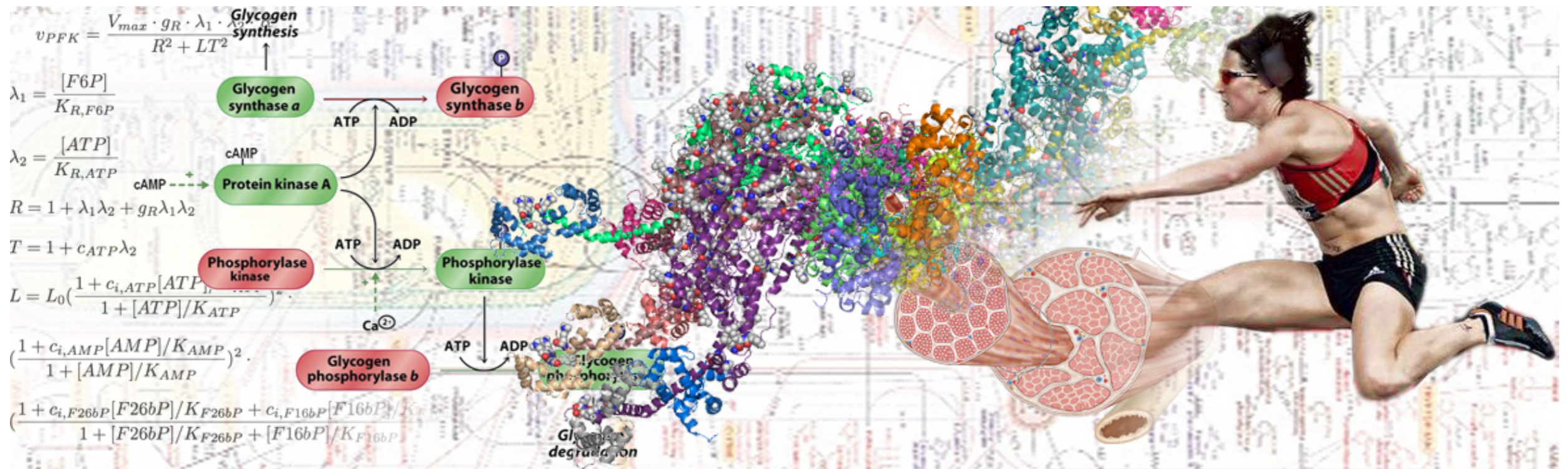


Mini-course: Molecular Systems Biology



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	<i>Human Rights Day</i>	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)				

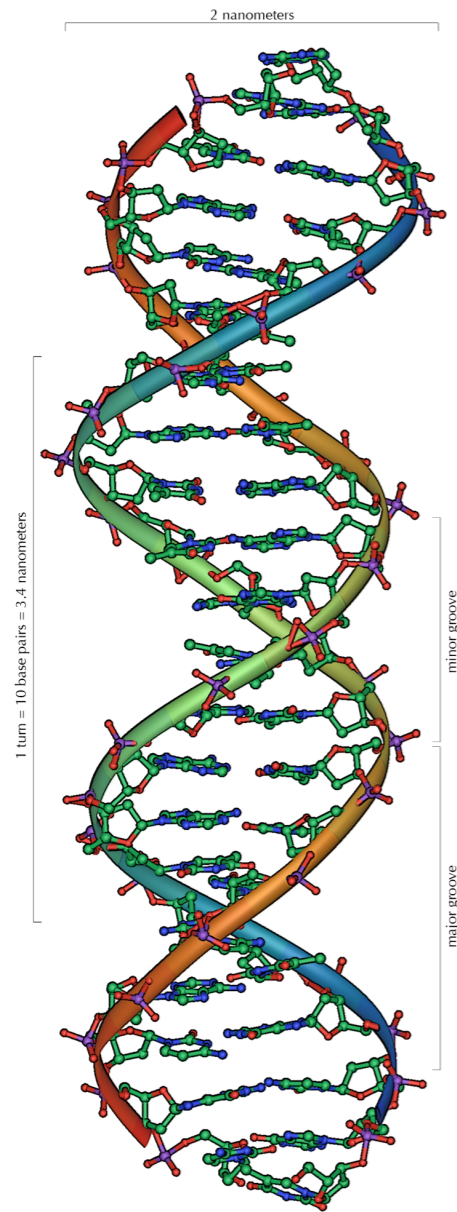
Course material

- Available at:

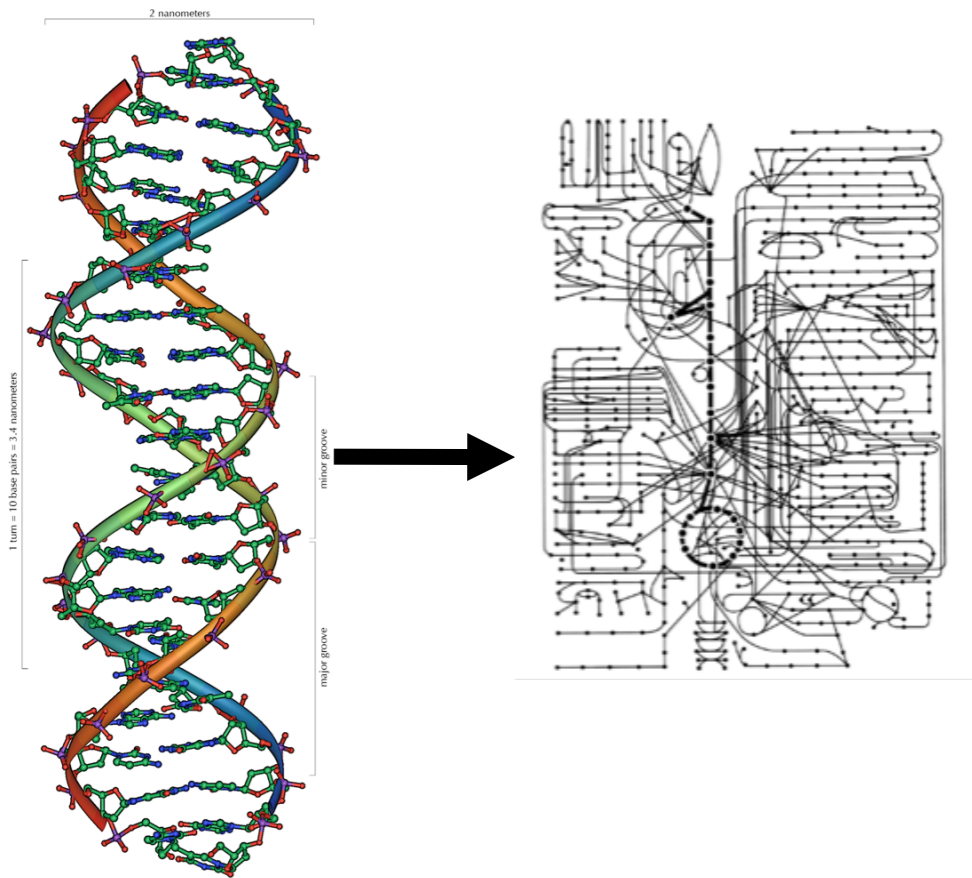
<http://glue.jjj.sun.ac.za/jjj/minicourse/>

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

The Ultimate Predictive Model



From Sequence to Network



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

\dot{s} $d(s)/d(t)$
 N stoichiometry matrix
 v reaction rates

Reconstruct reaction network using homologies between enzymes

Construct stoichiometry matrix from reaction network

Molecular Systems Biology

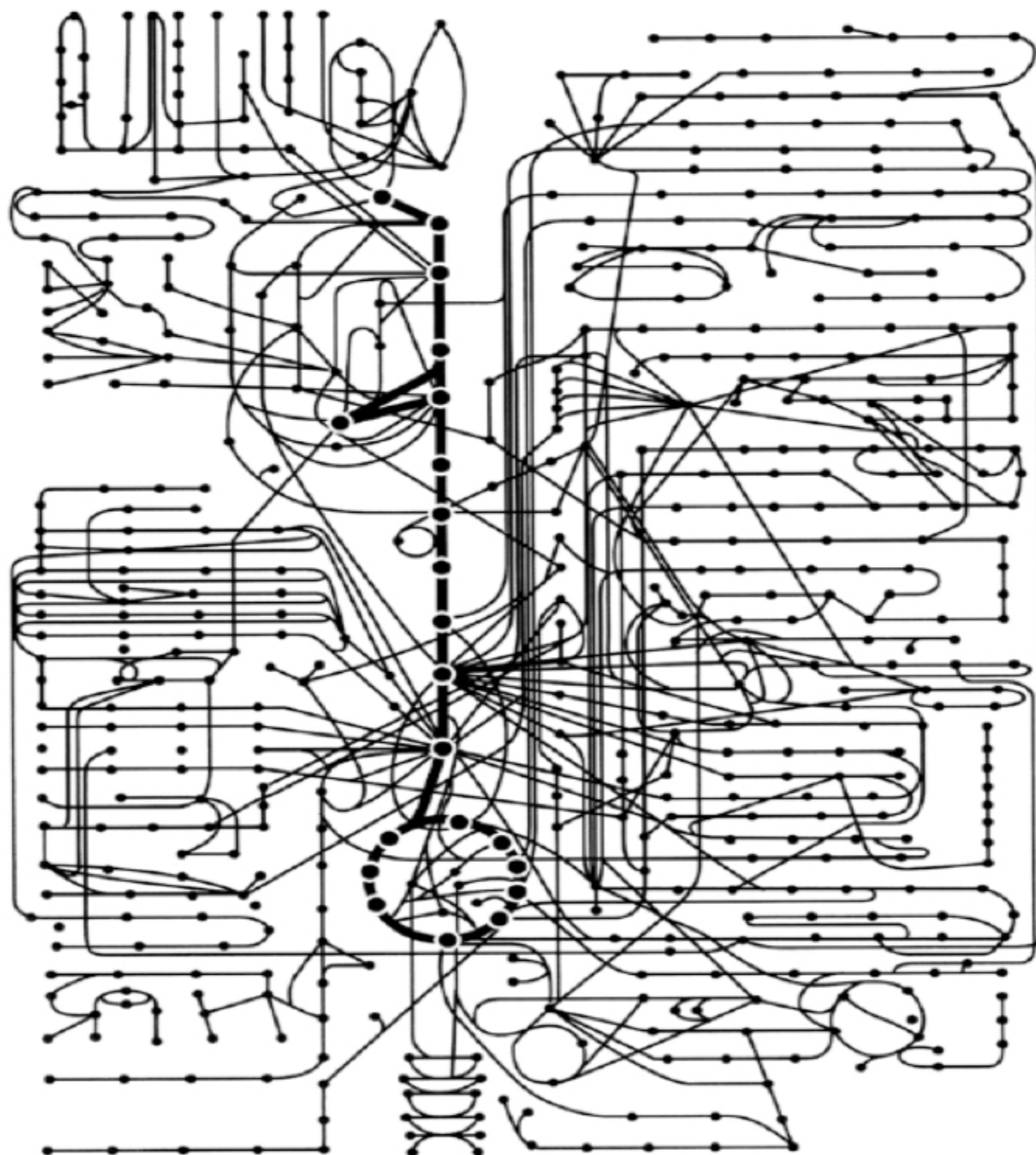
Topology studies show networks but are far removed from functional behaviour.

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

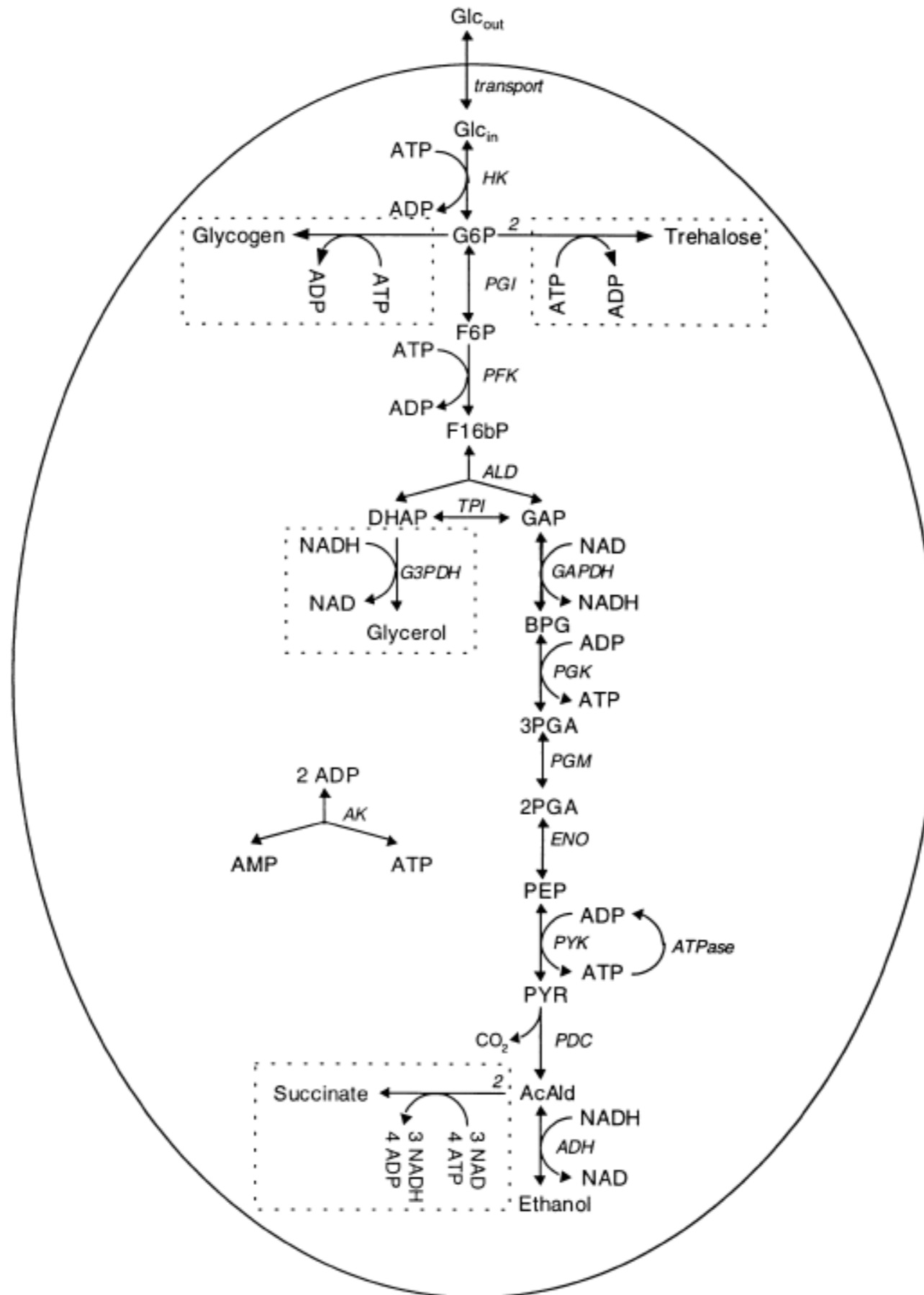
Applications in medicine (drug target identification) and biotechnology (metabolic engineering), need specific targets in the system (molecular mechanism).

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

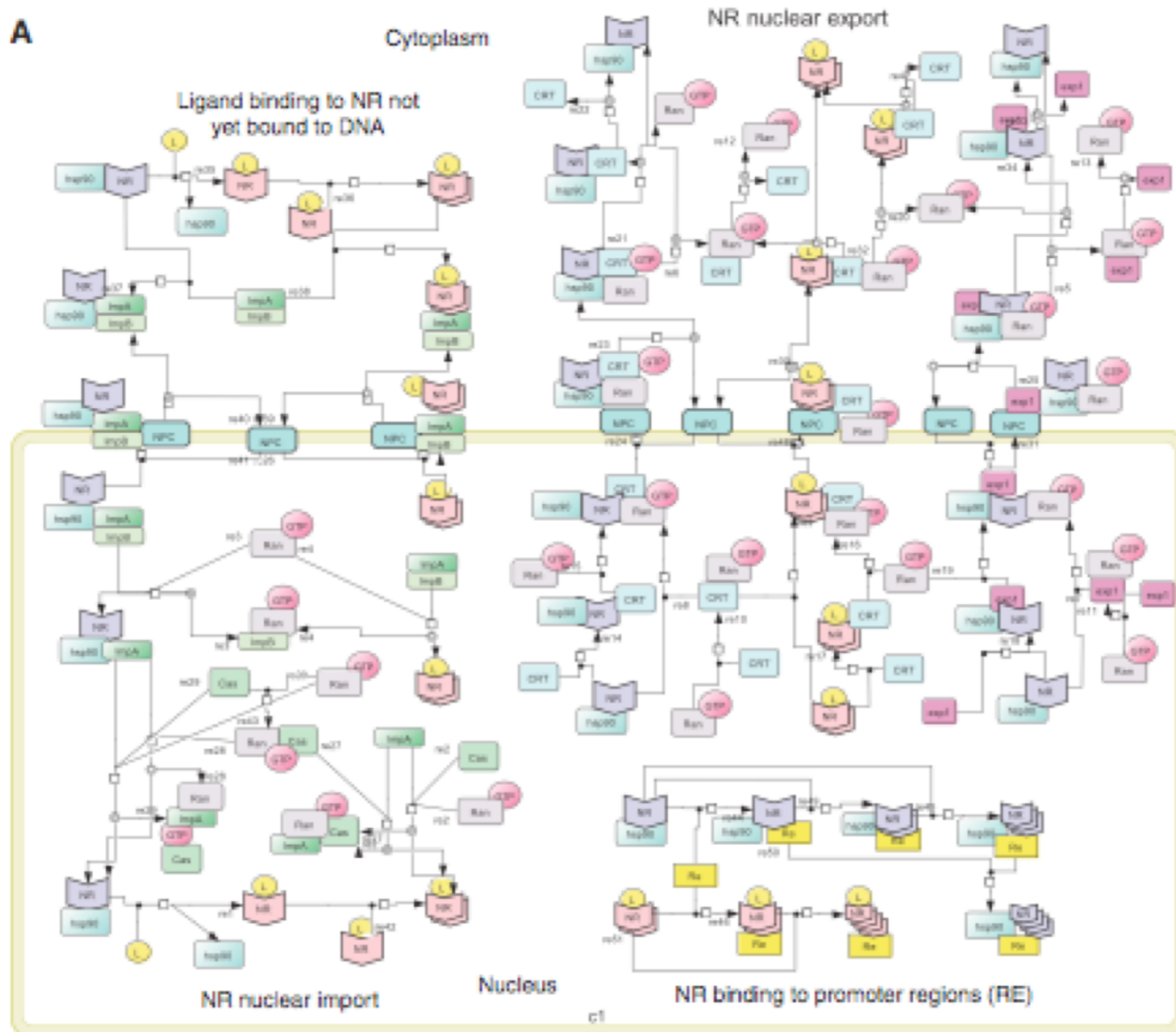
Metabolism



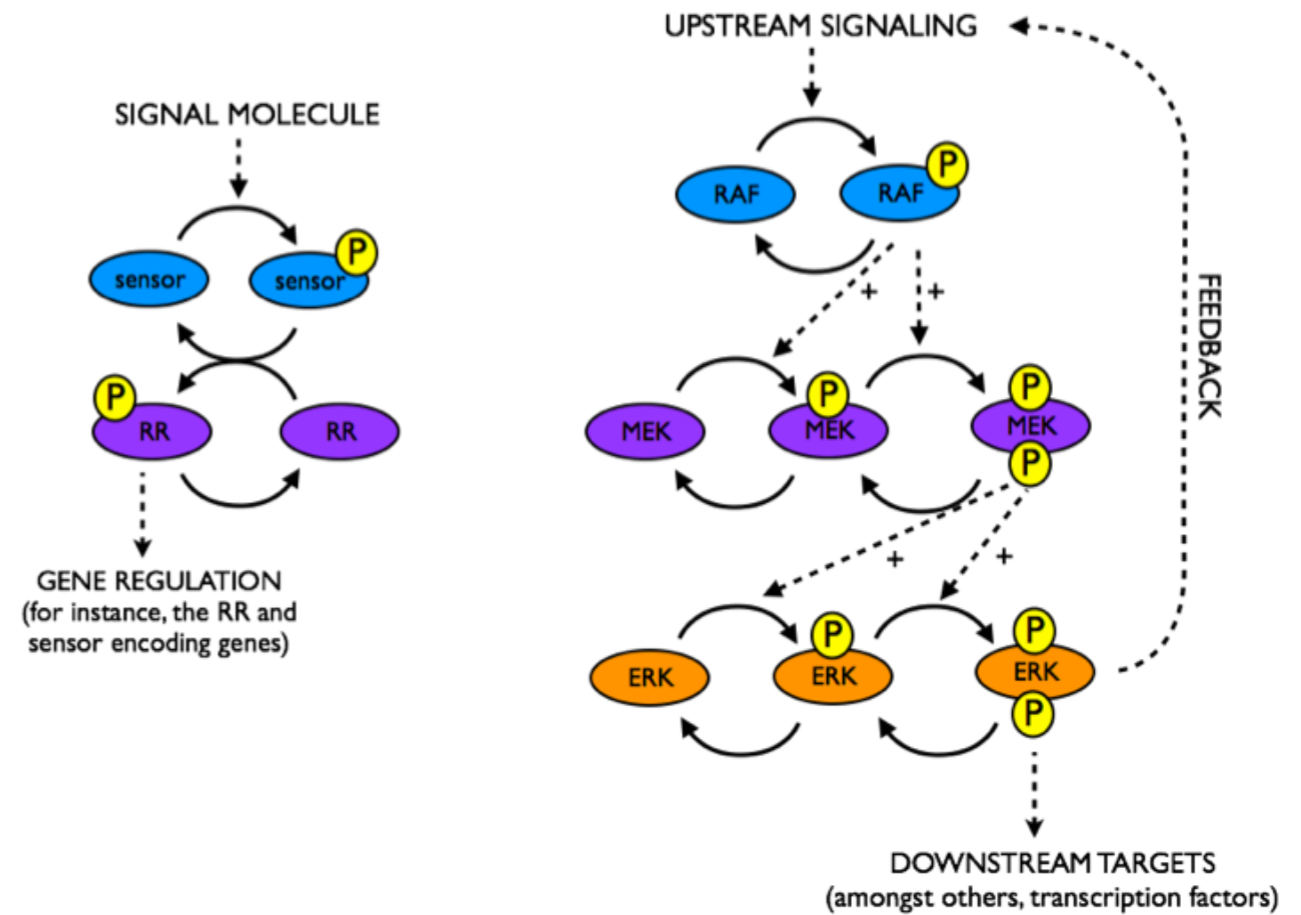
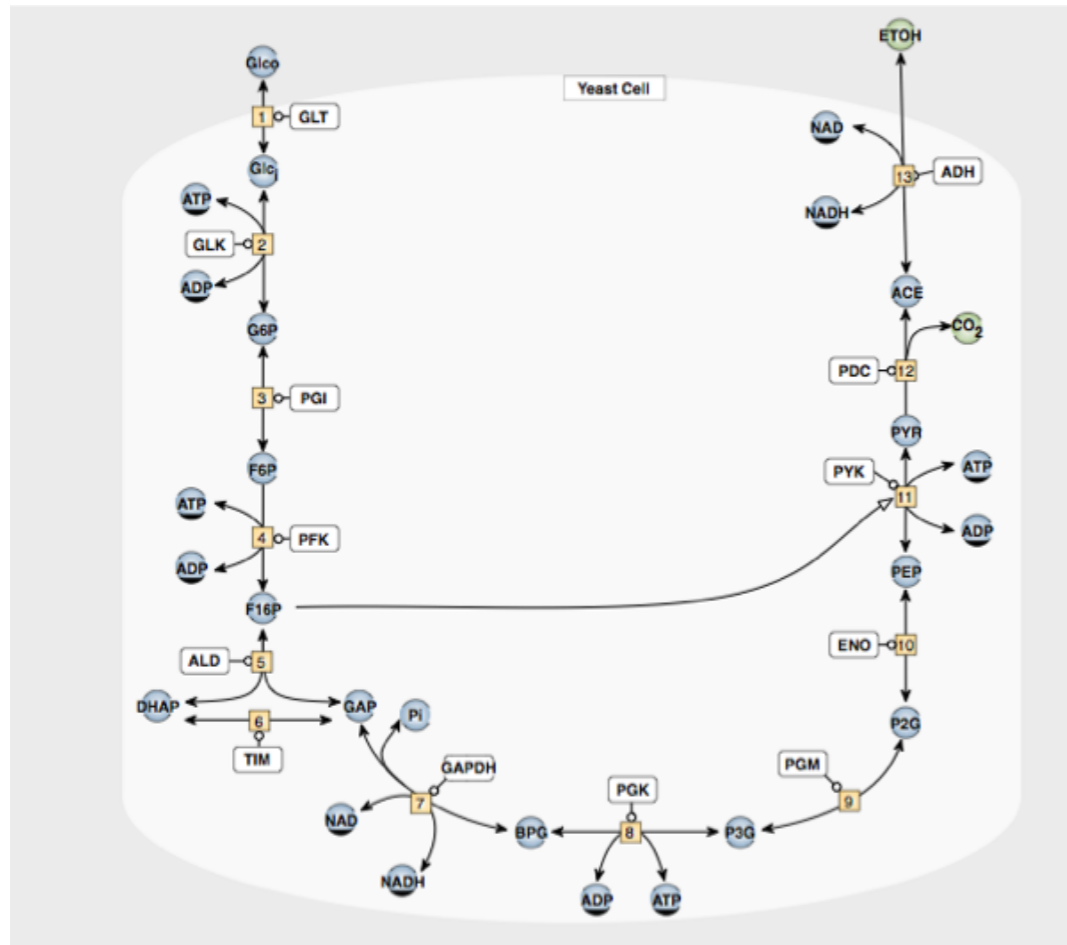
Glycolysis



Signalling



Metabolic and signal transduction networks

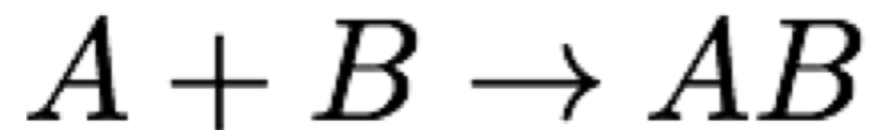


Metabolite concentrations, fluxes, time courses

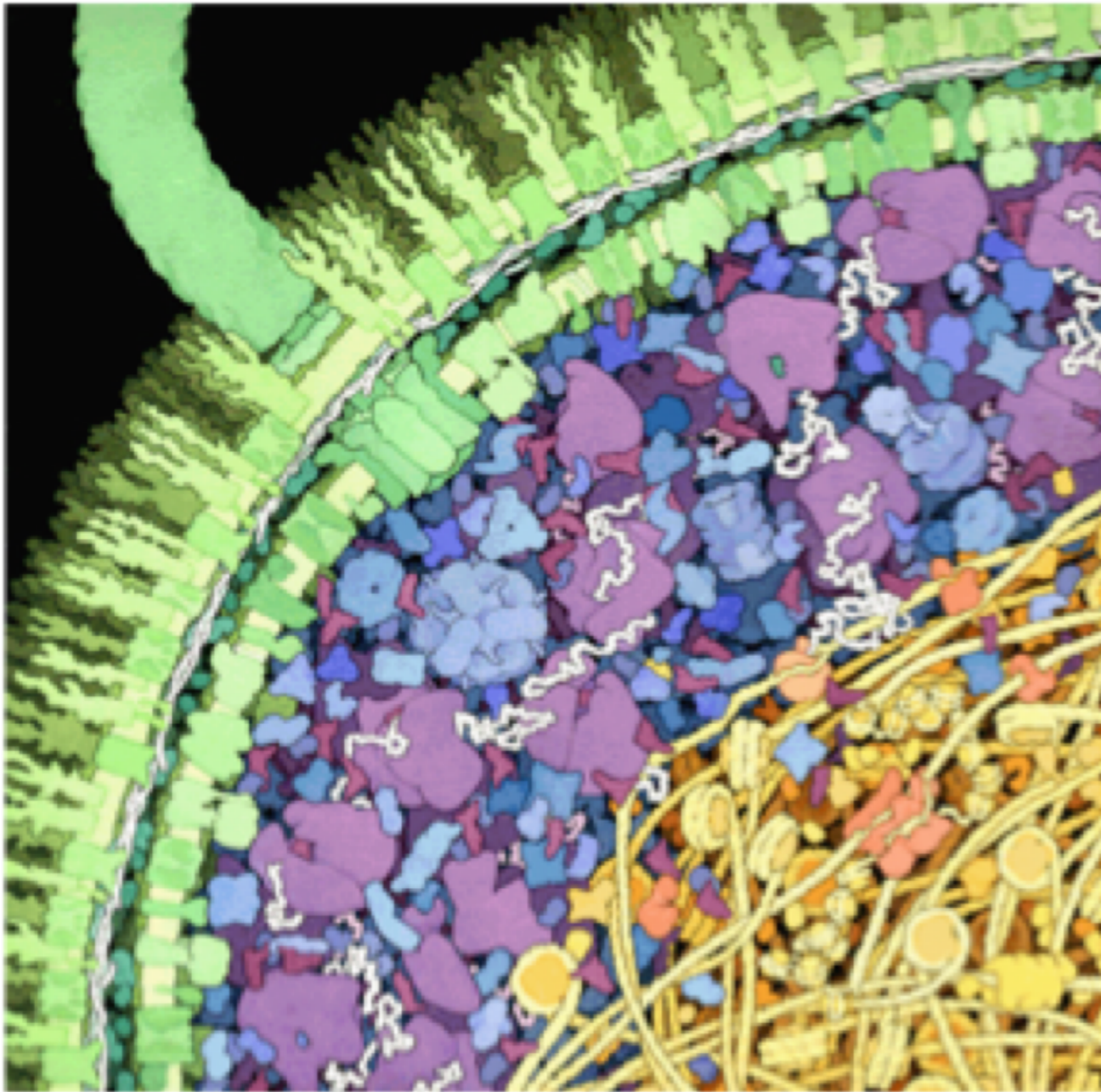
Response time, dose-response relation, ligand specificity

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



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



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

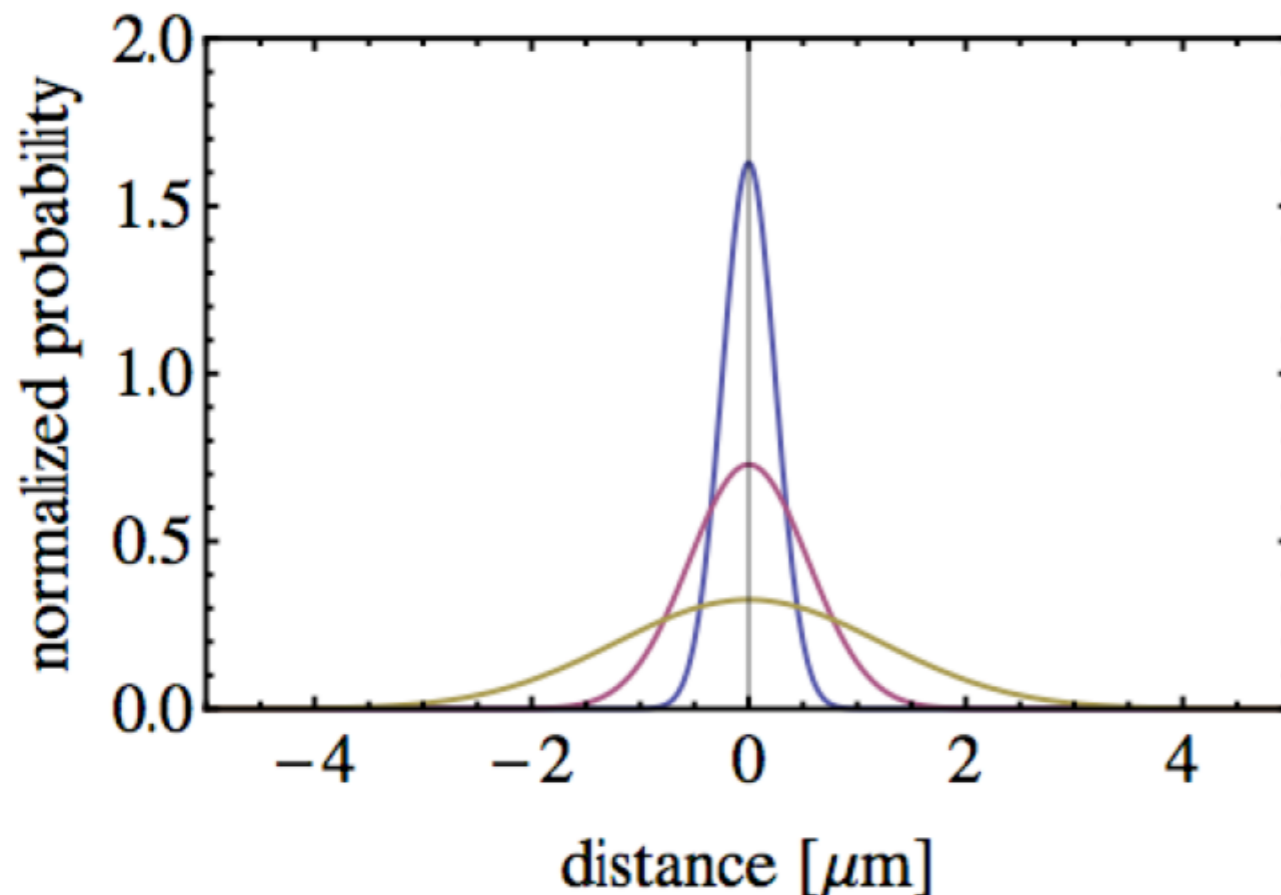
Diffusion and reactivity

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

- Distance from origin $\Delta = nR - nL$
- 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

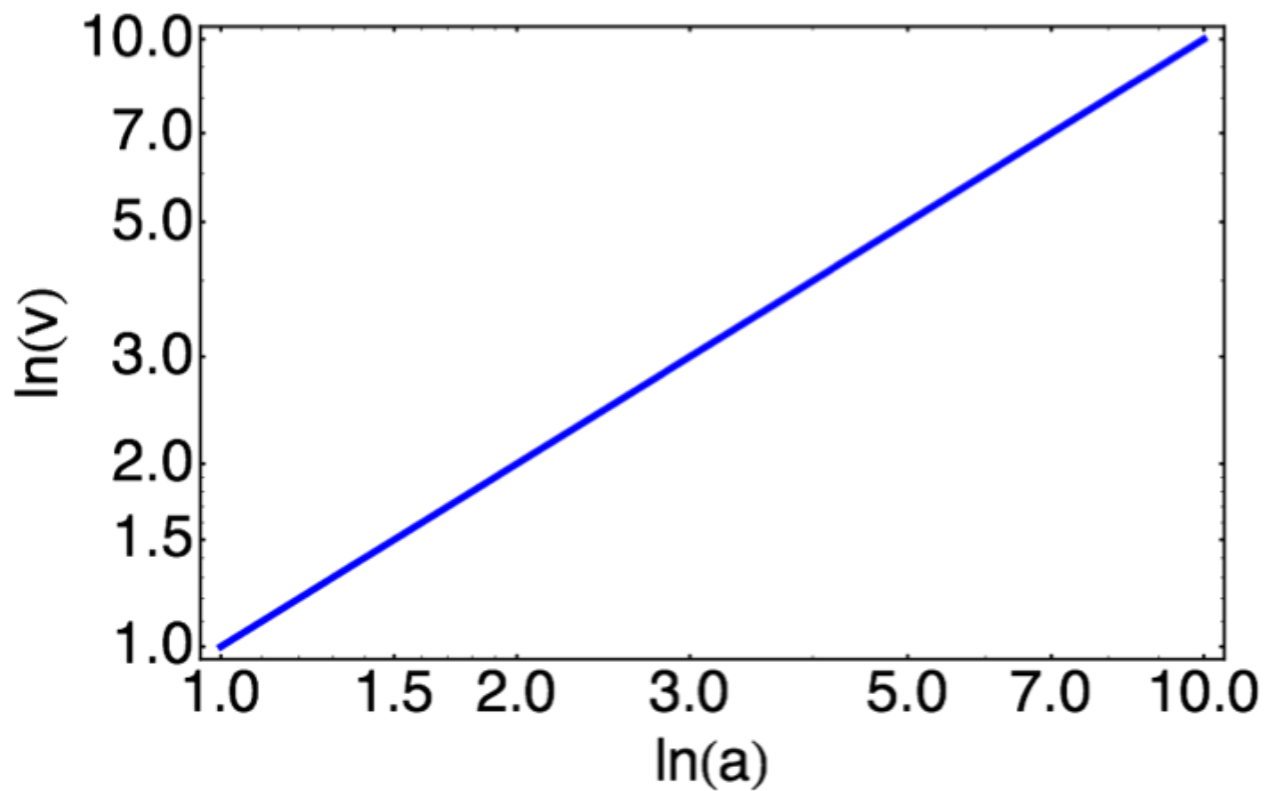
Kinetics of chemical reactions

- 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

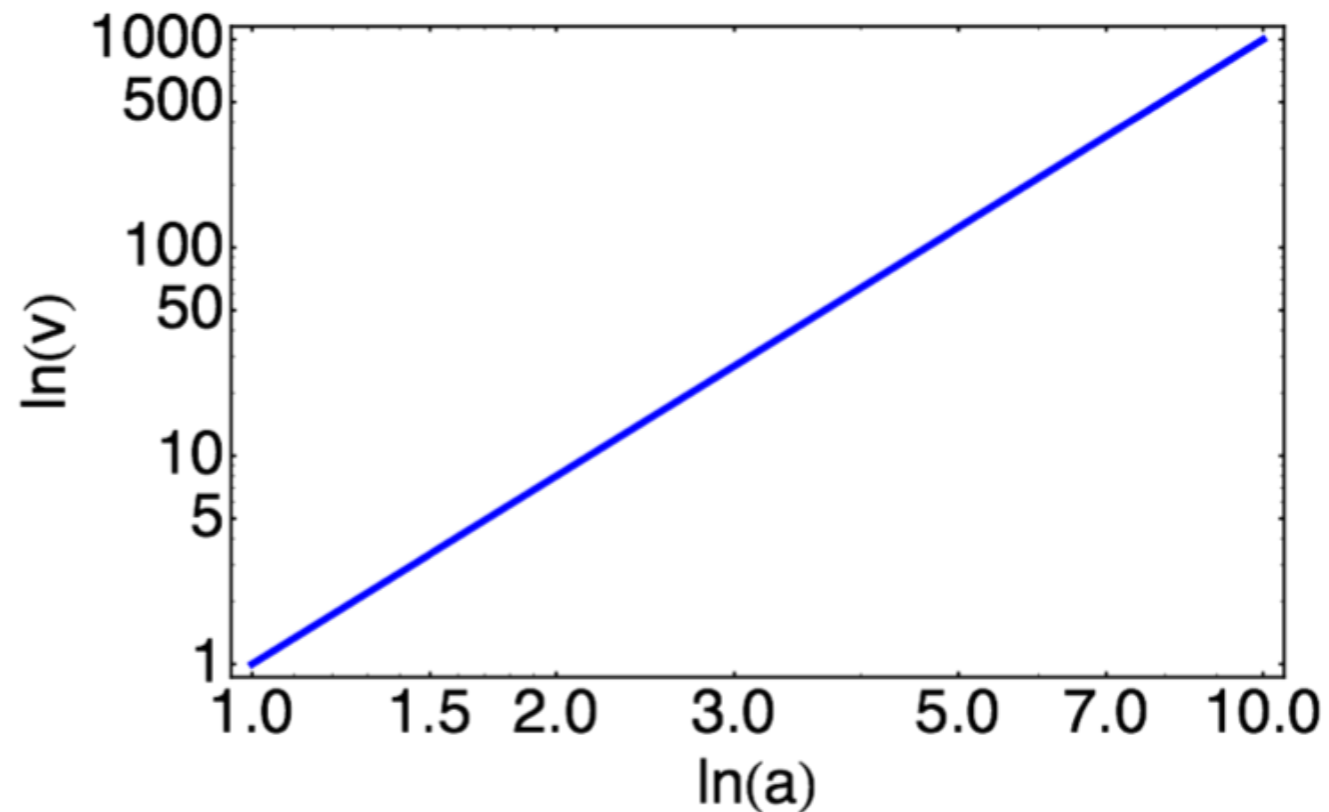
Driving force of a reaction

- 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

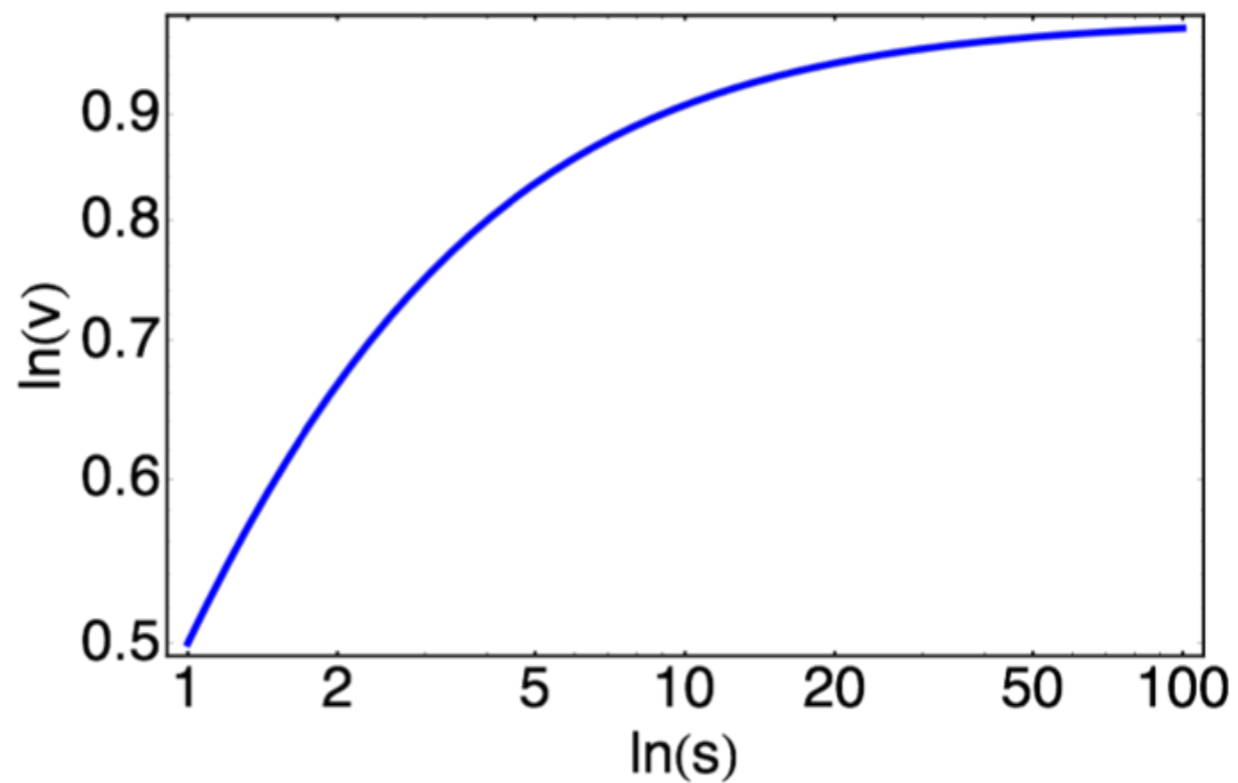
$$v=ab; b=1$$



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

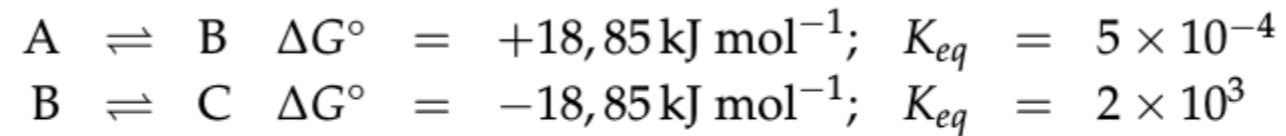


$$v = \frac{V_m \times s}{K_s + s}$$

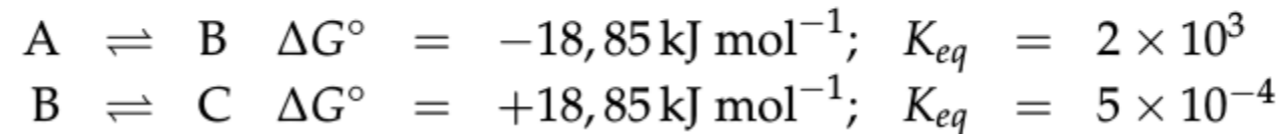


- Example: suppose you start with 1M of A and no B and C. Which of the following series will lead to the greatest $[C]_{eq}$?
- (Remember $K_{eq_T} = K_{eq_1} \times K_{eq_2}$)

- Series 1



- Series 2



Series 1:

At equilibrium:

$$[B]_{eq} = 5 \times 10^{-4} \cdot [A]_{eq}$$

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

$$[A]_{eq} + [A]_{eq} + 5 \times 10^{-4} \cdot [A]_{eq} = 1 \text{ M}$$

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

Series 2:

At equilibrium:

$$[B]_{eq} = 2 \times 10^3 \cdot [A]_{eq}$$

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

$$[A]_{eq} + [A]_{eq} + 2 \times 10^3 \cdot [A]_{eq} = 1 \text{ M}$$

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

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

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

Exercise I: Experimental data

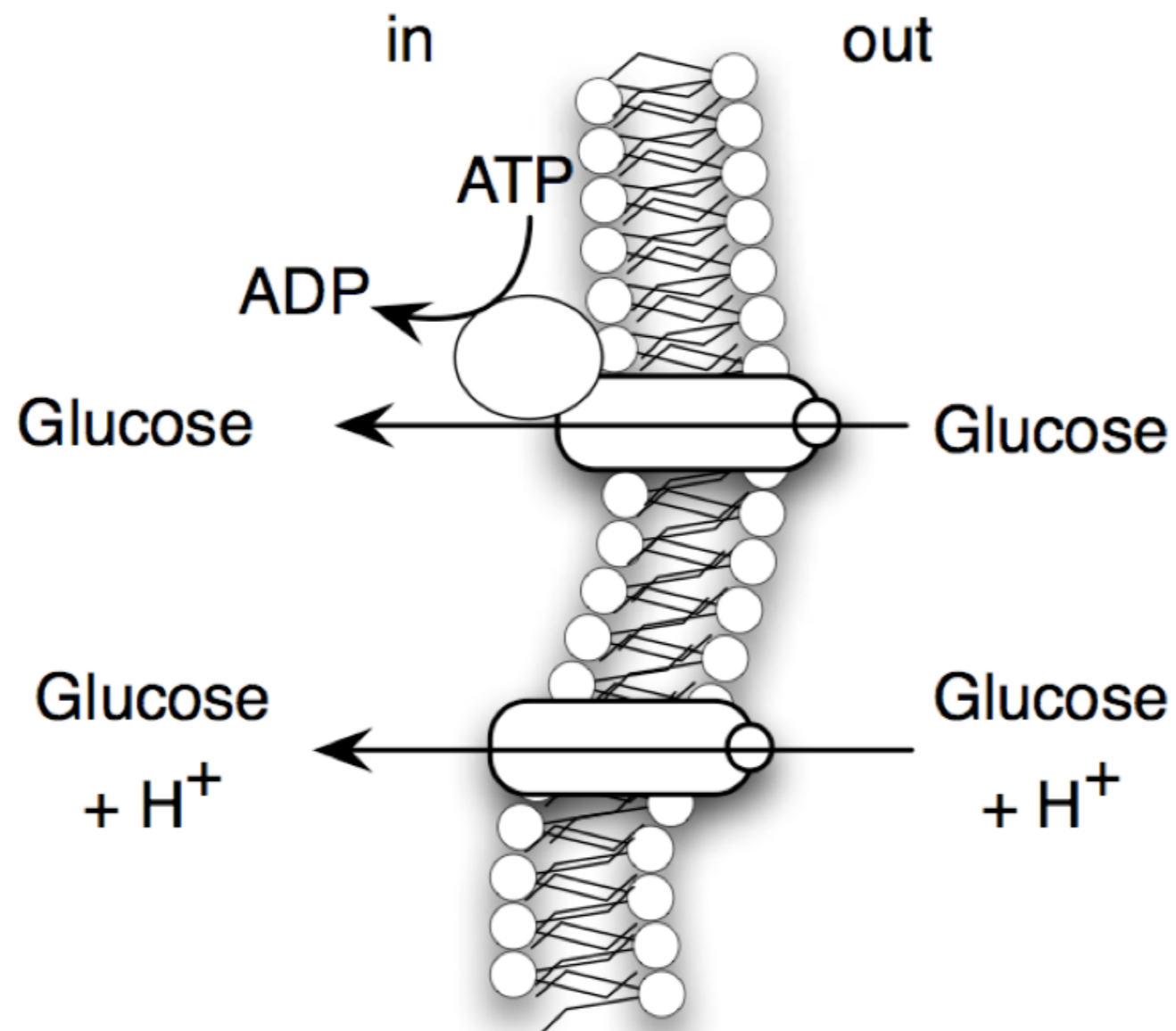
For a non catalyzed, chemical reaction;
 $A \rightleftharpoons B$ the following experimental data
were obtained:

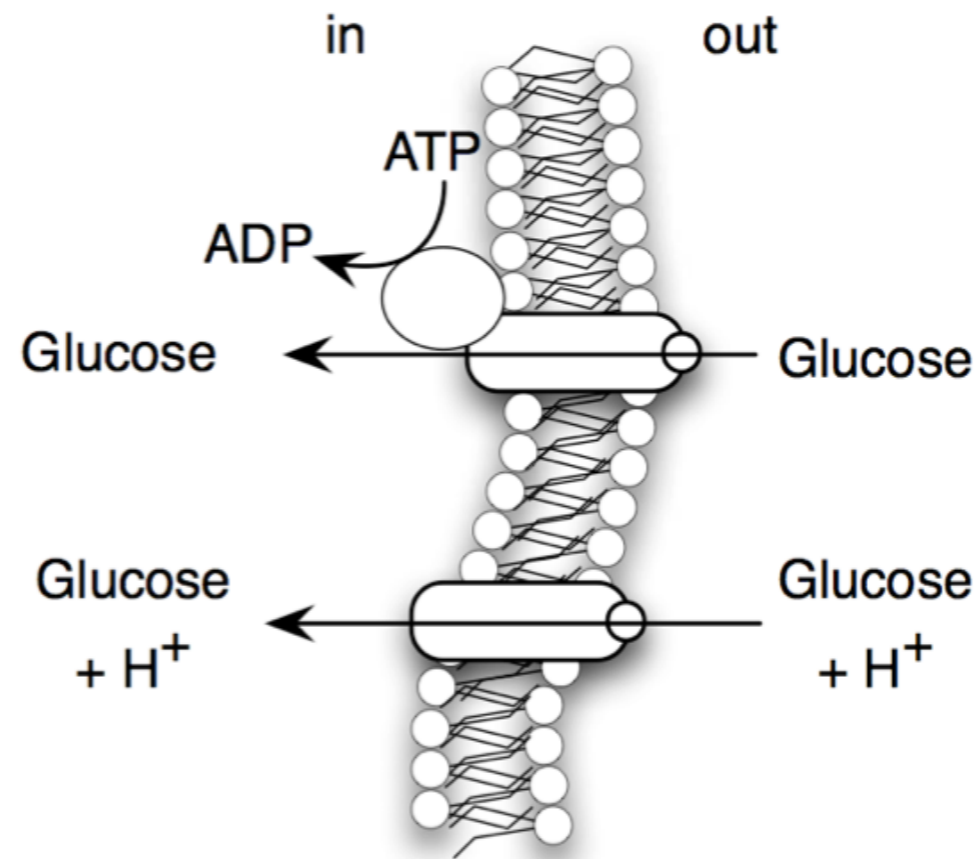
t (s)	a (mM)	b (mM)
0	10.	0.
1	7.5274	2.4726
2	5.86997	4.13003
3	4.75896	5.24104
4	4.01422	5.98578
5	3.51501	6.48499
6	3.18038	6.81962
7	2.95607	7.04393
8	2.80572	7.19428
9	2.70493	7.29507
10	2.63737	7.36263
11	2.59208	7.40792
12	2.56172	7.43828
13	2.54137	7.45863
14	2.52773	7.47227
15	2.51859	7.48141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

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