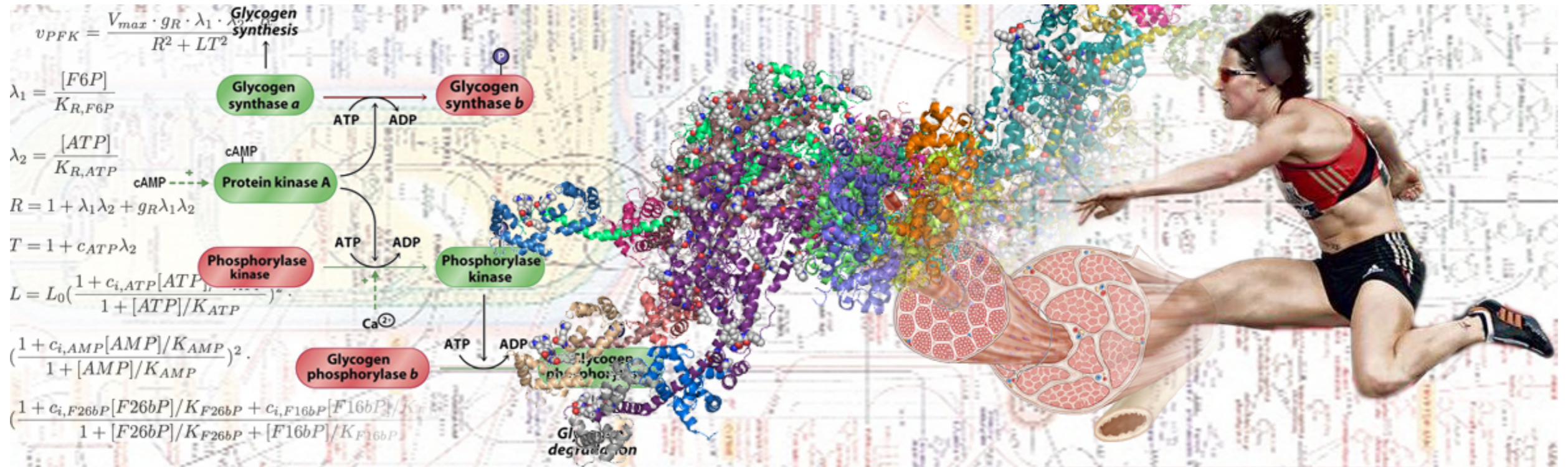


Biochemistry 714 Mini-course: Molecular Systems Biology



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

March - April 2024

Programme

	Mon	Tue	Wed	Thu	Fri
18 – 22 Mar	<p>09:30 Welcome & intro Lecture 1 (Mass action kinetics) JLS</p> <p>14:00 Lecture 2 (Enzyme catalysed reactions) JLS</p>	<p>09:30 Lecture 3 (Coupled reactions) JLS</p> <p>14:00 Tut 1 Intro JMR</p>	<p>09:30 Lecture 4 (Networks) JLS</p> <p>14:00 Tut 2 (Mass action kinetics) JMR</p>	<p>Human Rights Day</p>	<p>09:30 Lecture 5 (MCA) JLS</p> <p>14:00 Tut 3 (Enzyme catalysed reactions) JMR</p>
25 – 29 Mar	<p>Seminar Allocations (online)</p> <p>10:00 Tut 4 (Networks) JMR</p>	<p>09:30 Tut 5 (MCA) JMR</p> <p>Afternoon: Seminar</p>	<p>09:30 Tut 6 (Kinetic model) JMR</p> <p>14:00 Assessment 1 JMR & DvN</p>	<p>09:30 Research lect. DvN</p> <p>11:00 Peer grading of Assessment 1 JMR & DvN</p> <p>14:00 Introduction to Practical DvN</p>	<p>Good Friday</p>
1 – 5 April	Recess				
8 – 12 April	<p>09:00 Practical (whole day) DvN</p>	<p>09:00 Practical (morning) DvN</p> <p>Afternoon: Seminar</p>	<p>09:00 Practical / Data analysis (whole day) DvN & JMR</p>	<p>09:00 Research lect. JMR</p> <p>11:00 Data analysis / Practical (whole day) JMR / DvN</p>	<p>09:00 Data analysis (whole day) JMR</p>
15 – 19 April	<p>10:30 Data analysis (whole day) JMR</p>	<p>09:00 Research lect. JLS</p> <p>Hand in final report (16/04/2024 23:59)</p>			

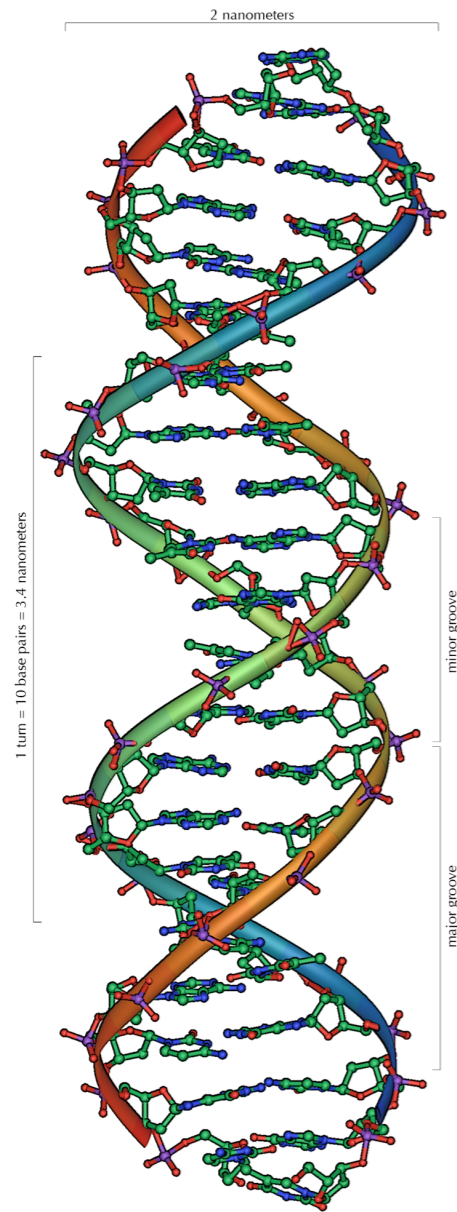
Course material

- Available at:

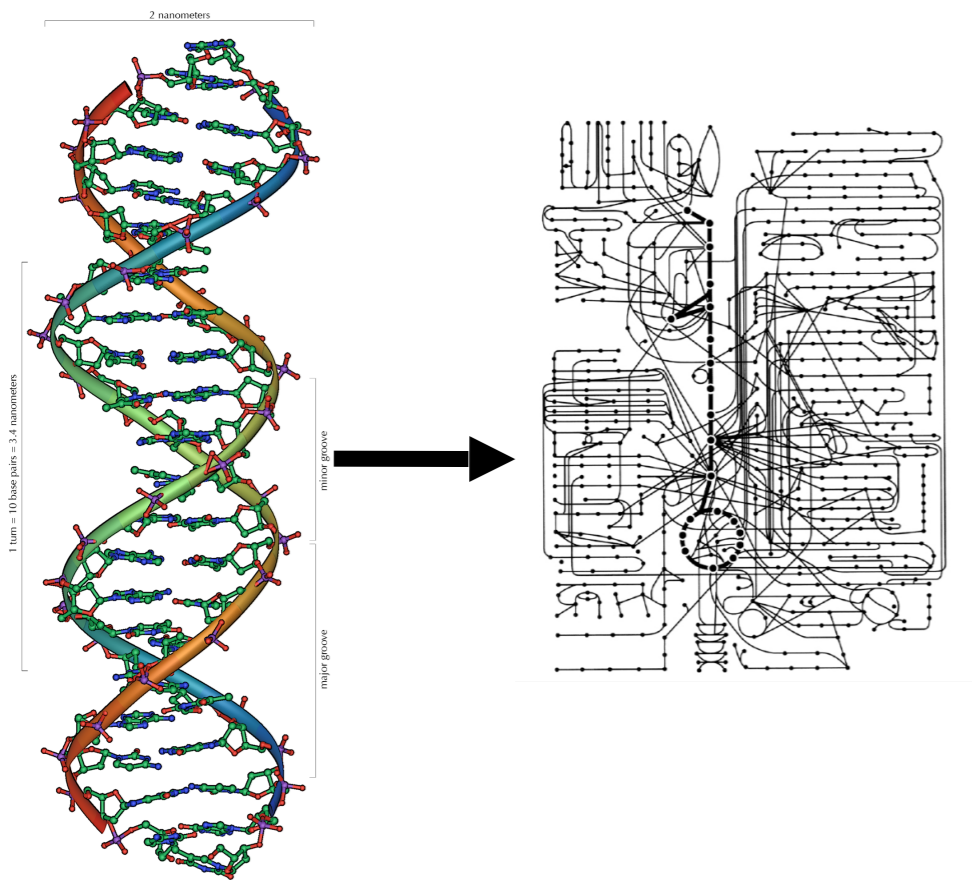
<http://glue.jjj.sun.ac.za/jjj/minicourse/>

- Molecular Systems Biology textbook available as pdf
- Lecture notes Biochem 324
- Lecture slides
- Tutorials & Python notebooks

The Ultimate Predictive Model



From Sequence to Network



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

\dot{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

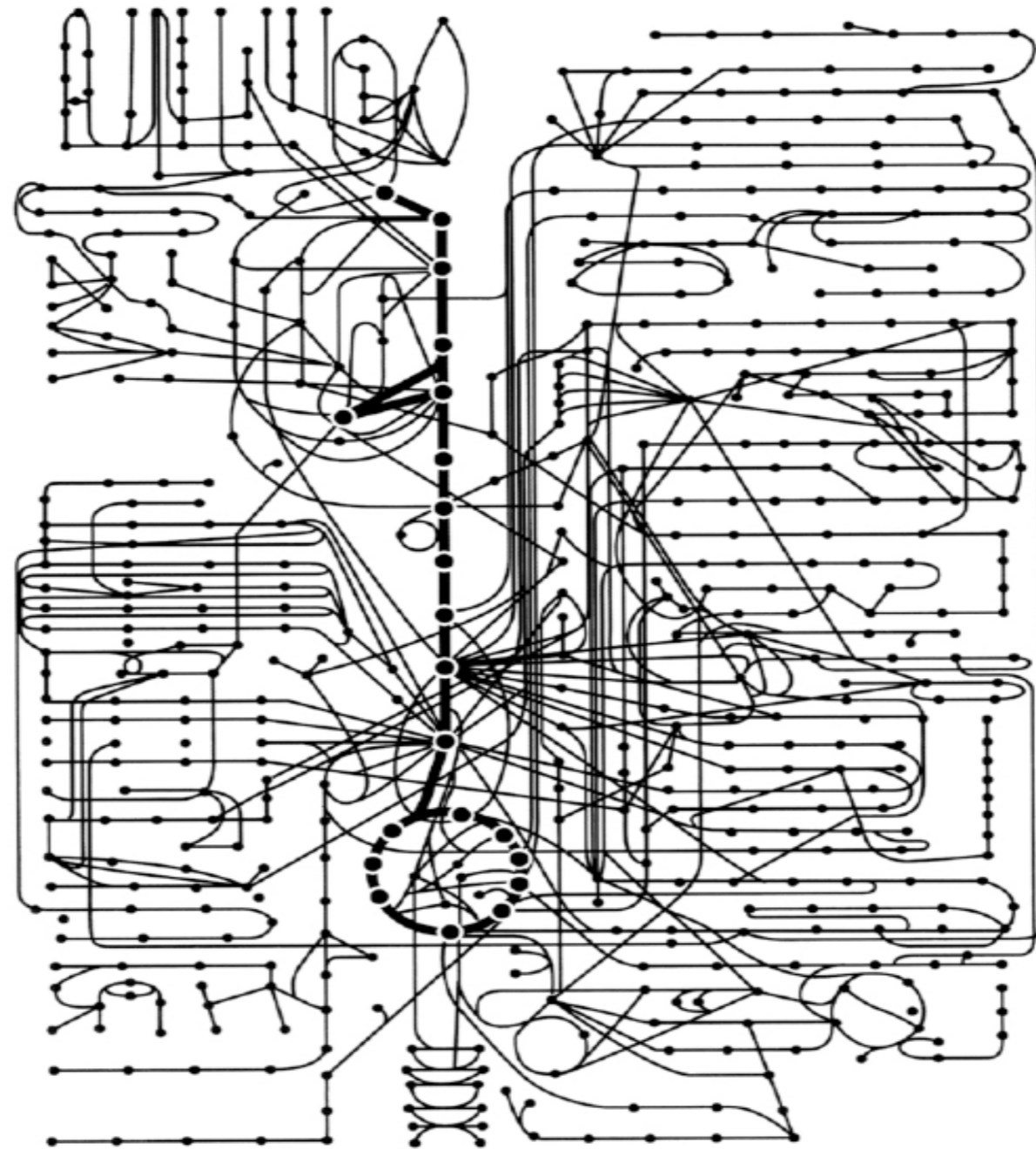
Topology studies show networks but are far removed from functional behaviour.

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

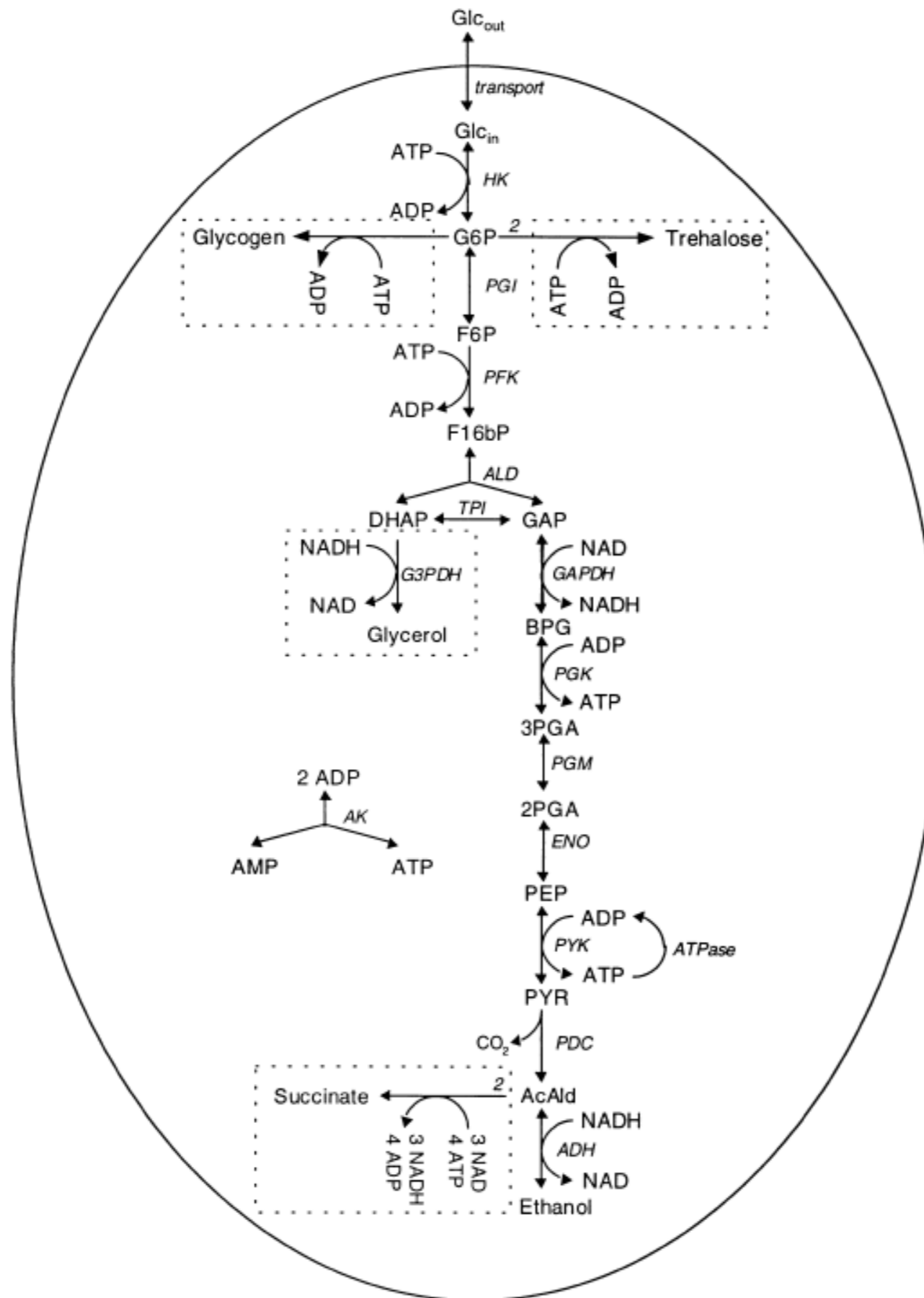
Applications in medicine (drug target identification) and biotechnology (metabolic engineering), need specific targets in the system (molecular mechanism).

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

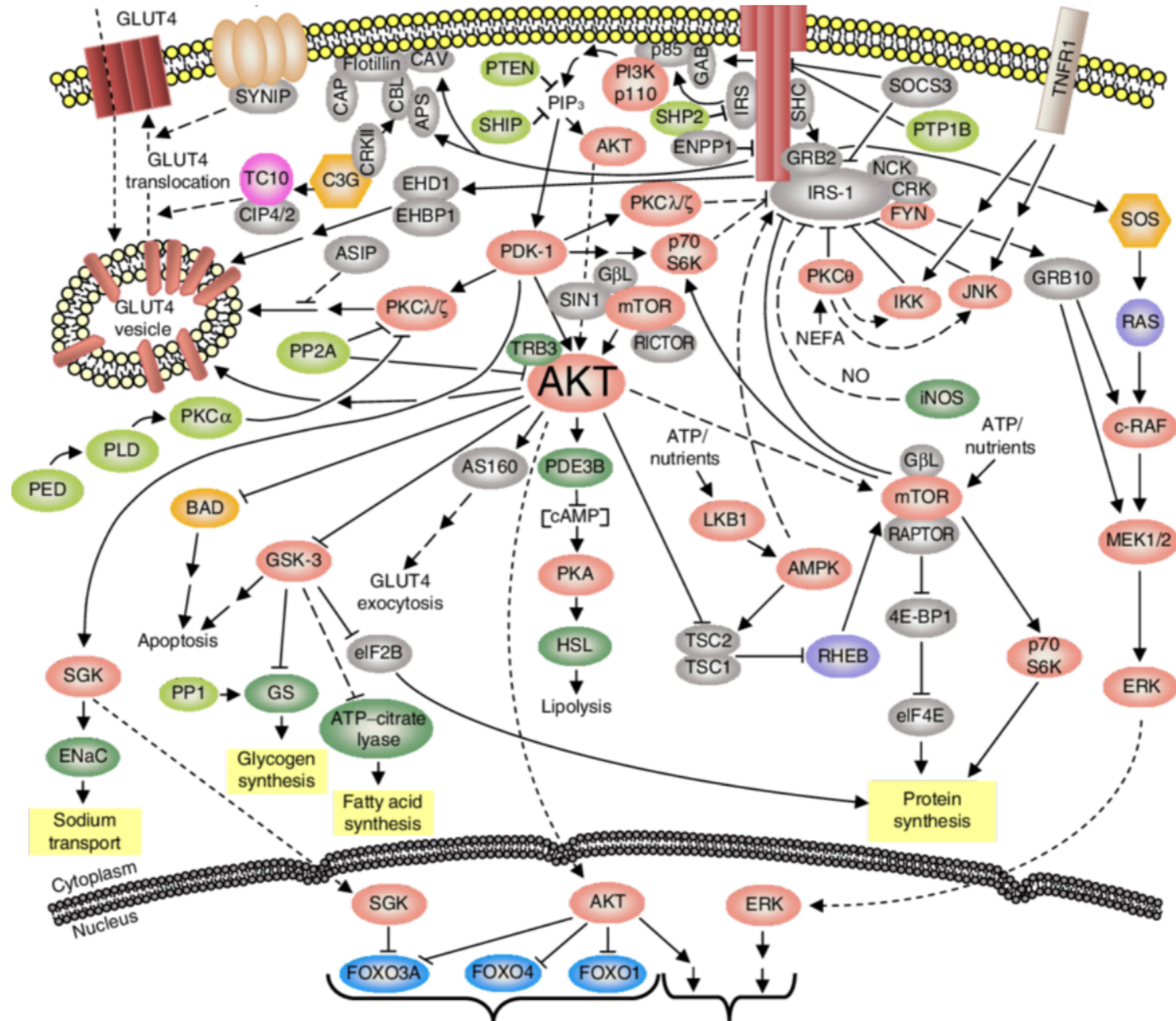
Metabolism



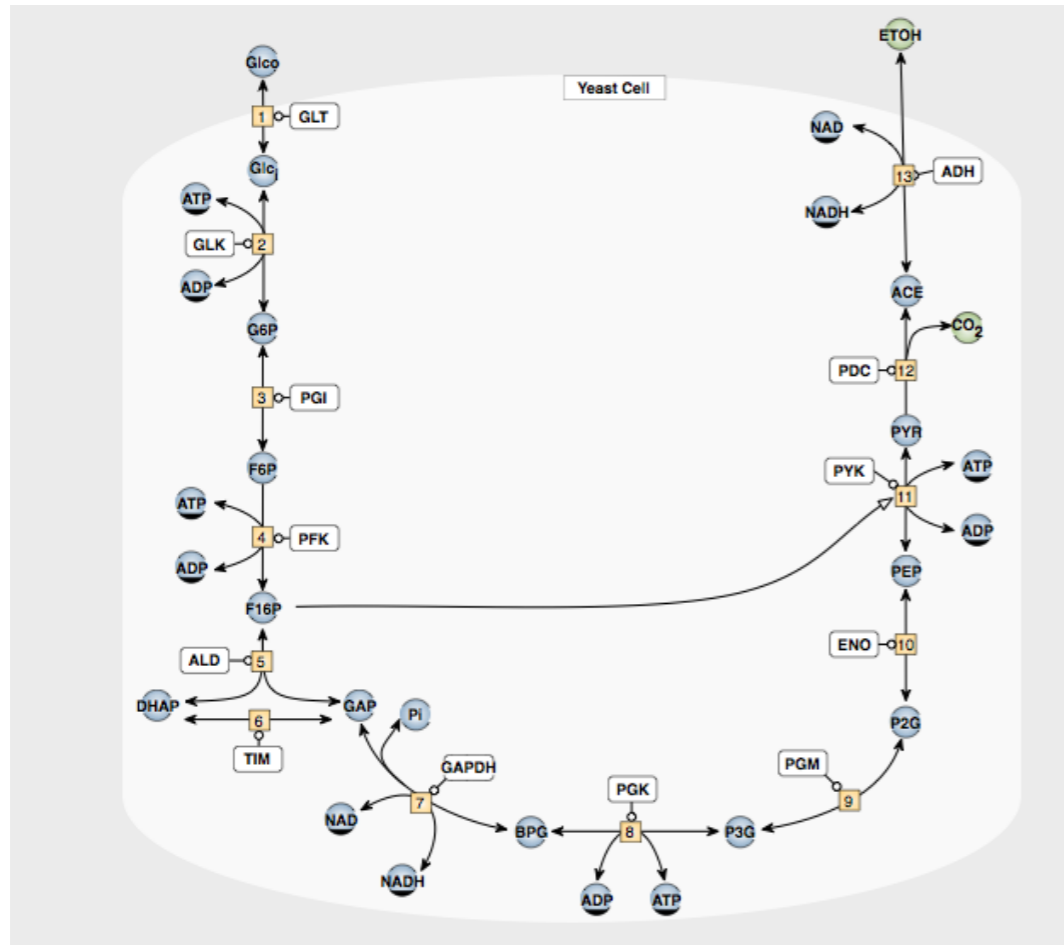
Glycolysis



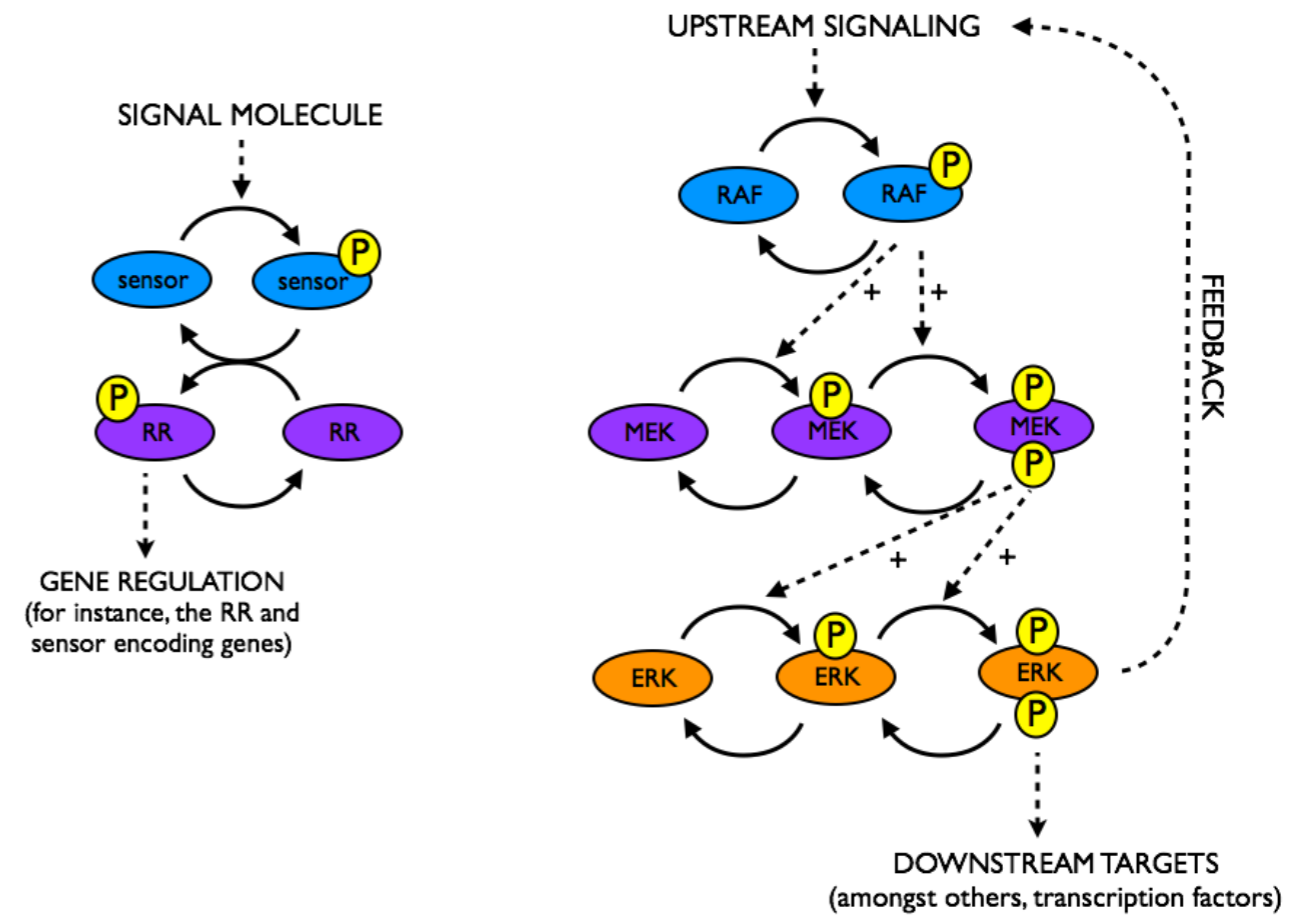
Signalling



Metabolic and signal transduction networks



Metabolite concentrations, fluxes, time courses

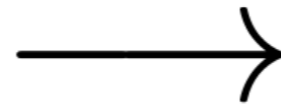
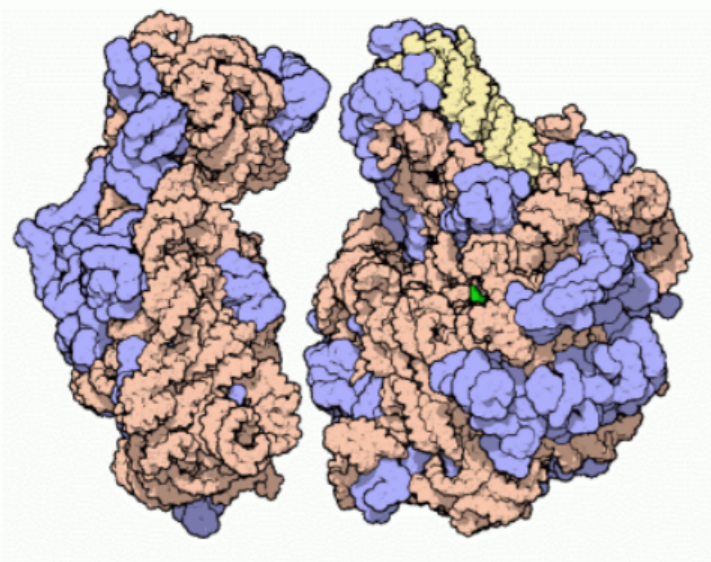


Response time, dose-response relation, ligand specificity

Systems Biology

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

Macromolecules
(dead)



Interaction

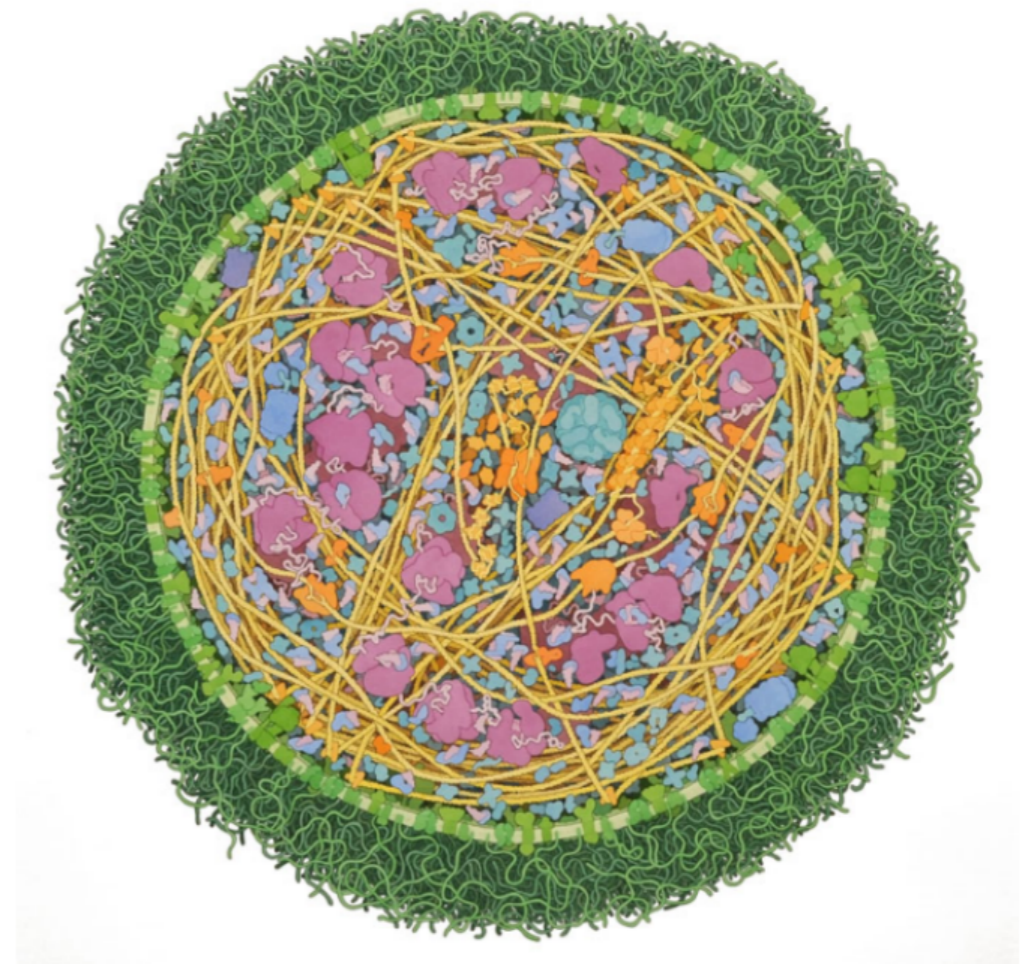


Function



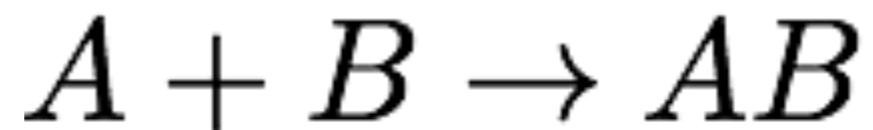
Functional
organisation

Cell
(alive)

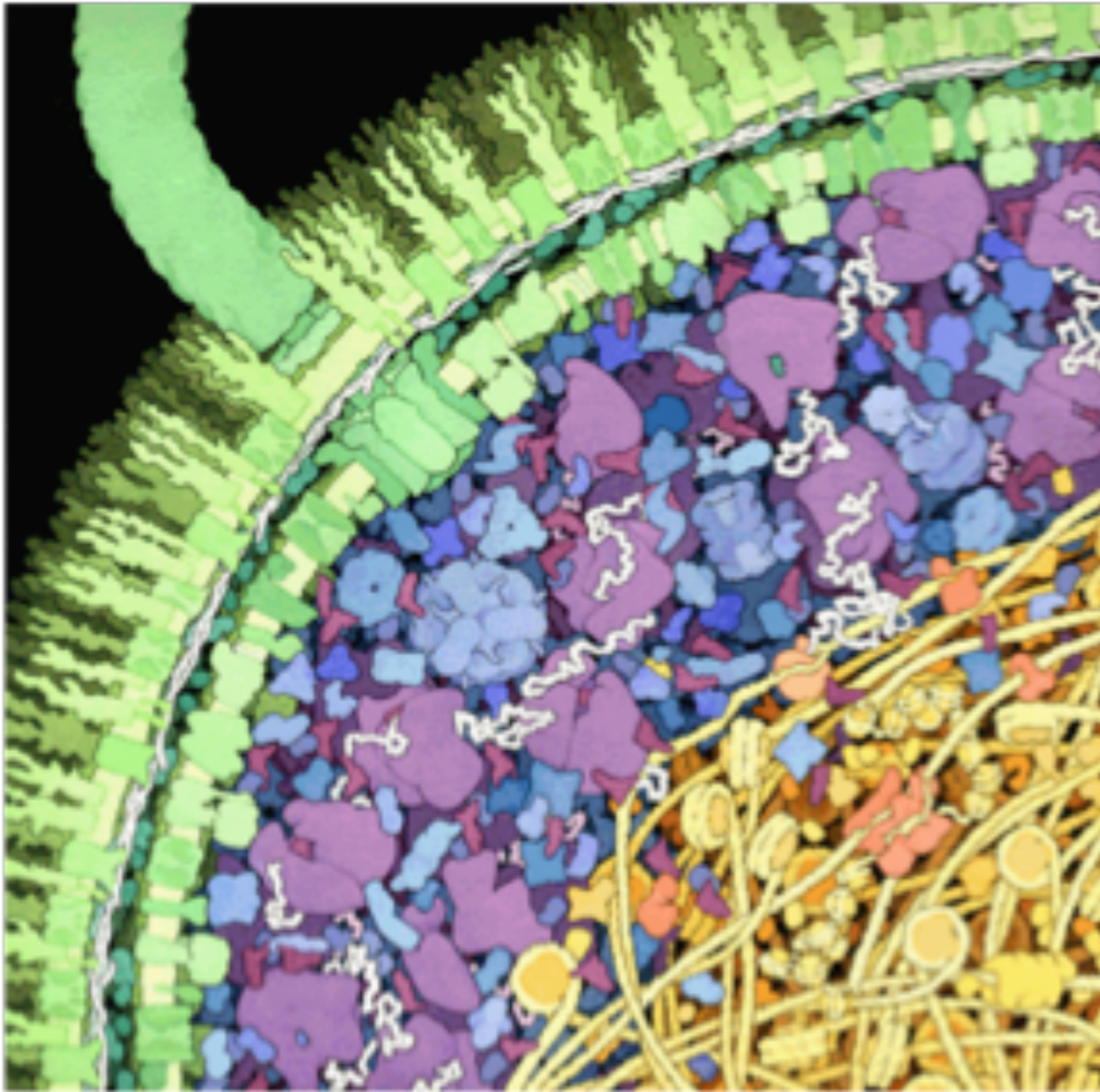


Networks and reactivity

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



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



- 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 catalyzed by enzymes
- We start first with non-catalyzed, chemical kinetics and then move to enzyme catalyzed reactions

Kinetics of chemical reactions

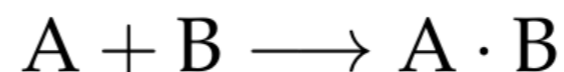
- Why does a reaction occur?
- What determines the direction of a reaction, i.e. forward or reverse?
- What determines the rate of a reaction?
- When does a reaction rate go to zero?
- How do the molecules know whether they should react or not?
- Net reaction rate, micro-reversibility, statistics

Driving force of a reaction

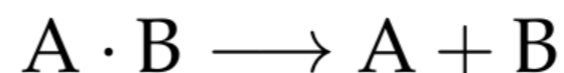
- A reaction will only occur if the Gibbs free-energy content of the products is less than that of the substrates, i.e. $\Delta G < 0$
- Gibbs free energy change determines the direction of the reaction
- The rate at which a reaction occurs is dependent on both thermodynamics and kinetics

Three types of elementary reactions

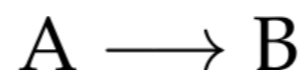
1. an *association* between two molecules to form a non-covalently bound complex,



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



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



Reaction mechanism

Breaking a reaction up into irreversible elementary reactions:



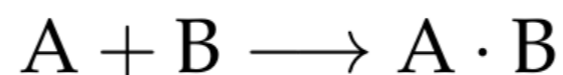
could have the mechanism



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.,



the reaction rate is proportional to concentration

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

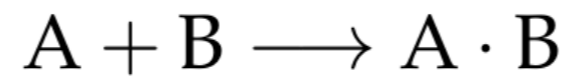
where

v is the rate of reaction

a and b are the concentrations of A and B.

$$v \propto ab$$

Reaction rate, v

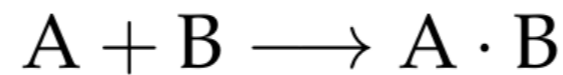


$$v = -\frac{da}{dt} = -\frac{db}{dt} = \frac{d(a \cdot b)}{dt}$$

The reaction rate v thus has units of concentration·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*:



$$v = kab$$

Reaction order

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

Determining reaction order



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

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

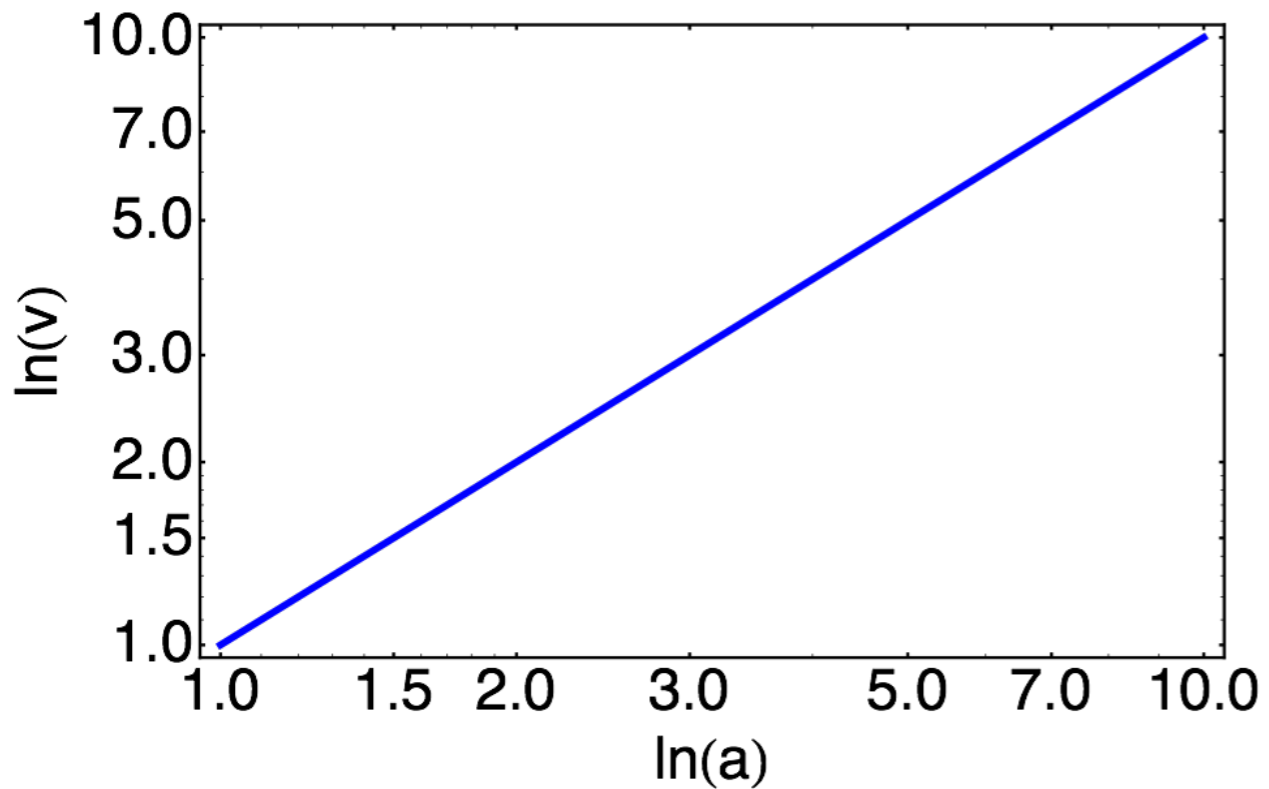
$$\frac{d \ln v}{d \ln a}$$

evaluated at a given a .

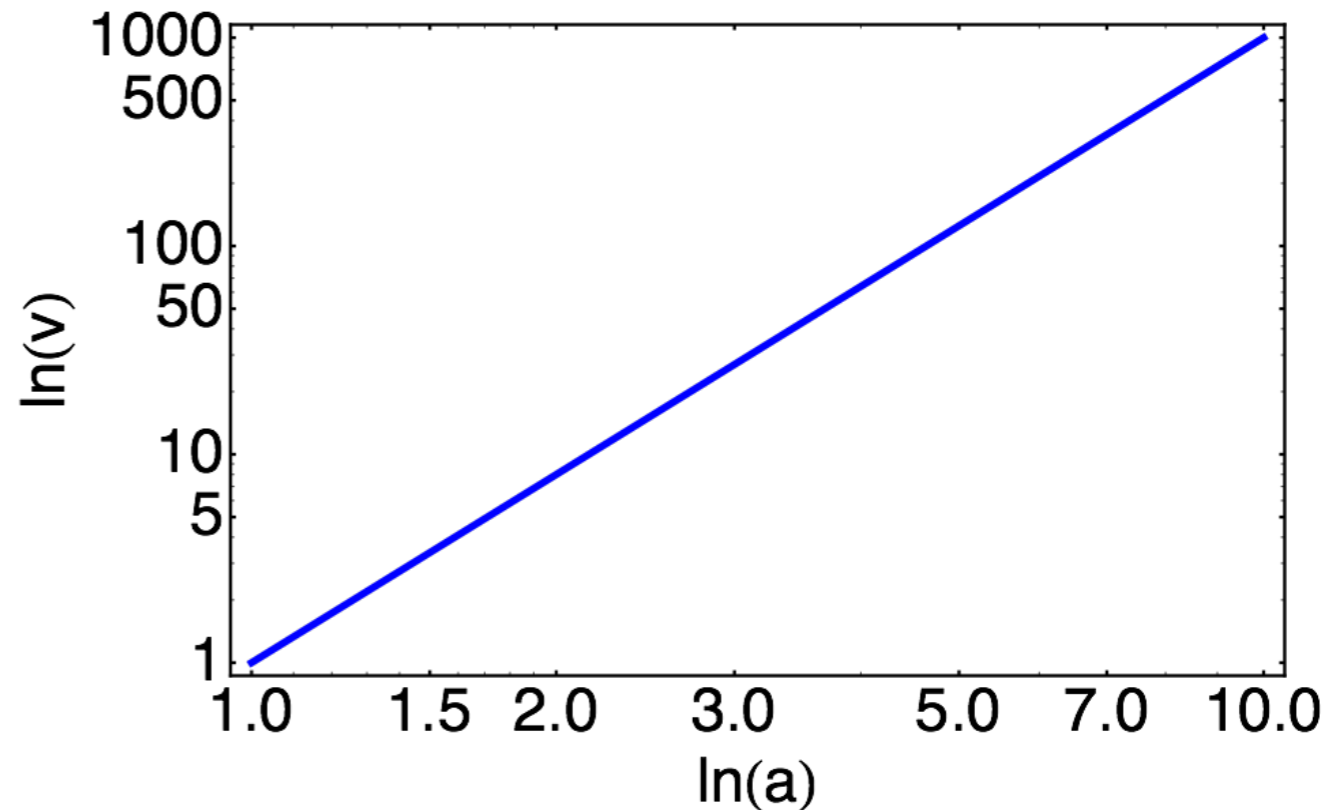
More correctly, because v is a function of both a and b ,

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

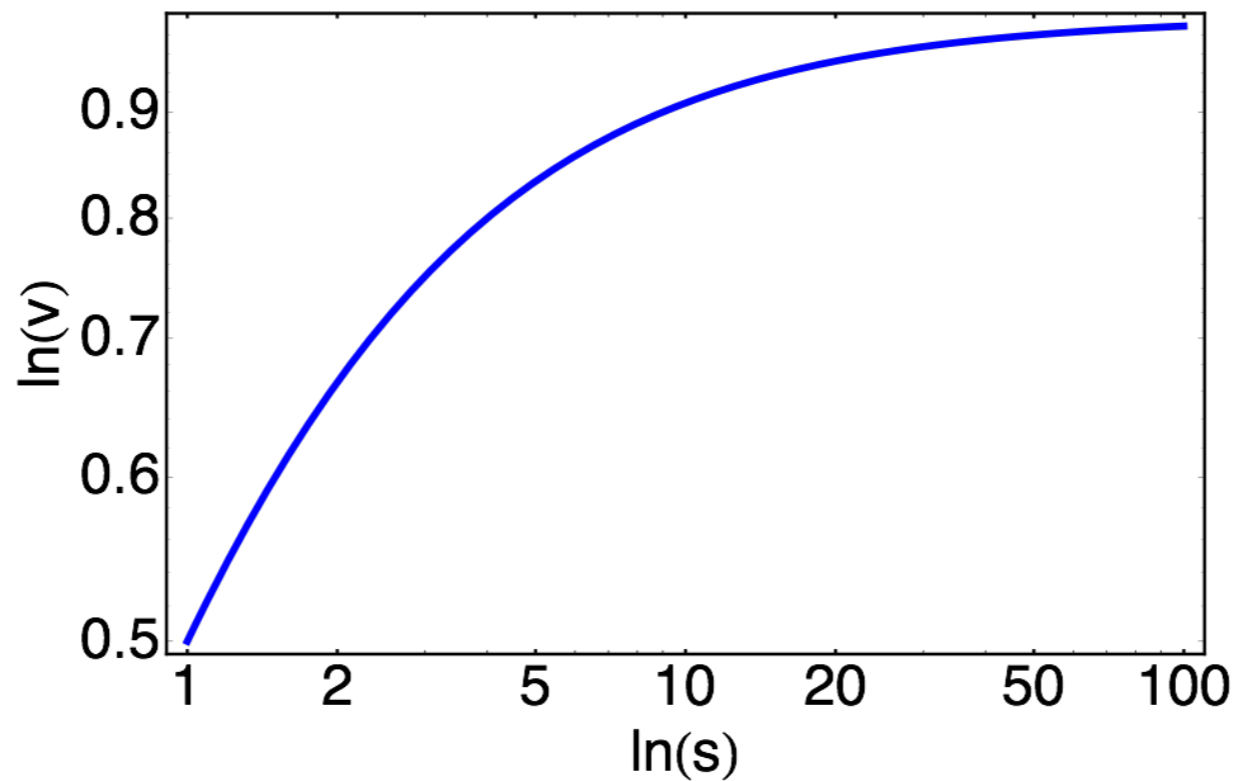
$$v=ab; b=1$$



$$v=a^3b; b=1$$



$$v = \frac{V_m \times s}{K_s + s}$$



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



is a combination of the *forward* reaction



and the *reverse* reaction



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

$$v = v_f - v_r = k_f ab - k_r c$$

The equilibrium constant

At equilibrium:

$$v = v_f - v_r = 0$$

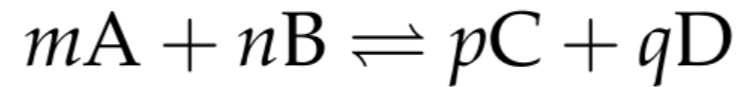
Therefore

$$k_f(a)_{eq}(b)_{eq} - k_r(c)_{eq} = 0$$

so that

$$\frac{k_f}{k_r} = \frac{(c)_{eq}}{(a)_{eq}(b)_{eq}} = K_{eq}$$

General reaction



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

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

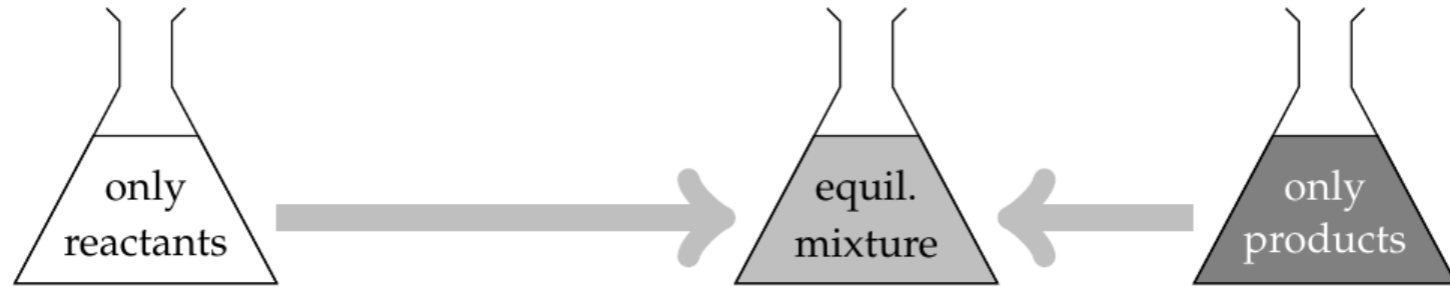
and using the equilibrium condition we obtain

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

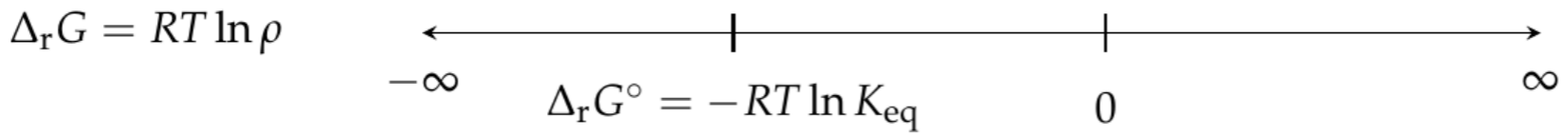
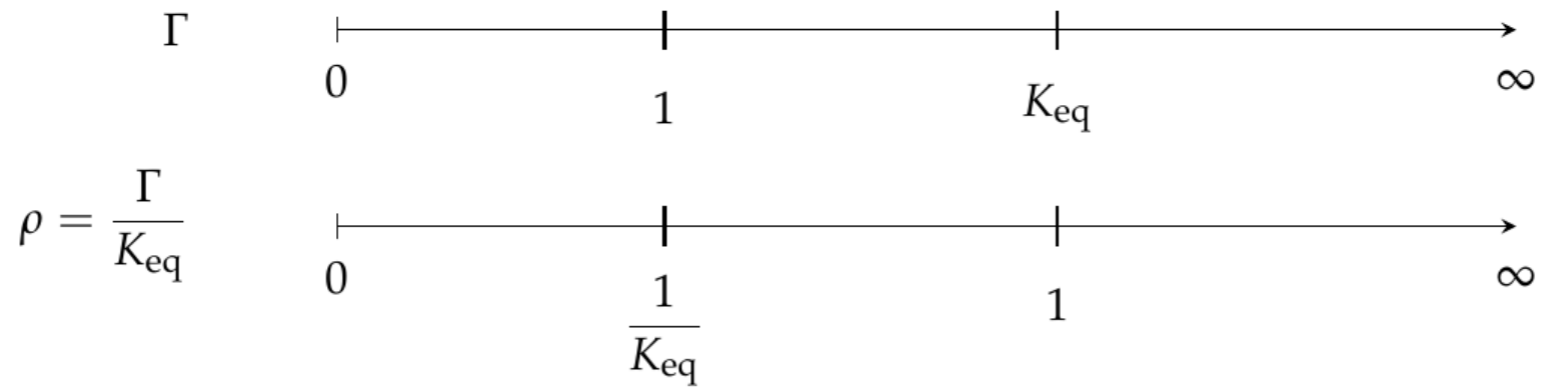
The mass-action ratio and the distance from equilibrium

$$\frac{v_r}{v_f} = \frac{k_r c}{k_f ab} = \left(\frac{c}{ab} \right) / K_{\text{eq}}$$

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).



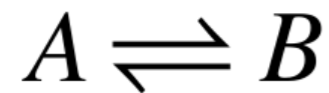
Standard conditions Equilibrium



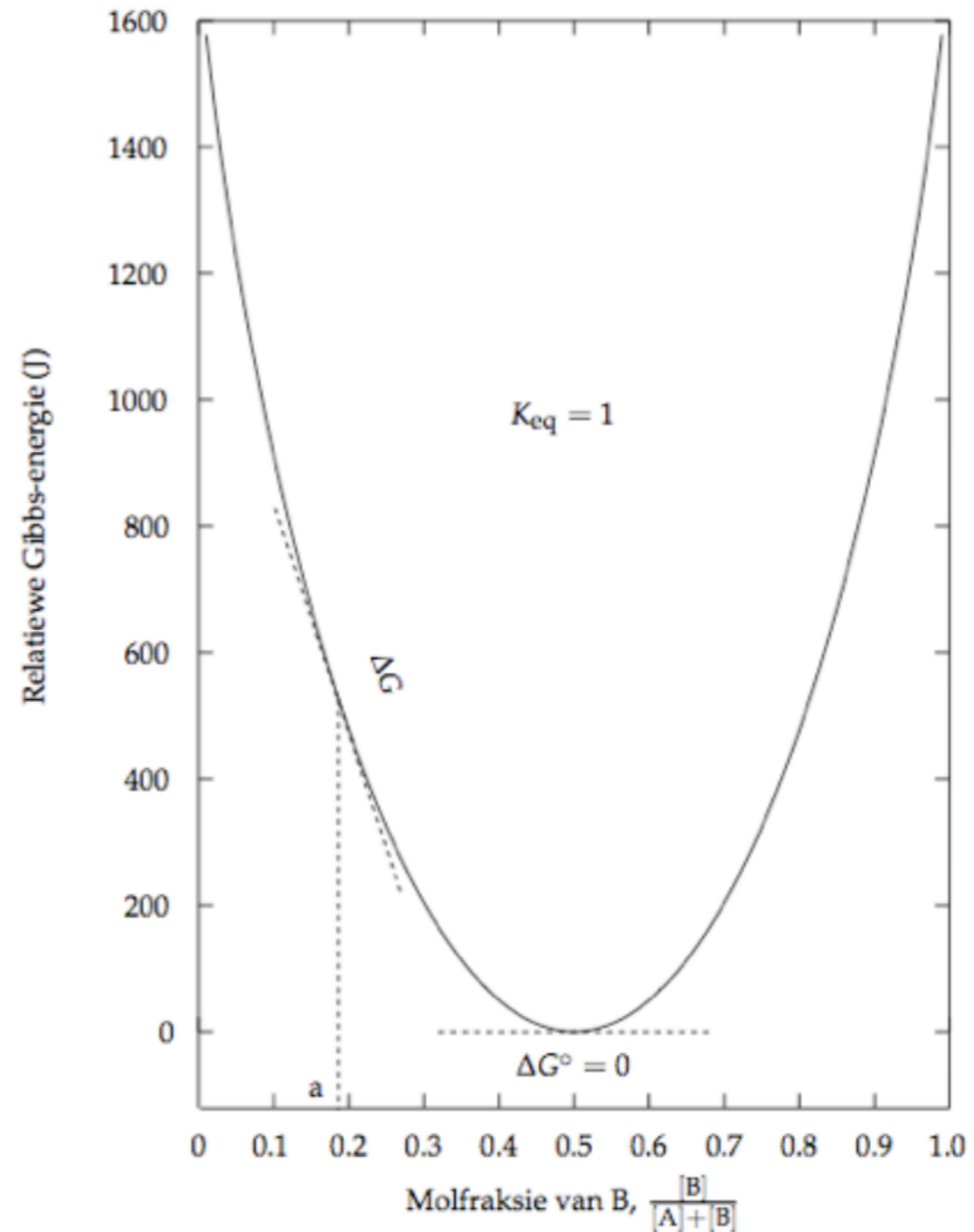
The Gibbs energy

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

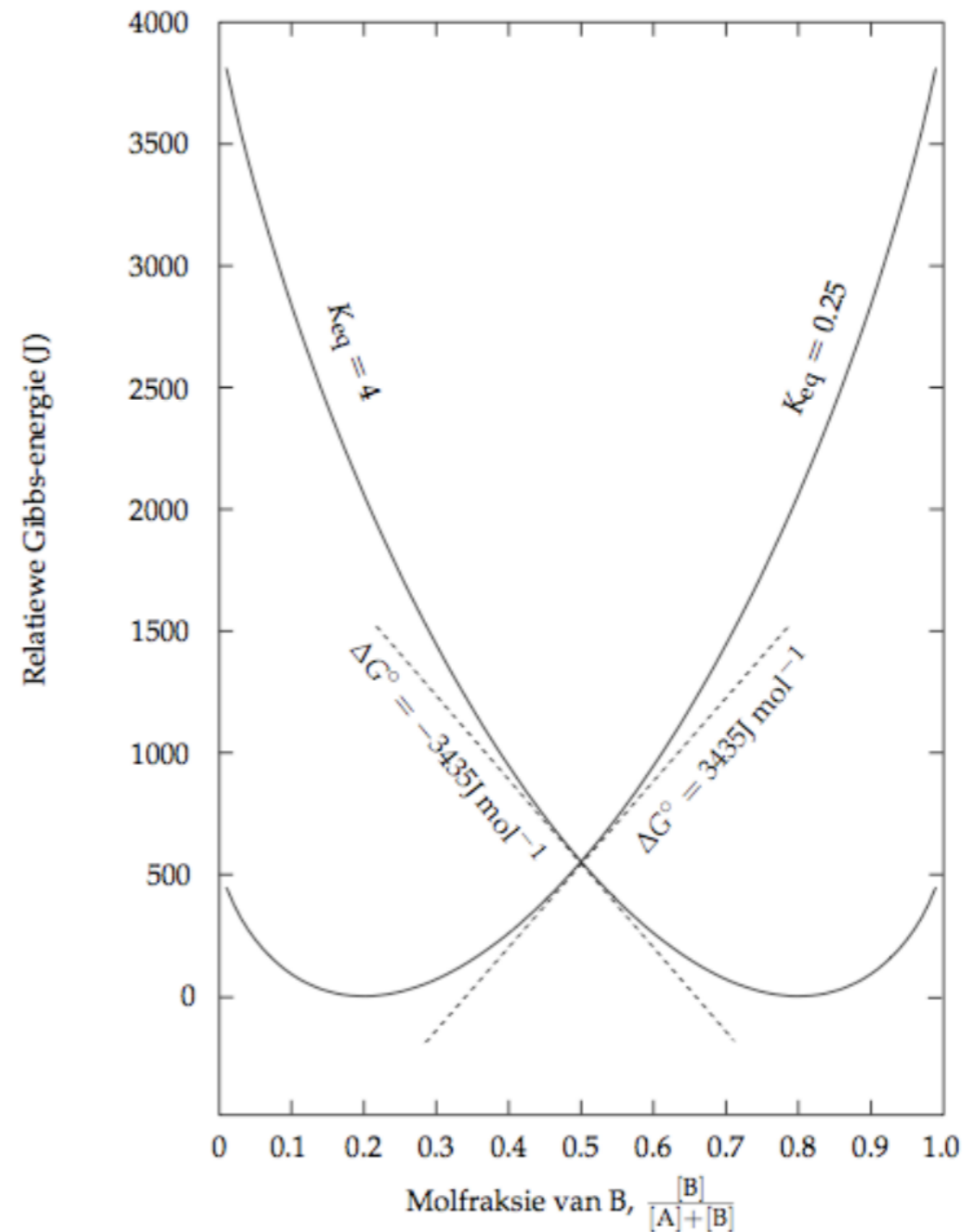
$$\Delta G^{\circ} = RT \ln \frac{1}{K_{\text{eq}}} = -RT \ln K_{\text{eq}}$$



- gradient increases as reaction moves further away from eq
- equilibrium $\Delta G = 0$
 - ▶ gradient = 0
 - ▶ $K_{eq} = 1 \Rightarrow [A]_{eq} = [B]_{eq}$
- at standard conditions $\Gamma=1$
 - ▶ $[A] = [B] \Rightarrow$ for this example equilibrium is at standard conditions
➔ $\Delta G^0 = 0$



- as $K_{eq} = 4 \Rightarrow$ at equilibrium:
 - ▶ $[B]_{eq} = 4[A]_{eq} \Rightarrow$ mole fraction = **0.8**
- as $K_{eq} = 0.25 \Rightarrow$ at equilibrium:
 - ▶ $[B]_{eq} = 0.25[A]_{eq} \Rightarrow$ mole fraction = **0.2**
- $\Delta G^0 =$ gradient at $\Gamma=1$
- convention that $G = 0$ at equilibrium



Coupled reactions



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

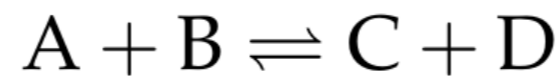
$$K_{eq12} = \frac{(c)_{eq}}{(a)_{eq}}$$

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



$$\begin{aligned}v &= k_f ab - k_r cd \\&= k_f ab \left(1 - \frac{k_r cd}{k_f ab} \right) \\&= k_f ab \left(1 - \frac{1}{K_{eq}} \frac{cd}{ab} \right) \\&= k_f ab \left(1 - \frac{\Gamma}{K_{eq}} \right)\end{aligned}$$

$\Gamma / K_{eq} = 1$: Equilibrium ($v = 0$)

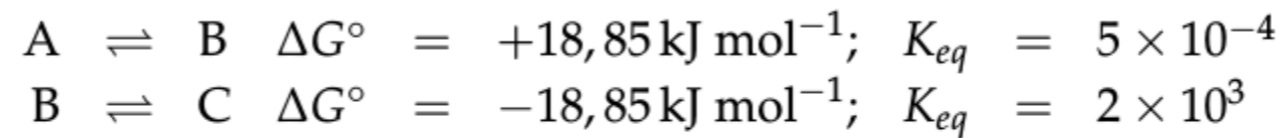
$\Gamma / K_{eq} < 1$: Reaction proceeds forward ($v > 0$)

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

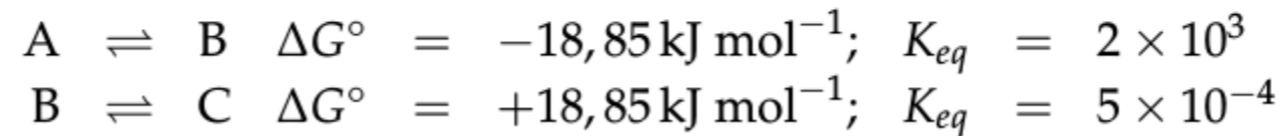
Exercise I

- 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 $K_{eq_T} = K_{eq_1} \times K_{eq_2}$)

- Series 1



- Series 2



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

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

Exercise 2: Experimental data

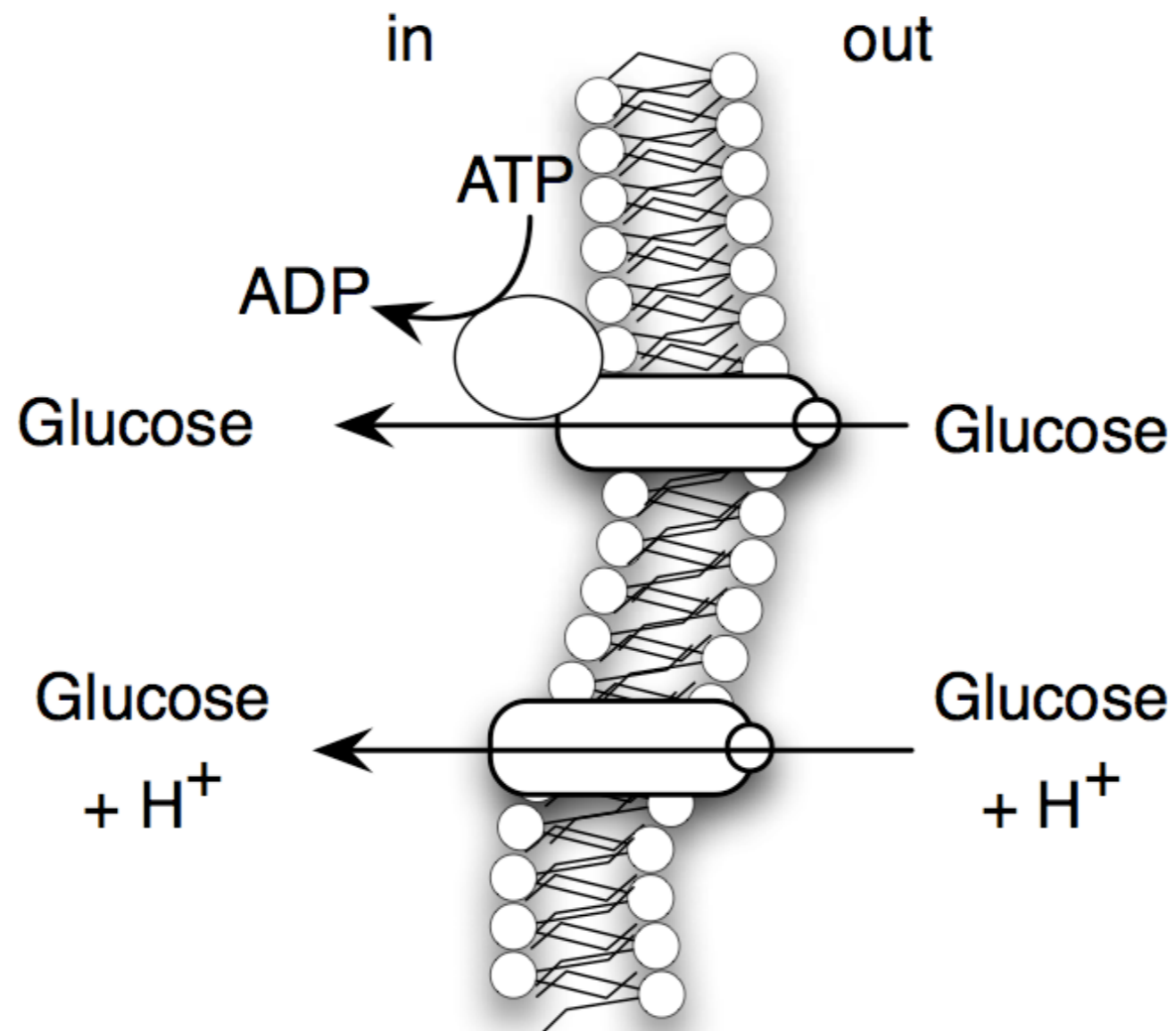
For a non catalyzed, chemical reaction;
 $A \rightleftharpoons B$ the following experimental data
were obtained:

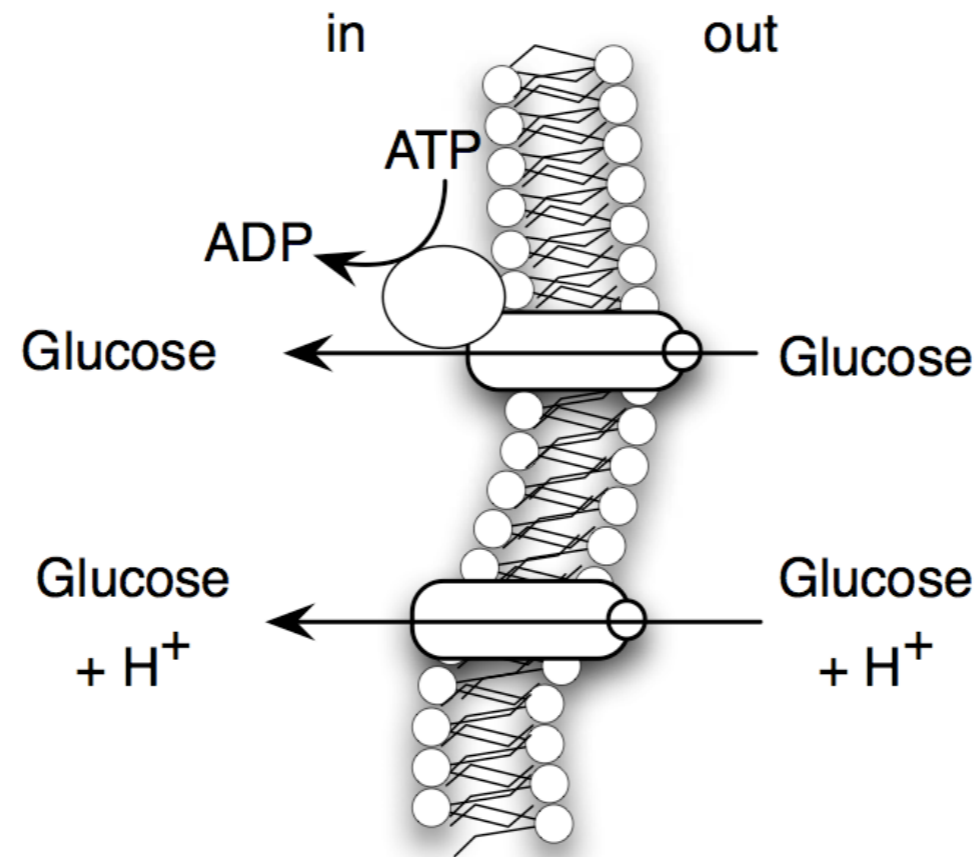
t (s)	a (mM)	b (mM)
0	10.	0.
1	7.5274	2.4726
2	5.86997	4.13003
3	4.75896	5.24104
4	4.01422	5.98578
5	3.51501	6.48499
6	3.18038	6.81962
7	2.95607	7.04393
8	2.80572	7.19428
9	2.70493	7.29507
10	2.63737	7.36263
11	2.59208	7.40792
12	2.56172	7.43828
13	2.54137	7.45863
14	2.52773	7.47227
15	2.51859	7.48141
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

Calculate:

- reaction rate at $t=5$ s
- K_{eq}
- $k(\text{forward})$
- $k(\text{reverse})$
- 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):

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

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).
- $R=8.31447 \text{ J/K/mol}$, $T=298.17 \text{ K}$,
 $F=96.485 \text{ kJ/V}$