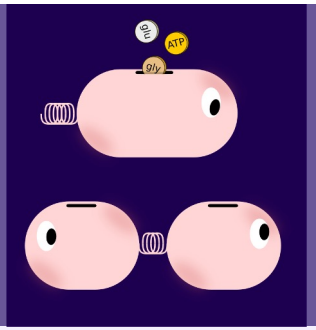
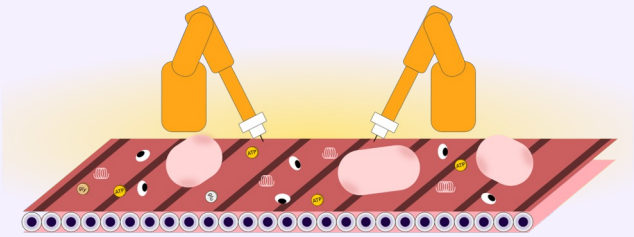
# Economic Principles in Cell Physiology

Paris, July 4-6, 2022




# Dynamics of Cell Metabolism

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



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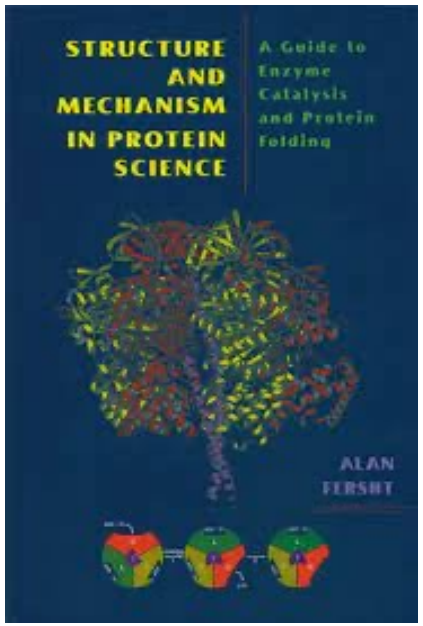
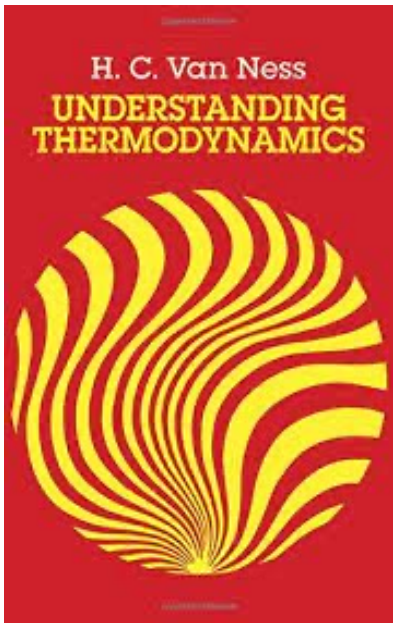
# Metabolism is chemistry, is physics, is mathematics….

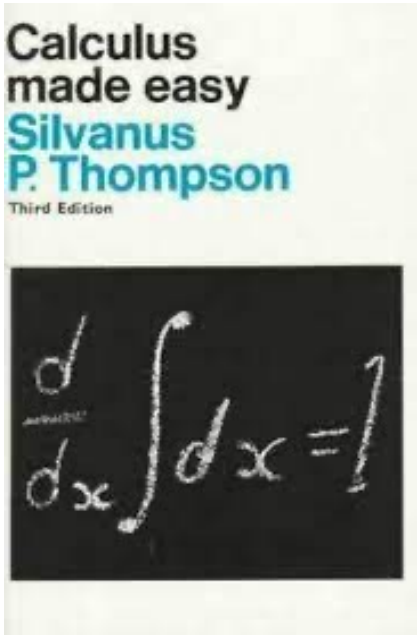
Biochemistry

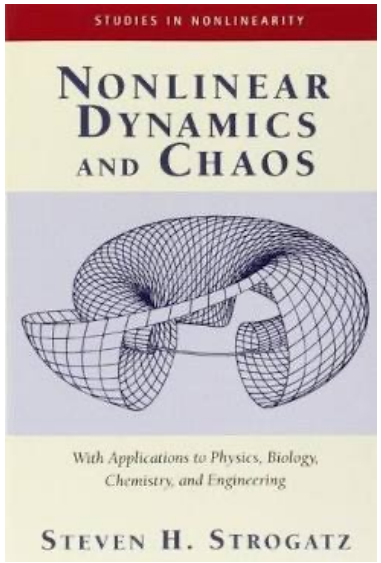



Thermodynamics



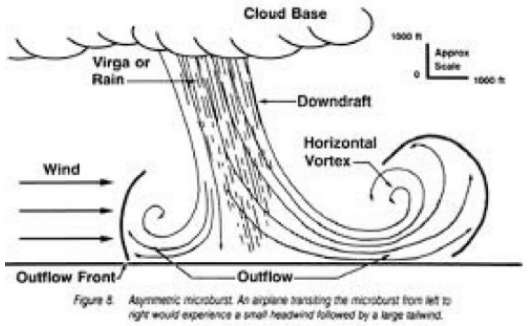

Calculus & Systems Dynamics

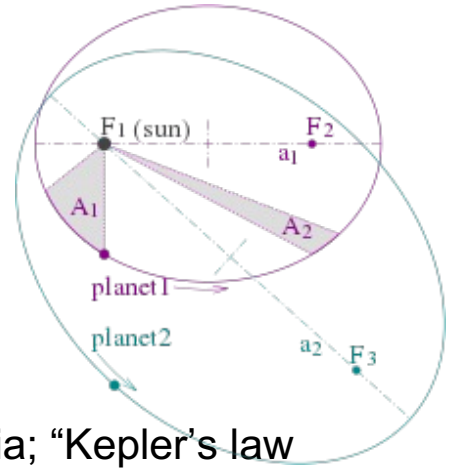


# Cell metabolism is dynamical

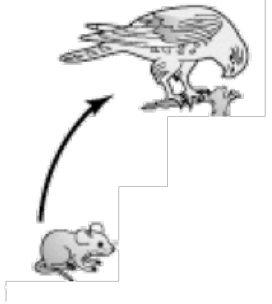
Weather - dynamic




Wikipedia; “Kepler’s law of planetary motion”

Populations - dynamic



See figure from Frenkel R, *et al* showing oscillations of various metabolites in beef extract

**Metabolite concentrations - dynamic**

Frenkel R, *Arch Biochem Biophy* 125 (1968)

# Calculus and dynamical systems theory

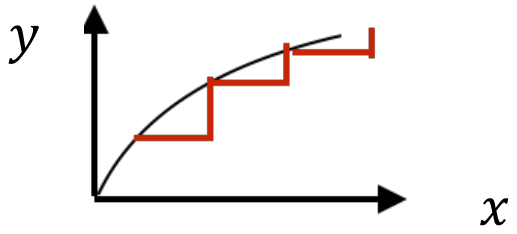
$$y = f(x) = x^2 \quad \underset{\text{integration}}{\overset{\text{differentiation}}{\rightleftharpoons}} \quad f'(x) = \frac{dy}{dx} = 2x$$

Function f(x) gives the relation between two variables

Know this and you can **calculate** the value of one variable given another!

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

Know this and you can **trace** how one variable would change given some changes in another variable!



# Time derivatives allow 'predicting' the future

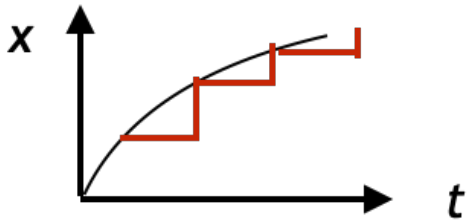
Consider we had a derivative where the independent variable is time and the dependent variable was a physical entity…

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

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

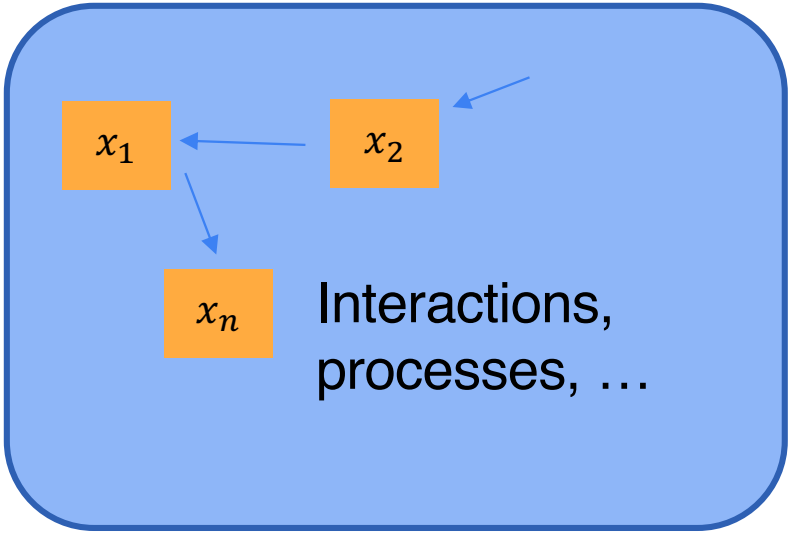
Derivative f'(x) gives the relation between small **changes in variables**

Know this and you can **trace** how one variable would change given some changes in another variable!



# Ordinary differential equations (ODEs)

System of interest




n-dimensional system of ODEs

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

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

$$\vdots$$

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

**Linear:** All the *x* on the right side appear to the first power only

**Nonlinear:** The right hand side contains products or higher powers of *x*

# My first ODE 'model' – population dynamics

A model of a mice population:

$$\frac{dN}{dt} = r \cdot N \xrightarrow{\textit{integration}} N_t = N_0 \cdot e^{r \cdot t}$$

Change in variable *N* – the population size of mice - with respect to time

A process – mating? - that **increases** *N* and that has a value dependent on the *N* at a given time…. more mice should make it easier to find a mate!

**Is this a good model?**



# A more ‘realistic’ ODE model for population dynamics

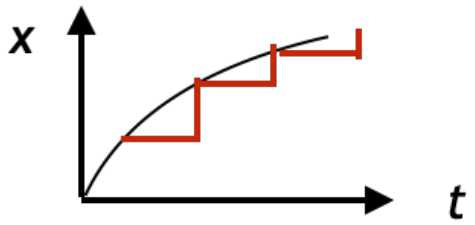
A ‘better’ model of a mice population:

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

***integration***

Not so easy!!!

Can we use ‘tracing’ ??




A new process – death? - that **decreases** *N* and that has a value dependent on the *N* at a given time…. more mice make it harder to find food!

# Time tracing – a.k.a. ‘numerical integration’

A ‘better’ model of a mice population:

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

Not so easy!!!

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

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

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

***Keep iterating***

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

# Time tracing – a.k.a. ‘numerical integration’

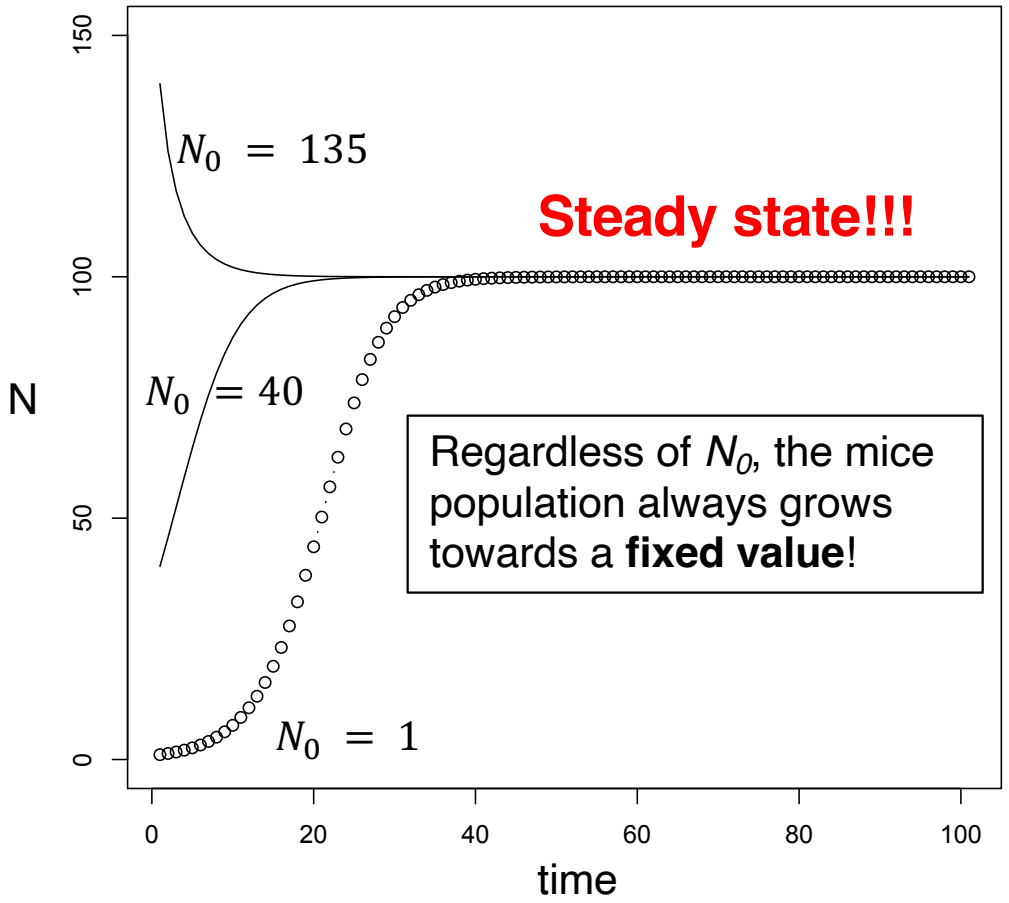
$$N_{0+dt} = N_0 + dN$$

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

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

***Keep iterating***. Computers are good at this!

Try this in MATLAB or R



# Steady state

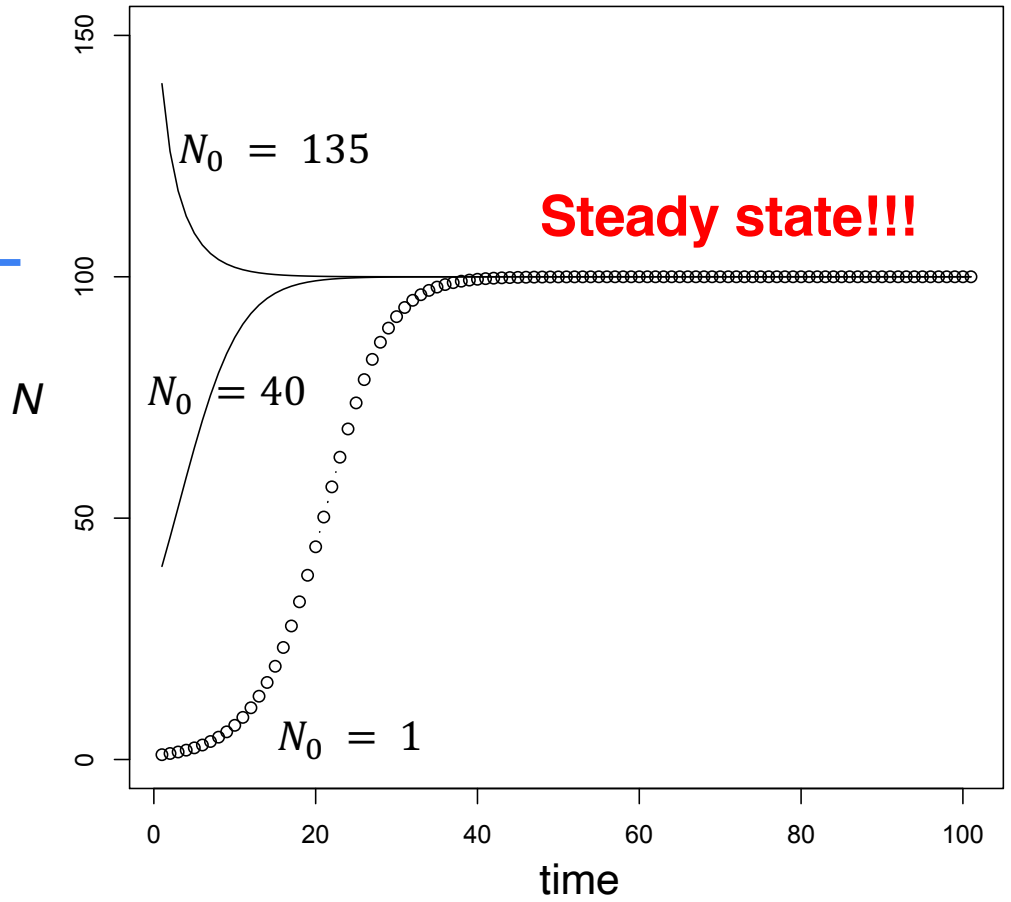
At long time, the population stabilised at a fixed value. There seems to be **no change** in *N* with time!

$$\frac{dN}{dt} = 0$$

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

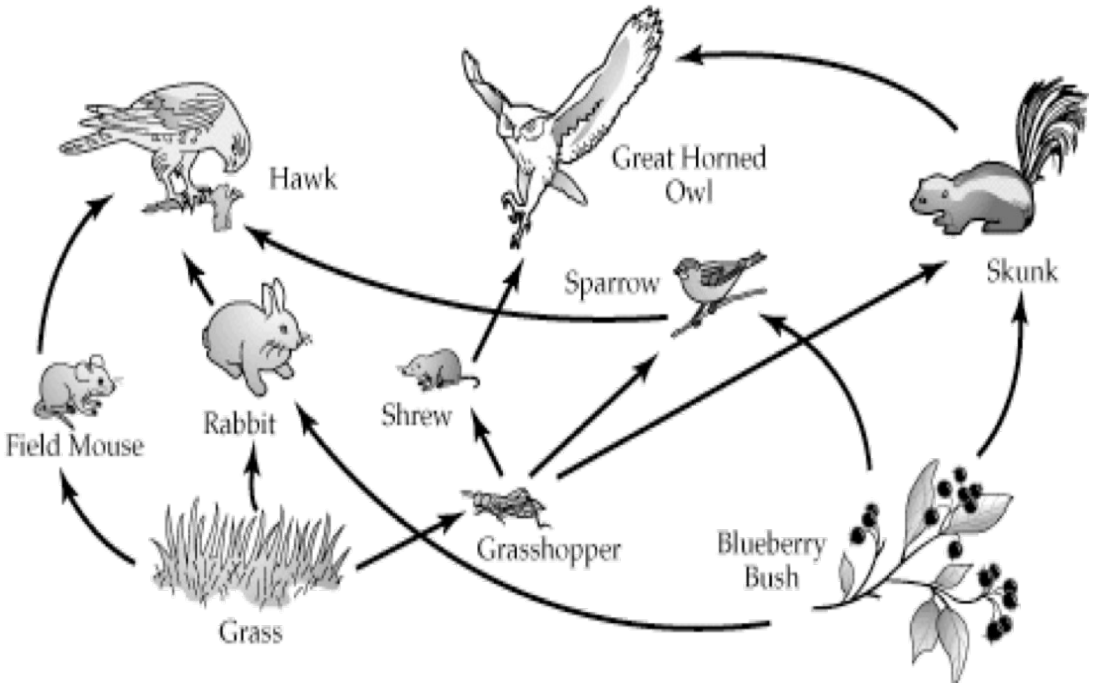
$$\frac{r \cdot N^2}{K} = r \cdot N \quad \rightarrow \quad \boxed{N = K}$$

The situation, where the differential equation is 0, is known as the **steady state**.



# Interim summary

System of interest
Interactions, processes…




'Modelling' → **ODEs** → integrate → Functional relation

**ODEs** → Numerical integration ↔ Parameter 'sweeping'

**ODEs** → Steady state analysis → Stability analysis

$$\frac{dx}{dt} = 0$$

'Insights'

# Chemical reactions and thermodynamics

A generic reversible chemical reaction….

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

'reactants' 'products'

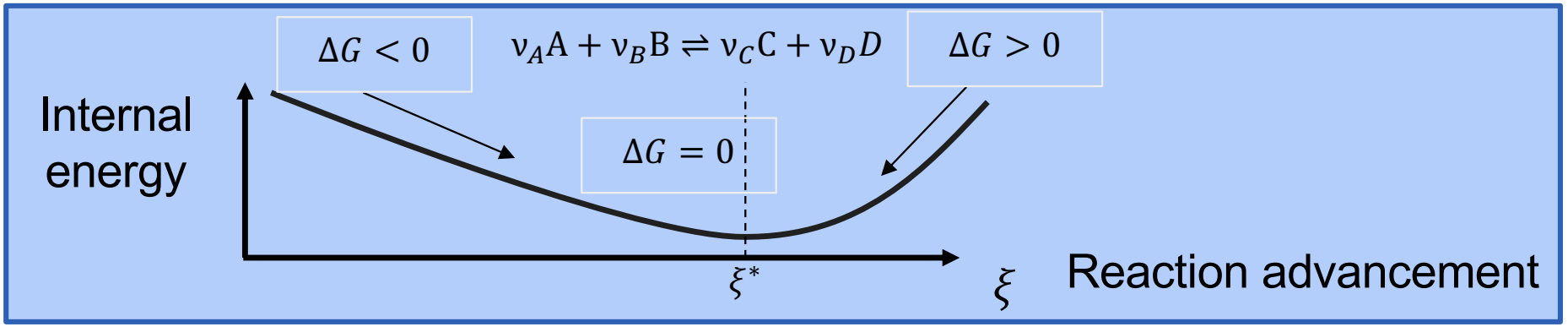
…. under constant temperature and pressure:

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

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

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

# Law of Mass Action




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

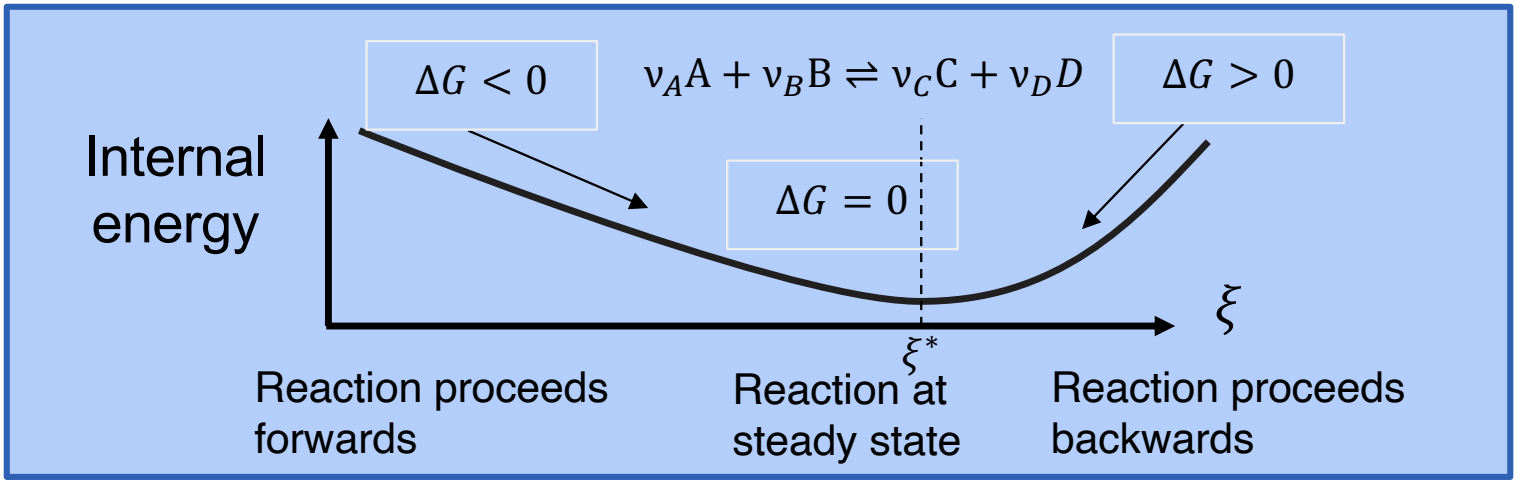
$$\Delta G^0 = -R \cdot T \cdot ln\left(\frac{[C]_{eq}^{\nu_C}[D]_{eq}^{\nu_D}}{[A]_{eq}^{\nu_A}[B]_{eq}^{\nu_B}}\right)$$

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

**Law of mass action**

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

# Law of Mass Action – A process (rate) based view




Forward reaction **rate**: $k_+[A]^{\nu_A}[B]^{\nu_B}$

Backward reaction **rate**: $k_-[C]^{\nu_C}[D]^{\nu_D}$

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

**Law of mass action**

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

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.

# Mass action model

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

Ordinary differential equations (ODEs) for this 'system':

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

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

Remember that, according to thermodynamics, $k_+$ and $k_-$ are related. We can not choose them freely!

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

***Reversible mass action model***

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

# Interim summary

A generic chemical reaction model:

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

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

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

### *Irreversible mass action model*

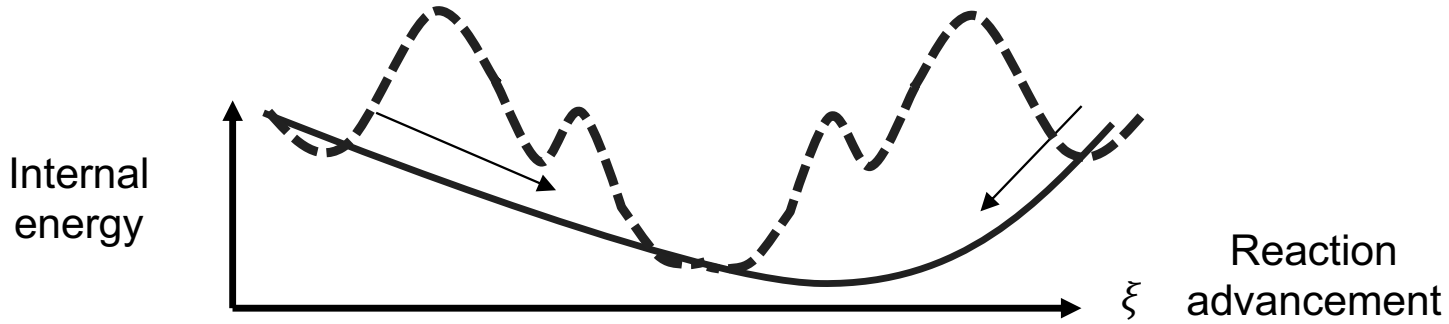
- Inconsistent with thermodynamics, but might be fine for far from equilibrium reactions.
- Net flux not zero at equilibrium.
- Flux only depends on substrates' concentrations.

### *Reversible mass action model*

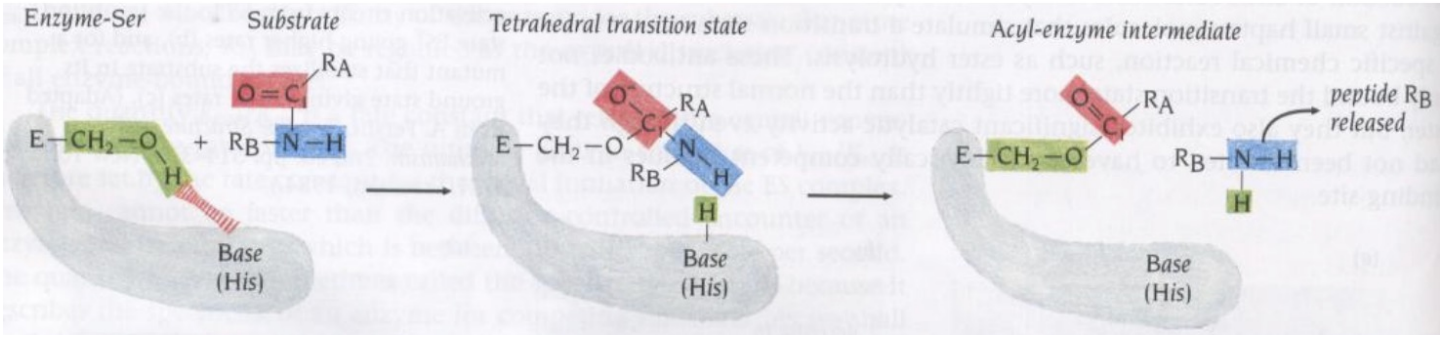
- Consistent with thermodynamics.
- Net flux reaches zero at equilibrium.
- Flux is a function of both substrates' and products' concentrations



# Biochemical reactions are **enzymatic**




Accounting for enzyme activity (function):

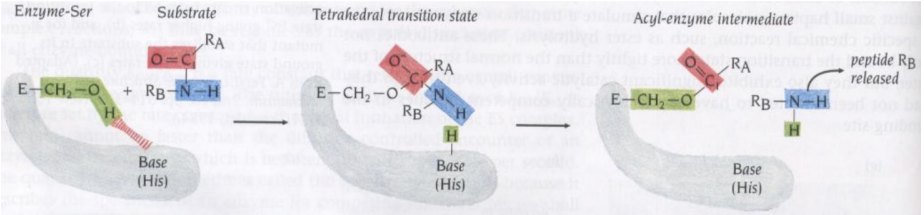



Substrate(s) and 'free' enzyme  Substrate(s) 'bound' on enzyme  Products(s) and free enzyme

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

# 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 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.* $[E] + [ES] = const.$

Outlined approach can be applied to any biochemical reaction scheme, no matter how complex!

*e.g.*

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



# Enzymatic reaction dynamics – example

1. Enzyme with single binding site and 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. Make assumptions: $k_6 = 0; k_3, k_4\ very\ large \rightarrow ES \rightleftharpoons EP\ instantenous$

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**:

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

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



# Enzymatic reaction dynamics – example

6. Make further assumptions:

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

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

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

Quasi steady state assumption:

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

Model reduction

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

$$\frac{d[P]}{dt} = k_{cat}[ES] \Rightarrow \frac{d[P]}{dt} = \frac{k_{cat} E_0 [S]}{[S] + (k_- + k_{cat})/k_+} \Rightarrow \frac{d[P]}{dt} = J = \frac{v_{max}\,[S]}{[S] + K_m}$$

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



# A reversible model of enzymatic reaction dynamics

1. Enzyme with single binding site and substrate
2. Elementary (bio)chemical reactions:
3. Make assumptions:

…..

Try this derivation!

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

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

$$\vdots$$



# A reversible model of enzymatic reaction dynamics

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

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

$$k_{cat}^{+} = \frac{k_3 k_5}{k_3+k_4+k_5};\ k_{cat}^{-} = \frac{k_2 k_4}{k_2+k_3+k_4};\ K_S = \frac{k_2k_4+k_2k_5+k_3k_5}{k_1(k_3+k_4+k_5)};\ K_P = \frac{k_2k_4+k_2k_5+k_3k_5}{k_6(k_2+k_3+k_4)}$$

**At Equilibrium:**

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

**Haldane relation**

$$\frac{k_{cat}^{+}\cdot K_P}{k_{cat}^{-}\cdot K_S} = \frac{k_1k_3k_5}{k_2k_4k_6} = \left(\frac{[P]}{[S]}\right)_{eq} = K_{eq}$$

As expected from **principle of equilibrium:**

Lewis, G.N. *PNAS* 11:3, 1925



# A reversible model of enzymatic reaction dynamics

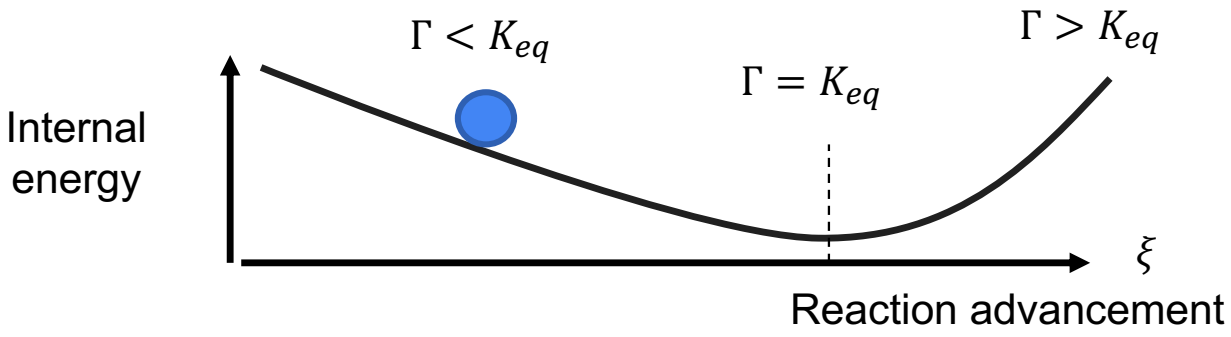
$$E + S \underset{k_2}{\overset{k_1}{\rightleftharpoons}} ES \qquad ES \underset{k_4}{\overset{k_3}{\rightleftharpoons}} EP \qquad EP \underset{k_6}{\overset{k_5}{\rightleftharpoons}} E + P$$

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

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

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

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



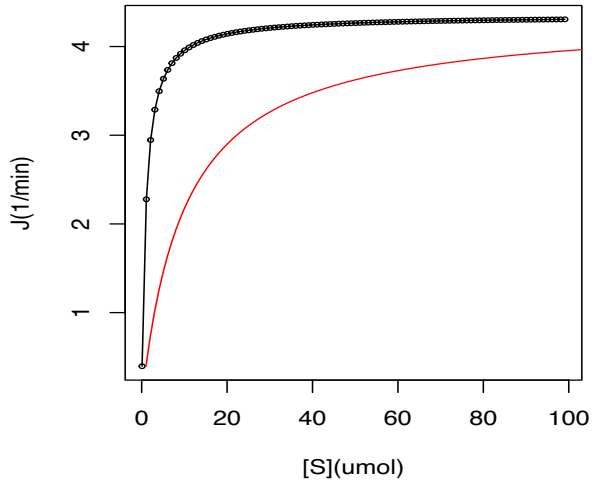

Noor et al. 2013
(10.1016/j.febslet.2013.07.028)

# Interim summary

## *Irreversible enzymatic model*

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

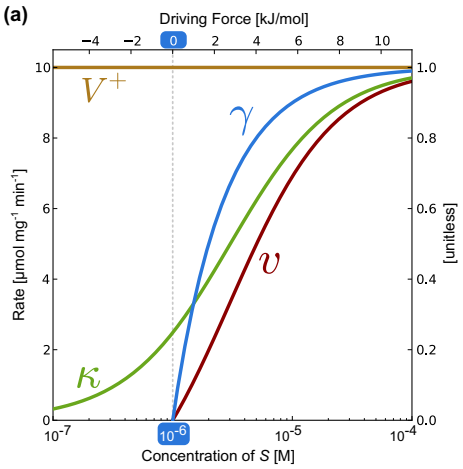
$$J = v_{max} \cdot \left(\frac{[S]}{[S] + K_m}\right)$$




## *Reversible enzymatic model*

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

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



# Modelling metabolic systems

**Toy models** mimicking aspects of metabolism

Re-occurring **motifs** and their dynamics

**Partial, but detailed, models** of specific pathways

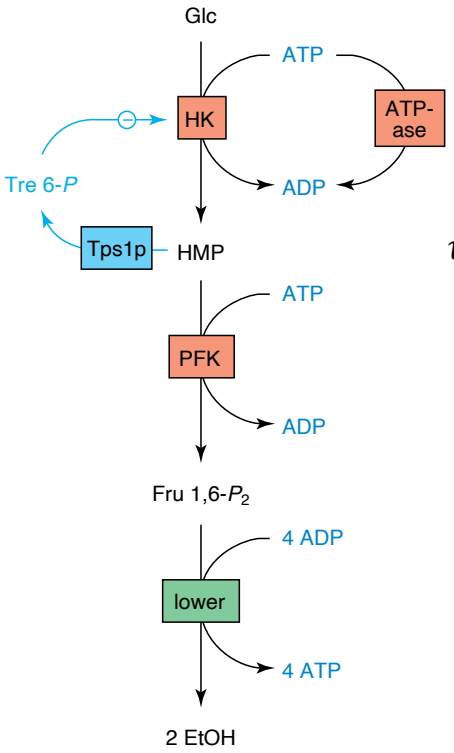
**Large-scale models** with much coverage as possible

*"All models are wrong, some are useful"*

attributed to a 1976 paper by George Box (statistician)



# Toy model of (upper) glycolysis




**Model without feedback**

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

**with 'Trehalose' feedback**

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

See Figures from van Heerden JD *et al* showing model behavior with metabolite accumulation and not.

HMP and Fru accumulate without bound!

All metabolites reach steady state

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

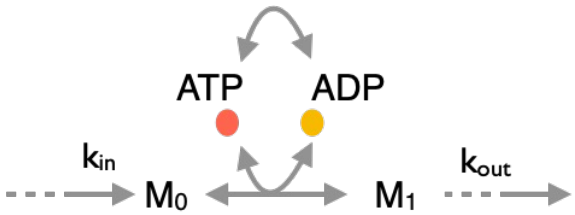


# Co-substrate cycling motif – a simpler model


$$\frac{d[M_0]}{dt} = k_{in} - \frac{v_{max} \cdot [M_0]}{K + [M_0]}$$

$$[M_0] = \frac{k_{in} \cdot K}{v_{max} - k_{in}}$$

$M_0$ accumulates towards infinity as $k_{in}$ approaches $V_{max}$




$$C = [ATP] + [ADP]$$

$$\frac{d[M_0]}{dt} = k_{in} - \frac{v_{max} \cdot [M_0] \cdot [ATP]}{K + [M_0] \cdot [ATP]}$$

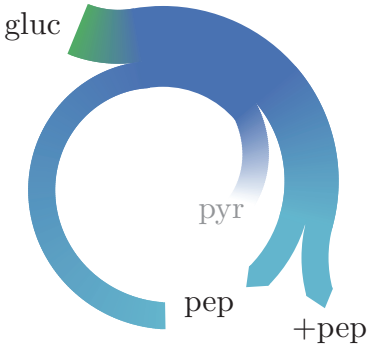
$$[M_0] = \frac{k_{in} \cdot K \cdot (k_5 + k_6)}{v_{max} \cdot (C \cdot k_6 - k_{in}) \cdot (v_{max} - k_{in})}$$

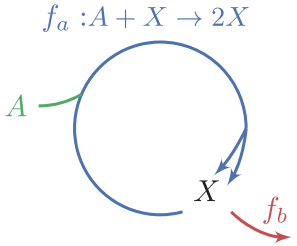
West, R. and Delattre. H. et al. *unpublished results*



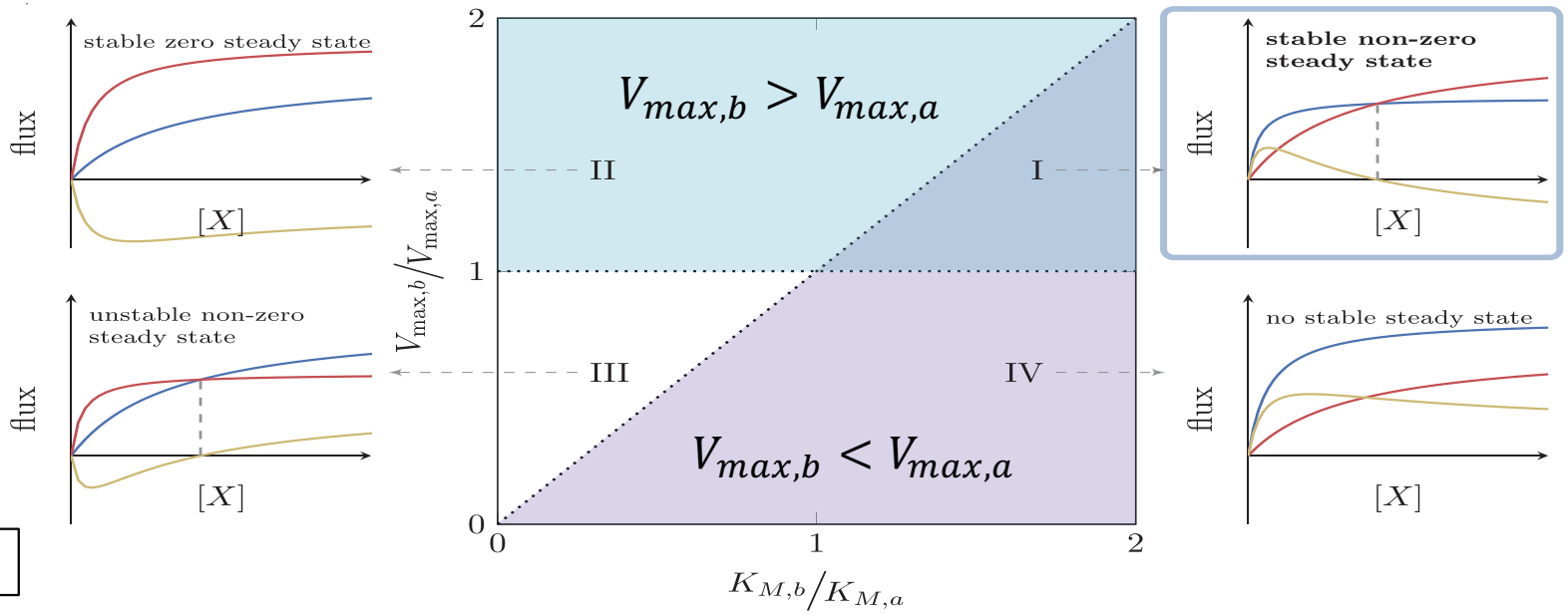
# Autocatalytic cycling motif

pep + gluc → 2 pep + pyr



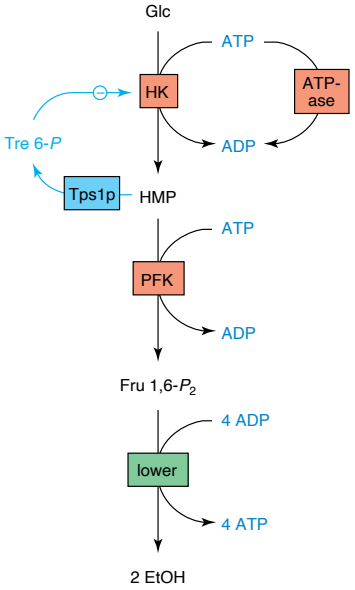



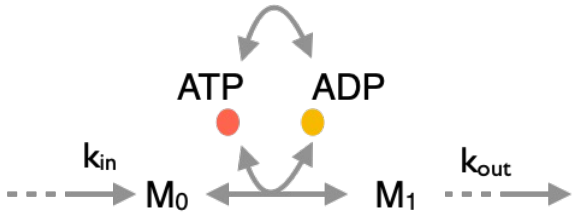

$$\frac{d[X]}{dt} = v_a - v_b \qquad v_a = \frac{V_{max,a} \cdot A \cdot X}{K_a + A \cdot X} \qquad v_b = \frac{V_{max,b} \cdot X}{K_b + X}$$

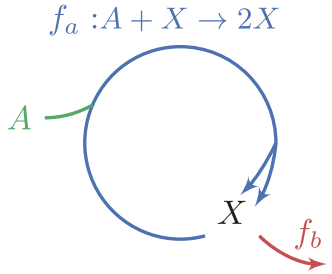



Barenholz. U. et al. *eLife* 6 (2017)

# Metabolic motifs suggest constraints on metabolic fluxes



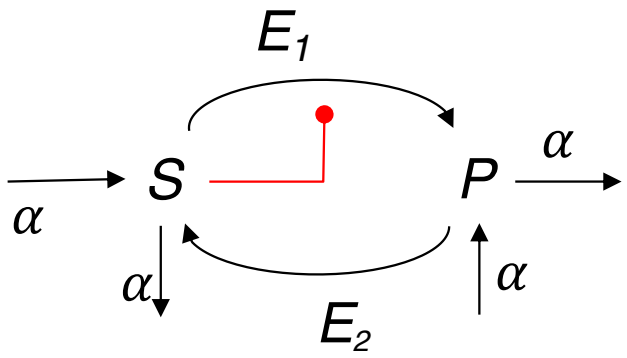




Different models, **same insight:** Avoiding metabolite accumulation **requires balance of fluxes** (i.e. enzyme capabilities)

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

# A cycle model with feedback

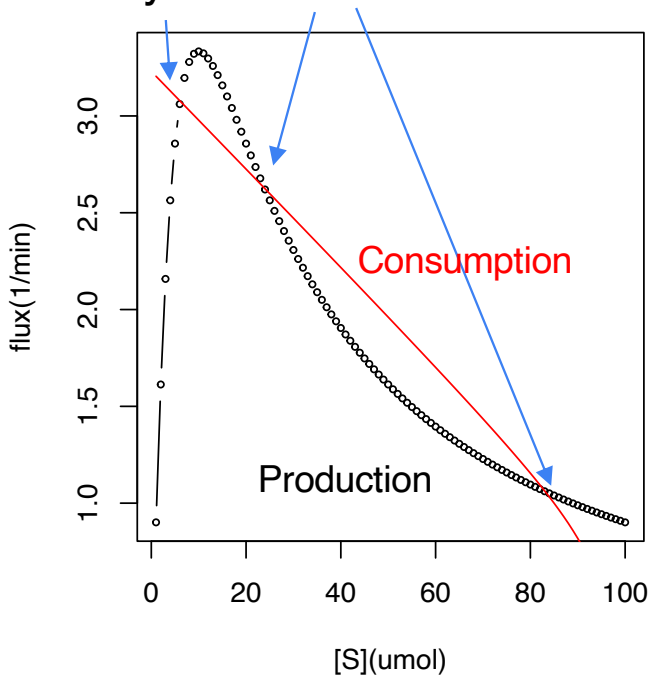
## Allosteric regulation in cycles




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

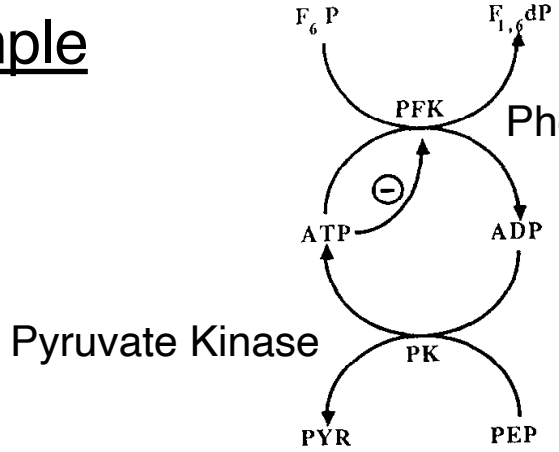
Intersections are the steady states of the system!




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

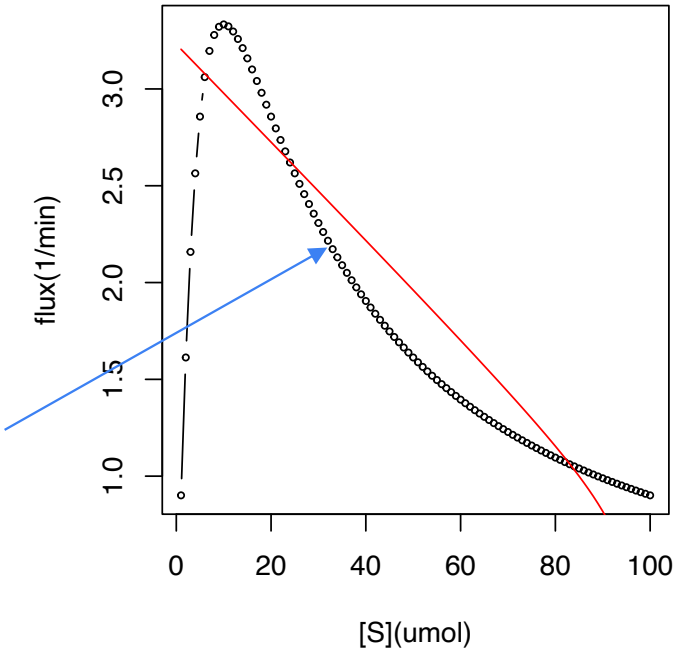
# **Bistability!** – in a cycle model with feedback

## A real example




Nonlinear P production function is essential for bistability, i.e. existence of three steady states!

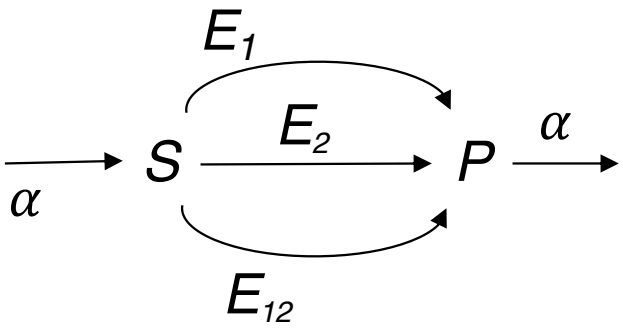
$$\frac{V_1 \cdot [S]}{K_1 + [S] + {[S]^2}/{K_3}}$$

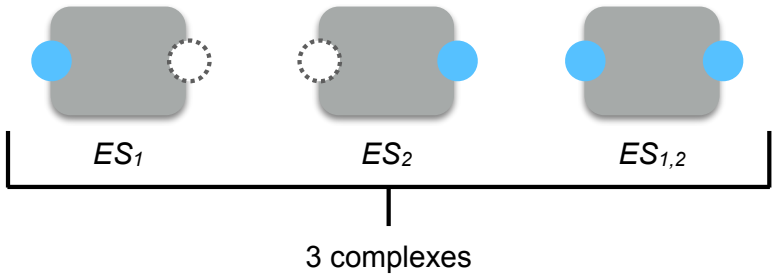



**What could be the biochemical basis of nonlinearity?**

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

# **Bistability!** – from multi-site enzyme structure




## Assumptions

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

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

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

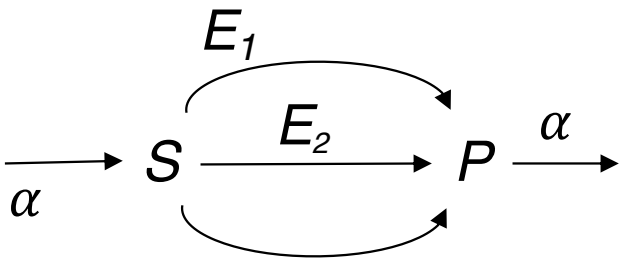
$$E + S \underset{b_{0,2}}{\overset{a_{0,2}}{\leftrightarrows}} SE \xrightarrow{c_{0,2}} E + P$$

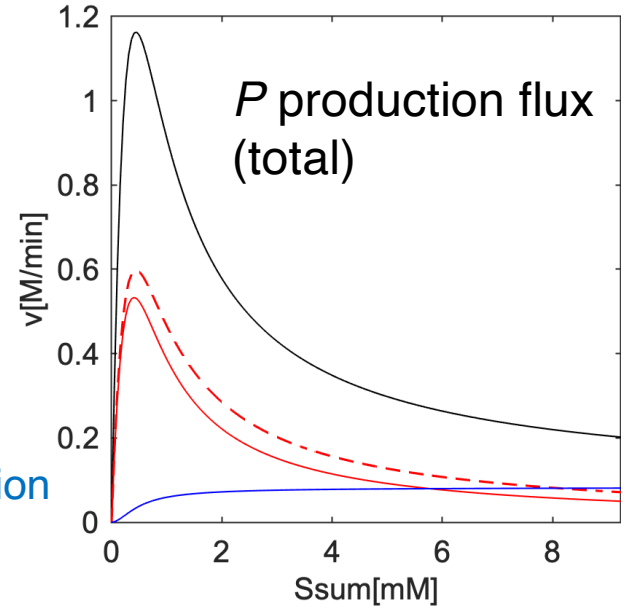
$$ES + S \underset{b_{1,[1,2]}}{\overset{a_{1,[1,2]}}{\leftrightarrows}} SES \xrightarrow{c_{1,[1,2]}} ES + P$$

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

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

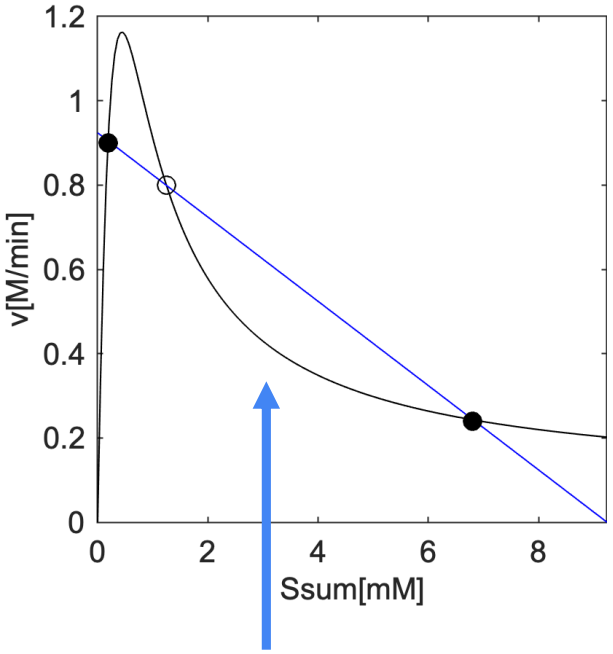
# **Bistability!** – from multi-site enzyme structure




*P* production flux (E12)

*P* production flux (E1 and E2)

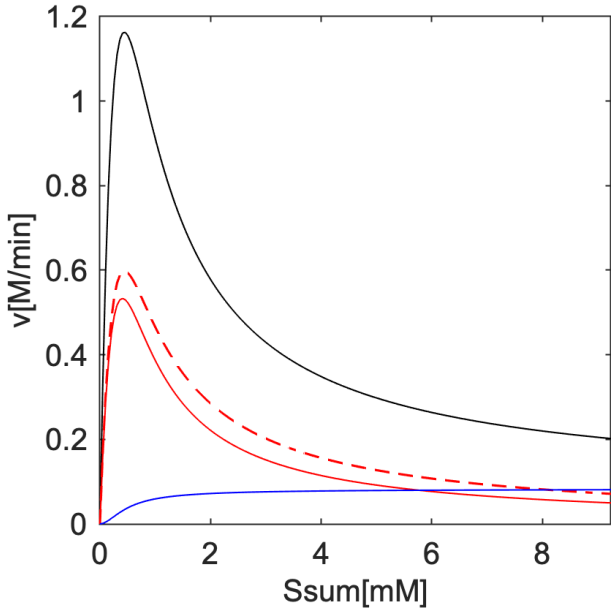


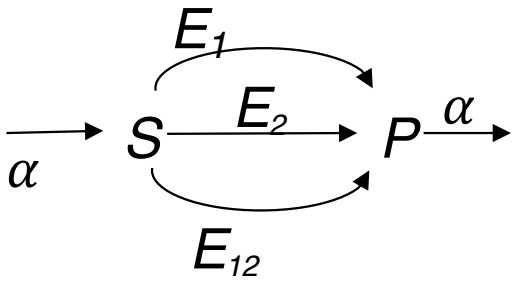

*P* production flux

*P* consumption flux

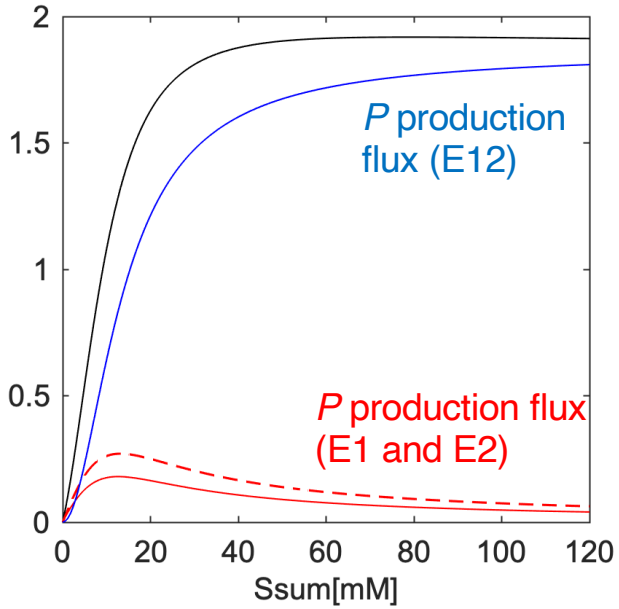
**Nonlinear production flux arises from dynamics of substrate-enzyme complexes**

# **Bistability!** – from multi-site enzyme structure




*P* production flux (total)




**Low *E12* catalysis :**
Bistable dynamics

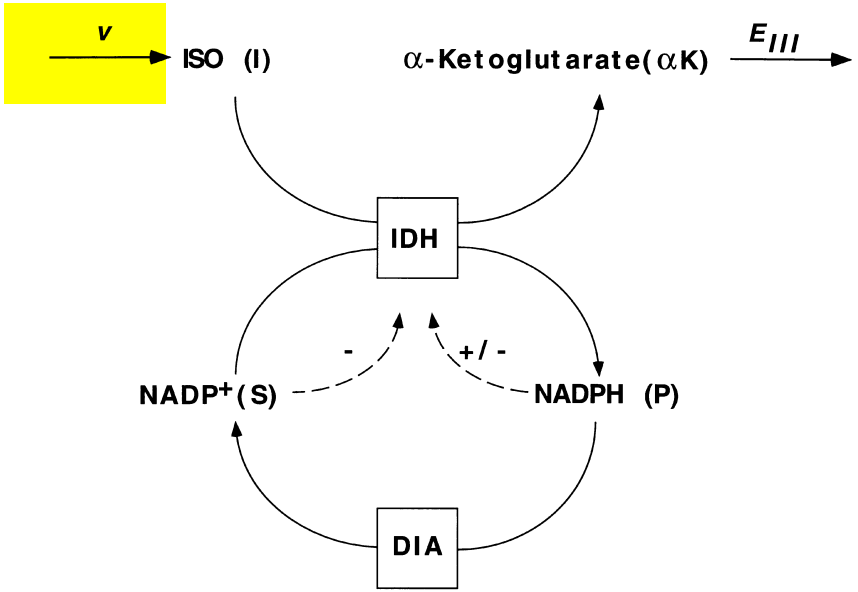
**High *E12* catalysis:**
Monostable dynamics

Same conclusion as from ‘substrate inhibition’ model

# Rich dynamics from simple models

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

See Figure from Guidi, GM *et al* showing oscillatory behavior from this model




See Figure from Guidi, GM *et al* showing bistable behavior from this model

High *v* gives rise to oscillations

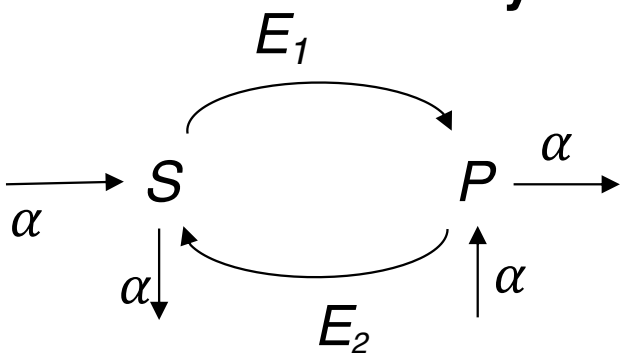
Low *v* gives rise to bistability

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

# Dynamical models and parameters

$10^{-8}$ – $10^{-4}$ M

**Enzyme levels**




**Fluxes** $10^{-2}$ – $10^{1}$ (M · min)$^{-1}$

**Enzyme kinetic parameters** $\frac{V_{max} \cdot [S]}{K_m + [S]}$

**Substrate levels**

$10^{-6}$ – $10^{-2}$ M

**CAUTION:** Mostly based on in vitro enzymology!

$k_{cat}$: $10^{1}$ – $10^{7}$ (min)$^{-1}$

$K_m$: $10^{-6}$ – $10^{-2}$ M

Binding/unbinding
$10^{7}$ – $10^{10}$ (M · min)$^{-1}$
$10^{2}$ – $10^{6}$ (min)$^{-1}$

**BRENDA database: www.brenda-enzymes.org**



# Dynamical models and experiments

*A model is something no one believes except the creator of the model, while an experiment is something everyone believes except the experimenter*

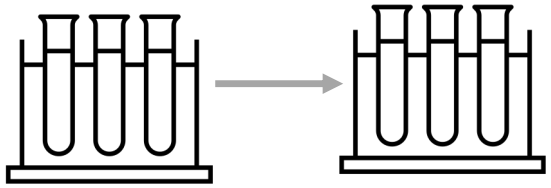
quote attributed to A. Einstein



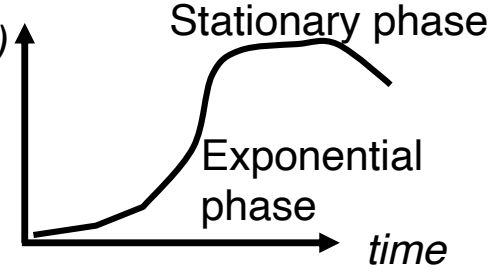
# Dynamical models and experiments

Population of cells

**Batch**



…




<u>Uptake:</u>
13C or other isotopes

<u>Internal metabolites:</u>

- Fluorescent microscopy + fluorescent reporters
- Cell lysis + mass spec or chemical detection

<u>Output:</u>
Filtration + mass spec or chemical detection
'Online' detection, e.g. gases, pH, etc.

Media inflow

Media and cell outflow, *d*

**Chemostat**

$$\frac{dN}{dt} = r \cdot N - d \cdot N$$

$$\frac{dN}{dt} = 0 \qquad \boxed{\boldsymbol{r = d}}$$

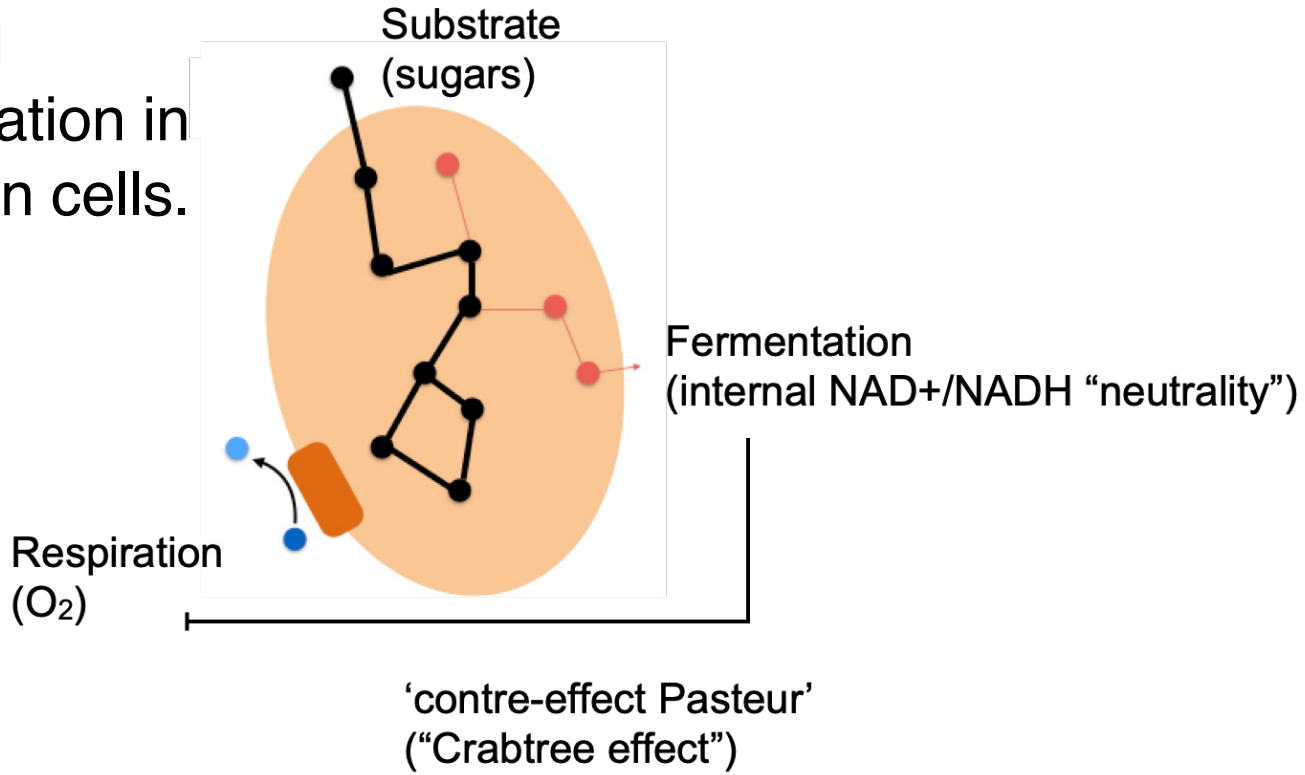


# Dynamical observations – flux changes

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.*



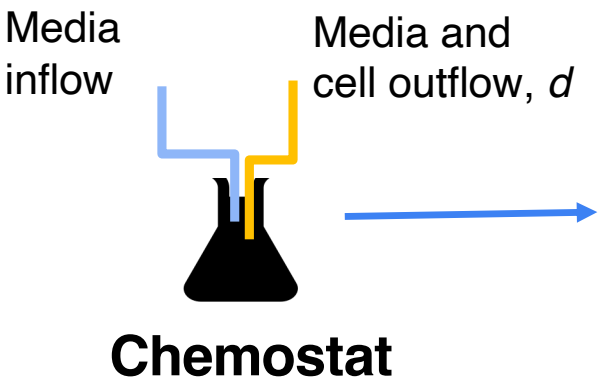

Crabtree H. G. *Biochem. J.*, 23 (1929)

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

# Dynamical observations – flux changes

M9 with 1gr/1l Glucose (~5mM)

See Figure 3 from Nanchen, A *et al* showing measured glucose consumption / acetate production in chemostats




$$\frac{dN}{dt} = r \cdot N - d \cdot N$$

$$\frac{dN}{dt} = 0$$

$$\boldsymbol{r = d}$$

Nanchen A. *et al. Appl Environ Microbiol*, 72:2 (2006)

# Dynamical observations – heterogeneity (bistability?)

~2500 cells observed, while fed with fluorescent glucose analog 2NBDG:

Dynamics of individual cells metabolising NBDG

Dynamics of individual cells not metabolising NBDG

See Figure from Simsek E *et al* showing glucose consumption behavior of individual cells

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



# Dynamical observations – oscillations

Yeast cells in a chemostat with pH control. Dilution rate was maintained at 0.085 $h^{-1}$.

After reaching sustained oscillation, chemostat was switched to an ethanol-based medium (15 $gL^{-1}$ ~ 300mM). The population is seemingly **synchronised** under these conditions!

See Figure from Keulers M *et al* showing oscillatory behavior of glucose consumption and oxygen respiration in yeast populations

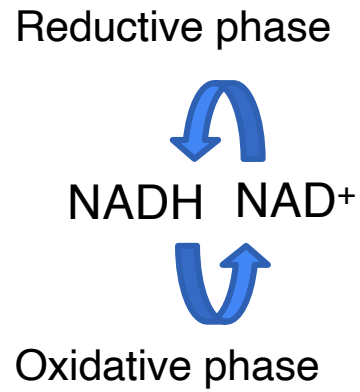
Keulers, M., et al. *FEMS Mirobiol. Lett.*, 142 (1996)



# Dynamical observations – oscillations

Truncated list….oscillations observed for most metabolites!

See Figure from Murray D *et al* showing oscillatory behavior of glucose consumption, oxygen respiration, and various metabolite concentrations in yeast populations




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

# 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.



# 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?



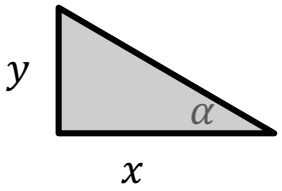
# Additional slides



# 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**:




$$y = f(x)$$

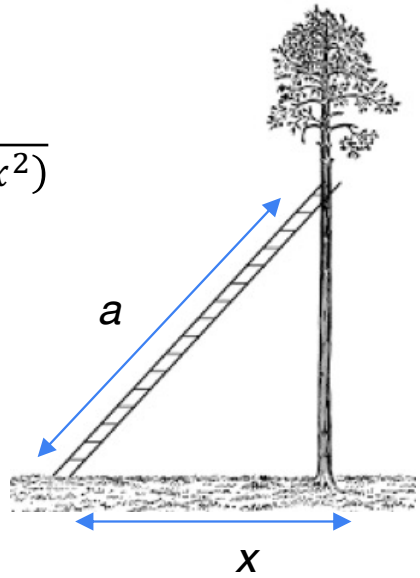
$$y = x \cdot \tan(\alpha)$$

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

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>**

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

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

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

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

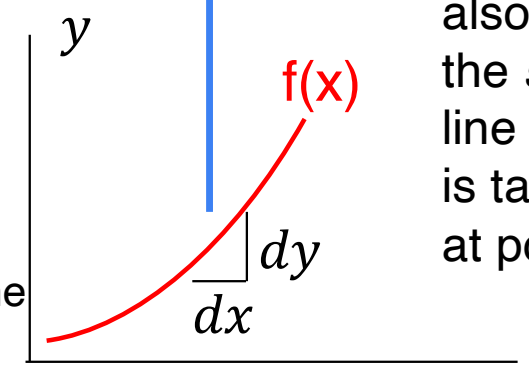
$$dy = dx(2x - dx)$$

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)**

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

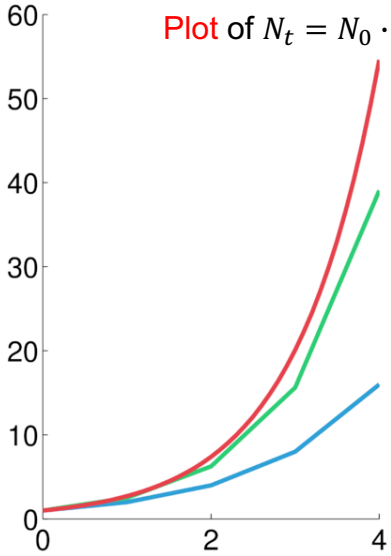
$dy = dx(2x - dx)$

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

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

Assume $2x - dx \approx 2x$

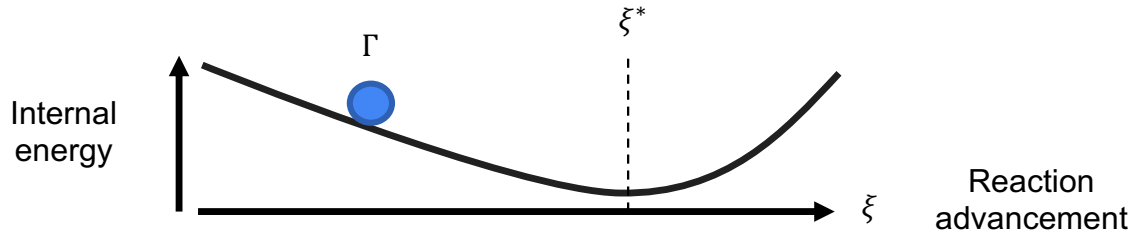
Remember this assumption? It can, and will always, cause inaccuracies in numerical integration.




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



# Chemical reactions and thermodynamics

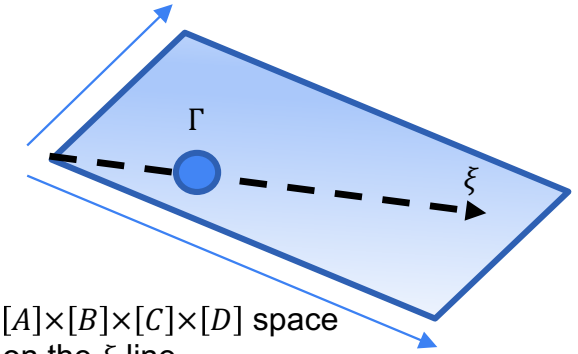



$$\nu_A \mathrm{A} + \nu_B \mathrm{B} \rightleftharpoons \nu_C \mathrm{C} + \nu_D D$$

The position of the reaction along axis $\xi$ is usually denoted as the **mass action ratio** $\Gamma$;

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

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




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

$\Gamma$ is a point in the $[A]\times[B]\times[C]\times[D]$ space instead of a point on the $\xi$ 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

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

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

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

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

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

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

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

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

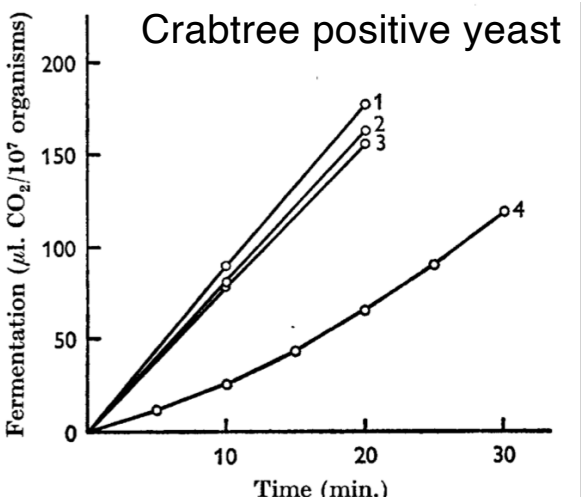
$$\boxed{\frac{J_-}{J_+} = \frac{\Gamma}{K_{eq}} = e^{\frac{\Delta G}{RT}}}$$

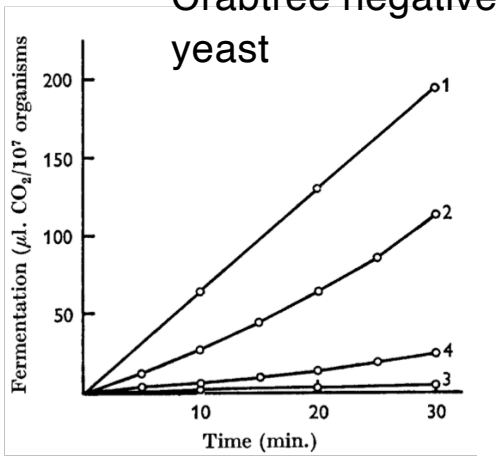
**Flux-Force relation**

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).

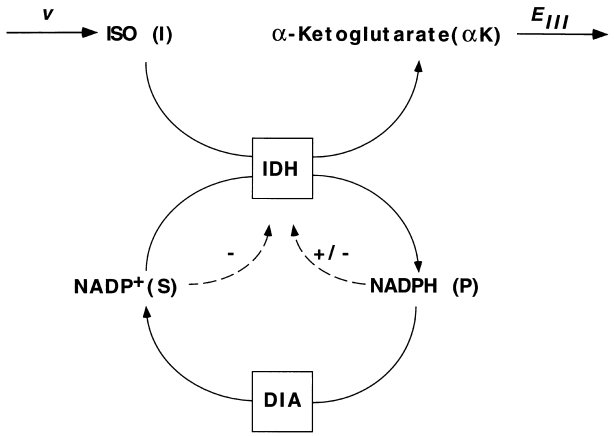
On the converse, Crabtree positive yeast always seems to use fermentative metabolism, even under conditions where respiration should be perfectly fine.

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?

# Biochemical basis of oscillations?

## Re-cap from lecture 7.

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




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

Other models of oscillation also exists, e.g.

Wolf J., Heinrich R. *Biochem. J.* 345 (2000)

# Oscillations: cells breathing in and out

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

See Figure from Papagiannakis A *et al* showing oscillatory behavior oxygen respiration and NAD(P)H, as well as cell cycle markers in yeast populations

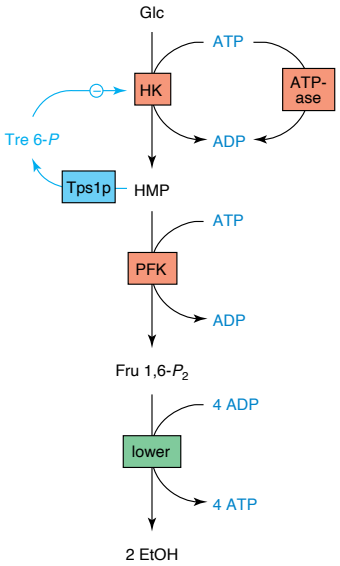
Papagiannakis, A., et al. *Mol Cell*, 65:2 (2017)

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

Cells incubated in a microfluidic device. Possible caveats: Oscillations induced by microfluidic pumps? Imaging of NAD(P)H causing cell damage?



# Metabolic bistability?




**Model without trehalose feedback**

**Model with 'Trehalose' feedback**

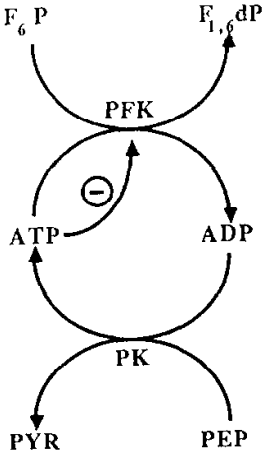
See Figures from van Heerden JD *et al* showing model behavior with metabolite accumulation and not, and also growth of different mutants

Presence of an imbalanced state is observed in yeast trehalose mutants:

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

# Metabolic bistability?

While several modelling and experimental papers indicate potential for bistability in metabolic systems, 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)

See Figure from Cimino A *et al* showing bistability behavior from different starting points (initial concentrations)