Cellular signalling

Spring semester 2021
Monday 8:15-10:00 CH-B3-31 => ZOOM
Ruud Hovius

Course material:

- Hand-out of lectures
- Articles
- Lecture notes

Moodle CH-411

- Text books :
  - Stryer's *Biochemistry*
  - Alberts' *Molecular Biology of the Cell*
- Milo & Philips' *Cell Biology by the Numbers*
  books.bionumbers.org

Contact:

- office: CH-B3-424
- e-mail: Ruud.Hovius@epfl.ch

Cellular signalling - Course layout

- *i* General concepts
- *ii* Organisation through molecular interactions
- *iii* Membranes, lipids, domains and phases
- *iv* Channels
- *v* Receptor-activated signalling cascades
- *vi* Items related to numbers, space and time
- *vii* New & Small things

- Oral exam or take home exam

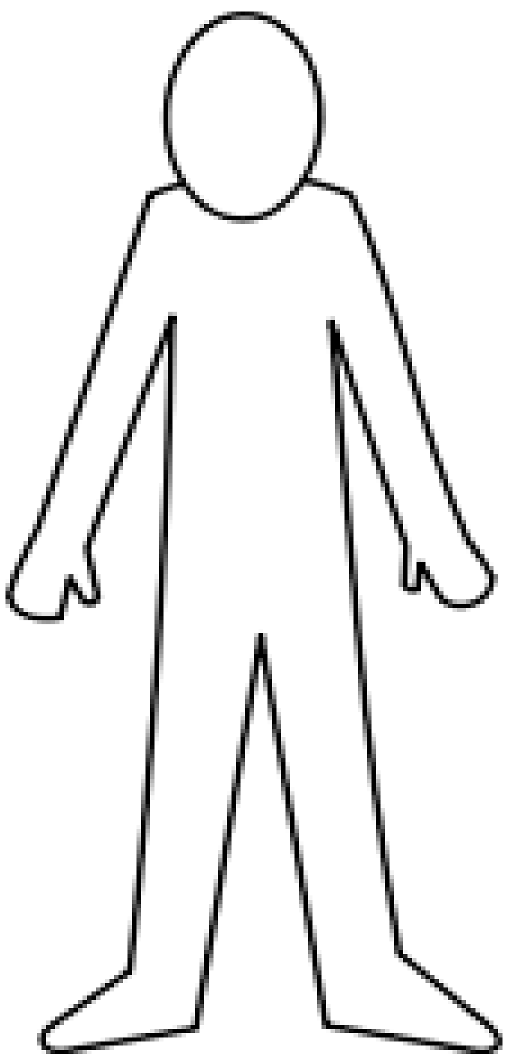
Cellular signalling - General concepts

- Sending & receiving
- A more quantitative look to biology
- Tiny numbers
- Crowding

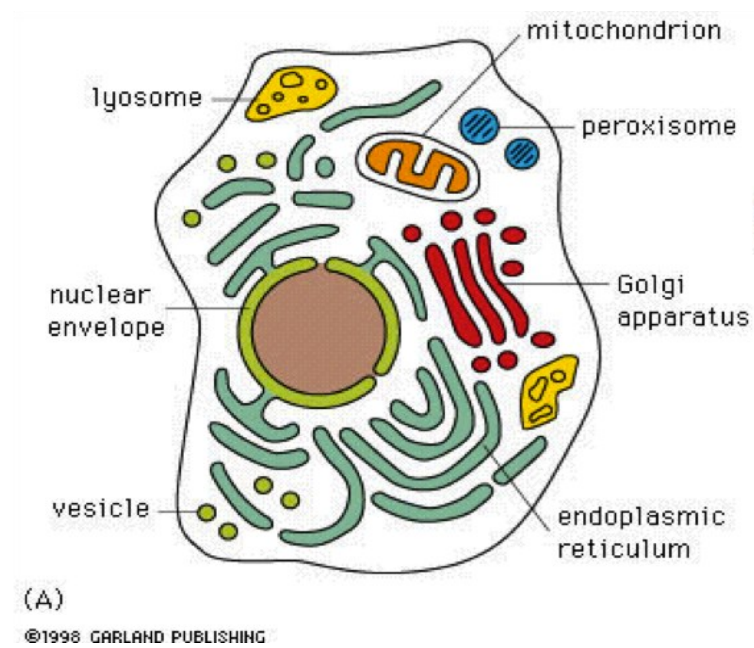
Cell Talk

Communication between the cells that constitute our body

1 human is composed of~10’000’000’000’000 (10^{13}) cells.



2 meters



~0.01 millimeter

Cellular signalling

- Sending out messages
- Receiving messages
- Signal conversion
- Integration of signals

Vital importance: - Knowing what is going on in surroundings
- Coordination with other cells and organisms

Sensing the environment

Light
Food
Temperature
Danger
...

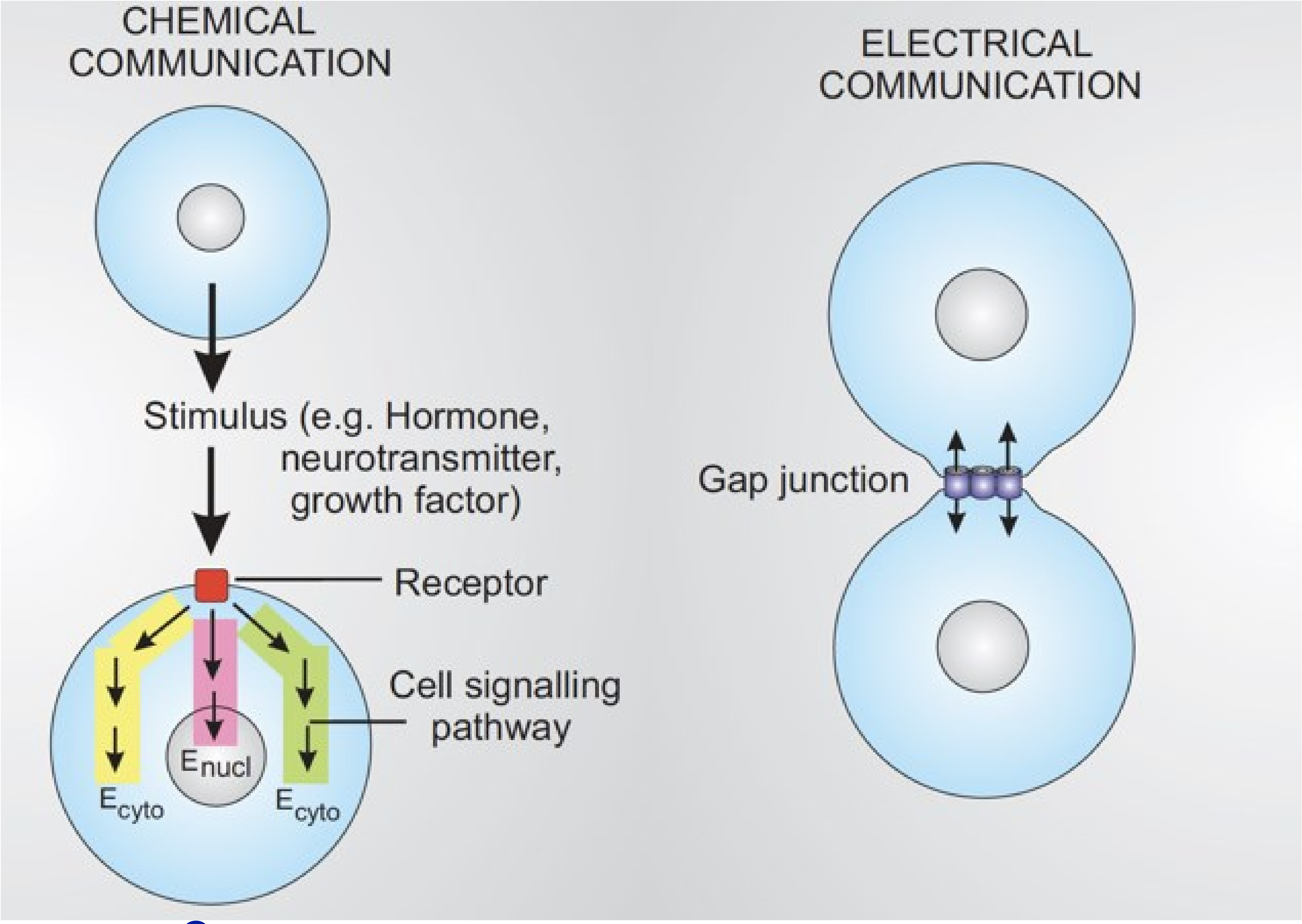
Communication between cells

Synchronisation
Recognition
S.O.S.
Death
...

Cellular signalling

Criteria for good signalling:

How do cells communicate ?

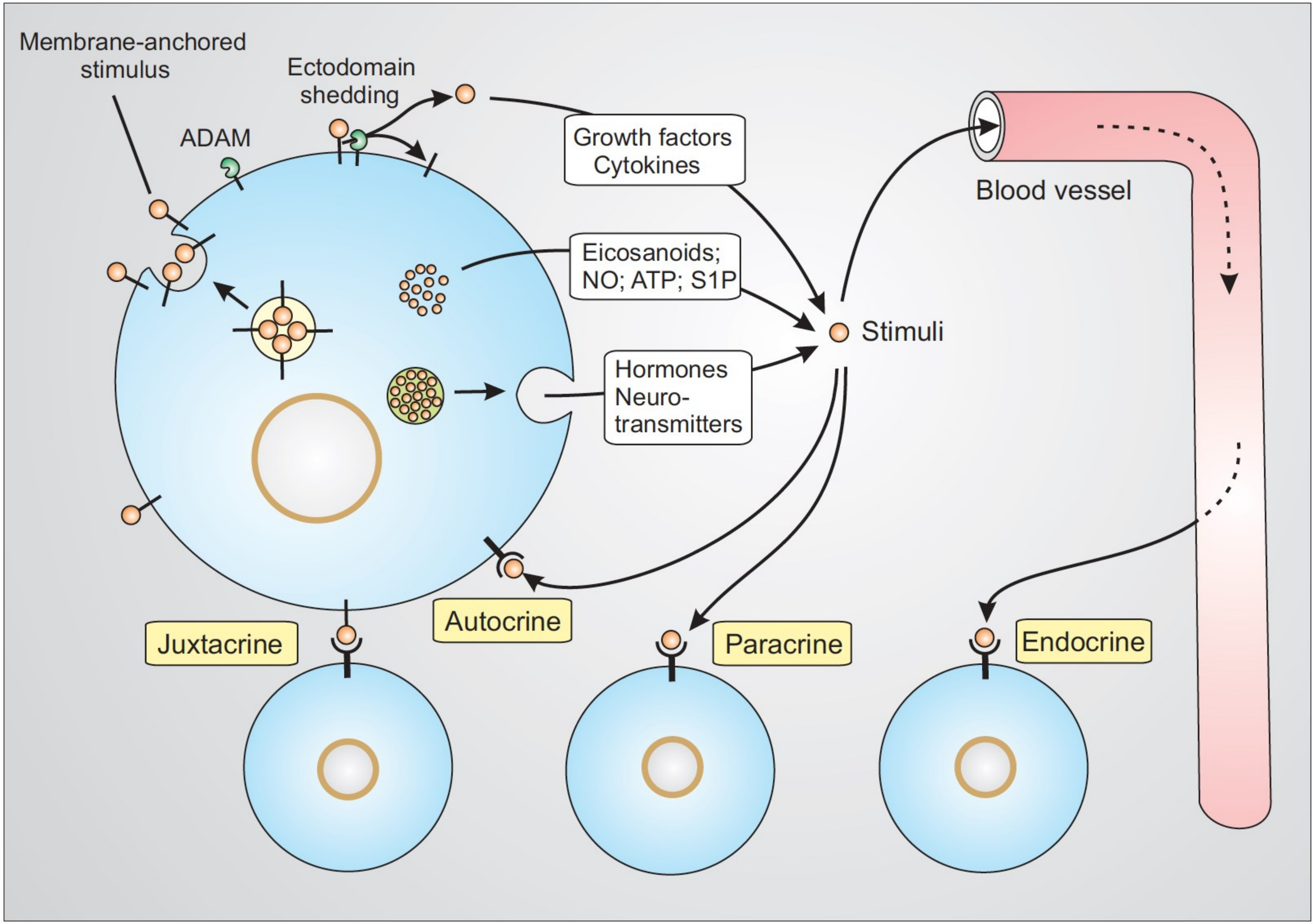


- Sending chemical components
- Electrical coupling
- Exchange of content
- Contact or force

- Sending & receiving

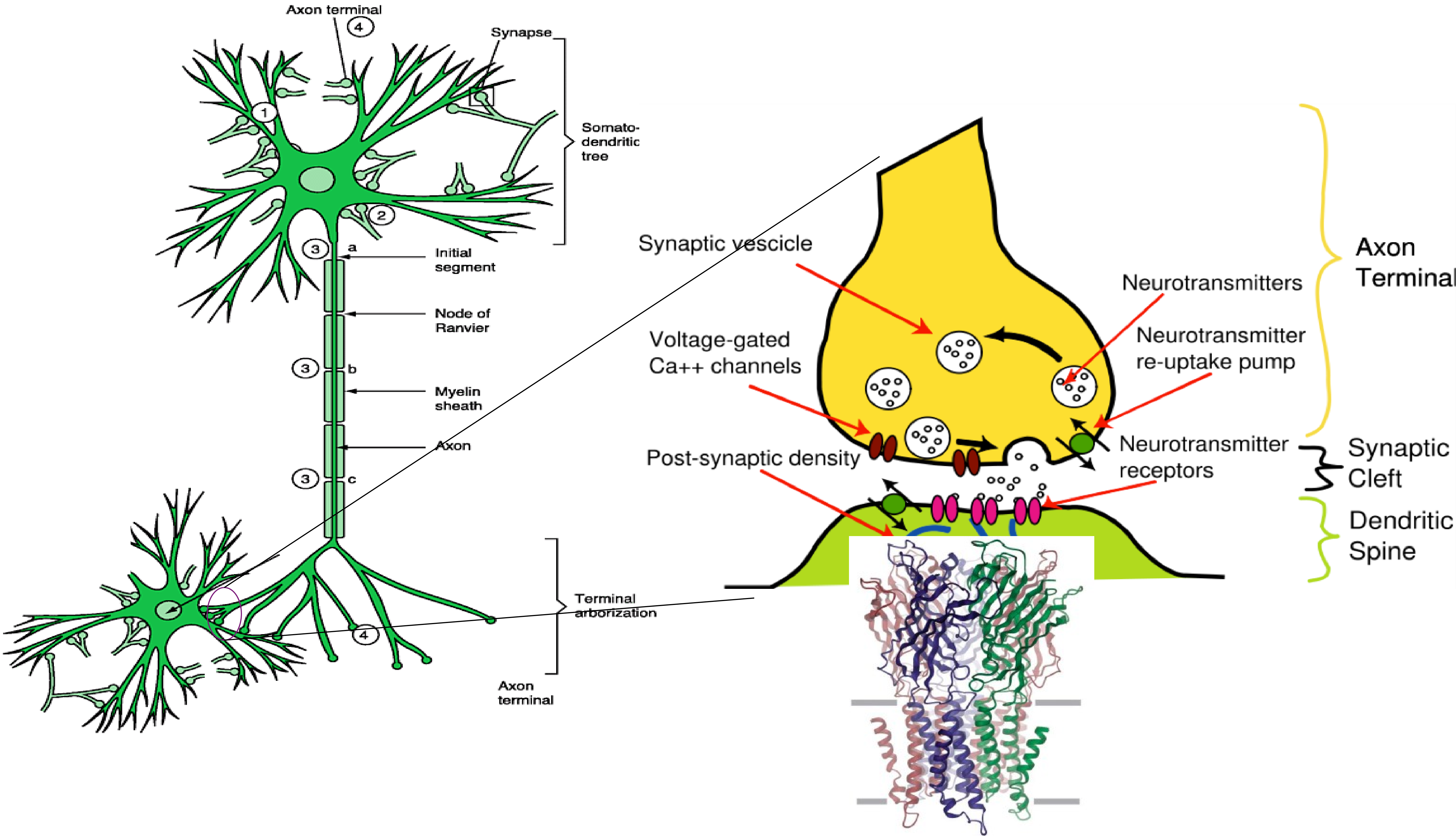
Sending chemicals components

- Excretion : to the exterior of the organism
- Secretion :



Sending chemicals via synapses

- The detection of compounds is done by receptors; these are proteins



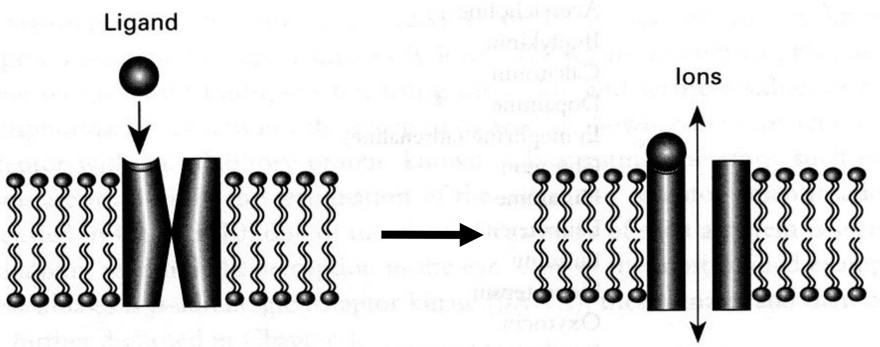
Receptors: General scheme

Receptors classes

Membrane-located

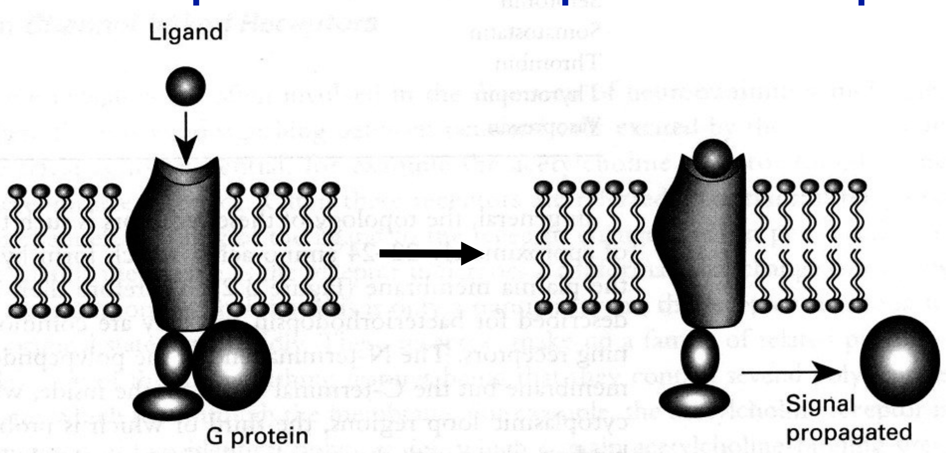
(a)

Ligand-gated ion channels



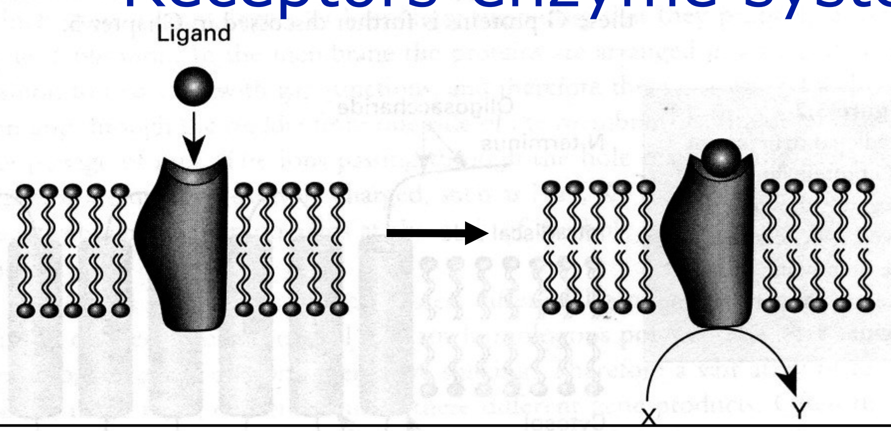
(b)

G protein-coupled receptors

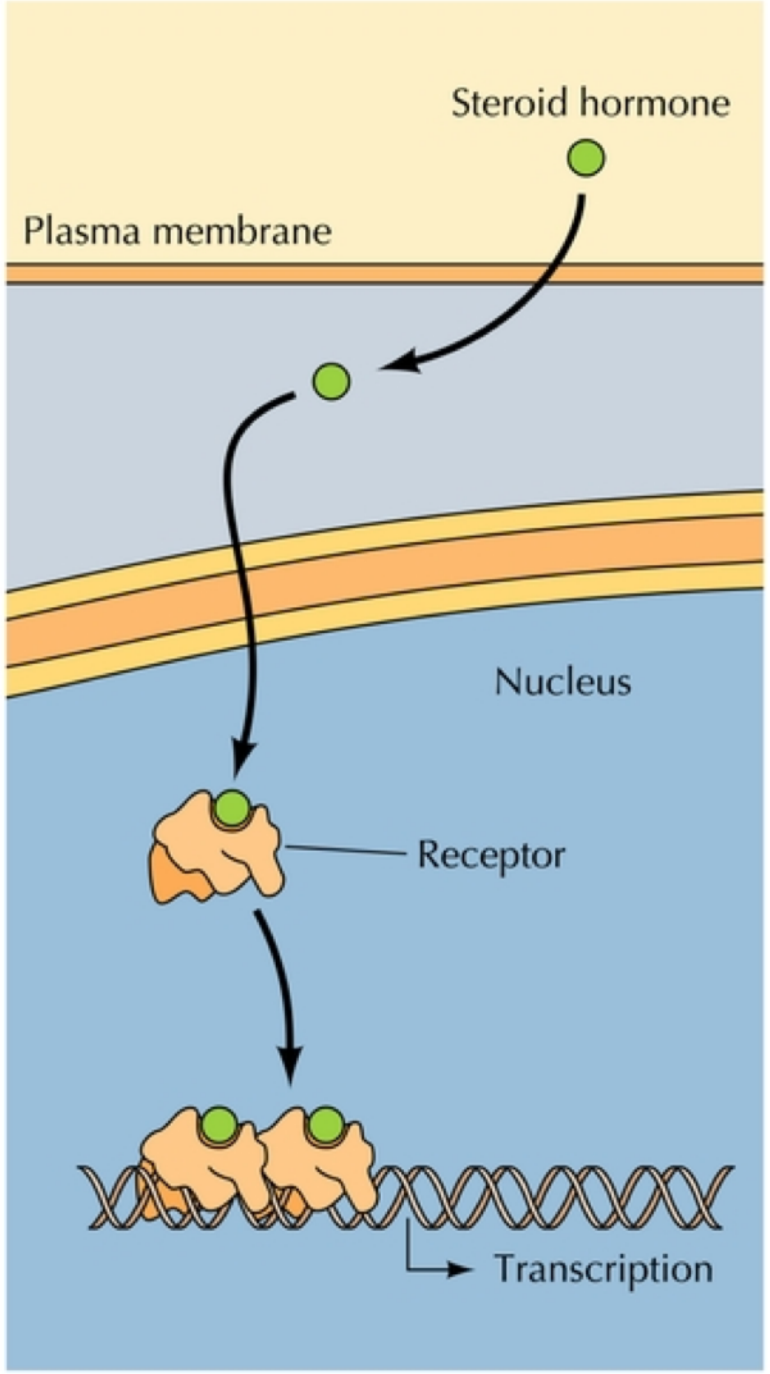


(c)

Receptors-enzyme systems



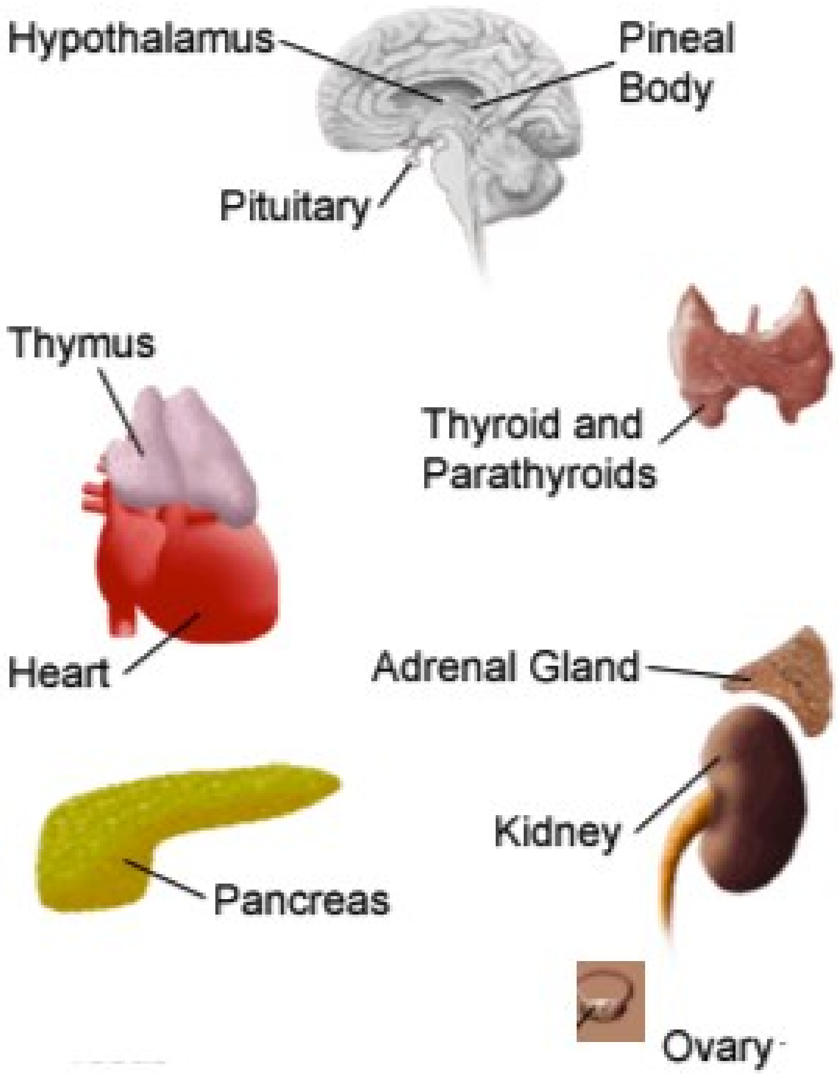
Nuclear receptors



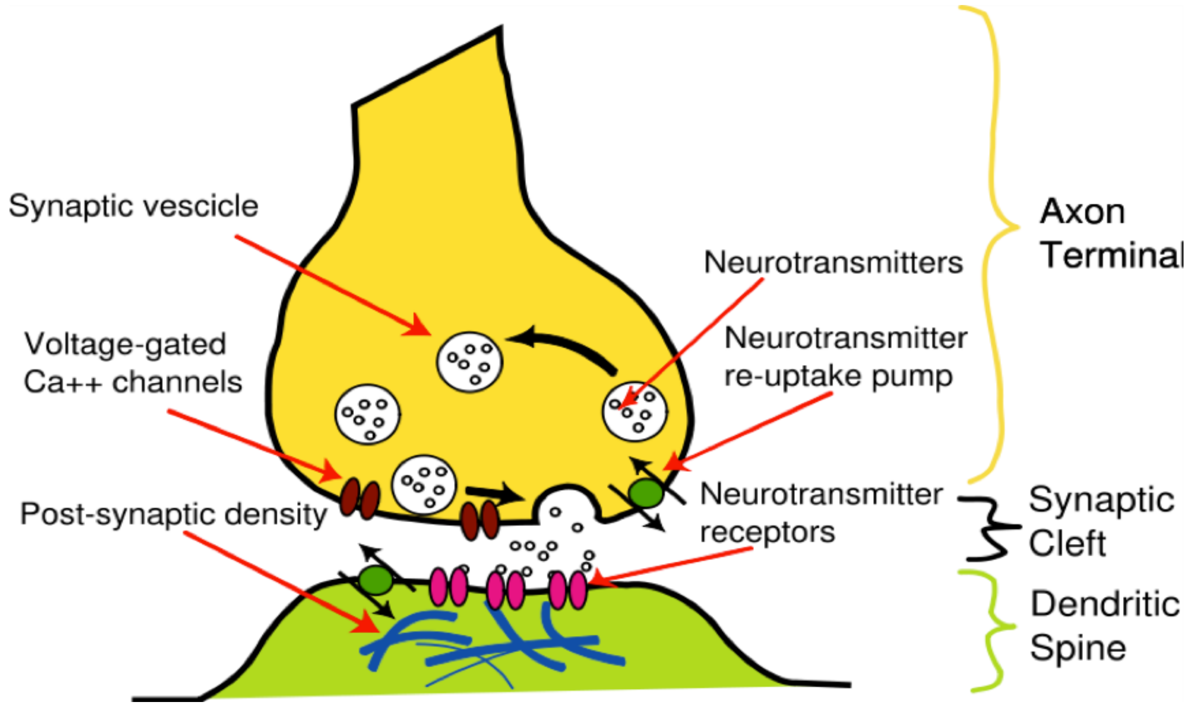
Scale of distance

The distance between sending and receiving cells can differ dramatically

÷ Endocrine



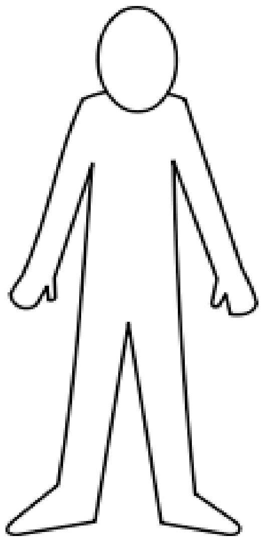
÷ Synaptic contact between neurons

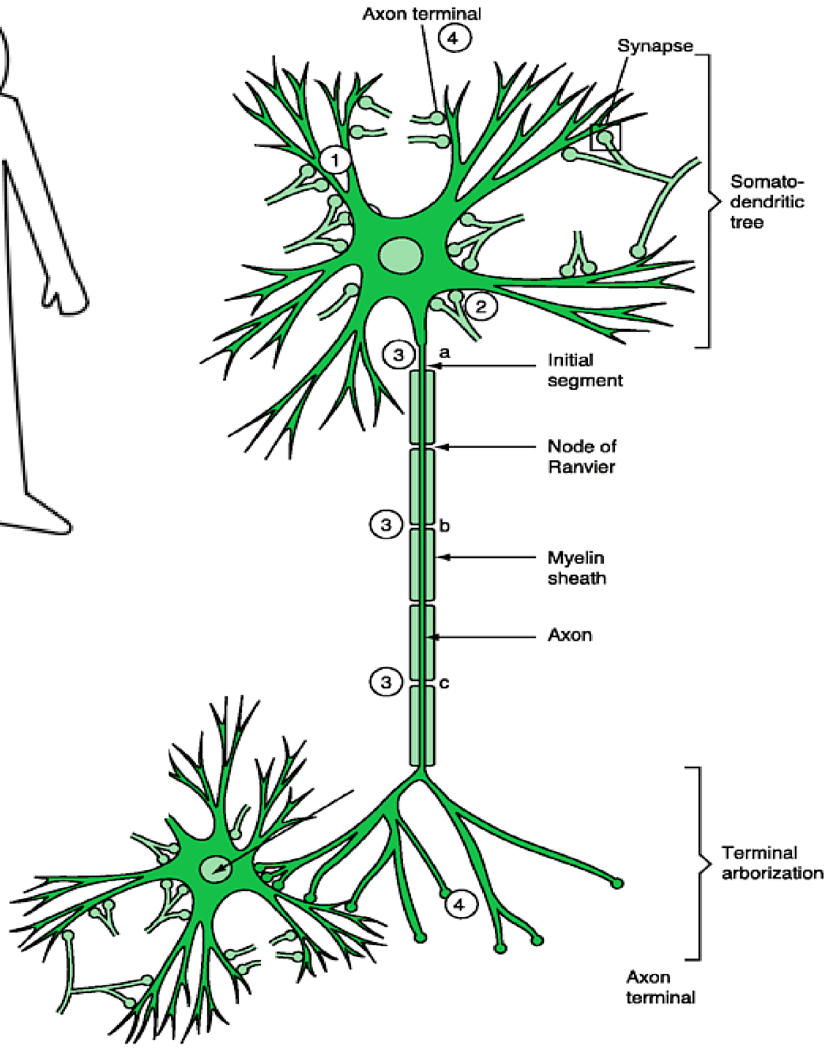


Long-distance signalling

Two complementary solutions:

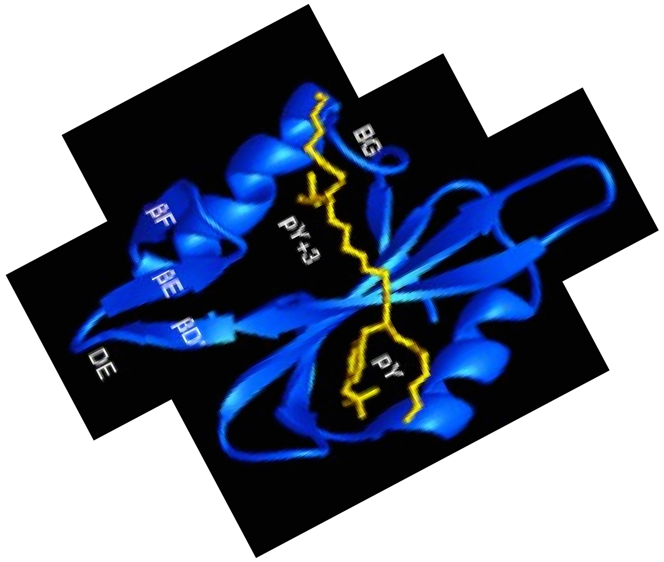
÷ Long nerve cells
up to 1 meter





÷ Precise targeting
combined with
highly sensitive detection

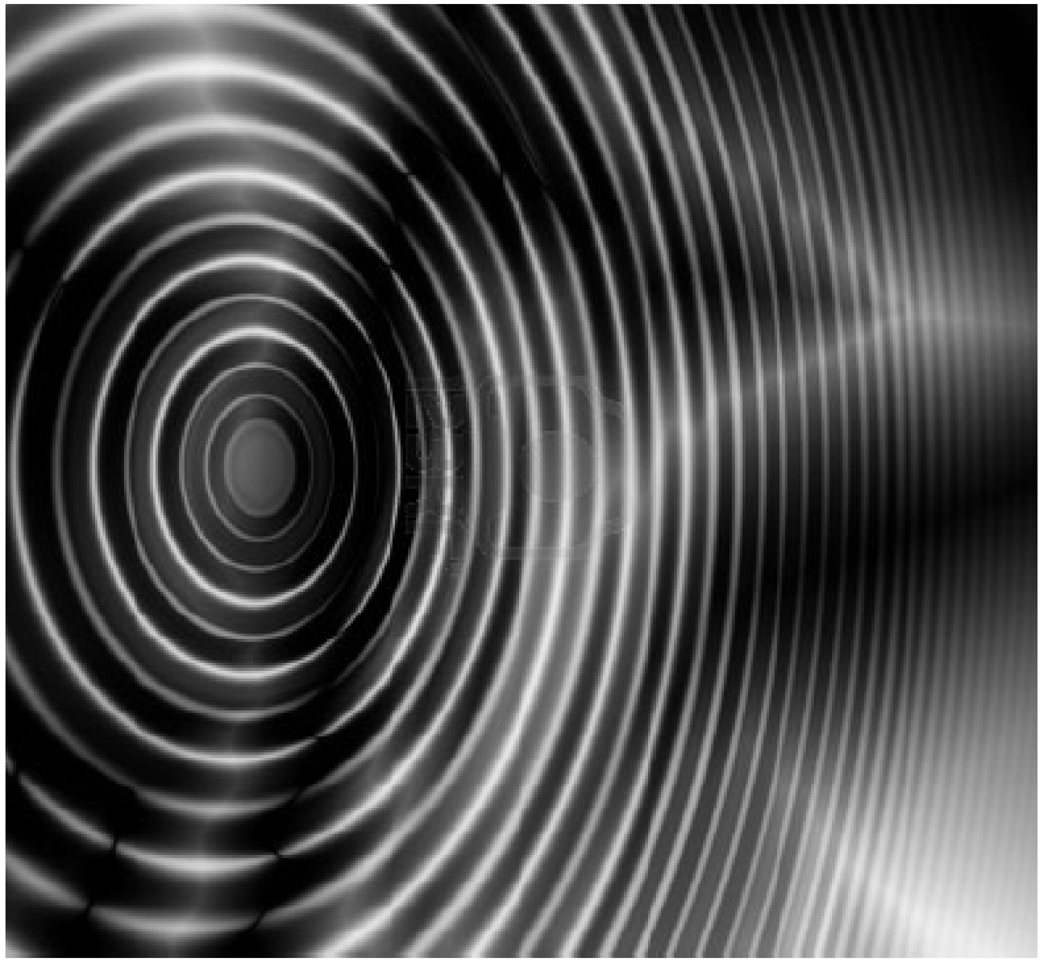
=> Message & receptor with complex and complementary structures



÷ Comparison: telephone number
00 41 21 123 4567

Consequence of long-distance signalling

The signal diminishes with the distance **r**



In quantitative terms :
When one is at a distance **r**
from the source

÷ in two dimensions
the signal diminishes with $\mathbf{r}^2$

÷ in three dimensions
the signal diminishes with $\mathbf{r}^3$

Thus: at 50 nanometers vs at 2 meter

=> strong signal => extremely weak signal

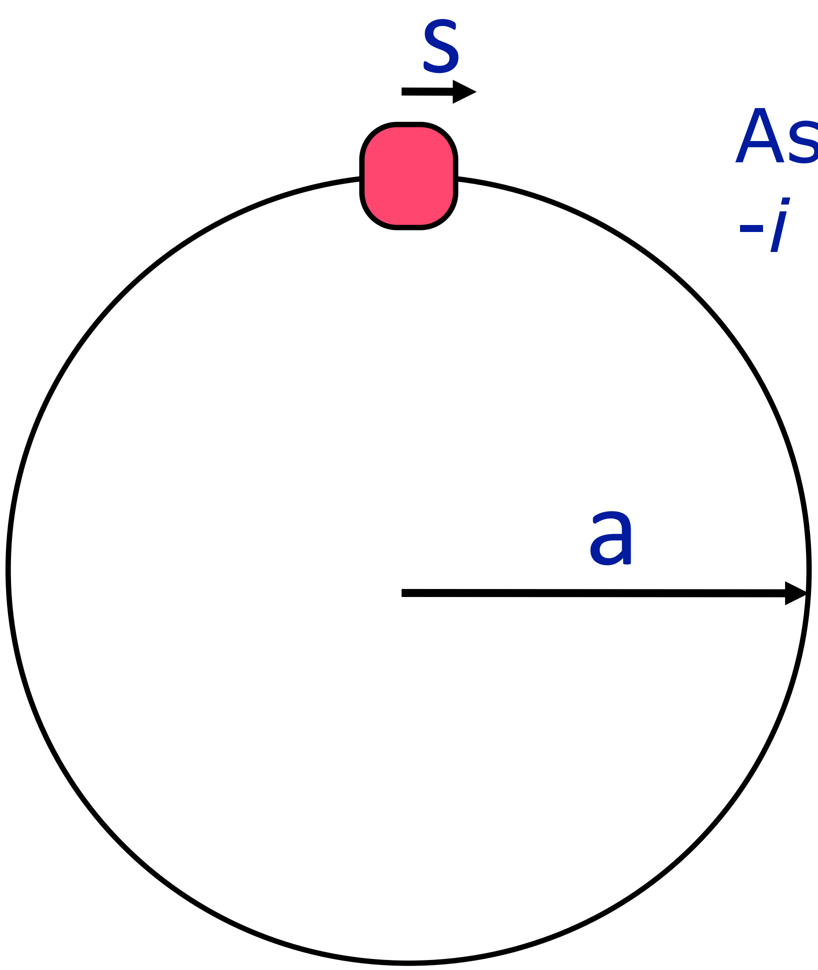
=> High concentration => highly diluted

Receptors sample extra-cellular ligands

Or rather fluctuation in the concentration ***c*** of a ligand.

How many receptors does a cell need to do this accurately?

Berg & Purcell, 1977



Assumptions:

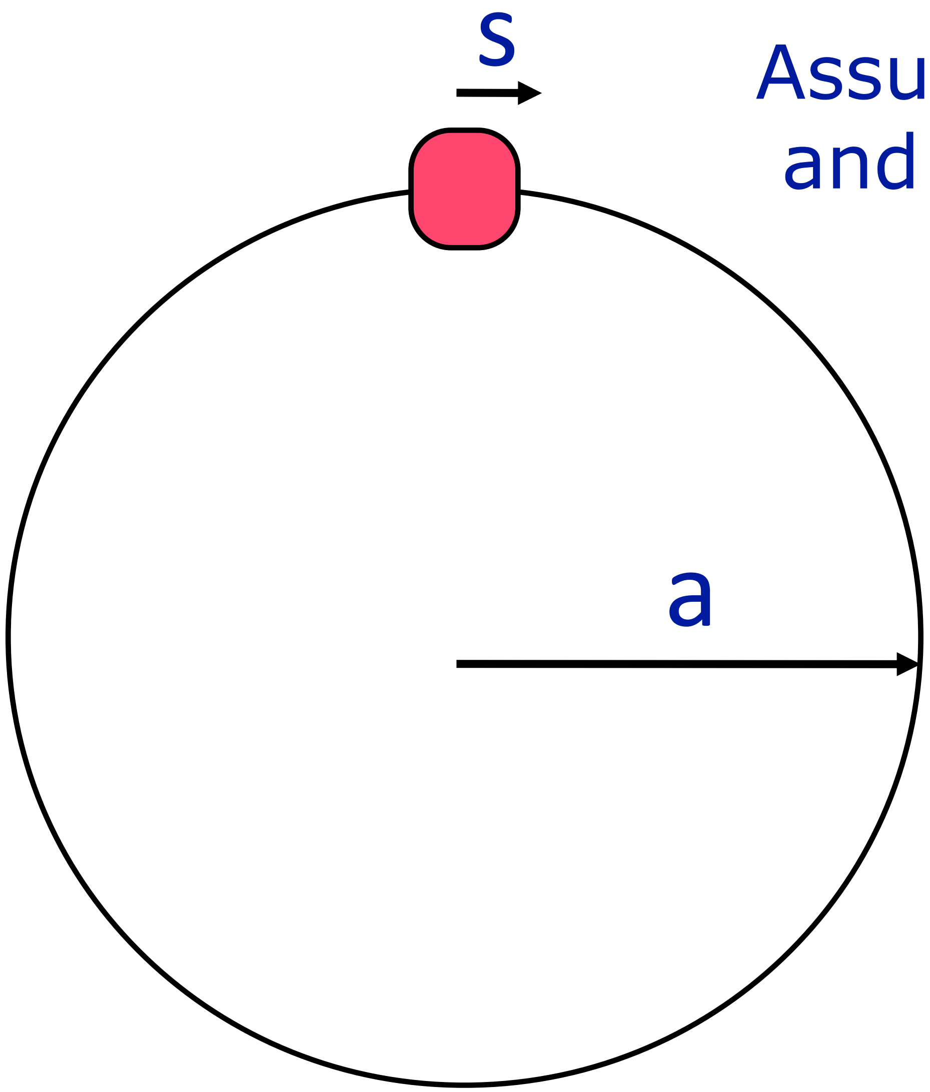
-*i* Cellular response **scales** with receptor occupancy

-*ii* Ligand binding events are **independent**

? How fast can the error **Δc** be detected ?

? How many receptors are needed per cell ?

How fast can receptors quantify ligands ?



Assume a cell has ***N=a/s*** receptors on its surface and a 1% precision is needed

$$\frac{\Delta c}{c} \cong \frac{1}{\sqrt{T \cdot c \cdot a \cdot D}}$$

where: ***D*** is the ligand's diffusion coefficient

c is [ligand] in molecule.cm^{-3}

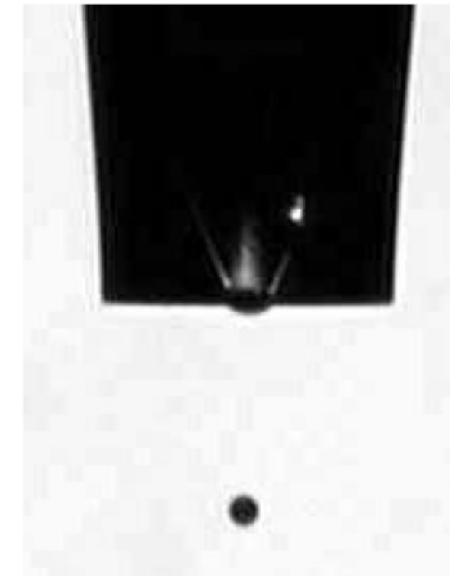
Time scales of signalling processes

Receptor - ligand binding	10^{-6} - 1 s
Receptor activation	< 10^{-6} - 10^{-3} s
Second-messenger response	10^{-3} - 10^{1} s
…	
Gene expression	10^{2} - 10^{4} s
Growth	10^{5} - >10^{6} s

=> More than 12 orders of magnitude !!!

The "n" in biology

	Metric world	=>	"Bio" world
Length	decimeter		1 μm
Volume	litre		10^{-15} L



$\geq 10^{-11}$ L
www.microfab.com

- Effect of scale on number of solute molecules

	Metric world	=>	"Bio" world

The "n" in biology

Some effects of small numbers

÷ Precision of detection of the properties depends on the number N of molecules

Signal $\propto N$ Standard deviation $\propto \sqrt{N}$

÷ Individual properties can be hidden within the average

- instantaneous but also temporal
- The smaller N the more obvious become individual properties

  dynamic properties

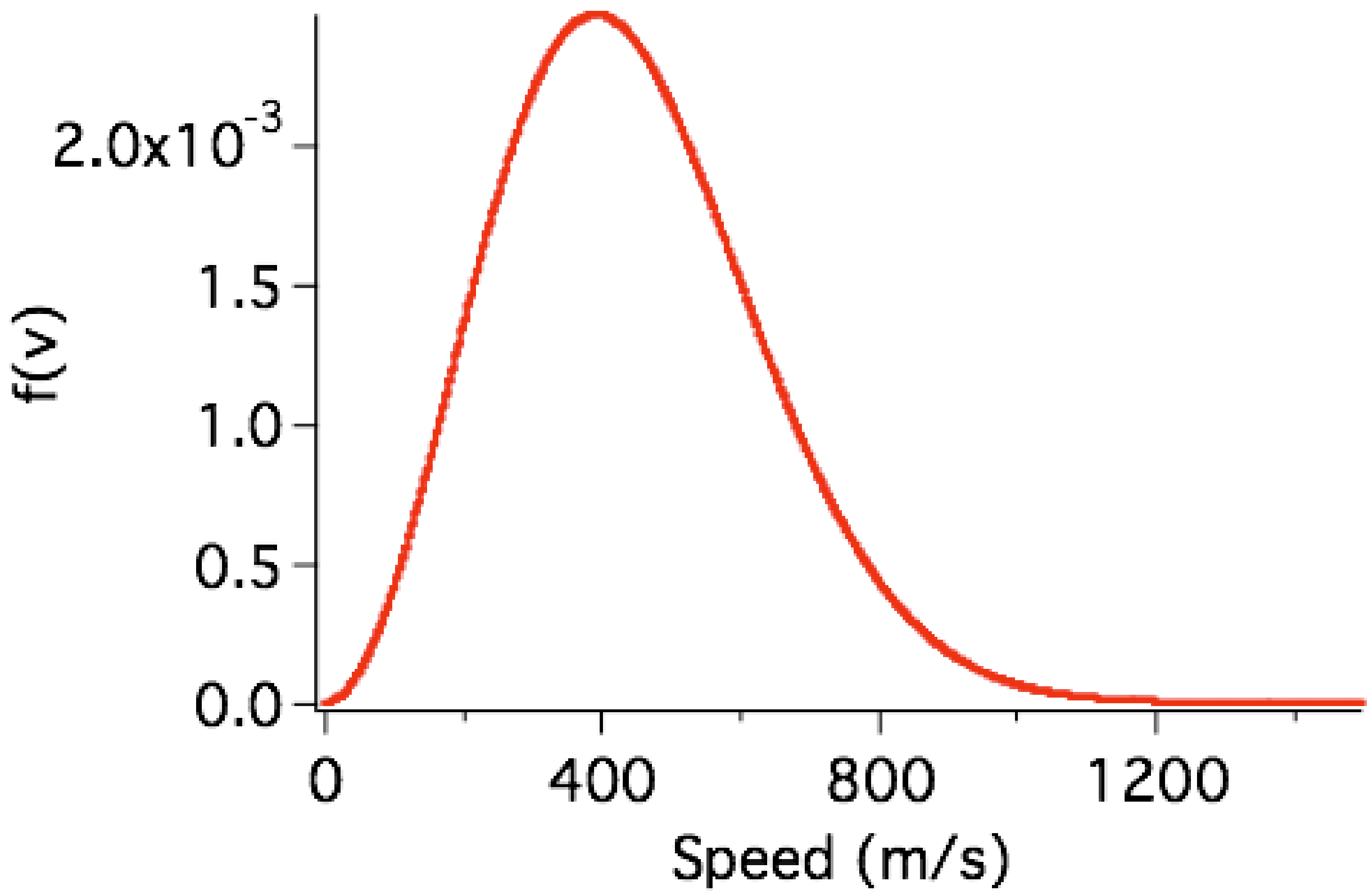
Individual molecules are not identical

Some effects of small numbers

- Distribution of molecular properties

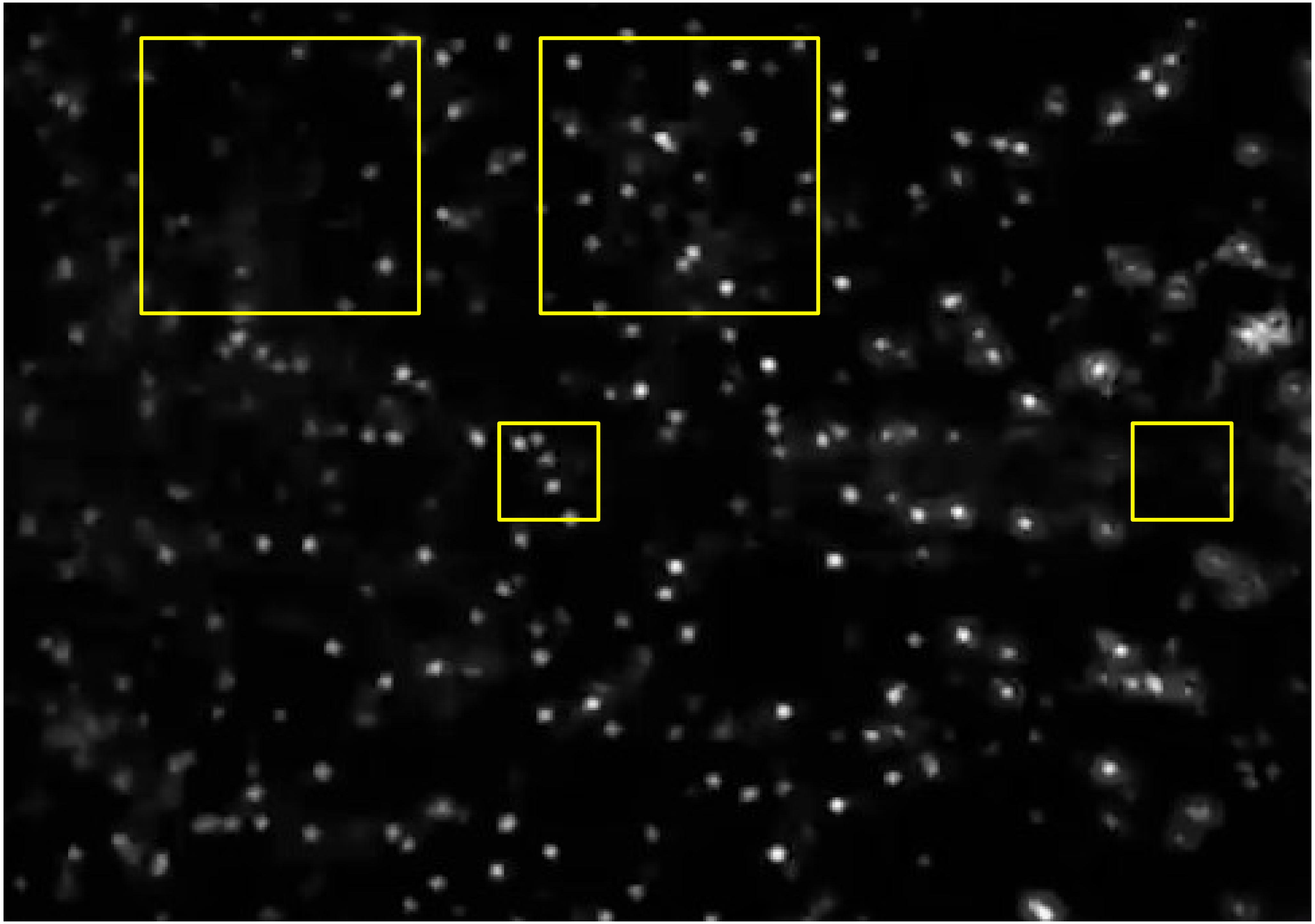
  e.g. speed of gase molecules – (*Boltzmann – Maxwell*)

  fraction *f(v)* of molecules with a speed v: $f(v) = 4\pi \left(\frac{m}{2\pi kT}\right)^{3/2} v^2 \exp\left[\frac{-mv^2}{2kT}\right]$



Consequence of being "small"

A solution filled with particles – How many particles are there in a given volume ?



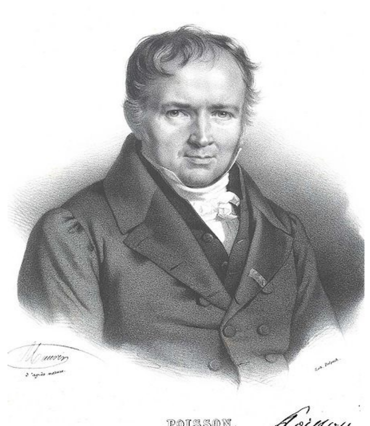
- Tiny numbers

Small numbers are Poisson distributed

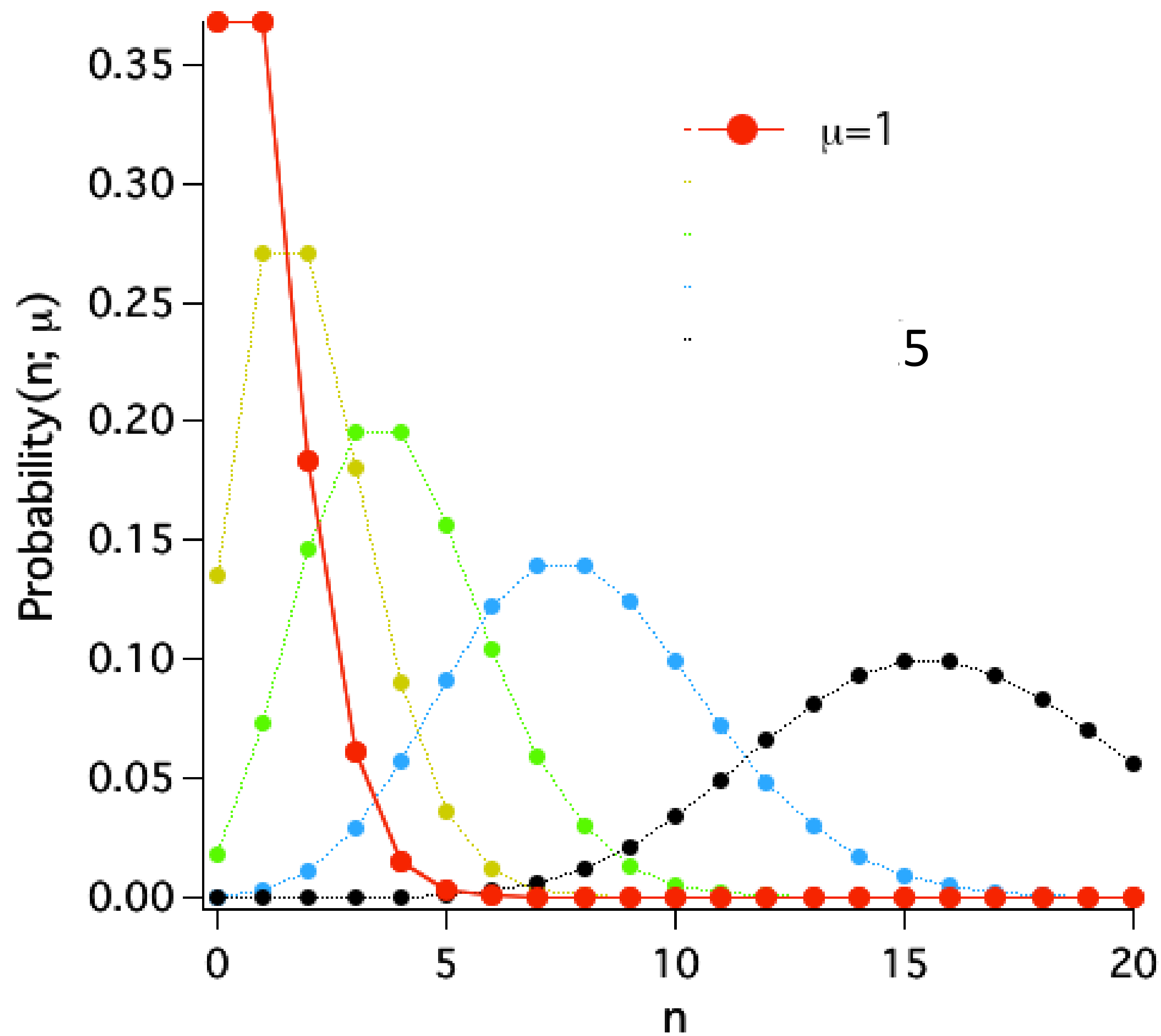
Poisson law of small numbers is a discrete probability distribution that expresses the probability ***p*** of a number of events ***n*** occurring in a fixed period of time if these events happen on the average ***μ*** times in such a period in an independent manner

free interpretation of Wikipedia

$$p(n;\mu) = \frac{\mu^n e^{-\mu}}{n!}$$



Siméon Denis Poisson
1781 - 1840

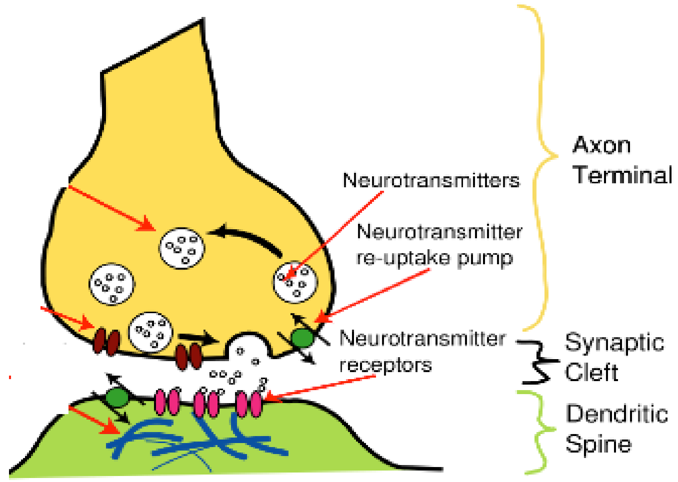


Small numbers : A consequence

Average behaviour does not always apply !!

An example:

Neuronal synapses are small and contain few receptors.



Suppose, 10 ligand-gated ion channels with each an open probability of 10 %

Then, upon presynaptically released agonist => on average 1 channel open

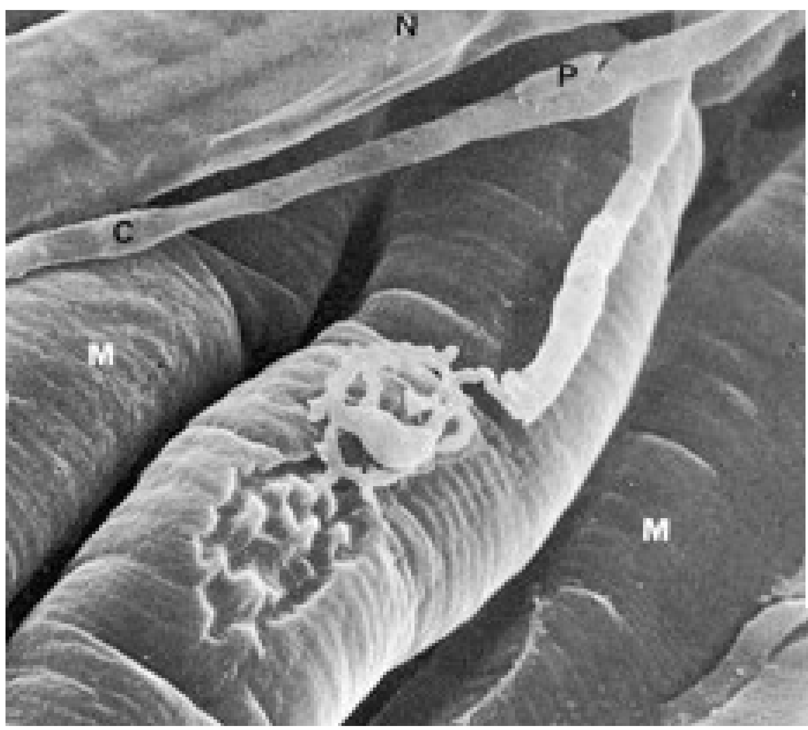
- How to make a synapse that always works ?
  or
- How to design as full proof system ?

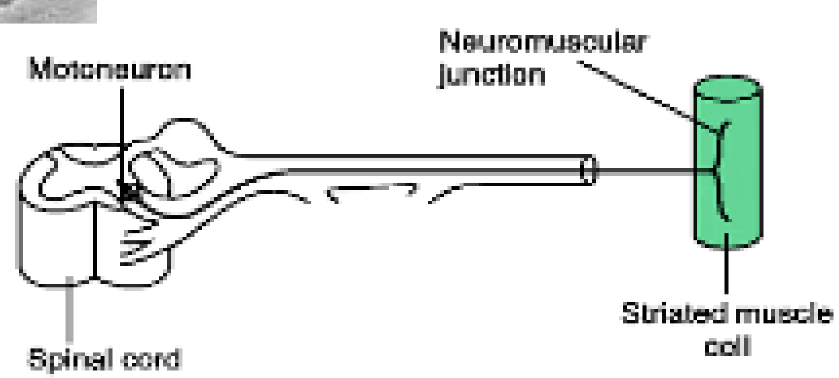
A synapse design to avoid Poissonian failure

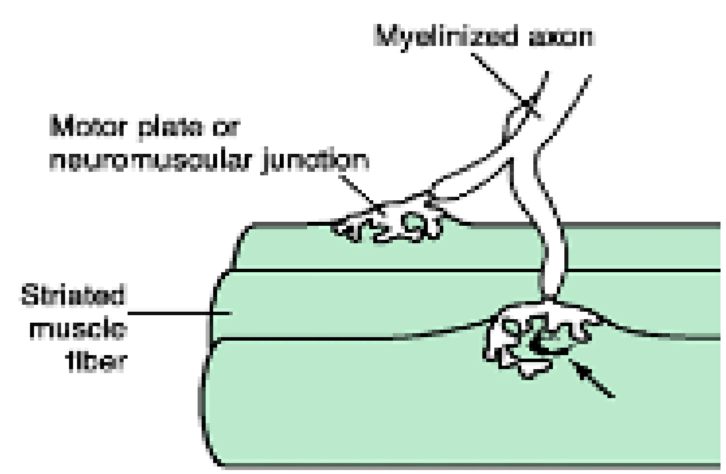
How to make a synapse that always works ?

=> Many receptors & high concentration of transmitter

Text book example: the neuro-muscular junction.

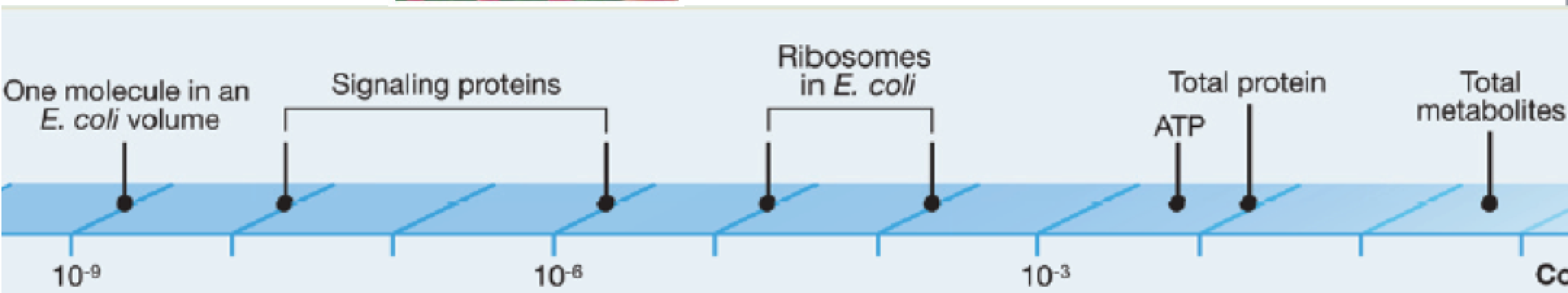






The “n” in biology

Escherichia coli:
Volume 10^{-15} L



Chromosome

DNA Polymeras

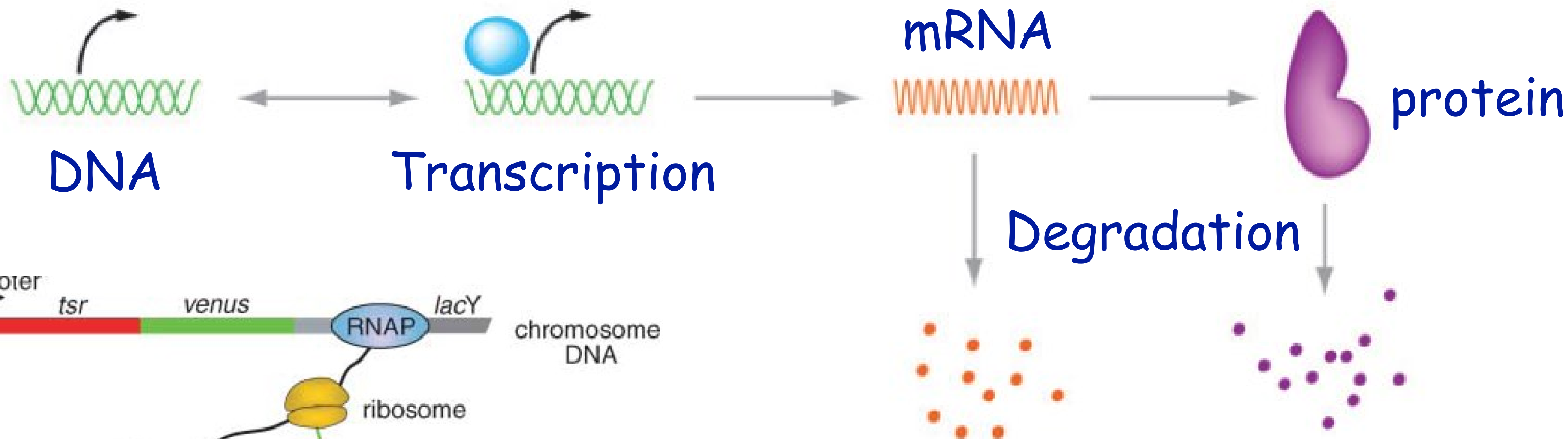
Concentration (Molar)

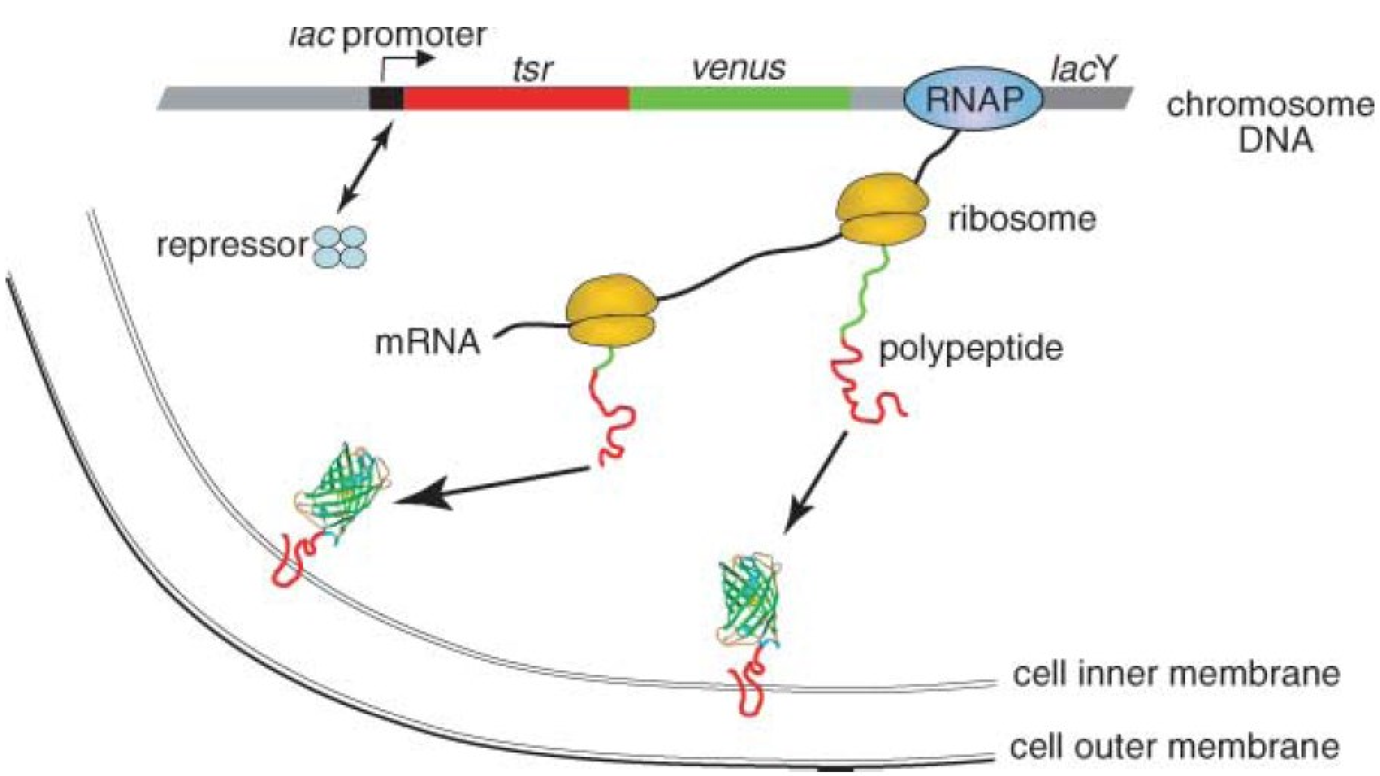
=> Several important molecules are rare, almost alone

The "n" in biology

Probing Gene Expression in Live Cells, One Protein Molecule at a Time

Ji Yu,[1*] Jie Xiao,[1*] Xiaojia Ren,[1] Kaiqin Lao,[2] X. Sunney Xie[1]†





Quantifying the expression of the fluorescent protein *Venus* in *E. coli*

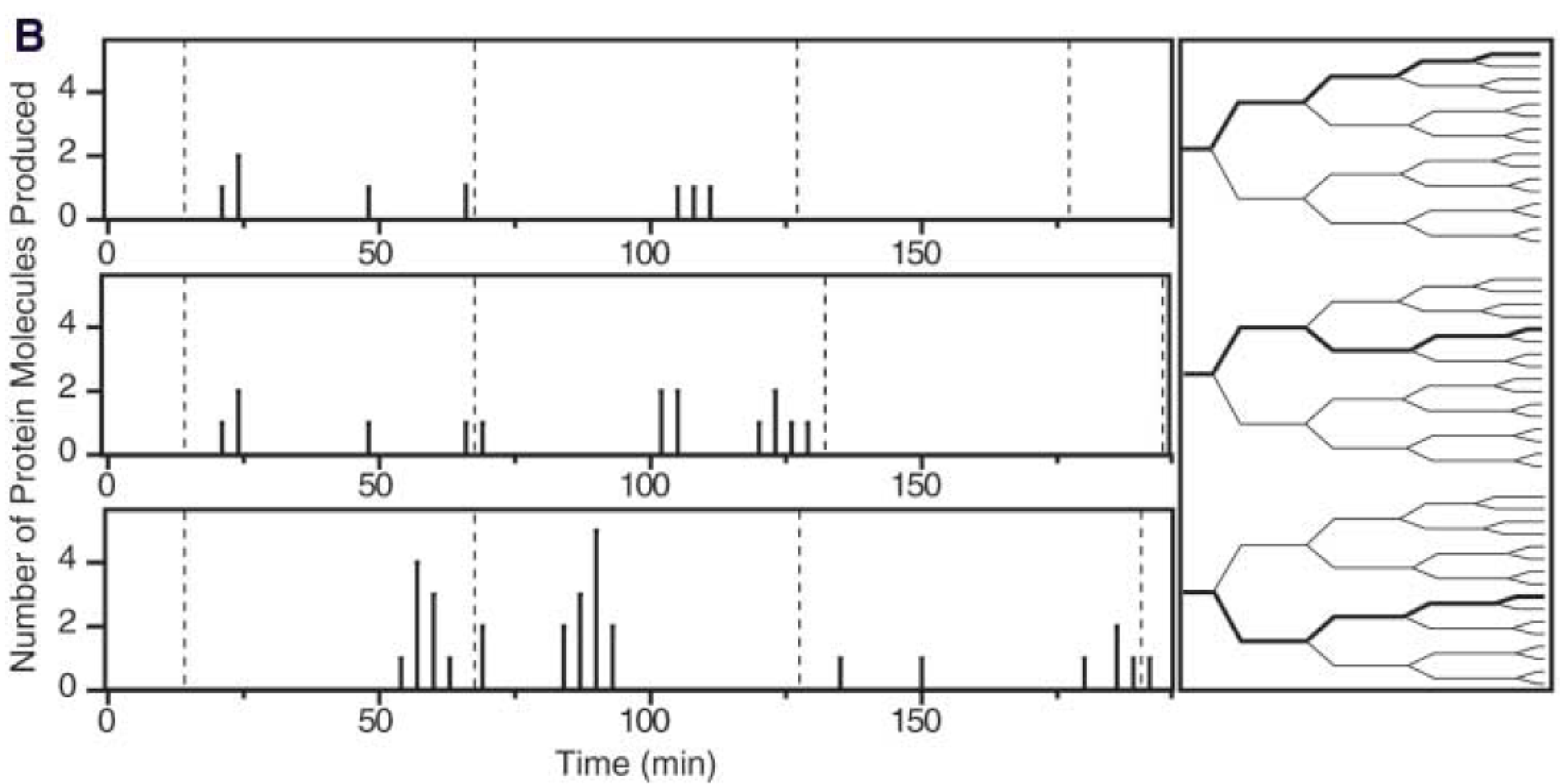
17 MARCH 2006 VOL 311 SCIENCE www.sciencemag.org

The “n” in biology

Quantifying the expression of a fluorescent protein in single *E. coli* cells

Images were collected every 3 min with 100 ms acquisition followed by 1100 ms photo-bleaching. The total elapsed time were 210 min (left) and 170 min (right).

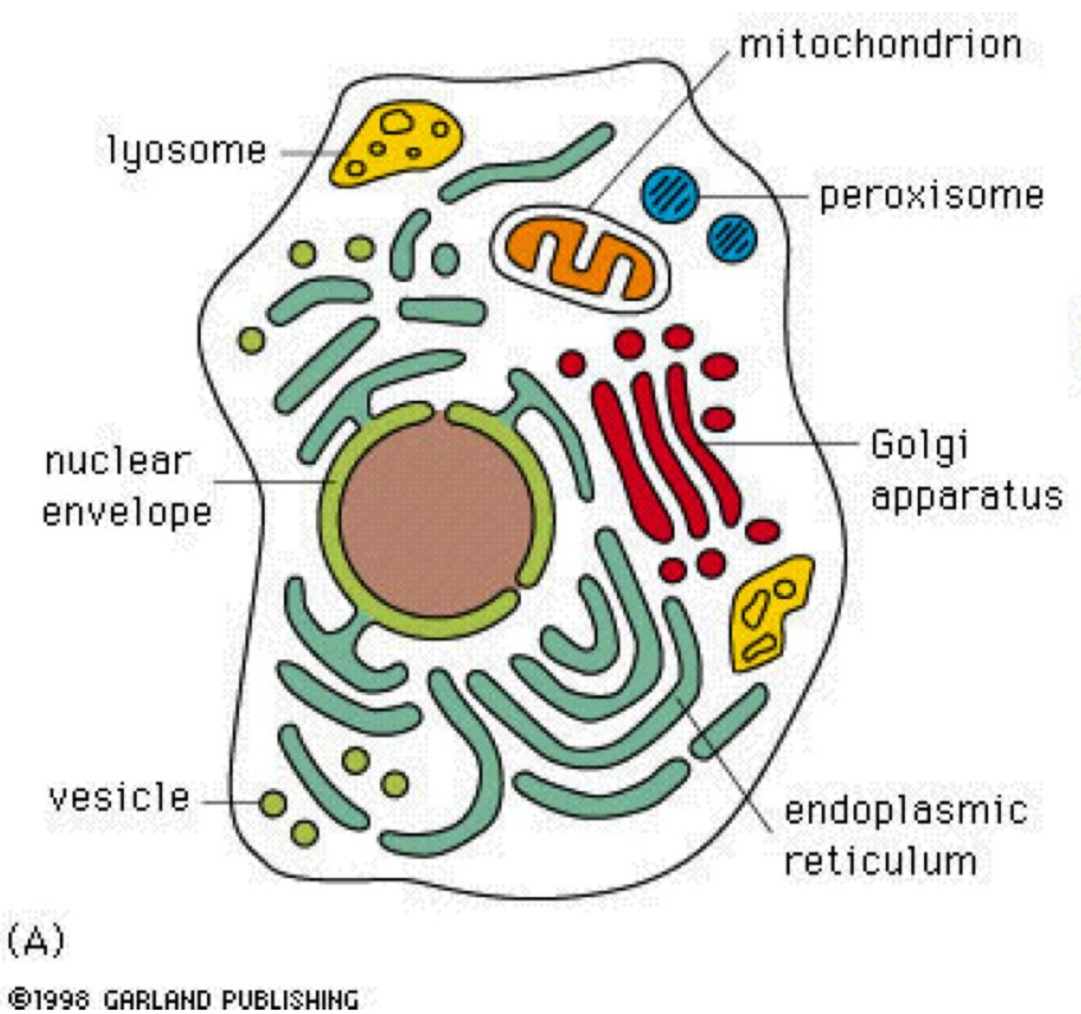
Fluorescent events in time for 3 cells; vertical lines mark cell divisions



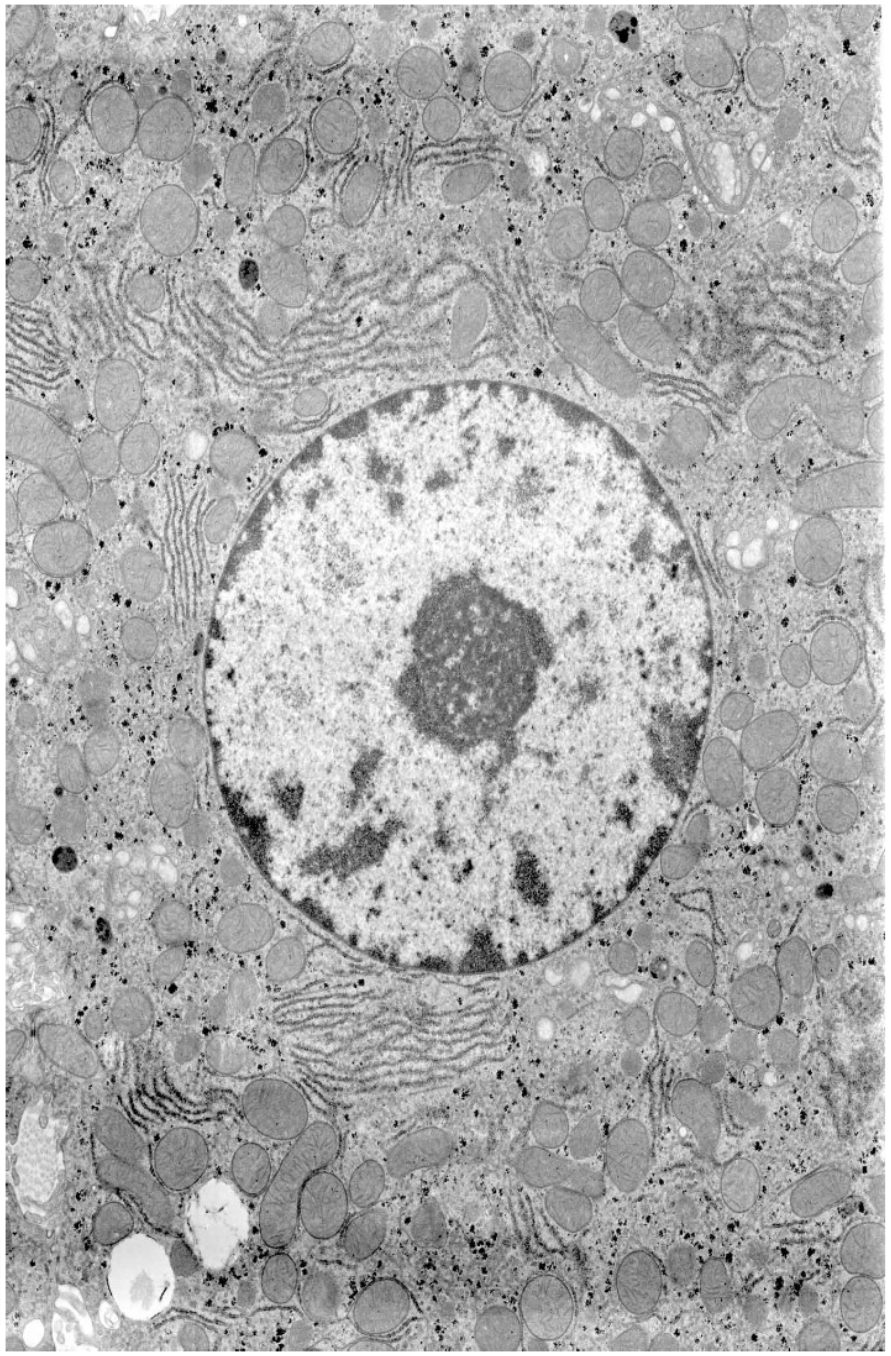
Cells: more than a small test tube

Many compartments => - Highly heterogeneous
- Transport and separation

Schematic drawing vs Electron micrograph



Modified From Figure 1-17, Page 15 from: *Essential Cell Biology: An introduction to the Molecular Biology of the Cell* by Alberts, Bray, Johnson, Lewis, Raff, Roberts and Walter 1997, Garland Publishing Inc. New York, NY

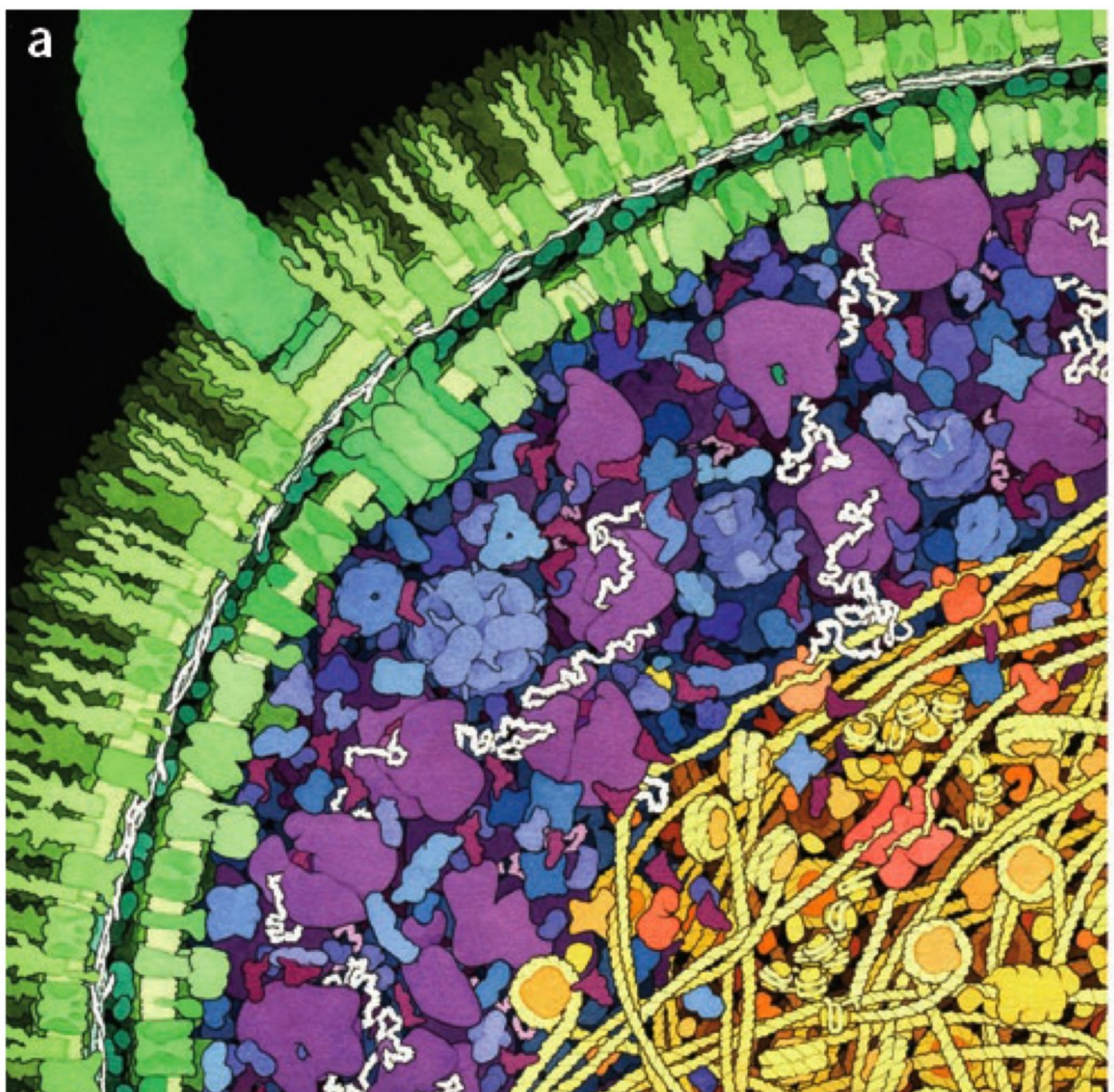


The Cell

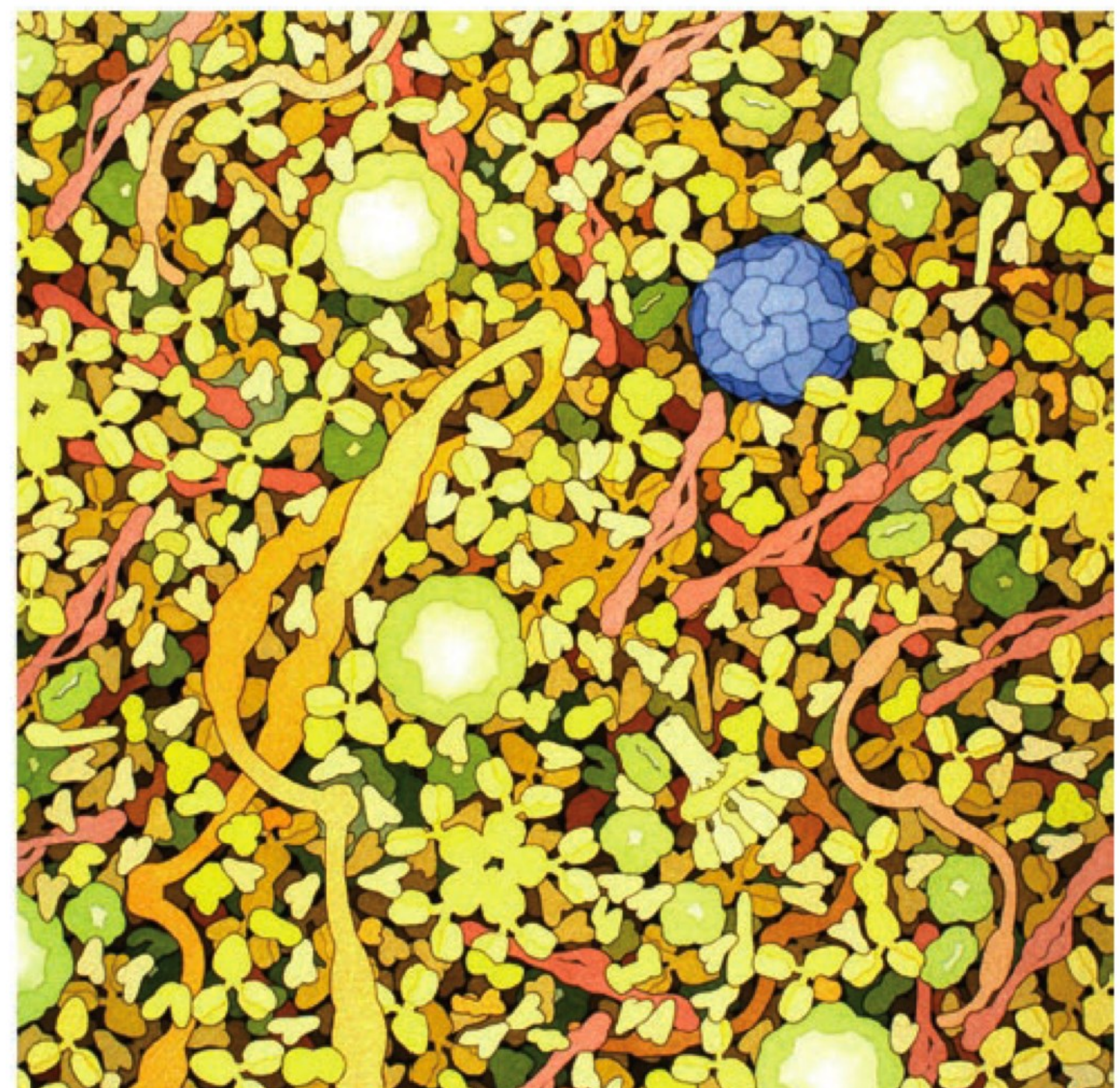
Cells: Highly crowded

Biochemical information is used to determine the concentration and location of the many different molecules, and diverse structural information from crystallography, NMR spectroscopy, electron microscopy or biophysical analysis is used to determine the shape and size of the molecules.

E. coli



Blood serum



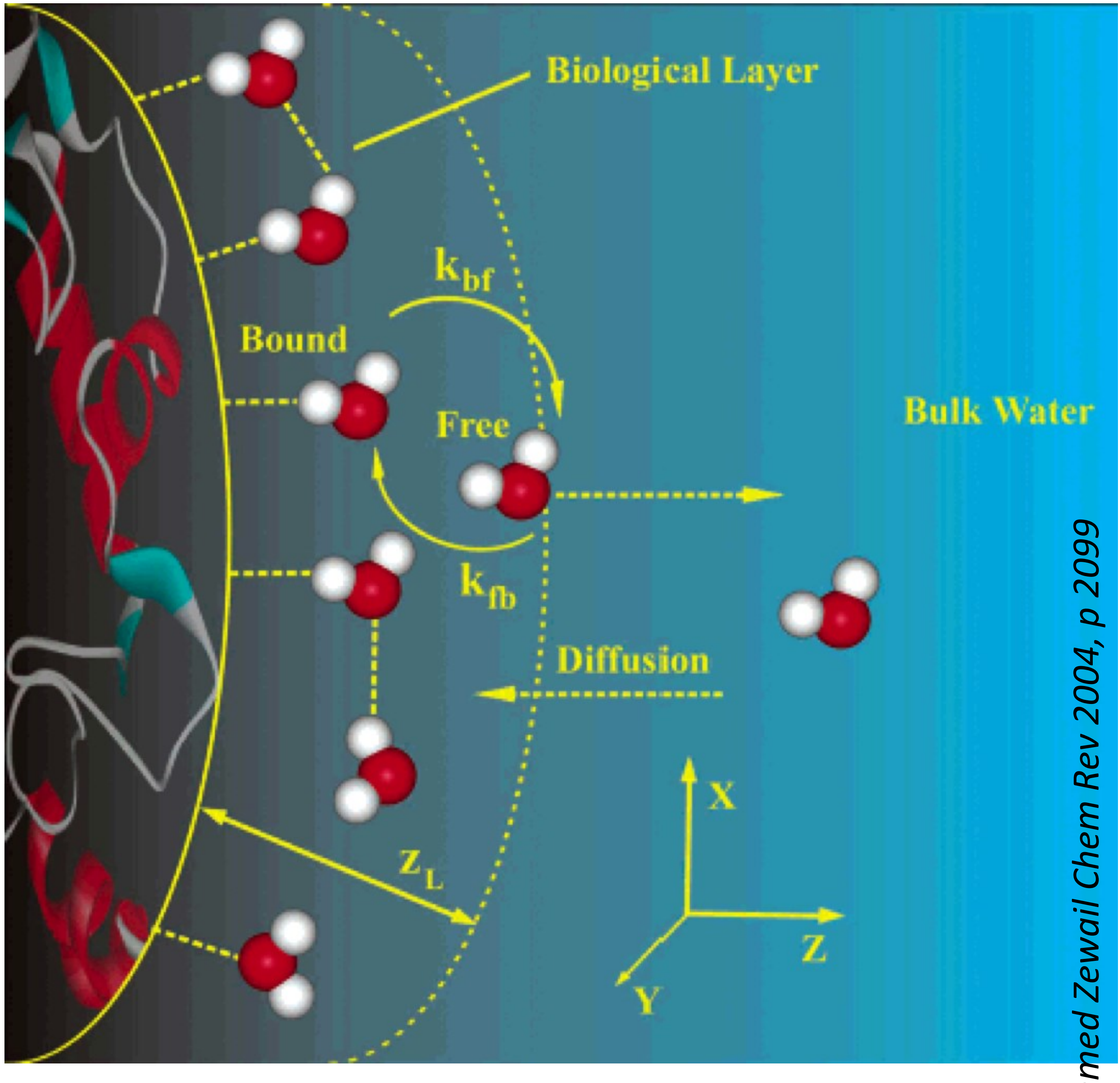
D.S. Goodsell, Nat Chem Biol 3 (2007) p.681

Cells: More than a small test tube

All these molecules interact with water.

For example: hemoglobin in a red blood cell:
- 35 % mass percent of erythrocyte
- binds about 400 water molecules
- several layers of perturbed water

Only ~10% of total water has the properties of bulk water.



Ahmed Zewail Chem Rev 2004, p 2099

Cells: very complicated reaction conditions

- Highly heterogeneous - compartments with very different contents
- Transport and separation
- Size-dependent effects on diffusion, kinetics and thermodynamics
- Non-equilibrium state
- Many components - 5’000 different gene products
  - Polymers like proteins, nucleotides, sugars
  - Solutes, like salts,metabolites.,..

  - Often at high concentrations

=> Biological fluids are highly non-ideal

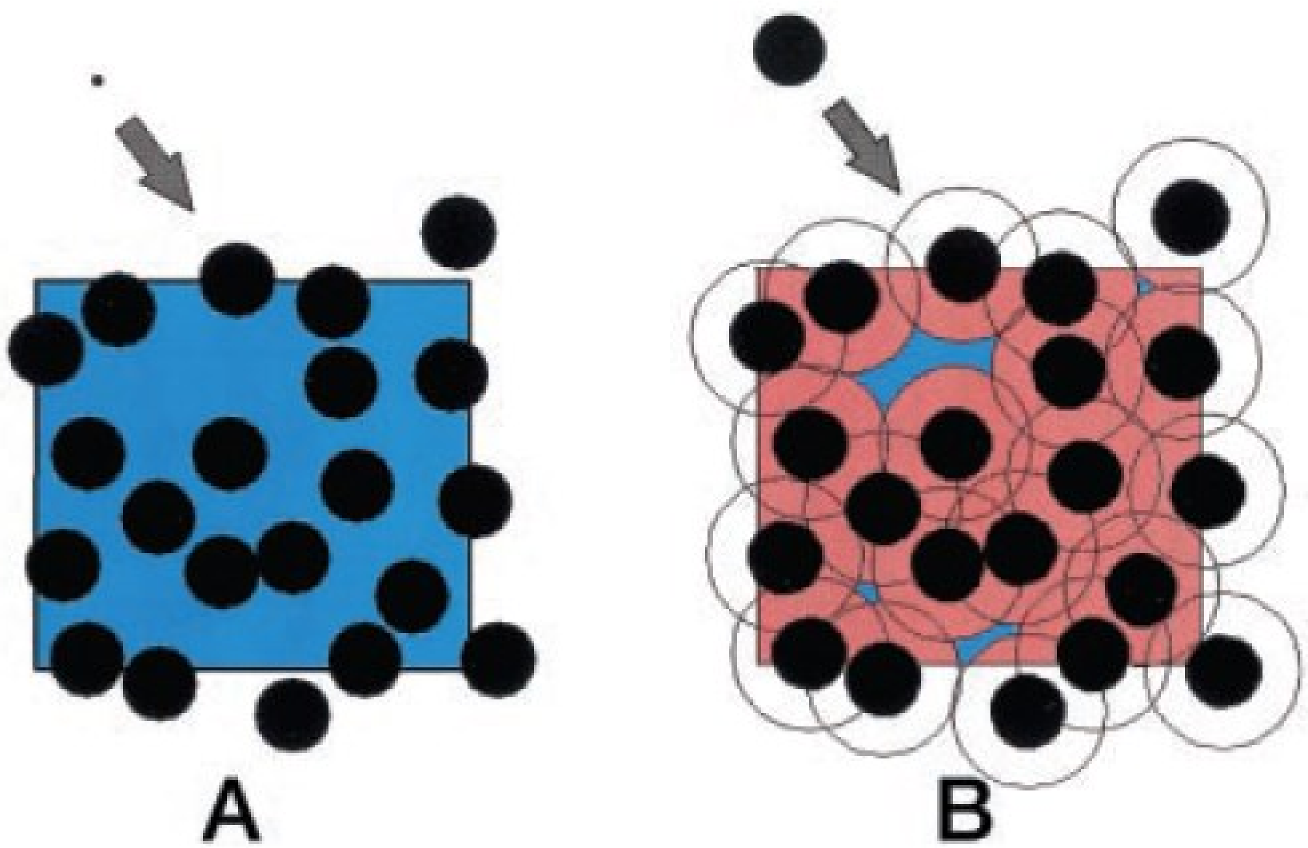
÷ The far aim of quantitative biology :
Description in space (x,y,z) and time (t) of a cell, an organism.
or even biotope

Cells: Highly crowded

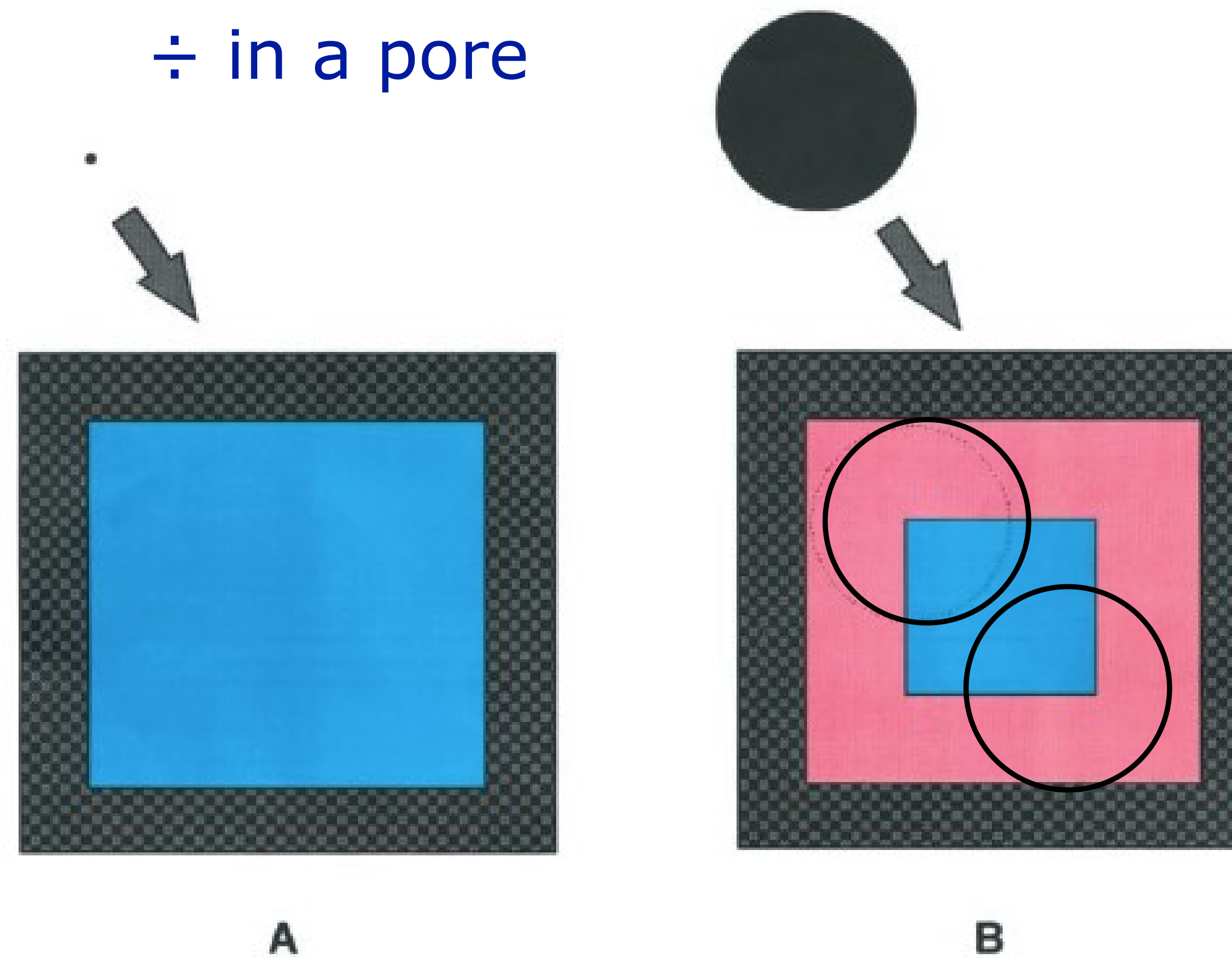
Size matters !!

Compare a small (A) and a large (B) molecule for the volume accessible (blue) or excluded (pink) to the centre of mass within a solution containing large solutes (left) or within a pore (right).

÷ in bulk solution



÷ in a pore



Minton, JBC, 2001

Effects of crowding: Slower diffusion

versus

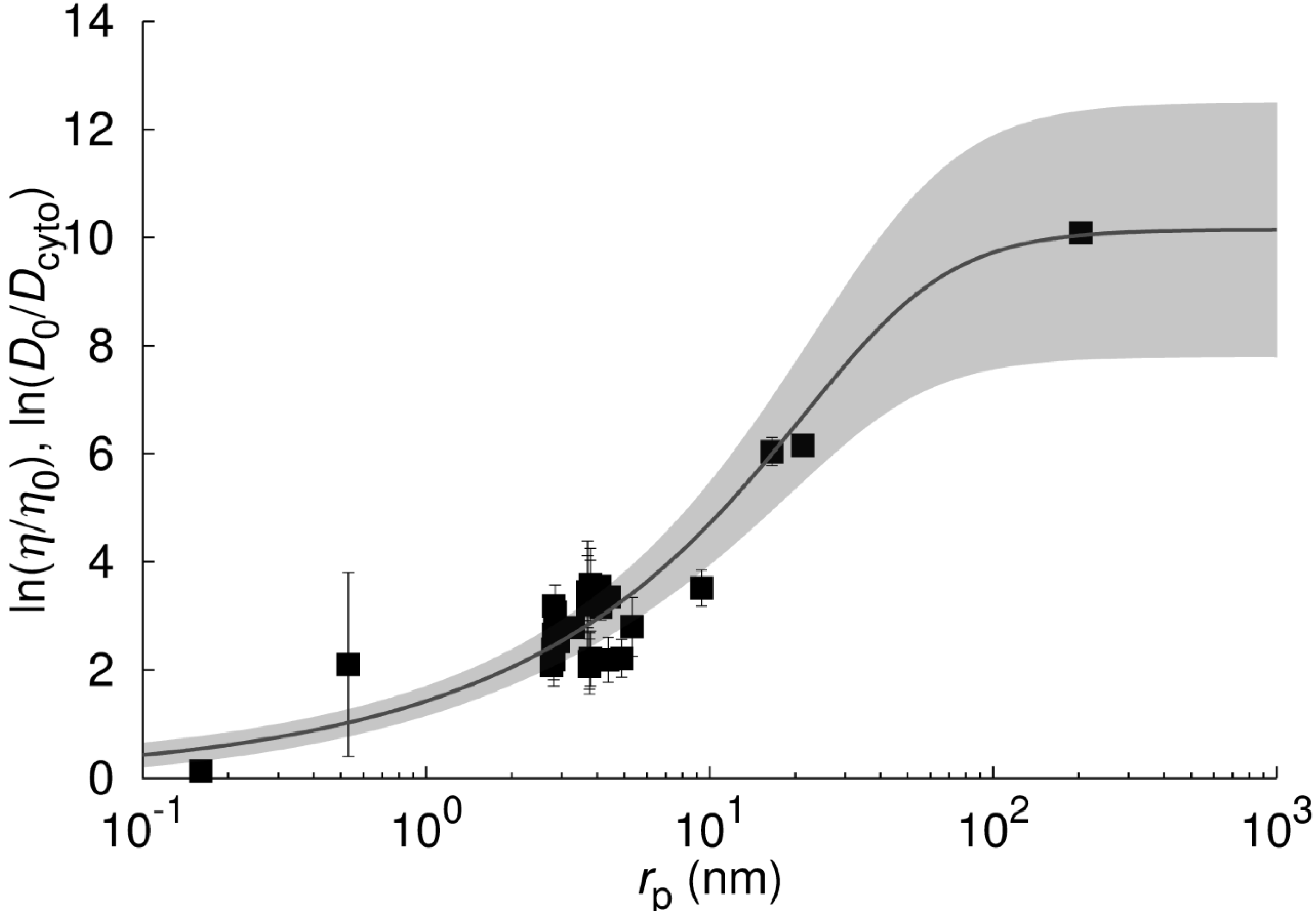
Macroscopically, the diffussion slows down
=> the diffusion coefficient ***D*** decreases strongly

The time ***t*** to travel a certain distance scales as $\boldsymbol{D^{-1}}$

e.g. GFP diffuses 10 times slower in the cytosol than in aqueous buffer

Diffusion in the cytosol : Size effects

The apparent viscosity in the cytosol depends on the size of the object:



=> the bigger the more is diffusion hampered !!

Kalwarczyk, Bioinformatics, 2012

Effects of crowding: Chemical equilibria

Ideal system: No interactions between molecules

This condition is met in systems like :
- gases at low pressure
- very diluted solutions

Imagine a polymerisation reaction: $nA \rightleftharpoons A_n$

characterized by a dissociation constant $K_d = \dfrac{[A]^n}{[A_n]}$

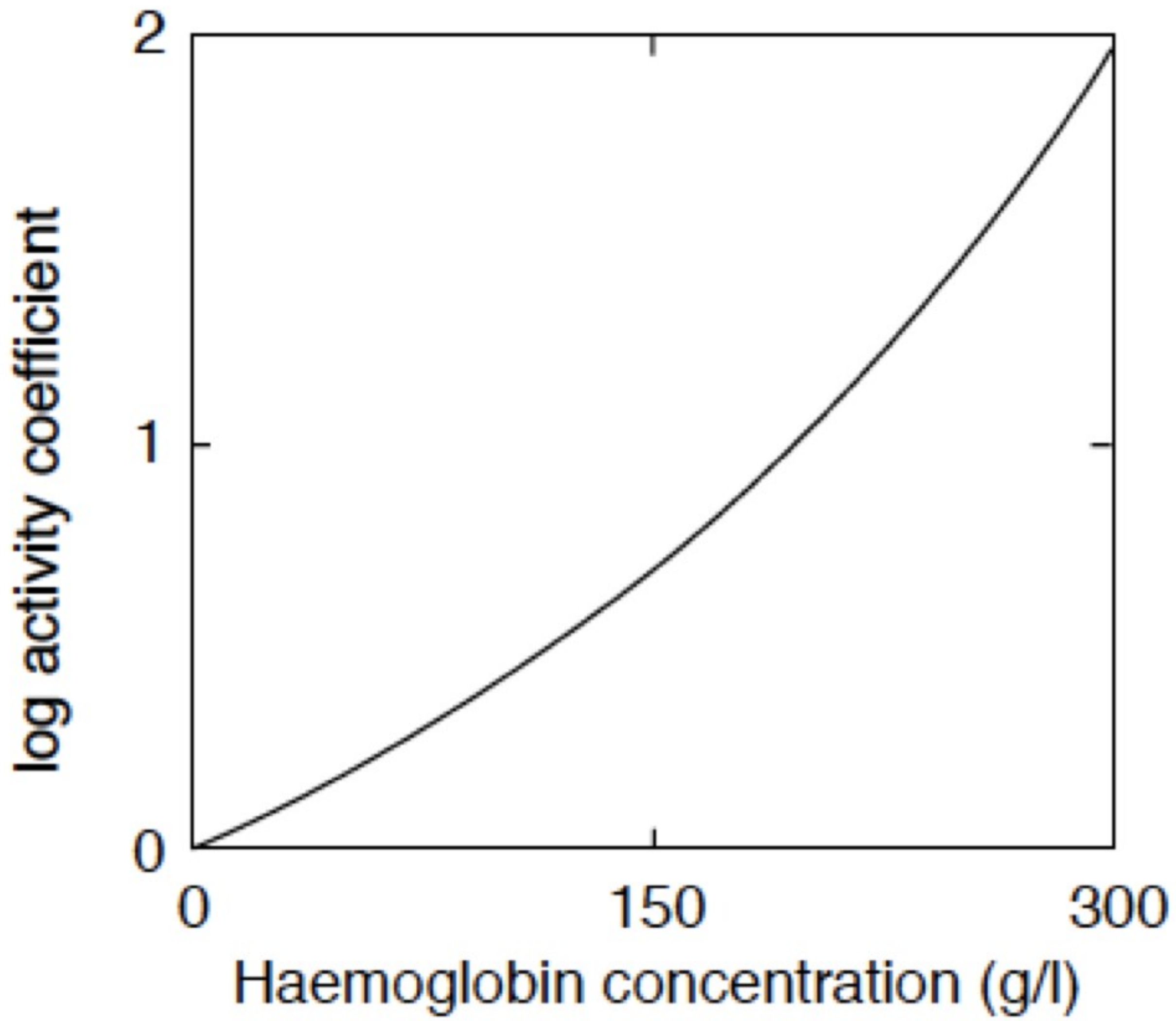
Under non-ideal conditions the concentrations of each species has to be multiplied by a correction factor - the activity constant $\boldsymbol{a}$

$$K_d = \frac{a_A{}^n \bullet [A]^n}{a_{An} \bullet [A_n]}$$

Effects of crowding: Chemical equilibria

Activity coefficient of a species depends on molecular size & total solute density

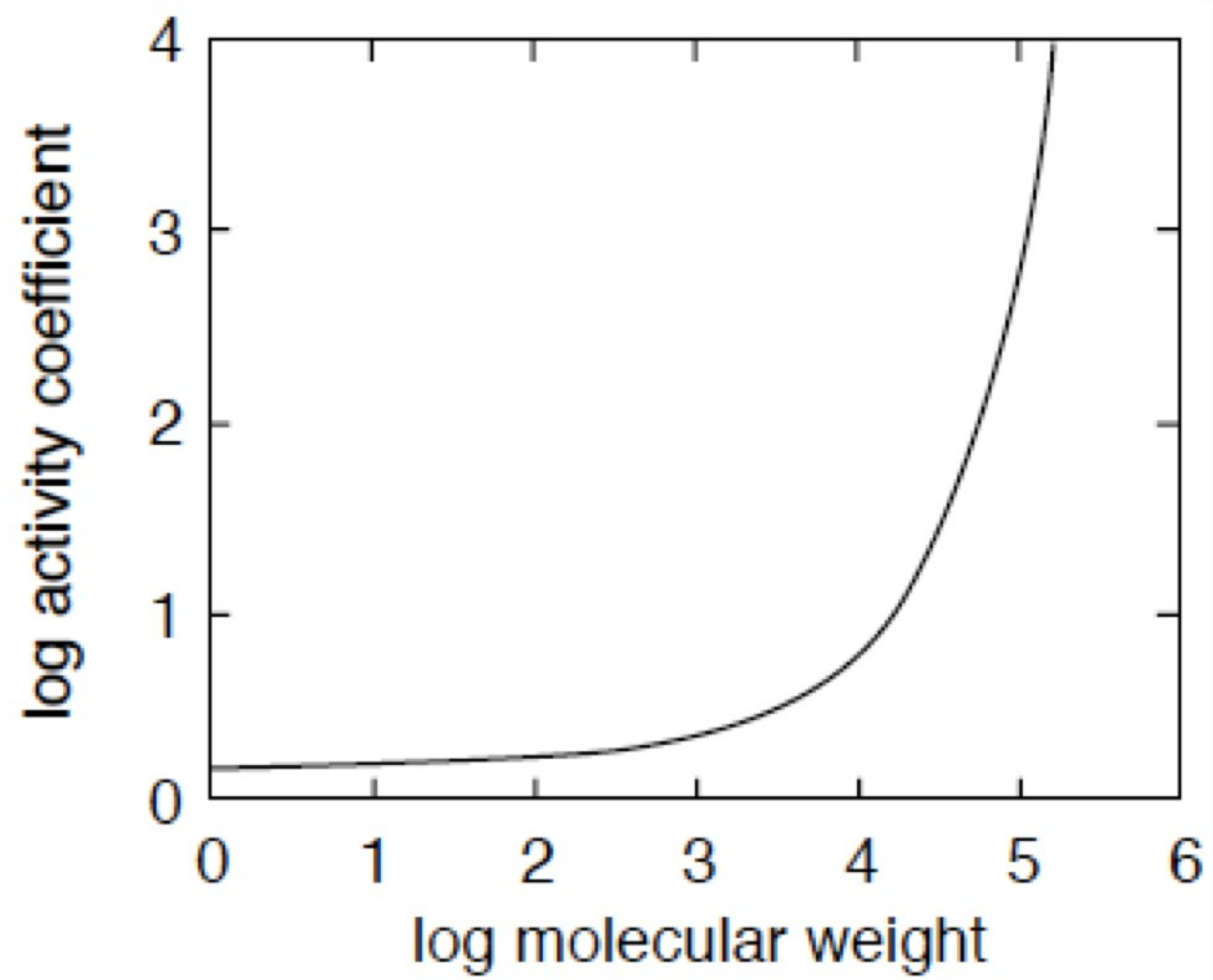
- Haemoglobin in water



at low conc. $a = 1$

in blood cell $a \approx 100$

- Molecules dissolve in a solution of haemoglobin of 300 g/l



at low MW $a = 1$

at high MW a explodes

R.J. Ellis, Curr Op Struc Biol 11 (2001) p.114

Effects of crowding: Chemical kinetics

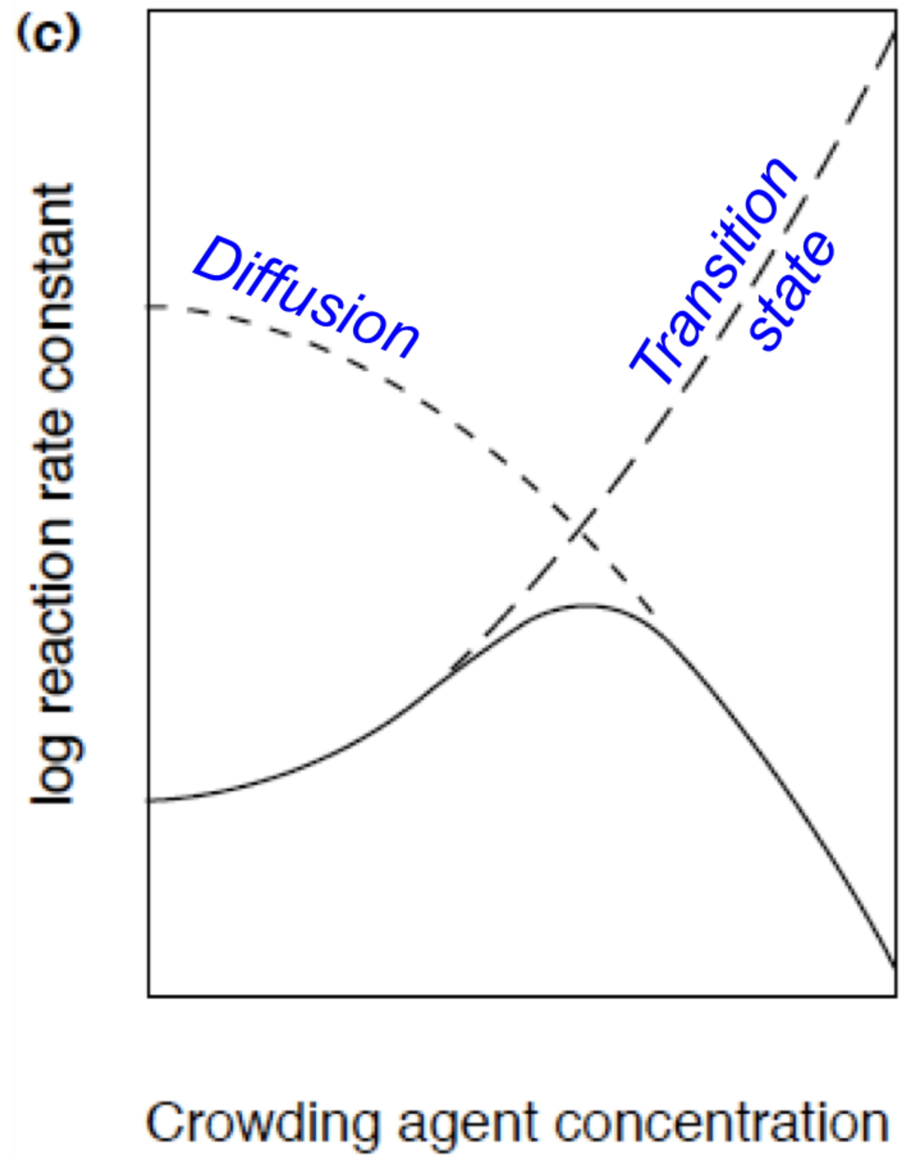
For a reaction to happen 2 important steps have to happen:

i) molecules have to meet,

&

ii) stay together for reaction to happen, passing the transition state

i) and *ii)* depend inversely on diffusion:

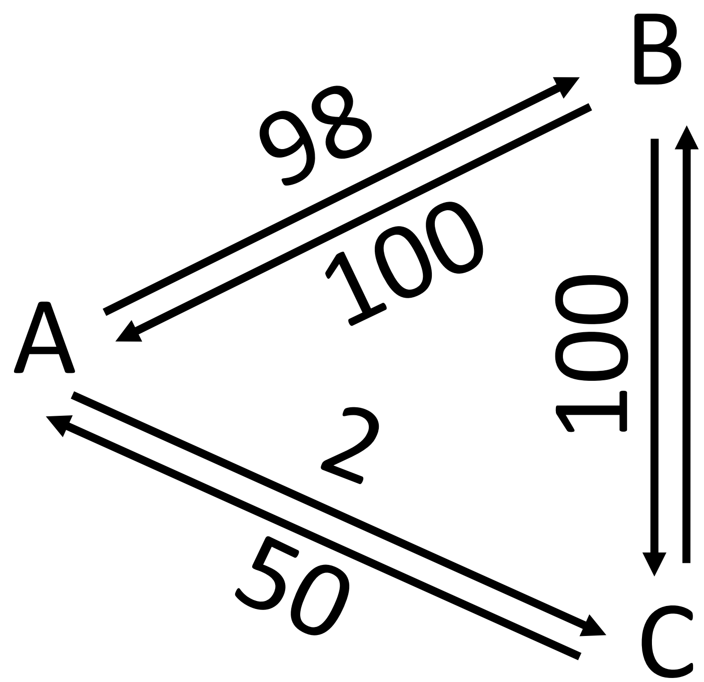


R.J. Ellis, Curr Op Struc Biol 11 (2001) p.114

Chemical reactions

$$A \underset{k_-}{\overset{k_+}{\rightleftharpoons}} B$$

- The rate constants are s^{-1}



Waterfall, M.C. Escher 1961

Further reading:

"Biochemistry" by Stryer e.a.

"The cell" by Alberts e.a.

Minton JBC 2001 : Macromolecular crowing

SnapShot Cell 2010 : Key numbers in biology

Next week(s):

Receptor-ligand interaction

Ligand-gated ion channels