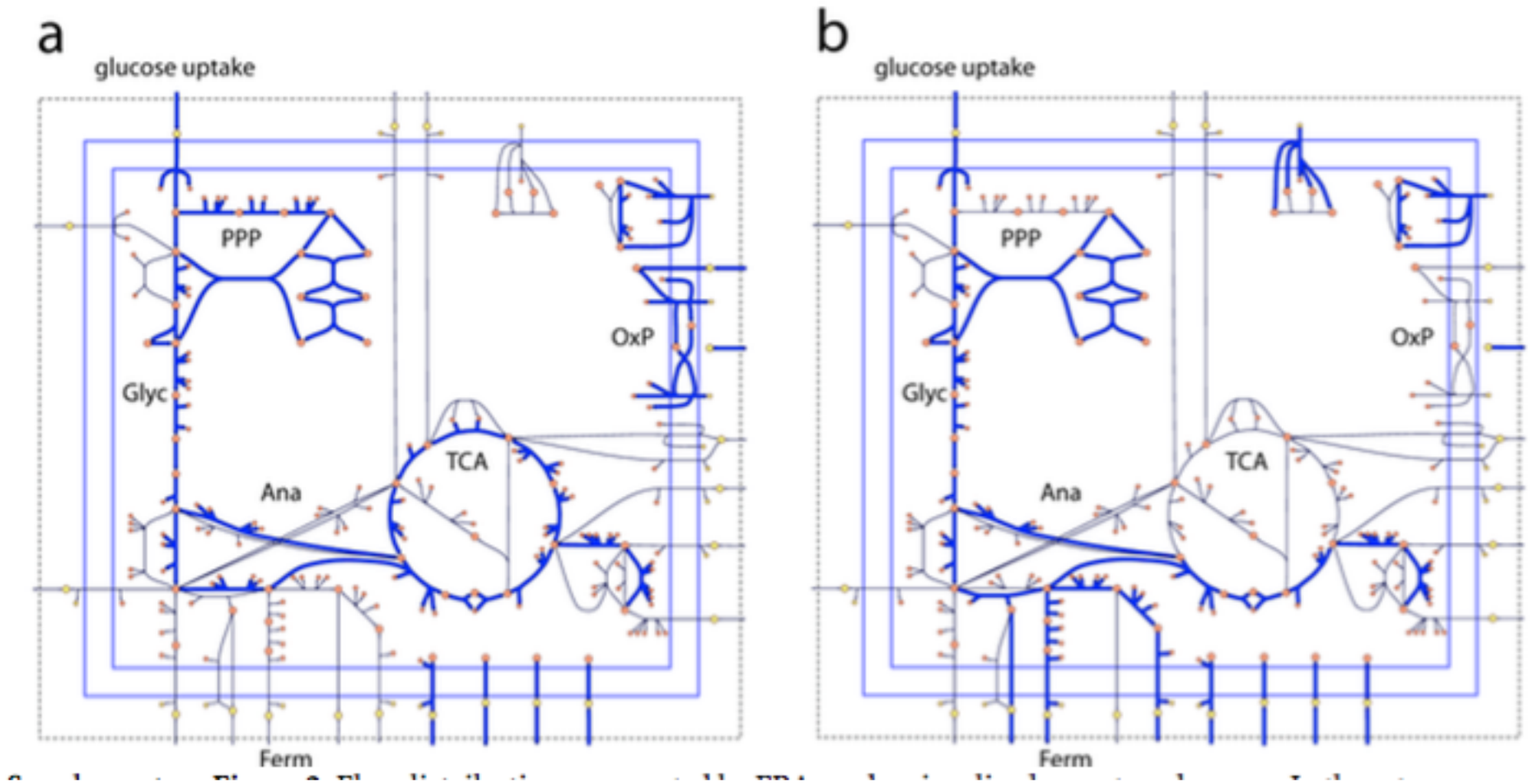


Supplementary Figure 1 Map of the core *E. coli* metabolic network. Orange circles represent cytosolic metabolites, yellow circles represent extracellular metabolites, and the blue arrows represent reactions. Reaction name abbreviations are uppercase and metabolite name abbreviations are lowercase.

CORE E COLI METABOLIC NETWORK

E coli central metabolism includes 72 different metabolites, 95 reactions, and 137 enzyme coding genes.

FBA ANALYSIS



AEROBIC AND ANAEROBIC CONDITIONS

FBA predictions for growth with and without oxygen. Blue lines indicate the nonzero fluxes.

GROWTH ON DIFFERENT MEDIA

	Growth Rate (hr^{-1})	
Substrate	Aerobic	Anaerobic
acetate	0.3893	0
acetaldehyde	0.6073	0
2-oxoglutarate	1.0982	0
ethanol	0.6996	0
D-fructose	1.7906	0.5163
fumarate	0.7865	0
D-glucose	1.7906	0.5163
L-glutamine	1.1636	0
L-glutamate	1.2425	0
D-lactate	0.7403	0
L-malate	0.7865	0
pyruvate	0.6221	0.0655
succinate	0.8401	0

GROWTH RATES W/ & W/O O2

Prediction of growth on different media in the presence and absence of oxygen.

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

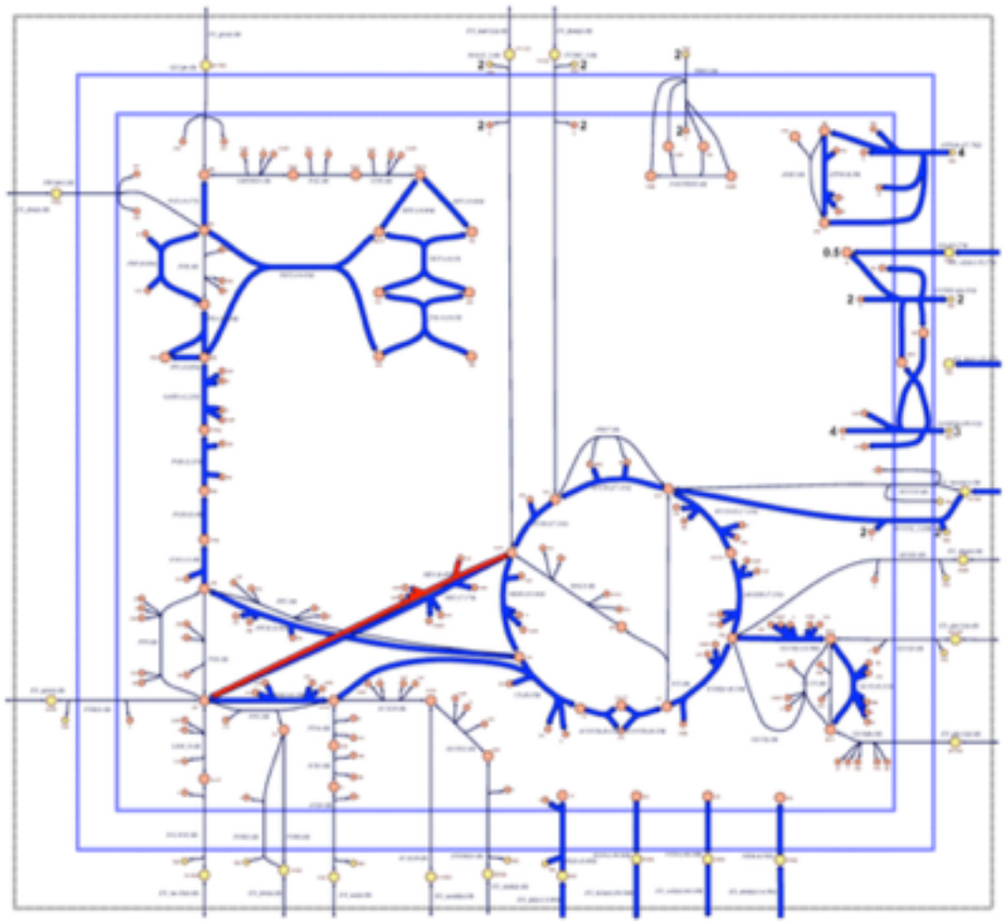
$$c^T r = c^T r^*$$
$$0 = Sr\,(x)$$
$$r \leq r^+$$
$$r \geq r^-$$

ROBUSTNESS OF FLUXES

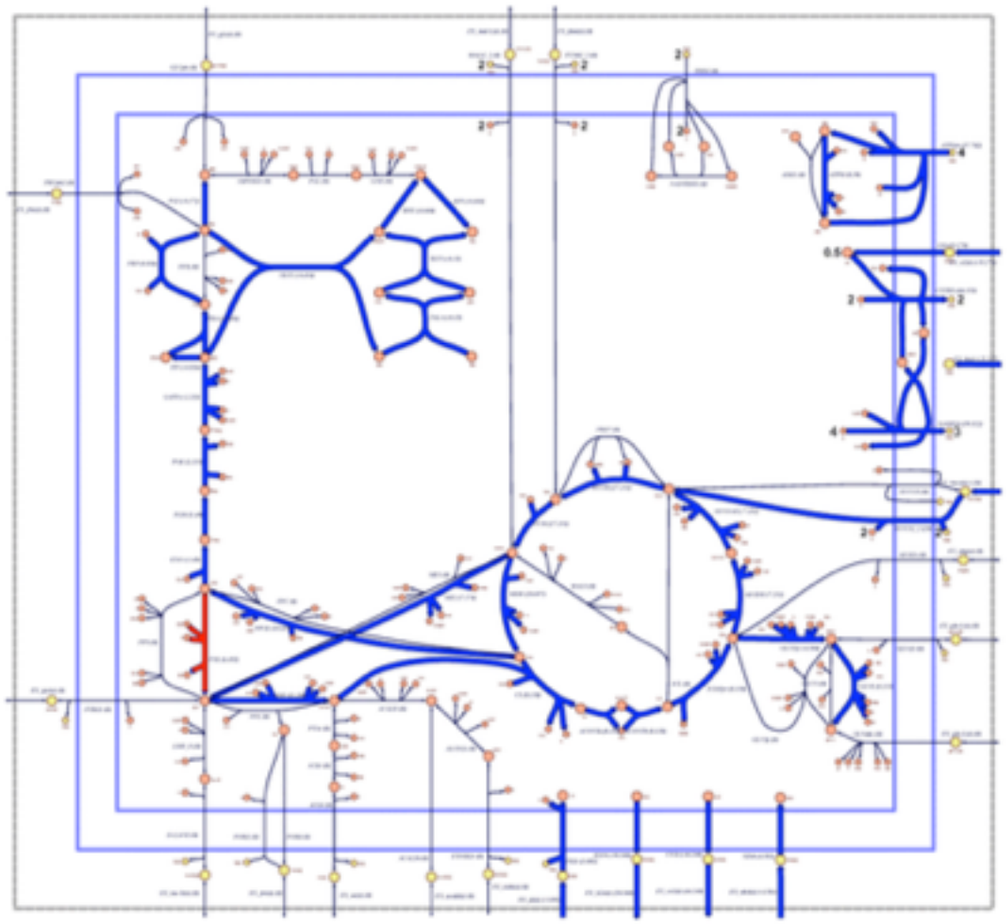
Usually, the optimal solution r^* is not unique. One can ask how much a given flux is allowed to vary without violating the metabolic optimum.

VARIABILITY EXAMPLES

a



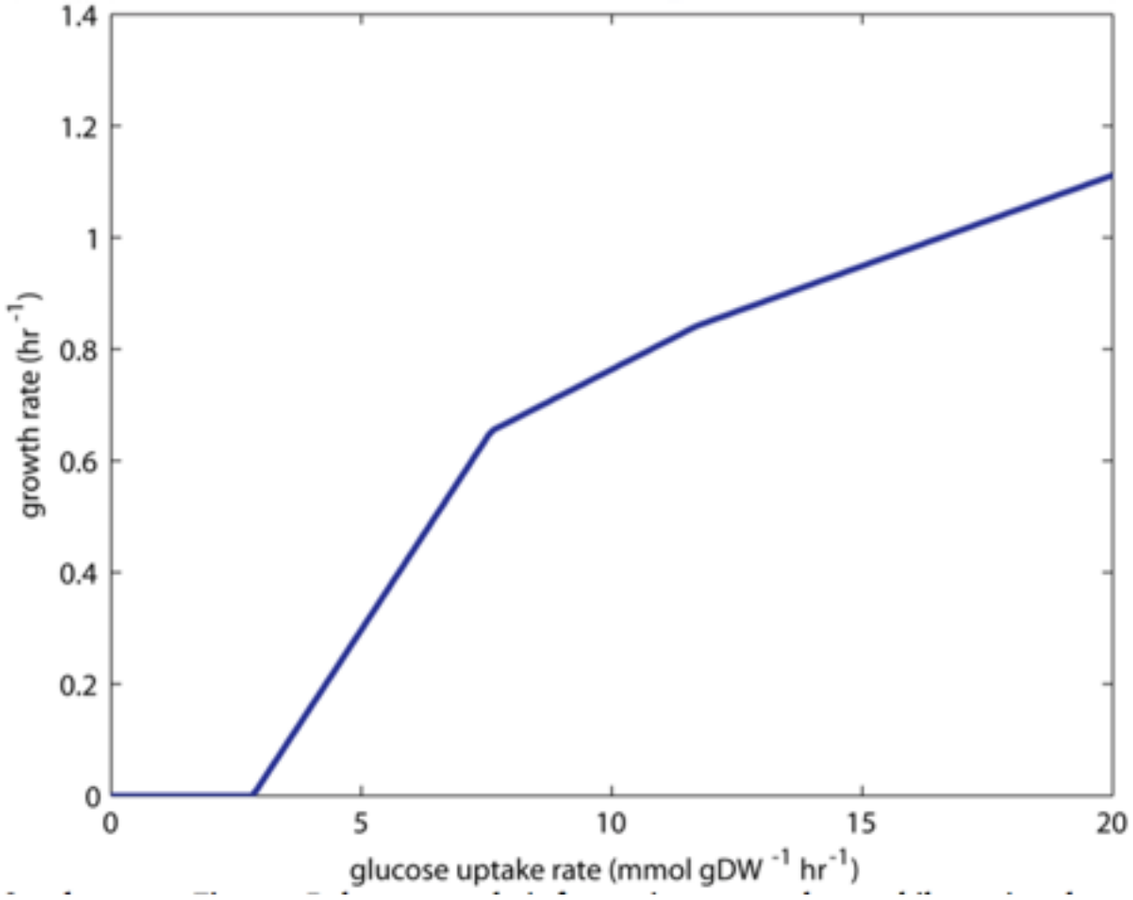
b



GROWTH ON SUCCINATE

ME1 is used to convert L-malate to pyruvate. Alternatively pyruvate kinase reaction is used.

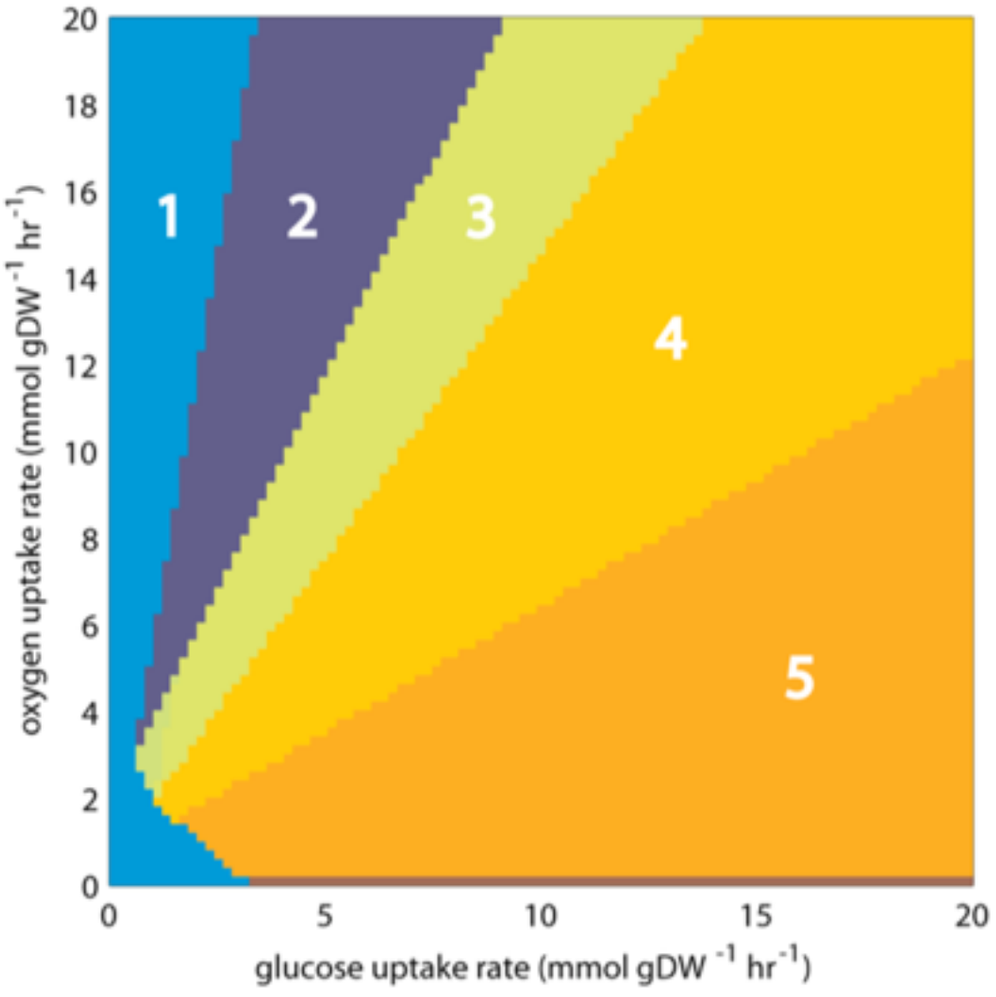
GROWTH RATE DEPENDENCE



GROWTH ON GLUCOSE

The dependence of growth on glucose concentration manifests in three phases. In Phase 1 growth is zero as basic energy needs are not met. In Phase 2 growth increases linearly. In Phase three growth increases at a lower rate due to oxygen limitation.

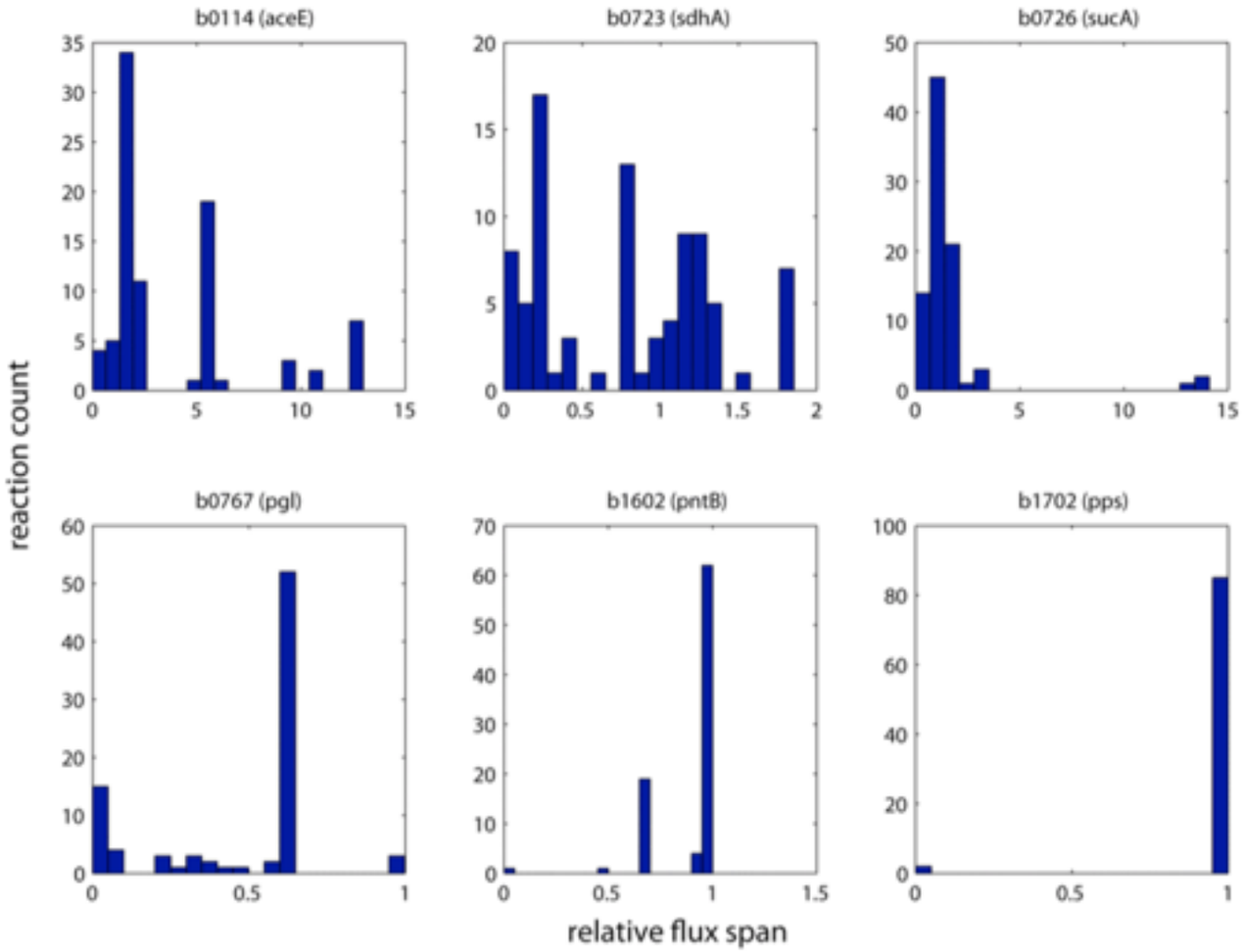
PHASE PLANE ANALYSIS



GROWTH ON GLUCOSE & O2

The dependence of growth on glucose concentration and O2 exhibits the mutual balance necessary to achieve maximal growth in different conditions.

GENE KNOCKOUT ANALYSIS



RELATIVE FLUX OF KNOCKOUTS

Most gene knockouts decrease optimal flux. Some gene knockouts, however, increase flux through certain pathways. Such knockouts are useful in metabolic engineering to increase yields.