Cell metabolism

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Glossary

Metabolite:
a small (<1 kDa) molecule, usually organic

Reaction:
a conversion of molecules by breaking and making chemical bonds

Catalyst:
a molecule that speeds up the reactions, but is not consumed itself. In biochemical systems, catalysts are either metal ions and/or proteins - enzymes

Cofactor:
a [small] molecule, essential to the catalytic activity of the enzyme

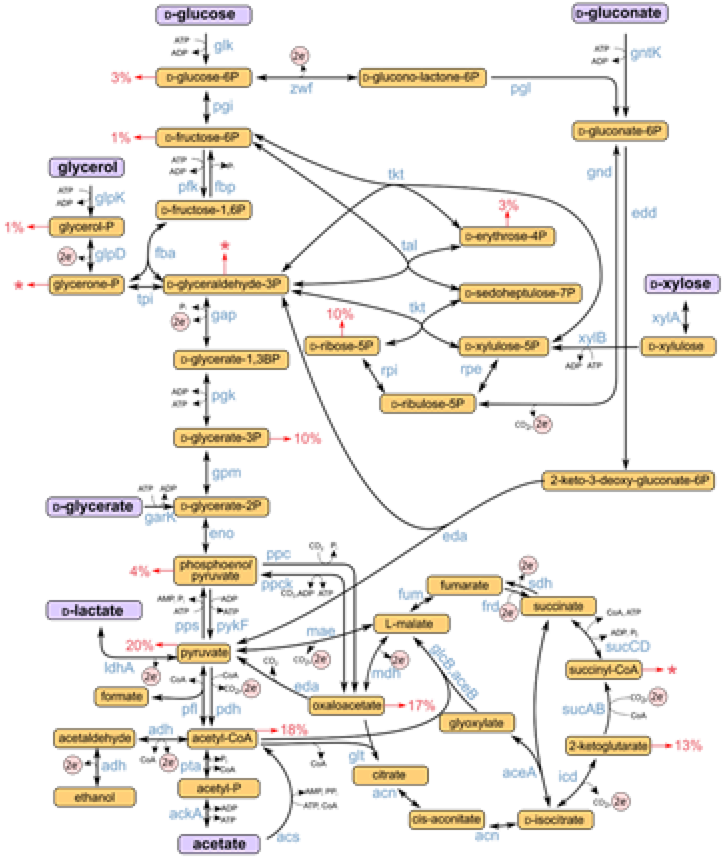
Central Carbon Metabolism as a puzzle

Metabolites

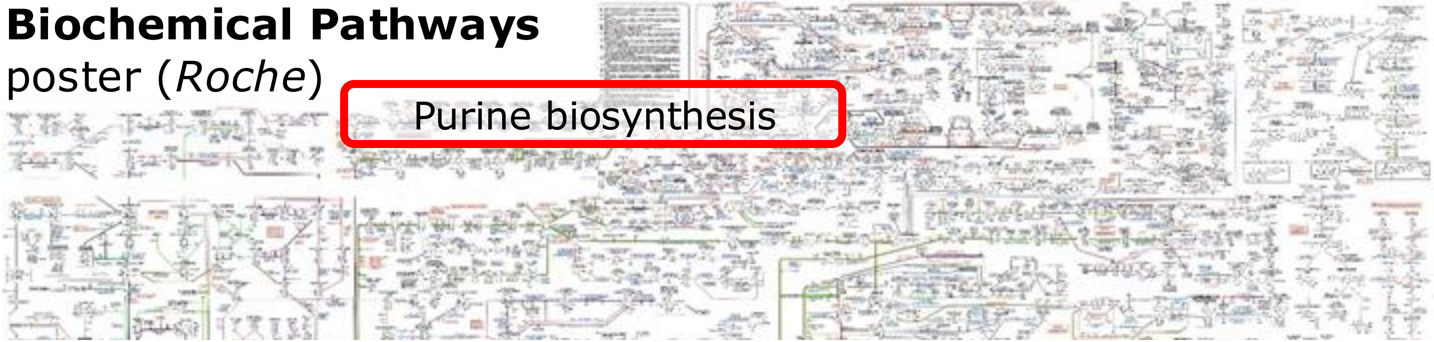
Enzymes

Co-factors

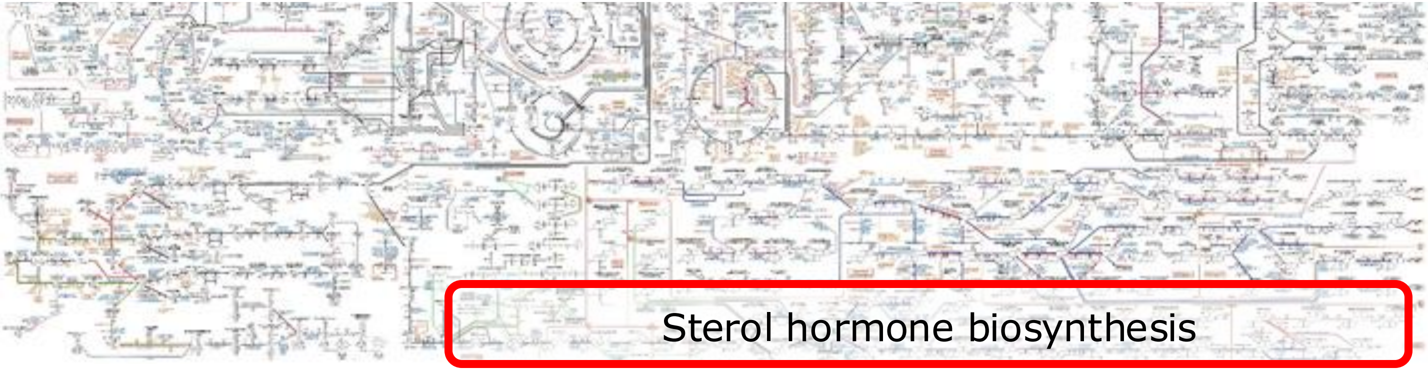
Drain for new cells (biomass)



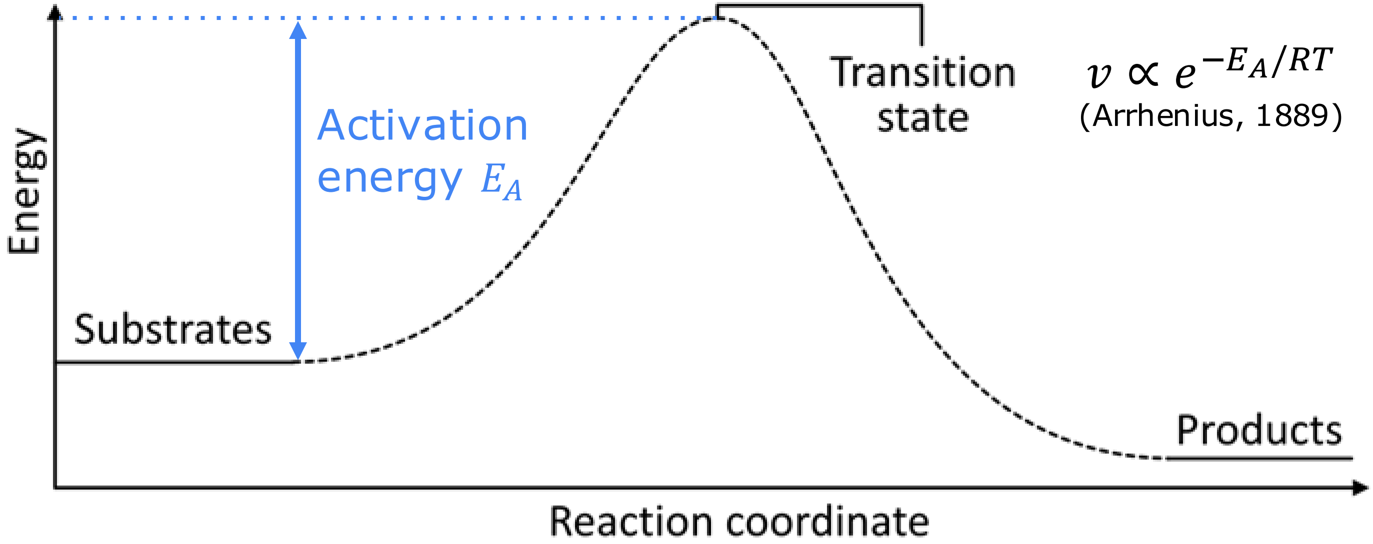
Biochemical Pathways
poster (*Roche*)



A metabolic **network** is a patchwork of metabolic **pathways**



How to go from substrate to a product?



$$v \propto e^{-E_A/RT}$$

(Arrhenius, 1889)

Mass-action kinetics

A generic, reversible chemical reaction:

$$n_A A + n_B B \leftrightarrow n_C C + n_D D$$

'reactants' 'products'

Forward reaction **rate**:

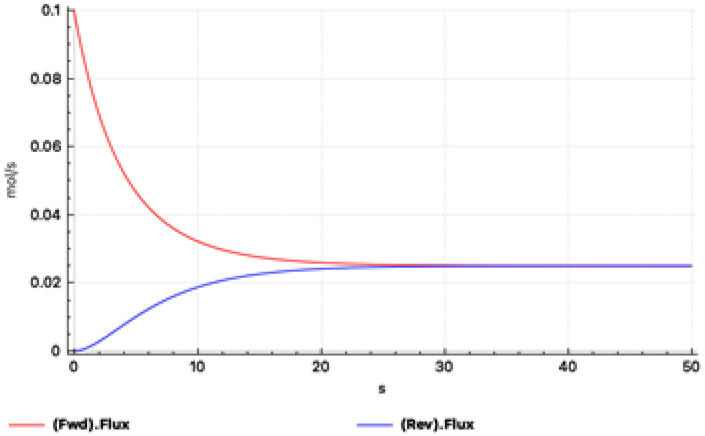
$$v_+ = k_+[A]^{n_A}[B]^{n_B}$$

Backward reaction **rate**:

$$v_- = k_-[C]^{n_C}[D]^{n_D}$$

The rate of a chemical reaction is **proportional** to the probability of collision of the reactants, which is in turn proportional to the **concentration of reactants to the power of their stoichiometry**.

Law of mass action



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$$n_A A + n_B B \leftrightarrow n_C C + n_D D$$

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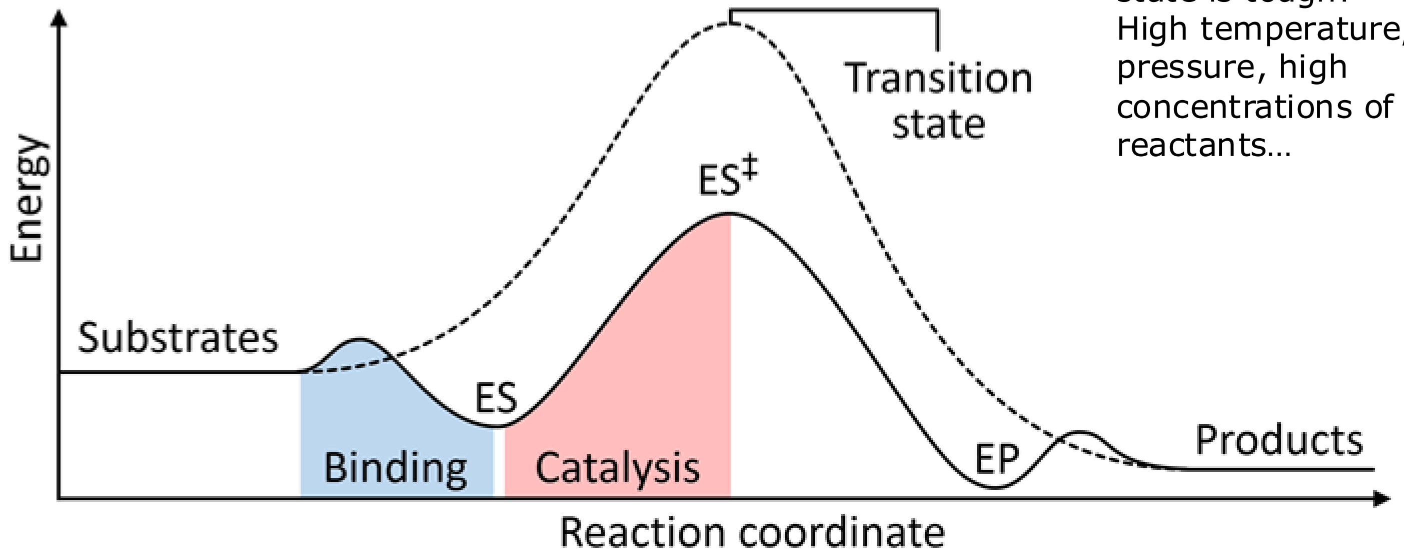
As t→∞, reaction reaches an equilibrium. How does it look like?

$$\frac{[C]_{eq}^{n_C}[D]_{eq}^{n_D}}{[A]_{eq}^{n_A}[B]_{eq}^{n_B}} = K_{eq}$$

Law of mass action

Empirically derived (aka law of Nature!)

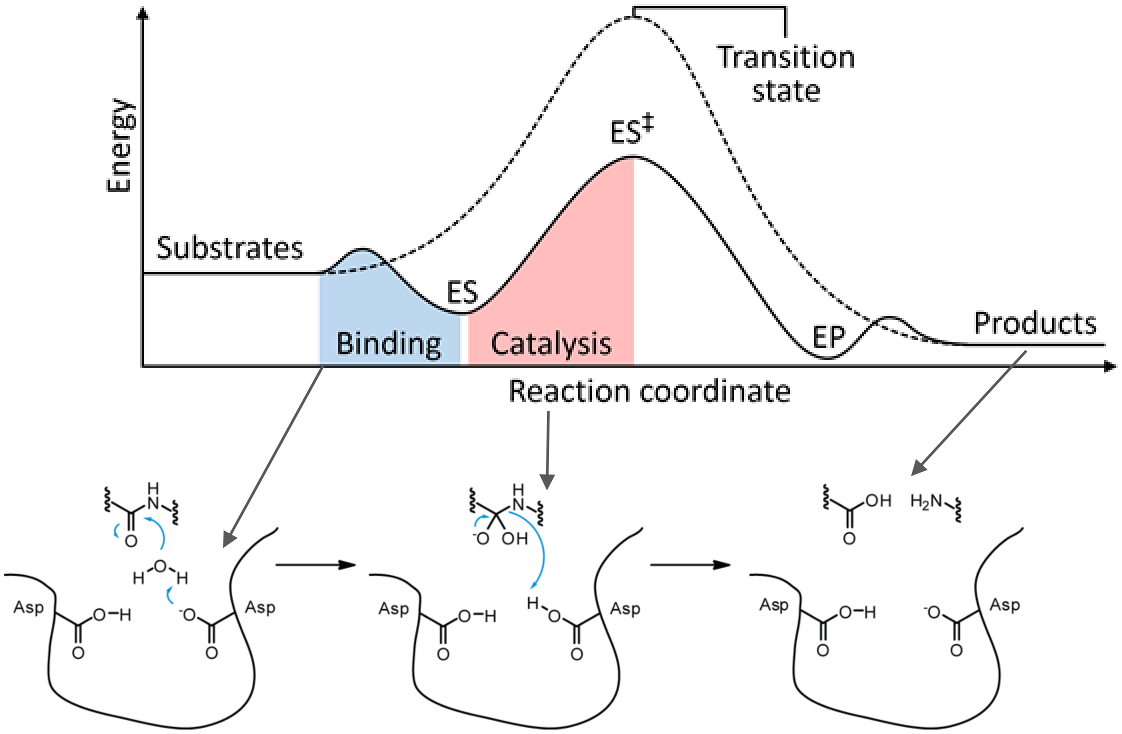
Why do we need catalysts?



Reaching the transition state is tough!
High temperature, pressure, high concentrations of reactants…

Lowering the activation energy by an alternative reaction mechanism!

Many biochemical reactions are catalyzed by enzymes



Note: Catalysts do not change the K_{eq}!

Substrate(s) and 'free' enzyme

Substrate(s) 'bound' on enzyme

Products(s) and free enzyme

$$E + S \rightleftharpoons ES$$

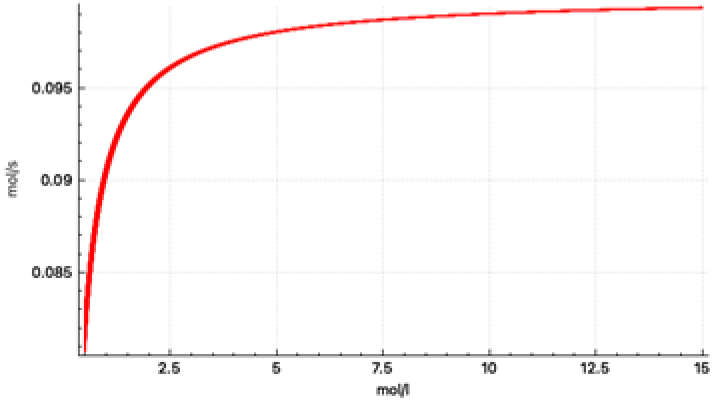
$$ES \rightleftharpoons E + P$$

Michaelis-Menten kinetic rate laws

Irreversible

$$E + S \underset{k_-}{\overset{k_+}{\rightleftharpoons}} ES \qquad ES \xrightarrow{k_{cat}} E + P$$

$$v = v_{max}\left(\frac{[S]}{[S] + K_M}\right)$$

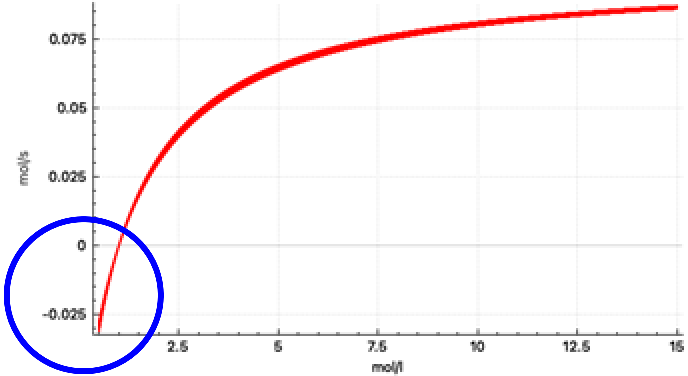


Reversible

Reaction quotient $\Gamma = \frac{P}{S}$

$$E + S \underset{k_2}{\overset{k_1}{\rightleftharpoons}} ES \qquad ES \underset{k_4}{\overset{k_3}{\rightleftharpoons}} EP \qquad EP \underset{k_6}{\overset{k_5}{\rightleftharpoons}} E + P$$

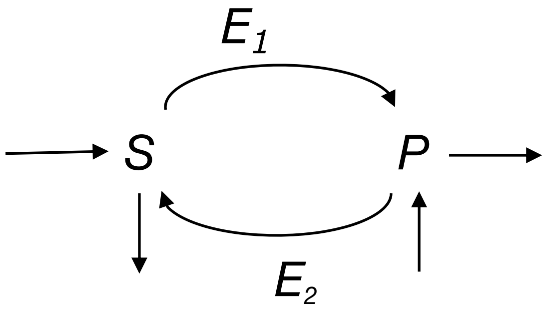
$$v = v_{max}\left(\frac{\frac{[S]}{K_S}}{1 + \frac{[S]}{K_S} + \frac{[P]}{K_P}}\right)\left(1 - \frac{\Gamma}{K_{eq}}\right)$$



note the flux is negative when P > S

Typical ranges of numbers in metabolic reactions

Fluxes: $10^{-1} - 10^{4}$ (mM · min)$^{-1}$
Substrate levels: $10^{-3} - 10$ mM



Enzyme levels: $10^{-5} - 10^{-1}$ mM

With $v_{max} = k_{cat}[E_{tot}]$, $v = \frac{k_{cat}[E_{tot}][S]}{[S] + K_M}$

Kinetic parameters:
k_{cat}: $10^{1} - 10^{7}$ (min)$^{-1}$
K_m: $10^{-3} - 10$ mM

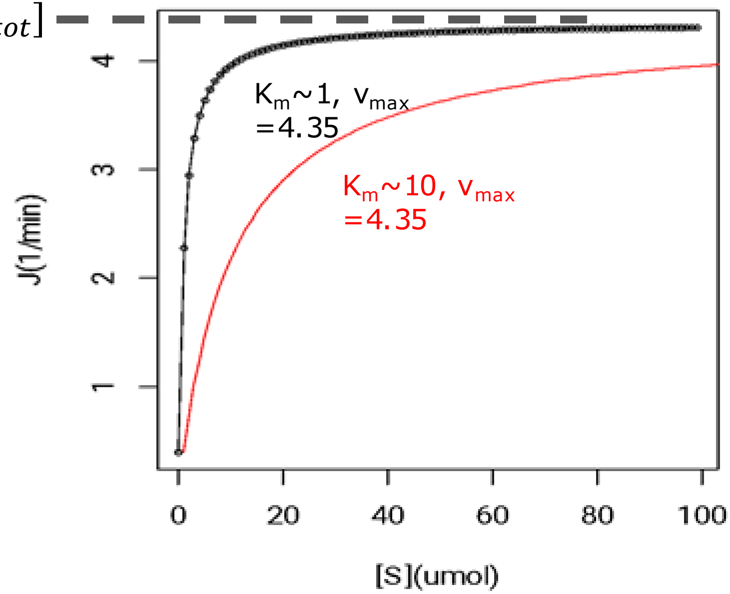
CAUTION: Mostly measured *in vitro*!

Databases for models and kinetic data
Equilibrator: https://equilibrator.weizmann.ac.il/
BIO-MODELS: https://www.ebi.ac.uk/biomodels/
BRENDA: www.brenda-enzymes.org
SABIO-RK: http://sabio.h-its.org/

Economic consideration: flux requires enzymes!

Flux limit due to total enzyme level → $v_{max} = k_{cat}[E_{tot}]$

$$v = v_{max}(\frac{[S]}{[S] + K_M})$$



Hypothesis: Constraints on metabolic fluxes are determined by enzyme levels, and therefore protein allocation to different pathways

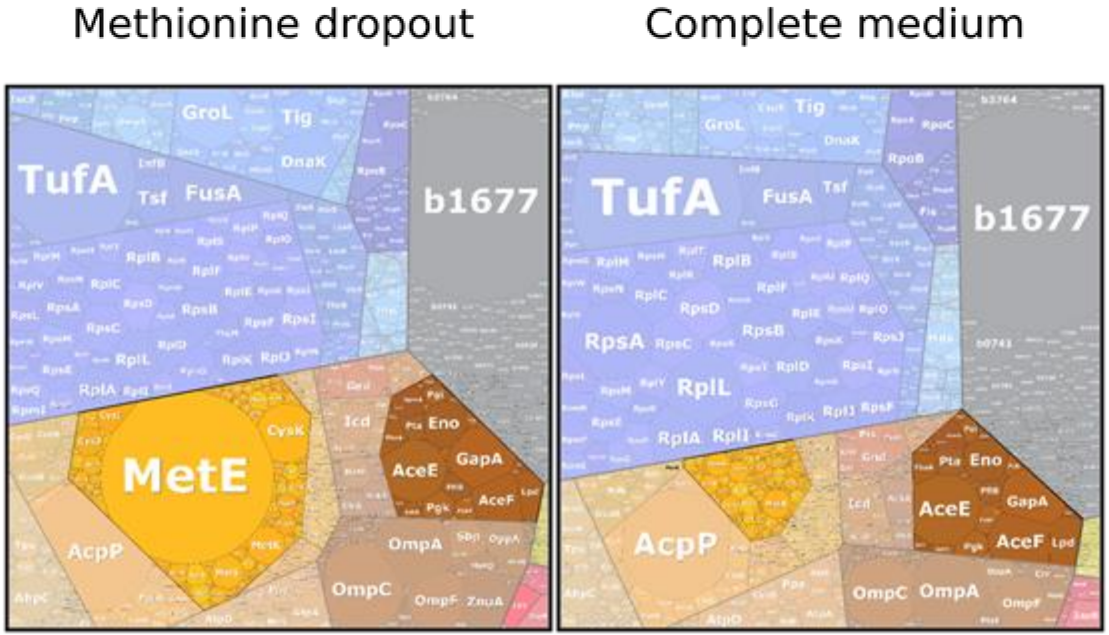
Molenaar, D. *Mol Syst Biol* 5 (2009)
Basan M. et al. *Nature* 528:7580 (2015)

Data/experiment support is limited...

Davidi D. et al. *PNAS* 113:12 (2016)

Metzl-Raz E. et al. *eLife* 6:e28034 (2017)

Economic consideration: flux requires enzymes!



The cell has an *enzyme budget* to spend, i.e. expression of one enzyme comes at the expense of another!

In general, we can note this as:

$$e_{tot} = \sum_i \frac{v_i}{k_{cat,i}}$$

Reaction equations and stoichiometric coefficients

- A metabolic reaction network is defined by a list of biochemical reaction equations:

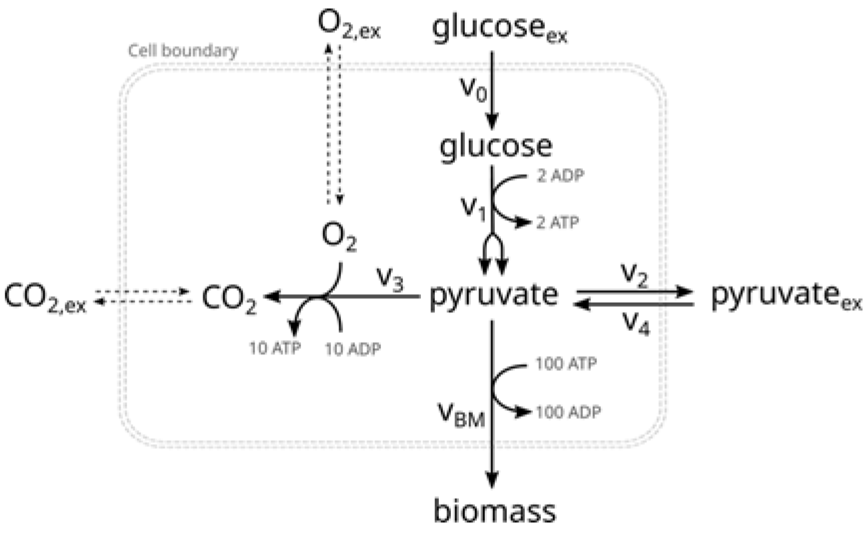
$$V_1 : n^r_{1,1} S_1 + n^r_{2,1} S_2 + \ldots \rightarrow n^p_{1,1} S_1 + n^p_{2,1} S_2 + \ldots$$
$$V_2 : n^r_{1,2} S_1 + n^r_{2,2} S_2 + \ldots \rightarrow n^p_{1,2} S_1 + n^p_{2,2} S_2 + \ldots$$
$$\vdots$$

- Consumption / production of metabolites in each reaction is quantified by the **stoichiometric coefficient**:

$n^r_{i,j}$	Reactant stoichiometric coefficient for metabolite i in reaction j
$n^p_{i,j}$	Product stoichiometric coefficient
$n_{i,j} = n^p_{i,j} - n^r_{i,j}$	Net stoichiometric coefficient

Simplest form of describing metabolism - stoichiometric matrix

All net stoichiometric coefficients are assembled in a matrix **N**:



The ***v***'s are **fluxes**

$$\begin{matrix} v_0 & v_1 & v_2 & v_3 & v_4 & v_{BM} \end{matrix}$$

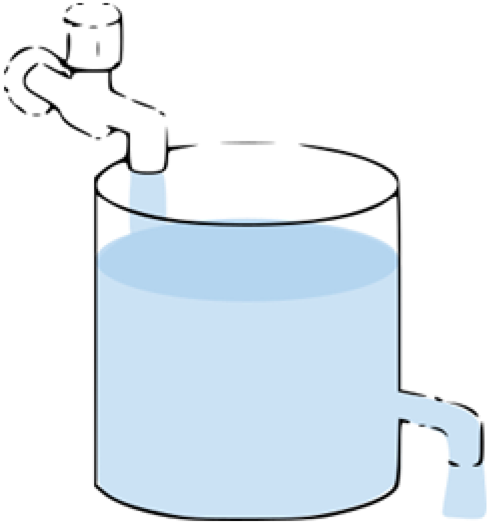
$$\mathbf{N} = \begin{pmatrix} 1 & -1 & 0 & 0 & 0 & 0 \\ 0 & 2 & -1 & -1 & 1 & -1 \\ 0 & 2 & 0 & 10 & 0 & -100 \\ 0 & -2 & 0 & -10 & 0 & 100 \end{pmatrix}, \qquad \mathbf{s} \equiv \begin{pmatrix} [G] \\ [P] \\ [ATP] \\ [ADP] \end{pmatrix}$$

Substrates take up **negative** coefficients, products - **positive**

Steady state and metabolite balancing

- A metabolic network is in steady state, if metabolite amounts do not change over time.
- This requires that
  production = consumption, or
  production – consumption = 0.
- To compute “production – consumption” for a metabolite, we can sum up the reaction fluxes with the net stoichiometric coefficients in the corresponding row of the stoichiometric matrix.
- Balancing glucose [G]:

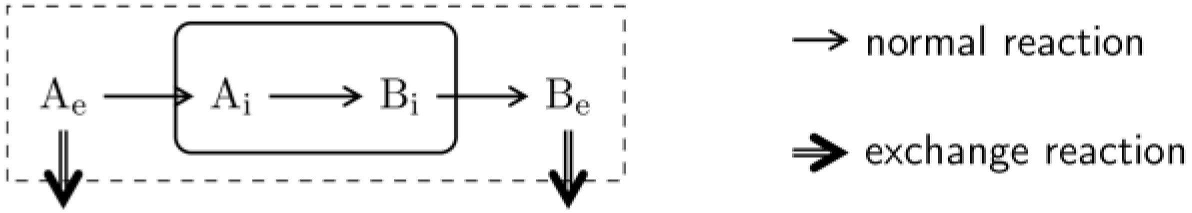
$$V_0 - V_1 = 0$$



Steady state:
inflow = outflow

Exchange reactions and internal vs. full stoichiometric matrix

- Transport processes also modelled with reaction equations
- The same metabolite is considered a different “chemical species” depending on the compartment it is in.
- Artificial exchange reactions model metabolite addition / removal across system boundary



No exchange reactions:

- Closed system
- Only trivial steady state possible

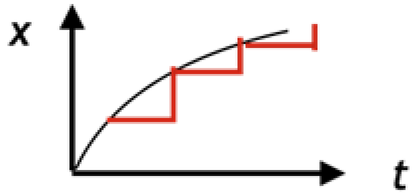
With exchange reactions:

- Open system
- Non-zero steady state possible

Differential equations allow to 'predict' the future

$$\frac{dx}{dt} = x/(b + x)$$

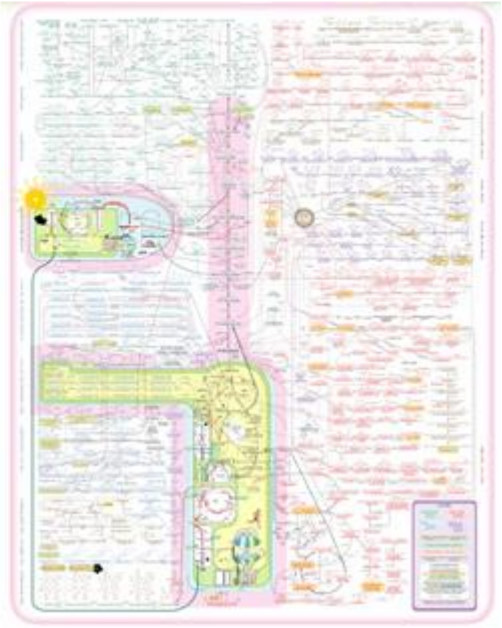
Derivative f′(x) (differential equation) gives the relation between small **changes in variables**



Consider we had a derivative where the independent variable is time and the dependent variable was a physical entity…

By 'tracing' the derivative, we could see how the variable changes over time!

System of interest
+
interactions, processes…



'Modelling'

ODEs

integration → Functional relation

Numerical integration

$$x_{n+dt} = x_n + f'(x_n) \cdot dt$$

Parameter 'sweeping'

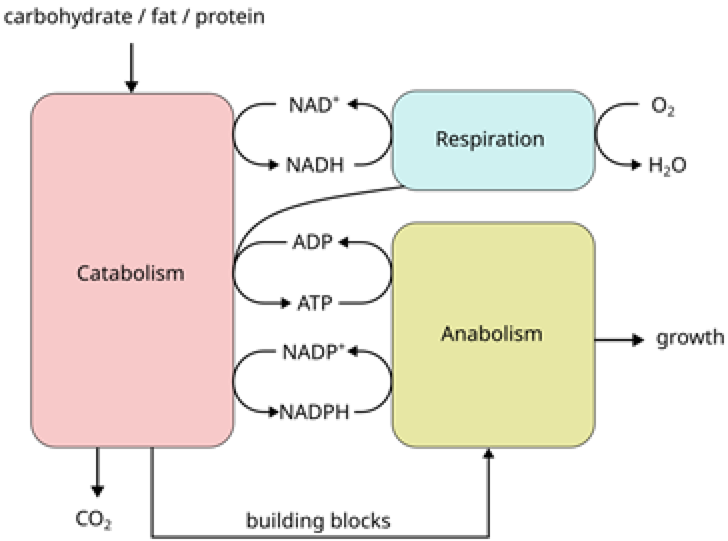
Steady state analysis $\frac{dx}{dt} = 0$

Stability analysis

'Insights'

The structure of overall cellular metabolism

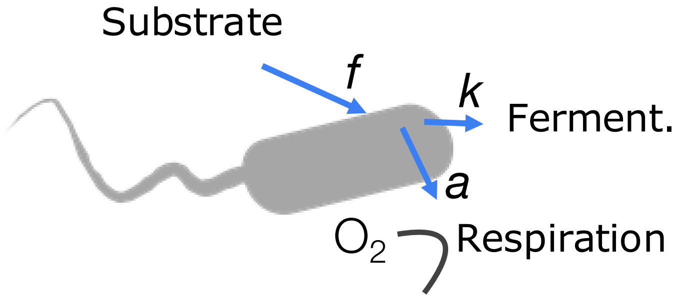
- **Catabolism**: Nutrients are broken down in smaller metabolites
  - Transfers chemical energy to ATP / NAD(P)H
  - Provides building blocks for biomass
- **Anabolism**: Synthesis of larger molecules / biomass



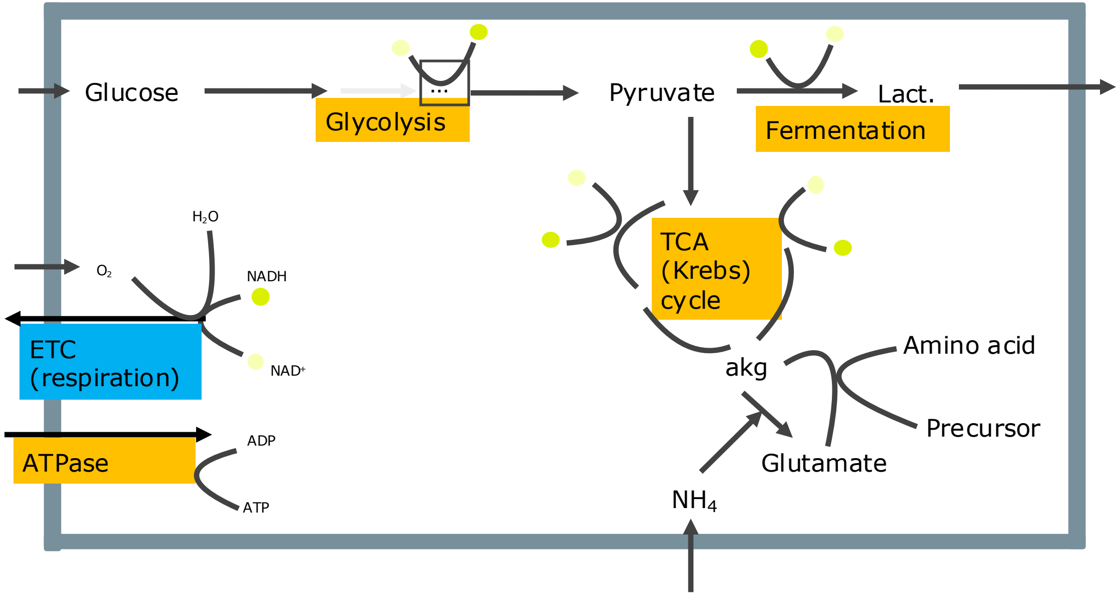
Metabolic shift between fermentation and respiration

Depending on conditions, many organisms can shift between fermentation and respiro-fermentation.

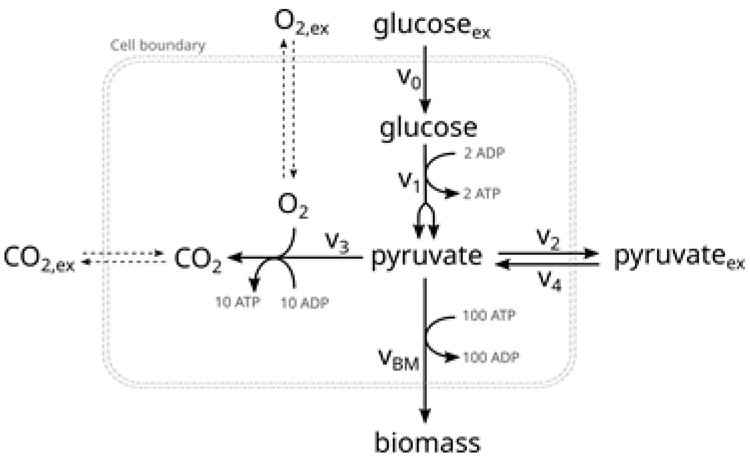
Warburg effect– in cancer, Crabtree effect – in yeast

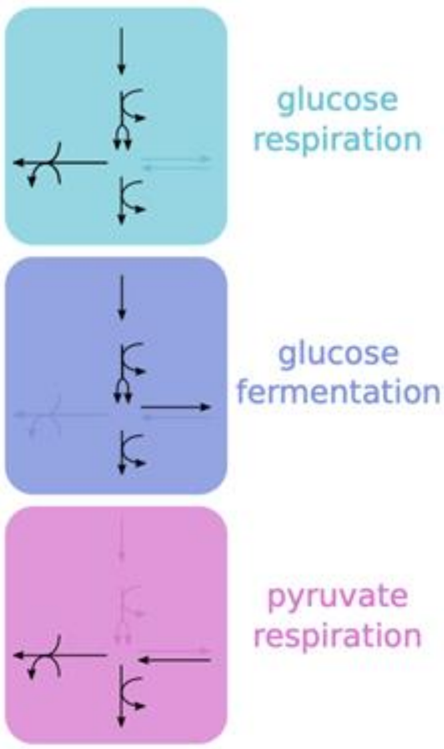


Respiration (high yield, low rate)
vs.
fermentation (low yield, high rate)



Even simple networks can contain multiple flux modules





Take-home messages

- Metabolism is a patchwork of multiple pathways
- Mass action- and Michaelis-Menten laws describe kinetics of most biochemical reactions
- A metabolic network is described by reaction stoichiometric coefficients assembled in the stoichiometric matrix
- Metabolite balancing gives a set of equations that describe a metabolic steady state
- On the organism / cell level, metabolism is structured in multiple functions (catabolism, anabolism) and the network can switch between different metabolic modes

Acknowledgements

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