Physical Biology of the Living Cell I.

Miklós Kellermayer

Topics - semester I

Qualitative and quantitative modelling in biology (Dr. Miklós Kellermayer)	Mar. 7
Microscopy studies of intracellular structures (Dr. Miklós Kellermayer)	Mar. 14
Formation of biological structures (Dr. Szabolcs Osváth)	Mar. 21
Dynamic intracellular protein structures (Dr. Miklós Kellermayer)	Mar. 28
Super-resolution microscopy (Dr. Szabolcs Osváth)	Apr. 4
Structural hierarchy of proteins (Dr. Schay Gusztáv)	Apr. 11
Stability of biological structures (Dr. Schay Gusztáv)	Apr. 18
Experimental methods to study biological structures - I (Dr. Schay Gusztáv)	Apr. 25
Visit to the research laboratories of the Dept. of Biophysics and Radiation biology of Semmelweis University (Dr. Szabolcs Osváth)	May 2
Experimental methods to study biological structures - II (Dr. Miklós Kellermayer)	May 9
Single molecule biological activity (Dr. Miklós Kellermayer)	May 16
Problem solving and consultation (Dr. Szabolcs Osváth)	May 23

Physical biology

- Today not only qualitative observations, but quantitative measurements are made (biological data → quantitative data).
- From quantitative data, quantitative models are built.
- Quantitative models are expected to provide with experimentally testable predictions.

"Make things as simple as possible, but not simpler."
Albert Einstein

Premises of model building

- What facts are available?
  a. Facts observable by anyone
  (e.g., the cell contains proteins)
  b. Facts accepted after extensive experimental testing
  (e.g., proteins are synthesized on the ribosome)
  c. Speculative statements
  (e.g., mitochondria are descendents of ancient bacteria)
- Is the problem interesting or important?
- Biological entities must not violate the laws of physics and chemistry.

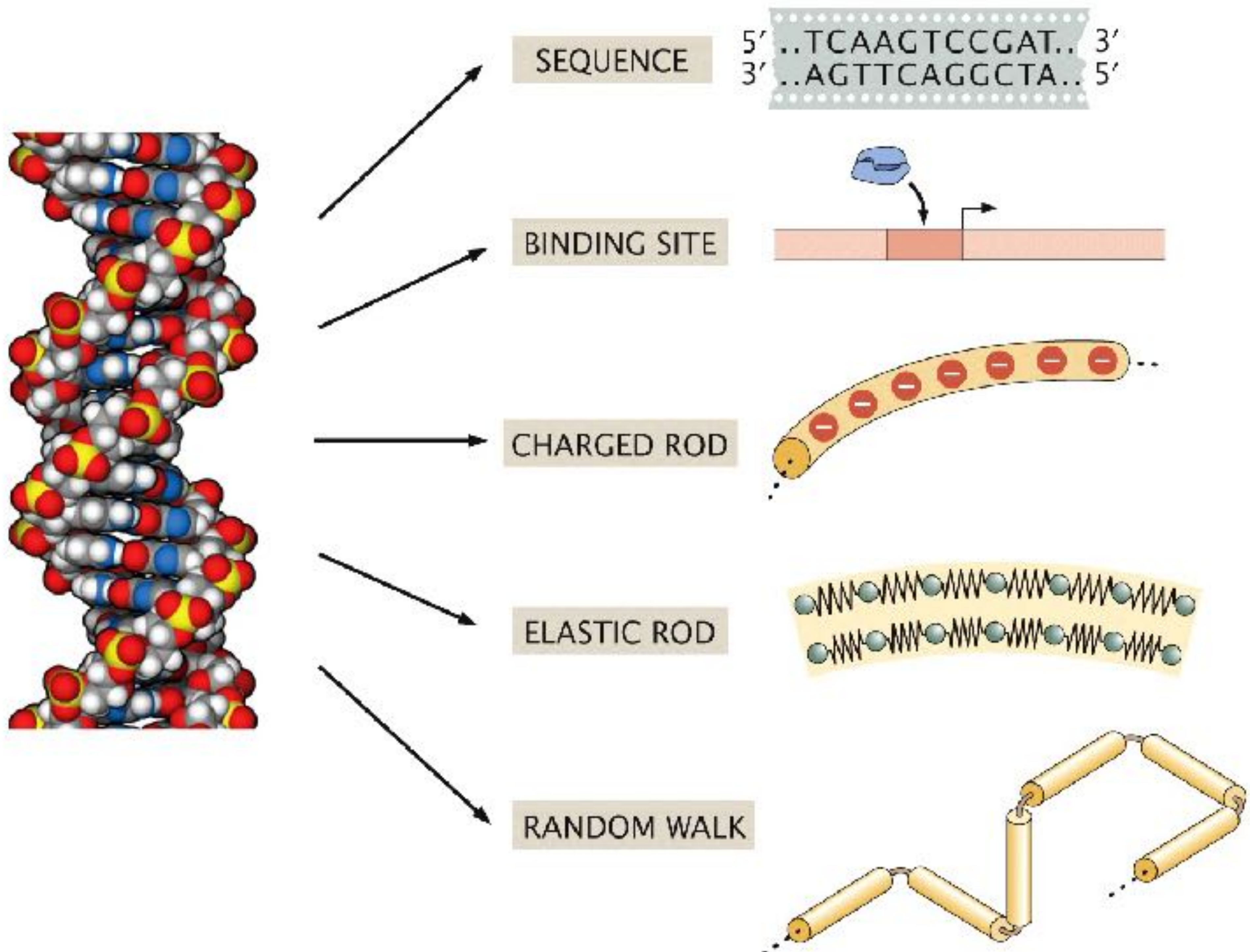
Why is life alive?

- Life is described through a collection of qualitative approximations
  -e.g., growth, energy utilization/transformation, reproduction
- The living cell is built of surprisingly few elements
- The cell contains structurally and functionally specialized macromolecules
  -proteins, nucleic acids, carbohydrates, lipids
  -macromolecules are formed by a combinatorial assembly of units
  -macromolecules encode information (in different "languages")

Biological model building

- Abstraction
- Simplification
- We cannot attain a complete atomic description of the macromolecules
- Projections are made, which reflect a certain property of the macromolecule
- Idealization

Idealization of the DNA molecule



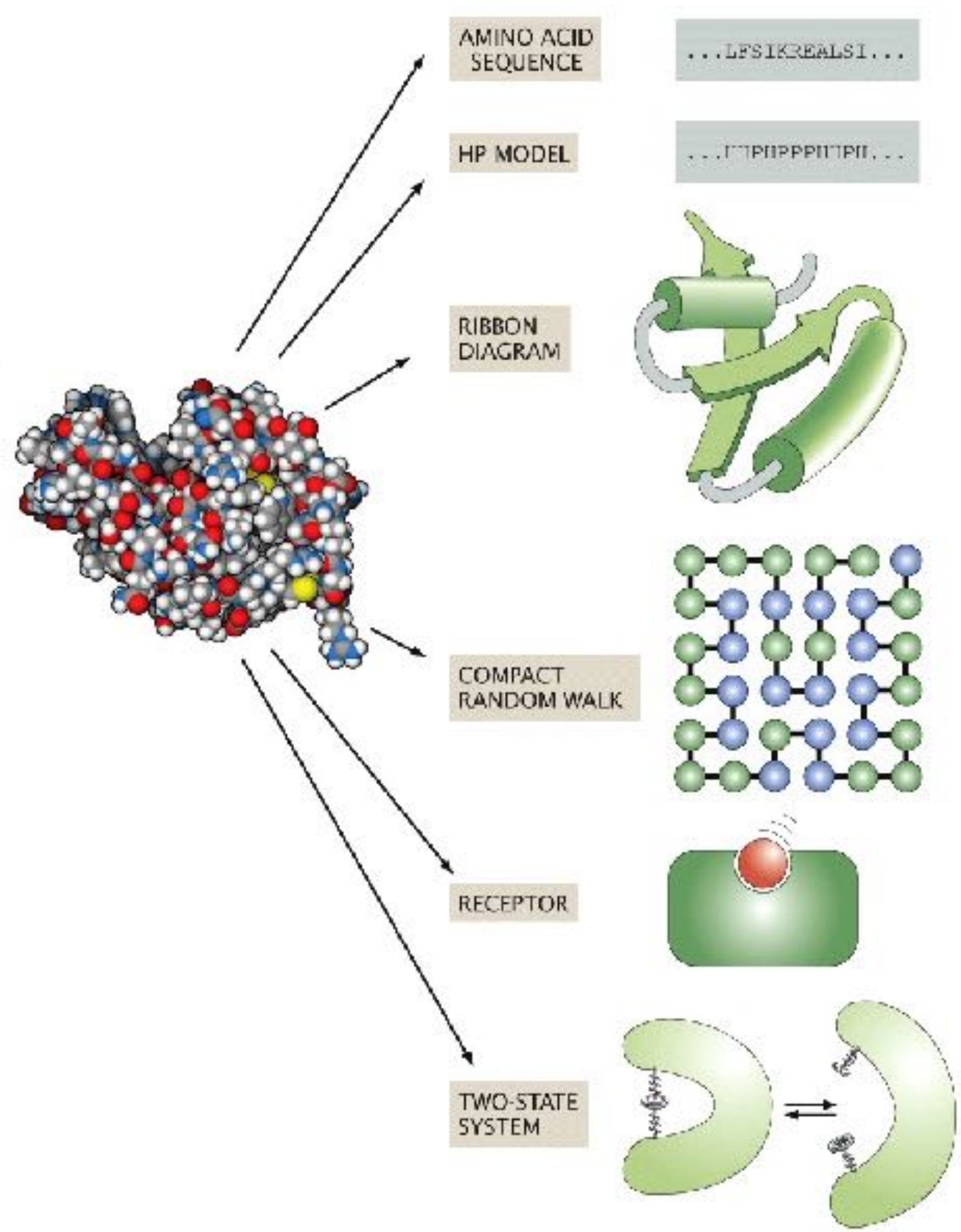
4 different bases

Ligand affinity varies along DNA

Axial and bending elasticity (rigidity)

Entropic elasticity, shape fluctuations

Idealization of a protein molecule



20 different AA

H=hydrophobic
P=polar

-α-helix
-β-chain
-random chain segment
-etc.

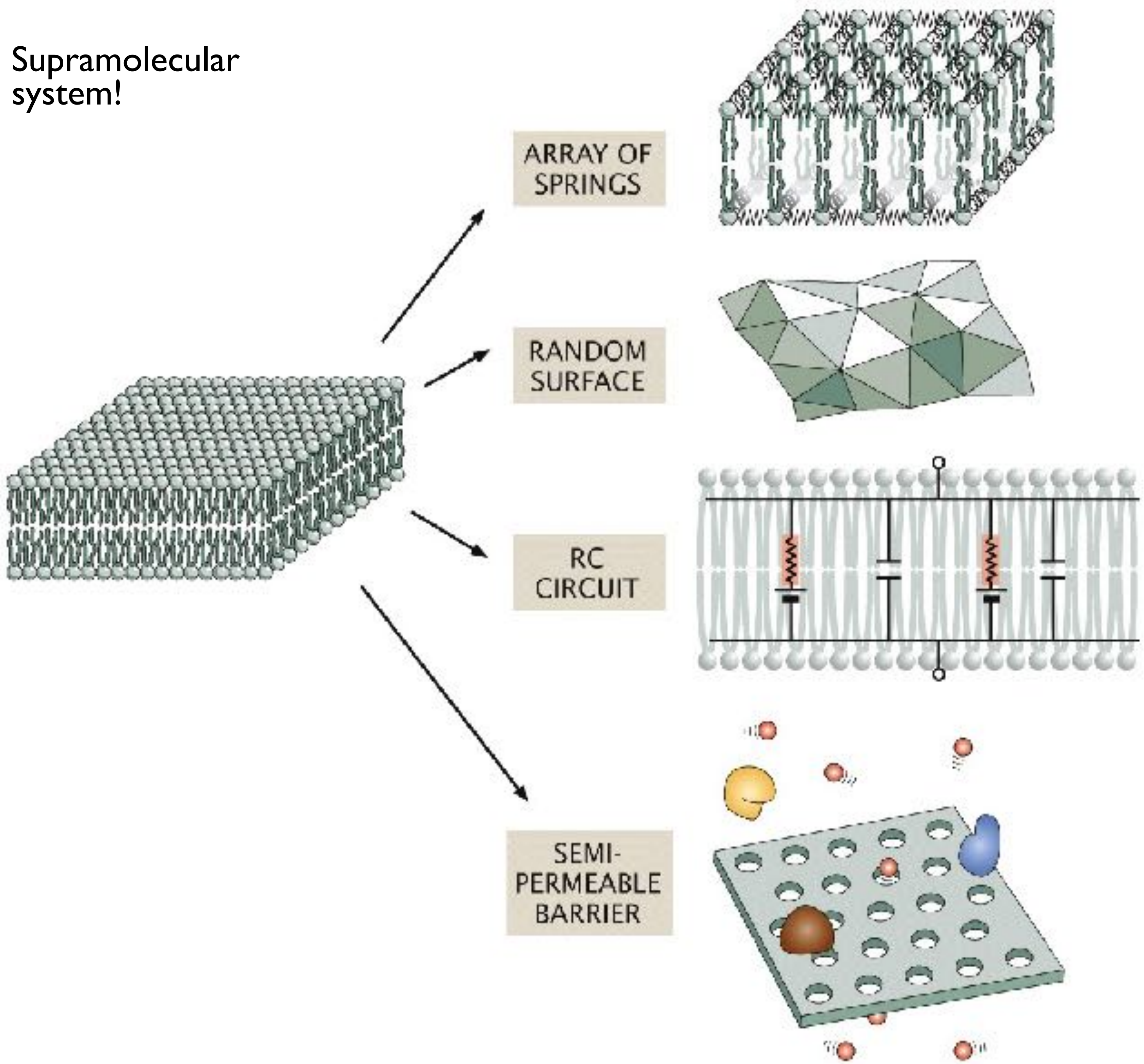
amino acids in the different lattice coordinates

Binding site - ligand

aktive-inactive
bound-dissociated
unfolded-folded
etc.

Idealization of lipids and membranes

Supramolecular system!



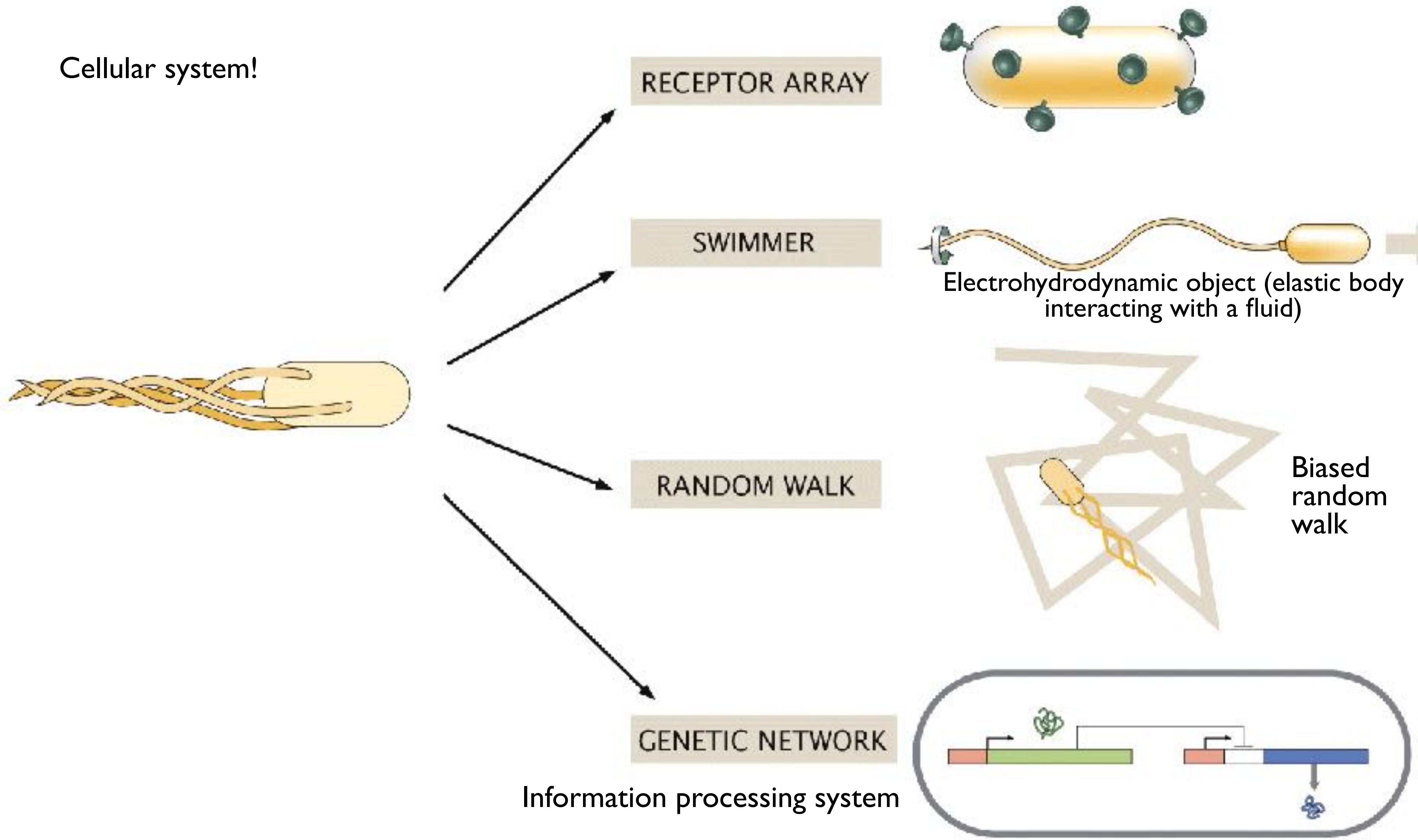
Elasticity

Fluidity

Electrical properties

Diffusion, osmosis, barrier functions

Idealization of an *Escherichia coli* cell

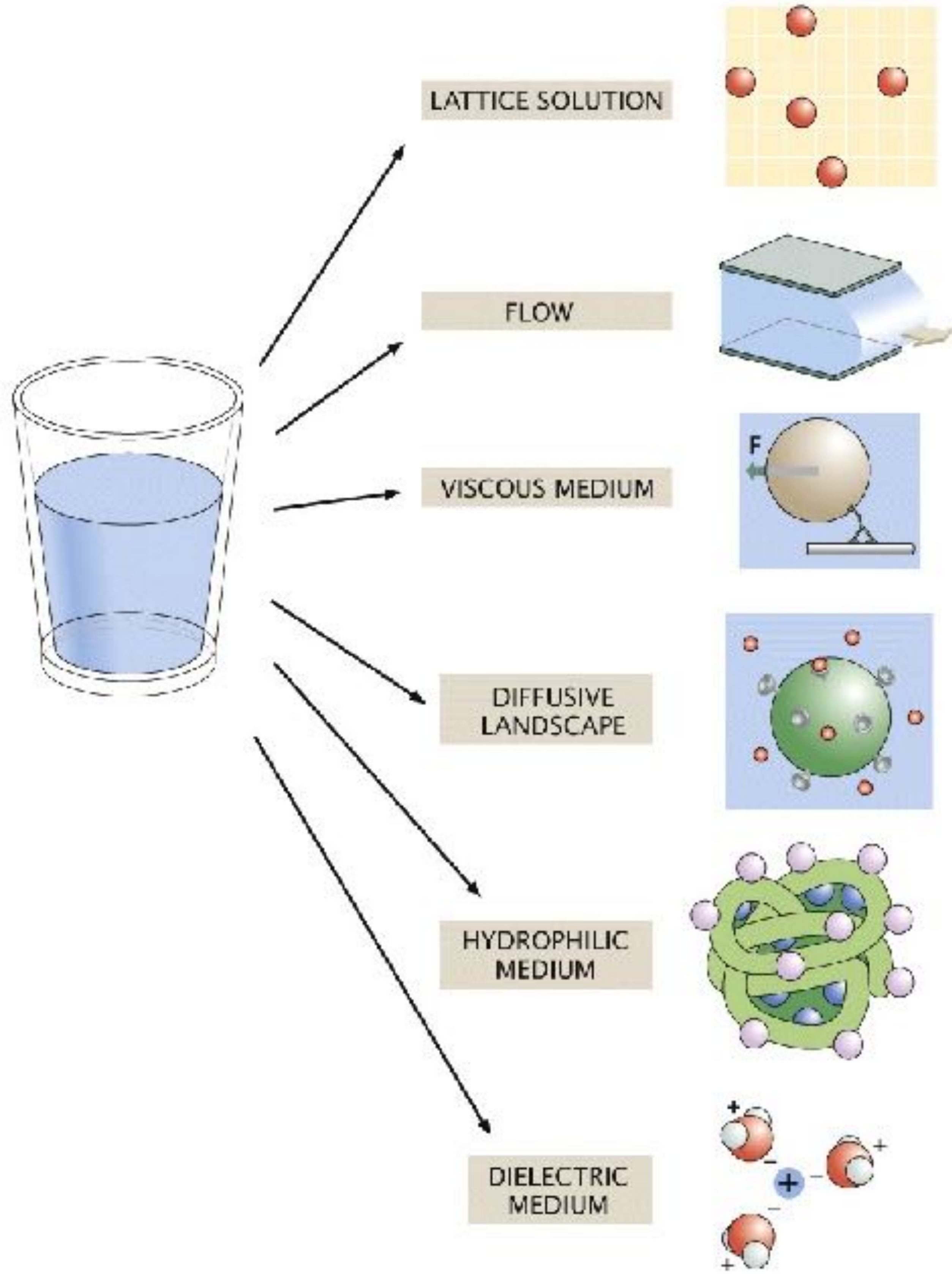


Idealization of a solution

Living cell as an ideal solution(?)

Homogenous

Isotropic



Idealization, expansion and application of the concept of elasticity

Average deviation from equilibrium position:

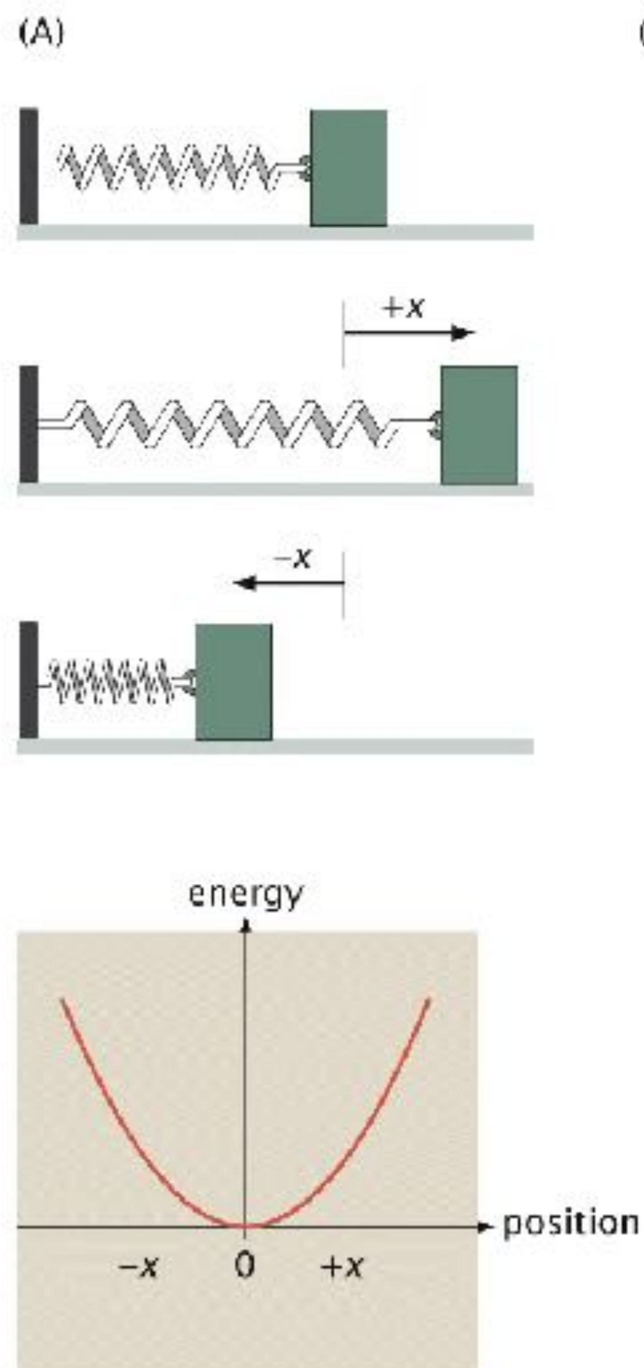
$$Energy = \frac{1}{2}\kappa x^2$$

κ: spring constant - energetic cost of deviating from equilibrium

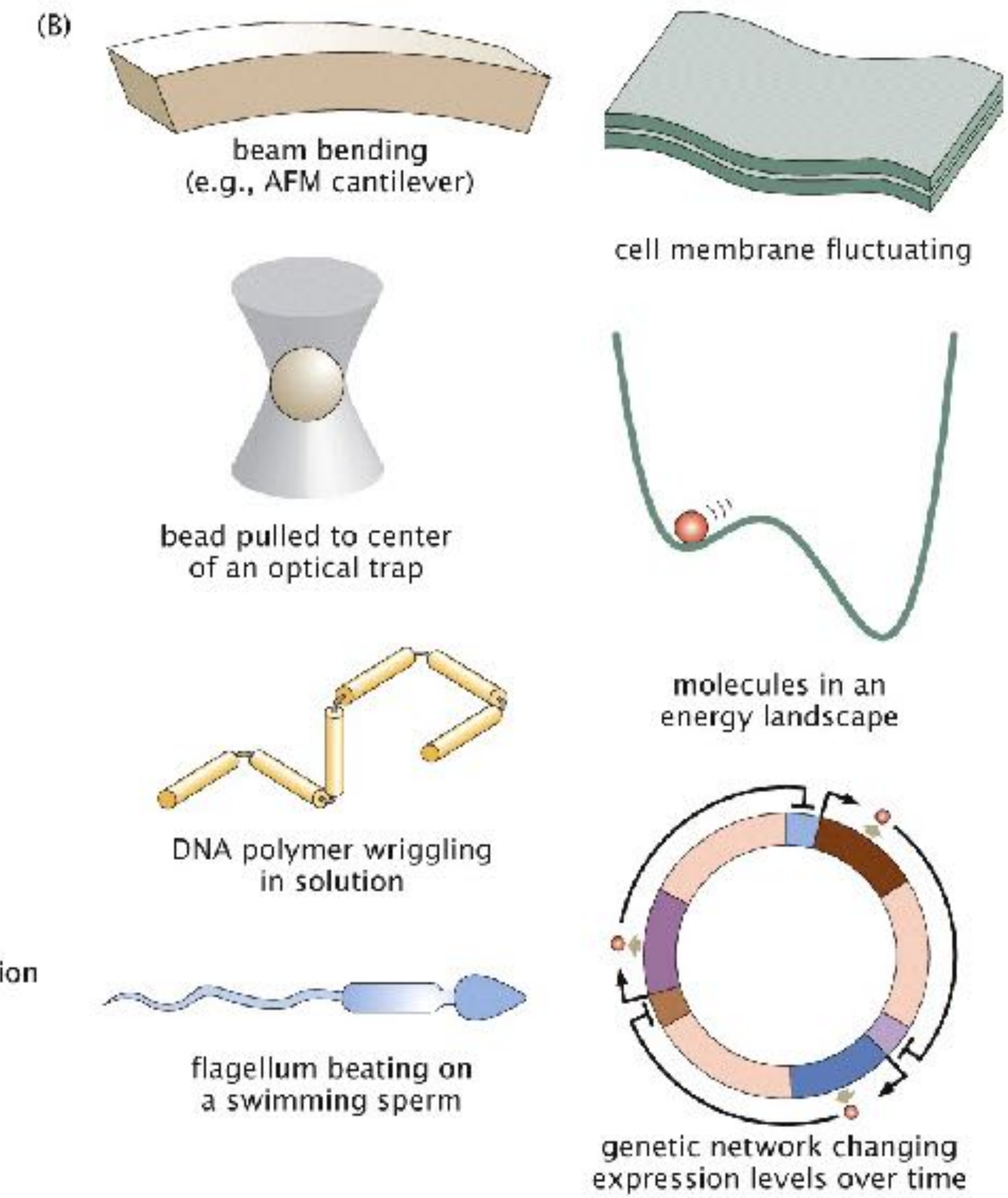
Recovery force:

$$F = -\kappa x$$

Harmonic oscillation



Biological, biophysical examples



Scaling in biology

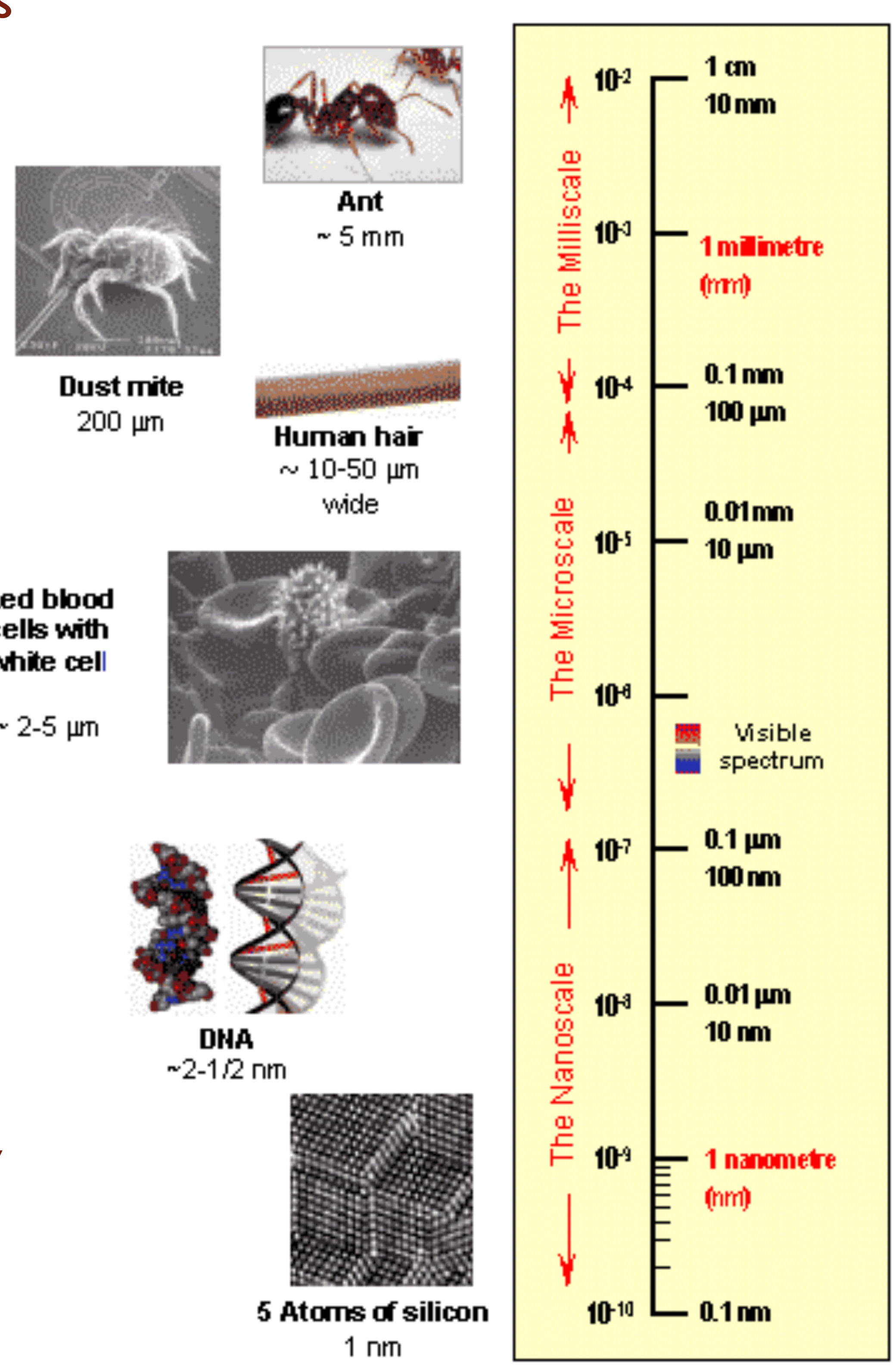
Size of biomolecular systems

Thermodynamics

Mesoscale

Quantum chemistry

Quantum physics



10^{23} Atoms

10^{10} Atoms

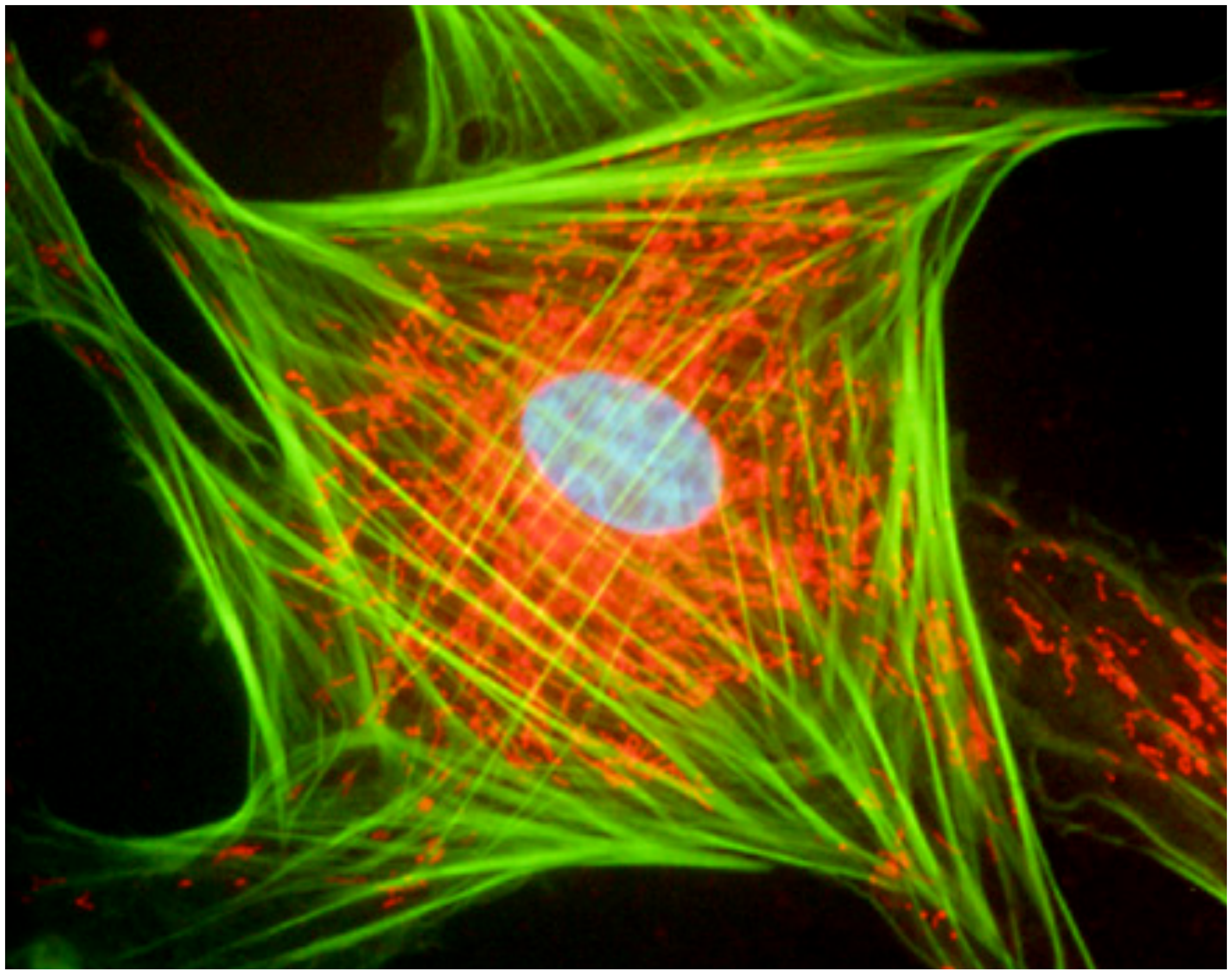
10^{3} Atoms

10^{1} Atoms

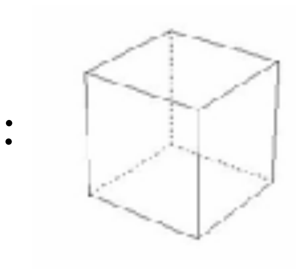
10^{0} Atoms

Length scale of the living cell

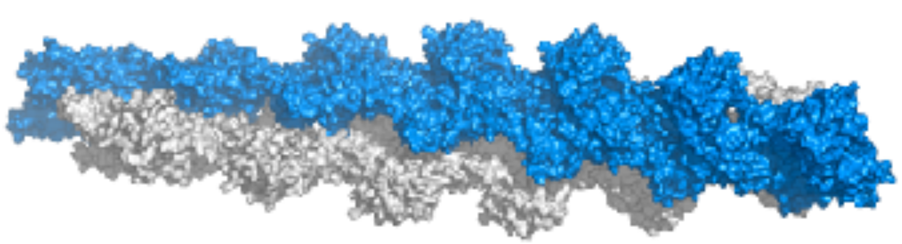
Rudolf Virchow (1855): *"Omnis cellula e cellula"*



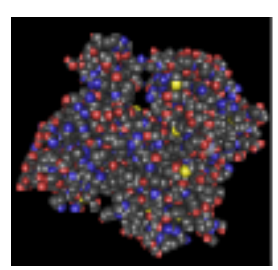
Simplified cell model: cube



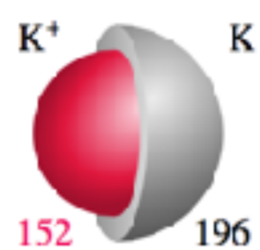
	Cell: cube with 20 μm edge	Analogue - **Lecture hall:** cube with 20 m edge
Size of actin molecule	5 nm	5 mm
Number of actin molecules	~500 million	~500 million
Average distance between actins	~25 nm	~25 mm
Size of potassium ion	0.15 nm	0.15 mm
Number of potassium ions	$\sim 10^9$	$\sim 10^9$
Average distance between K^+ ions	~20 nm	~2 cm



Actin filament (d=7 nm)



G-actin (d=5 nm, cc~100 μM)

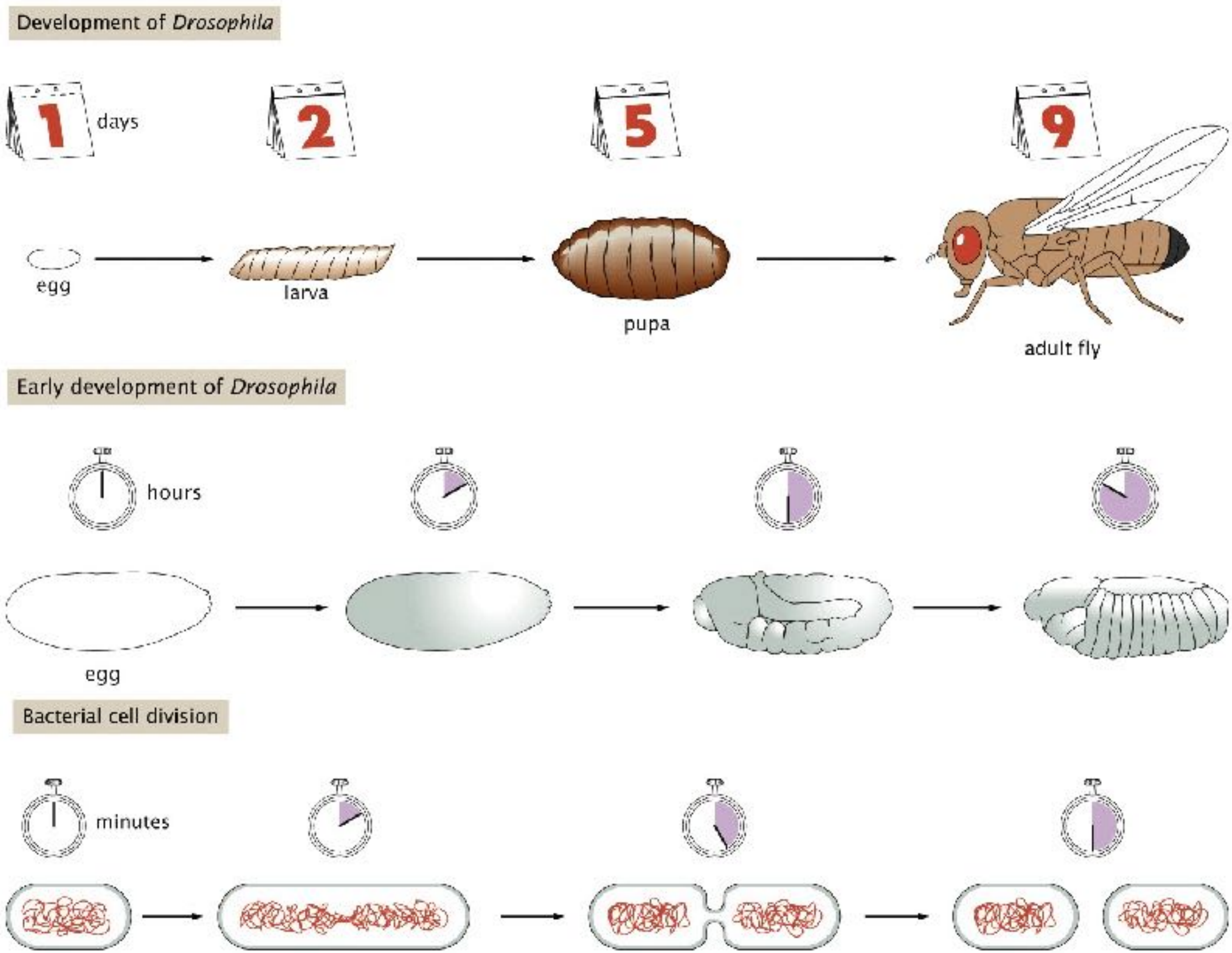


Potassium ion (d=0.15 nm, cc~150 mM)

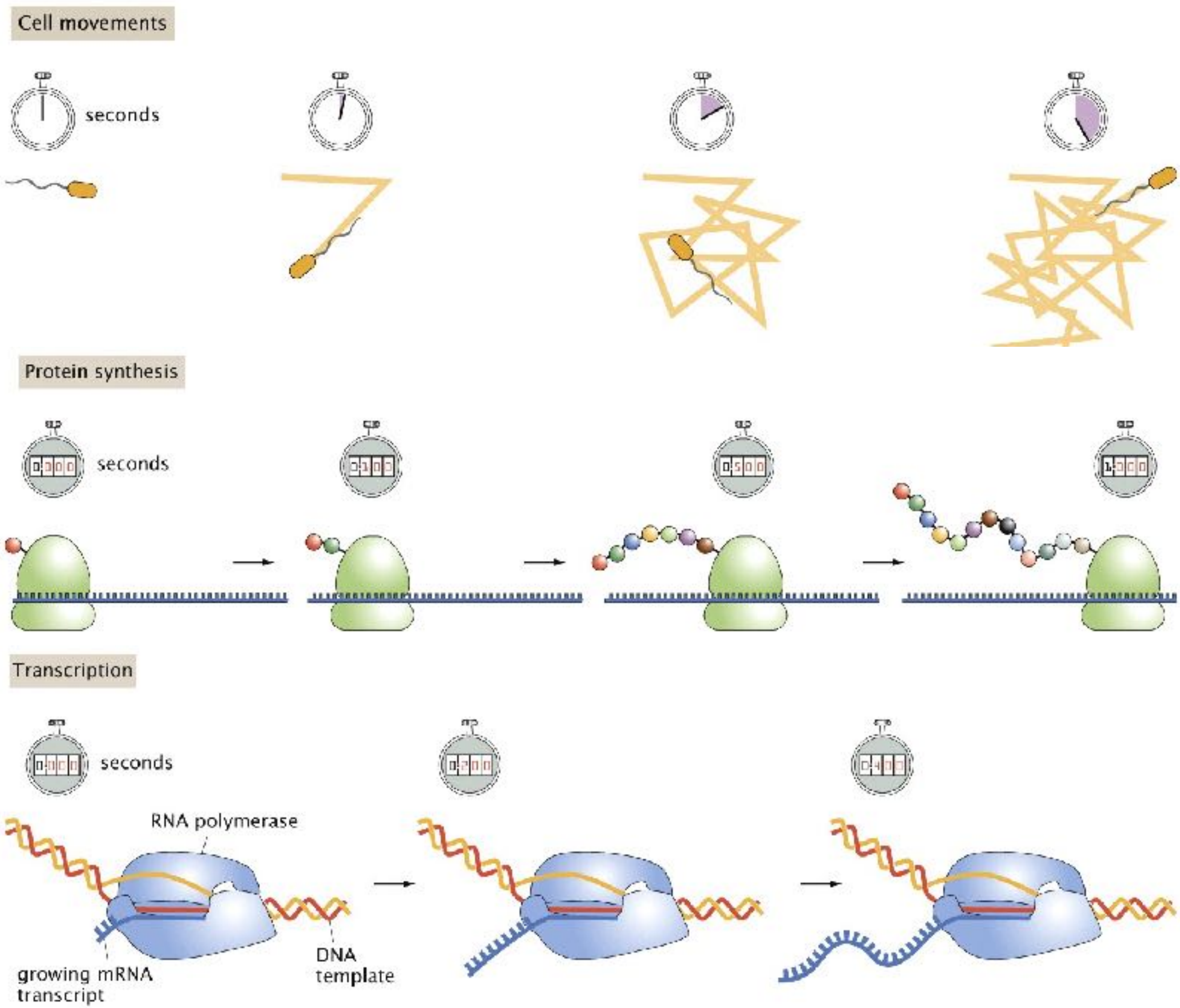
Deficiencies of the model:
- concentrations vary locally
- dynamics: constant motion and collisions
- interactions, many types due to dynamics

Scaling in biology

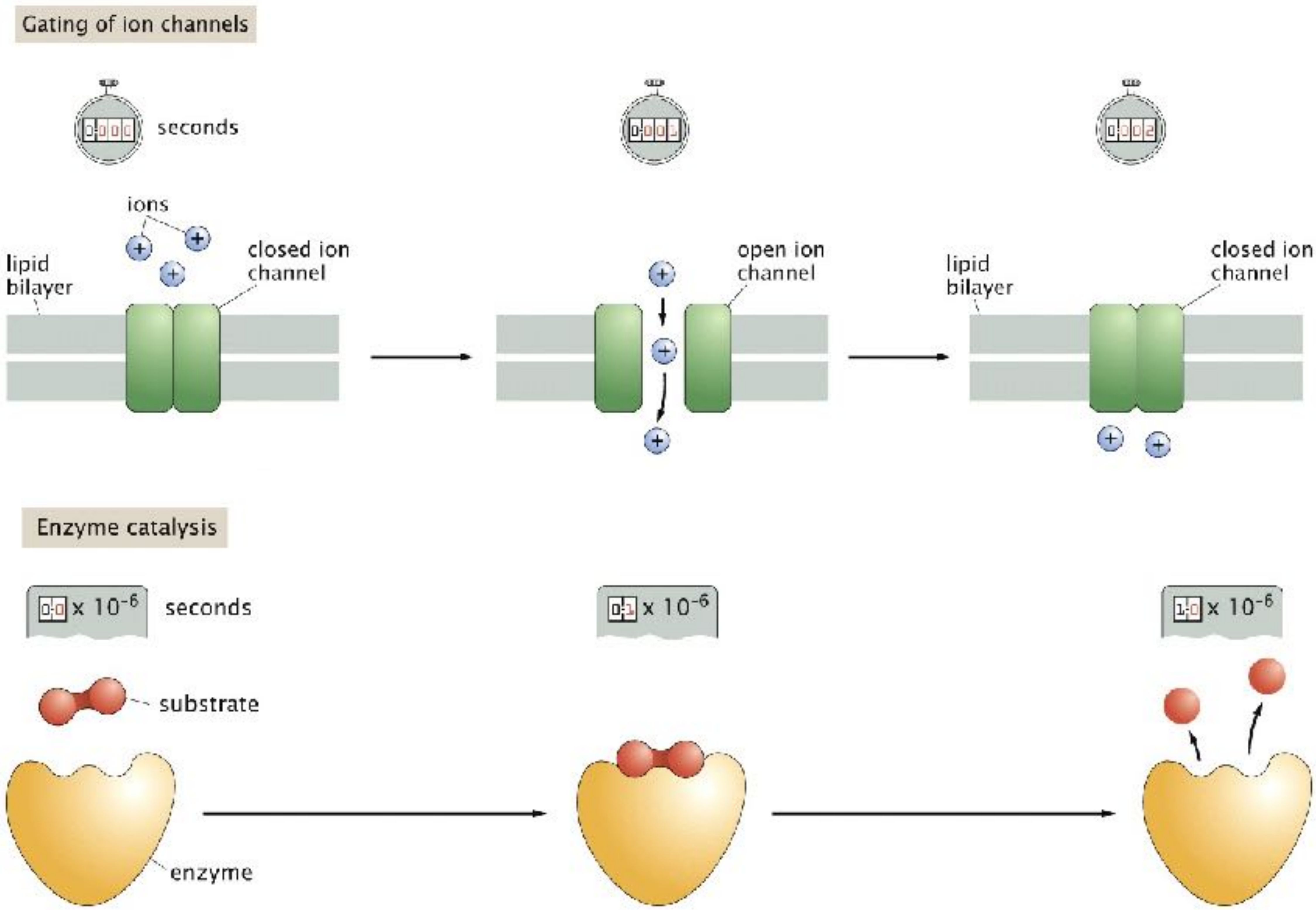
Biological time scale I.



Biological time scale II.



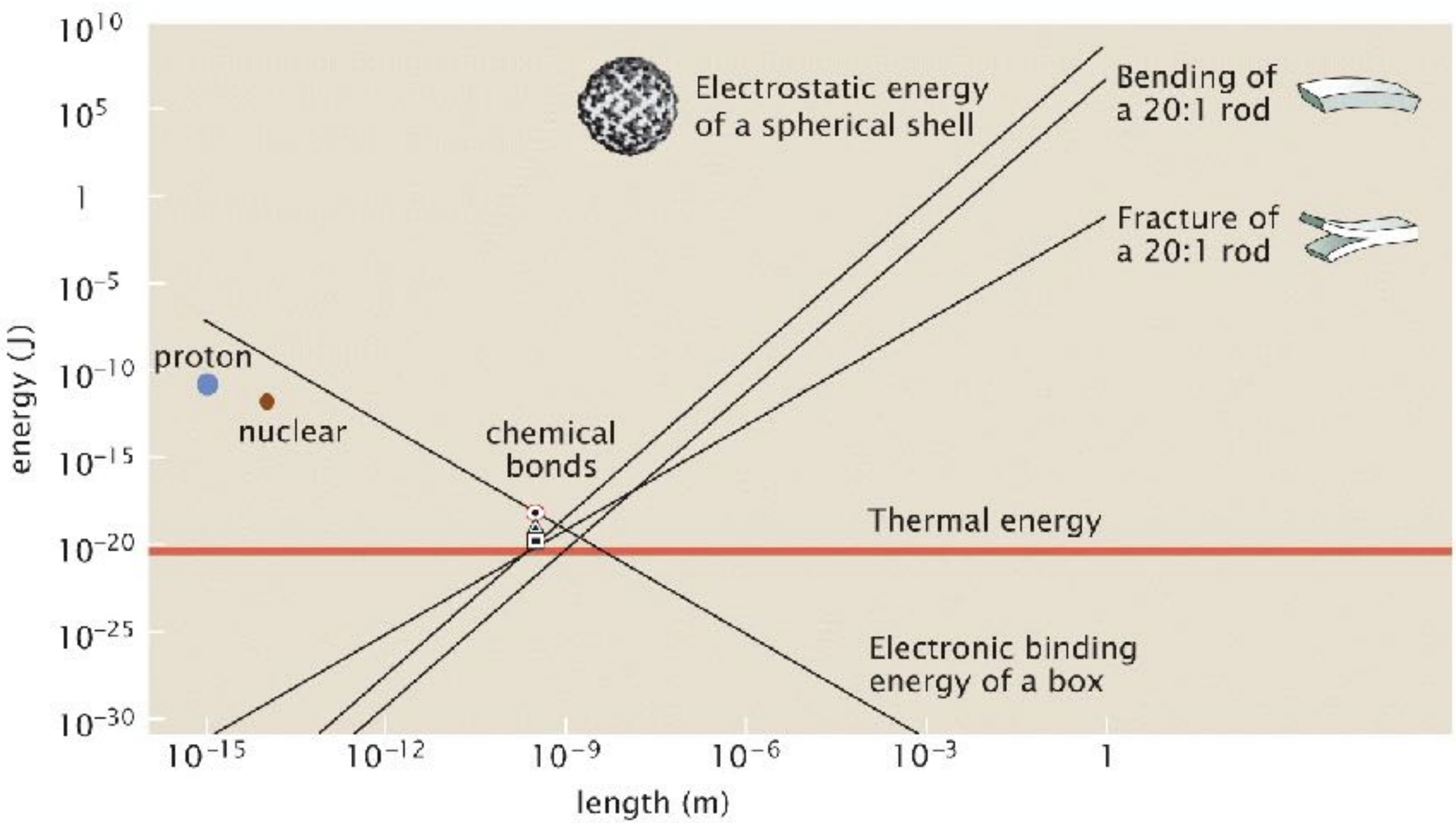
Biological time scale III.



Furthermore, light absorption 10^{-15} s!

Correlation of energy and size scales

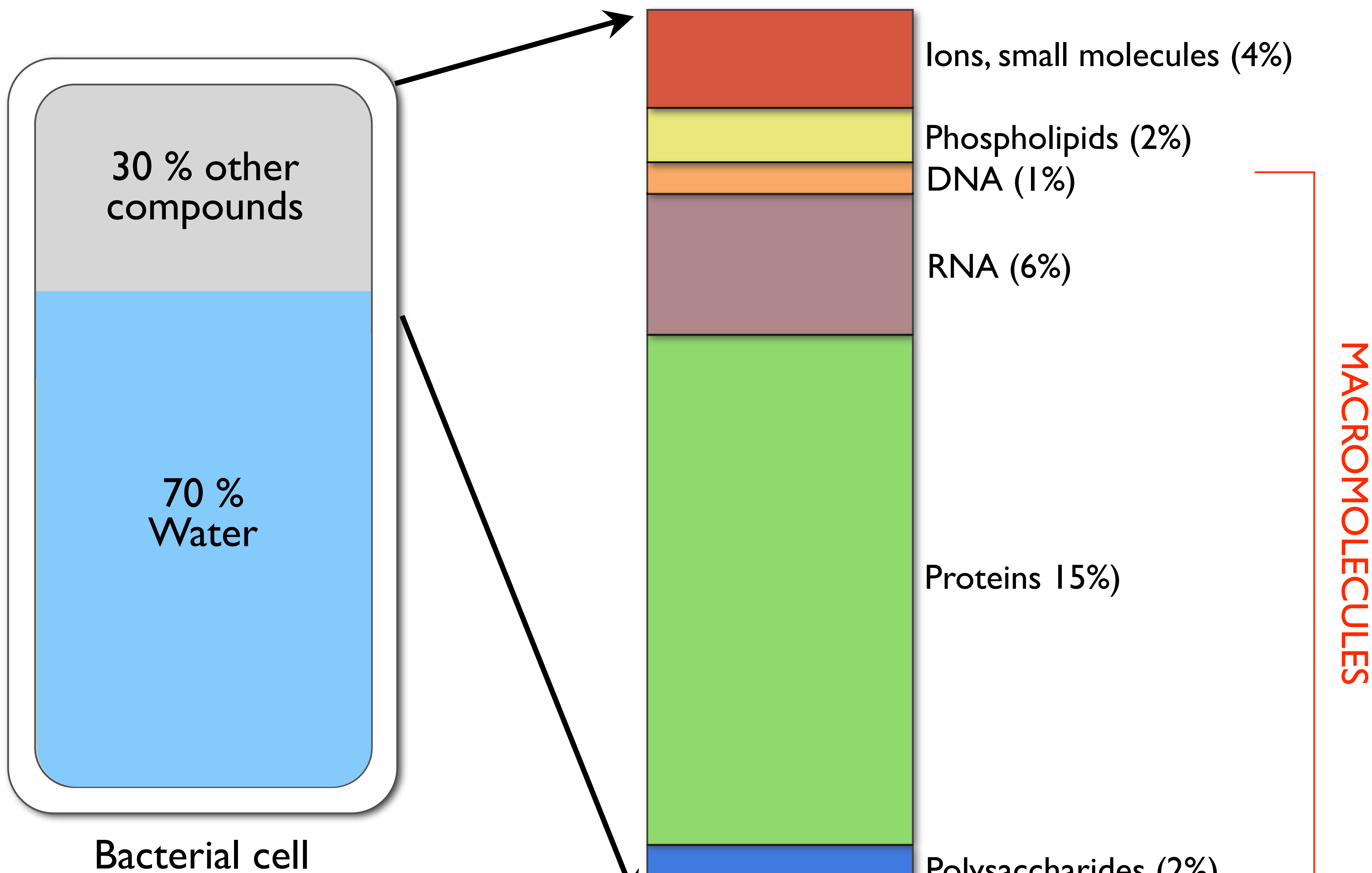
- "Deterministic" (chemical, mechanical, electromagnetic) vs. "thermal" energies
- Thermal energy unit: $k_B T = 4.1 \times 10^{-21}$ J = 4.1 pNnm
- Relevant scaling - Boltzmann factor: $\exp(-E_{det}/k_B T)$
- Thermal energy = $k_B T = 4.1 \times 10^{-21}$ J = 4.1 pNnm = 0.6 kcal/mol = 2.5 kJ/mol (biochemical reactions) = 25 meV (charge transfer)



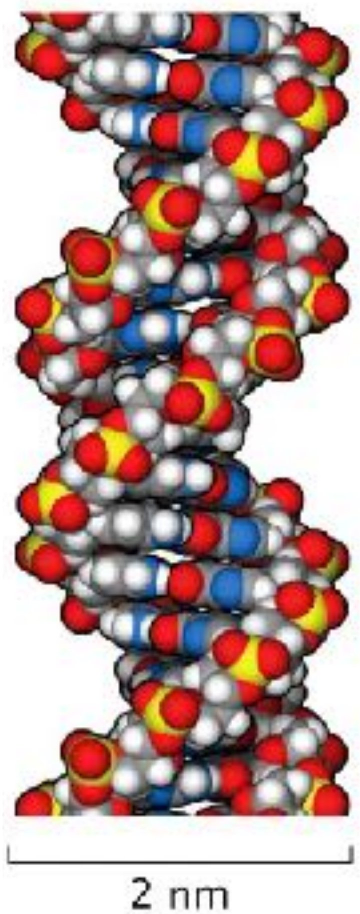
Some rules of thumb in quantitative biology

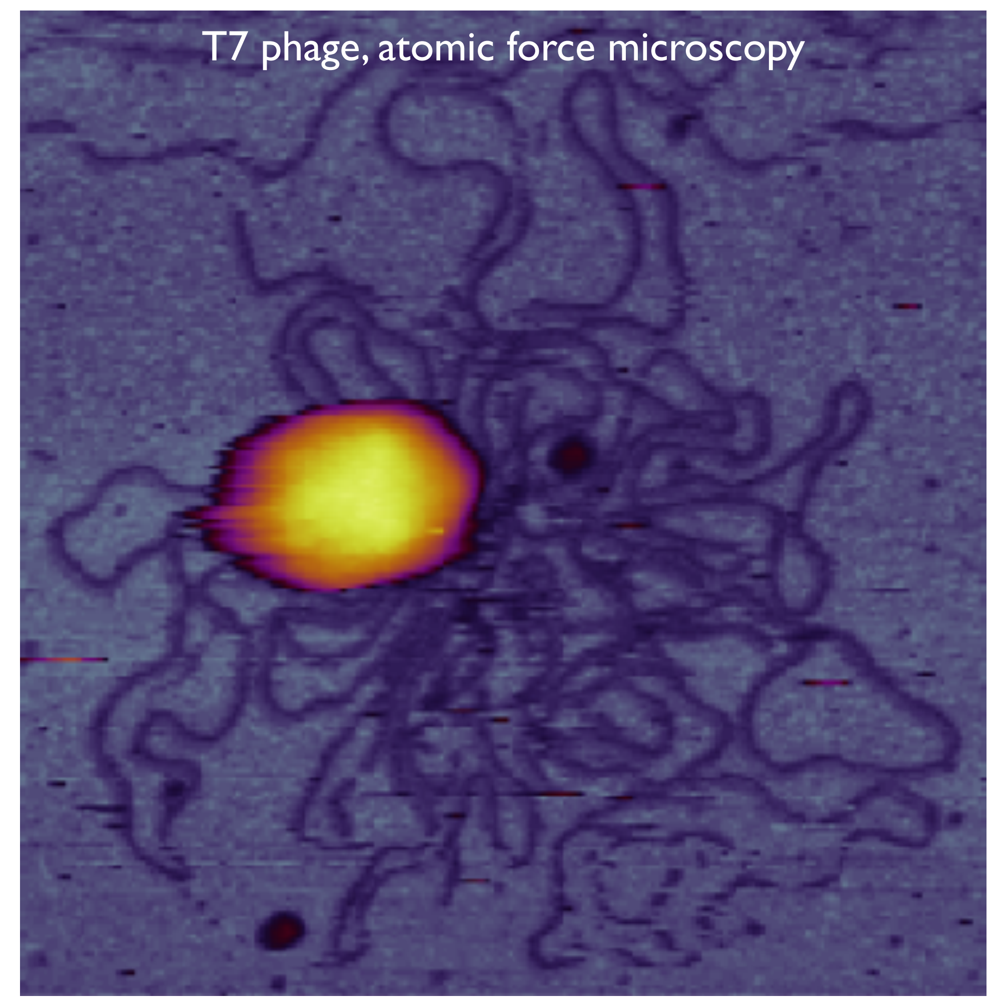
- 1 dalton (Da) = 1g/mol ≈ 1.6×10^{-24} g
- 1 nM ≈ 1 molecule/bacterium ≈10^3-10^4 molecule/eukaryotic cell
- 1 M ≈ 1/nm^3
- Cellular protein concentration ≈ 2-4 million/μm^3
- 1 mg of 1 kb DNA fragment ≈ 1 pmol ≈ 10^{12} molecules
- Mean distance between molecules at 1 M concentration ≈ 1 nm
- Molecular mass of a typical amino acid ≈ 100 Da
- Water concentration/density ≈ 55 M ≈ 1000 kg/m^3
- Volume of a water molecule ≈ 0.03 nm^3
- Length of a base pair (along DNA) ≈ 0.3 nm
- Volume of a base pair ≈ 1 nm^3

Proportion of macromolecules in the cell by mass is large



Biological macromolecules are giant molecules

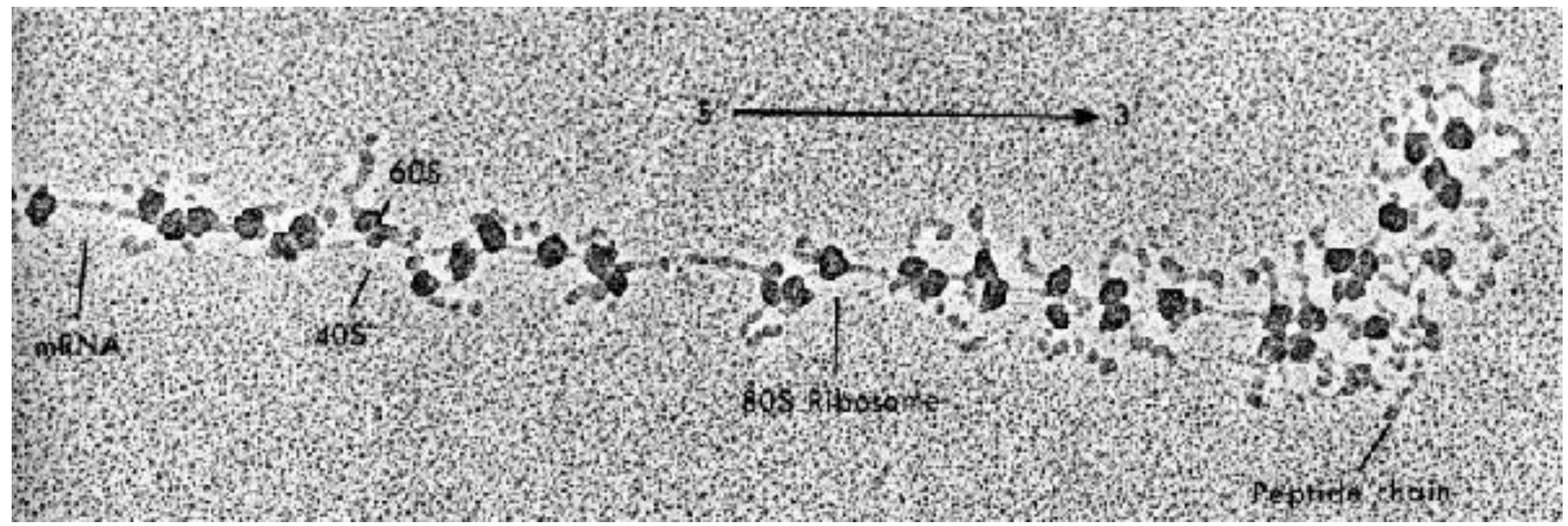




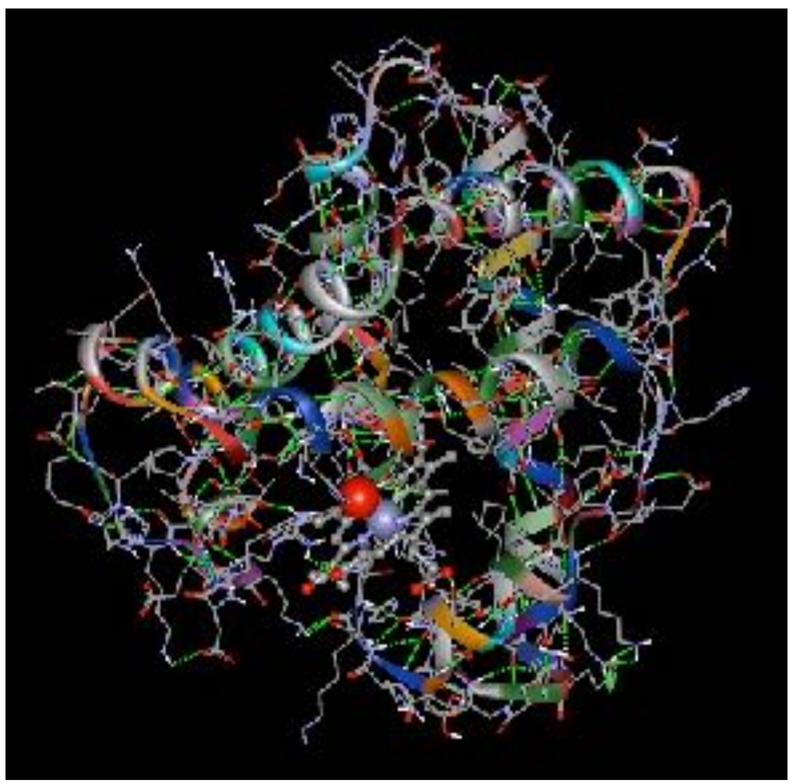
DNA double helix

DNA released from bacteriophage head

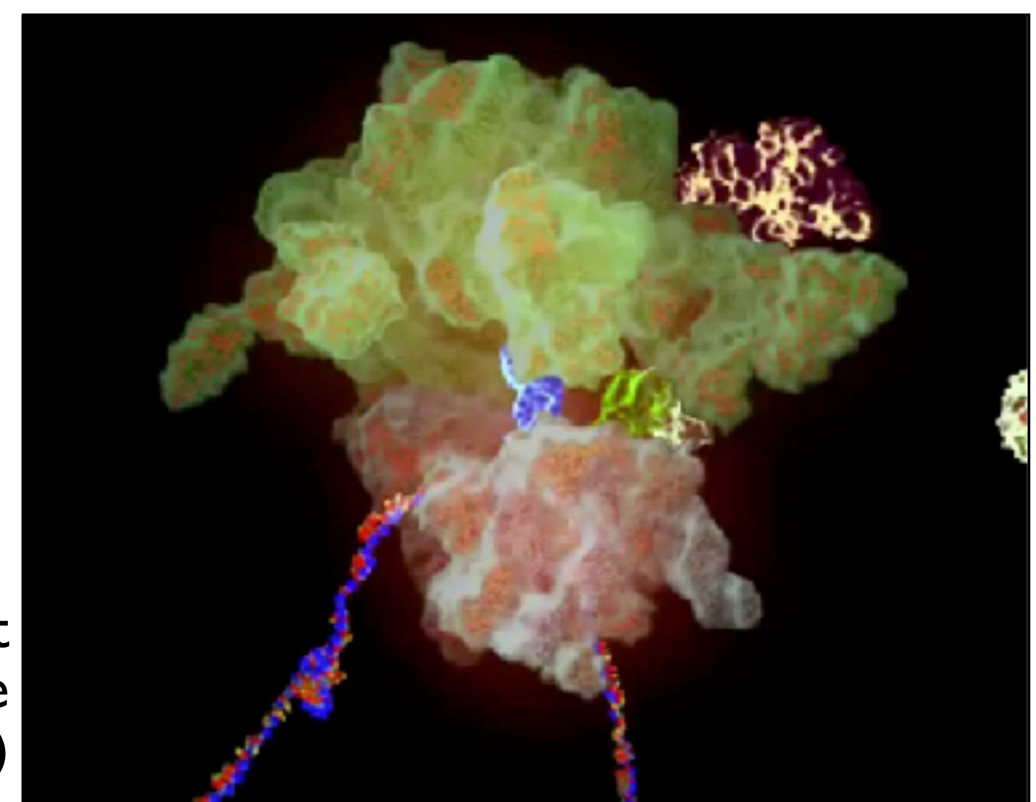
Biological macromolecules are exciting molecules



Newly synthesized protein (silk fibroin)



Structure of hemoglobin subunit



Folding of nascent protein (on the ribosome)

Biological macromolecules: biopolyers

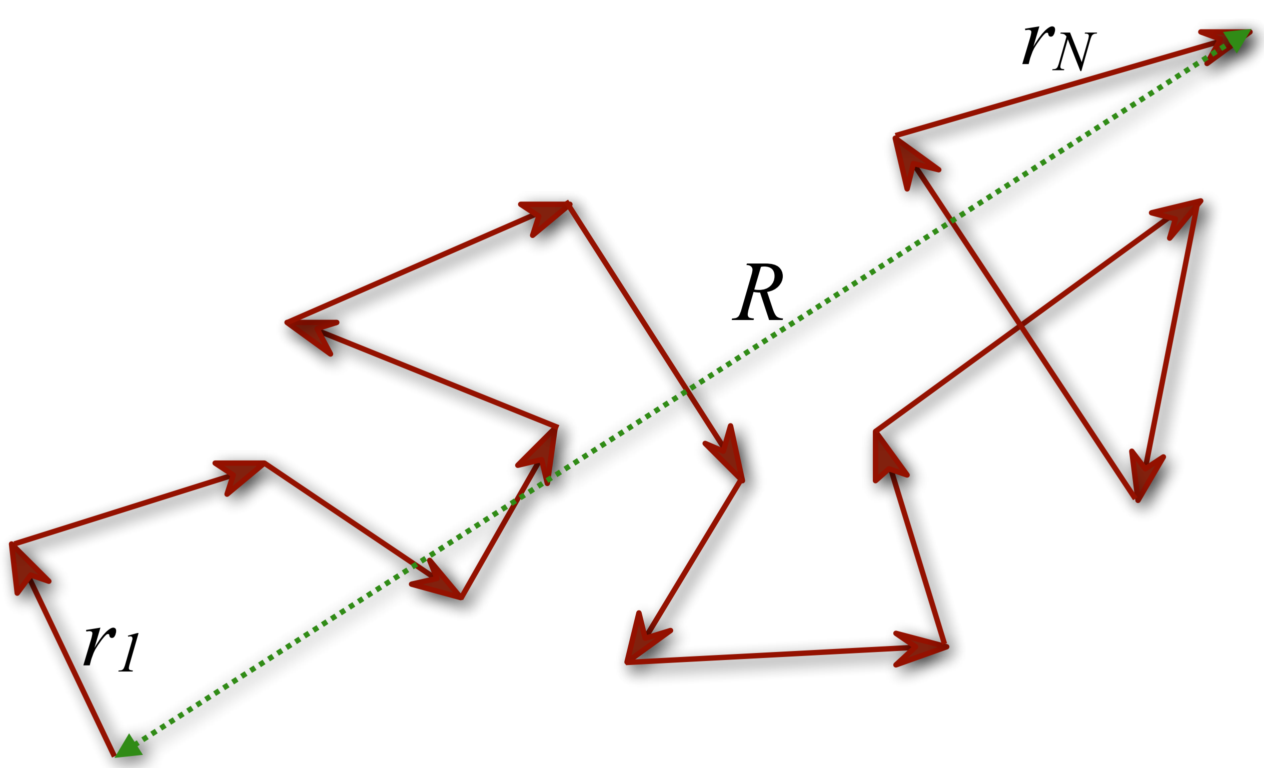
Polymers:
chains built up from monomers

Number of monomers: N>>1;
Typically, $N \sim 10^2 - 10^4$,
but, in DNA, e.g.: $N \sim 10^9 - 10^{10}$

Biopolymer	**Monomer**	**Bond**
Protein	Amino acid	Covalent (peptide bond)
Nucleic acid (RNA, DNA)	Nucleotide (CTUGA)	Covalent (phosphodiester)
Polysaccharide (e.g., glycogen)	Sugar (e.g., glucose)	Covalent (e.g., α-glycosidic)
Protein polymer (e.g., microtubule)	Protein (e.g., tubulin)	Secondary

Shape of the polymer chain resembles random walk

Brownian-movement - "random walk"



"Square-root law":

$$\langle R^2 \rangle = Nl^2 = Ll$$

R = end-to-end distance

N = number of elementary vectors

$l = |\vec{r}_i| =$ correlation length

r_i = elementary vector

$Nl = L$ = contour length

l is related to **bending rigidity**.

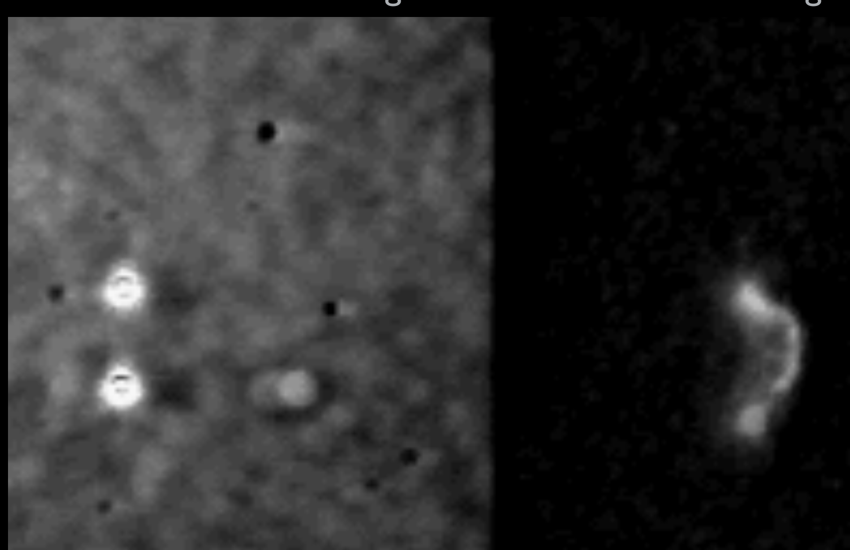
In case of Brownian-movement R = displacement, N = number of elementary steps, L = total path length, and l = mean free path length.

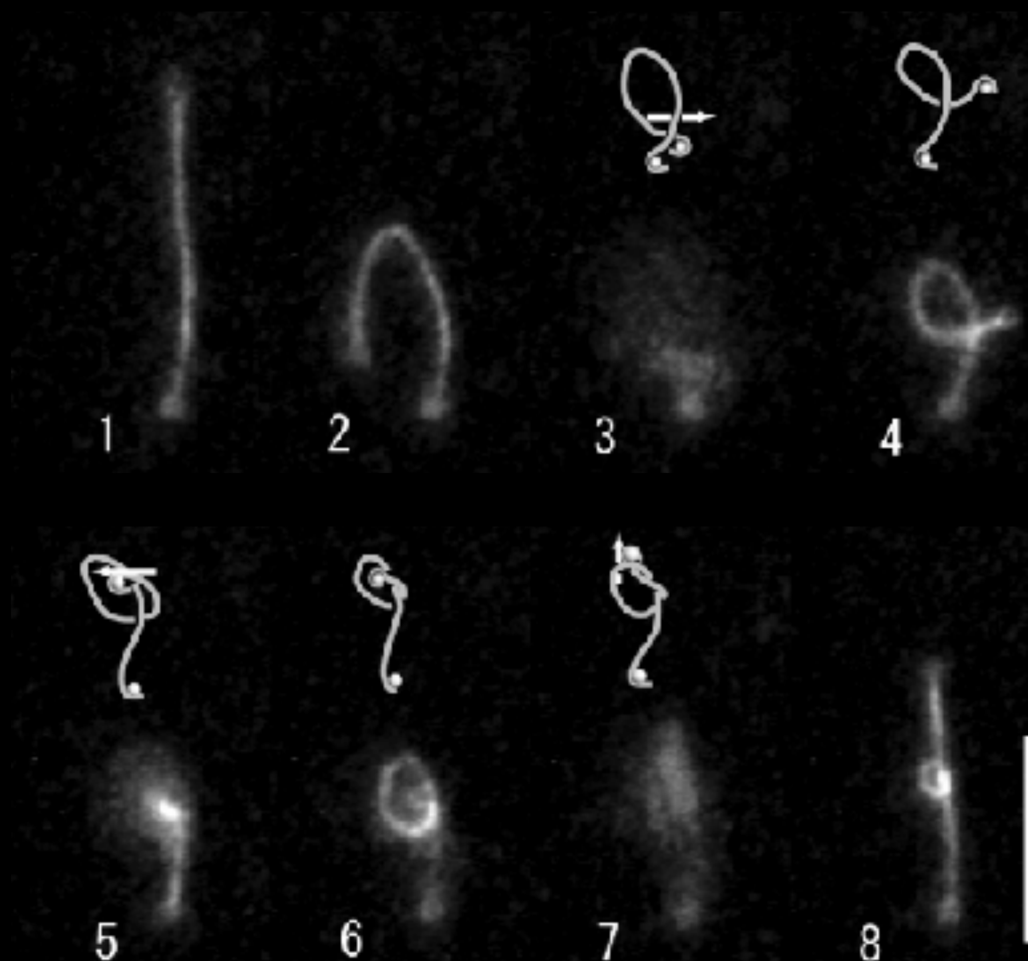
Visualization of a random chain

Tying a knot on a single DNA chain

Phase contrast image

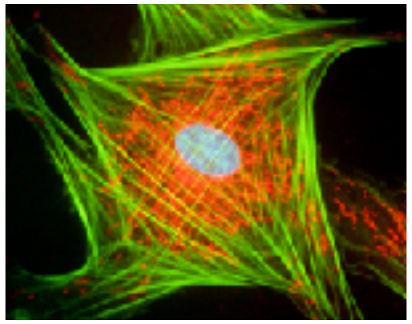
Fluorescence image

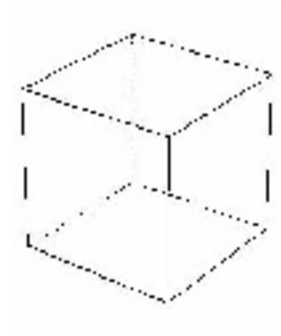




Kinosita Group

Physical size of the human genome



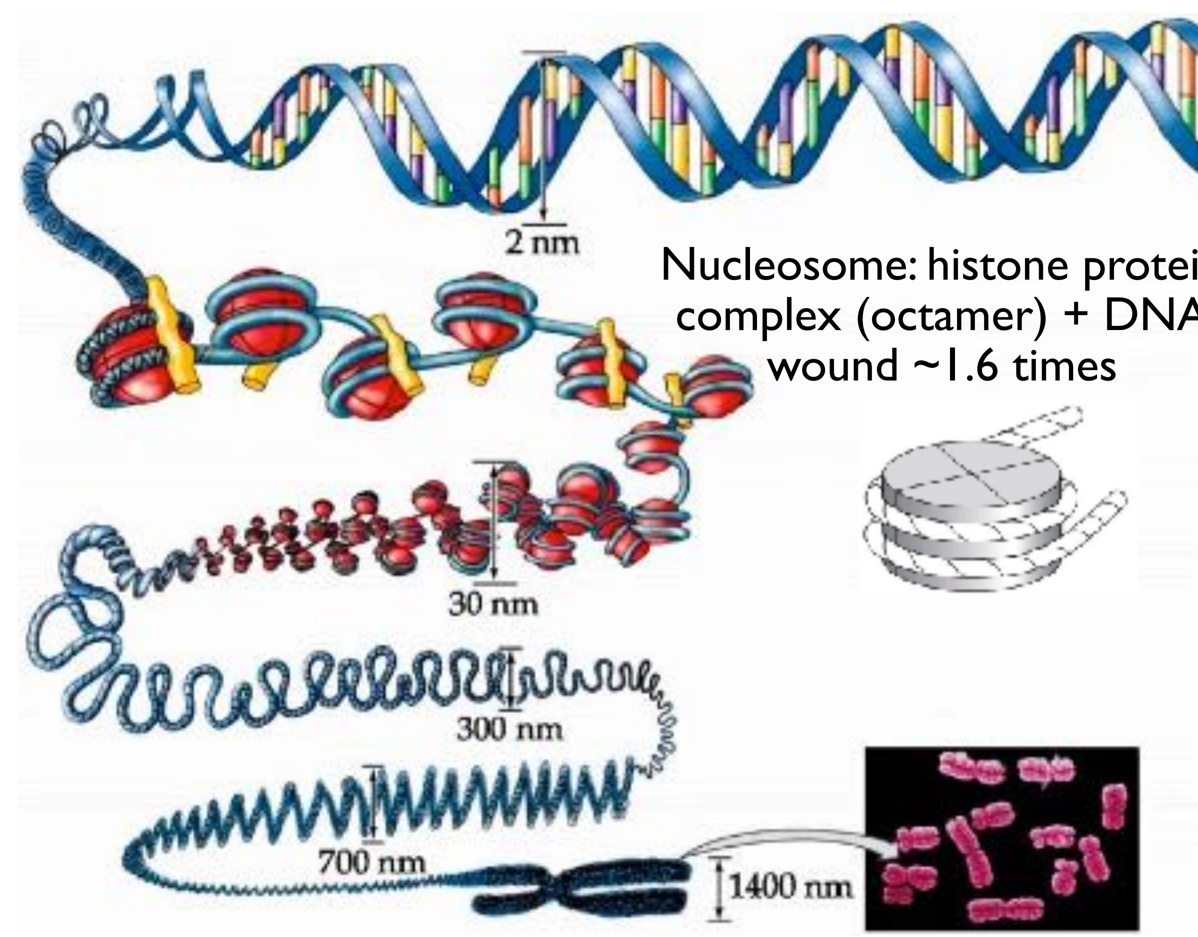


Simplified cell model: cube

Solution: DNA needs to be packed!

	Cell: 20 μm edge cube	Analog - **Lecture hall:** 20 m edge cube
DNA thickness	2 nm	2 mm
Full length of human DNA	~2 m	~2000 km (!!!) (Perimeter of Hungary: ~2200 km)
Persistence length of dsDNA (L_P)	~50 nm	~50 cm
Mean end-to-end length $\sqrt{\langle R^2 \rangle} = \sqrt{L_C L_P}$	~350 μm (!)	~350 m (!)
Radius of gyration (R_G) $R_G = R/\sqrt{6}$	130 μm	130 m
Volume of fully compacted DNA	~2 x 2 x 2 μm³	~2 x 2 x 2 m³ (= 8 m³)

Chromosome condensation



- ***Condensins*** play a role in high-order DNA packaging
- DNA chain: complex linear path with roadblocks!