Physical Biology of the Living Cell - II. semester

Wednesdays 11:15-13:00

Diffusion, polymerization, reptation (Dr. Miklós Kellermayer)	Sept. 15
Motor proteins, out of equilibrium processes (Dr. Miklós Kellermayer)	Sept. 22
The second law of thermodynamics in small systems, Evans-Searles fluctuation theorem (Dr. Szabolcs Osváth)	Sept. 29
Crooks fluctuation theorem (Dr. Szabolcs Osváth)	Oct. 6
Jarzinski equality (Dr. Szabolcs Osváth)	Oct. 13
Thermodynamics of molecular motors (Dr. Szabolcs Osváth)	Oct. 20
Protein structure prediction, use of structural databases (Dr. Tamás Hegedűs)	Nov. 3
Modeling protein folding and conformational changes (Dr. Tamás Hegedűs)	Nov. 10
Protein - protein interactions and protein networks (Dr. Tamás Hegedűs)	Nov. 17
Thermodynamic characterization of protein - protein and protein-ligand interactions (Dr. György Ferenczy)	Nov. 24
Computer modeling of protein - ligand binding I. Calculating thermodynamic quantities (Dr. György Ferenczy)	Dec. 1
Computer modeling of protein - ligand binding II. Estimating thermodynamic quantities using approximations (Dr. György Ferenczy)	Dec. 8
Problem solving and consultation (Dr. Szabolcs Osváth)	Dec. 15

Physical Biology of the Living Cell II.

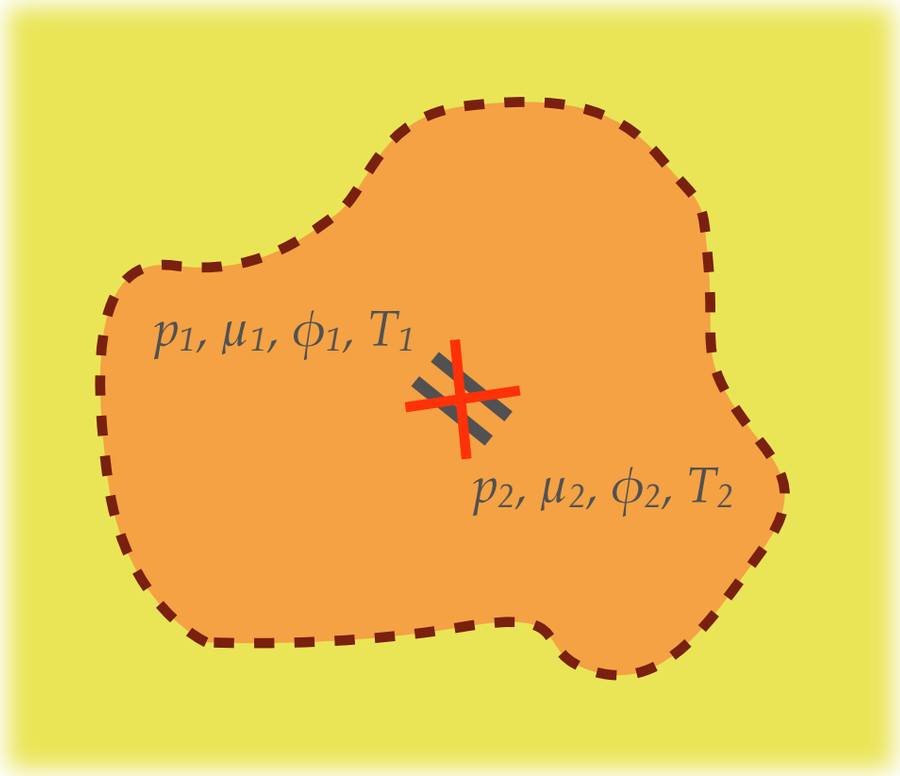
Diffusion, polymers, reptation

Miklós Kellermayer

- Diffusion, diffusion-driven processes
- Shape of polymers
- Diffusion of polymers, reptation.

THERMODYNAMIC CURRENTS

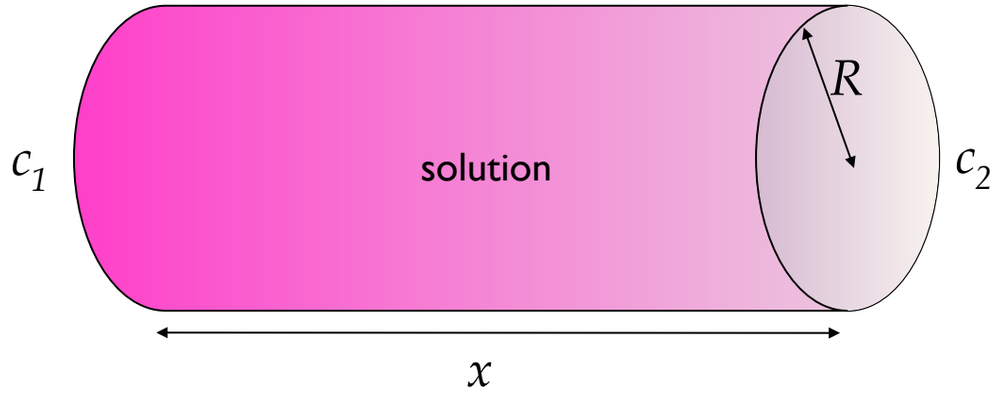
- Natural processes are rarely reversible.
- If there are inequalities in the intensive variables at different locations within the system, thermodynamic currents arise.
- Thermodnamic currents (irreversible processes) aim at the restoration of equilibrium.
- Irreversible processes are described by irreversible thermodynamics.



Thermodynamic current	Relevant intensive variable (its difference maintains current)	Current density	Physical law
Heat flow	Temperature (T)	$J_E = -\lambda \frac{\Delta T}{\Delta x}$	Fourier
Volumetric flow	Pressure (p)	$J_V = -\frac{R^2}{8\eta} \frac{\Delta p}{\Delta x}$	Hagen-Poiseuille
Electric current	Electric potential (φ)	$J_Q = -\frac{1}{\rho} \frac{\Delta \varphi}{\Delta x}$	Ohm
Material transport (diffusion)	Chemical potential (μ)	$J_n = -D \frac{\Delta c}{\Delta x}$	Fick

MATERIAL TRANSPORT (DIFFUSION)

Thermodynamic current	Relevant intensive variable (its gradient drives current)	Current density	Law
Material transport (diffusion)	Chemical potential (μ)	$J_n = -D\frac{\Delta c}{\Delta x}$	Fick

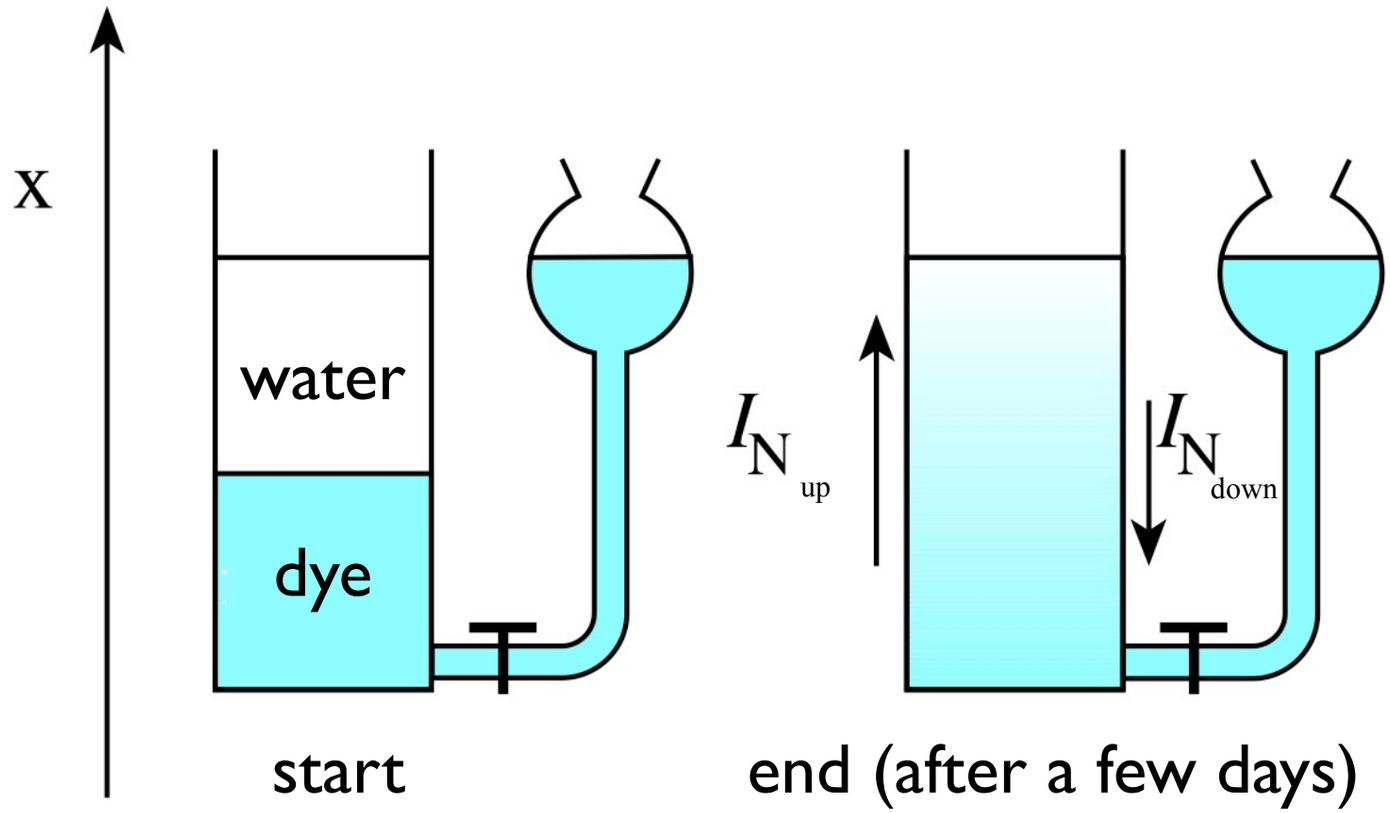


m = amount of material transported
t = time
R = tube radius
x = tube length
($\Delta c/\Delta x$ = concentration gradient, maintained by c_1-c_2)
A = cross-sectional area of tube
J_n = material transport current density
D = diffusion coefficient

$$\frac{m}{tA} = J_n = -D\frac{\Delta c}{\Delta x}$$

DIFFUSION

- Spontaneous mixing, distribution concentration-equilibration of particles driven by thermal motion.

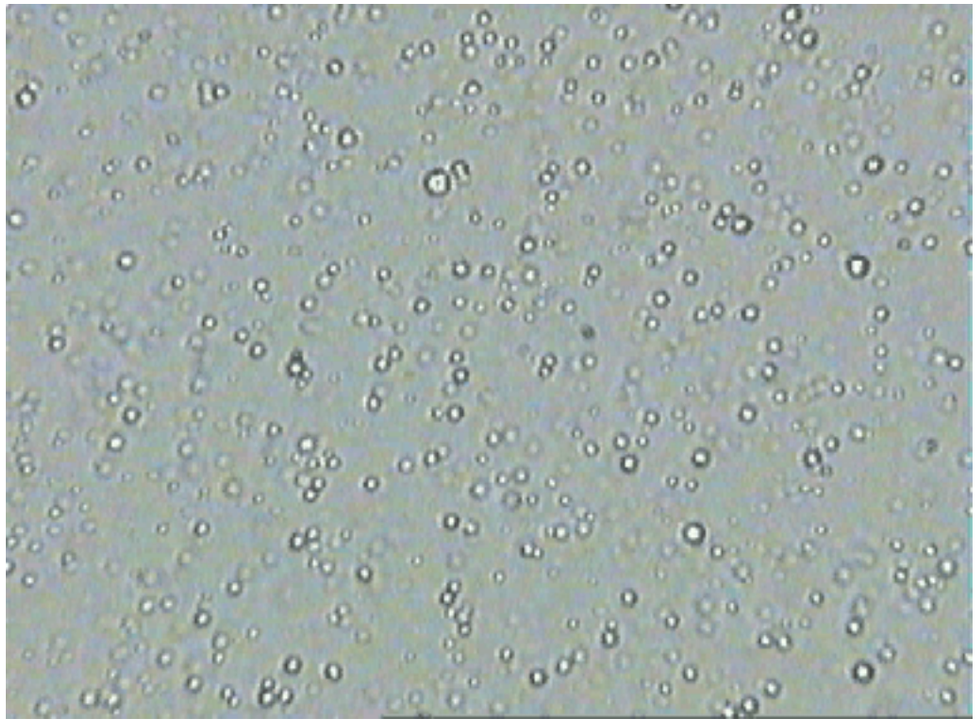


$$x^2 = 2Dt$$

x = displacement of boundary (in reality, it is the smearing of the boundary)
t = time
D = constant (diffusion coefficient)

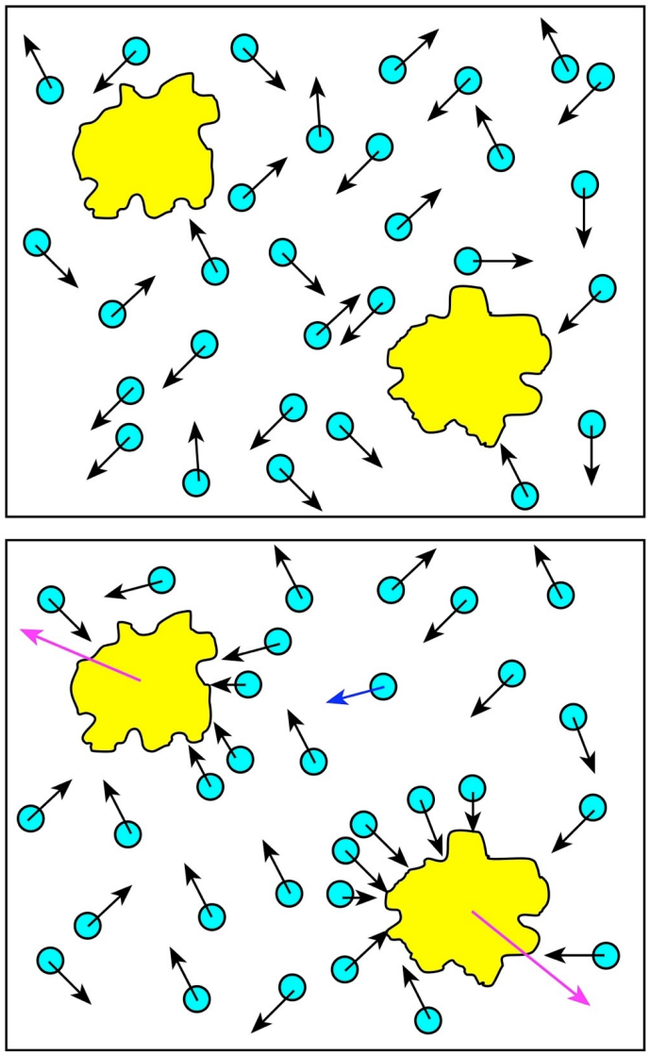
Microscopic manifestation of diffusion: Brownian motion

Robert Brown
(1773-1858)

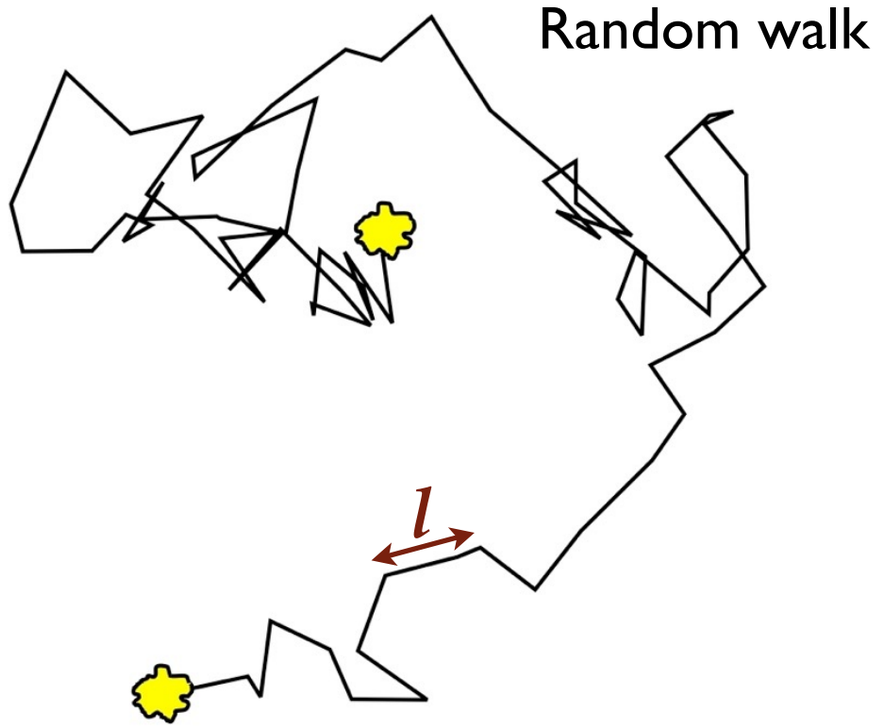


Lipid droplets suspended in milk
(droplet size 0.5 - 3 μm)

Brownian-motion



Random motion of the microscopic (Brownian) particle is the result of stochastic collisions with molecules.



l = mean free path (average distance between consecutive collisions)

v = average velocity of the thermally propelled particle

DIFFUSION

- *Fick's I. law:* material flow density is the product of the evoking concentration gradient and the diffusion coefficient.

Material flow density (material transport):

$$J_n = -D\frac{\Delta c}{\Delta x}$$

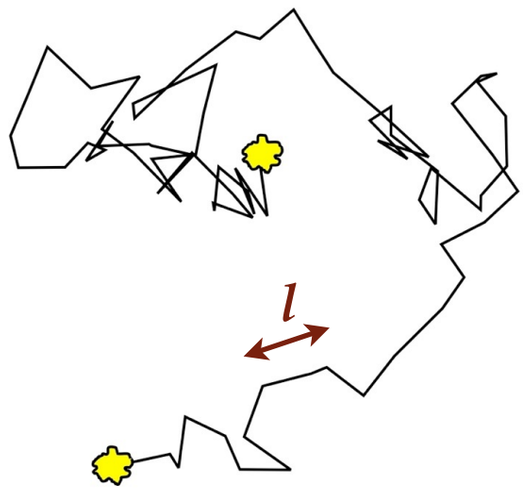
J_n = material flow density
$\Delta c/\Delta x$ = drop in concetration (gradient)
D = constant (diffusion coefficient)

Diffusion coefficient:

$$D = \frac{1}{3}vl$$

v = average velocity of thermally propelled particle
l = mean free path (average distance between consecutive collisions)
D = amount of material transported across unit cross-sectional area per unit time (m^2/s) (at unit concentration drop).

Brownian motion



Diffusion coefficient for spherical particle:

$$D = \frac{k_B T}{6\pi\eta r}$$

Einstein-Stokes equation:
k_B = Boltzmann's constant
T = absolute temperature
η = viscosity
r = radius of particle

DIFFUSION

- *Fick's II. law:* instantaneous material flow density depends on the temporal change of the evoking concentration gradient.

Material flow:

$$-\frac{\Delta J_n}{\Delta x} = \frac{\Delta c}{\Delta t}$$

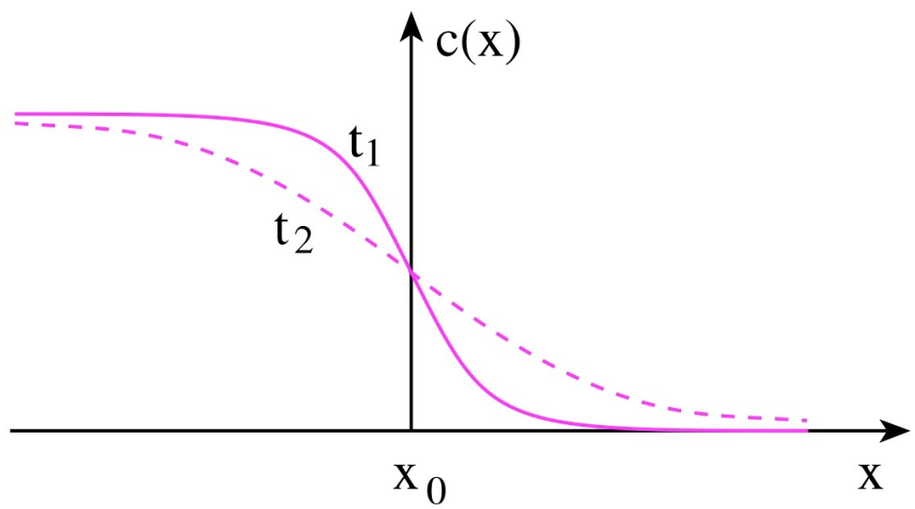
J_n = flow density
x = distance
t = time

Diffusion coefficient:

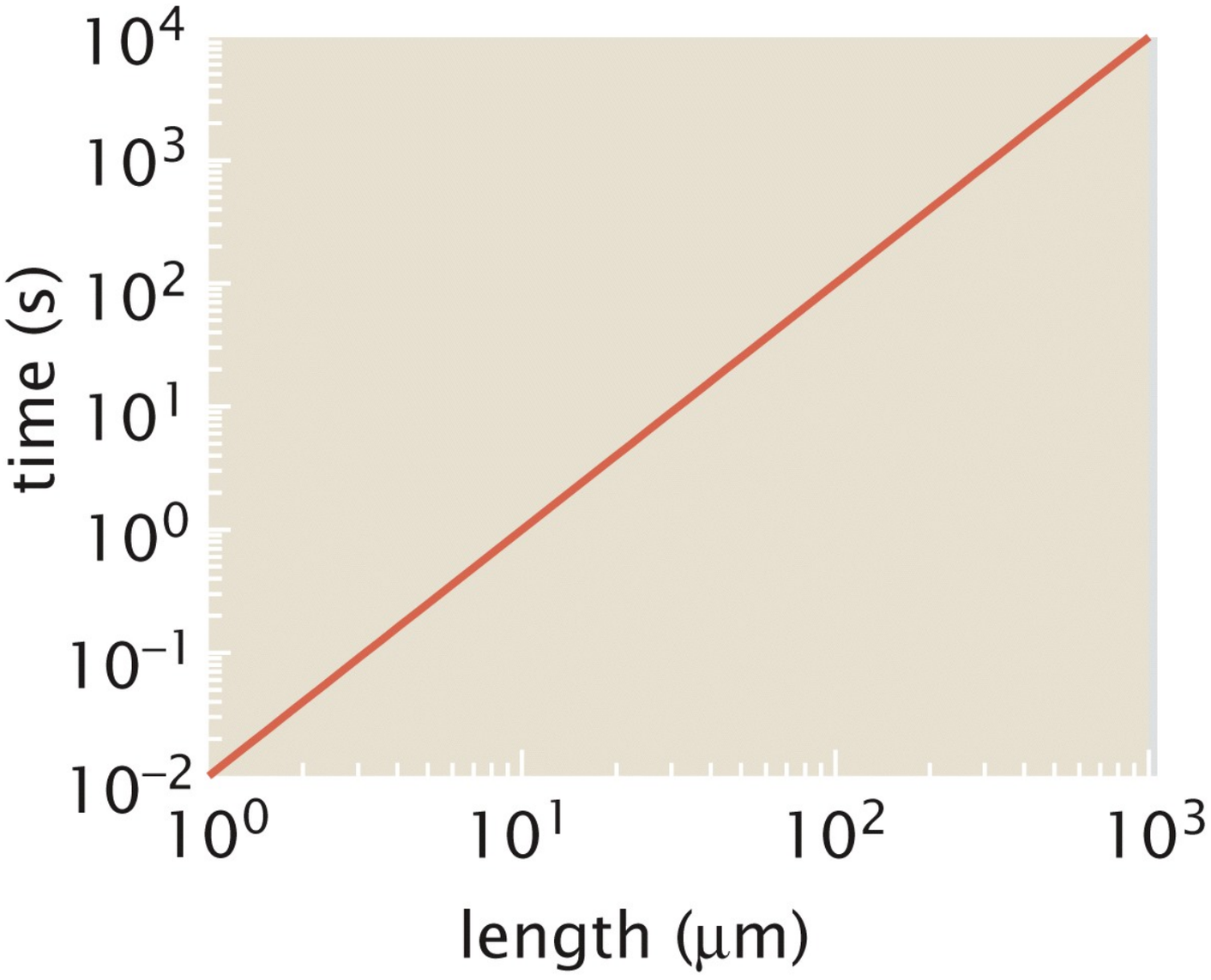
$$D\frac{\Delta\left(\frac{\Delta c}{\Delta x}\right)}{\Delta x} = \frac{\Delta c}{\Delta t}$$

D = diffusion coefficient.

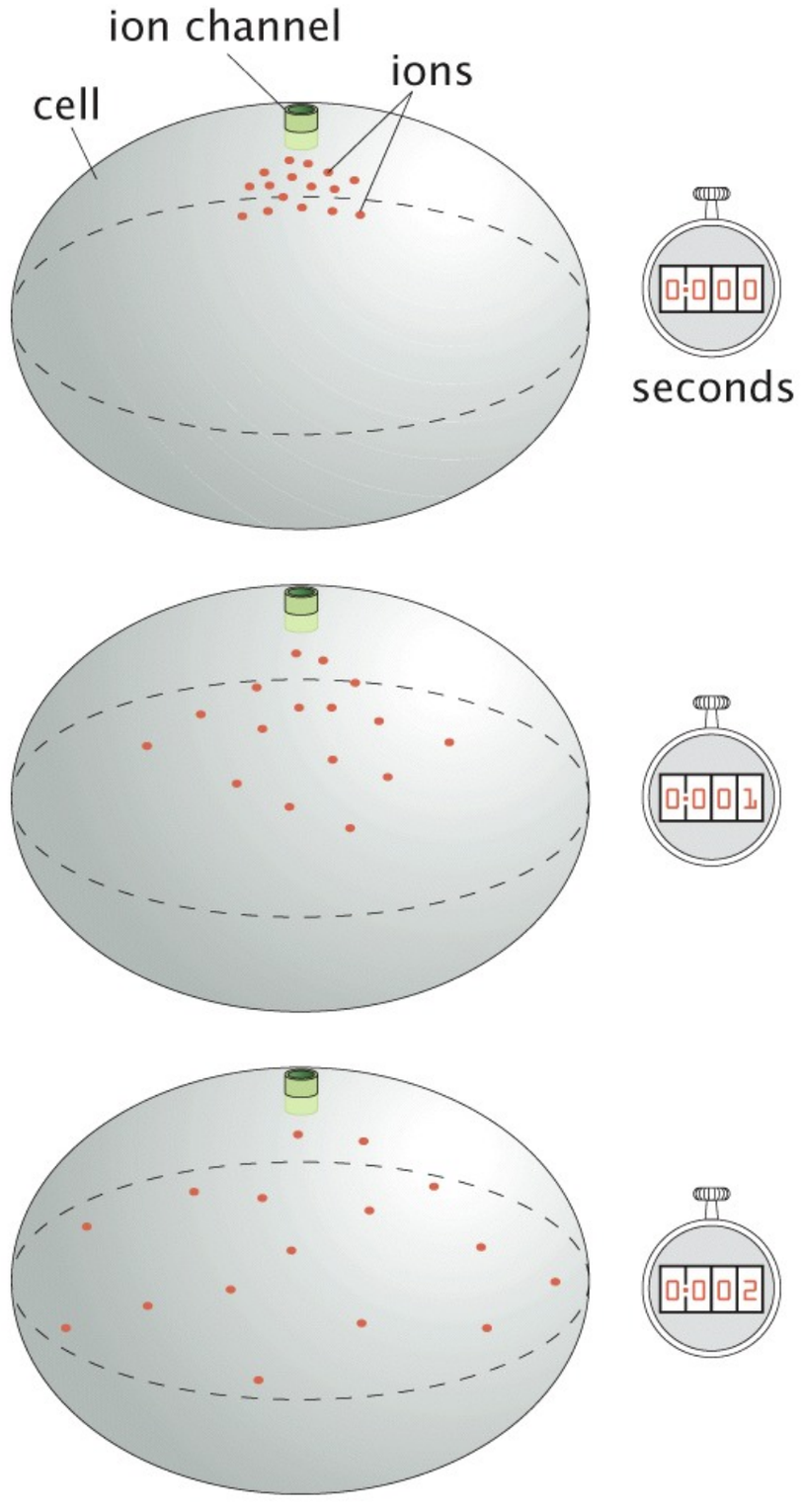
Concentration gradient decreases with time (boundary becomes smeared)



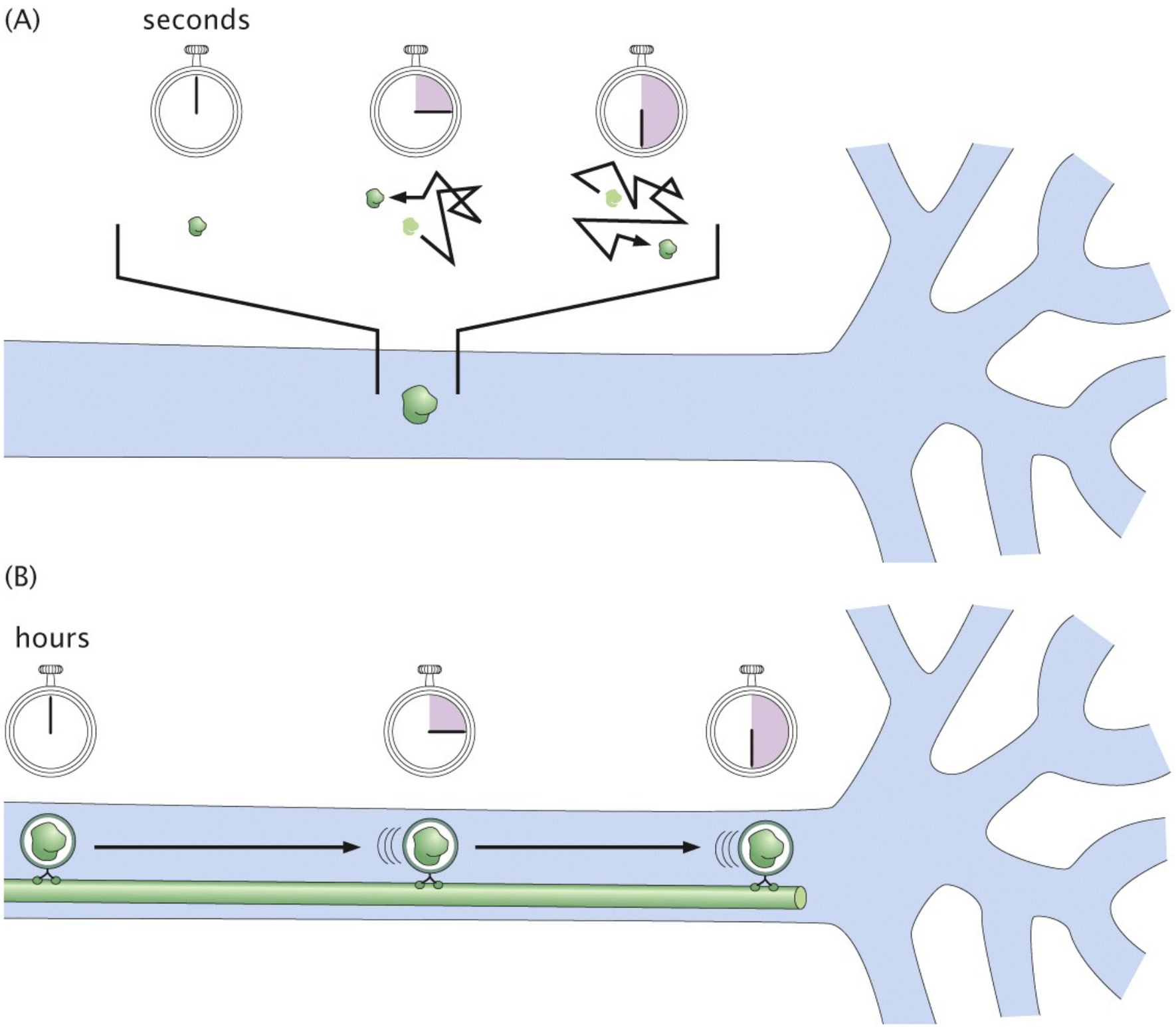
Diffusion provides rapid transport only on a short length scale



Power (square) relationship: slope = 2



Diffusion provides rapid transport only on a short length scale



Biopolymers (biological polymers)

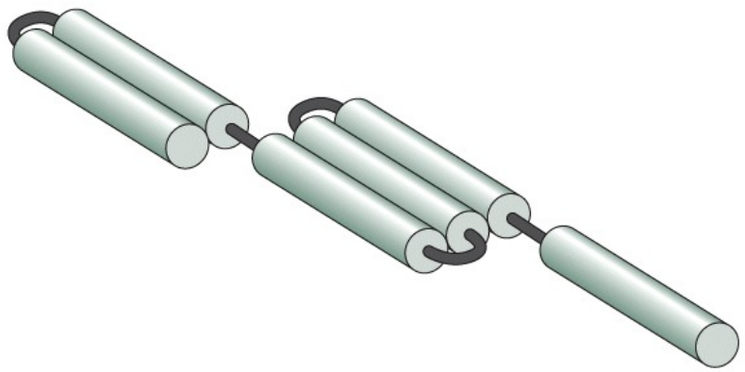
Polymers:
chains built up from monomers

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

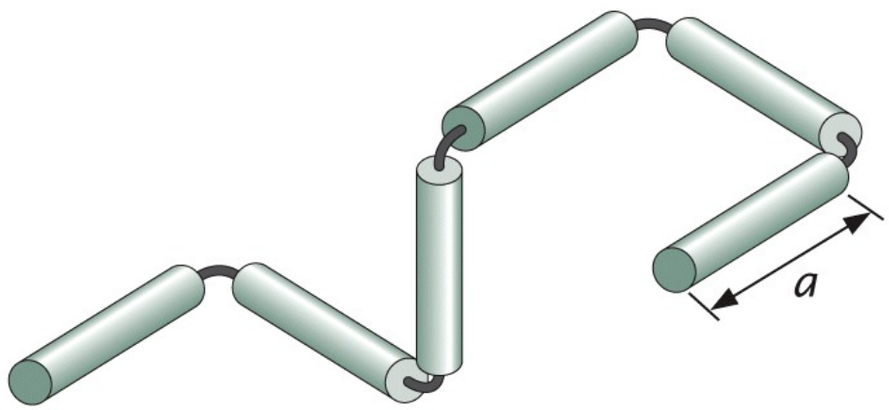
Biopolymer	Monomer	Bond
Protein	Amino acid	Covalent (peptide bond)
Nucleic acid (RNA, DNA)	Nucleotide (CTUGA)	Covalent (phosphodiester)
Polysaccharide (e.g., glycogen)	Sugar (e.g., glucose)	Covalent (e.g., α-glycosidic)
Protein polymer (e.g., microtubule)	Protein (e.g., tubulin)	Secondary

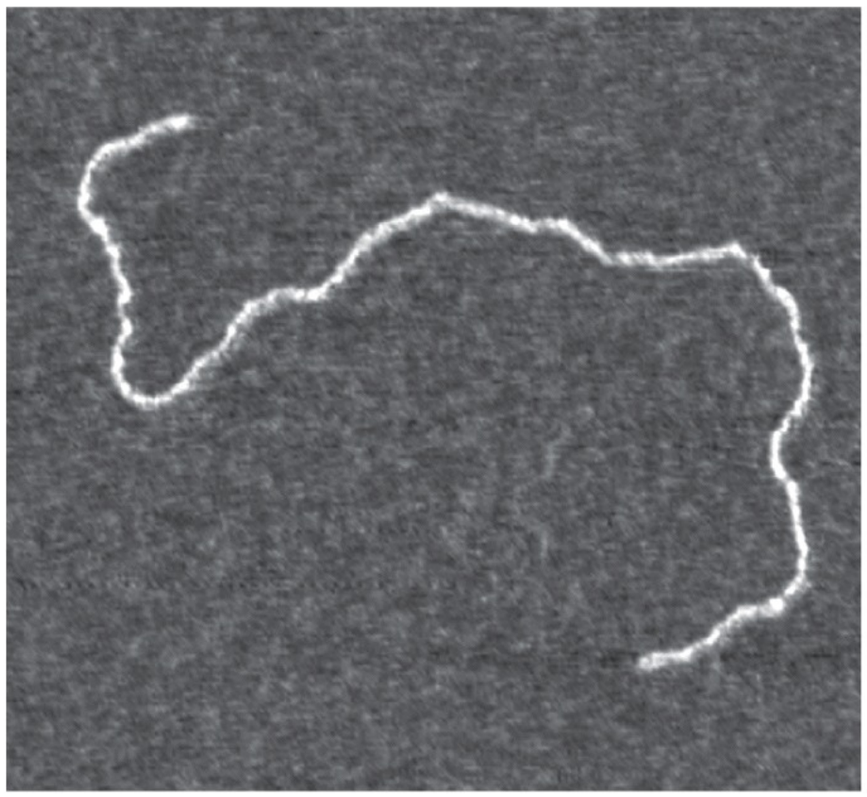
Shape of the polymer chain resembles random walk

1D random walk

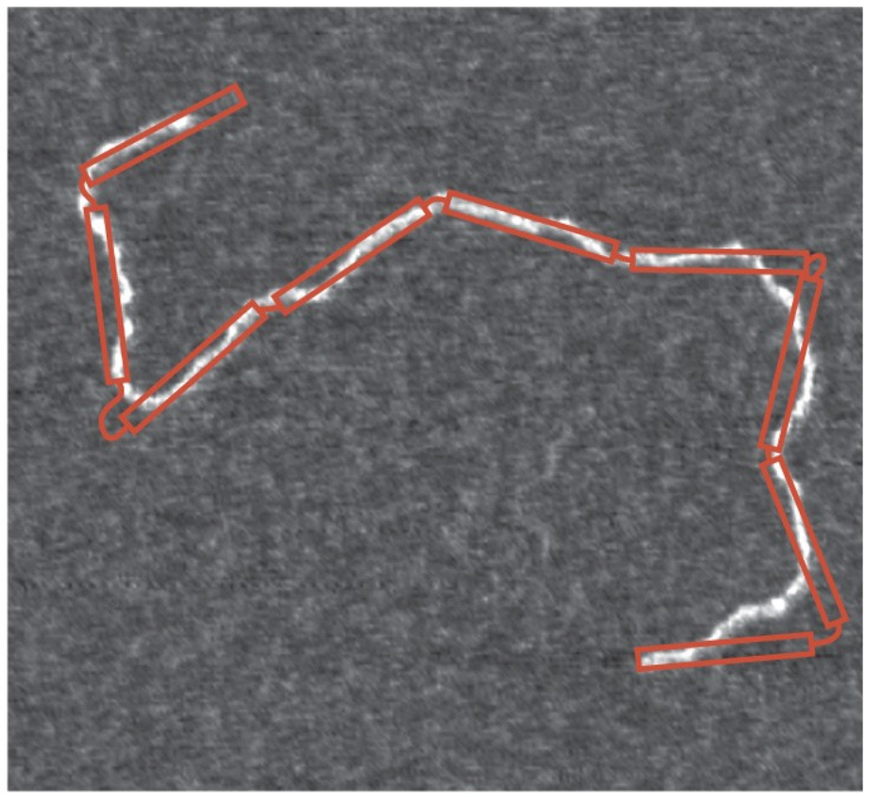


3D random walk





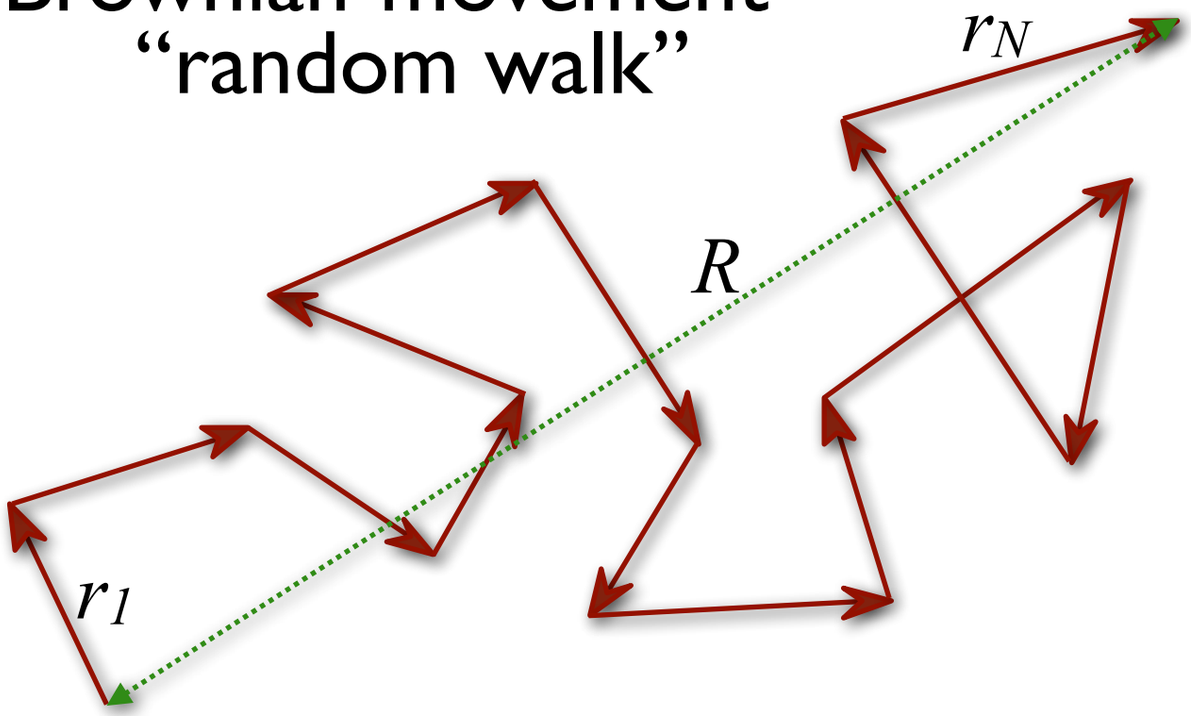
100 nm



dsDNA molecule

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.

Average particle velocity: $v = \frac{l}{\tau}$

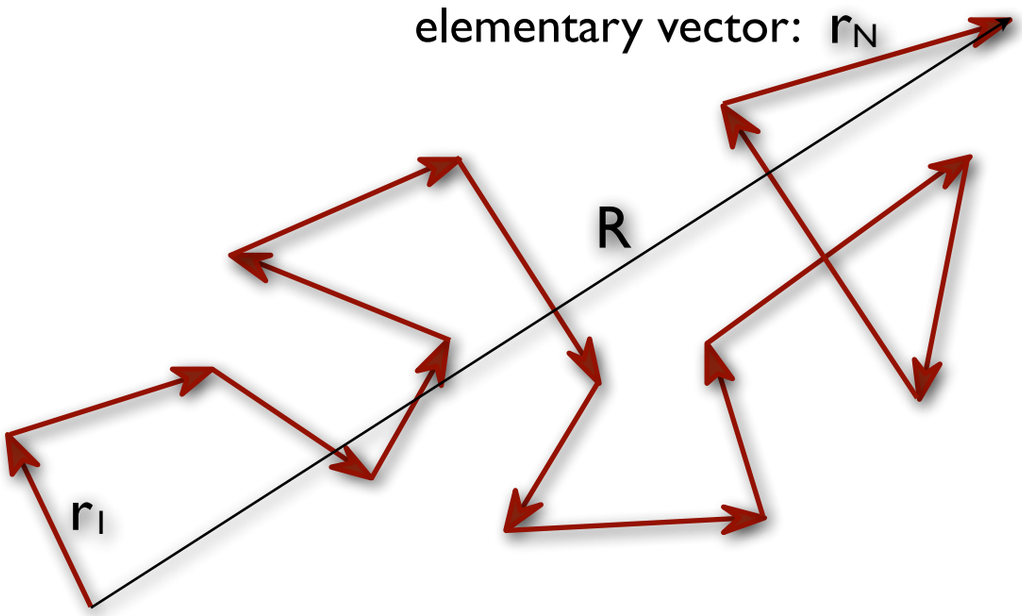
Total diffusion time: $t = N\tau$

Diffusion coefficient: $D = \frac{1}{3}vl$

$$\langle R \rangle = \sqrt{Nl^2} = \sqrt{\frac{t}{\tau}l^2} = \sqrt{tvl} = \sqrt{3Dt}$$

Shape and shape change of a random polymer chain

Brownian motion (random walk)



Square-root law:

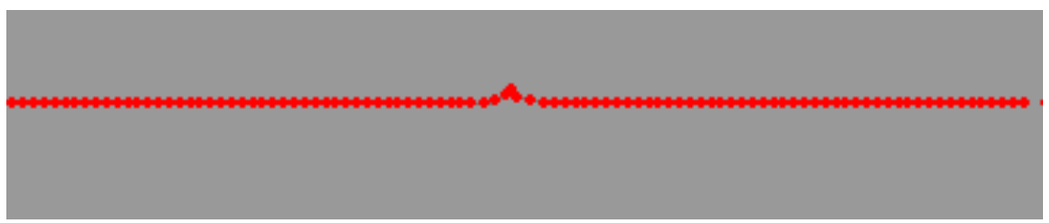
$$\langle R^2 \rangle = Nl^2 = Ll$$

The tendency of maximizing the orientational disorder (entropy) of elementary vectors leads to elastic behavior

Entropic elasticity:
Upon thermal excitation, the polymer chain goes through random fluctuations, shape changes.

Conformational entropy of the chain (orientational entropy of the elementary vectors) increases.

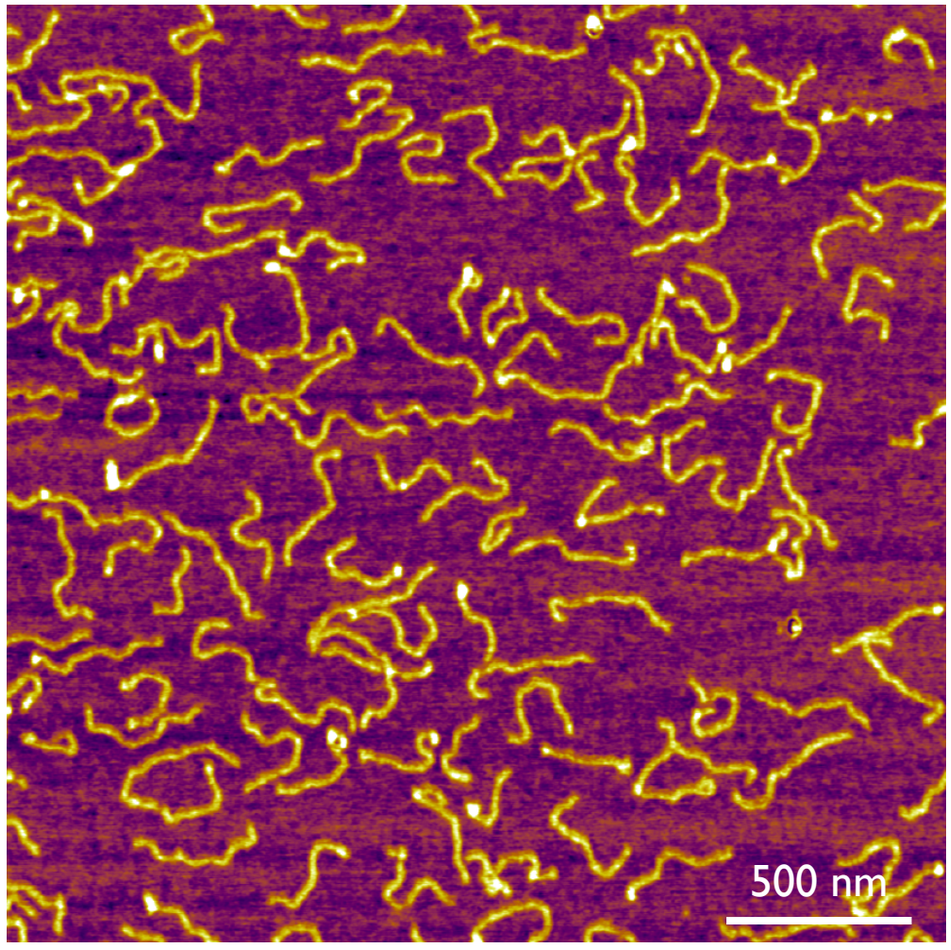
The polymer chain contracts.



Equilibrium shape of a random polymer

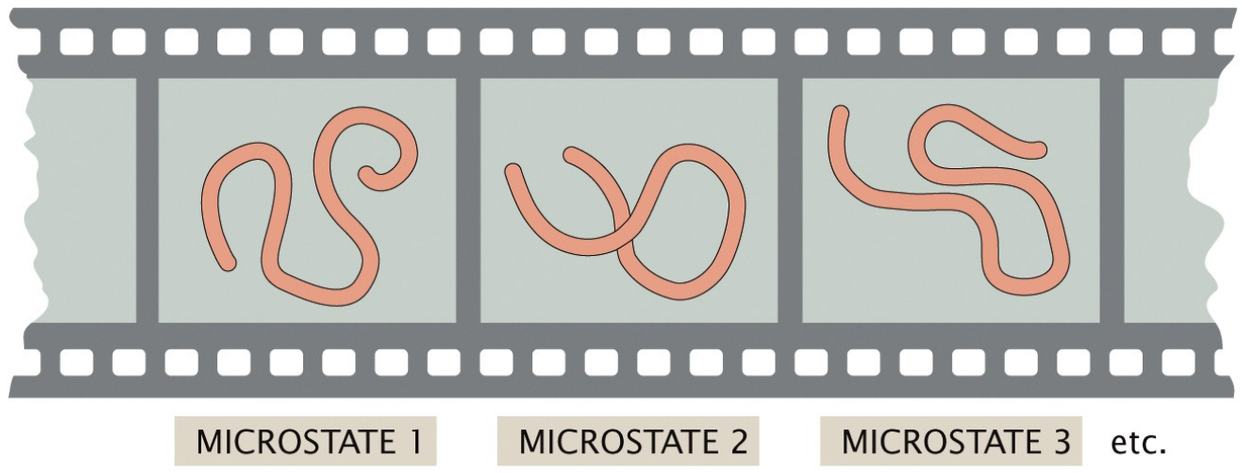
The macrostate which can be realized by the largest number of microstates (the most probable state)

Spatial distribution of microstates



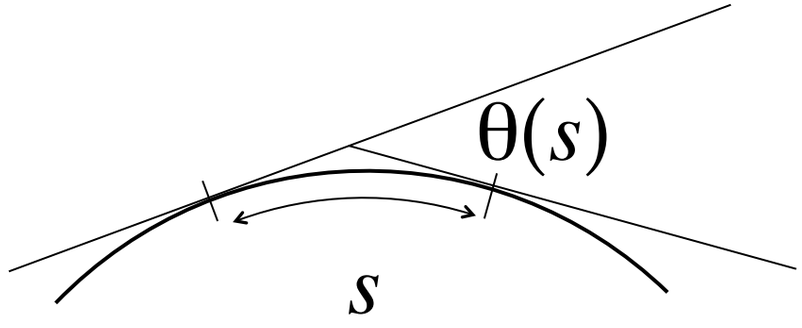
AFM image of surface-adsorbed dsDNA molecules (identical contour length, PCR product)

Temporal distribution of microstates



Wormlike chain polymer model

WLC (wormlike chain):



if s is large enough, then $\langle \cos\theta(s) \rangle$ decays as a function of s: $\langle \cos\theta(s) \rangle = \exp\left(-\frac{s}{l_p}\right)$

l_p=persistence length

If $s<<l_p$, then $\langle \cos\theta(s) \rangle \sim 1$, and $\theta(s)$ fluctuates around 0.

If $s>>l_p$, then $\langle \cos\theta(s) \rangle \sim 0$,

that is, $\theta(s)$ may be between 0° and 360° with equal probabilities.

Meaning of persistence length:

statstical distance across which the chain retains its orientation (remembers it).

I.e., the orientation "persists".

Beyond the persistence length the chain forgets its orientation.

$$l_p = \frac{EI}{k_B T}$$

EI = flexural ridity (E = Young's modulus - material dependent, I = second moment of cross section - shape dependent); $k_B T$ = thermal energy

Meaning: the more rigid the chain, the greater the distance (l_p) beyond which the thermal fluctuations become detectable.

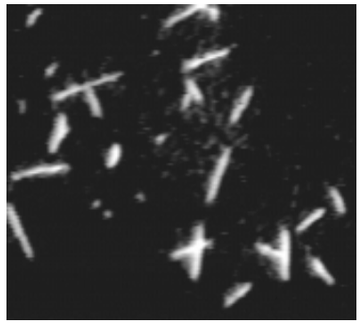
Relationship between the shape and elasticity of the polymer chain

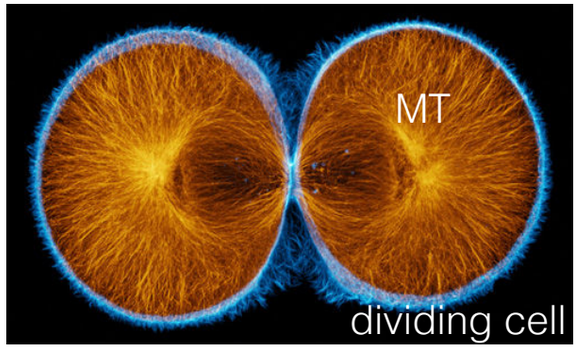
Rigid chain

$L_p >> L_c$

(mm >> 10 μm)

Microtubule



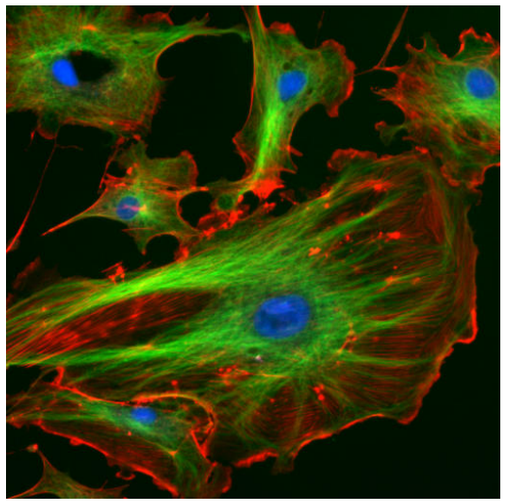


Semiflexible chain

$L_p \approx L_c$

(μm $\approx$ μm)

Microfilament (actin)

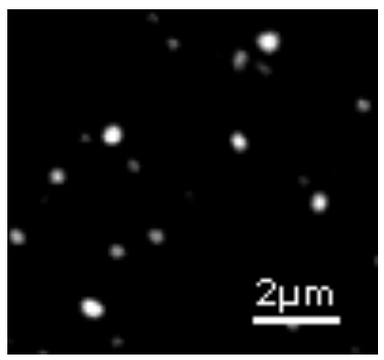


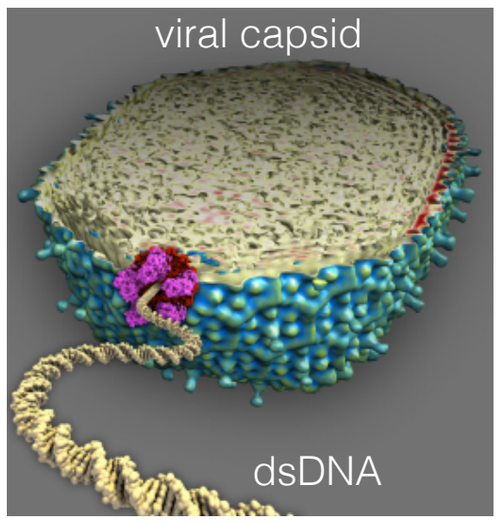
Flexible chain

$L_p << L_c$

(50 nm << cm)

dsDNA





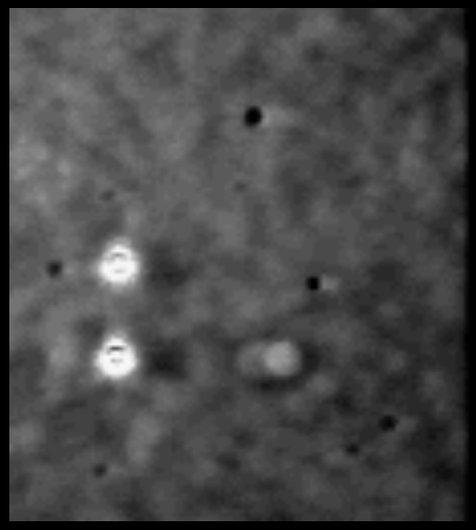
L_p = persistence length

L_c = contour length

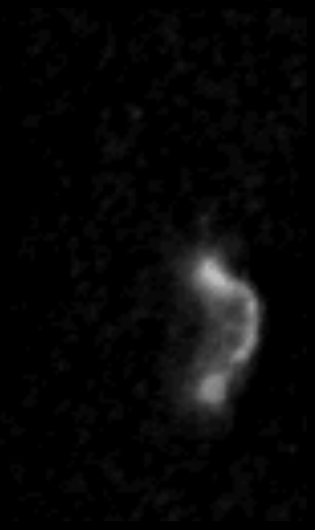
Visualization of a random (entropic) chain

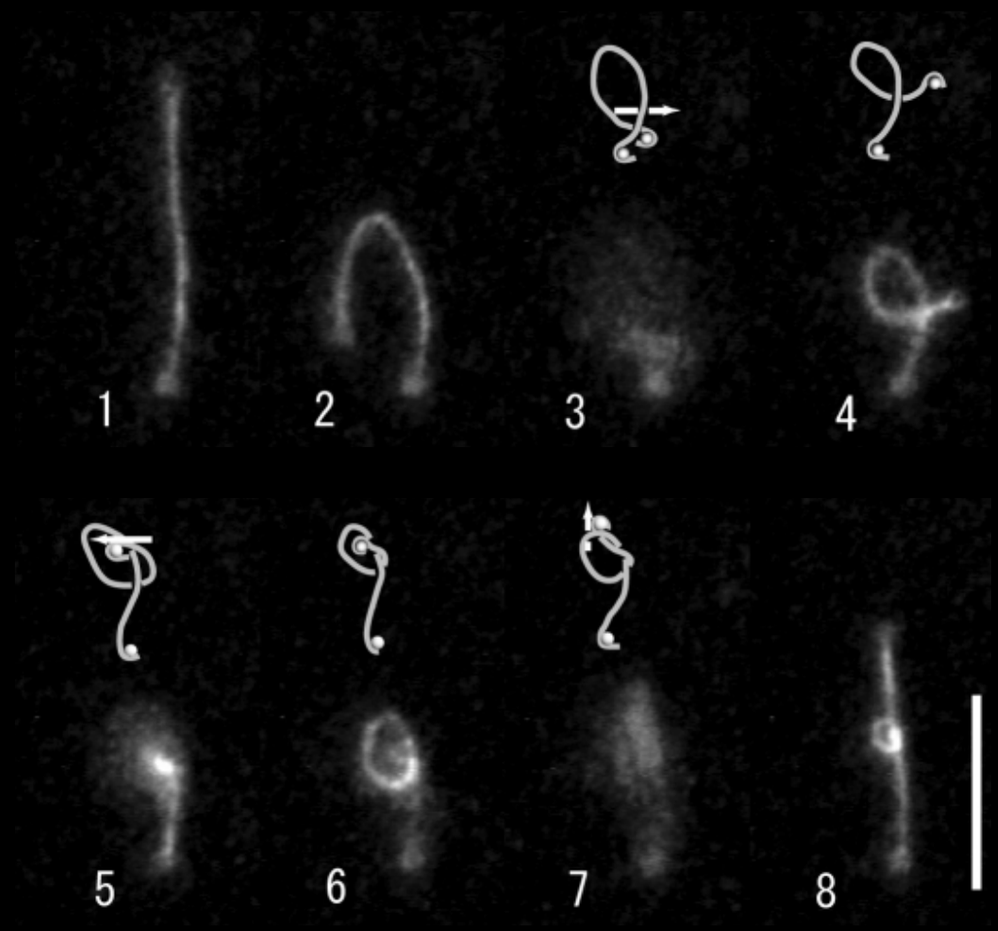
Tying a knot on a single dsDNA molecule

Phase contrast image



Fluorescence image



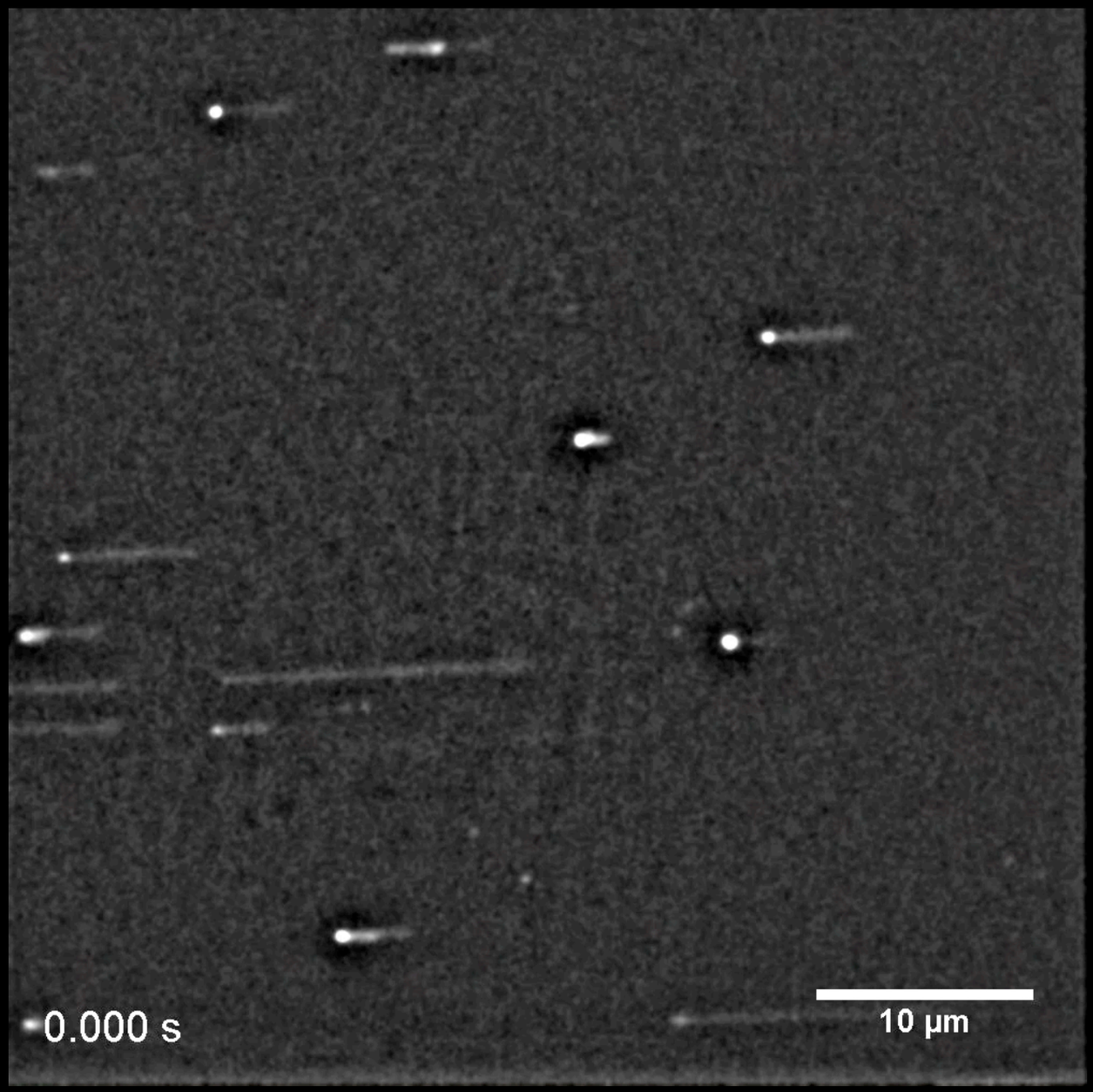


Kinosita Group

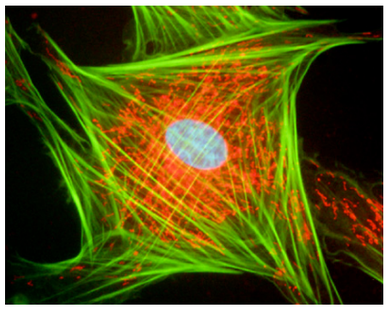
Visualization of a random (entropic) chain

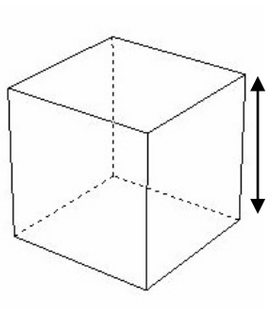
Extending dsDNA with drag force (fluid flow)

dsDNA molecules ejected from T7 bacteriophages and labeled, instantaneously, with sytox orange



Physical size of the human genome



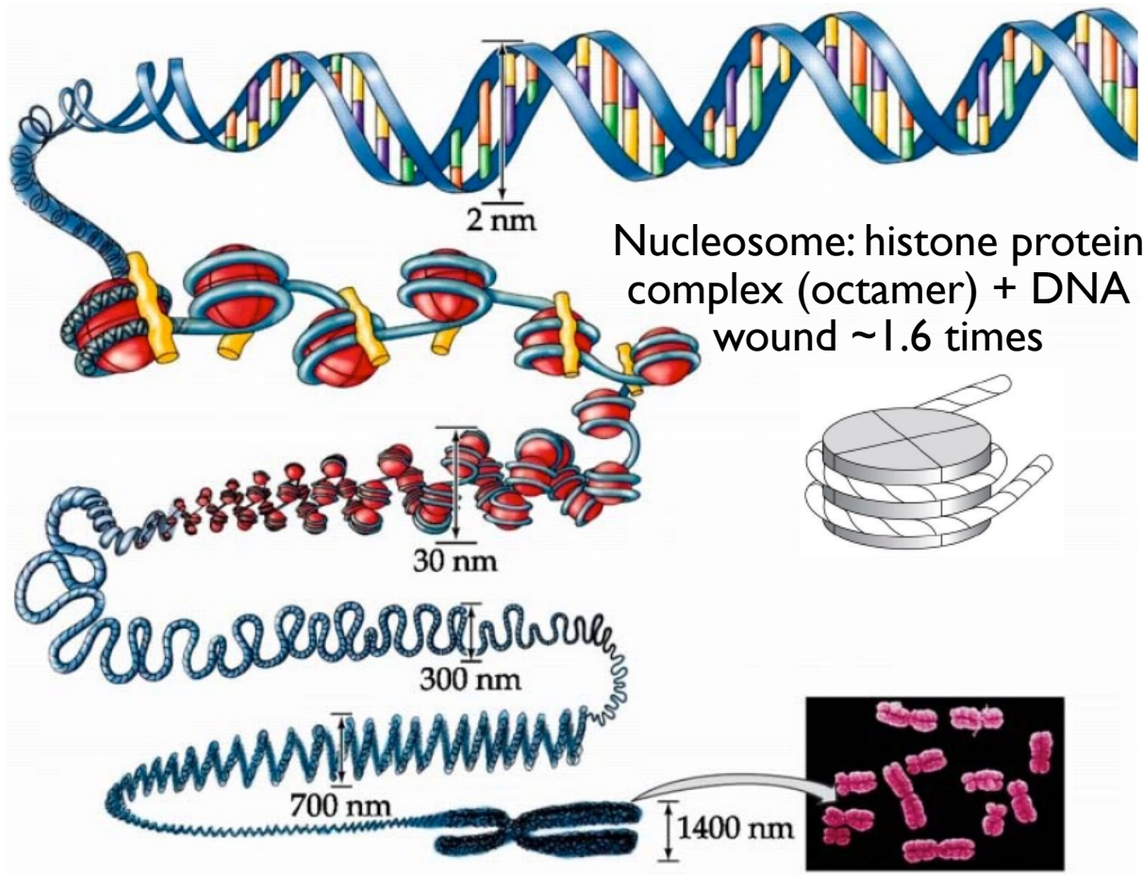


Simplified cell model: cube

	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)

Solution: DNA needs to be packed!

Chromosome condensation



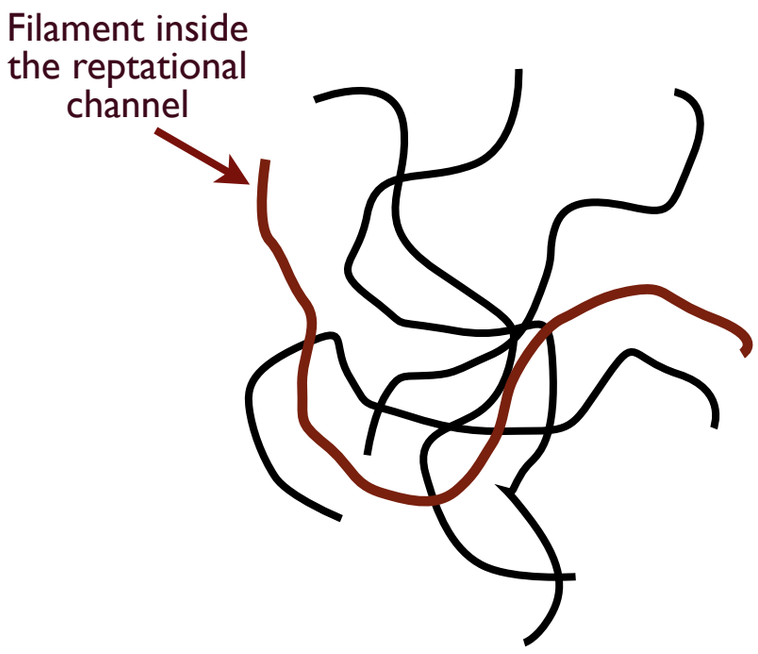
Nucleosome: histone protein complex (octamer) + DNA wound ~1.6 times

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

SPECIAL CASE OF DIFFUSION: REPTATION

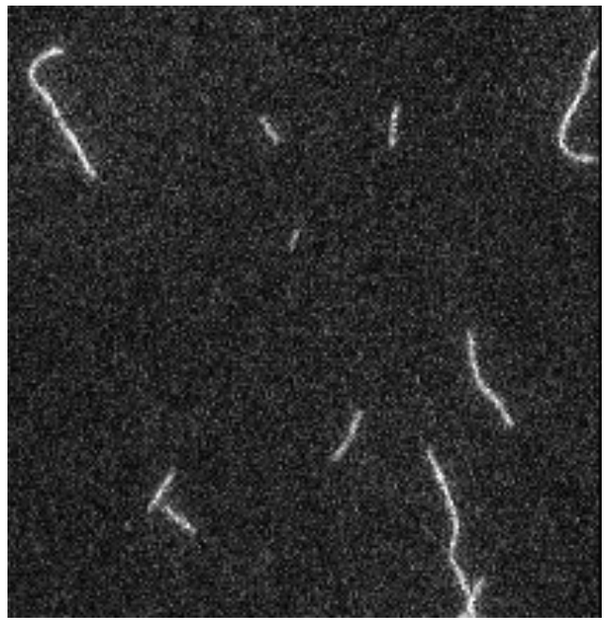
- *Reptation:* "snakelike" diffusional motion within polymer network ("*Reptilia*")

Polymer matrix:
"entanglement"



Indiana Jones and
Raiders of the Lost Ark

Actin filaments in methyl-
cellulose matrix.
Unidirectionalized diffusion



$$\tau_r = \frac{L^2 \cdot N}{\mu \cdot k \cdot T}$$

τ_r = Reptation time: time required for traveling a distance equivalent to one contour length; L = contour length; N = number of elementary segments; μ = chain mobility; kT = thermal energy

$$D_r = \frac{\left(a \cdot \sqrt{N}\right)^2}{\tau_r}$$

D_r = Reptation diffusion coefficient; N = number of elementary segments; a = length of elementary segment (~persistence length); τ_r = reptation time. N.B.: numerator is analogous to the mean-square-displacement.