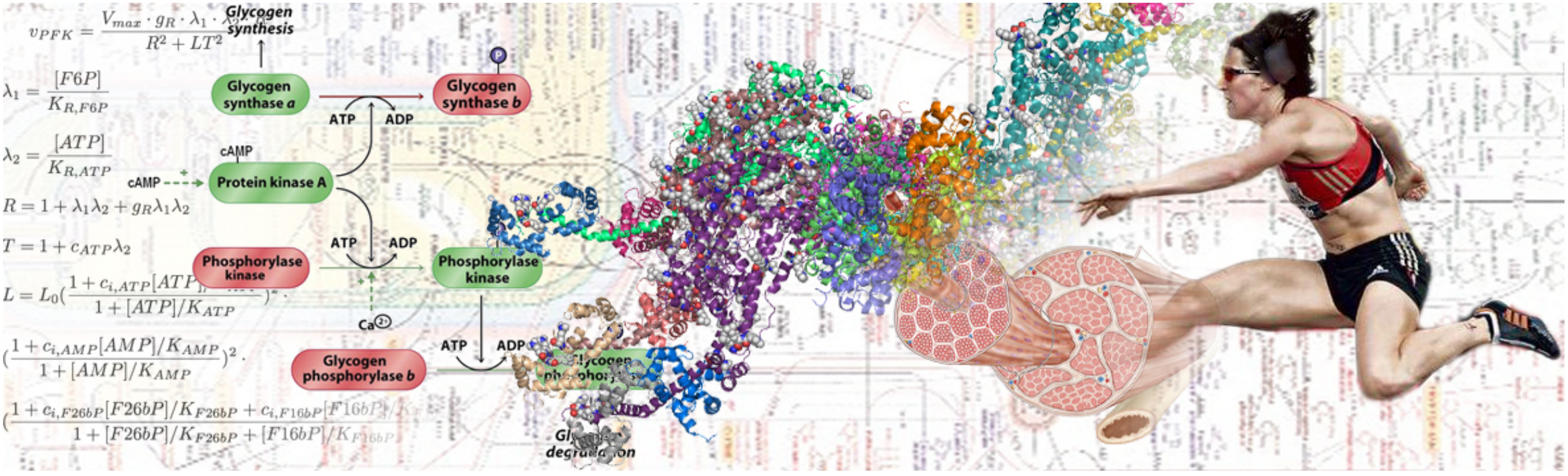
Mini-course: Molecular Systems Biology



Prof Jacky Snoep (Lectures and Practical) and Prof Johann Rohwer (Tutorials and Data analysis)

March 2018

Programme

	Mon	Tue	Wed	Thu	Fri
5 – 9 Mar	**Morning: Revise Biochem 324** **Tut 1 (14:00) Introduction to Python** JR	**Lect. 1 (09:30)** JLS **Afternoon: Seminar**	**Lect. 2 (09:30)** JLS **Tut 2 (14:00)** JR	**Research Lecture (09:00)** Dr A Botes **Lect. 3 (11:00)** JLS **Tut 3 (14:00)** JR	**Lect. 4 (09:30)** JLS **Tut 4 (14:00)** JR
12 - 16 Mar	**Lect. 5 (09:30)** JLS **Tut 5 (14:00)** JR	**Tut 6 (09:30)** JR **Afternoon: Seminar**	**Practical (09:30)** whole day JLS	**Research Lecture (09:30)** JLS **Practical (10:30)** rest of day JLS	**Practical (09:30)** whole day JLS
19 - 23 Mar	**Practical (09:30)** JLS **Data analysis (14:00)** JR	**Data analysis (09:30)** JR **Afternoon: Seminar**	***Human Rights Day***	**Research Lecture (09:30)** JR **Data analysis (10:30)** rest of day JR	**Data Analysis (09:30)** whole day JR
26 Mar	**Finish final report** rest of day **Hand in final report (16:30)**				

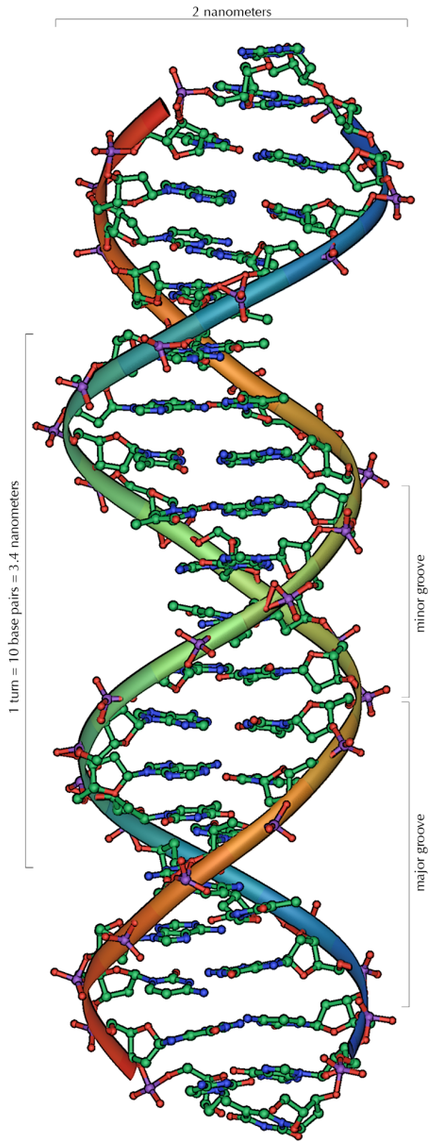
Course material

- Available at:

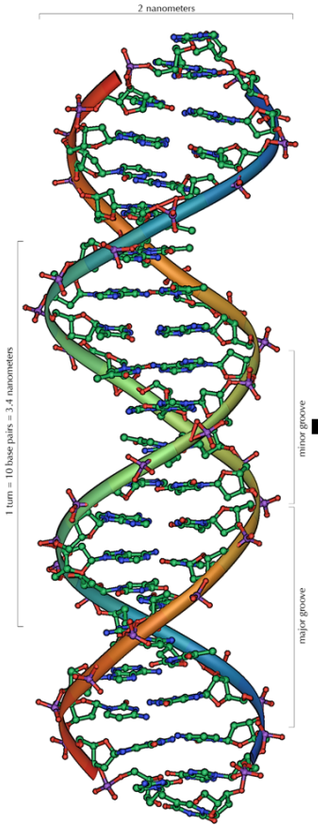
  http://glue.jjj.sun.ac.za/jjj/minicourse/

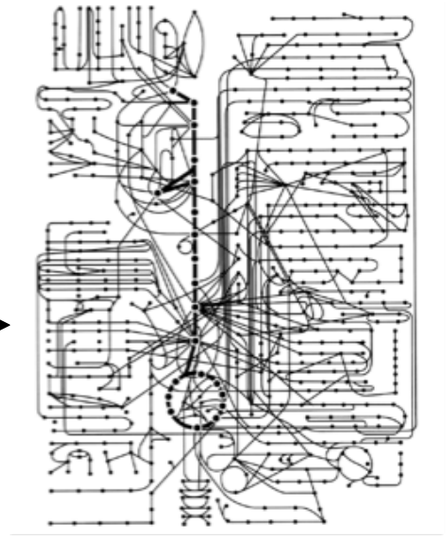
- Molecular Systems Biology textbook available as pdf
- Lecture notes Biochem 324
- Lecture slides
- Tutorials

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

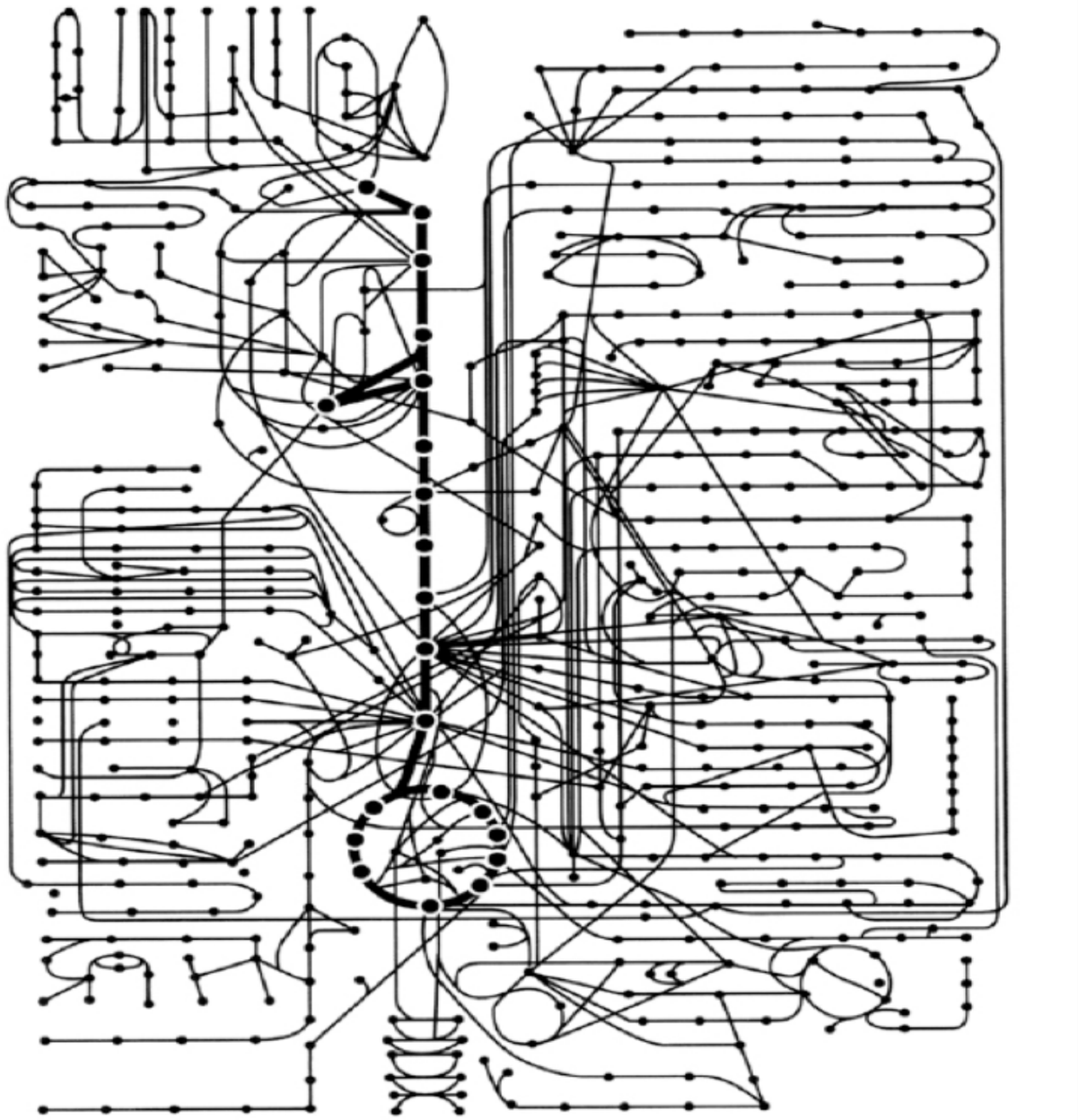
Topology studies show networks but are far removed from functional behaviour.

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

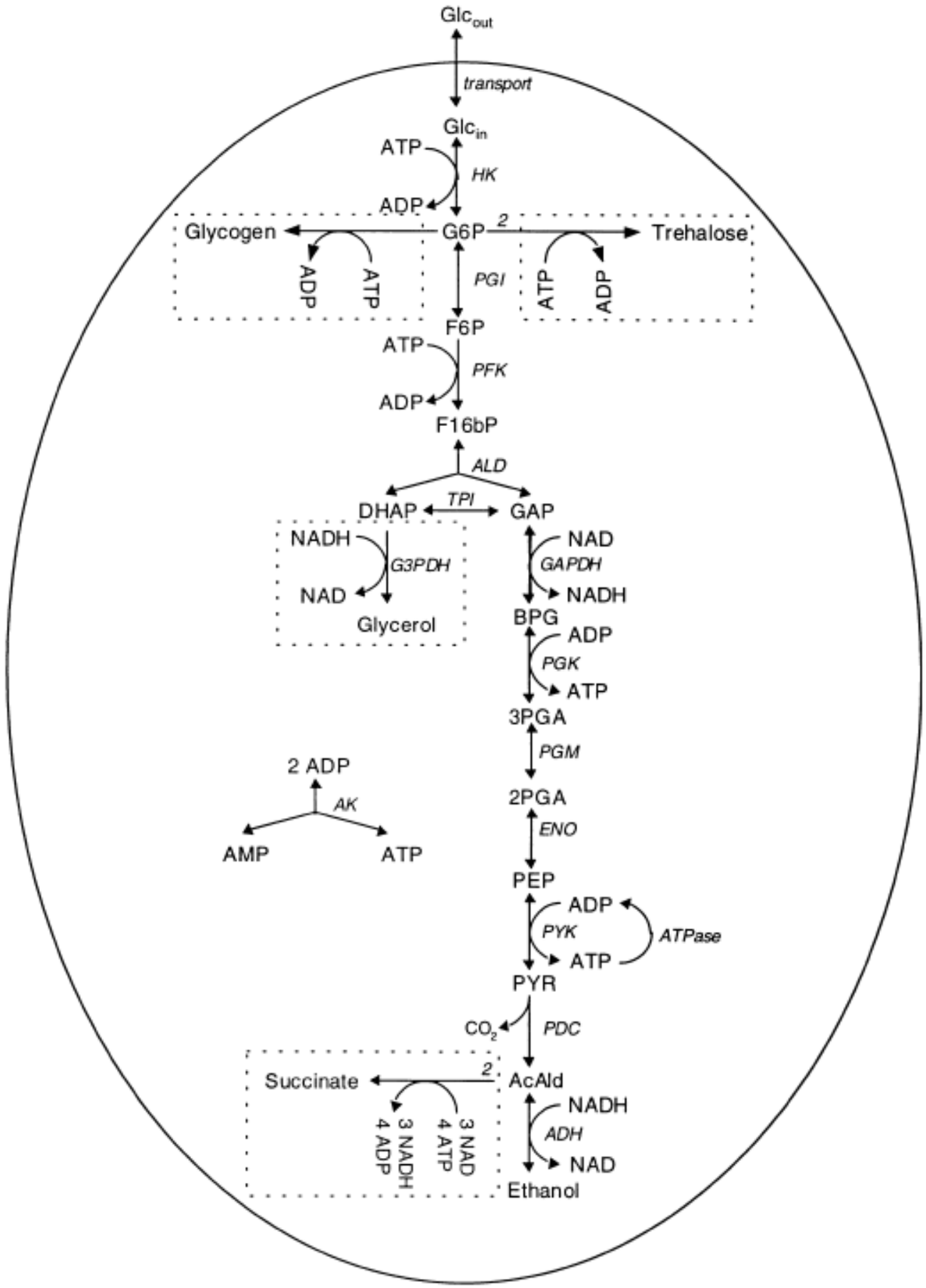
Applications in medicine (drug target identification) and biotechnology (metabolic engineering), need specific targets in the system (molecular mechanism).

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

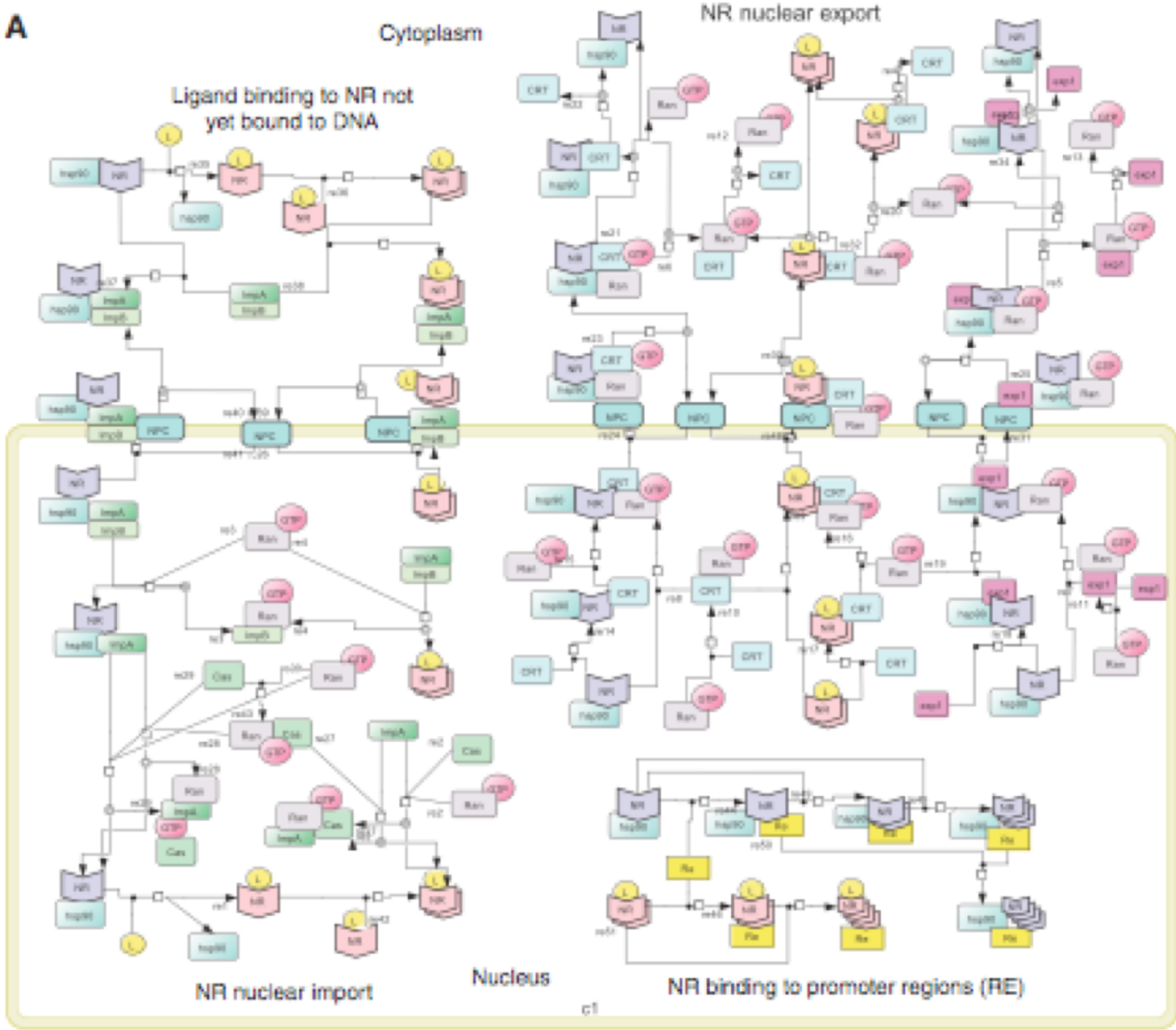
Metabolism



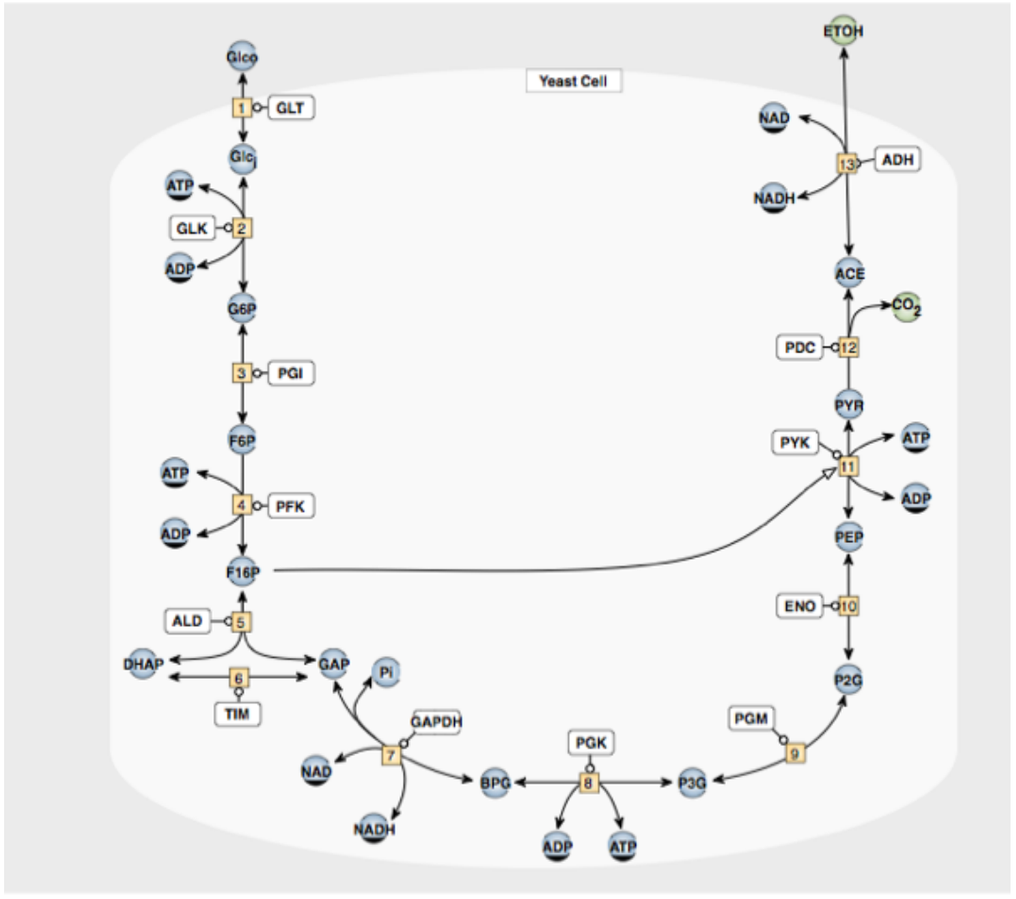
Glycolysis

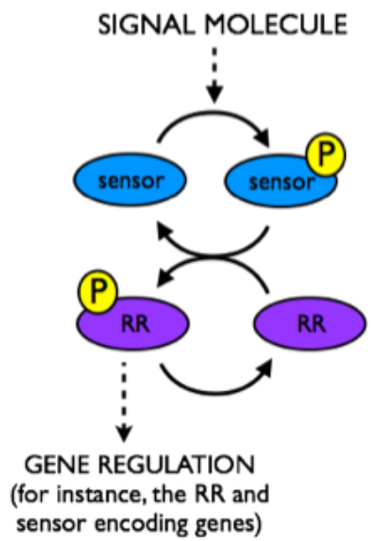

Glc$_{out}$
transport
Glc$_{in}$
ATP
HK
ADP
Glycogen
G6P
2
Trehalose
ADP
ATP
PGI
ATP
ADP
F6P
ATP
PFK
ADP
F16bP
ALD
DHAP
TPI
GAP
NADH
G3PDH
NAD
Glycerol
NAD
GAPDH
NADH
BPG
ADP
PGK
ATP
3PGA
PGM
2 ADP
AK
AMP
ATP
2PGA
ENO
PEP
ADP
PYK
ATP
ATPase
PYR
CO$_2$
PDC
Succinate
2
AcAld
3 NADH
4 ADP
3 NAD
4 ATP
NADH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

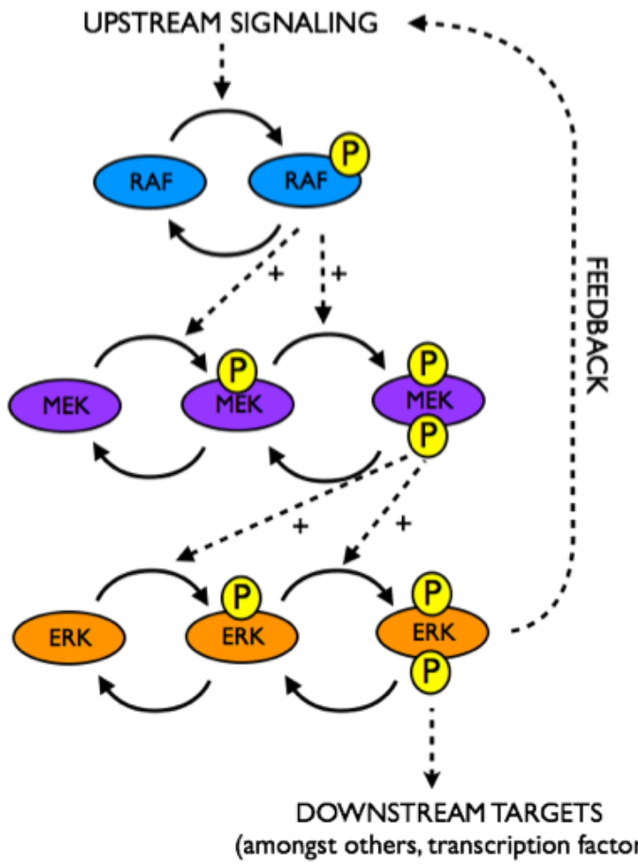
Signalling


A
Cytoplasm
Ligand binding to NR not yet bound to DNA
NR nuclear export
NR nuclear import
Nucleus
NR binding to promoter regions (RE)

Metabolic and signal transduction networks







Metabolite concentrations, fluxes, time courses

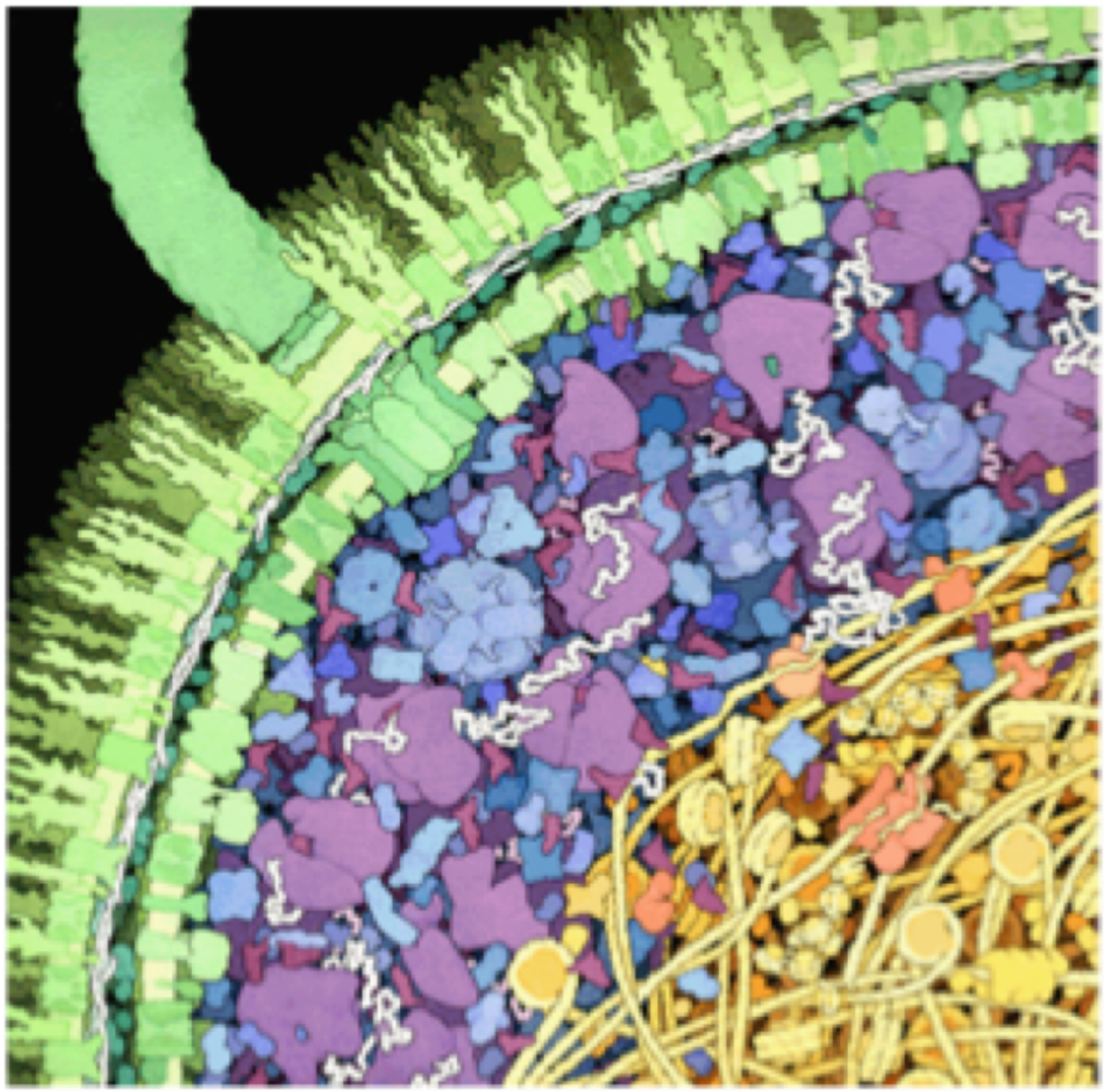
Reponse time, dose-response relation, ligand specificity

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

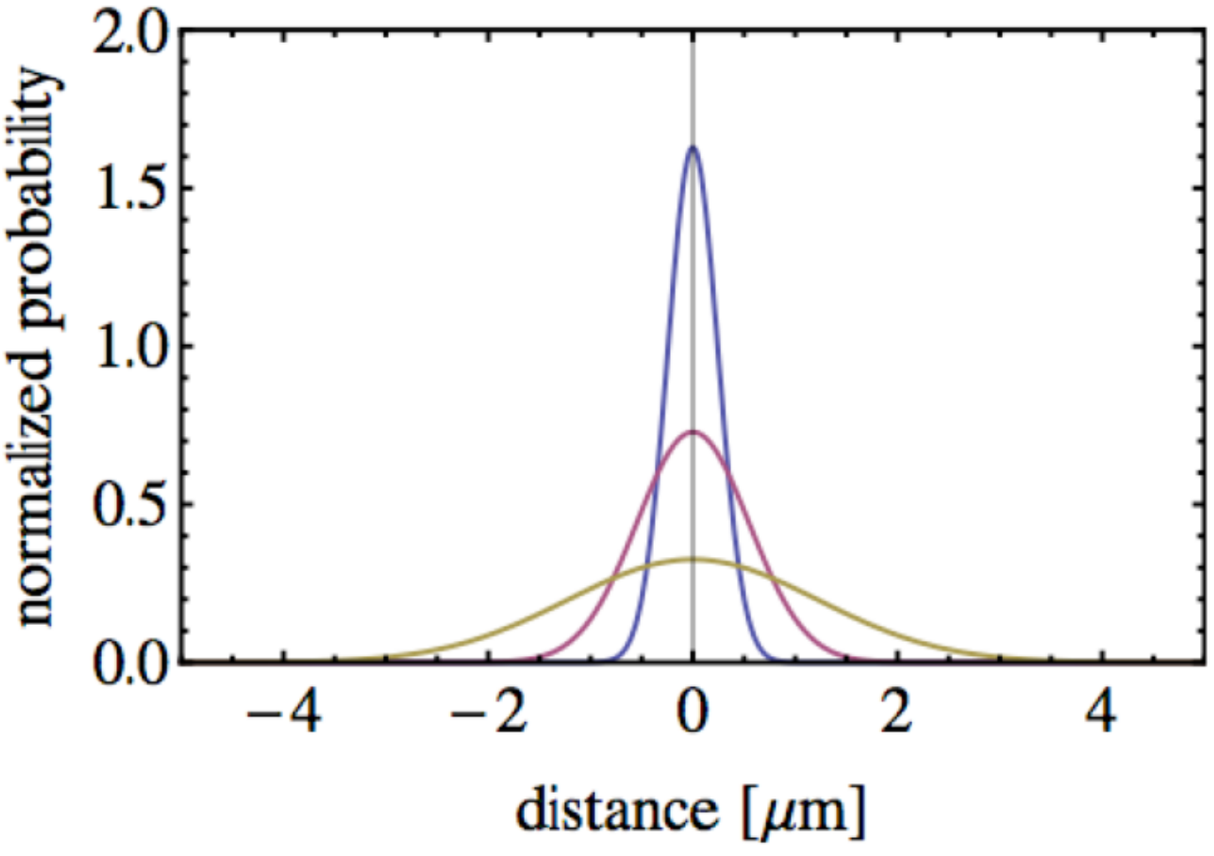
Diffusion and reactivity

Consider a molecule that has moved nR steps to the right and nL steps to the left after N total steps:

- Distance from origin Δ = nR - nL
- For large N probability that molecule has moved distance:

$$p(\Delta, t) = \frac{1}{\sqrt{4\pi Dt}} e^{\frac{-\Delta^2}{4Dt}}$$

Gaussian approximation of binomial distribution



$$D = \frac{\delta^2}{2\tau}$$

δ = average distance per step

τ = average time per step

$$[D] = \frac{\text{area}}{\text{time}}$$

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

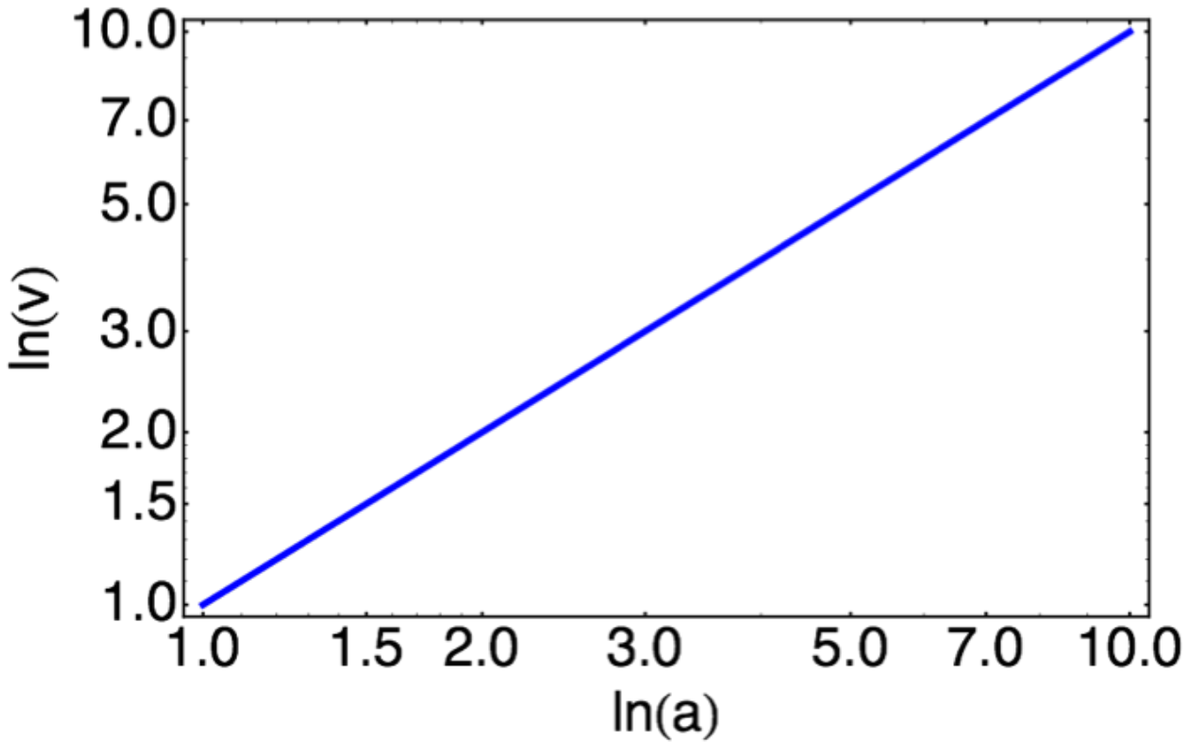
Kinetics of chemical reactions

- 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

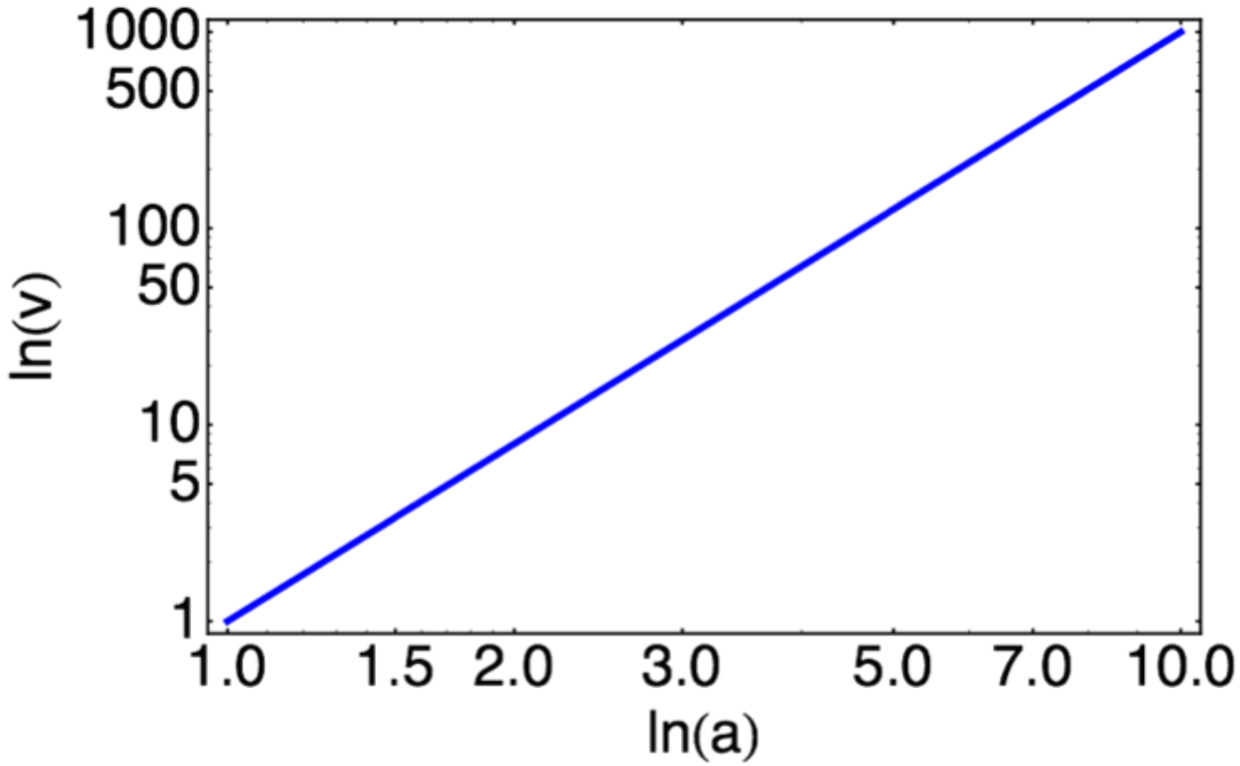
Driving force of a reaction

- 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

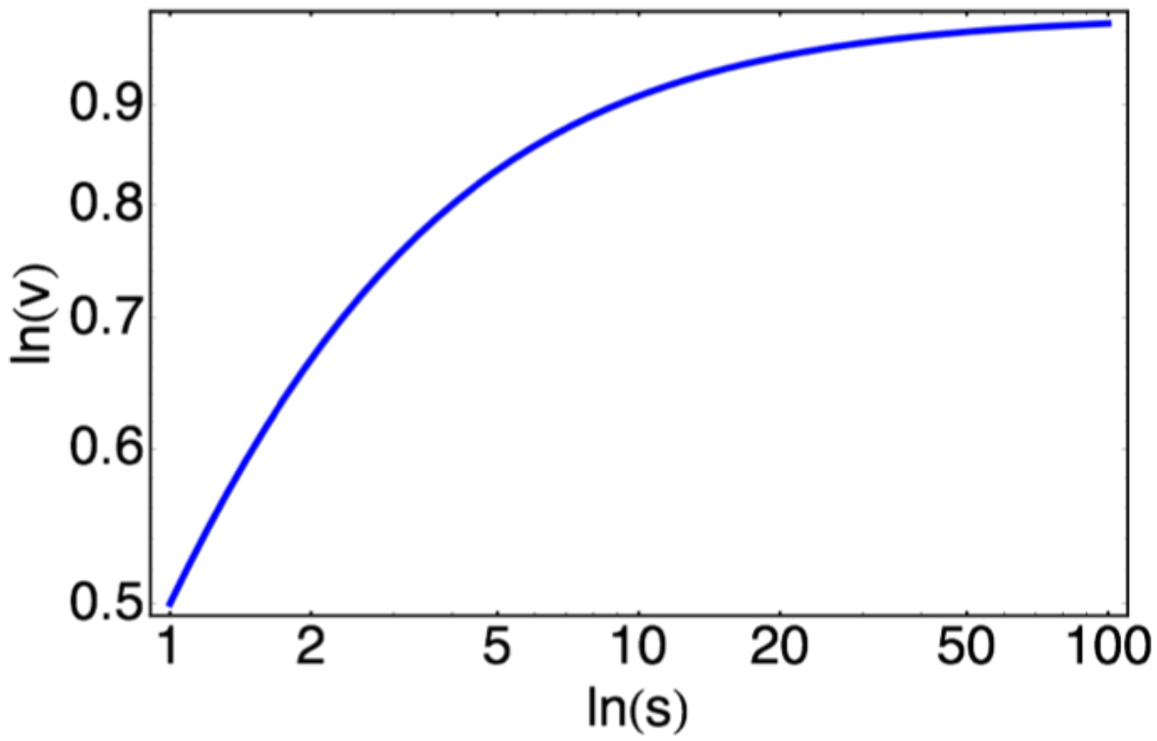
v=ab; b=1



v=a^3b; b=1



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



- 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

$$\begin{aligned} A &\rightleftharpoons B \quad \Delta G^\circ = +18,85\,\mathrm{kJ\,mol^{-1}};\ K_{eq} = 5\times10^{-4} \\ B &\rightleftharpoons C \quad \Delta G^\circ = -18,85\,\mathrm{kJ\,mol^{-1}};\ K_{eq} = 2\times10^{3} \end{aligned}$$

  - Series 2

$$\begin{aligned} A &\rightleftharpoons B \quad \Delta G^\circ = -18,85\,\mathrm{kJ\,mol^{-1}};\ K_{eq} = 2\times10^{3} \\ B &\rightleftharpoons C \quad \Delta G^\circ = +18,85\,\mathrm{kJ\,mol^{-1}};\ K_{eq} = 5\times10^{-4} \end{aligned}$$

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

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

Series 1:

At equilibrium:

$[B]_{eq} = 5\times10^{-4}.[A]_{eq}$

$[C]_{eq} = [A]_{eq}$

$[A]_{eq} + [A]_{eq} + 5\times10^{-4}.[A]_{eq} = 1\ M$

$\Rightarrow [A]_{eq} = 0.5\ M;\quad [B]_{eq} = 0.00025\ M;\quad [C]_{eq} = 0.5\ M$

Series 2:

At equilibrium:

$[B]_{eq} = 2\times10^{3}.[A]_{eq}$

$[C]_{eq} = [A]_{eq}$

$[A]_{eq} + [A]_{eq} + 2\times10^{3}.[A]_{eq} = 1\ M$

$\Rightarrow [A]_{eq} = 0.0005\ M;\quad [B]_{eq} = 0.999\ M;\quad [C]_{eq} = 0.0005\ M$

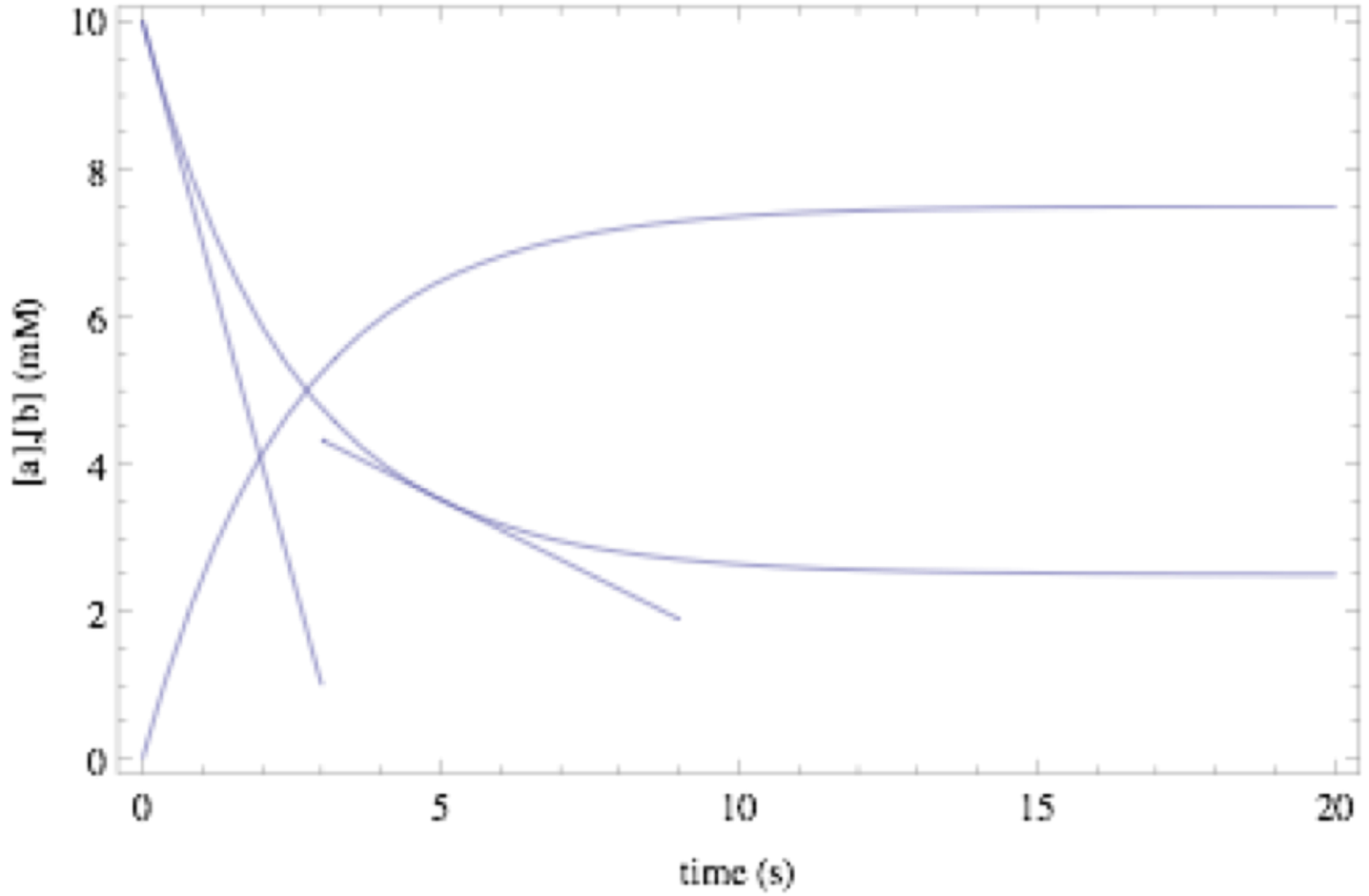
Exercise 1: Experimental data

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 catalyzed, 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



v -d[A]/dt = slope of tangent at t=5
= 0.4 mM/s

K_{eq} Equilibrium ratio of [B]/[A] = 7.5/2.5 = 3

$v = k_f*a - k_r*b$;
rate of reaction (d[A]/dt) at t=0;

k_f = -d[A]/dt
= slope of tangent at t=0
= 3.0 mM/s;
k_f = 3.0/10 = 0.3 1/s

k_r $K_{eq} = k_f/k_r$;
$k_r = 0.3/3 = 0.1$ 1/s

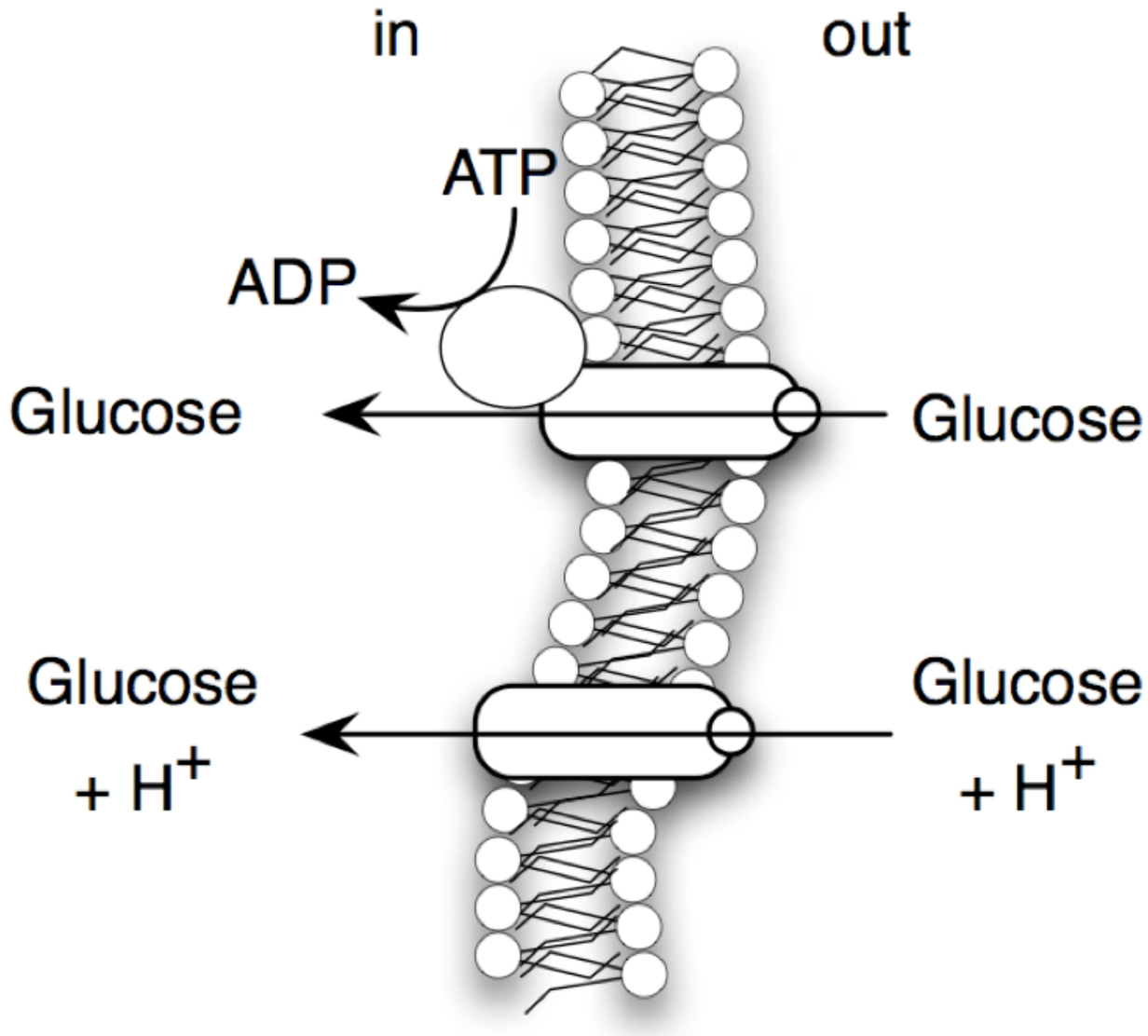
Γ [B]/[A] at t=5, 6.48/3.52 = 1.84

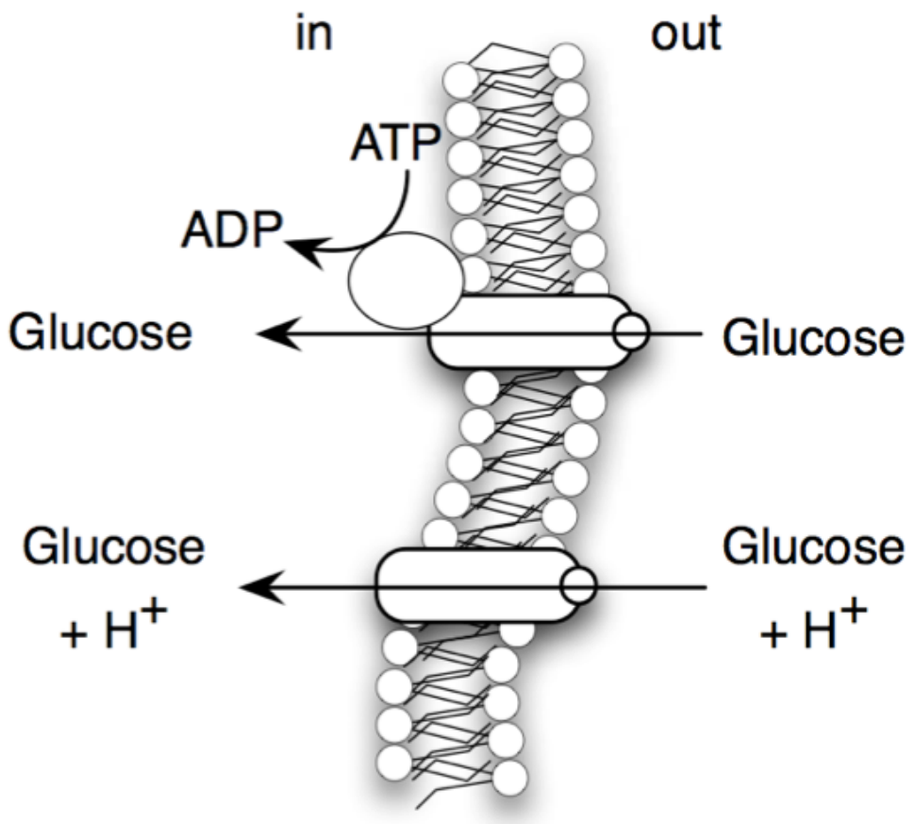
v_{20} at t=20 the reaction is very close to equilibrium as is evident from the constant concentrations of A and B, the velocity is close to 0

$v_{f,\ t=20}$ at t=20 $v_f = 0.3*2.5 = 0.75$ mM/s

$v_{r,\ t=20}$ at t=20 $v_r = 0.1*7.5 = 0.75$ mM/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

- 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

Solution

- 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)

$$
\begin{aligned}
pH &= -^{10}log[H^+] \\
pH = 7 &-> [H^+] = 10^{-7} \\
pH = 6.7 &-> [H^+] = 10^{-6.7} = 1.995 \cdot 10^{-7}
\end{aligned}
$$

$$
\begin{aligned}
\Delta G_{H^+} &= RTln\frac{[H^+]_{in}}{[H^+]_{out}} + ZF \cdot \Delta\psi \\
\Delta G_{H^+} &= 8.31447 \cdot 298.17 \cdot ln(1.995) + 96.485 \cdot 10^3 \cdot -0.2 \\
\Delta G_{H^+} &= 1712.19 - 19297 \\
\Delta G_{H^+} &= -17584.8\,\mathrm{J/mol\ H^+}
\end{aligned}
$$

$$
\begin{aligned}
\Delta G_{Glc} &= RTln\frac{[Glc]_{in}}{[Glc]_{out}} \\
2 \cdot 17584.8 &= 8.31447 \cdot 298.17 \cdot ln\frac{[Glc]_{in}}{[Glc]_{out}} \\
\frac{[Glc]_{in}}{[Glc]_{out}} &= 1.45 \cdot 10^6
\end{aligned}
$$