

# Physical Biology of the Living Cell - II. semester

Tuesdays 13:15-15:00

<b>Diffusion, polymerization, reptation</b> (Dr. Miklós Kellermayer)	Sept. 13
<b>Motor proteins, out of equilibrium processes</b> (Dr. Miklós Kellermayer)	Sept. 20
<b>The second law of thermodynamics in small systems, Evans-Searles fluctuation theorem</b> (Dr. Szabolcs Osváth)	Sept. 27
<b>Crooks fluctuation theorem</b> (Dr. Szabolcs Osváth)	Oct. 4
<b>Jarzynski equality</b> (Dr. Szabolcs Osváth)	Oct. 11
<b>Thermodynamics of molecular motors</b> (Dr. Szabolcs Osváth)	Oct. 18
<b>Protein structure prediction, use of structural databases</b> (Dr. Tamás Hegedűs)	Oct. 25
<b>Modeling protein folding and conformational changes</b> (Dr. Tamás Hegedűs)	Nov. 8
<b>Protein - protein interactions and protein networks</b> (Dr. Tamás Hegedűs)	Nov. 15
<b>Thermodynamic characterization of protein - protein and protein-ligand interactions</b> (Dr. György Ferenczy)	Nov. 22
<b>Computer modeling of protein - ligand binding I. Calculating thermodynamic quantities</b> (Dr. György Ferenczy)	Nov. 29
<b>Computer modeling of protein - ligand binding II. Estimating thermodynamic quantities using approximations</b> (Dr. György Ferenczy)	Dec. 6
<b>Problem solving and consultation</b> (Dr. Szabolcs Osváth)	Dec. 13

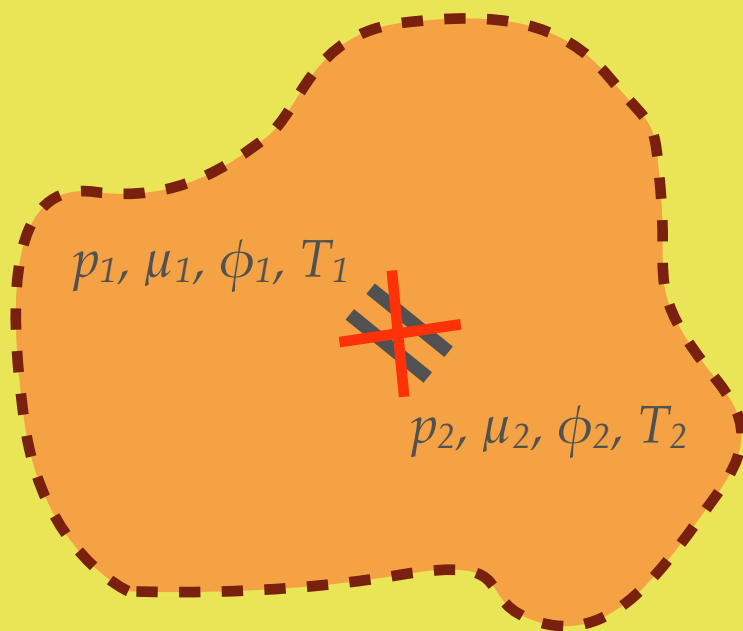
PHYSICAL BIOLOGY OF THE  
LIVING CELL II.  
DIFFUSION, POLYMERS, REPTATION

MIKLÓS KELLERMAYER

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

# THERMODYNAMIC CURRENTS

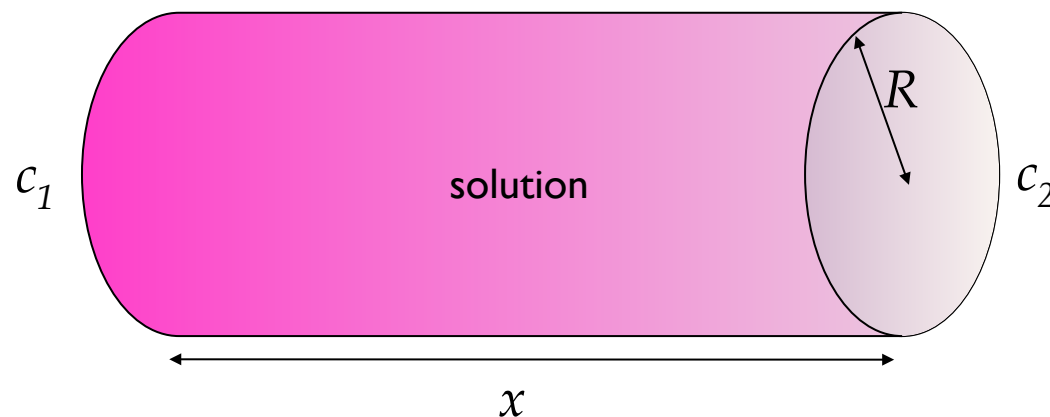
- Natural processes are rarely reversible.
- If there are inequalities in the intensive variables at different locations within the system, thermodynamic currents arise.
- Thermodynamic currents (irreversible processes) aim at the restoration of equilibrium.
- Irreversible processes are described by 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 ( $\varphi$ )	$J_Q = -\frac{1}{\rho} \frac{\Delta \varphi}{\Delta x}$	Ohm
Material transport (diffusion)	Chemical potential ( $\mu$ )	$J_n = -D \frac{\Delta c}{\Delta x}$	Fick

# MATERIAL TRANSPORT (DIFFUSION)

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

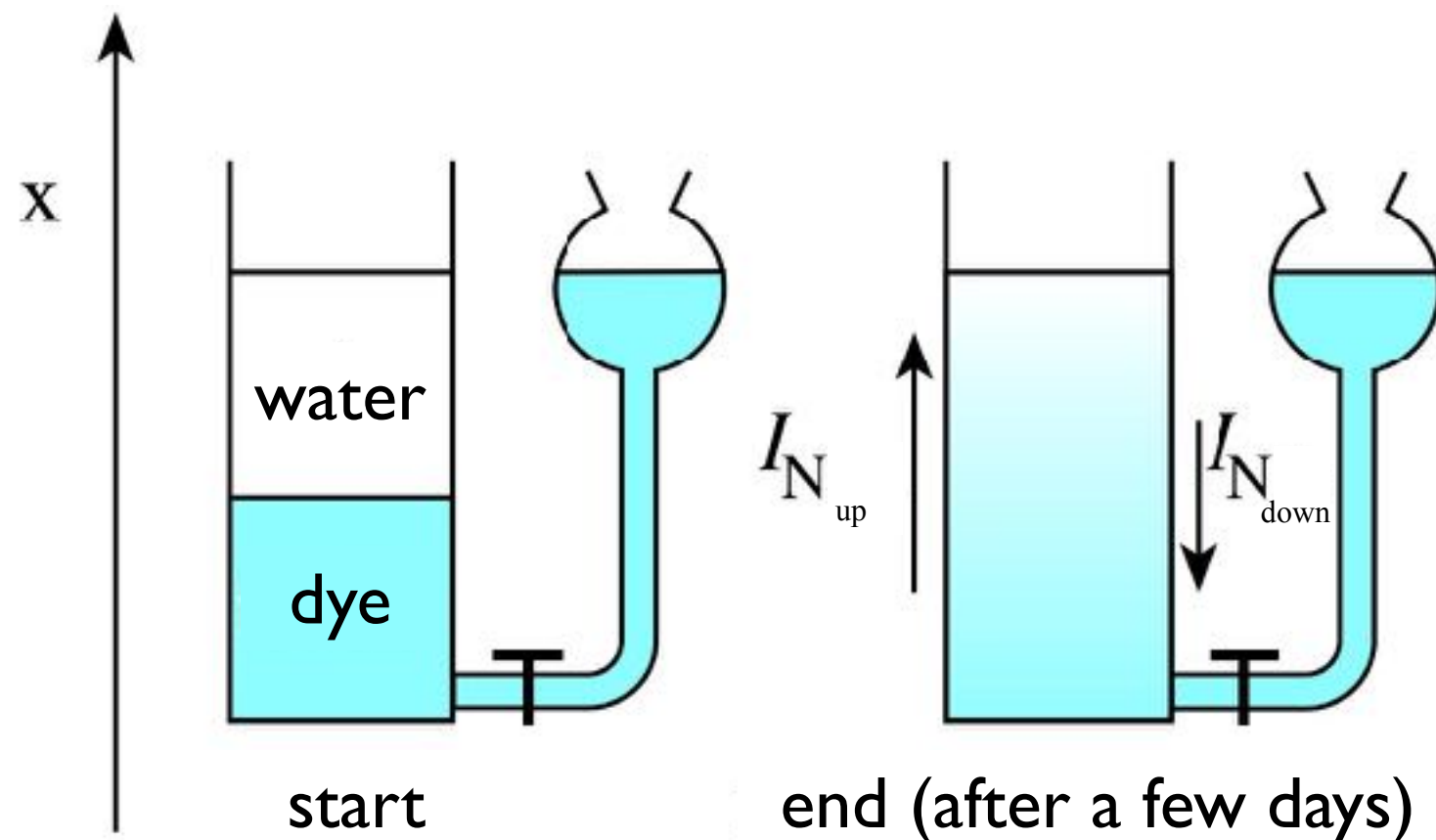


$m$  = amount of material transported  
 $t$  = time  
 $R$  = tube radius  
 $x$  = tube length  
 $(\Delta c / \Delta x = \text{concentration gradient, maintained by } c_1 - c_2)$   
 $A$  = cross-sectional area of tube  
 $J_n$  = material transport current density  
 $D$  = diffusion coefficient

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

# 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

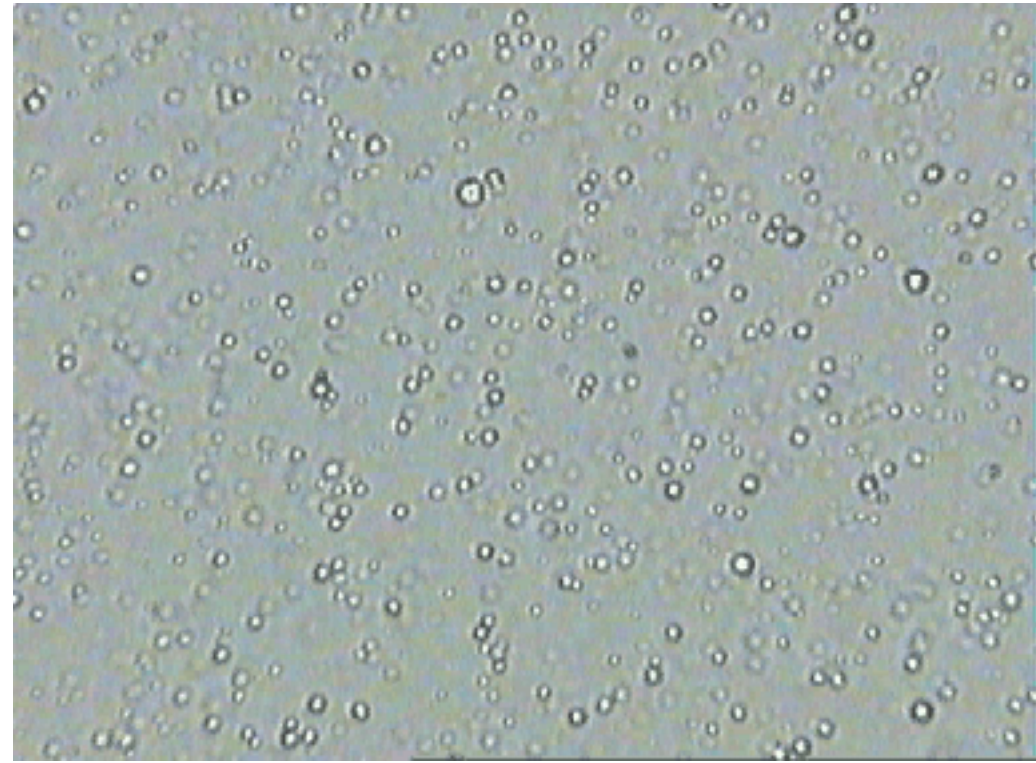
$D$  = constant (diffusion coefficient)



# Microscopic manifestation of diffusion: Brownian motion

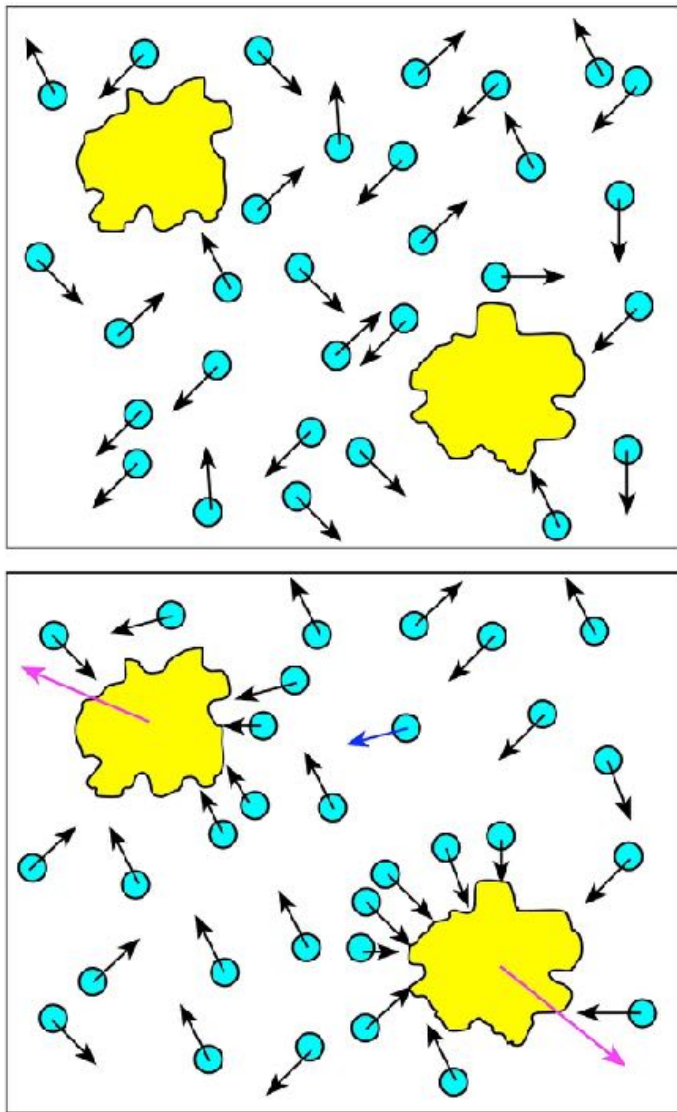


Robert Brown  
(1773-1858)

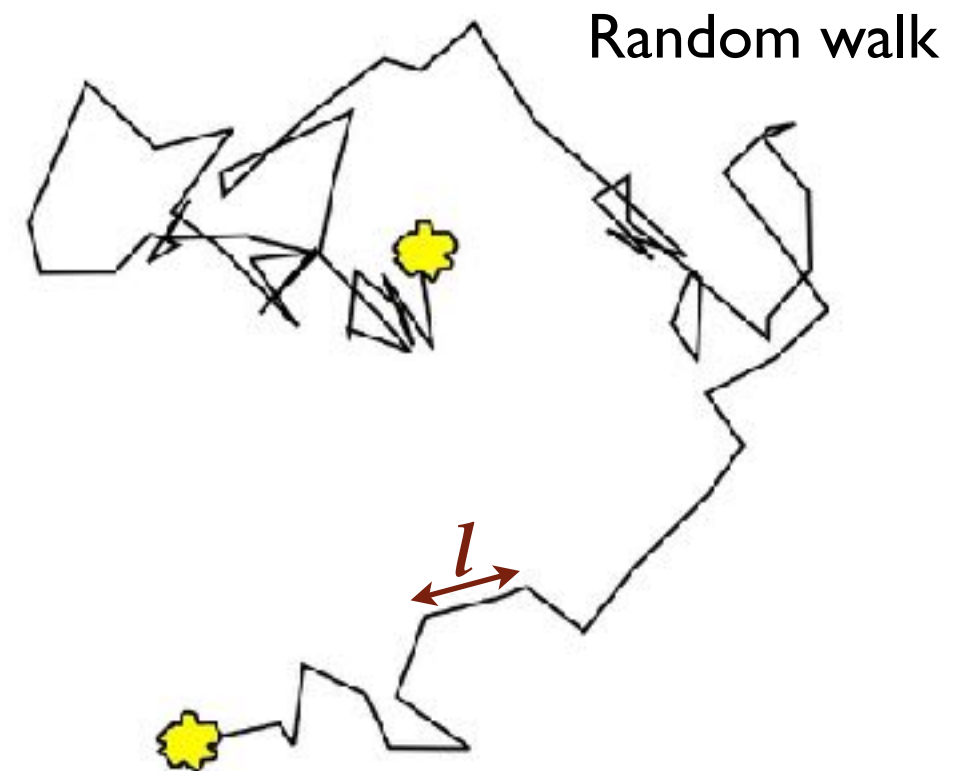


Lipid droplets suspended in milk  
(droplet size 0.5 - 3  $\mu\text{m}$ )

# 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 1. 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 concentration (gradient)  
 $D$  = constant (diffusion coefficient)

Diffusion coefficient:

$$D = \frac{1}{3} v l$$

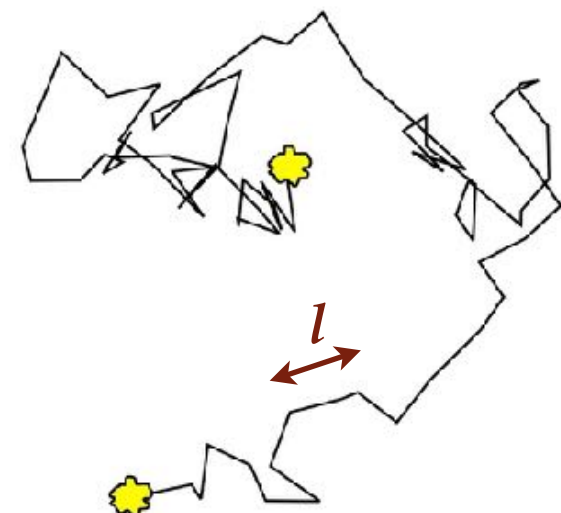
$v$  = average velocity of thermally propelled particle  
 $l$  = mean free path (average distance between consecutive collisions)  
 $D$  = amount of material transported across unit cross-sectional area per unit time (m<sup>2</sup>/s) (at unit concentration drop).

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  
 $\eta$  = viscosity  
 $r$  = radius of particle

Brownian motion



# DIFFUSION

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

Material flow:

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

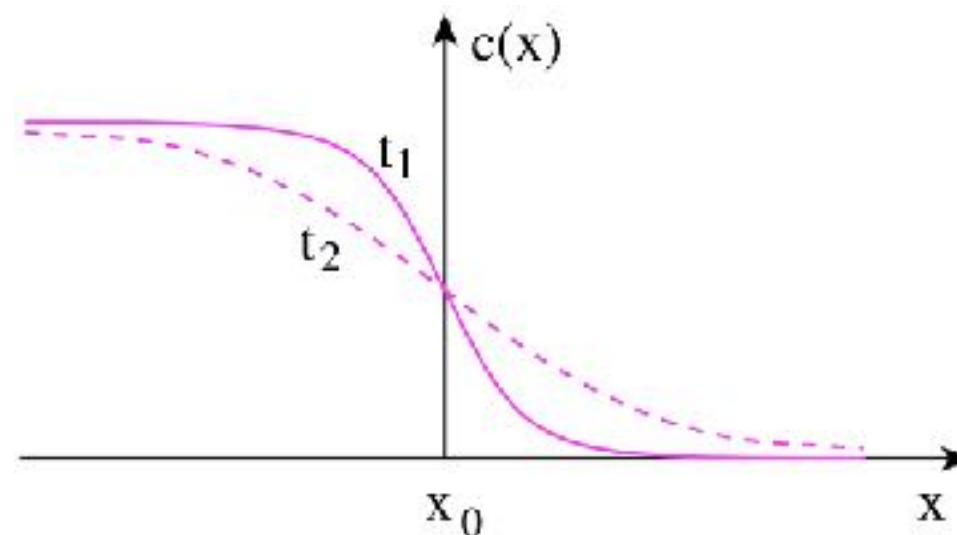
$J_n$  = flow density  
 $x$  = distance  
 $t$  = time

Diffusion coefficient:

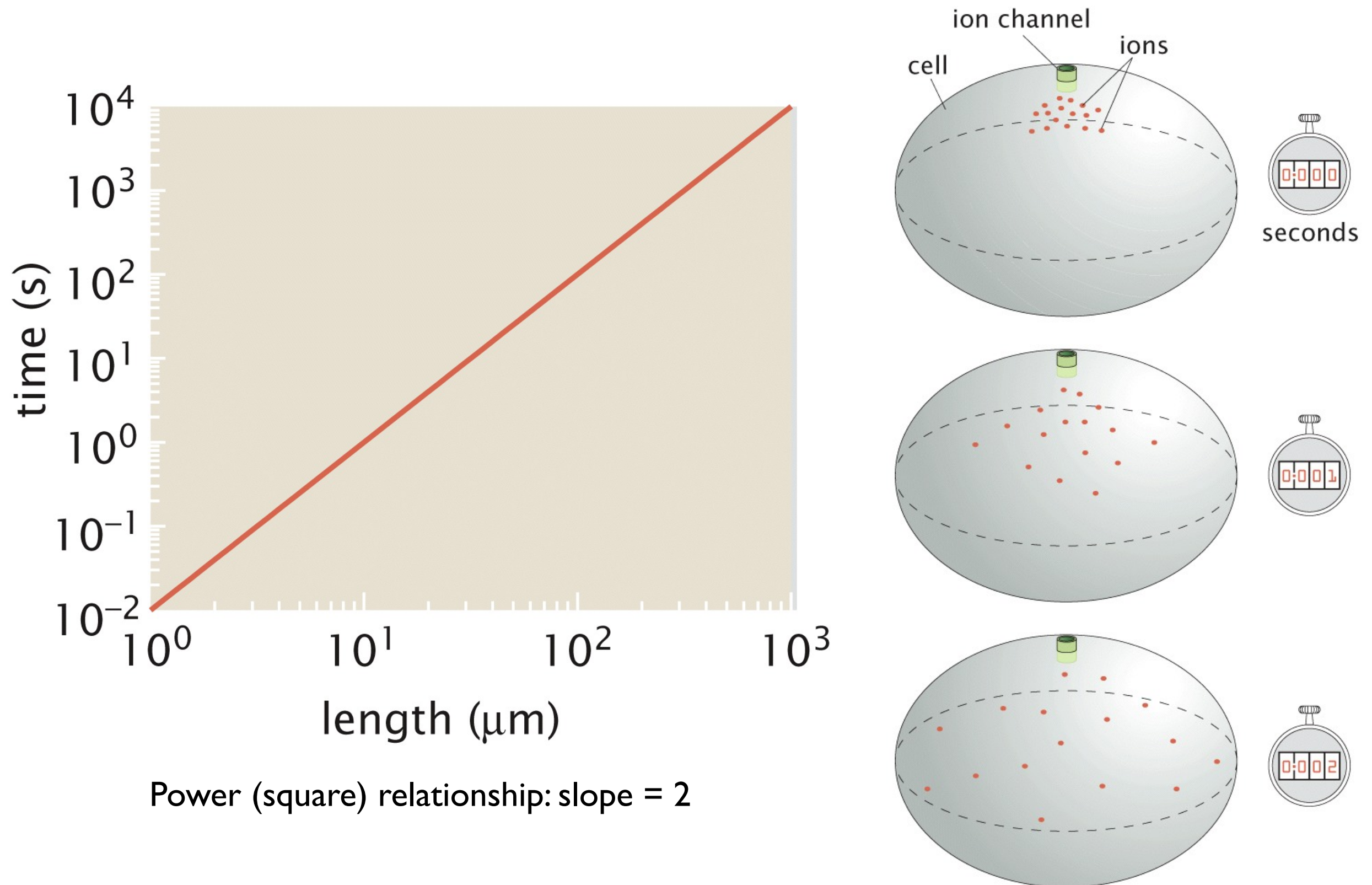
$$D \frac{\Delta \left( \frac{\Delta c}{\Delta x} \right)}{\Delta x} = \frac{\Delta c}{\Delta t}$$

$D$  = diffusion coefficient.

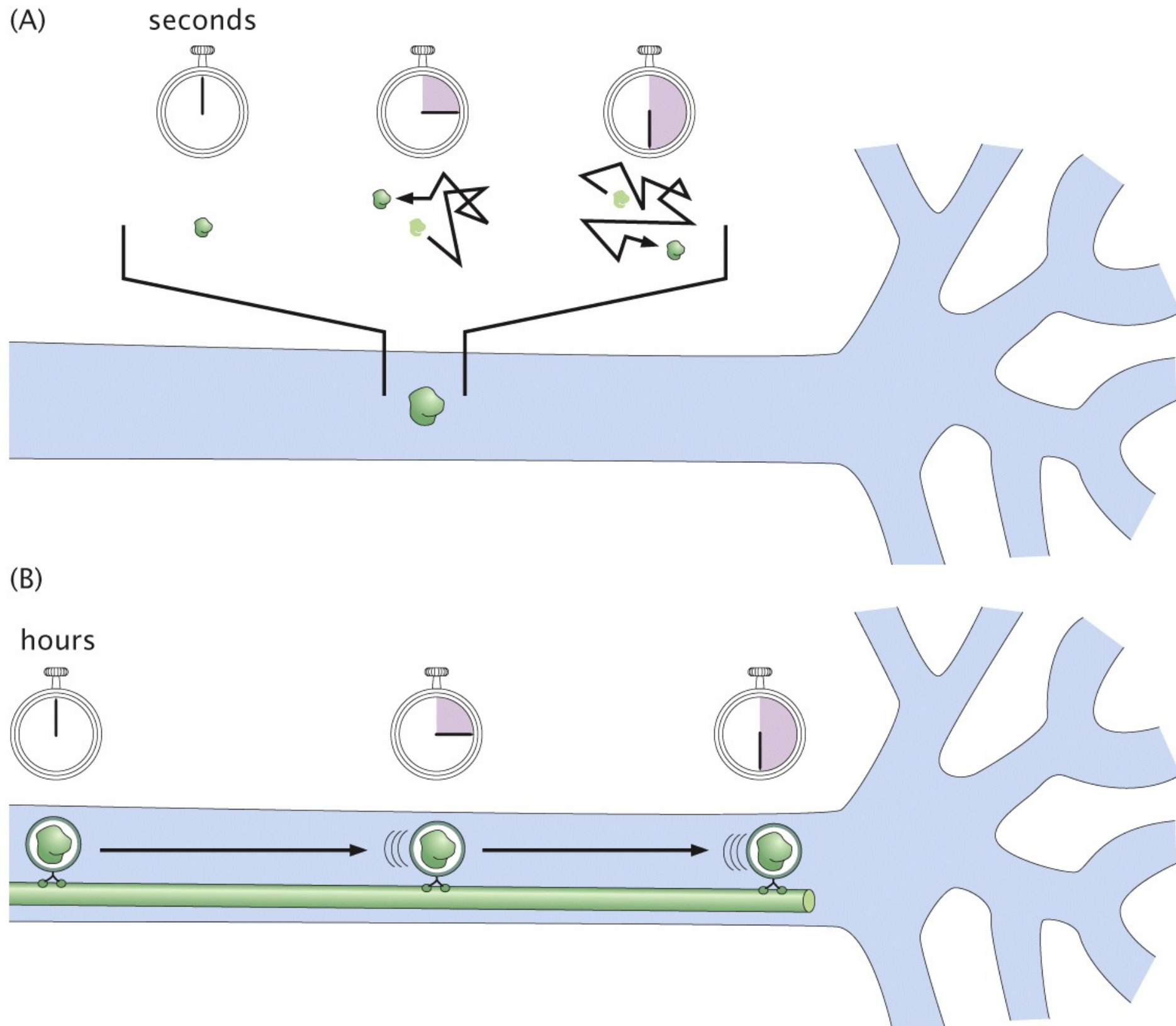
Concentration gradient decreases with time (boundary becomes smeared)



# Diffusion provides rapid transport only on a short length scale



# Diffusion provides rapid transport only on a short length scale



# Biopolymers (biological polymers)

Polymers:  
chains built up from monomers

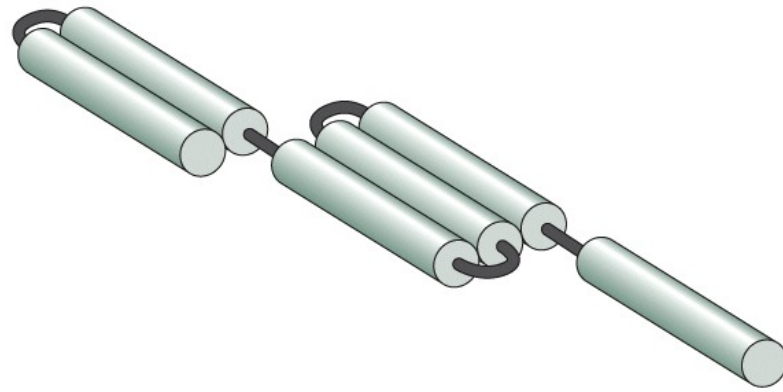
Number of monomers:  $N \gg 1$ ;  
Typically,  $N \sim 10^2 - 10^4$ ,  
but, in DNA, e.g.:  $N \sim 10^9 - 10^{10}$

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., $\alpha$ -glycosidic)
Protein polymer (e.g., microtubule)	Protein (e.g., tubulin)	Secondary

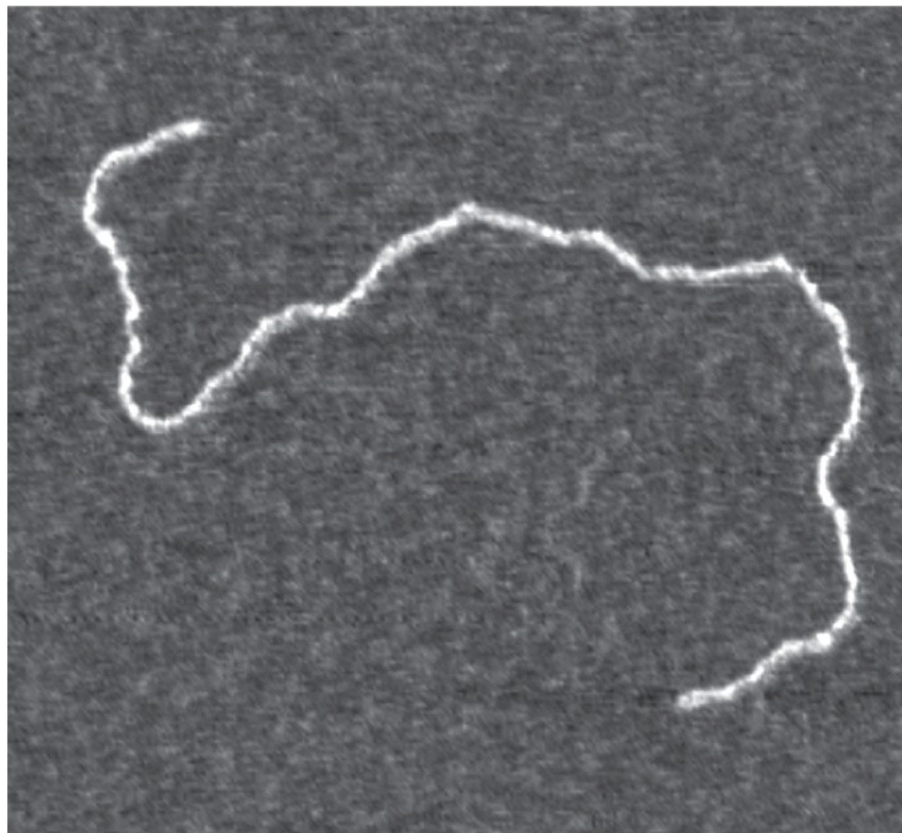
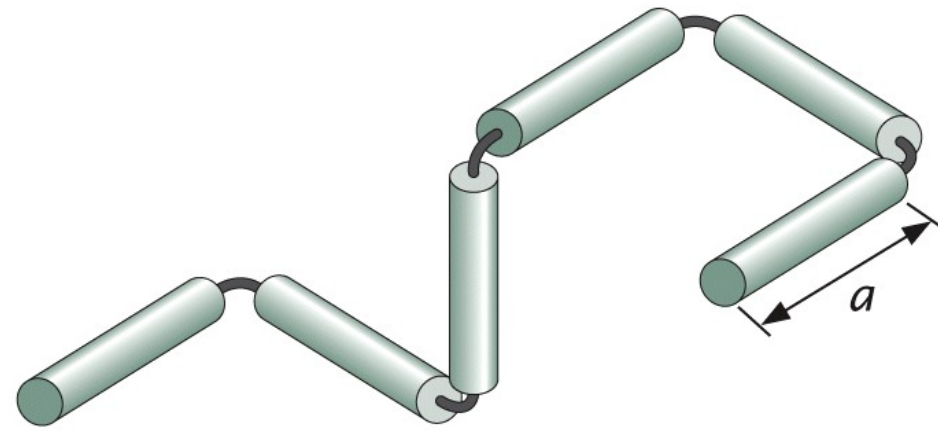


# Shape of the polymer chain resembles random walk

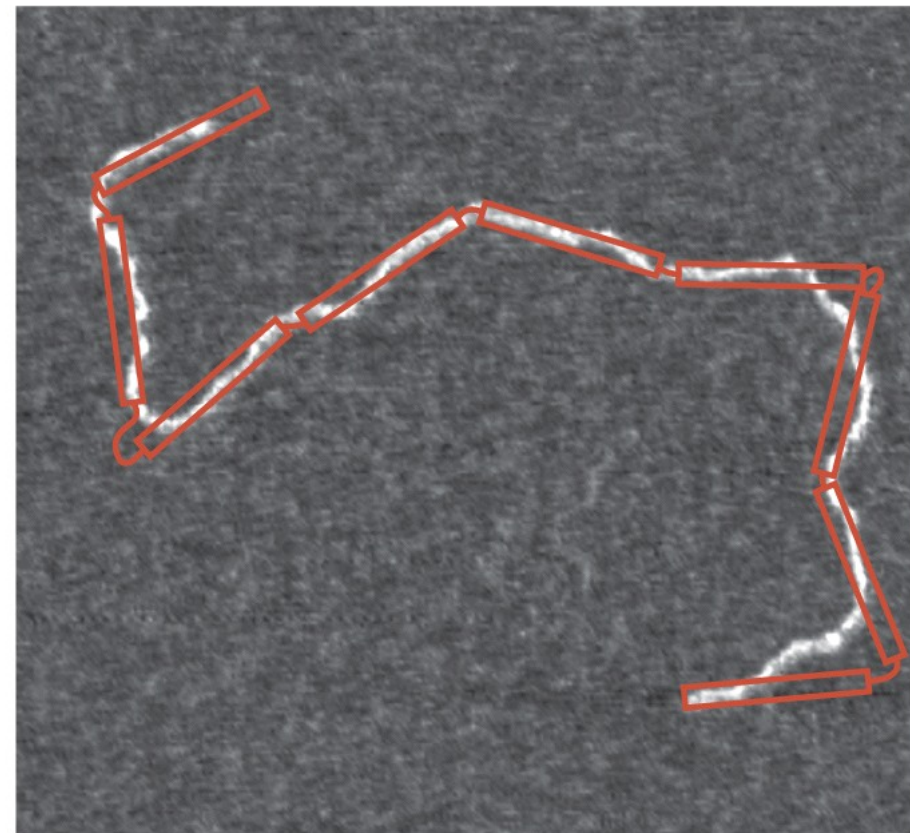
1D random walk



3D random walk



100 nm

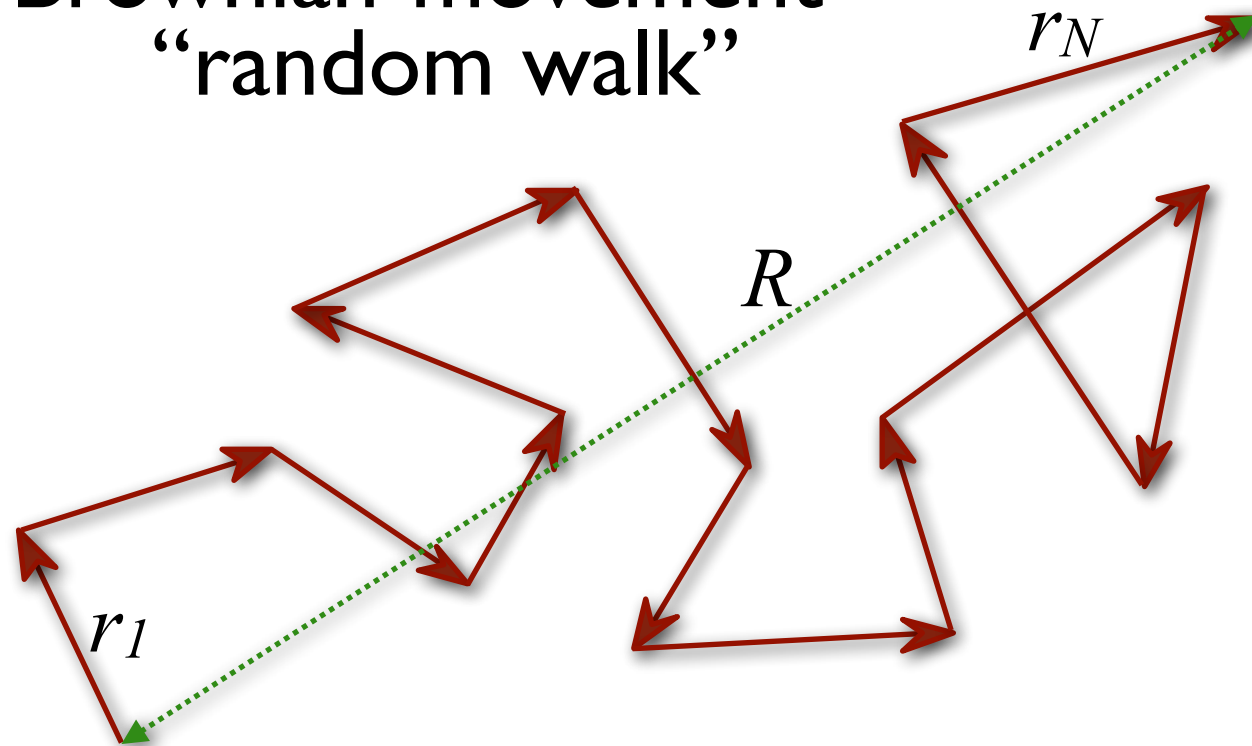


dsDNA molecule



# 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:  $v = \frac{l}{\tau}$

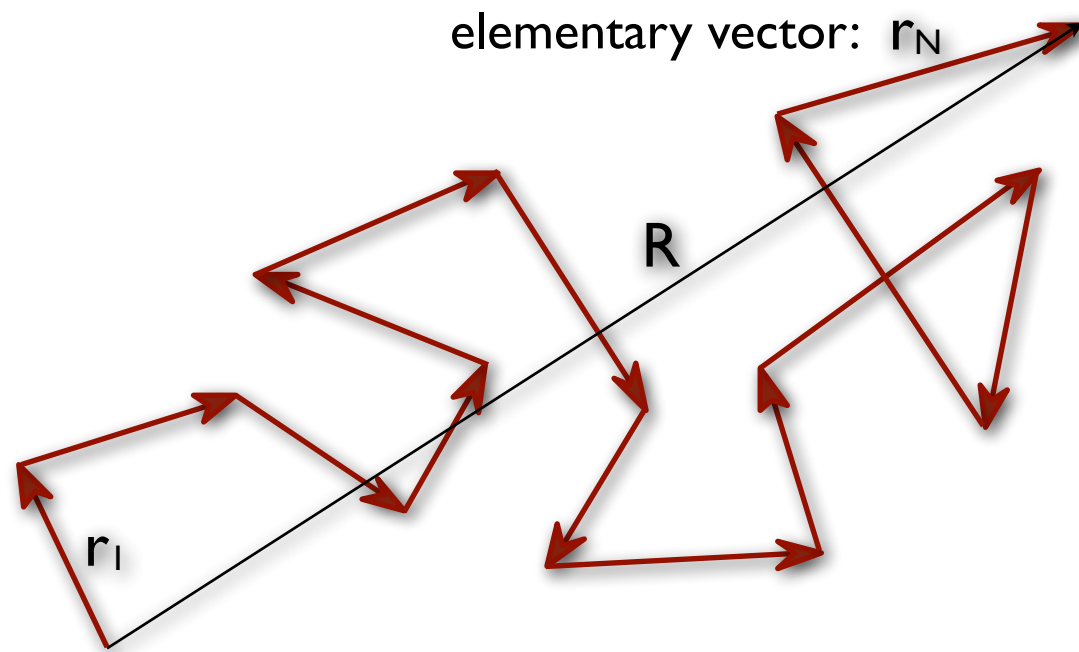
Total diffusion time:  $t = N\tau$

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

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

# 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:**

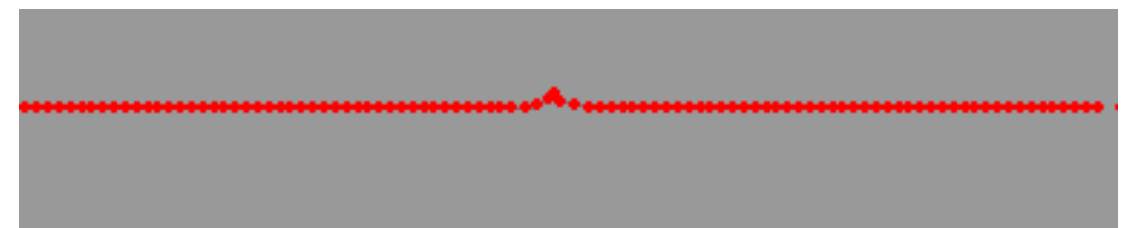
Upon thermal excitation, the polymer chain goes through random fluctuations, shape changes.



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



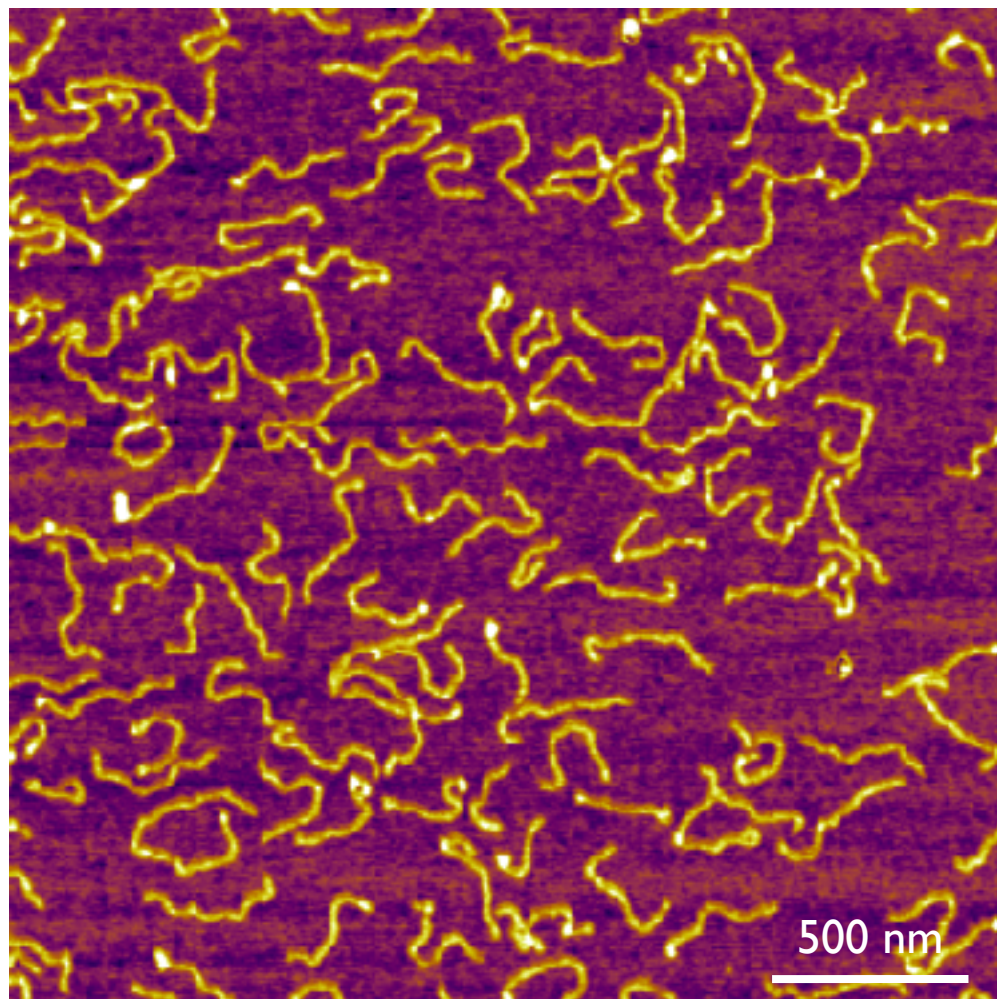
The polymer chain contracts.



# Equilibrium shape of a random polymer

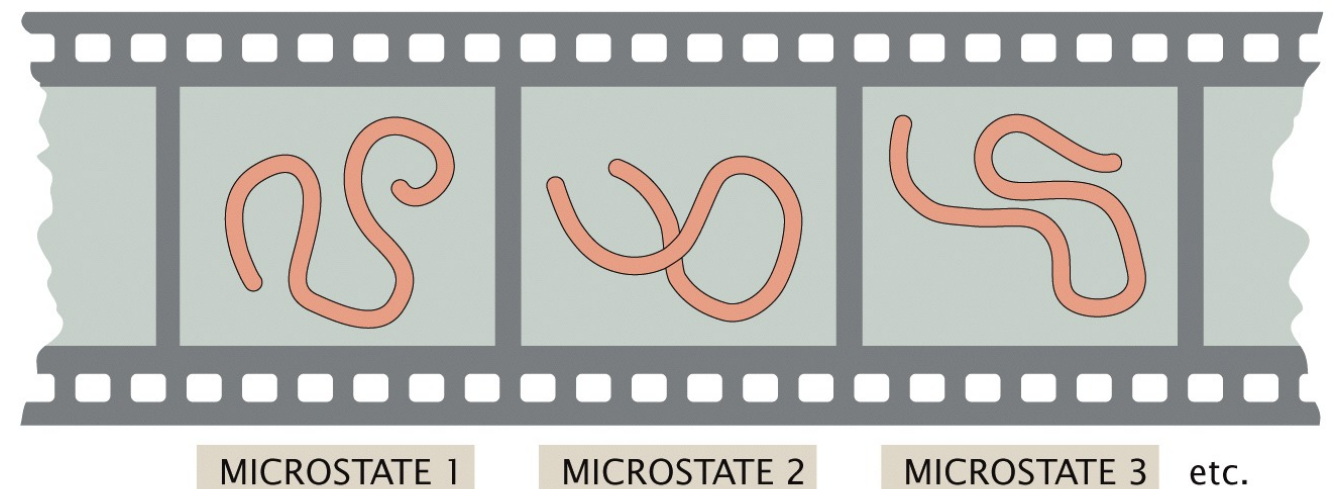
The macrostate which can be realized by the largest number of microstates (the most probable state)

Spatial distribution of microstates



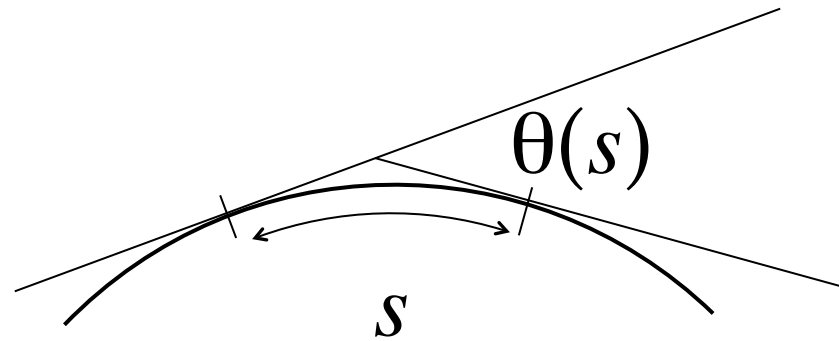
AFM image of surface-adsorbed dsDNA molecules (identical contour length, PCR product)

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

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

that is,  $\theta(s)$  may be between  $0^\circ$  and  $360^\circ$  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.



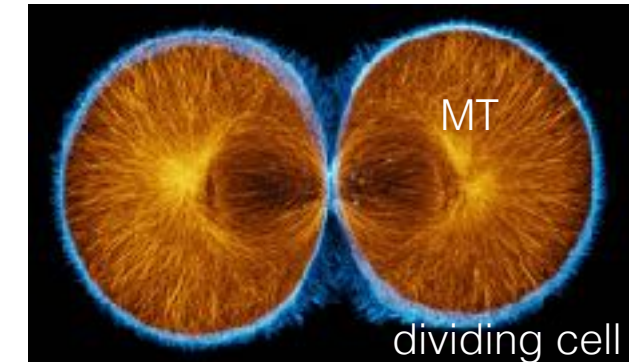
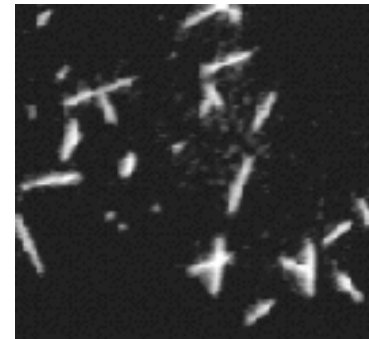
# Relationship between the shape and elasticity of the polymer chain

## Rigid chain

$$L_p \gg L_c$$

(mm  $\gg$  10  $\mu$ m)

Microtubule

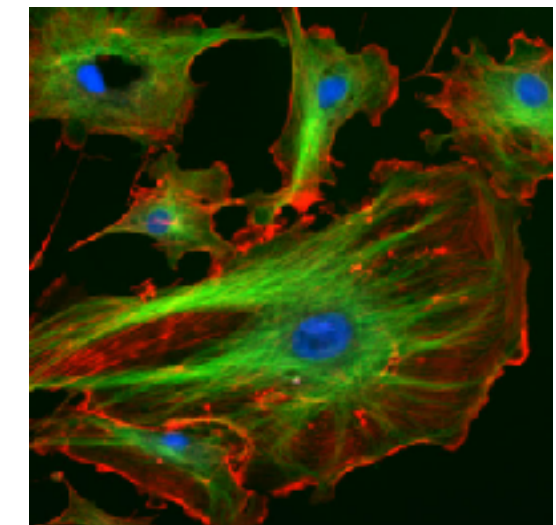


## Semiflexible chain

$$L_p \approx L_c$$

( $\mu$ m  $\approx$   $\mu$ m)

Microfilament (actin)

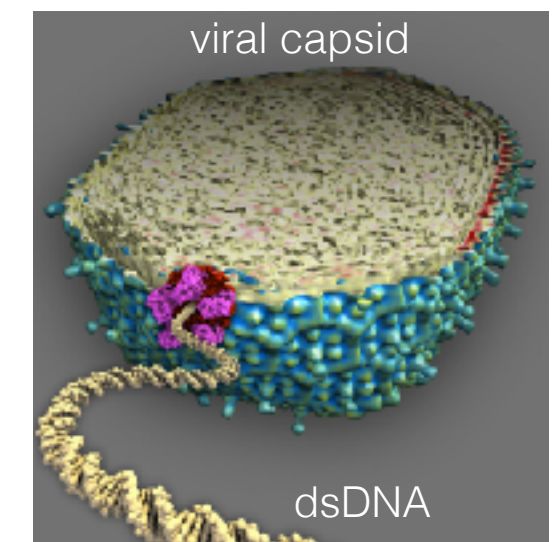
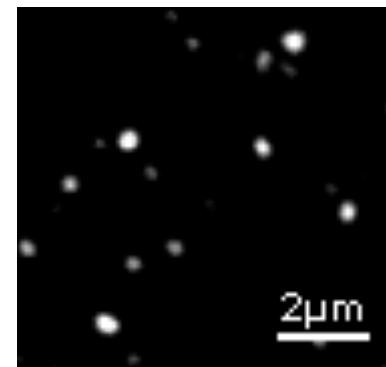
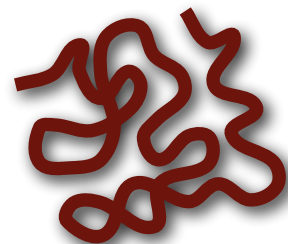


## Flexible chain

$$L_p \ll L_c$$

(50 nm  $\ll$  cm)

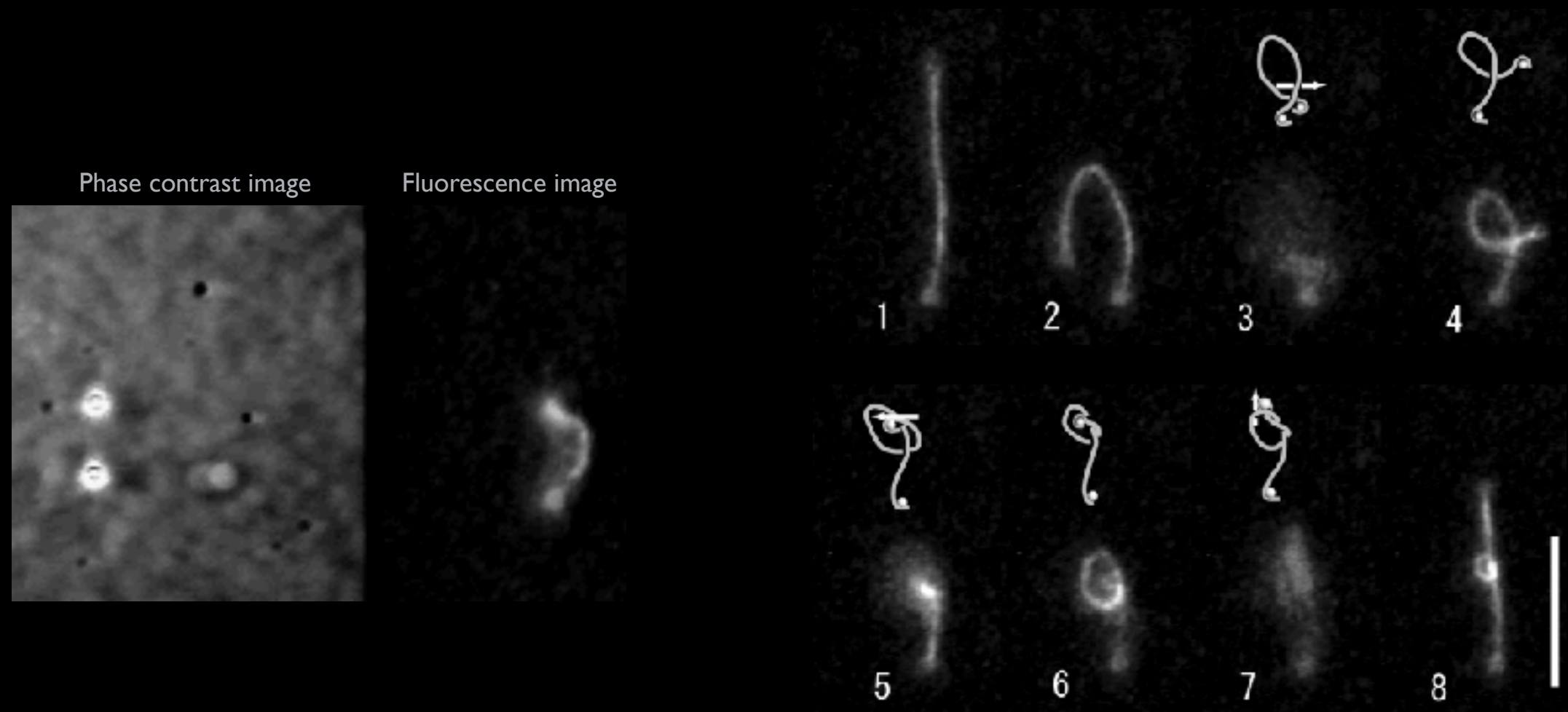
dsDNA



$L_p$  = persistence length  
 $L_c$  = contour length

# Visualization of a random (entropic) chain

Tying a knot on a single dsDNA molecule

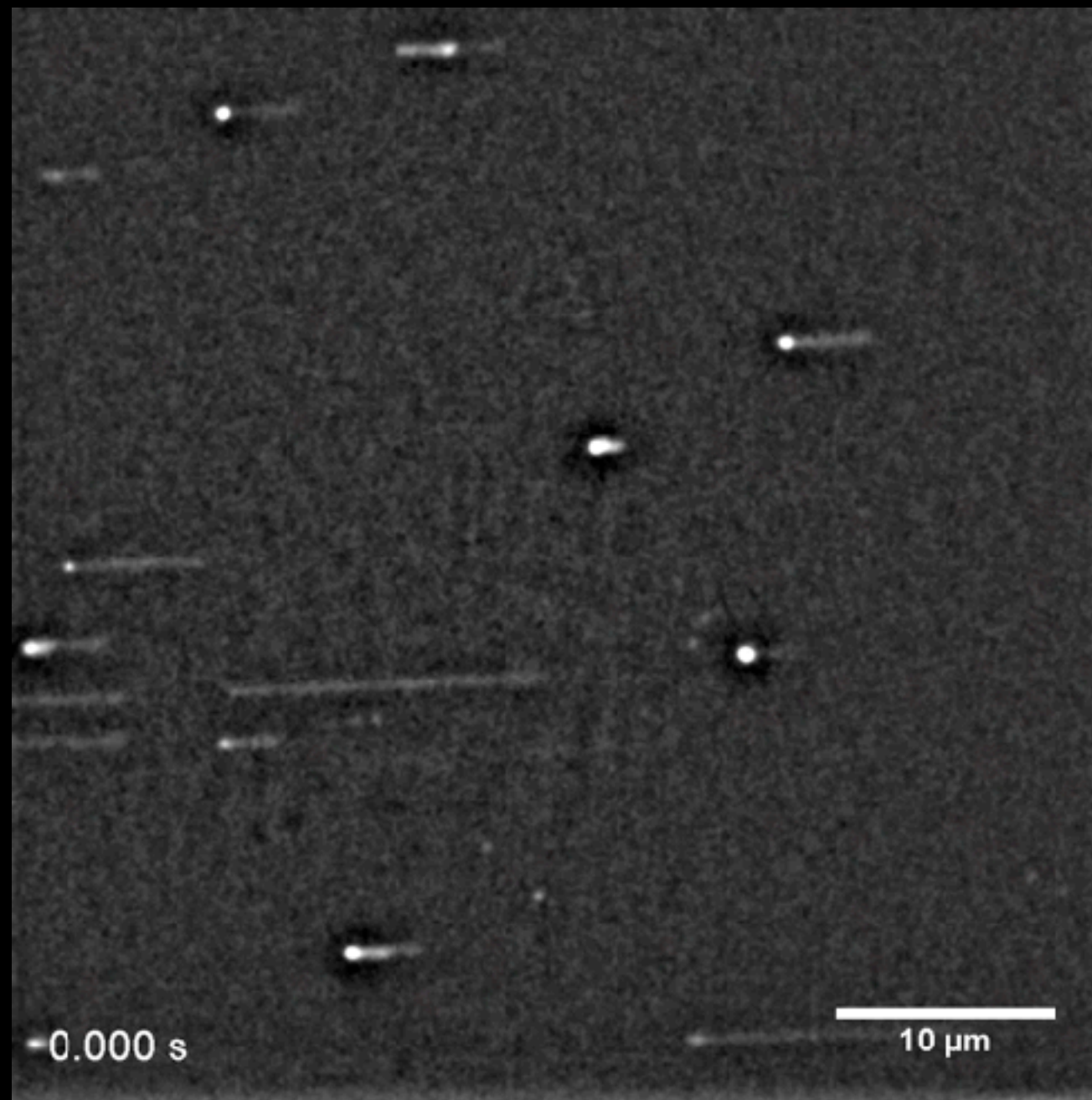




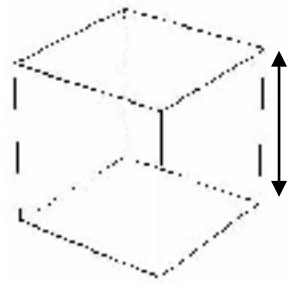
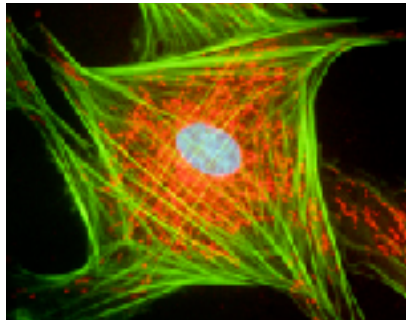
# Visualization of a random (entropic) chain

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

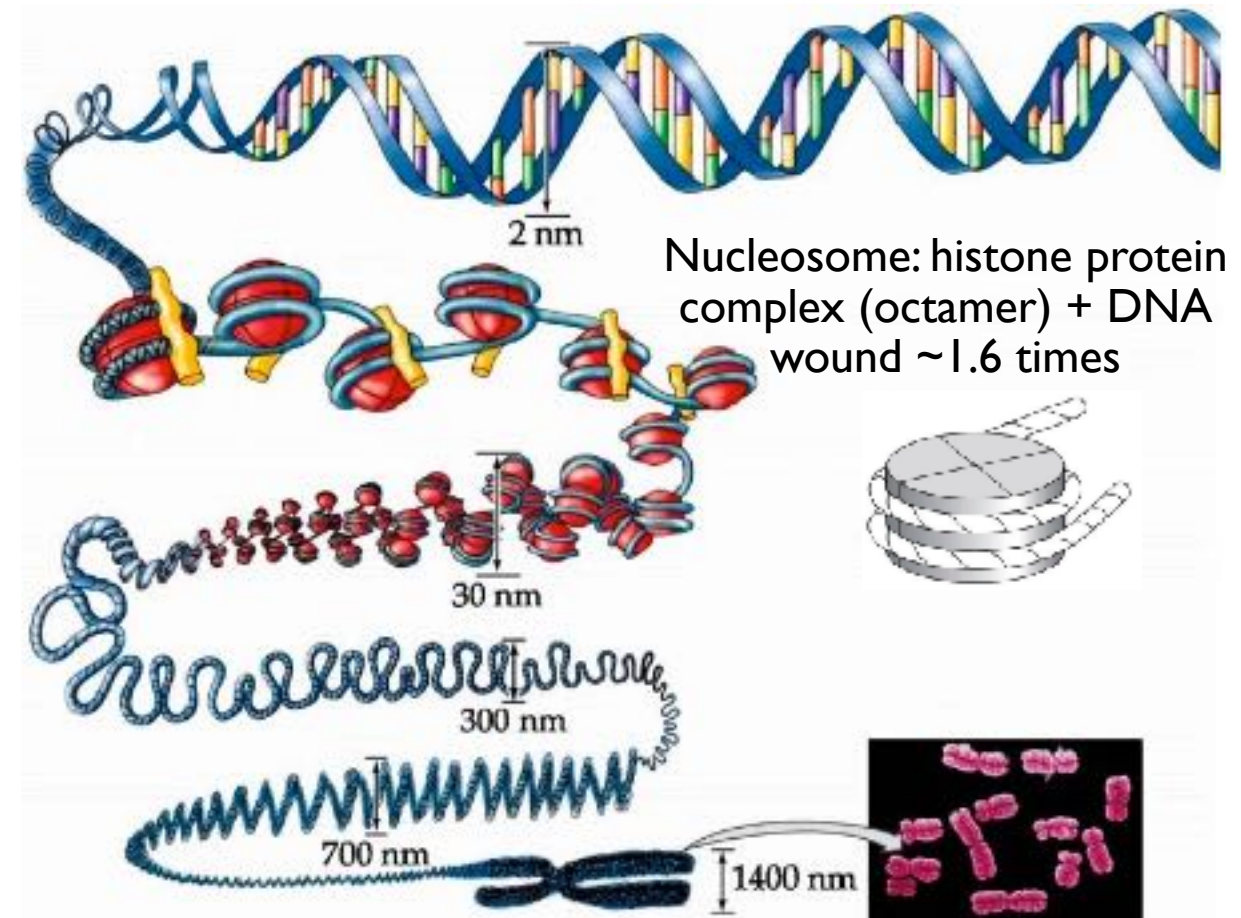


20  $\mu\text{m}$   
(20 m) Simplified cell  
model: cube

**Solution:** DNA needs  
to be packed!

	<b>Cell:</b> 20 $\mu\text{m}$ edge cube	Analog - <b>Lecture hall:</b> 20 m edge cube
DNA thickness	2 nm	2 mm
Full length of human DNA	$\sim 2$ m	$\sim 2000$ km (!!!) (Perimeter of Hungary: $\sim 2200$ km)
Persistence length of dsDNA ( $L_P$ )	$\sim 50$ nm	$\sim 50$ cm
Mean end-to-end length $\sqrt{\langle R^2 \rangle} = \sqrt{L_C L_P}$	$\sim 350$ $\mu\text{m}$ (!)	$\sim 350$ m (!)
Radius of gyration ( $R_G$ ) $R_G = R/\sqrt{6}$	130 $\mu\text{m}$	130 m
Volume of fully compacted DNA	$\sim 2 \times 2 \times 2$ $\mu\text{m}^3$	$\sim 2 \times 2 \times 2$ $\text{m}^3$ (= 8 $\text{m}^3$ )

## Chromosome condensation



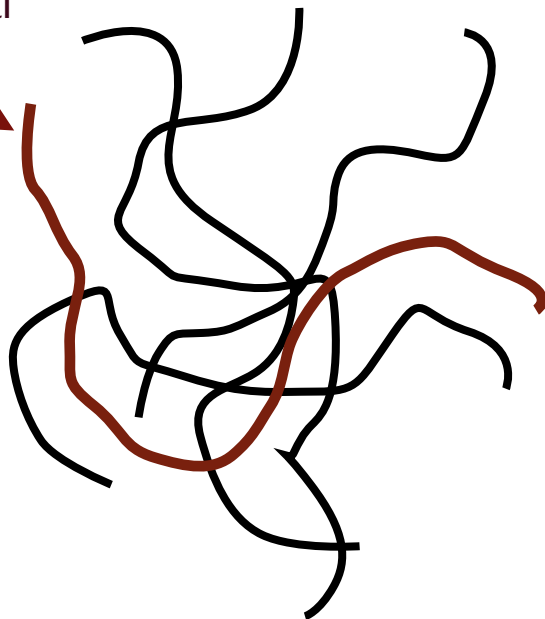
- **Condensins** play a role in high-order DNA packaging
- DNA chain: complex linear path with roadblocks!

# SPECIAL CASE OF DIFFUSION: REPTATION

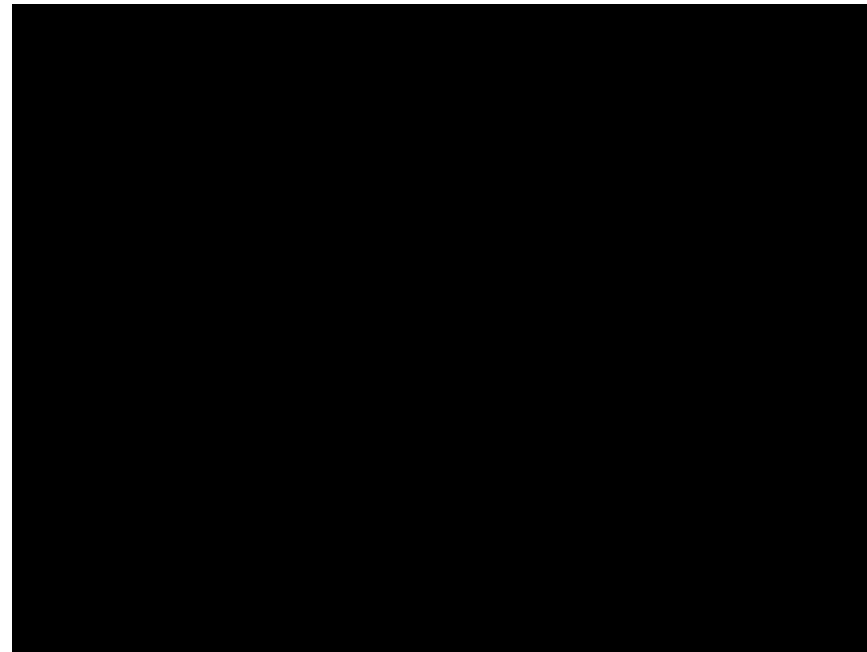
- *Reptation*: “snakelike” diffusional motion within polymer network (“*Reptilia*”)

Polymer matrix:  
“entanglement”

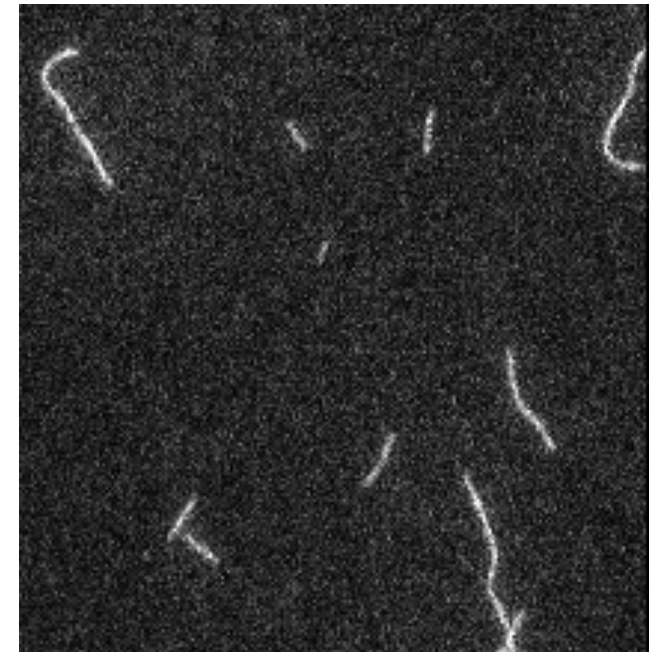
Filament inside  
the reptational  
channel



Indiana Jones and  
Raiders of the Lost Ark



Actin filaments in methyl-  
cellulose matrix.  
Unidirectional diffusion



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

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

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

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