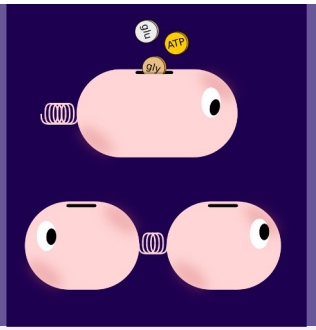
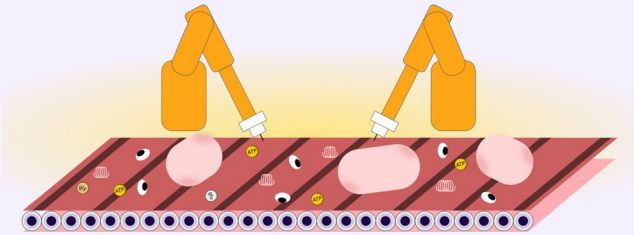
Economic Principles in Cell Physiology

Paris, July 8-10, 2024



Dynamics of Cell Metabolism

Orkun S Soyer, Robert West, Hadrien Delattre, Elad Noor, Herbert Sauro

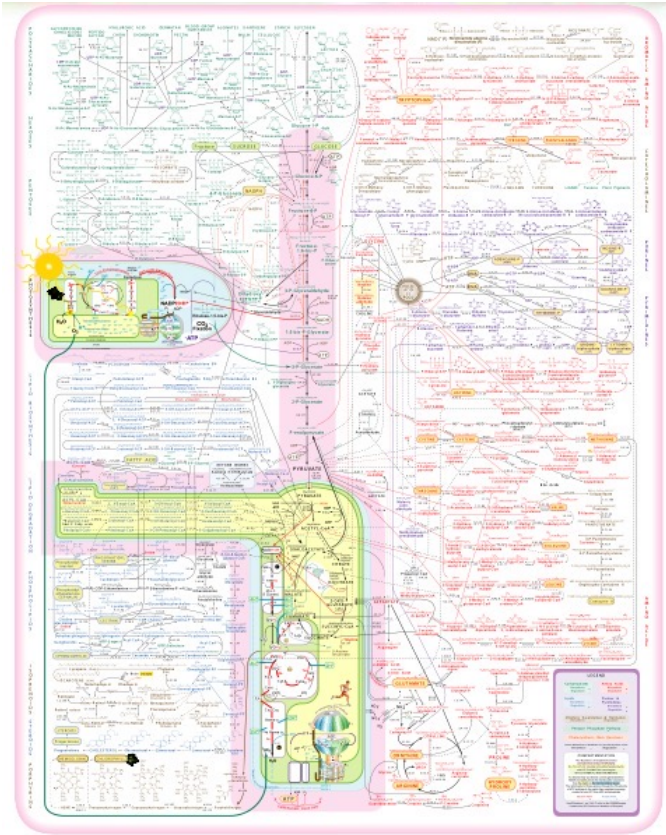


LEARNING PLANET INSTITUTE

PART 1

INTRODUCTION & MOTIVATION

Cracking metabolism

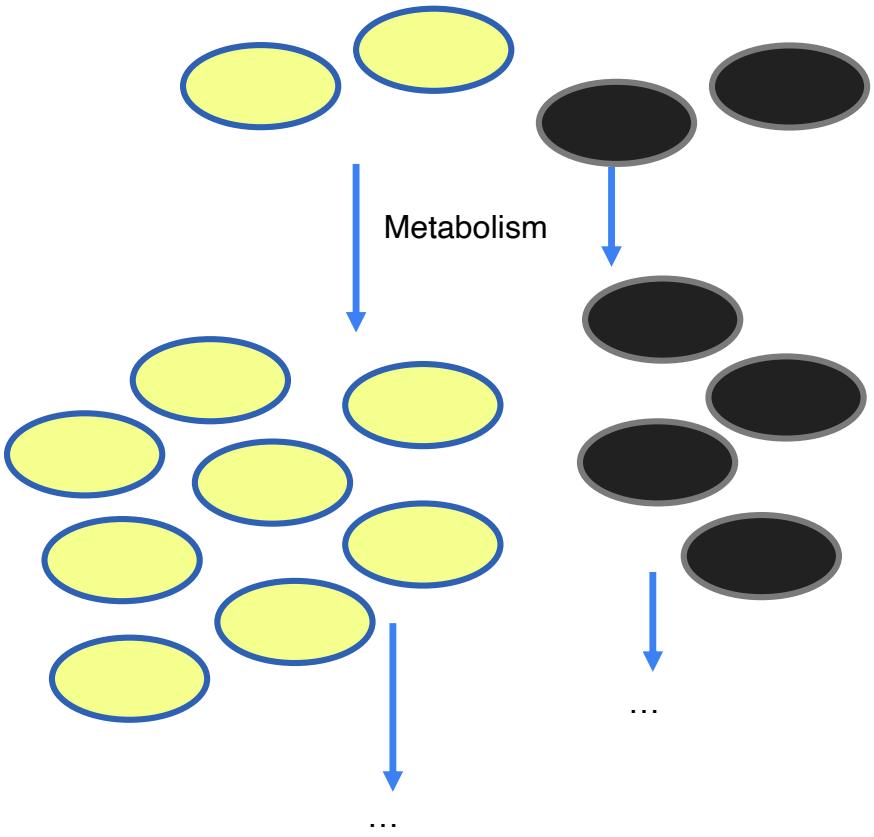


Complex, dynamical system presenting many <u>open questions</u>

Any underpinning functional/structural principles?

How to predict temporal dynamics?

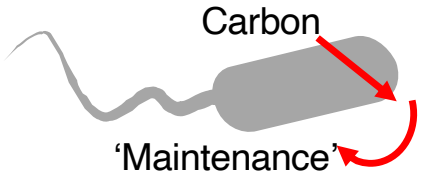
Metabolism as ‘optimal biomass generator’



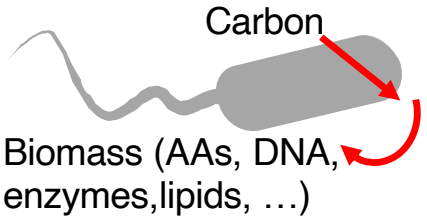
One view posits that metabolism is the process through which cells **acquire energy to make biomass**

It follows that through evolution, metabolism should (might**??**) have been ***optimised*** for ***efficient*** (yield) or ***fast*** (rate) biomass generation

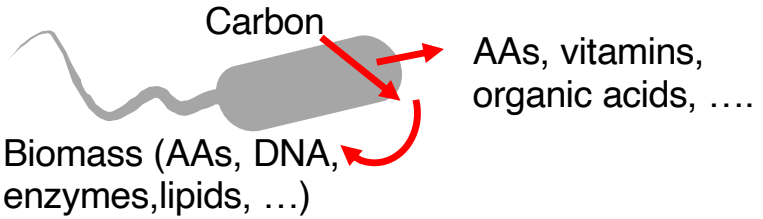
Diverse metabolic dynamics & behaviors



'No growth' metabolism



'Normal' (high yield?) metabolism



'Overflow' (fast?) metabolism

De Deken R. *J. Gen. Microbiol.*, 44 (1966)

Warburg I Crabtree effect

Metabolic oscillations

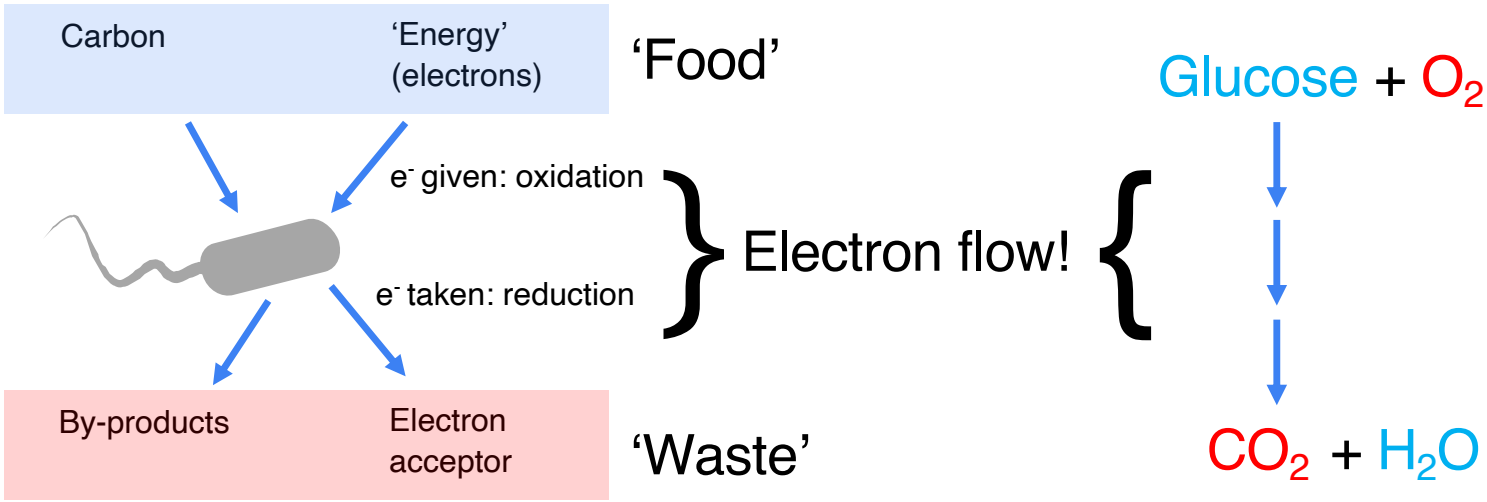
Murray, D., et al. *PNAS*, 104:7 (2007)

Metabolic heterogeneity (bistability?)

Simsek E. & Kim M., *ISME J.* 12:5 (2018)

van Heerden J.D. et al., *Science* 343:6174 (2014)

Metabolism as electron flows



"Life is an electron looking for a place to rest"

quote from c. 1960 by Albert Szent-Györgyi (1893-1986). Nobel laureate (1937) and discoverer of Vitamin C. Studied TCA cycle.

Zerfass. C., Asally M., Soyer O.S. *Curr Opin Syst Biol* 13, 2019

Schoepp-Cothenet, B. et al. *Biochim Biophys Acta* 1827:2, 2013

SOME TAKE HOME MESSAGES BEFORE WE START

Cell metabolism & physiology presents many open questions

Models are ideas in need of experiments to revise them

Experiments report what is observed under a given condition

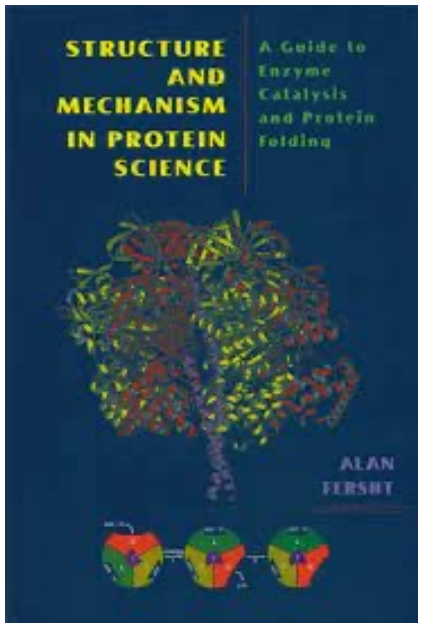
Genome Scale FBA ≠ Metabolic modelling

PART 2

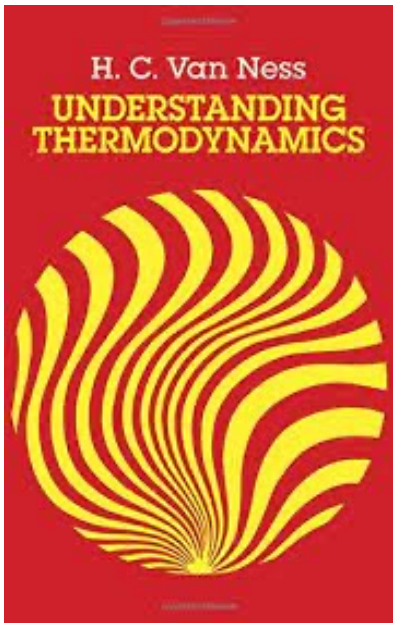
FOUNDATIONS

Metabolism is chemistry, is physics, is mathematics….

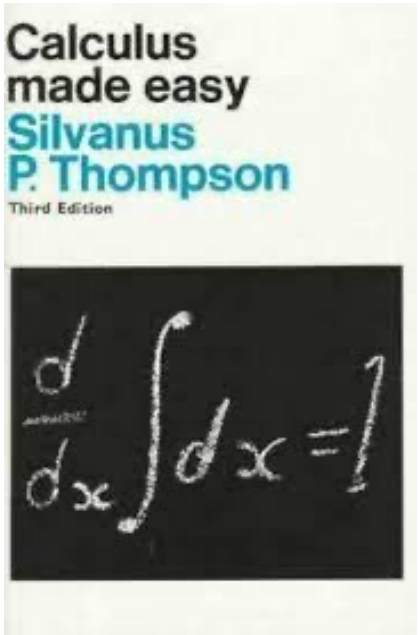
Biochemistry

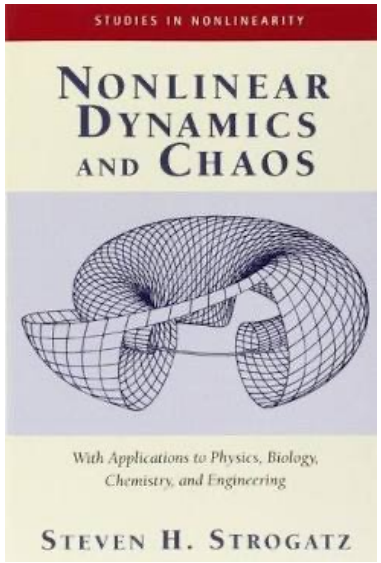


Thermodynamics



Calculus & Systems Dynamics





PART 2.1

DERIVATIVES (aka differential equations)

Differential equations allow 'predicting' the future

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

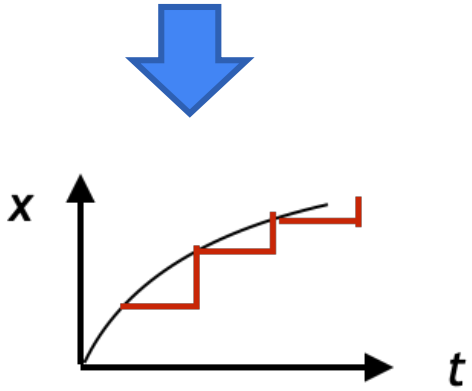
Derivative f'(x) (differential equation) gives the relation between small **changes in variables**

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

Differential equations allow 'predicting' 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!

My first (ordinary) differential equation model

ODE model of a mice population:

$$\frac{dN}{dt} = r \cdot N - \frac{r \cdot N^2}{K}$$

If we know the population size at an initial time point t_0, then we can ***predict*** the population size at time $t_0 + dt$ using our ODE!

$$N_{0+dt} = N_0 + dN$$

$$N_{0+dt} = N_0 + \frac{dN}{dt} \cdot dt$$

$$N_{0+dt} = N_0 + \left(r \cdot N_0 - \frac{r \cdot N_0^2}{K}\right) \cdot dt$$

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

Differential equations allow 'predicting' the future

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

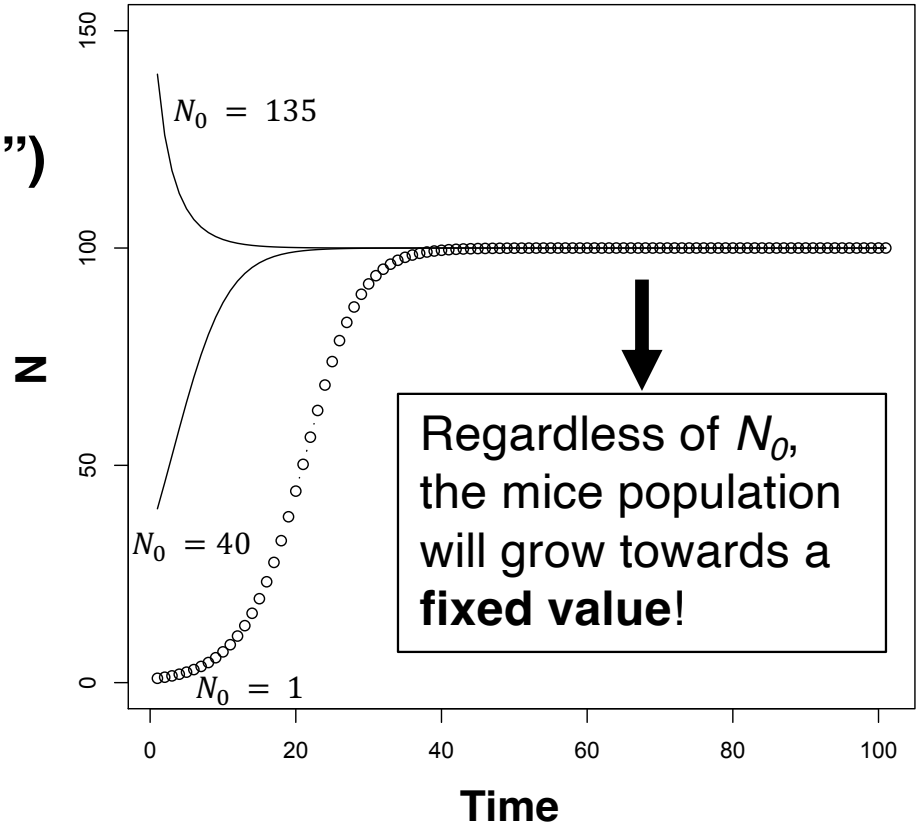
Keep iterating

ODE "simulation" (aka "numerical integration")

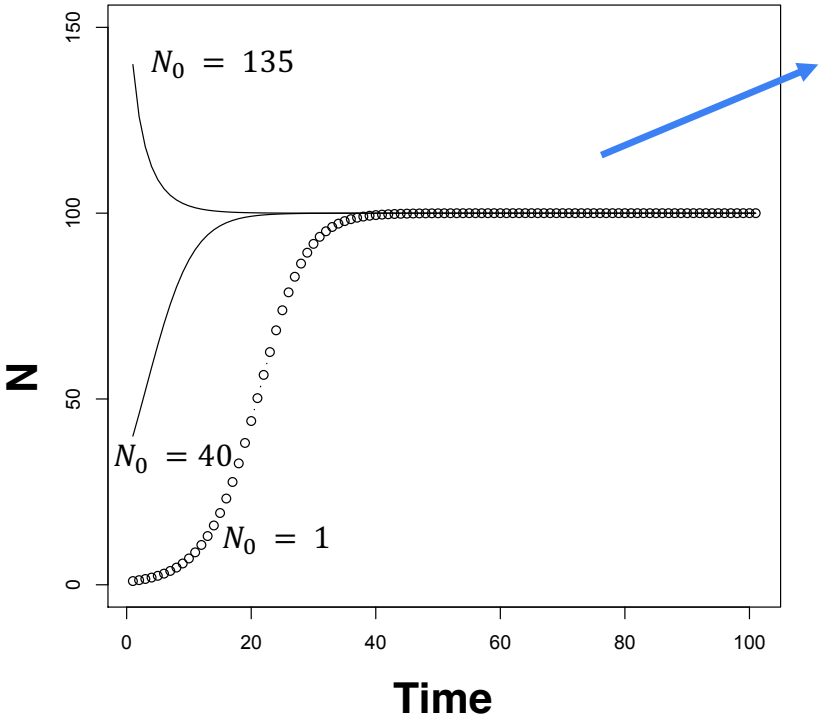
Do try this on your computer

```
K = 100; N0 = 1; r = 0.25;
N = rep(0,100);
N[1] = N0;
for (i in 2:length(N)) {
        Nch = N[i-1] * (r * (1 - N[i-1]/K))
        N[i] = N[i-1] + Nch;
}
plot(N);
```

ODE "simulation"



Differential equations allow 'predicting' the future



After a time, the population stabilised at a fixed value and there seems to be **no change** in N with time!

So, we could have predicted perhaps what that fixed value was going to be from the ODE:

$$\frac{dN}{dt} = 0 = r \cdot N - \frac{r \cdot N^2}{K}$$

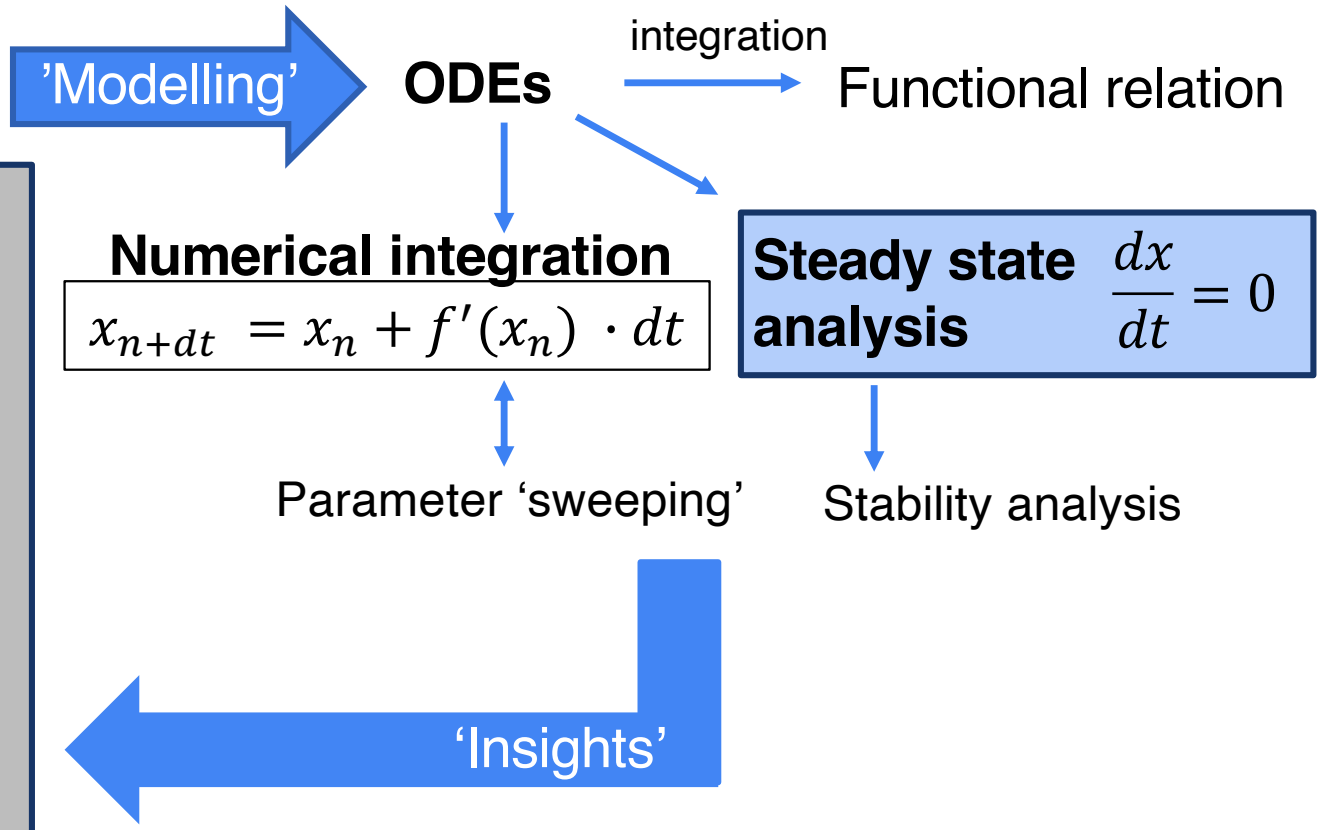
$$\frac{r \cdot N^2}{K} = r \cdot N \quad \Rightarrow \quad \boxed{N = K}$$

Steady state

Systems dynamics - toolset

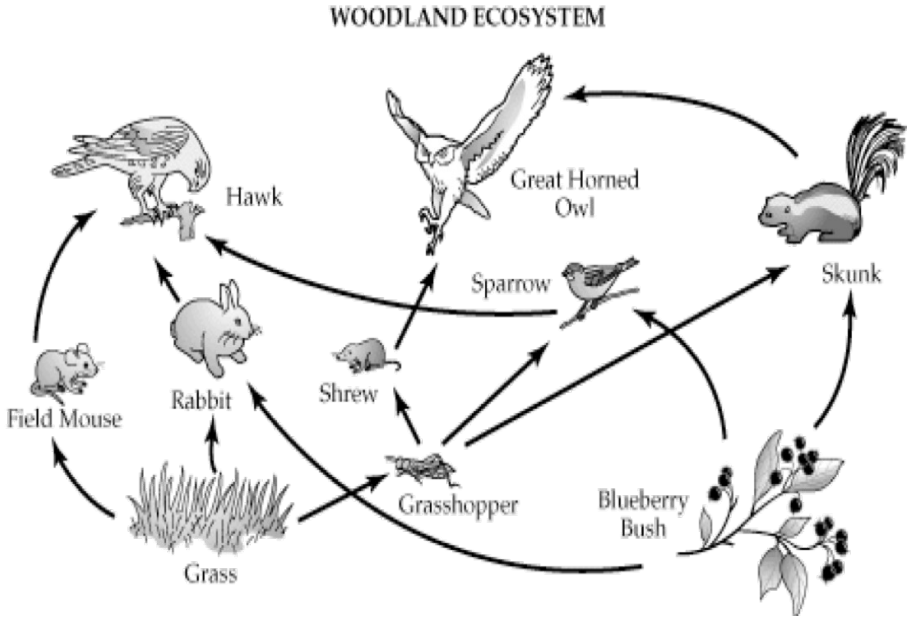
System of interest
Interactions, processes…

$$\frac{dx_1}{dt} = x_1' = f(x_1, x_2, \ldots, x_n)$$
$$\frac{dx_2}{dt} = x_2' = f(x_1, x_2, \ldots, x_n)$$
$$\vdots$$
$$\frac{dx_n}{dt} = x_n' = f(x_1, x_2, \ldots, x_n)$$



Systems dynamics - toolset

System of interest
Interactions, processes…



'Modelling' → **ODEs** —integration→ Functional relation

Numerical integration

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

Parameter 'sweeping'

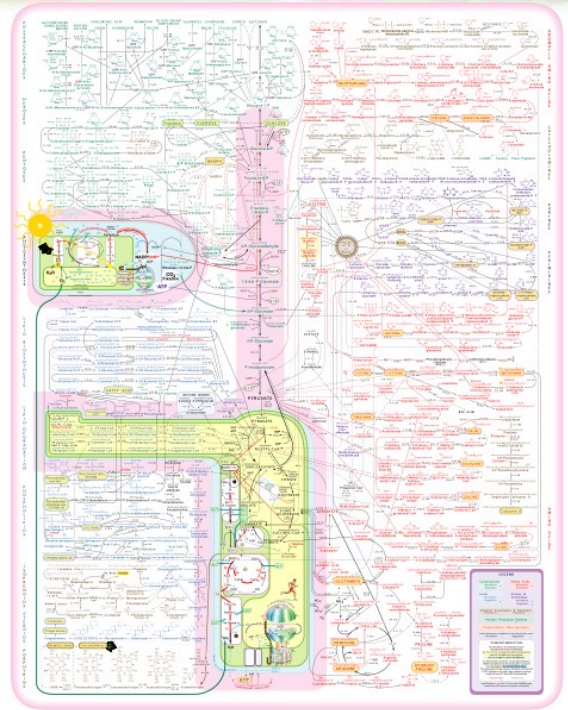
Steady state analysis $\frac{dx}{dt} = 0$

Stability analysis

'Insights'

Maths don’t care about “details”!

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’

PART 2.2

REACTIONS, LAW OF MASS ACTION & THERMODYNAMICS

Metabolic systems involve chemical reactions

A generic <u>reversible</u> chemical reaction….

$$\nu_A \mathrm{A} + \nu_B \mathrm{B} \rightleftharpoons \nu_C \mathrm{C} + \nu_D D$$

'reactants' 'products'

A given reaction always reaches same equilibrium!

$$\frac{[C]_{eq}^{\nu_C}[D]_{eq}^{\nu_D}}{[A]_{eq}^{\nu_A}[B]_{eq}^{\nu_B}} = K_{eq}$$

Law of mass action

Empirically derived (aka law of Nature!)

Recommended reading: "Textbook errors: IX. More about the laws of reaction rates and of equilibrium", Guggenheim, E.A., *J Chem Educ* 33:11 (1956)

Thermodynamic explanation for Law of Mass Action

A generic <u>reversible</u> chemical reaction….

$$\nu_A A + \nu_B B \rightleftharpoons \nu_C C + \nu_D D$$

'reactants' 'products'

…. under <u>constant</u> temperature and pressure:

$$\Delta G = \Delta G^0 + R \cdot T \cdot ln\left(\frac{[C]^{\nu_C}[D]^{\nu_D}}{[A]^{\nu_A}[B]^{\nu_B}}\right)$$

$$\Delta G^0 = \Delta G^0(C) + \Delta G^0(D) - (\Delta G^0(A) + \Delta G^0(B))$$

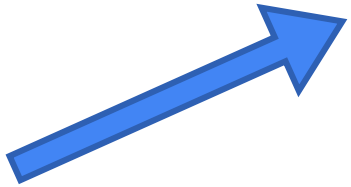
Sometimes ΔG is given as $\Delta_{rxn} G$. The subscript, e.g ΔG^0 , refers to standard states (chemicals at 1M). To refer to biochemical standard conditions, i.e. all at 1M, but pH=7, use; $\Delta G^{0\prime}$

Thermodynamic explanation for Law of Mass Action

$$\Delta G = 0 = \Delta G^0 + R \cdot T \cdot ln\left(\frac{[C]_{eq}^{\nu_C}[D]_{eq}^{\nu_D}}{[A]_{eq}^{\nu_A}[B]_{eq}^{\nu_B}}\right)$$

$$\Delta G^0 = -R \cdot T \cdot ln\left(\frac{[C]_{eq}^{\nu_C}[D]_{eq}^{\nu_D}}{[A]_{eq}^{\nu_A}[B]_{eq}^{\nu_B}}\right)$$

$$\frac{-\Delta G^0}{R \cdot T} = ln\left(\frac{[C]_{eq}^{\nu_C}[D]_{eq}^{\nu_D}}{[A]_{eq}^{\nu_A}[B]_{eq}^{\nu_B}}\right)$$



$$e^{\frac{-\Delta G^0}{R \cdot T}} = \frac{[C]_{eq}^{\nu_C}[D]_{eq}^{\nu_D}}{[A]_{eq}^{\nu_A}[B]_{eq}^{\nu_B}} = K_{eq}$$

Law of mass action

Thermodynamic explanation for Law of Mass Action

$$\Delta G = 0 = \Delta G^0 + R \cdot T \cdot ln\left(\frac{[C]_{eq}^{\nu_C}[D]_{eq}^{\nu_D}}{[A]_{eq}^{\nu_A}[B]_{eq}^{\nu_B}}\right)$$

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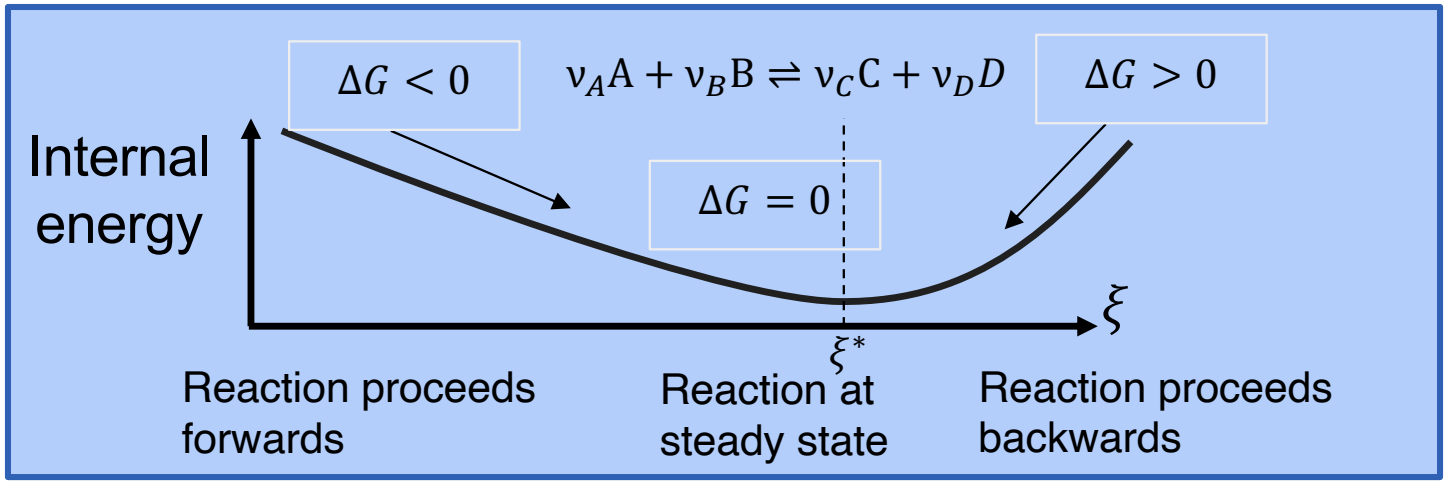
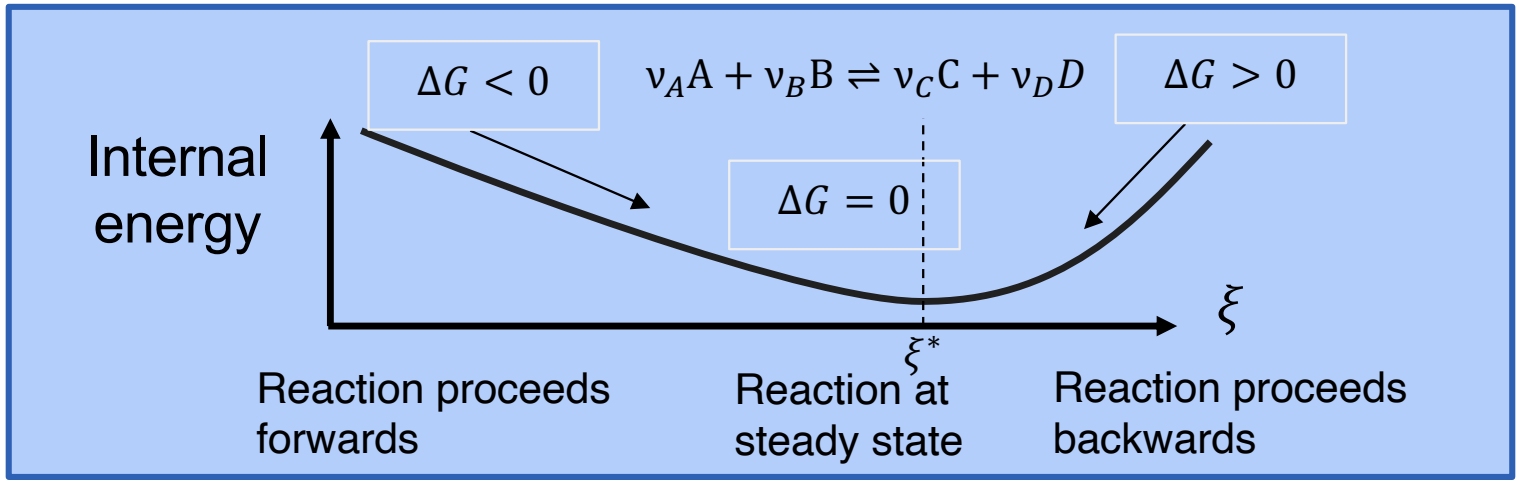
Law of mass action



Process (rate) based explanation for Law of Mass Action



Forward reaction **rate**:

$$\boldsymbol{k_+[A]^{\nu_A}[B]^{\nu_B}}$$

Backward reaction **rate**:

$$\boldsymbol{k_-[C]^{\nu_C}[D]^{\nu_D}}$$

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**.

Process (rate) based explanation for Law of Mass Action

Mass action rate model

At equilibrium: $k_+[A]^{\nu_A}[B]^{\nu_B} = k_-[C]^{\nu_C}[D]^{\nu_D}$

Thermodynamic model

At equilibrium: $\Delta G = 0 = \Delta G^0 + R \cdot T \cdot ln\left(\frac{[C]_{eq}^{\nu_C}[D]_{eq}^{\nu_D}}{[A]_{eq}^{\nu_A}[B]_{eq}^{\nu_B}}\right)$

Law of mass action

$$\frac{k_+}{k_-} = \frac{[C]_{eq}^{\nu_C}[D]_{eq}^{\nu_D}}{[A]_{eq}^{\nu_A}[B]_{eq}^{\nu_B}} = K_{eq} = e^{\frac{-\Delta G^0}{R \cdot T}}$$

Reversible mass action model of a (chemical) reaction

$$\nu_A \mathrm{A} + \nu_B \mathrm{B} \underset{k_-}{\overset{k_+}{\rightleftharpoons}} \nu_C \mathrm{C} + \nu_D D$$

ODEs for this 'system':

$$\frac{d[A]}{dt} = -k_+[A]^{\nu_A}[B]^{\nu_B} + k_-[C]^{\nu_C}[D]^{\nu_D}$$

$$J = \frac{d[C]}{dt} = k_+[A]^{\nu_A}[B]^{\nu_B} - k_-[C]^{\nu_C}[D]^{\nu_D}$$

Steady state:

$$\frac{k_+}{k_-} = \frac{[C]_{eq}^{\nu_C}[D]_{eq}^{\nu_D}}{[A]_{eq}^{\nu_A}[B]_{eq}^{\nu_B}} = K_{eq} = e^{\frac{-\Delta G^0}{R \cdot T}}$$

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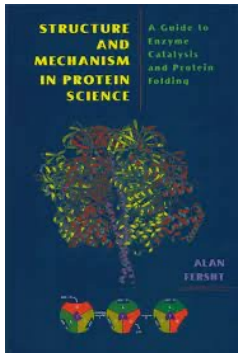
Remember that, according to thermodynamics, k_+ **and** k_- **are related. We cannot choose them freely!**

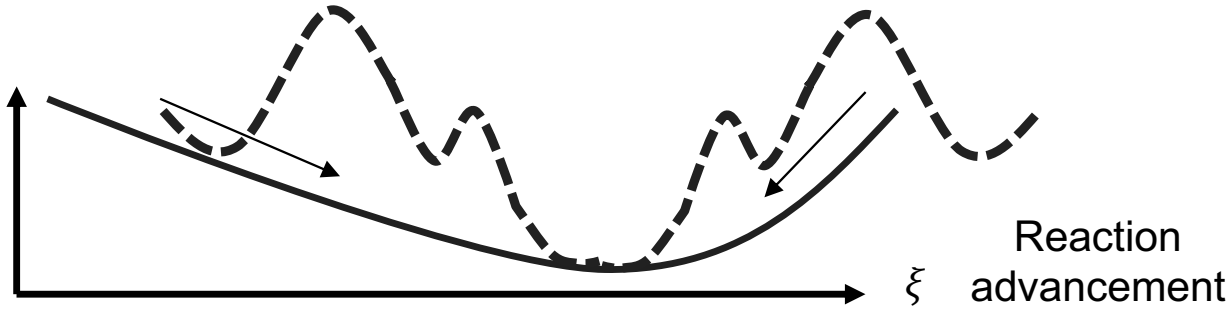
$$J = k_+[A]^{\nu_A}[B]^{\nu_B} - \frac{k_+}{K_{eq}}[C]^{\nu_C}[D]^{\nu_D}$$

PART 2.3

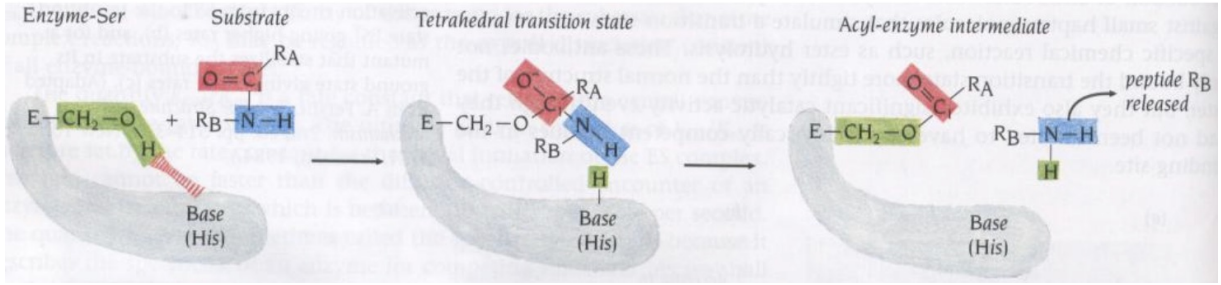
ENZYMATIC RATE MODELS

Biochemical reactions are enzymatic





Accounting for enzyme activity (function) in reaction dynamics:



Substrate(s) and 'free' enzyme

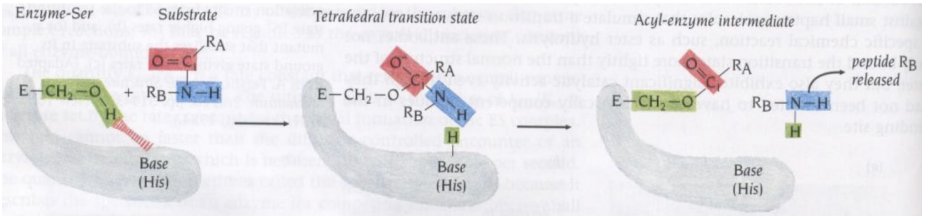
Substrate(s) 'bound' on enzyme

Products(s) and free enzyme

$$\mathrm{E} + S \rightleftharpoons ES \qquad ES \rightleftharpoons E + P$$

Enzymatic reaction dynamics – modelling strategy

1. Create ‘cartoon’ model of enzyme ‘mechanism’:



2. Convert mechanism into **elementary** (bio)chemical reactions:

e.g. $E + S \rightleftharpoons ES$

3. Write ODEs for elementary reactions by assuming **law of mass action**:

e.g. $\frac{d[S]}{dt} = -k_+[S][E] + k_-[ES]$

4. Make further assumptions to create **simplifications**:

e.g. $[E] + [ES] = const.$

Enzymatic reaction dynamics – example

1. Enzyme with single binding site & substrate

2. Elementary (bio)chemical reactions:

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

3. Simplifying assumptions:

$$k_6 = 0; k_3, k_4 \; very \; large$$
$$ES \rightleftharpoons EP \; instantenous$$

4. New reaction scheme:

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

5. Write ODEs by assuming **law of mass action**:

$$\frac{d[ES]}{dt} = k_+[S][E] - k_-[ES] - k_{cat}[ES]$$

$$\frac{d[S]}{dt} = -k_+[S][E] + k_-[ES] \qquad \boxed{\frac{d[P]}{dt} = k_{cat}[ES]}$$

Enzymatic reaction dynamics – example

6. Make further assumptions:

$$k_+, k_- \gg k_{cat}$$

$$[E] + [ES] = const. = E_0$$

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

Model reduction

Quasi steady state assumption:

$$\frac{d[ES]}{dt} \approx 0$$

$$\frac{d[ES]}{dt} = 0 = k_+[S](E_0 - [ES]) - k_-[ES] - k_{cat}[ES] \Rightarrow [ES] = \frac{k_+ E_0 [S]}{k_+[S] + k_- + k_{cat}}$$

$$\frac{d[P]}{dt} = k_{cat}[ES]$$

$$\frac{d[P]}{dt} = \frac{k_{cat} E_0 [S]}{[S] + (k_- + k_{cat})/k_+}$$

$$\boldsymbol{\frac{d[P]}{dt} = J = \frac{v_{max}\,[S]}{[S] + K_m}}$$

Irreversible Michaelis – Menten model for the reaction flux of an enzymatic reaction!

A reversible enzymatic reaction model

1. Enzyme with single binding site & substrate

2. Elementary (bio)chemical reactions:

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

3. Simplifying assumptions:

4. New reaction scheme:

$$\frac{d[ES]}{dt} = \frac{d[EP]}{dt} = 0$$

5. Write ODEs by assuming **law of mass action**:

$$\vdots$$

Try this derivation!

A reversible enzymatic reaction model

$$J = [E_0]\frac{k_{cat}^{+}{}^{[S]}/_{K_S} - k_{cat}^{-}{}^{[P]}/_{K_P}}{1 + {}^{[S]}/_{K_S} + {}^{[P]}/_{K_P}}$$

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

$$k_{cat}^{+} = \frac{k_3 k_5}{k_3 + k_4 + k_5};\ k_{cat}^{-} = \frac{k_2 k_4}{k_2 + k_3 + k_4};\ K_S = \frac{k_2 k_4 + k_2 k_5 + k_3 k_5}{k_1(k_3 + k_4 + k_5)};\ K_P = \frac{k_2 k_4 + k_2 k_5 + k_3 k_5}{k_6(k_2 + k_3 + k_4)}$$

Steady State:

$$\frac{[E_0] \cdot k_{cat}^{+} \cdot {}^{[S]}/_{K_S}}{1 + {}^{[S]}/_{K_S} + {}^{[P]}/_{K_P}} = \frac{[E_0] \cdot k_{cat}^{-} \cdot {}^{[P]}/_{K_P}}{1 + {}^{[S]}/_{K_S} + {}^{[P]}/_{K_P}}$$

Haldane relation

$$\frac{k_{cat}^{+} \cdot K_P}{k_{cat}^{-} \cdot K_S} = \frac{k_1 k_3 k_5}{k_2 k_4 k_6} = \left(\frac{[P]}{[S]}\right)_{eq} = K_{eq}$$

As expected from **principle of equilibrium:** Lewis, G.N. *PNAS* 11:3, 1925

A reversible model of enzymatic reaction dynamics

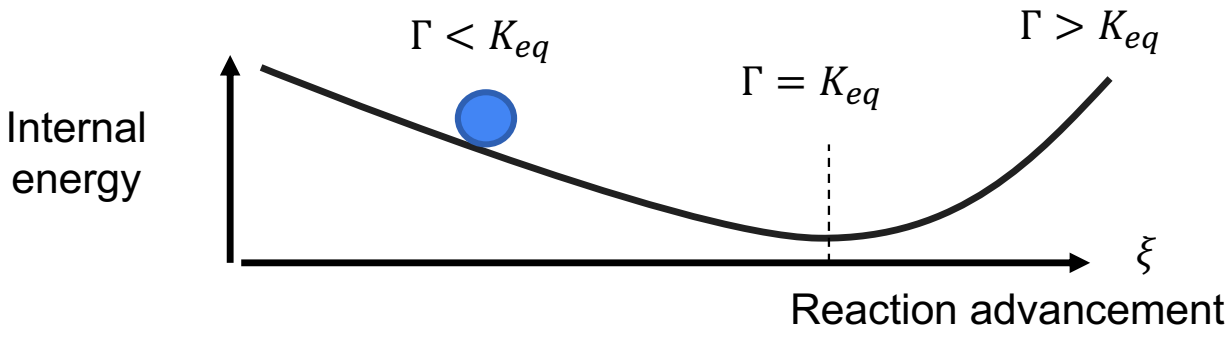
$$J = [E_0]\frac{k_{cat}^{+}{}^{[S]}/_{K_S} - k_{cat}^{-}{}^{[P]}/_{K_P}}{1 + {}^{[S]}/_{K_S} + {}^{[P]}/_{K_P}}$$

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

$$J = [E_0] \cdot k_{cat}^{+}\left(\frac{{}^{[S]}/_{K_S}}{1 + {}^{[S]}/_{K_S} + {}^{[P]}/_{K_P}}\right)\left(1 - \frac{{}^{[P]}/_{[S]}}{K_{eq}}\right)$$

$$J = v_{max} \cdot \kappa \cdot \left(1 - \frac{\Gamma}{K_{eq}}\right)$$

$$J = v_{max} \cdot \kappa \cdot \left(1 - e^{\frac{\Delta G}{RT}}\right)$$



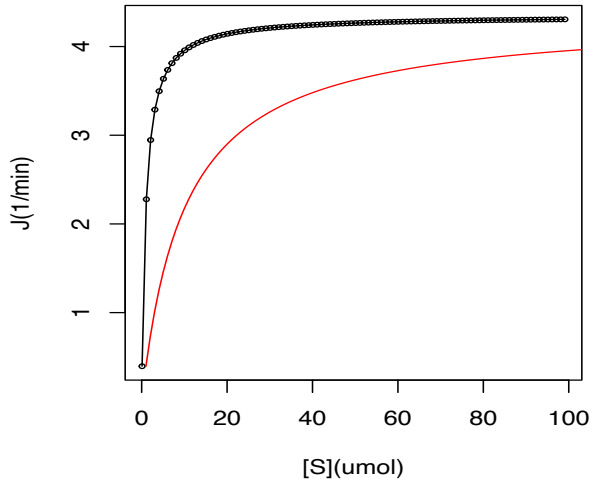
Noor et al. 2013
(10.1016/j.febslet.2013.07.028)

Enzymatic reaction models summary

Irreversible enzymatic model

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

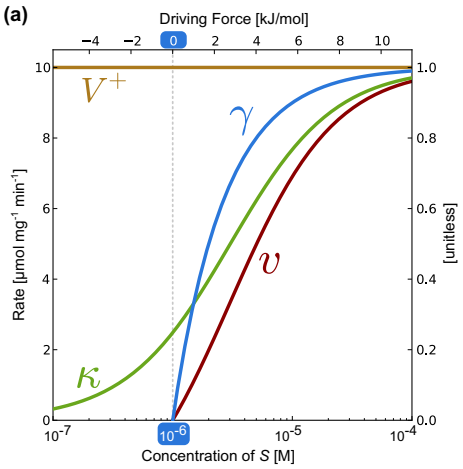
$$J = v_{max} \cdot \left(\frac{[S]}{[S] + K_m} \right)$$



Reversible enzymatic model

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

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

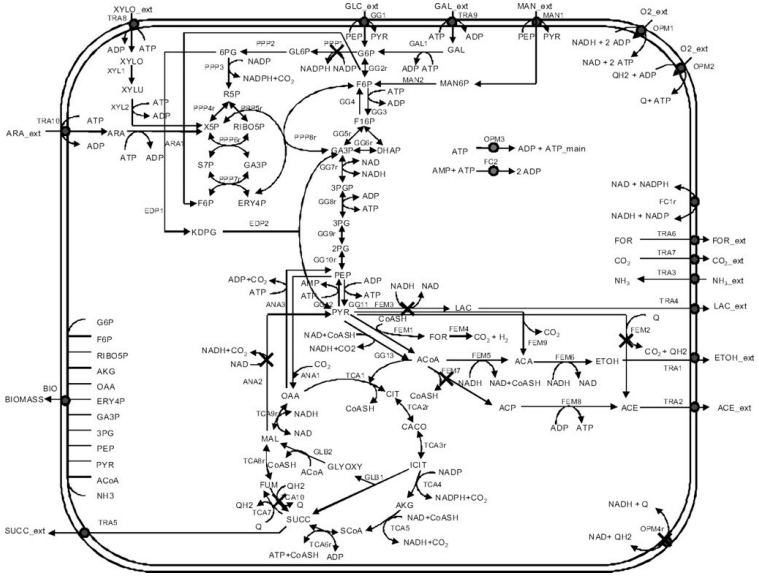


PART 3

MODELS, DATA & EXPERIMENTS

Modelling metabolic systems

Central carbon metabolism



Partial, but detailed, models of pathways

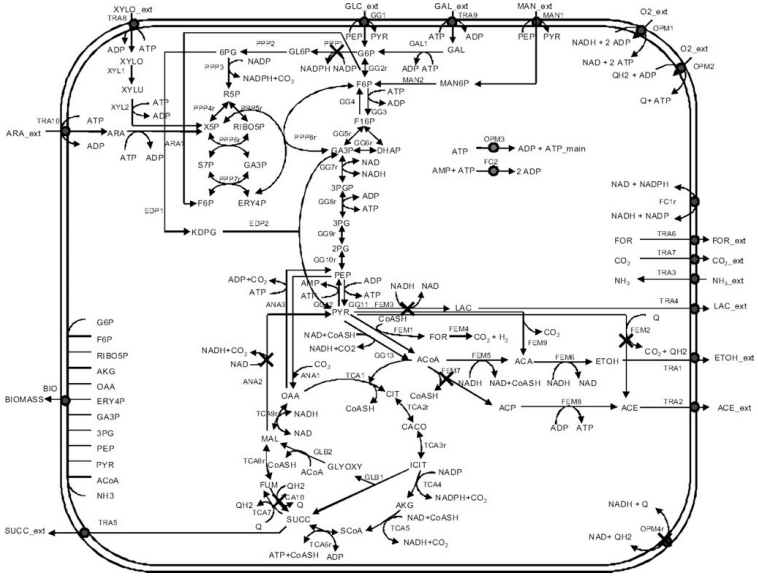
Re-occurring **motifs** and their dynamics

Toy models mimicking aspects of metabolism

Large-scale models

Modelling metabolic systems

Central carbon metabolism



Partial, but detailed, models of pathways

Re-occurring **motifs** and their dynamics

Toy models mimicking aspects of metabolism

Large-scale models

"All models are wrong, some are useful"
attributed to a 1976 paper by George Box (statistician)

A model is something no one believes except the creator of the model, while an experiment is something everyone believes except the experimenter
quote attributed to A. Einstein

Dynamical models and parameters

Fluxes:

10^{-1} – 10^{4} (mM · min)$^{-1}$

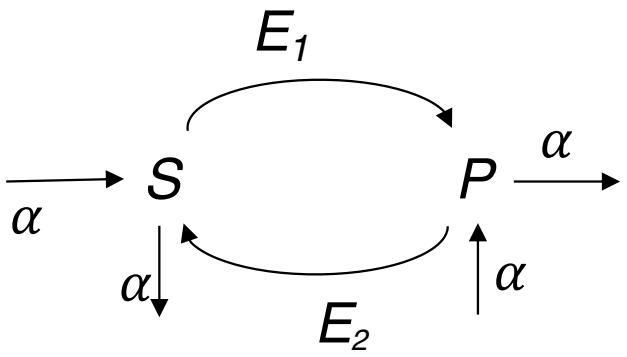
Enzyme levels:

10^{-5} – 10^{-1} mM

$$\frac{k_{cat} \cdot [E_{tot}][S]}{K_m + [S]}$$

Substrate levels:

10^{-3} – 10 mM



Enzyme kinetics:

k_{cat}: 10^{1} – 10^{7} (min)$^{-1}$

K_m: 10^{-3} – 10 mM

Binding/unbinding
10^{10} – 10^{13} (mM · min)$^{-1}$
10^{2} – 10^{6} (min)$^{-1}$

CAUTION: Mostly based on *in vitro* enzymology!

Equilibrator: https://equilibrator.weizmann.ac.il/
BIO-MODELS: https://www.ebi.ac.uk/biomodels/
BRENDA: www.brenda-enzymes.org

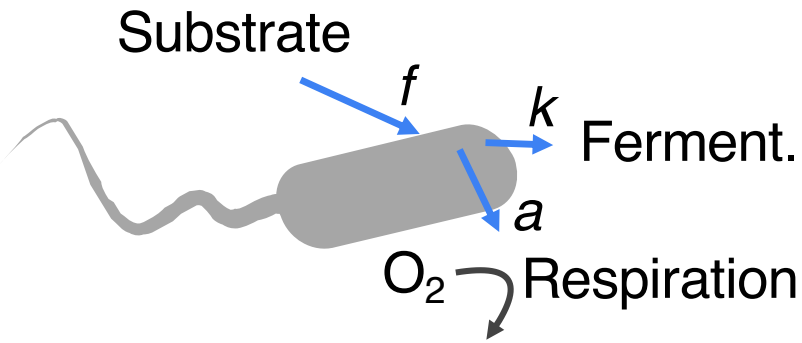
PART 3.1

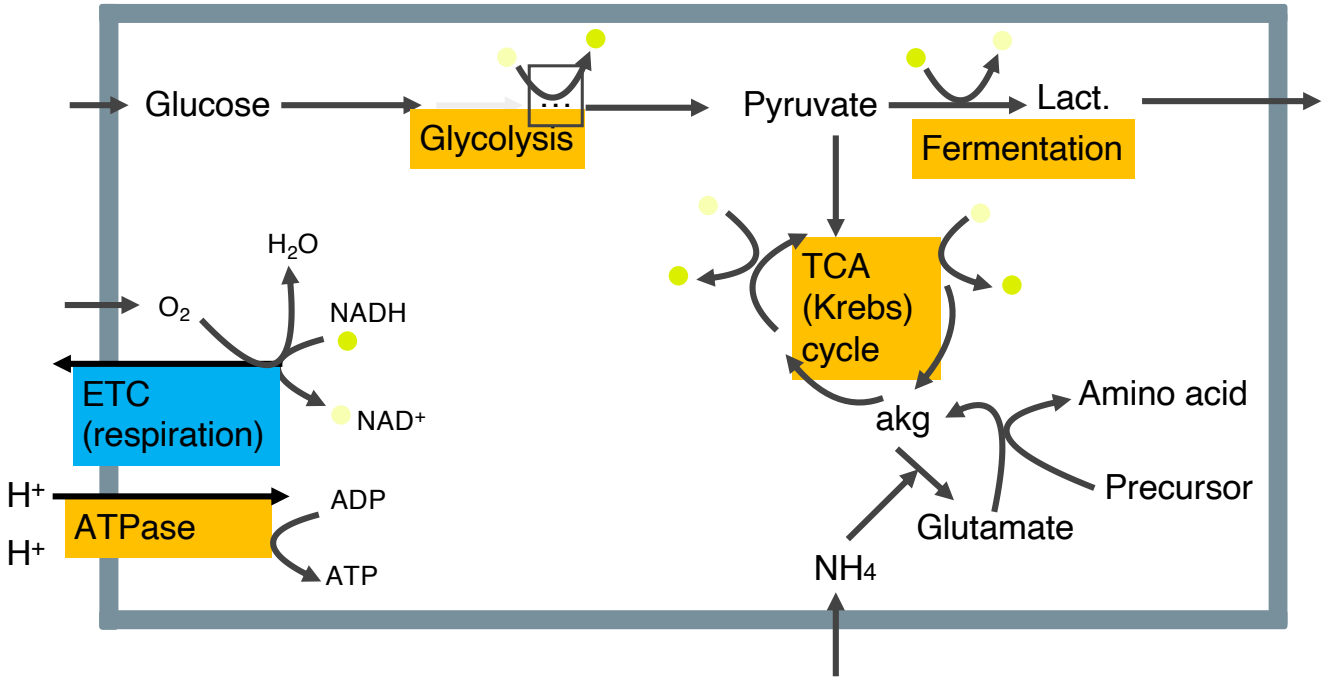
METABOLIC OVERFLOW

Metabolic flux shift under high glucose

Metabolic ‘overflow’: Shift between fermentation and respiro-fermentation in yeast, bacteria, and mammalian cells.

Warburg effect– in cancer, Crabtree effect – in yeast

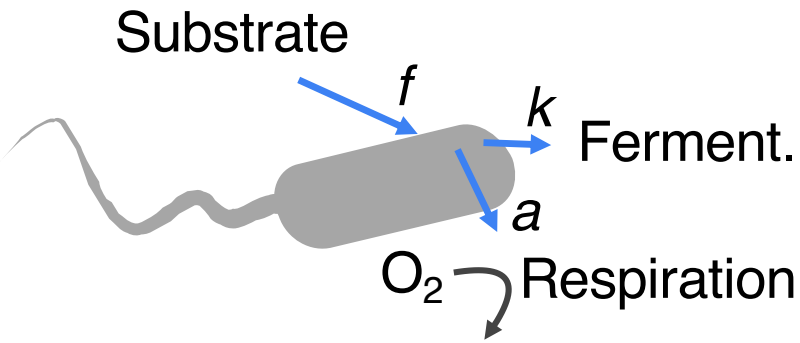




Respiration (high energy yield) vs. fermentation (low yield)

Metabolic flux shift under high glucose

<u>Simple Hypothesis:</u> Cells must 'switch' to fermentation because of **constraints on metabolic fluxes** (of respiration)



Yield: $Y = a/f$

At steady state: $f = a + k$

$Y = (f - k)/f$

Simple Constrained-Optimization View of Acetate Overflow in *E. coli*

R. A. Majewski and M. M. Domach*
Department of Chemical Engineering, Carnegie Mellon University, Pittsburgh, Pennsylvania 15213

Accepted for publication September 1, 1989

Majewski, R. A. & Domach, M. M. *Biotech. & Bioeng.* 35 (1990)

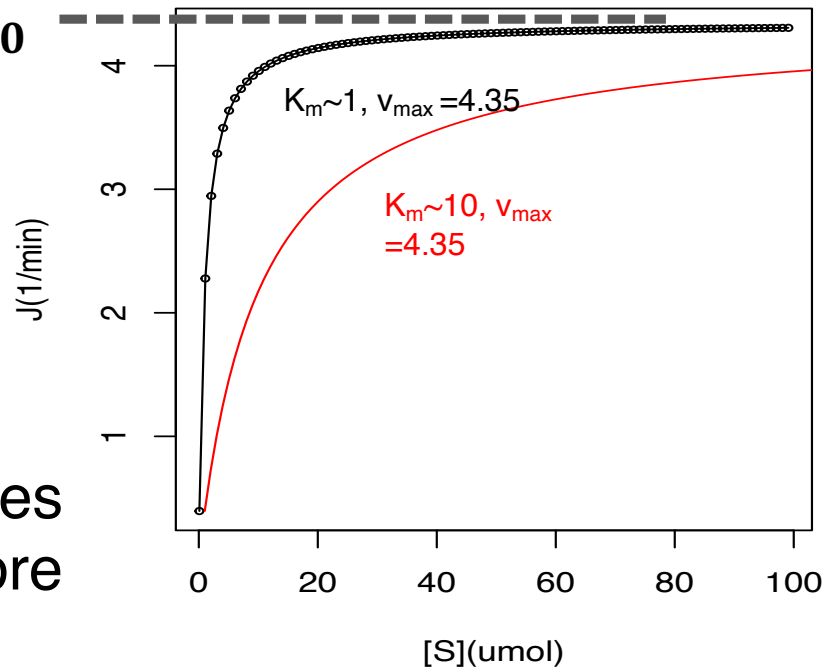
Yield is maximised by $k = 0$, but if there are limits (i.e. constraints) on a, then k needs to be non-zero as f increases

What constraints metabolic fluxes?

Flux limit due to total enzyme level

$$v_{max} = k_{cat}\, E_0$$

$$\frac{d[P]}{dt} = J = \frac{v_{max}\,[S]}{[S] + K_m}$$



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

Basan M. et al. *Nature* 528:7580, 2015

Molenaar, D. *Mol Syst Biol* 5 (2009)

Data/experiment support is limited*

Davidi D. et al. *PNAS* 113:12, 2016

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

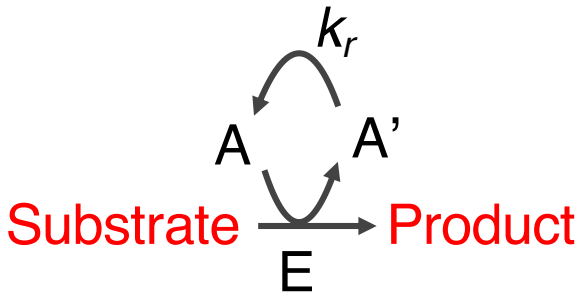
What constraints metabolic fluxes?

'ordinary' reaction

Max flux is determined by $k_{cat} * [E_{tot}]$

$v = k_{cat} * [E_{tot}] * [S] / (K_m + [S])$

Co-substrate reaction

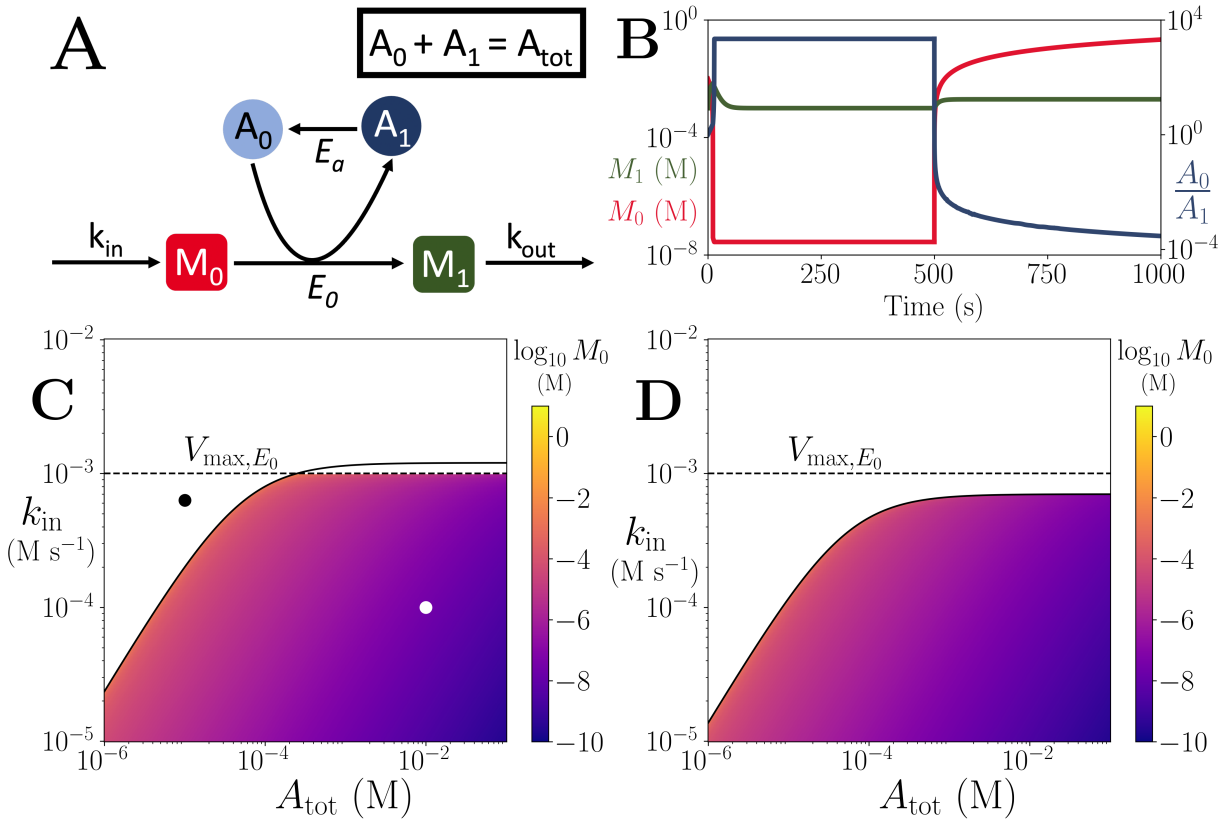


Max flux is determined by $k_{cat} * [E_{tot}]$ & k_r and A_{tot}

$v = f(k_{cat}, [E_{tot}], [A_{tot}], [k_r])$

Hypothesis: Constraints on metabolic fluxes are determined by co-substrate pools and conversion dynamics, allowing an additional layer of regulation

Co-substrate constraint on single flux



$$m_0 = \frac{\alpha\, k_{in} K_{M,E_0}}{(V_{max,E_0} - k_{in})(V_{max,E_a} A_{tot} - k_{in}(K_{M,E_a} + A_{tot}))}$$

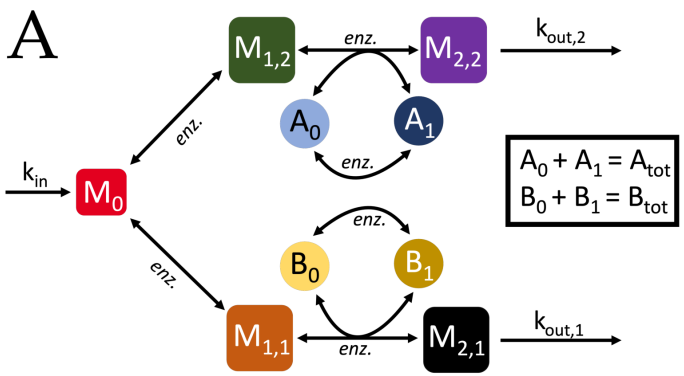
$$k_{in} < V_{max,E_0} \quad \text{and} \quad k_{in} < \frac{A_{tot} V_{max,E_a}}{K_{M,E_a} + A_{tot}}.$$

Enzyme constraint

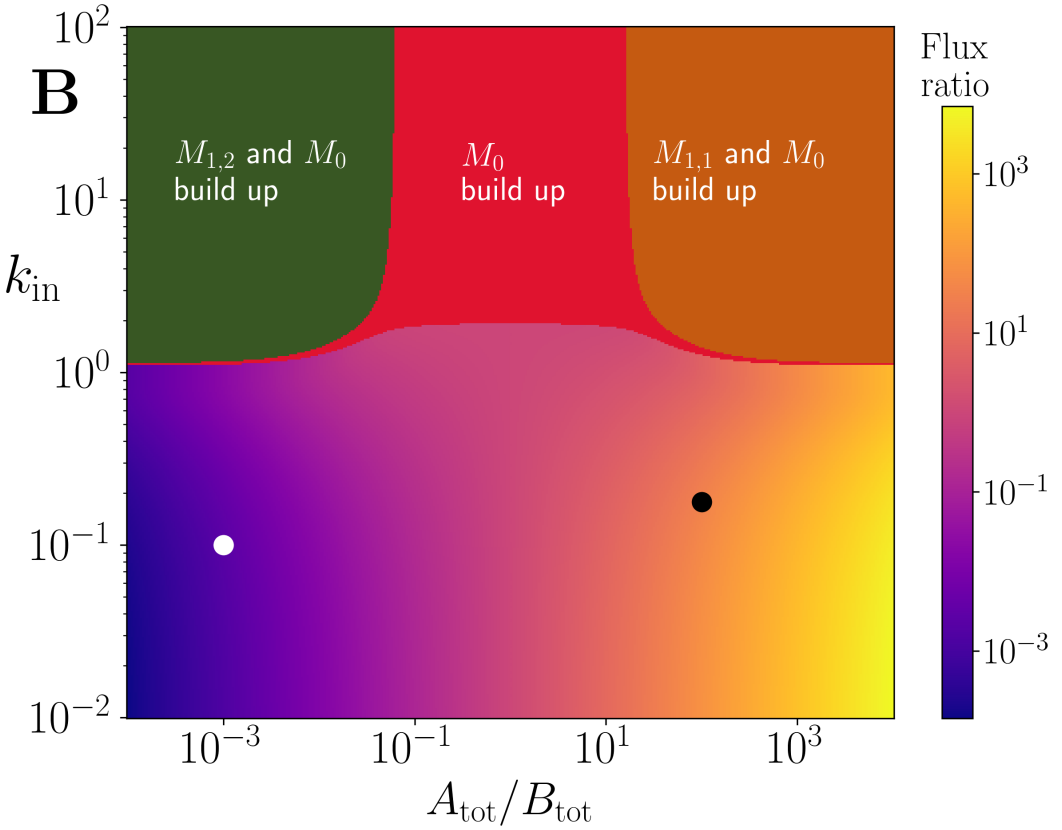
Co-substrate constraint

West, R., et al. *eLife* 19:1, 2023

Constraint = potential for control

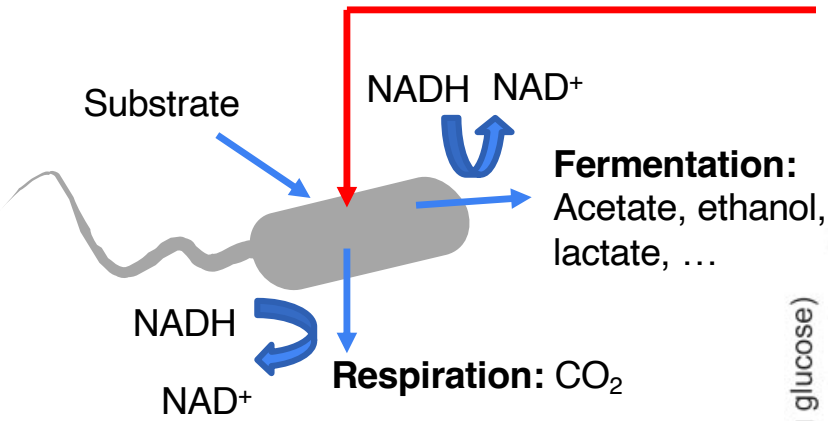


Increase A_{tot} and decrease B_{tot} to take flux to upper branch, and vice versa.

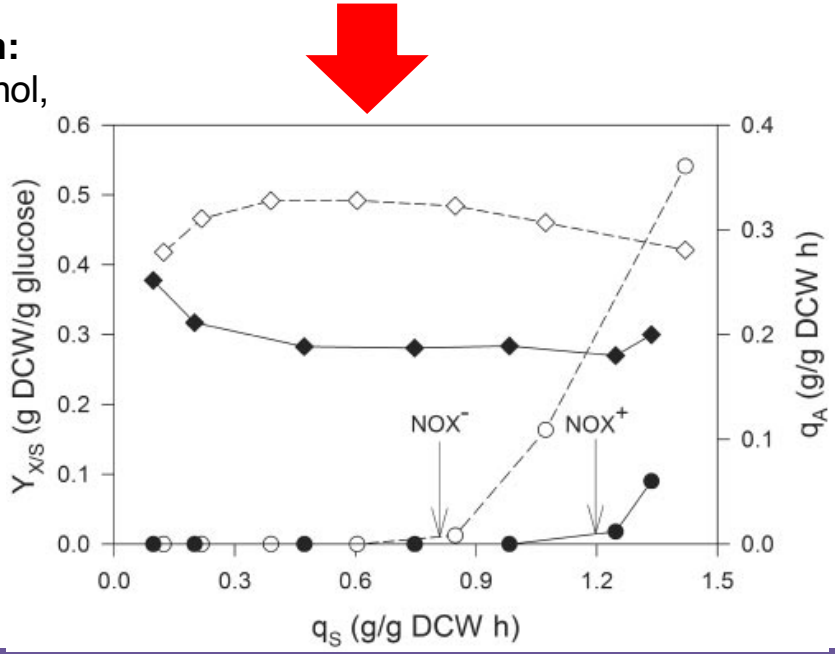


Co-substrate reactions as regulatory points

The respiration-fermentation ‘switch’ relates to NADH dynamics in *E. coli* and yeast cells:



Synthetically introducing NADH oxidising NOX gene in *E. coli* shifts overflow point!



Vemuri, G. N., *et al. Appl Environ Microbiol* 72:5, 2006

Vemuri, G. N., *et al. PNAS* 104:7, 2007

PART 3.2

BISTABILITY IN METABOLISM

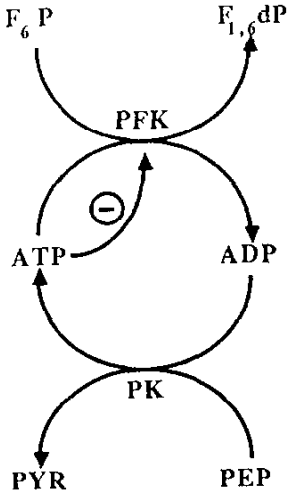
Bistability in metabolic systems

$$\frac{dN}{dt} = 0 = r \cdot N - \frac{r \cdot N^2}{K}$$

$$\frac{r \cdot N^2}{K} = r \cdot N$$

1 Steady state

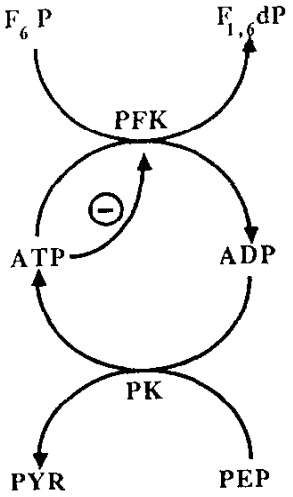
$$N = K$$



Can metabolic reaction systems lead to ODEs with **multiple steady states**?

Hervagault JF., Cimino A. *J. Theor. Biol.* 140 (1989)

Bistability in metabolic systems

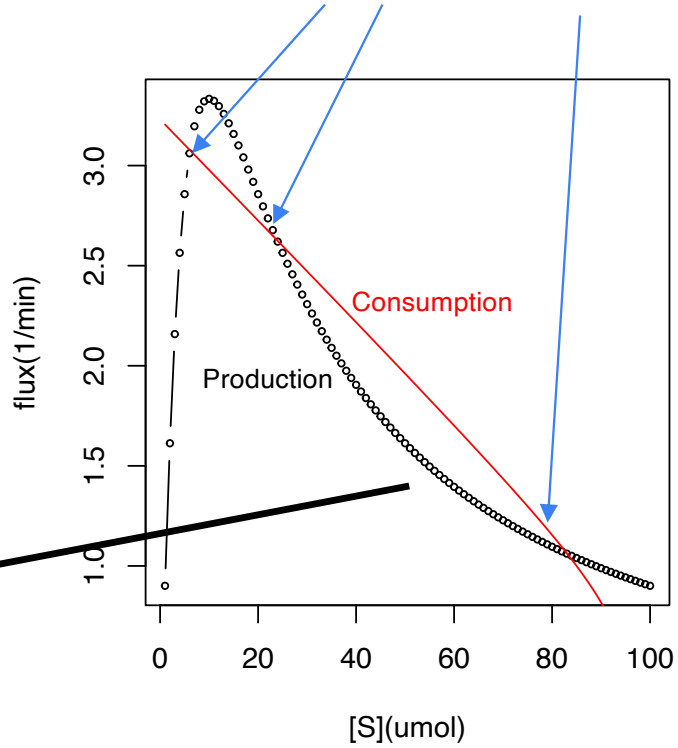


At steady state:

$$\frac{V_1 \cdot [S]}{K_1 + [S] + {[S]^2}/{K_3}} = \frac{V_2 \cdot (C - [S])}{K_2 + (C - [S])} + \alpha \cdot (S_0 - [S])$$

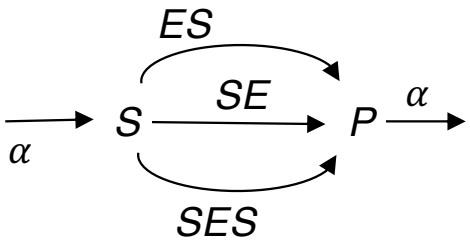
P production **P consumption**

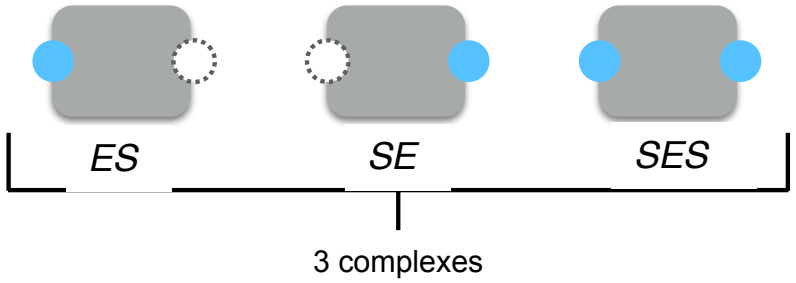
Intersections are the steady states of the system!



What are the possible biochemical basis of this nonlinearity?

Bistability in multi-site enzymes





Assumptions

$$[S_{tot}] = [S] + [ES] + [SE] + 2[SES] + [P]$$

$$[E_{tot}] = [E] + [ES] + [SE] + [SES]$$

Relaxing these does not alter the key results that follows

$$E + S \underset{b_{0,1}}{\overset{a_{0,1}}{\leftrightarrows}} ES \xrightarrow{c_{0,1}} E + P$$

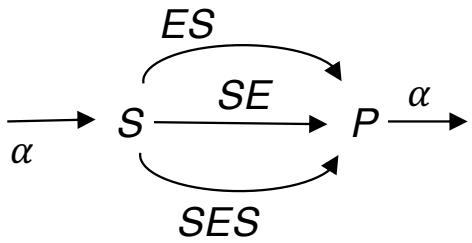
$$E + S \underset{b_{0,2}}{\overset{a_{0,2}}{\leftrightarrows}} SE \xrightarrow{c_{0,2}} E + P$$

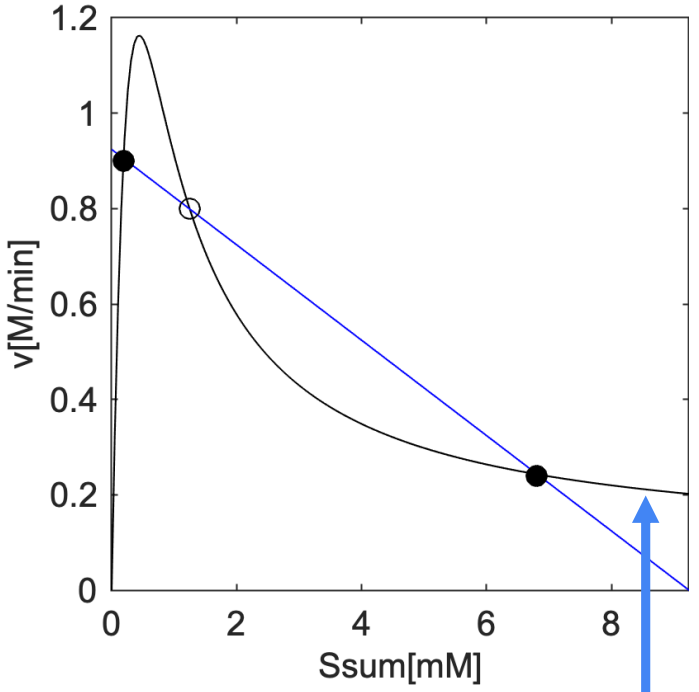
$$ES + S \underset{b_{1,[1,2]}}{\overset{a_{1,[1,2]}}{\leftrightarrows}} SES \xrightarrow{c_{1,[1,2]}} ES + P$$

$$SE + S \underset{b_{2,[1,2]}}{\overset{a_{2,[1,2]}}{\leftrightarrows}} SES \xrightarrow{c_{2,[1,2]}} SE + P$$

Hayes. C. et al. *ACS Syn Bio* (2021)

Bistability in multi-site enzymes





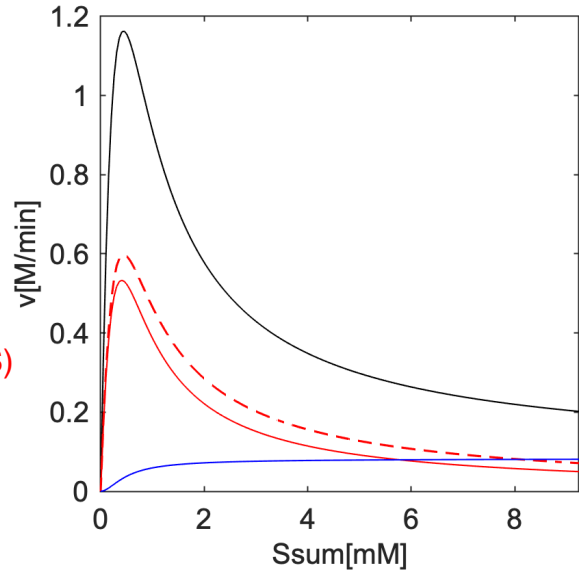
P production flux

P consumption flux

P production flux (total)

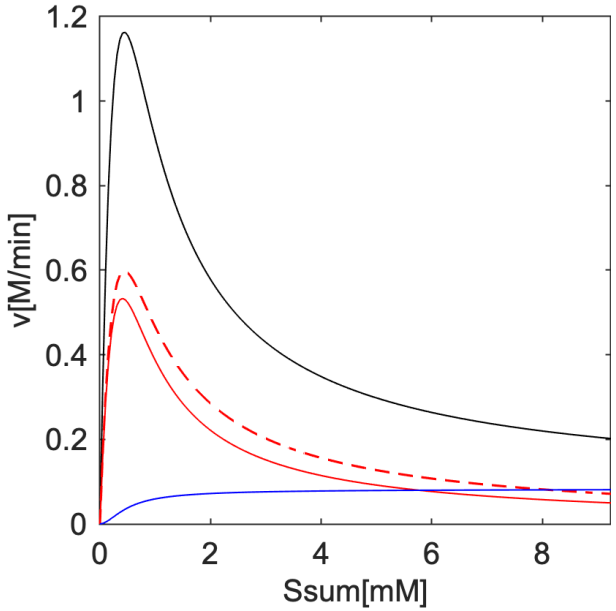
P production flux (SE and ES)

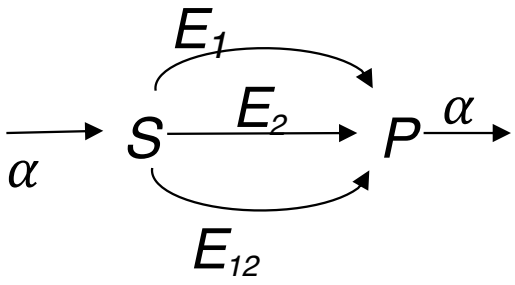
P production flux (SES)



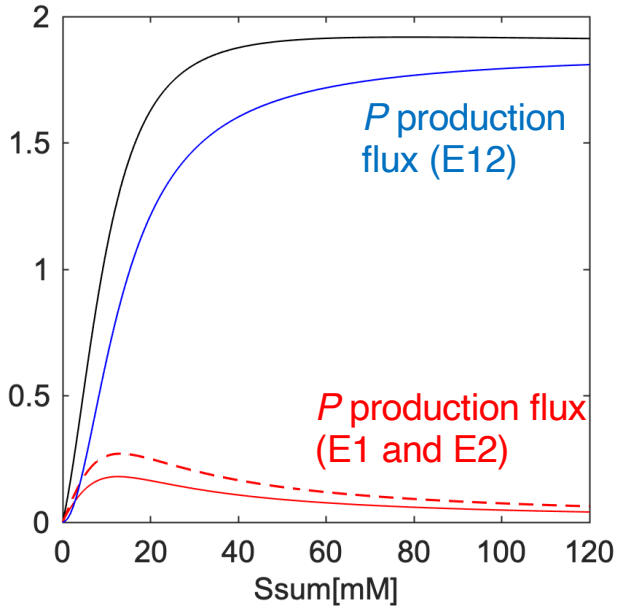
Nonlinear production flux arises from dynamics of substrate-enzyme complexes

Bistability! – from multi-site enzyme structure





P production flux (total)



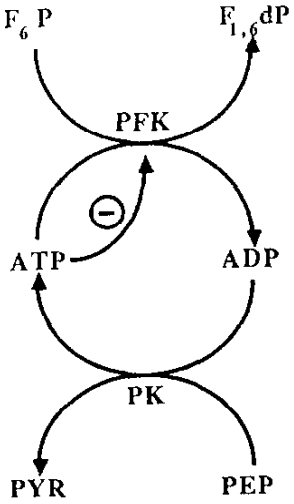
Low *E12* catalysis :
Bistable dynamics

High *E12* catalysis:
Monostable dynamics

Same conclusion as from ‘substrate inhibition’ model

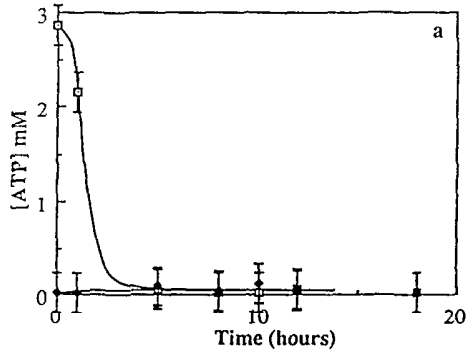
Experimental demonstration of bistability

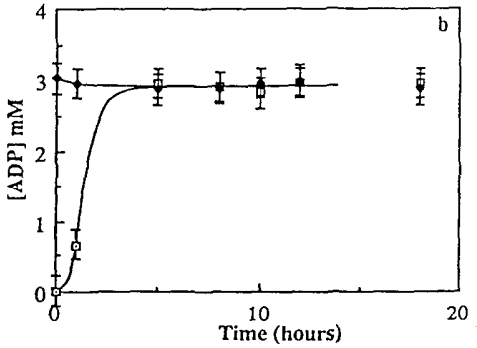
Clear experimental evidence for bistability is currently lacking. Bistability is observed, however, in enzymatic re-constitution experiments *in vitro*:

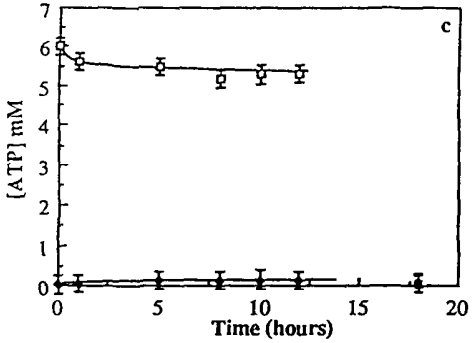


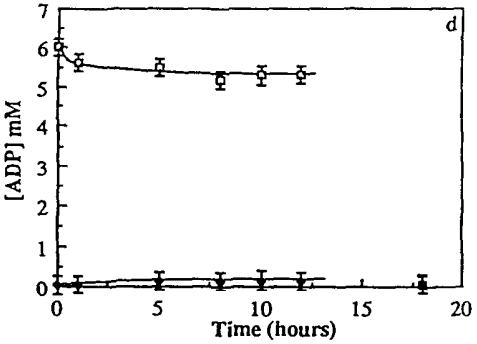
$[ATP]_{tot}$ = 3mM **One Steady State**

$[ATP]_{tot}$ = 6mM **Two Steady States**









Cimino A. & Hervagault J., *FEBS Lettr.* 263 (1990)

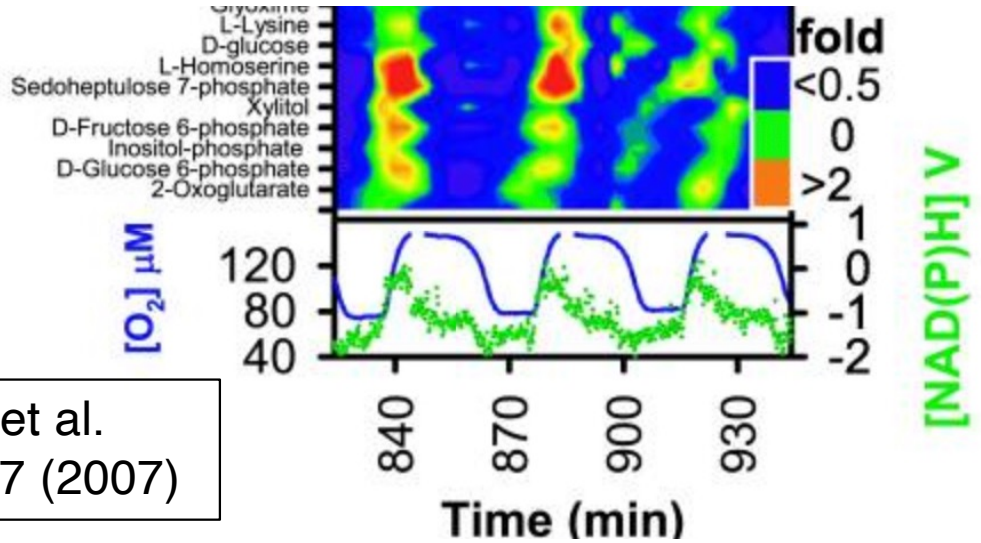
PART 3.3

OSCILLATIONS IN METABOLISM

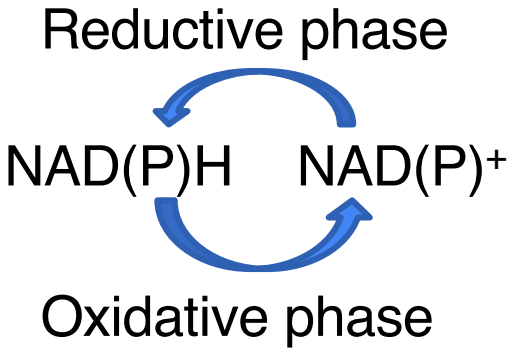
Oscillations: synchronised cells in a population

Yeast cells were grown on high glucose (20 gL^{-1} ~ 100mM + 1 gL^{-1} yeast extract), in a chemostat and the dilution rate was maintained at 0.087 h^{-1}. The population is seemingly **synchronised** under these conditions!

Truncated list….oscillations observed for most metabolites!

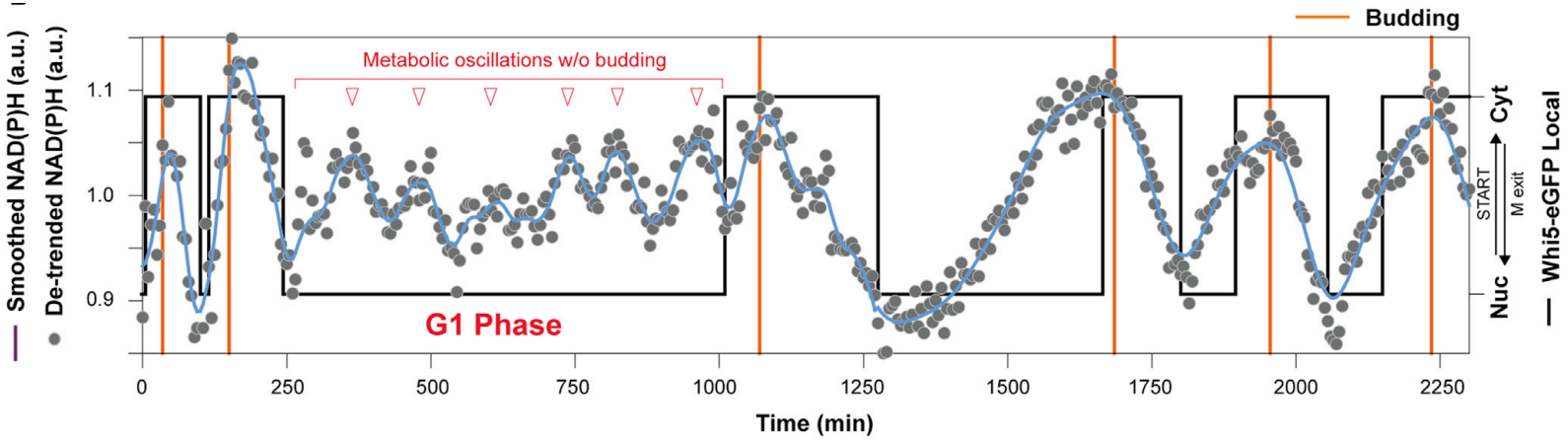


Murray, D., et al.
PNAS, 104:7 (2007)



Oscillations: single cells breathing in and out!

Metabolic oscillations in **single cells** are separate from, but coupled with, cell cycle oscillations.

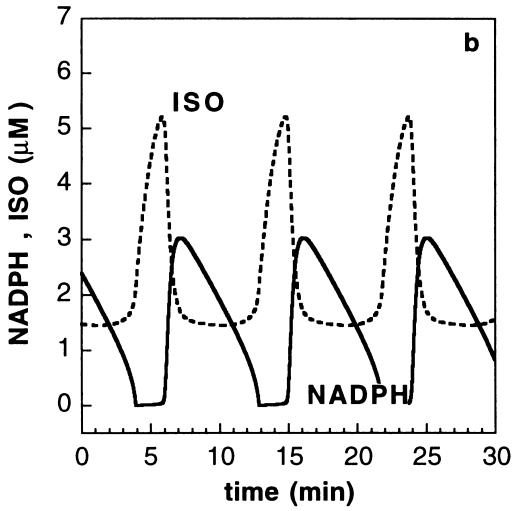


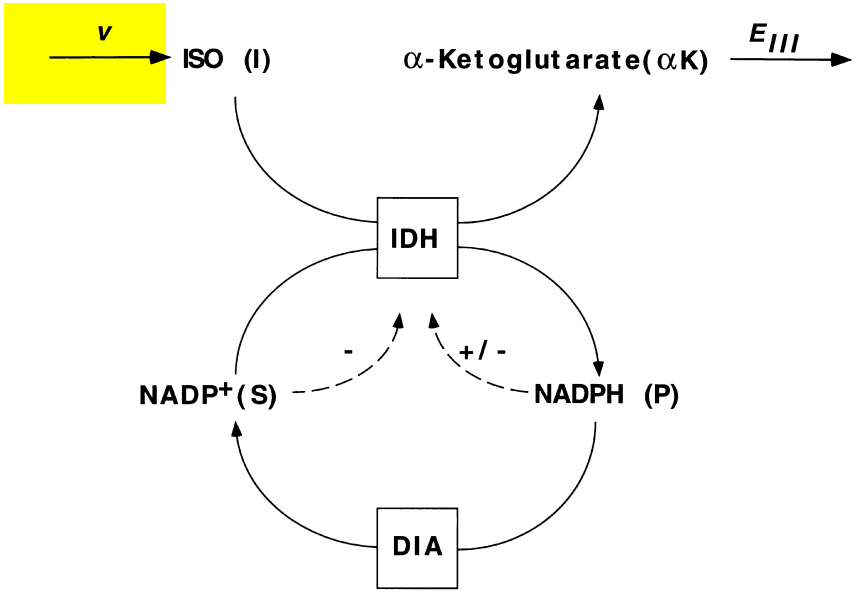
Papagiannakis, A., et al. *Mol Cell*, 65:2 (2017)

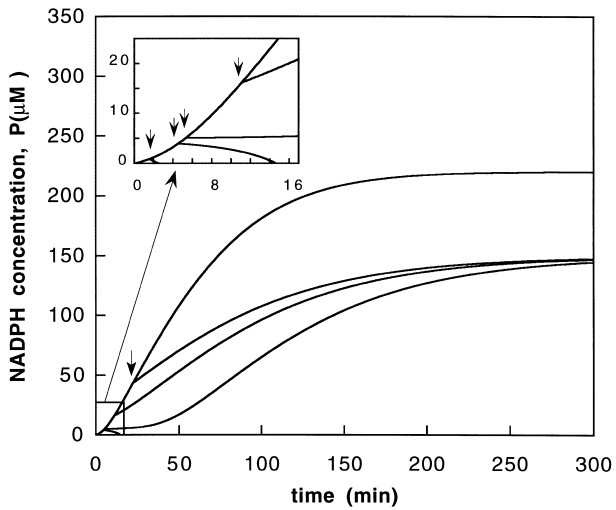
Yeast cells were grown on high glucose (10 gL^{-1} ~ 50mM). Single cell analysis in the absence of synchronization.

Oscillations: Many models can do it. Jury is out

Similar, cyclic motif as before, but with two allosteric regulation points:







High *v* gives rise to oscillations

Guidi G.M., Goldbeter A. *Biophy. Chem.* 72 (1998)

Low *v* gives rise to bistability

PART 4

SUMMARY & OUTLOOK

Summary

Metabolic systems are capable of **rich dynamics**, including bistability, oscillations, and hetereogenity.

These dynamic features are ‘expressed’ under some conditions and can **determine cell physiology** and higher level functions (e.g. dormancy).

ODE **models and assumptions can give us insights** independent of experimental data or explain specific experimental dynamics.

Multiple models can result in same behaviors and is **not always possible to distinguish** or disentangle these alternative explanations from each other.

The condition dependency of metabolic behaviors makes it important that each experimental finding is considered **in the context of the experimental setup** used.

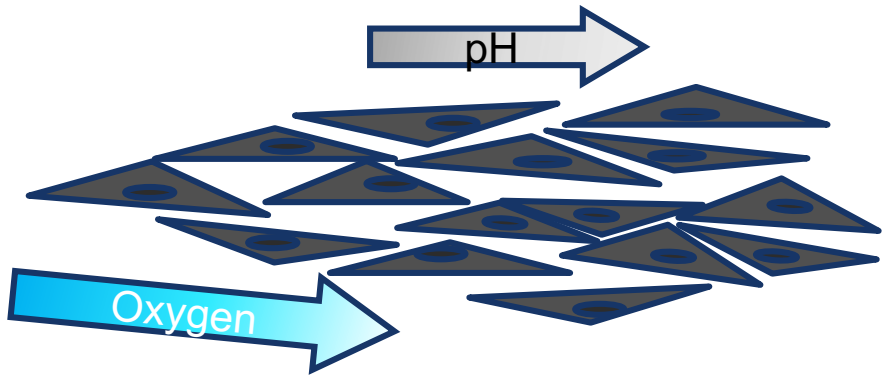
SOME OPEN AREAS OF INVESTIGATION

Bistability I Oscillations

Temporal flux / metabolite measurements

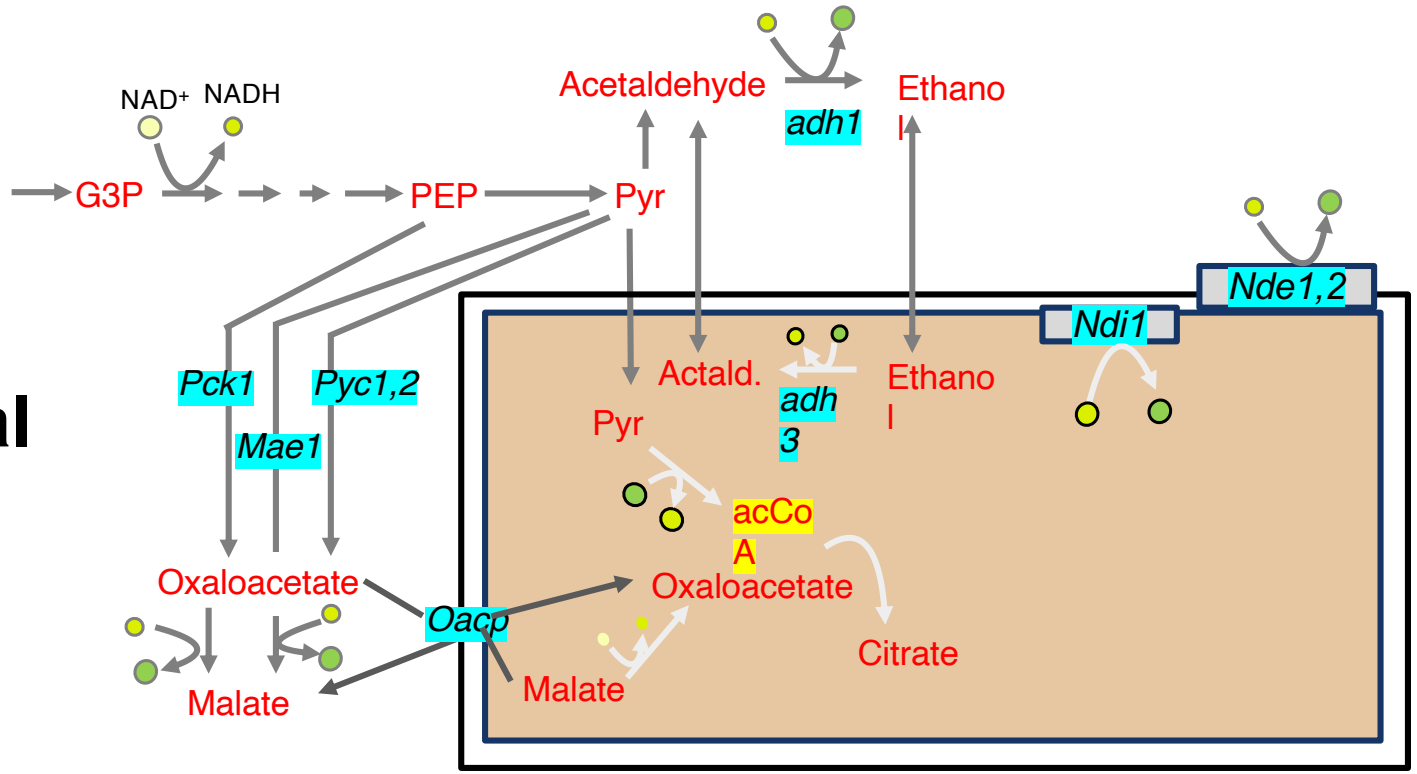
Compartments – how to combine metabolism, membrane potential, ionic fluxes, pH

Metabolism – microenvironment feedbacks



SOME OPEN AREAS OF INVESTIGATION

Compartments
Membrane potential
pH
….



Thank you for listening

Looking for PhDs & Postdocs

O.Soyer@warwick.ac.uk

Cell metabolism, spatial organization, microbial communities

OSS LAB

http://osslab.lifesci.warwick.ac.uk

Mary Coates
Sarah Duxbury
Kelsey Cremin

Robert West
Sonal

Collaborators: Marco Polin, Sebastien Ragidaeu, Chris Quince, Wenying Shou

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BBSRC bioscience for the future

GORDON AND BETTY MOORE FOUNDATION

PART 5

EXERCISES & EXTRAS

Additional reading and resources

Core reading:

- Ch. 1 in “Nonlinear Dynamics and Chaos with Applications to …”, Strogatz, S. Perseus Books (1994)
- Ch. 1-3 in “Calculus Made Easy”, Thompson, S. P. The Macmillan Company (1910)
- Ch. 2 and 3 in “Mathematical Modelling in Systems Biology: An Introduction”, Ingalls, B. at: https://www.math.uwaterloo.ca/~bingalls/MMSB/Notes.pdf

Recommended reading:

- Ch. 2 and 3 in “Principles and Problems in Physical Chemistry for Biochemists”, Price N. C., *et. al.* Oxford U. Press
- Ch. 3 and 4 in “Structure and mechanism in protein science” by Fersht, A. Freeman and Company

Optional, but fun reading:

- “Textbook errors: IX. More about the laws of reaction rates and of equilibrium”, Guggenheim, E.A., *J Chem Educ* 33:11 (1956)
- “A new principle of equilibrium”, Lewis G. N., *PNAS* 11:3 (1925).
- “On the validity of the steady state assumption of enzyme kinetics”, Segel. L. A. *Bull Math Bio* 50: 6 (1988)
- “A note on the kinetics of enzyme action”. Noor E. Flamholz, A., et al. *FEBS Lett* 587:17 (2013)
- Further chapters in Thompson’s and Strogatz’s books.
- “The growth of bacterial cultures” by Jacques Monod (Nobel laureate, 1965).

Optional resources:

Mathematical systems biology models: http://www.ebi.ac.uk/biomodels-main/
BRENDA database: www.brenda-enzymes.org
Database for models and experimental data**:** https://datanator.info

Questions & Exercises?

What is a ***function***? Plot the following function and consider how *y* and *x* relate to each other:

Explain the meaning of the ***derivative*** and ***slope***.

Develop an ODE model for the concentration of a protein, considering only its translation from mRNA and its degradation by proteases

What is the formula for K_{eq}? What does K_{eq} stand for, i.e what does it mean?

Can you state the 'rate based' formulation of the law of mass action? Can you explain what a 'rate coefficient' is in the context of law of mass action?

Write the ODEs for the following reactions based on reversible (irreversible) mass action models:

$$\mathrm{A} + \mathrm{B} \rightleftharpoons D$$

$$2\mathrm{A} + \mathrm{B} \rightleftharpoons D$$

Where does the following equation come from?
(the question is not to answer, but to encourage you to read more into thermodynamics – see 1st slide)

$$\Delta G = \Delta G^0 + R \cdot T \cdot ln\left(\frac{[C]^{\nu_C}[D]^{\nu_D}}{[A]^{\nu_A}[B]^{\nu_B}}\right)$$

Questions & Exercises?

What is the formula for Haldane relation? What does it stand for, i.e what does it mean?

Can you explain the assumptions made for obtaining this rate equation?

Write the reversible rate equation the following enzymatic reaction. $\mathrm{A} + \mathrm{B} \rightleftharpoons C$

Work out a model for a single substrate reaction mediated by an enzyme with two binding sites.

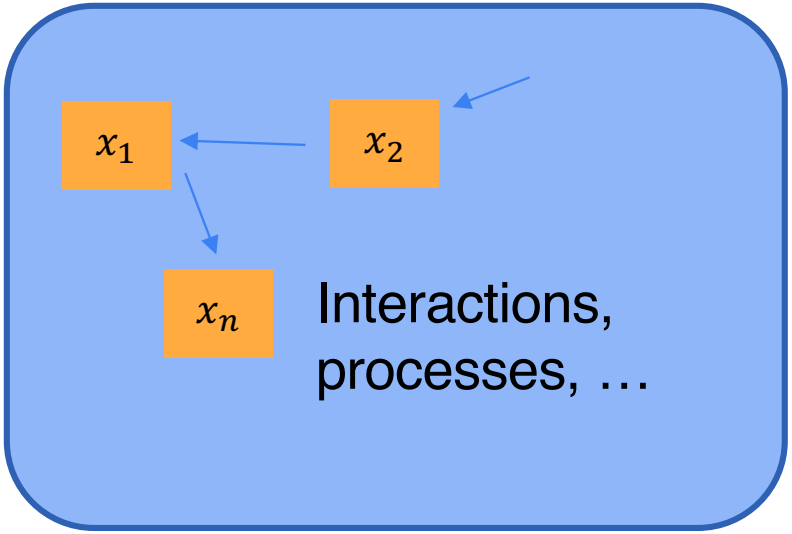
What is the 'principle of equilibrium'?
(don't have to answer for this module, but you are encouraged to take a look at the highly recommended Lewis paper!)

Can you develop a model to explain the observed oscillations in NAD(P)H?

Additional slides

Ordinary differential equations (ODEs)

System of interest



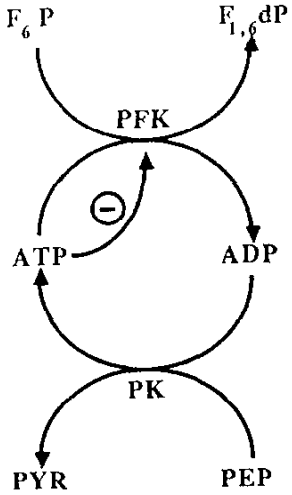
n-dimensional system of ODEs

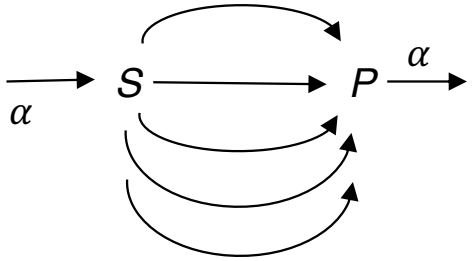
$$\frac{dx_1}{dt} = x_1' = f(x_1, x_2, \ldots, x_n)$$
$$\frac{dx_2}{dt} = x_2' = f(x_1, x_2, \ldots, x_n)$$
$$\vdots$$
$$\frac{dx_n}{dt} = x_n' = f(x_1, x_2, \ldots, x_n)$$

Multi-site enzymes and co-substrate cycles

Speculative hypothesis:

Co-substrate cycles regulate fluxes and allow for distinct 'flux states' via bi- / multi-stability





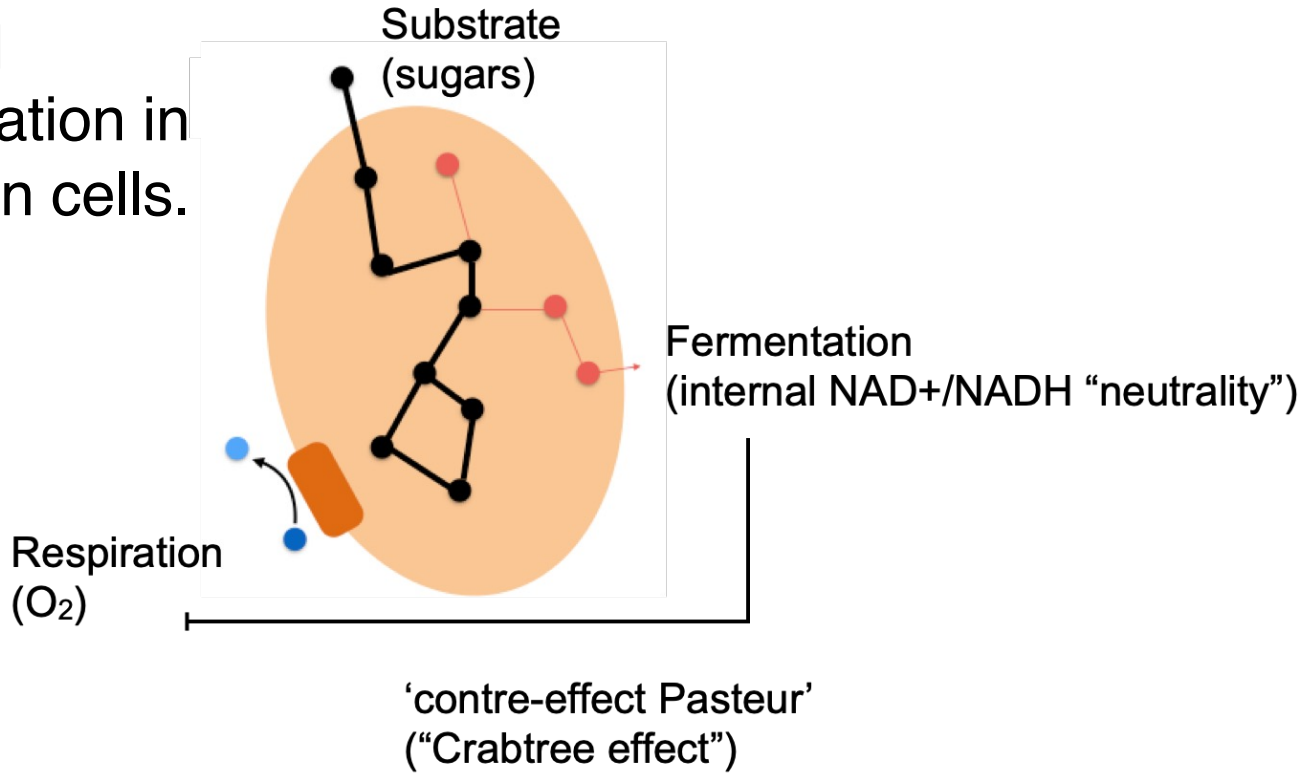
enzyme	EC number	enzyme oligomer structure	substrate (showing substrate inhibition)[a]
malate dehydrogenase	1.1.1.37	tetramer	oxaloacetate
lactate dehydrogenase	1.1.1.27	tetramer	pyruvate
D-3-phosphoglycerate dehydrogenase	1.1.1.95	tetramer	phosphohydroxypyruvate
isocitrate dehydrogenase	1.1.1.42	dimer	NADH
phosphofructokinase	2.7.1.11	tetramer	ATP

Dynamical observations – flux changes

Shift between fermentation and respiration and respiro-fermentation in **yeast**, bacteria, and mammalian cells.

$$U = Q_f - 2Q_r$$

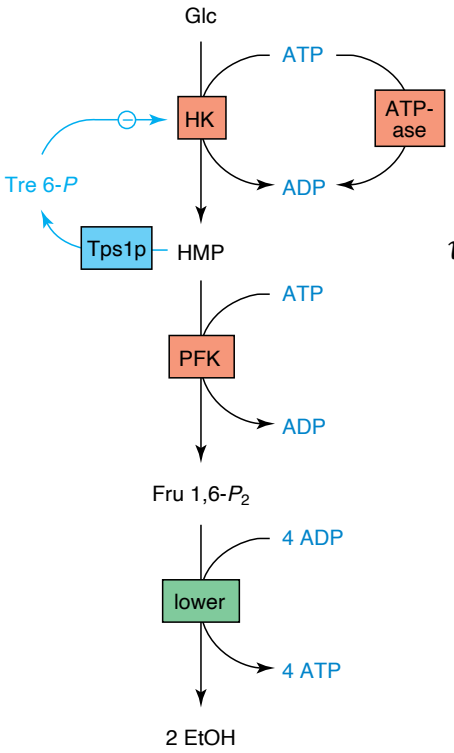
All the tumours grafted intraperitoneally show a carbohydrate metabolism conforming to that found by Warburg. A positive U, or excess fermentation, is a common property.



Crabtree H. G. *Biochem. J.*, 23 (1929)

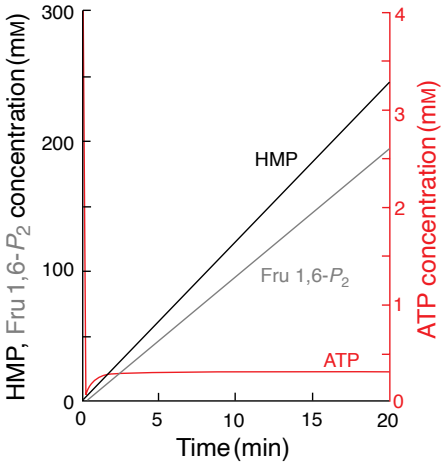
De Deken R. *J. Gen. Microbiol.*, 44 (1966)

Toy model of (upper) glycolysis



Model without feedback

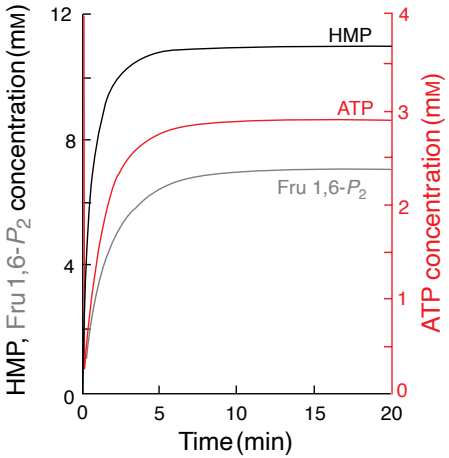
$$v_{HK} = \frac{v_{max,HK} \cdot {[Glc]}/{K_{Glc}} \cdot {[ATP]}/{K_{ATP}}}{\left(1 + {[Glc]}/{K_{Glc}}\right) \cdot \left(1 + {[ATP]}/{K_{ATP}}\right)}$$



HMP and Fru accumulate without bound!

with 'Trehalose' feedback

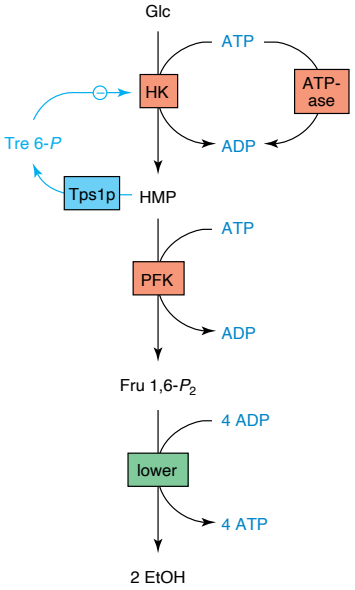
$$v_{HK} = \frac{v_{max,HK} \cdot {[Glc]}/{K_{Glc}} \cdot {[ATP]}/{K_{ATP}}}{\left(1 + {[Glc]}/{K_{Glc}} + {[HMP]^2}/{K_{Tre}}\right) \cdot \left(1 + {[ATP]}/{K_{ATP}}\right)}$$

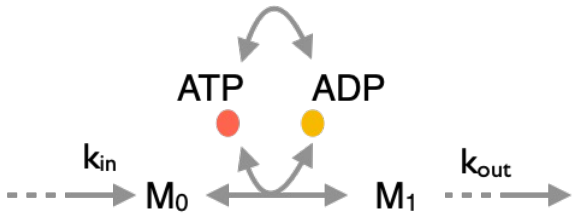


All metabolites reach steady state

Teusink. B. et al. *Trends Biochem Sci.* 23:5, (1998)

Metabolic motifs suggest constraints on metabolic fluxes



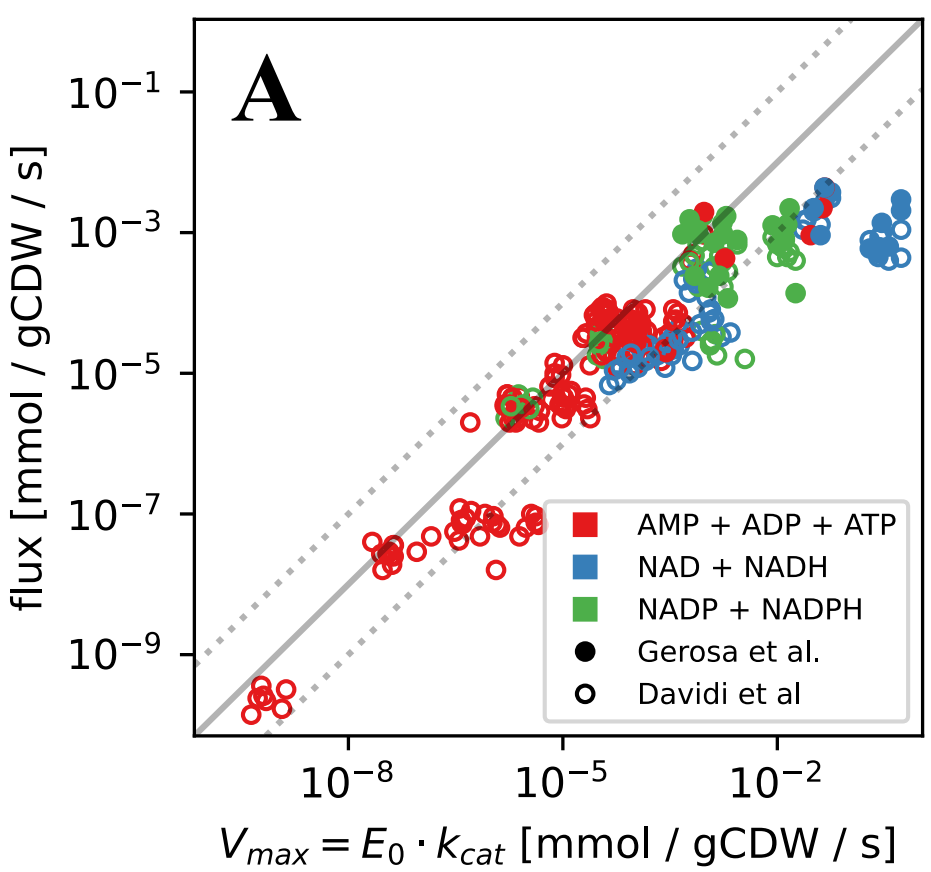


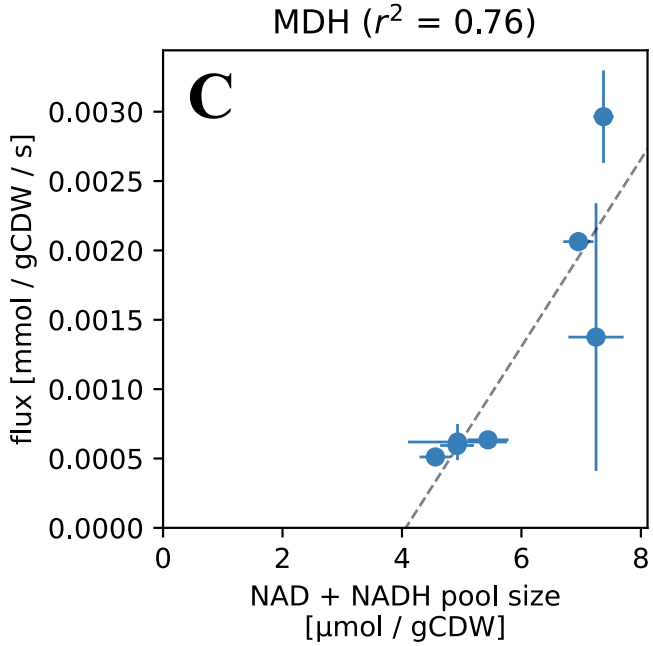
Different models, **same insight:** Avoiding metabolite accumulation **requires balance of fluxes** (i.e. enzyme capabilities)

The ability to provide a certain insight, does not necessarily require a complex model. It is a useful exercise, to ‘strip’ a model of complexity to see what elements of it lead to a specific phenomenon

Co-substrate reactions and measured fluxes

$v = \mathrm{f}\,(k_{cat}, [E_{tot}], [A_{tot}], [k_r])$



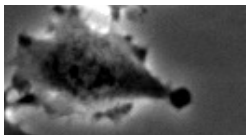


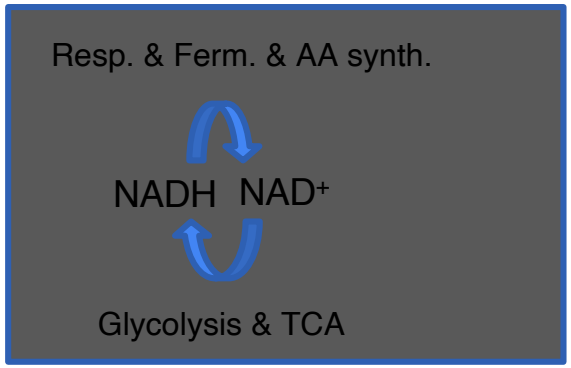
This analysis ignores substrate and thermodynamic effects, which could explain some of the low flux values

Co-substrate based regulation?

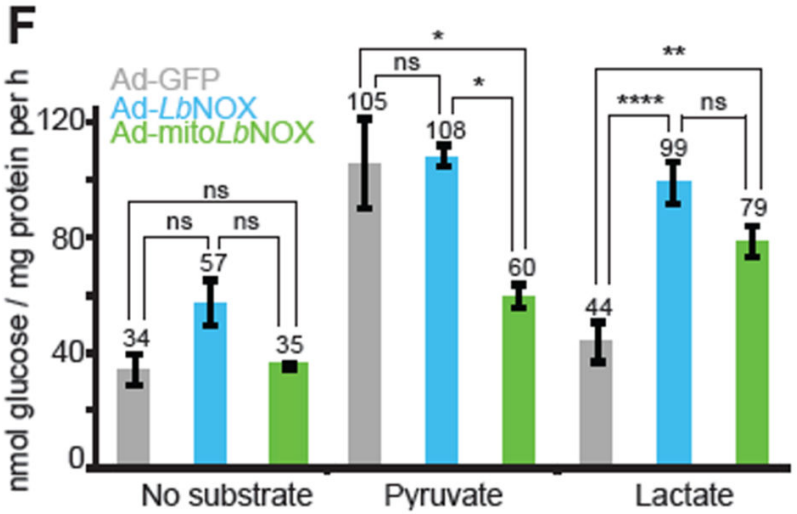
The central metabolism dynamics relate to NADH dynamics in mammalian cells:

Synthetically introducing NADH oxidising NOX gene in cytosol or mitochondria alters gluconeogenesis rate



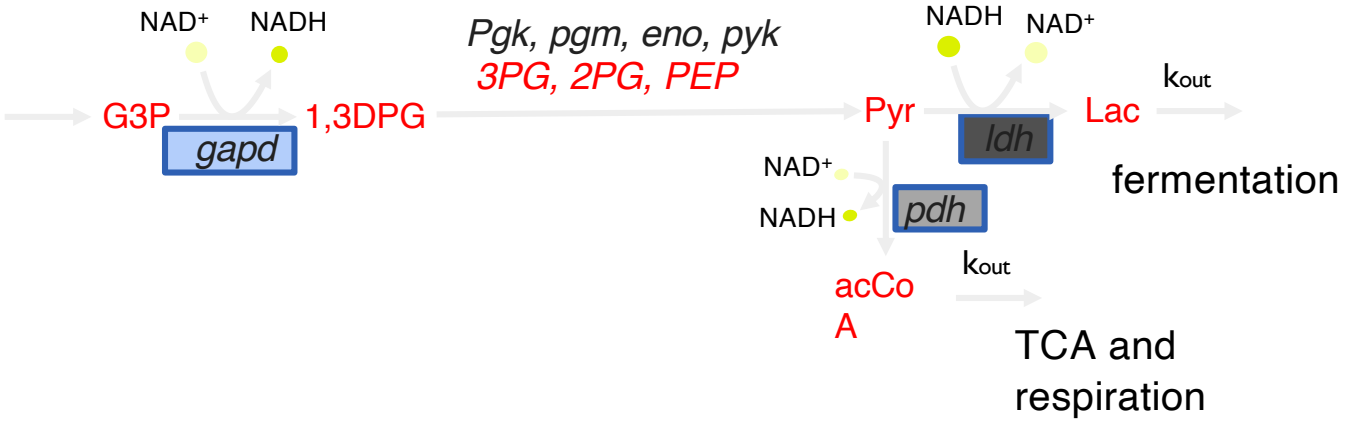


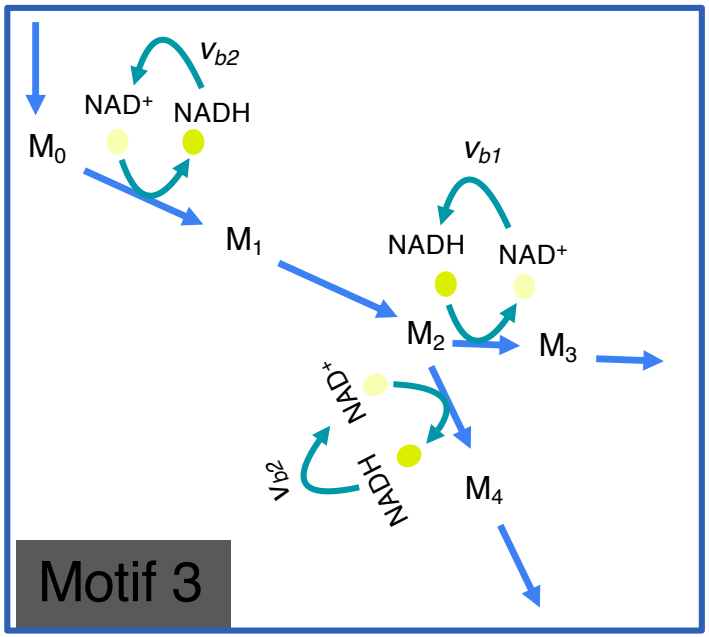
Titov, D. V., et al. *Science* 352:6282, 2016



Co-substrate based regulation?

Control of lower glycolysis and resp/fermentation branch:





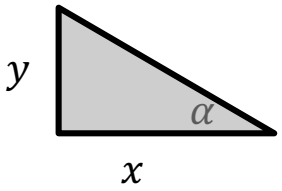
Self-regulation in metabolic systems?

"Energy metabolism of the cell: a theoretical treatise" by Reich J. G. and Sel'kov, E. E. Academic Press 1981

Calculus and dynamical systems theory

"What one fool can do, another can."
Ancient Simian(!) Proverb introduced by Silvanus Thompson

Function is a mathematical expression that states a relation between physical entities that can change, e.g. length and height of a triangle, position of a car, weight of a body. In other words, **a function defines the relation between variables**:



$$y = f(x)$$

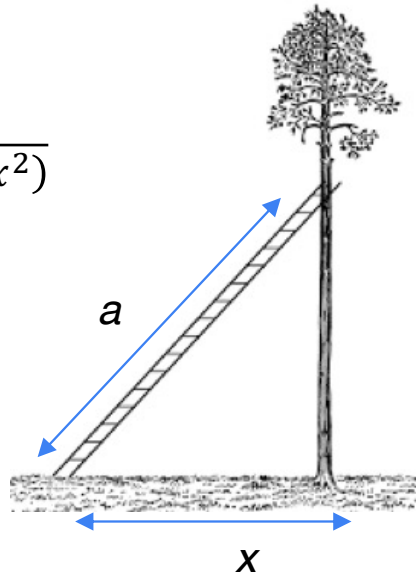
$$y = x \cdot \tan(\alpha)$$

$$y = \sqrt{(a^2 - x^2)}$$

Dependent variable

Independent variable

Constant



Calculus and dynamical systems theory

The derivative of a function simply provides the relation between a small change in one variable with regards to a small change in another. In other words, **a derivative defines the relation between <u>changes in variables</u>**:

<u>Function f(x)</u>

$$y = f(x) = x^2$$

$$dy = (x + dx)^2 - x^2$$

$$dy = x^2 + 2xdx + dx^2 - x^2$$

$$dy = 2xdx + dx^2$$

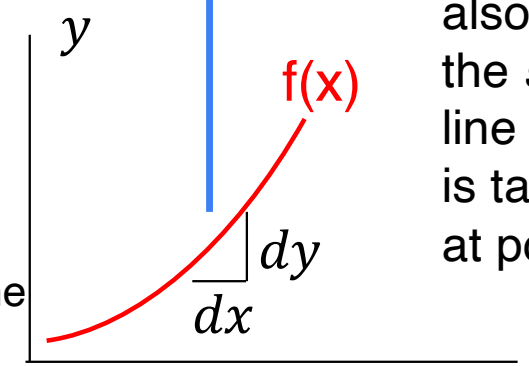
$$dy = dx(2x - dx)$$

Assume
$2x - dx \approx 2x$

The derivative is always an **approximation**! The smaller the step size, the more accurate

<u>Derivative of f(x): f'(x)</u>

$$f'(x) = \frac{dy}{dx} = 2x$$



The derivative is also known as the ***slope*** of the line segment that is tangent to f(x) at point x.

Derivative (i.e. differential equation) models

We can 'construct' differential equations, using time as an independent variable, for a system of multiple variables that all depend on time.

The 'construction' of derivatives should take into account *processes* that *affect* the variables!

An example:

$$\frac{dx}{dt} = k - a \cdot x$$

?? Can you guess how the function *t* vs. *x* would look like ??

Change in variable *x* with respect to time

A process that **increases** *x* and that has a constant value with respect to *x* and time

A process that **decreases** *x* and that has a value dependent on the value of *x* at a given time

A caution about the derivative and the numerical integration

An example and a visual help:

Function f(x)

$y = f(x) = x^2$

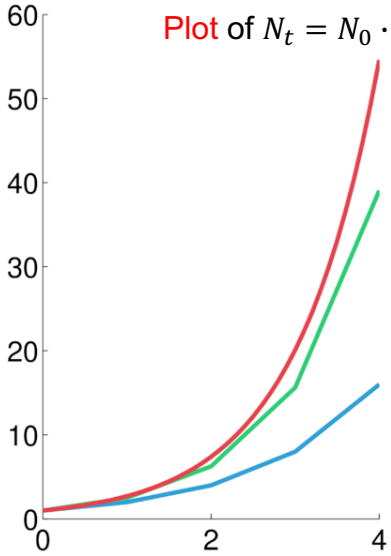
$dy = dx(2x - dx)$

Derivative of f(x): f'(x)

$f'(x) = \frac{dy}{dx} = 2x$

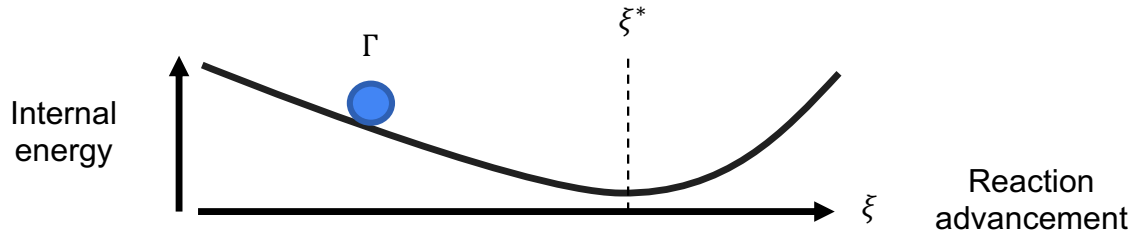
Assume $2x - dx \approx 2x$

Remember this assumption? It can, and will always, cause inaccuracies in numerical integration.



Numerical integration of $\frac{dN}{dt} = r \cdot N$.
Using Euler or Midpoint method.

Chemical reactions and thermodynamics

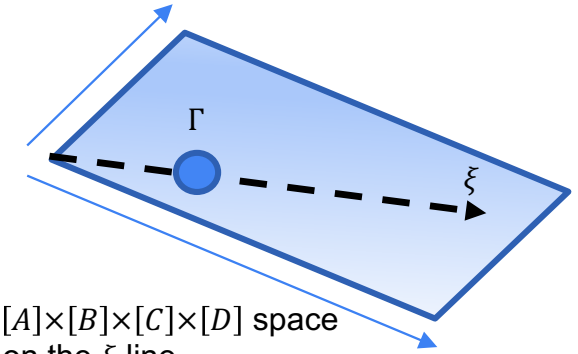


$$\nu_A \mathrm{A} + \nu_B \mathrm{B} \rightleftharpoons \nu_C \mathrm{C} + \nu_D D$$

The position of the reaction along axis ξ is usually denoted as the **mass action ratio** Γ;

$$\Delta G = \Delta G^0 + R \cdot T \cdot ln\left(\frac{[C]^{\nu_C}[D]^{\nu_D}}{[A]^{\nu_A}[B]^{\nu_B}}\right)$$

$$\Gamma = \frac{[C]^{\nu_C}[D]^{\nu_D}}{[A]^{\nu_A}[B]^{\nu_B}}$$



$$\Delta G = \Delta G^0 + R \cdot T \cdot ln(\Gamma)$$

Γ is a point in the $[A]\times[B]\times[C]\times[D]$ space instead of a point on the ξ line

A note about *assumptions*

Assumptions are usually made to achieve simpler models that are easier to understand.

Assumptions should rely on some actual physical or biochemical conditions. Hence, they have a direct relation to reality!

$$\mathrm{E} + S \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} ES \underset{k_{-2}}{\overset{k_2}{\rightleftharpoons}} E + P$$

$[E] + [ES] = E_0$ Reaction dynamics faster than gene expression dynamics

Irreversibility of step 1 or 2: $k_{-1} = 0, k_{-2} = 0$

$k_+, k_- \gg k_{cat}$ Instantaneous equilibrium of step 1: $k_1, k_{-1} \gg k_2$

$\frac{d[ES]}{dt} = 0$ Quasi Steady State of ES: $[E_0] \ll [S_0] + K_M$[1]

Segel. L. A. 1988. 10.1016/S0092-8240(88)80057-0

Reversible models and flux-force relation

$$\nu_A A + \nu_B B \underset{k_-}{\overset{k_+}{\rightleftharpoons}} \nu_C C + \nu_D D$$

$$J = k_+[A]^{\nu_A}[B]^{\nu_B} - \frac{k_+}{K_{eq}}[C]^{\nu_C}[D]^{\nu_D}$$

$$\boxed{J = J_+\left(1 - \frac{\Gamma}{K_{eq}}\right) = J_+\left(1 - e^{\frac{\Delta G}{RT}}\right)}$$

$$J_+ = k_+[A]^{\nu_A}[B]^{\nu_B}$$

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

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

$$\boxed{J = J_+\left(1 - \frac{\Gamma}{K_{eq}}\right) = J_+\left(1 - e^{\frac{\Delta G}{RT}}\right)}$$

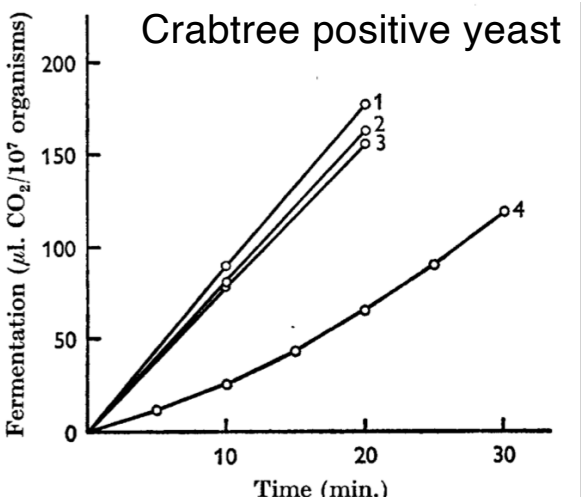
$$J_+ = \frac{[E_0] \cdot k_{cat}^+ \cdot [S]/K_S}{1 + [S]/K_S + [P]/K_P}$$

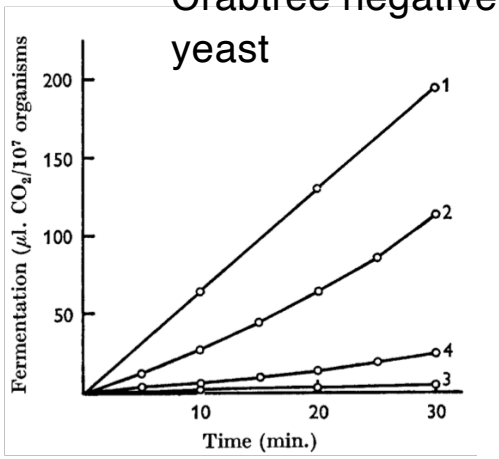
$$\boxed{\frac{J_-}{J_+} = \frac{\Gamma}{K_{eq}} = e^{\frac{\Delta G}{RT}}}$$

Flux-Force relation

D. A. Beard and H. Qian, PLoS One 2007 Vol. 2:1

Paradox of Crabtree effect?





Adaptation to a fermentative metabolism needs to happen in Crabtree negative yeast, but not in Crabtree positive yeast (unless it is fully enforced).

On the converse, Crabtree positive yeast always seems to use fermentative metabolism, even under conditions where respiration should be perfectly fine.

This is a paradox! Full respiration of glucose can generate about 20 ATP, while fermentation can generate 4. Why aren't all yeast simply Crabtree negative?