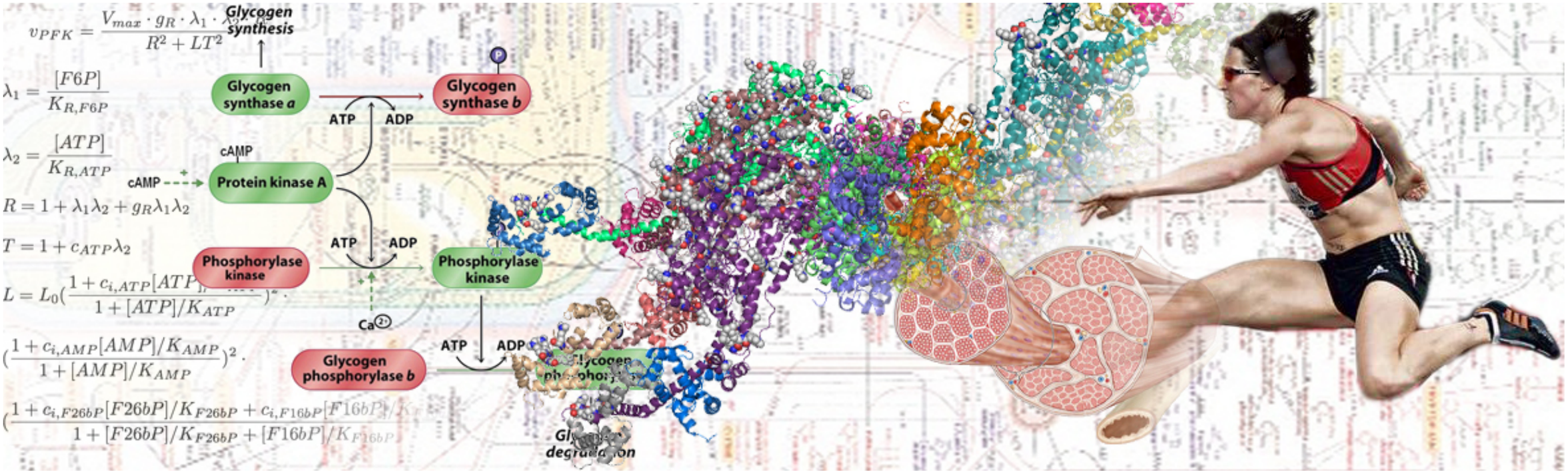
Biochemistry 714 Mini-course: Molecular Systems Biology



Prof Johann Rohwer (lectures), Dr Dawie van Niekerk (tutorials and data analysis), Prof Jacky Snoep (tutorials and practical)

March – April 2025

Programme

	Mon	Tue	Wed	Thu	Fri
17 – 21 Mar	**10:00** **Welcome & Intro** DvN **14:00** **Tut 1 (Intro)** DvN	**09:30** **Lecture 1 (Mass action kinetics)** JR **14:00** **Tut 2 (Mass action kinetics)** DvN & JLS	**09:30** **Lecture 2 (Enzyme catalysed reactions)** JR **14:00** **Tut 3 (Enzyme catalysed reactions)** DvN & JLS	**12:30** **Safety Lecture 2** ES **14:00** **Lecture 3 (Coupled reactions)** JR	**Human Rights Day**
24 – 28 Mar	**09:30** **Lecture 4 (Networks)** JR **14:00** **Tut 4 (Networks)** DvN & JLS	**11:00** **Tut 5 (Kinetic model)** DvN **Graduation**	**09:30** **Lecture 5 (MCA)** JR **14:00** **Tut 6 (MCA)** DvN & JLS	**09:30** **Research lecture** **14:00** **Assessment 1** DvN & JLS	**09:00** **Peer grading of Assessment 1** DvN & JLS **11:30** **Research lecture** **14:00** **Introduction to Practical** JLS
31 Mar – 4 April	**Recess**				
7 – 11 April	**09:00** **Practical** (whole day) JLS	**09:00** **Practical** (whole day) JLS	**09:00** **Practical / Data analysis** (whole day) JLS & DvN	**09:30** **Research lecture** **11:00** **Data analysis / Practical** (whole day) DvN & JLS	**09:30** **Data analysis** (whole day) DvN
14 – 18 April	**09:30** **Data analysis** (whole day) DvN	**Hand in final report** (15/04/2025 23:59)			

- Face-to-face **lectures** will take place in the **Biochemistry Seminar Room** (A111)
- Face-to-face **computer practicals** and data analysis will take place in the **computer lab** (JC Smuts B215, departmental computers or own laptops)
- Practical **enzyme kinetics experiments** will take place in the **molecular systems biology lab** (JC Smuts B203A)

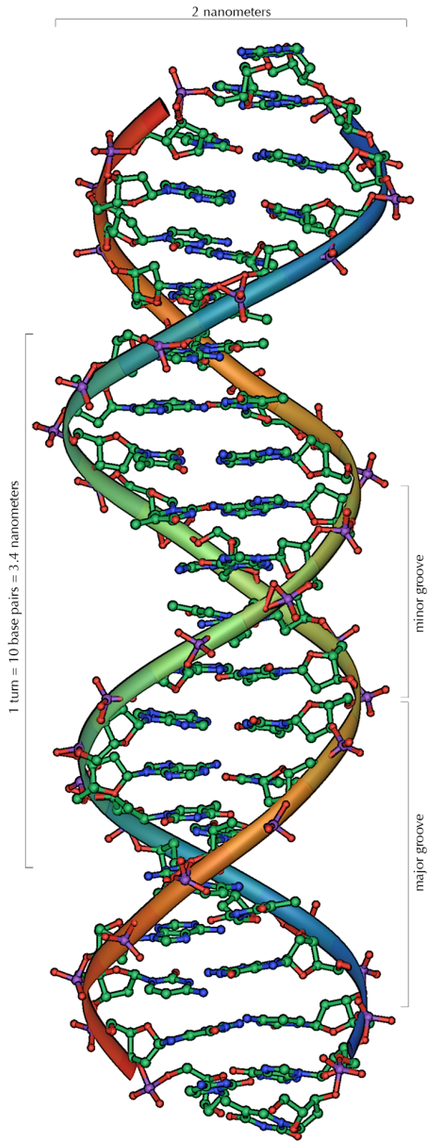
Course material

- Available at:

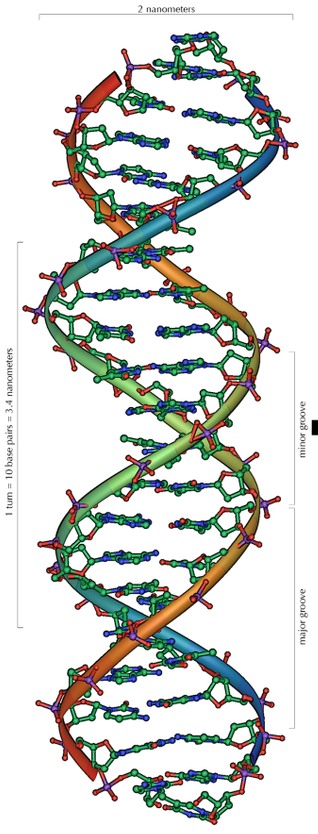
https://glue.jjj.sun.ac.za/jjj/minicourse/

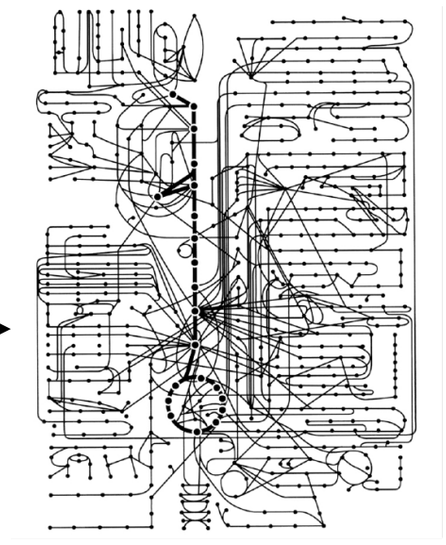
- Molecular Systems Biology textbook available as pdf
- Lecture notes Biochem 323
- Lecture slides
- Tutorials & Mathematica notebooks

The Ultimate Predictive Model



From Sequence to Network





$$\dot{\mathbf{s}} = \mathbf{N} * \mathbf{v}$$

$$\begin{pmatrix} \dot{DHAP} \\ \dot{F6P} \\ \dot{FBP} \\ \dot{G13DP} \\ \dot{G6P} \\ \dot{GAP} \\ \dot{NAD} \\ \dot{NADH} \\ \dot{P2G} \\ \dot{P3G} \\ \dot{PEP} \end{pmatrix} = \begin{pmatrix} 0 & 0 & 0 & 1 & 1 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\ 0 & 1 & -1 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\ 0 & 0 & 1 & -1 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & 0 & 1 & -1 & 0 & 0 & 0 & 0 & -1 & 0 \\ 1 & -1 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 1 & -1 & -1 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & 0 & -1 & 0 & 0 & 0 & 0 & 1 & 0 & 0 \\ 0 & 0 & 0 & 0 & 0 & 1 & 0 & 0 & 0 & 0 & -1 & 0 & 0 \\ 0 & 0 & 0 & 0 & 0 & 0 & 0 & 1 & -1 & 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & 0 & 0 & 1 & -1 & 0 & 0 & 0 & 0 & 1 \\ 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 1 & -1 & 0 & 0 & 0 \end{pmatrix} * \begin{pmatrix} v1 \\ v2 \\ v3 \\ v4 \\ v5 \\ v6 \\ v7 \\ v8 \\ v9 \\ v10 \\ v11 \\ v12 \\ v13 \end{pmatrix}$$

$\dot{\mathbf{s}}$ d(s)/d(t)

$\mathbf{N}$ stoichiometry matrix

$\mathbf{v}$ reaction rates

Reconstruct reaction network using homologies between enzymes

Construct stoichiometry matrix from reaction network

Molecular Systems Biology

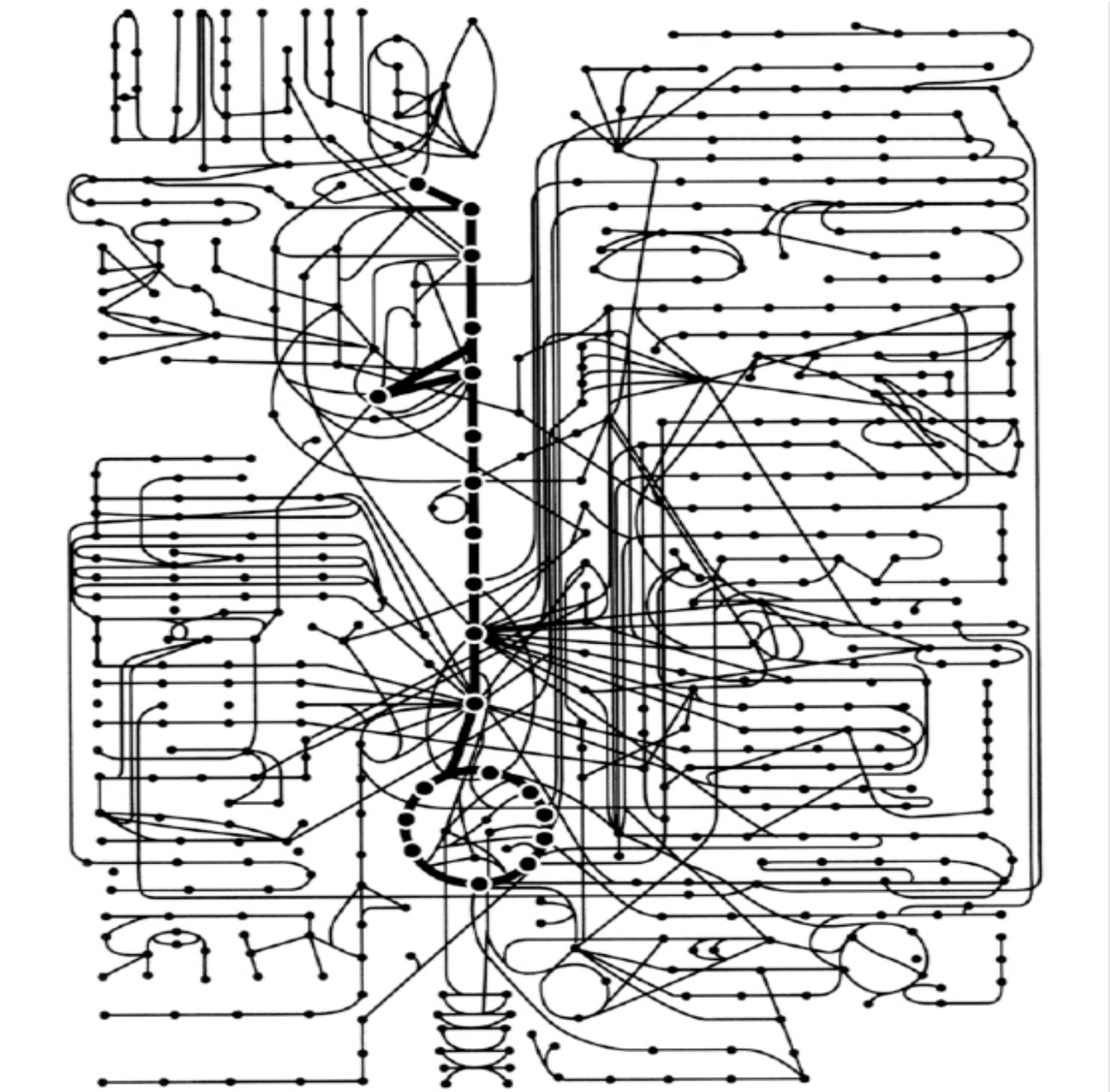
Topology studies show networks but are far removed from functional behaviour.

Classic analyses are qualitative and cannot relate the properties of a system to its components.

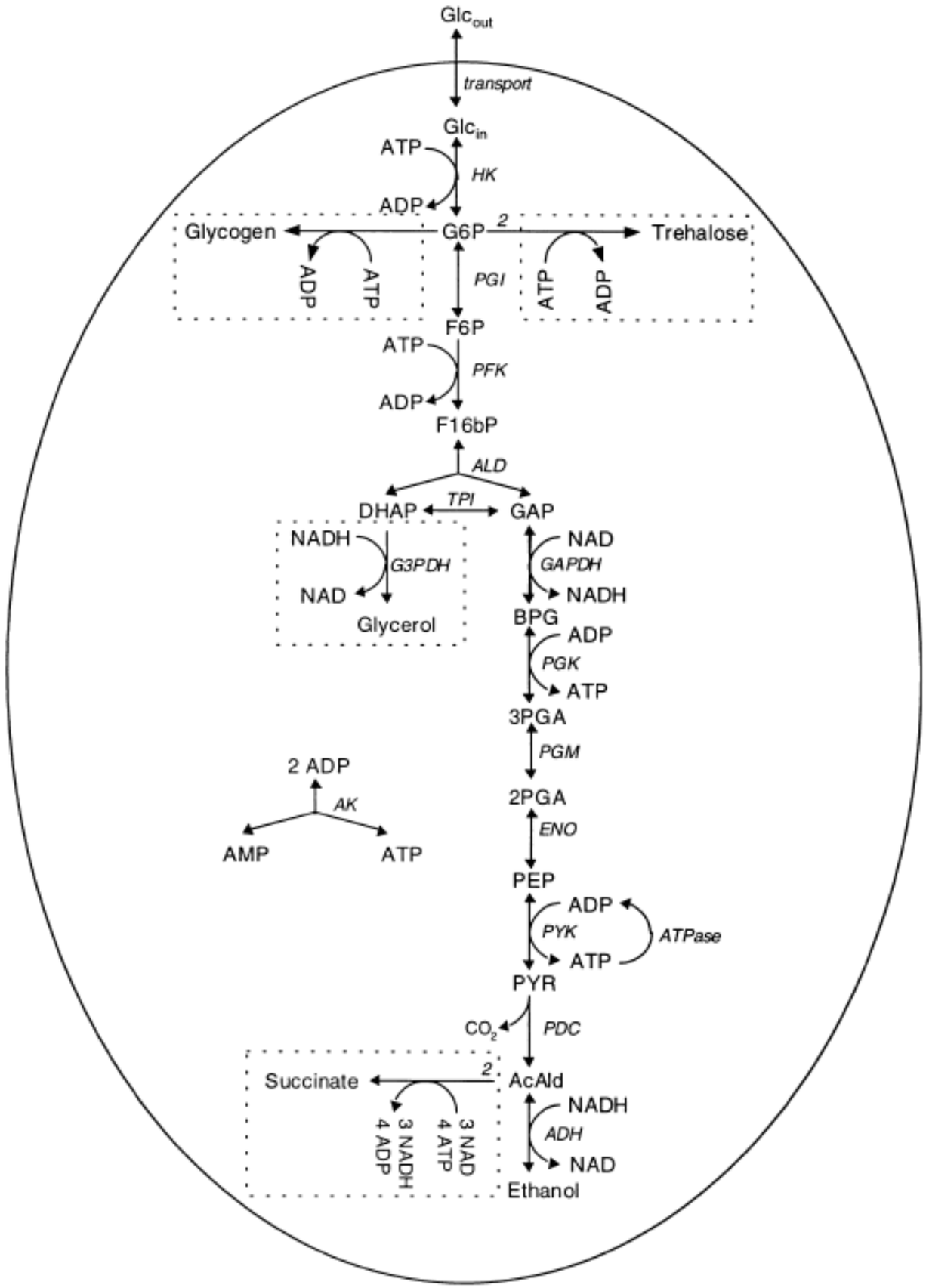
Applications in medicine (drug target identification) and biotechnology (metabolic engineering), need specific targets in the system (molecular mechanism).

With a molecular systems biology analysis we aim to understand systems on the basis of the characteristics of their components.

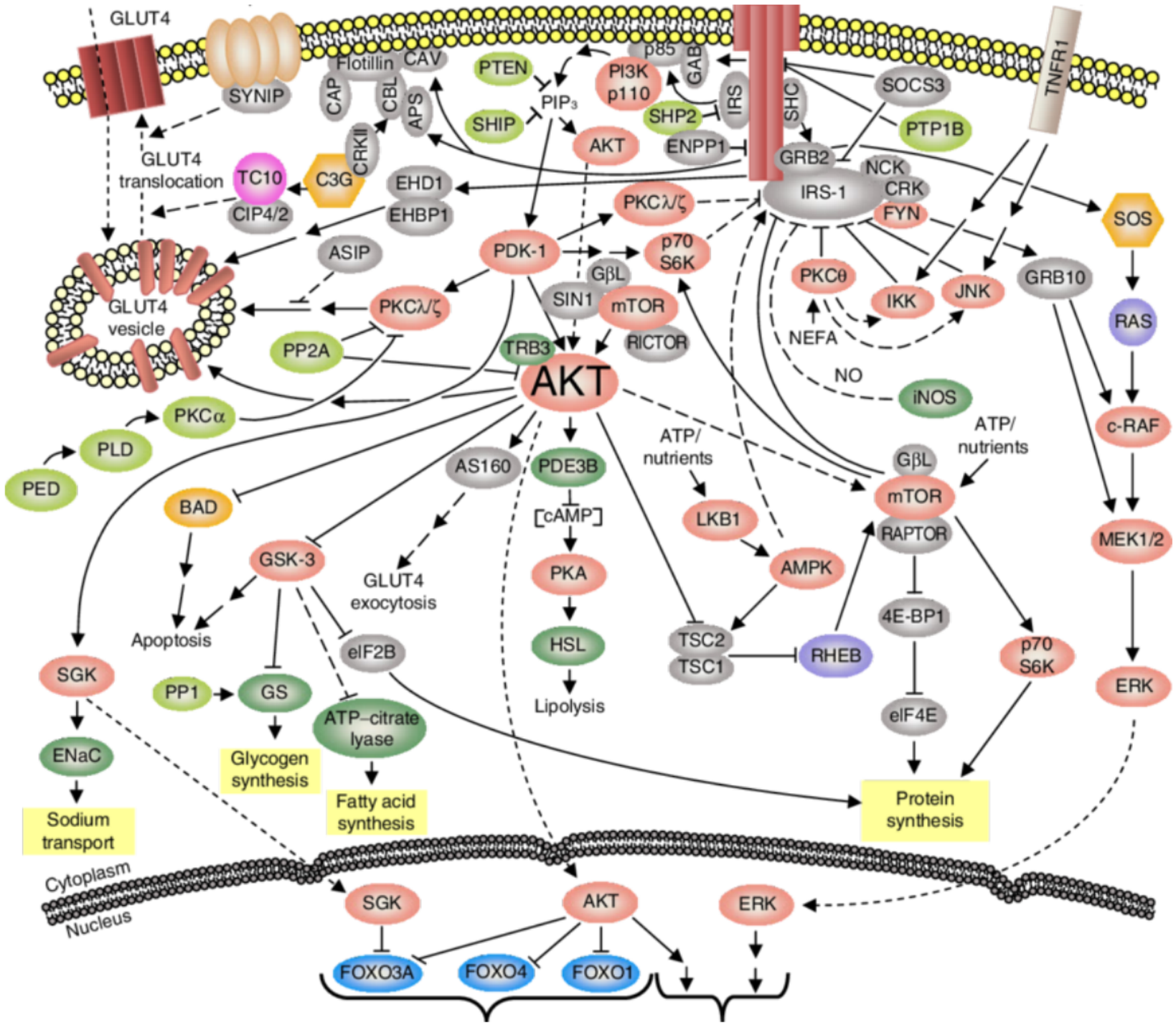
Metabolism



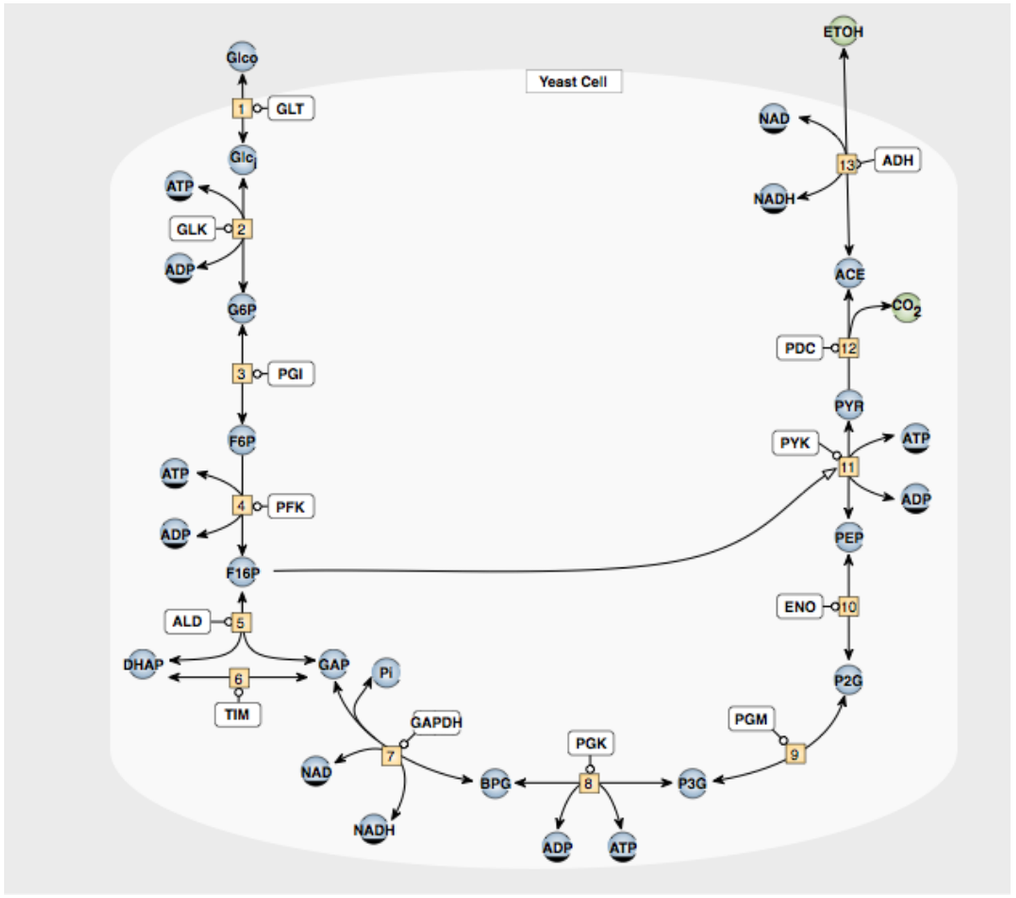
Glycolysis

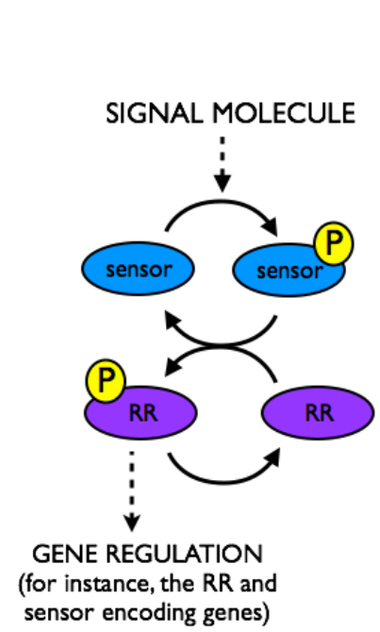

Glc$_{out}$
transport
Glc$_{in}$
ATP
HK
ADP
Glycogen
G6P
2
Trehalose
ADP
ATP
PGI
ATP
ADP
F6P
ATP
PFK
ADP
F16bP
ALD
TPI
DHAP
GAP
NADH
G3PDH
NAD
Glycerol
NAD
GAPDH
NADH
BPG
ADP
PGK
ATP
3PGA
PGM
2 ADP
AK
AMP
ATP
2PGA
ENO
PEP
ADP
PYK
ATP
ATPase
PYR
CO$_2$
PDC
2
Succinate
AcAld
3 NADH
4 ADP
3 NAD
4 ATP
NADH
ADH
NAD
Ethanol

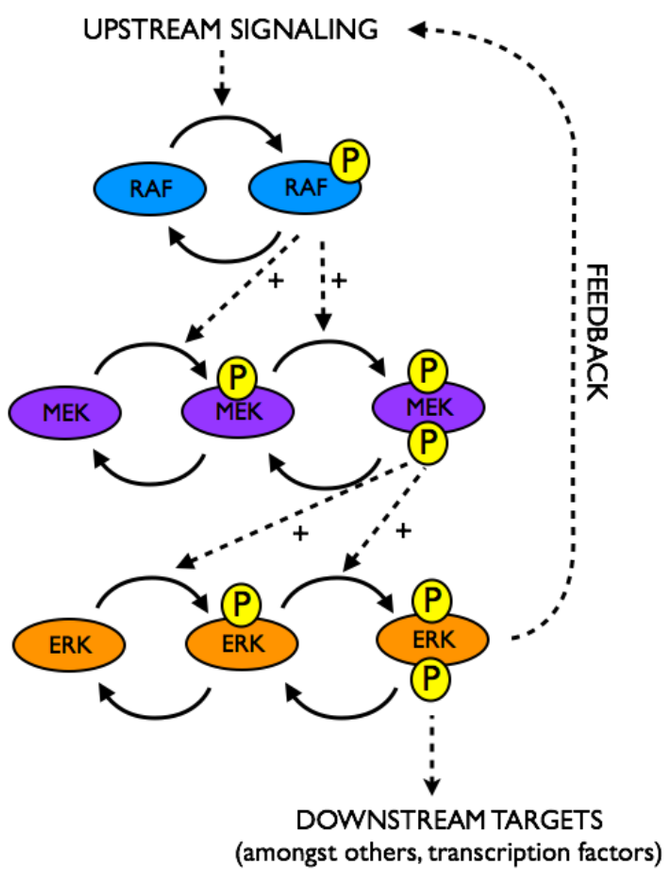
Signalling


GLUT4
SYNIP
Flotillin
CAV
CAP
CBL
APS
CRKII
C3G
TC10
CIP4/2
GLUT4 translocation
GLUT4 vesicle
EHD1
EHBP1
ASIP
PTEN
PIP3
SHIP
PI3K p110
p85
GAB
IRS
SHC
SHP2
AKT
ENPP1
SOCS3
PTP1B
TNFR1
GRB2
NCK
CRK
IRS-1
FYN
SOS
RAS
c-RAF
MEK1/2
ERK
PKCλ/ζ
PDK-1
p70 S6K
GβL
SIN1
mTOR
RICTOR
TRB3
AKT
PP2A
PKCλ/ζ
PKCθ
IKK
JNK
GRB10
NEFA
NO
iNOS
ATP/ nutrients
ATP/ nutrients
GβL
mTOR
RAPTOR
LKB1
AMPK
4E-BP1
eIF4E
TSC2
TSC1
RHEB
p70 S6K
Protein synthesis
PKCα
PLD
PED
BAD
Apoptosis
AS160
PDE3B
[cAMP]
PKA
HSL
Lipolysis
GLUT4 exocytosis
GSK-3
eIF2B
SGK
PP1
GS
Glycogen synthesis
ATP–citrate lyase
Fatty acid synthesis
ENaC
Sodium transport
Cytoplasm
Nucleus
SGK
AKT
ERK
FOXO3A
FOXO4
FOXO1

Metabolic and signal transduction networks







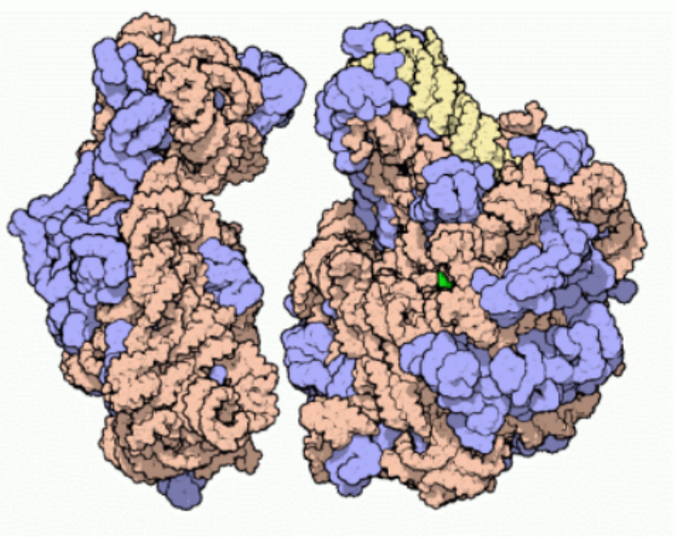
Metabolite concentrations, fluxes, time courses

Reponse time, dose-response relation, ligand specificity

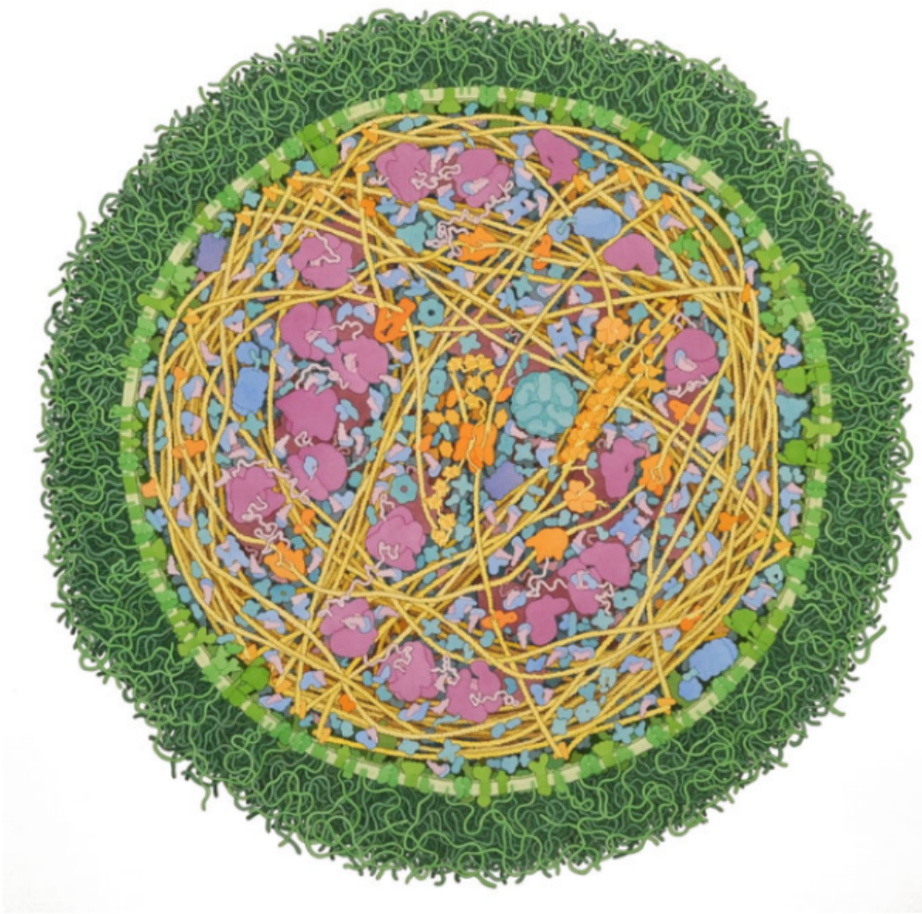
Systems Biology

is the science that studies how biological function emerges from the interactions between the components of living systems.

Macromolecules (dead) → Cell (alive)



Interaction
↓
Function
↓
Functional organisation

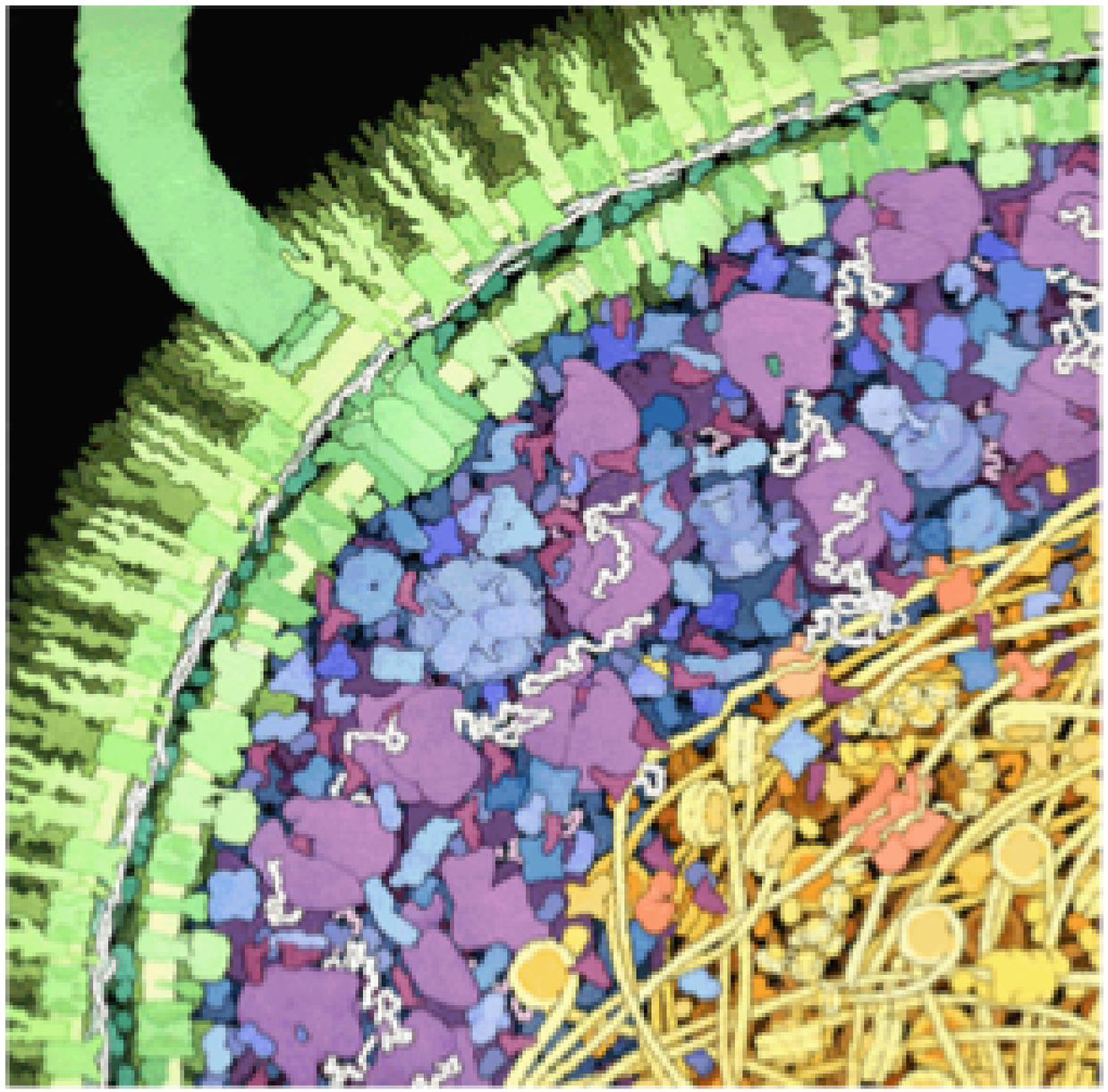


Networks and reactivity

- Links in networks indicate reactions, (association, dissociation, isomerization)
- To react (or bind) molecules need to first meet
- metabolites + enzymes or protein + protein

$$A + B \rightarrow AB$$

$$v = k \cdot a \cdot b$$



- crowded cellular environment
- molecules undergo random movement (walk)
- rate of diffusion related to rate of association

Diffusion and reactivity

- Diffusion coefficient (*D*) strongly dependent on size (metabolites fast, proteins slow).
- Diffusion sets an upper limit to reaction rates.
- MSB book: p. 22-23

Kinetics of individual reaction steps

- Individual reaction step is the lowest level of systems description in our approach
- Most reactions in biological systems are catalysed by enzymes
- We start first with non-catalysed, chemical (mass-action) kinetics and then move to enzyme-catalysed reactions

Kinetics of chemical reactions

- Why does a reaction occur?
- What determines the direction of a reaction, i.e. forward or reverse?
- What determines the rate of a reaction?
- When does a reaction rate go to zero?
- How do the molecules know whether they should react or not?
- Net reaction rate, micro-reversibility, statistics

Driving force of a reaction

- A reaction will only occur if the Gibbs free-energy content of the products is less than that of the substrates, i.e. $\Delta G<0$
- Gibbs free energy change determines the direction of the reaction
- The rate at which a reaction occurs is dependent on both thermodynamics and kinetics

Three types of elementary reactions

1. an *association* between two molecules to form a non-covalently bound complex,

$$A + B \longrightarrow A \cdot B$$

2. a *dissociation* of a complex into two molecules,

$$A \cdot B \longrightarrow A + B$$

3. an interconversion where one molecule is chemically transformed into another (an *isomerisation*).

$$A \longrightarrow B$$

Reaction mechanism

Breaking a reaction up into irreversible elementary reactions:

$$A + B \rightleftharpoons C$$

could have the mechanism

$$A + B \rightleftharpoons A \cdot B \rightleftharpoons C$$

Each half of the double arrow ($\rightleftharpoons$) denotes one of the elementary reactions.

The rate of a chemical reaction

The *law of mass action* states that for any elementary reaction, e.g.,

$$\mathrm{A} + \mathrm{B} \longrightarrow \mathrm{A} \cdot \mathrm{B}$$

the reaction rate is proportional to concentration

$$v \propto a \qquad \text{and} \qquad v \propto b$$

where
v is the rate of reaction
a and b are the concentrations of A and B.

$$v \propto ab$$

Reaction rate, v

$$\mathrm{A} + \mathrm{B} \longrightarrow \mathrm{A} \cdot \mathrm{B}$$

$$v = -\frac{da}{dt} = -\frac{db}{dt} = \frac{d(a \cdot b)}{dt}$$

The reaction rate v thus has units of concentration$\cdot$time^{-1}.

Rate equation

The proportionality between rate v and concentrations a and b is transformed into a *rate equation* by inserting a constant, called the *rate constant*:

$$\mathrm{A} + \mathrm{B} \longrightarrow \mathrm{A} \cdot \mathrm{B}$$

$$v = kab$$

Reaction order

- First-order with respect to A
- First-order with respect to B
- Overall order: 2

Determining reaction order

$$\mathrm{A} + \mathrm{B} \longrightarrow$$

$$v = ka^p b^q$$

p and q are the unknown orders.

Taking logarithms on both sides we obtain

$$\ln v = \ln k + p \ln a + q \ln b$$

Plot $\ln v$ against either $\ln a$ or $\ln b$ to obtain p or q.

General definition of reaction order

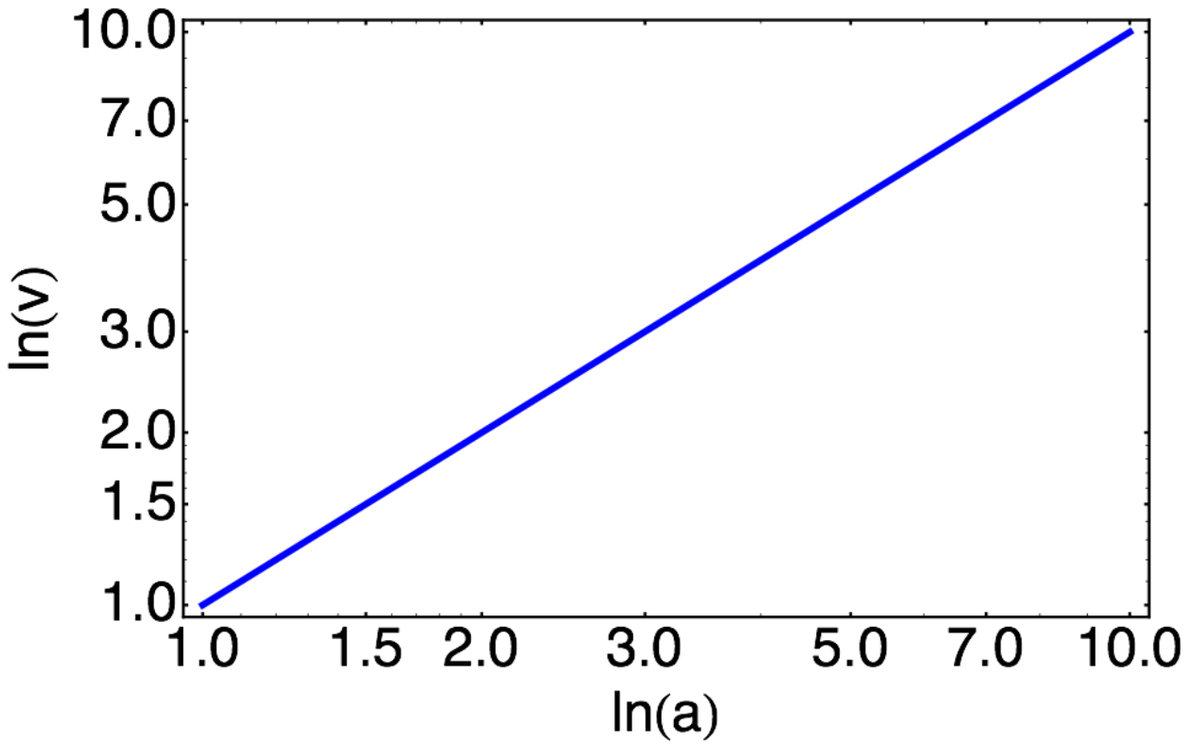
$$\frac{d \ln v}{d \ln a}$$

evaluated at a given a.

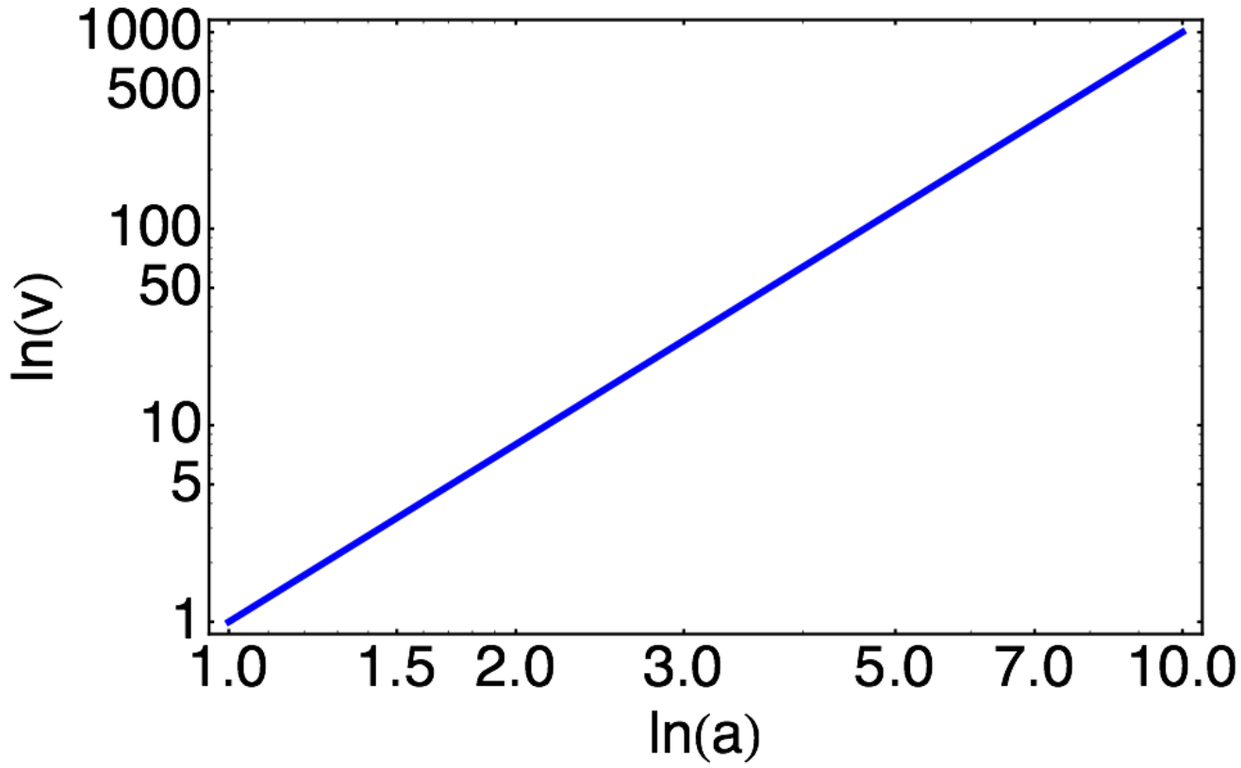
More correctly, because v is a function of both a and b,

$$\left(\frac{\partial \ln v}{\partial \ln a} \right)_b$$

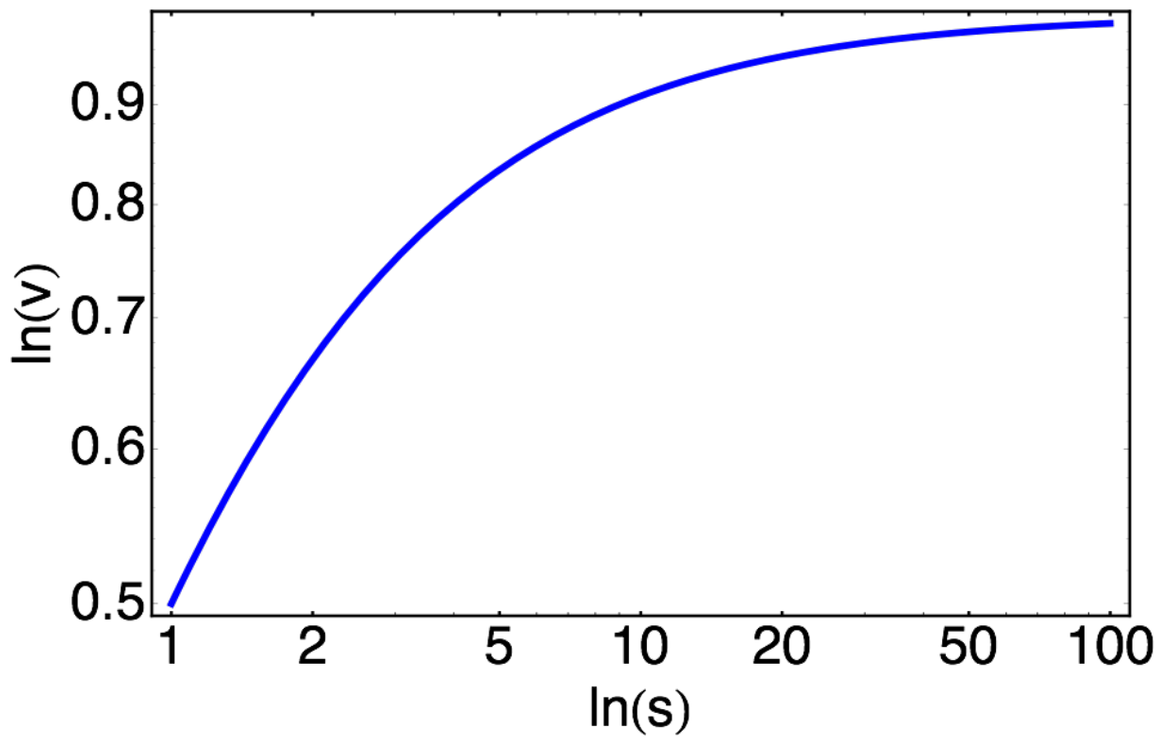
v=ab; b=1



v=a^3b; b=1



$$v=\frac{Vm\times s}{Ks+s}$$



Molecularity and reaction order

- *Molecularity:* the number of molecules that react (stoichiometric coefficients in a balanced reaction equation).
- *Reaction order:* experimentally determined quantity (generally not related to stoichiometric coefficients).

Mass-action

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

is a combination of the *forward* reaction

$$\mathrm{A} + \mathrm{B} \rightarrow \mathrm{C} \qquad \text{with rate equation} \qquad v_\mathrm{f} = k_\mathrm{f} a b$$

and the *reverse* reaction

$$\mathrm{C} \rightarrow \mathrm{A} + \mathrm{B} \qquad \text{with rate equation} \qquad v_\mathrm{r} = k_\mathrm{r} c$$

The *net rate* of reaction is the difference between the forward and reverse rates

$$v = v_\mathrm{f} - v_\mathrm{r} = k_\mathrm{f} a b - k_\mathrm{r} c$$

The equilibrium constant

At equilibrium:

$$v = v_{\mathrm{f}} - v_{\mathrm{r}} = 0$$

Therefore

$$k_{\mathrm{f}}(a)_{eq}(b)_{eq} - k_{\mathrm{r}}(c)_{eq} = 0$$

so that

$$\frac{k_{\mathrm{f}}}{k_{\mathrm{r}}} = \frac{(c)_{eq}}{(a)_{eq}(b)_{eq}} = K_{\mathrm{eq}}$$

General reaction

$$mA + nB \rightleftharpoons pC + qD$$

where m, n, p, and q are the stoichiometric coefficients. From the rate equations for the forward and reverse reactions

$$v_f = k_f a^m b^n \quad \text{and} \quad v_r = k_r c^p d^q$$

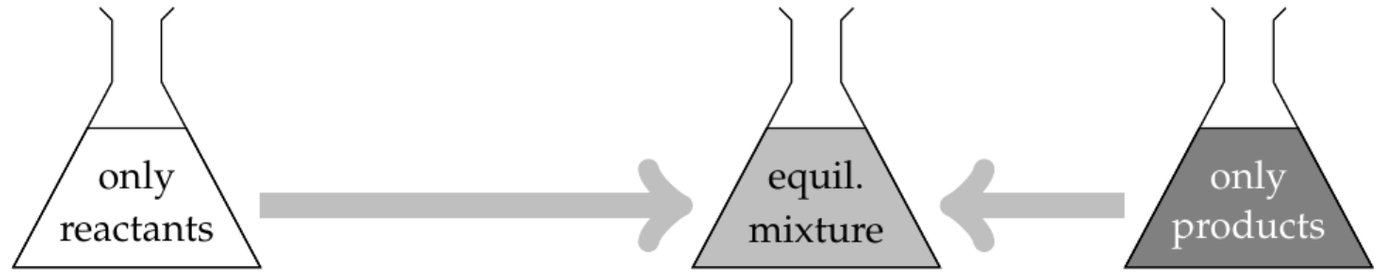
and using the equilibrium condition we obtain

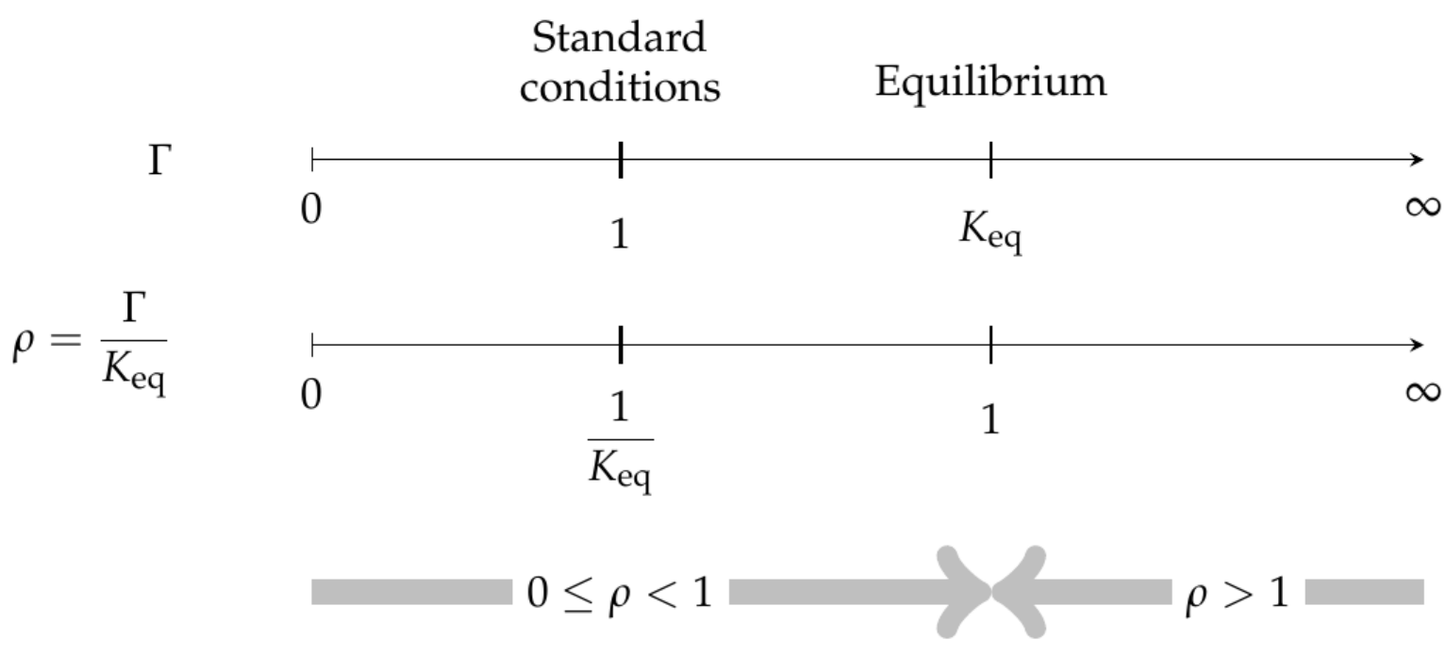
$$K_{eq} = \frac{(c)_{eq}{}^p (d)_{eq}{}^q}{(a)_{eq}{}^m (b)_{eq}{}^n}$$

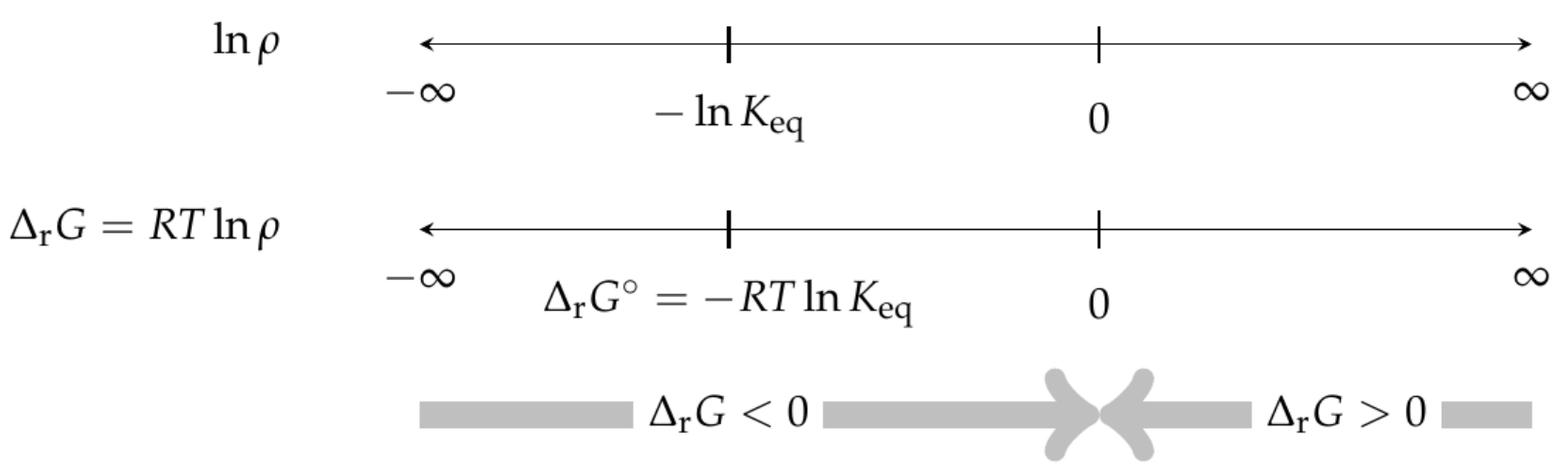
The mass-action ratio and the distance from equilibrium

$$\frac{v_\mathrm{r}}{v_\mathrm{f}} = \frac{k_\mathrm{r}c}{k_\mathrm{f}ab} = \left(\frac{c}{ab}\right) \Big/ K_\mathrm{eq}$$

The quantity c/ab is so important that it has been given a special name, the *mass-action ratio*, usually symbolised by Γ (capital Greek gamma).


only
reactants
equil.
mixture
only
products


Standard
conditions
Equilibrium
Γ
0
1
K_{eq}
∞
$\rho = \dfrac{\Gamma}{K_{\mathrm{eq}}}$
0
$\dfrac{1}{K_{\mathrm{eq}}}$
1
∞
$0 \leq \rho < 1$
$\rho > 1$


$\ln \rho$
$-\infty$
$-\ln K_{\mathrm{eq}}$
0
∞
$\Delta_{\mathrm{r}} G = RT \ln \rho$
$-\infty$
$\Delta_{\mathrm{r}} G^{\circ} = -RT \ln K_{\mathrm{eq}}$
0
∞
$\Delta_{\mathrm{r}} G < 0$
$\Delta_{\mathrm{r}} G > 0$

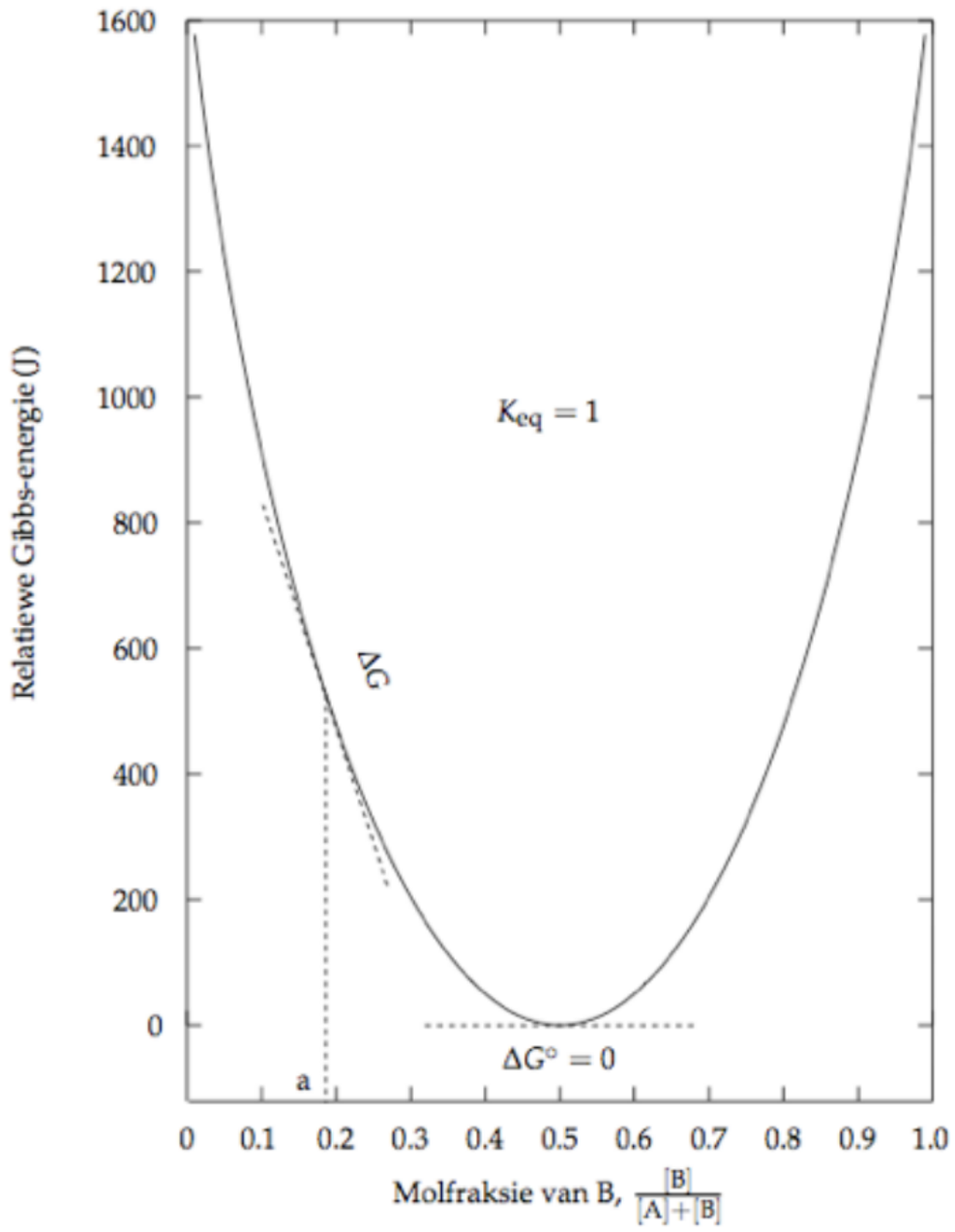
The Gibbs energy

$$\Delta G = RT \ln \frac{\Gamma}{K_{\mathrm{eq}}}$$

$$\Delta G^\circ = RT \ln \frac{1}{K_{\mathrm{eq}}} = -RT \ln K_{\mathrm{eq}}$$

$$A \rightleftharpoons B$$

- gradient increases as reaction moves further away from eq
- equilibrium $\Delta G = 0$
  - gradient = 0
  - Keq = 1 => [A]eq = [B]eq
- at standard conditions Γ=1
  - [A] = [B] => for this example equilibrium is at standard conditions
    - ➡ $\Delta G^0 = 0$

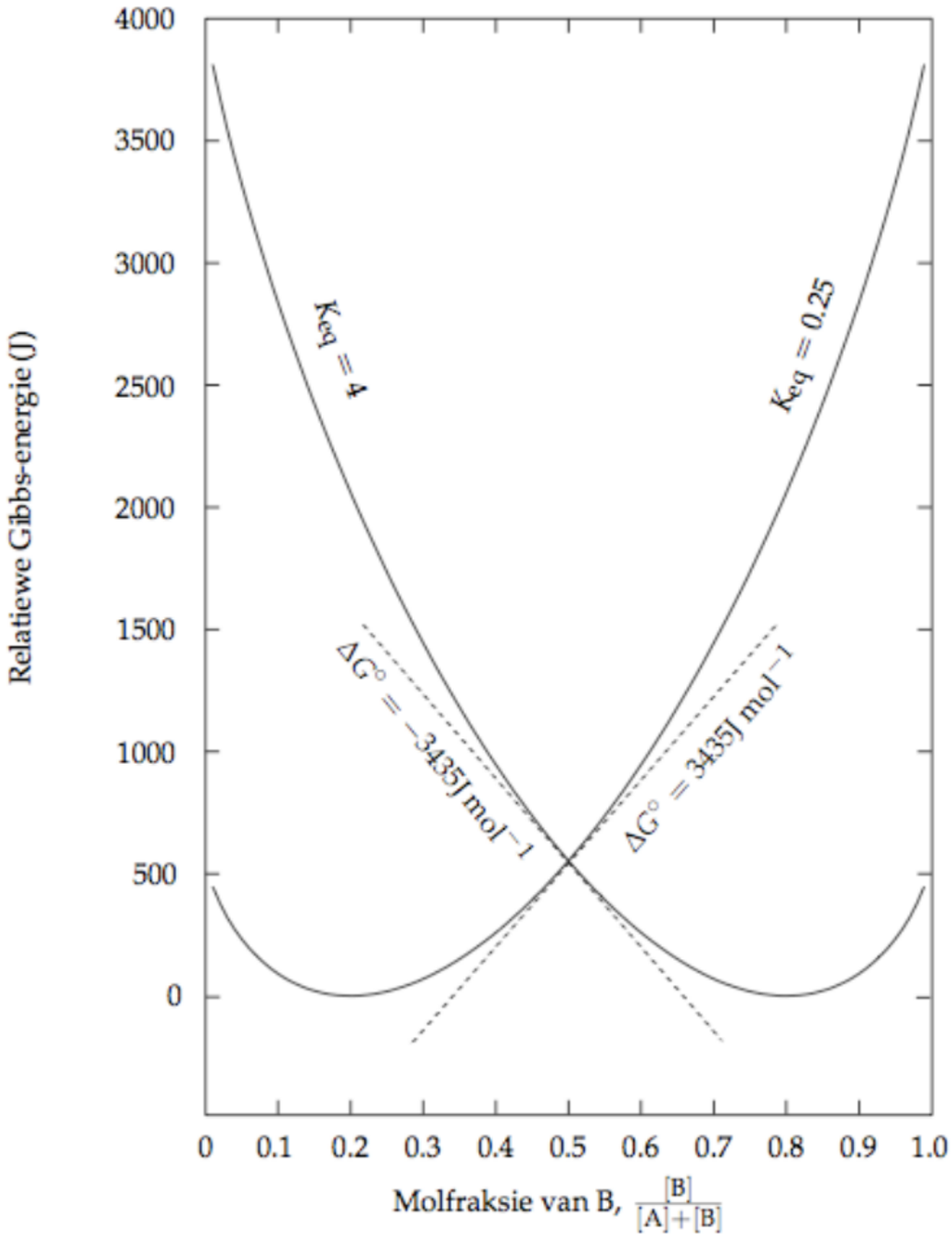


- as Keq = 4 => at equilibrium:
  - [B]eq = 4[A]eq => mole fraction = 0.8

- as Keq = 0.25 => at equilibrium:
  - [B]eq = 0.25[A]eq => mole fraction = 0.2

- ΔG^0 = gradient at Γ=I

- convention that G = 0 at equilibrium



Coupled reactions

$$\mathrm{A} \rightleftharpoons \mathrm{B} \rightleftharpoons \mathrm{C}$$

Individual equilibrium constants

$$K_{eq1} = \frac{(b)_{eq}}{(a)_{eq}} \quad \text{and} \quad K_{eq2} = \frac{(c)_{eq}}{(b)_{eq}}$$

For the sequence as a whole

$$K_{eq12} = \frac{(c)_{eq}}{(a)_{eq}}$$

It follows that:

$$K_{eq1}K_{eq2} = \frac{(b)_{eq}}{(a)_{eq}}\frac{(c)_{eq}}{(b)_{eq}} = \frac{(c)_{eq}}{(a)_{eq}} = K_{eq12}$$

Kinetic and energetic components

Consider a possible rate equation for the reaction

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

$$\begin{aligned} v &= k_\mathrm{f} ab - k_\mathrm{r} cd \\ &= k_\mathrm{f} ab \left(1 - \frac{k_\mathrm{r} cd}{k_\mathrm{f} ab}\right) \\ &= k_\mathrm{f} ab \left(1 - \frac{1}{K_\mathrm{eq}} \frac{cd}{ab}\right) \\ &= k_\mathrm{f} ab \left(1 - \frac{\Gamma}{K_\mathrm{eq}}\right) \end{aligned}$$

$\Gamma / K_\mathrm{eq} = 1$: Equilibrium ($v = 0$)
$\Gamma / K_\mathrm{eq} < 1$: Reaction proceeds forward ($v > 0$)
$\Gamma / K_\mathrm{eq} > 1$: Reaction proceeds backward ($v < 0$)

Exercise 1

- Example: suppose you start with 1M of A and no B and C. Which of the following series will lead to the greatest $[C]_{eq}$?
- (Remember $Keq_T = Keq_1 \times Keq_2$)
  - Series 1

$$\begin{array}{rcl} A \rightleftharpoons B & \Delta G^\circ = +18,85\,\mathrm{kJ\,mol^{-1}}; & K_{eq} = 5 \times 10^{-4} \\ B \rightleftharpoons C & \Delta G^\circ = -18,85\,\mathrm{kJ\,mol^{-1}}; & K_{eq} = 2 \times 10^{3} \end{array}$$

  - Series 2

$$\begin{array}{rcl} A \rightleftharpoons B & \Delta G^\circ = -18,85\,\mathrm{kJ\,mol^{-1}}; & K_{eq} = 2 \times 10^{3} \\ B \rightleftharpoons C & \Delta G^\circ = +18,85\,\mathrm{kJ\,mol^{-1}}; & K_{eq} = 5 \times 10^{-4} \end{array}$$

$$[A] + [B] + [C] = 1M$$

$$[A]_{eq} + [B]_{eq} + [C]_{eq} = 1M$$

Exercise 2: Experimental data

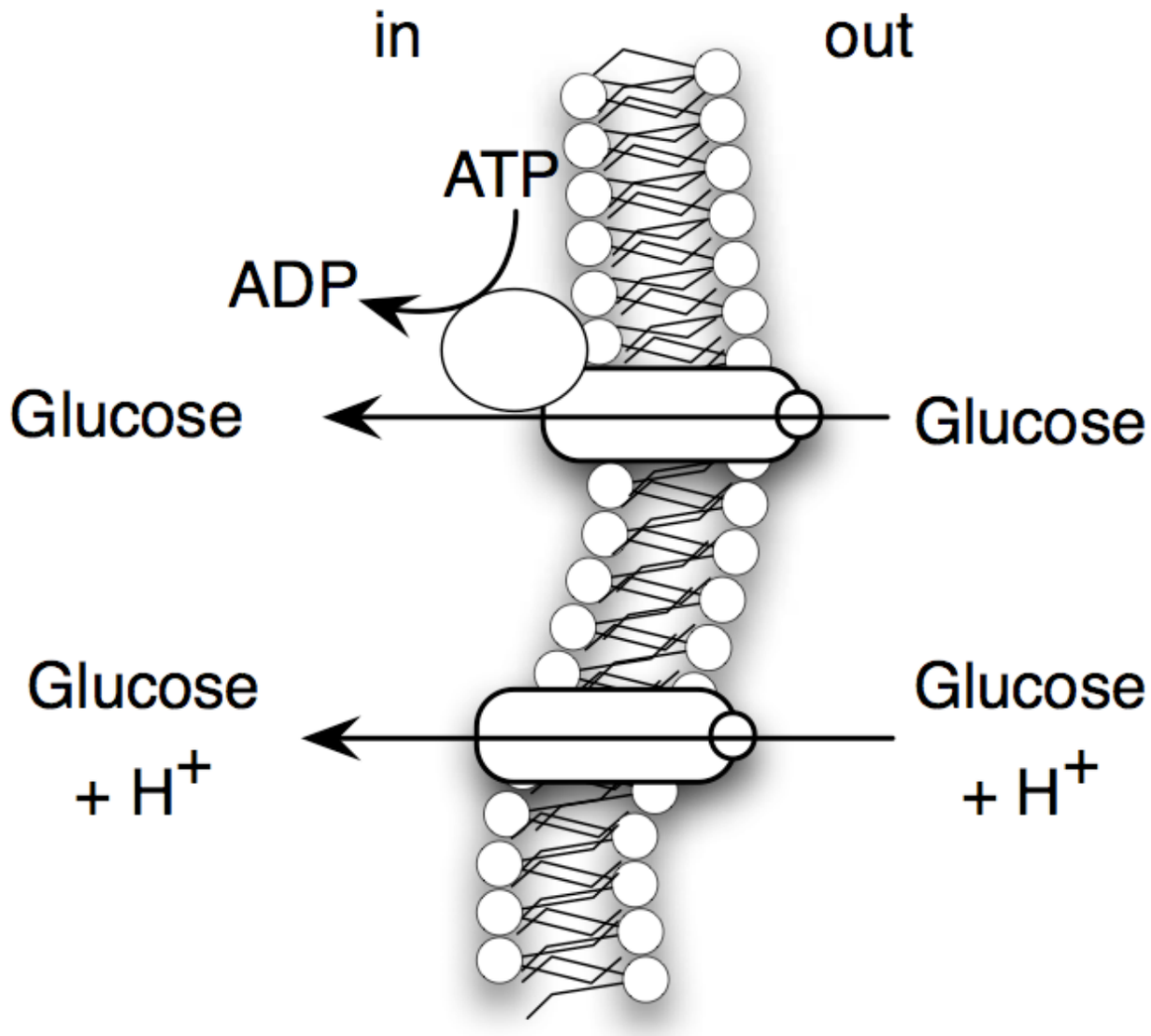
t (s)	a (mM)	b (mM)
0	10.	0.
1	7.5274	2.4726
2	5.86997	4.13003
3	4.75896	5.24104
4	4.01422	5.98578
5	3.51501	6.48499
6	3.18038	6.81962
7	2.95607	7.04393
8	2.80572	7.19428
9	2.70493	7.29507
10	2.63737	7.36263
11	2.59208	7.40792
12	2.56172	7.43828
13	2.54137	7.45863
14	2.52773	7.47227
15	2.51859	7.48141
16	2.51246	7.48754
17	2.50835	7.49165
18	2.5056	7.4944
19	2.50375	7.49625
20	2.50252	7.49748

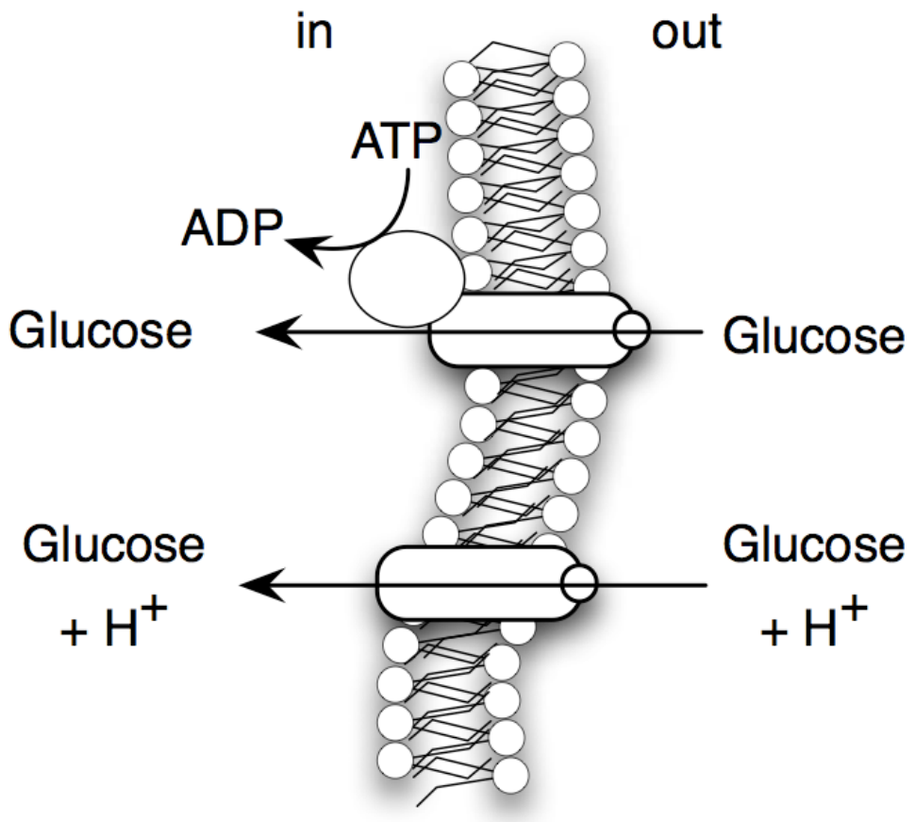
For a non-catalysed, chemical reaction;
A<->B the following experimental data were obtained:

Calculate:

- reaction rate at t=5 s
- Keq
- k(forward)
- k(reverse)
- mass action ratio at t=5 s
- reaction rate at t=20 s
- forward rate at t=20 s
- reverse rate at t=20 s

Coupling of processes





Example If we consider the ABC transporter as depicted in Fig. 15, and assume a ΔG_{ATP} for ATP hydrolysis of -57 kJ/mol, then we can calculate what the maximal glucose gradient would be at which the transporter could still import glucose, assuming 100 % efficiency of coupling between the two processes and a stoichiometry of 1 mol of glucose transported per mol of ATP hydrolysed (i.e. 57 kJ/mol is available per mol of glucose transported):

$$\begin{aligned} \Delta G_{Glc_{up}} &= RT \ln \frac{x_{in}}{x_{out}} \\ 57 \cdot 10^3 &= 8.31447 \cdot 298.17 \cdot \ln \frac{x_{in}}{x_{out}} \\ \frac{x_{in}}{x_{out}} &= 1 \cdot 10^{10} \end{aligned}$$

Exercise 3

- Calculate the maximal glucose gradient possible for a proton symport system with a stoichiometry of 2 protons per glucose molecule, if there is a pH difference of -0.3 (inside 6.7, outside 7.0), and the membrane potential is
-200mV (negative inside).

- R=8.31447 J/K/mol, T=298.17 K, F=96.485 kJ/V