

Diffusion:
the energy-saving way to move

30.10.2013

Diffusion

- Motivation:
Why is it interesting?
- Theory:
What is it?
- Application:
What can we learn from studying it?

Phenomenological definition

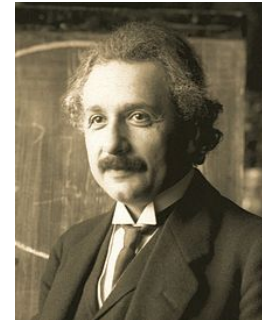
Diffusion

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Passive transport of particles due to
thermal energy

Important PIs

- Robert Brown,
botanist, 1773-1858
- Albert Einstein,
physicist, 1879-1955
- Marian Smoluchowski,
physicist, 1872-1917



Macroscopic visualization

- Pipette colored liquid in an agar plate and watch it over time

→ Video

Everything flows

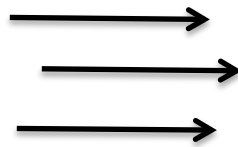
- Thermal energy:
 $k \cdot T = 4 \cdot 10^{-21} \text{ J}$
 - Electrostatic energy (2 charges at 3 nm):
 $q_1 q_2 / 4\pi \epsilon_0 \epsilon_r r = 1 \cdot 10^{-21} \text{ J}$
 - Gravitational energy (27 kD at 10 μm):
 $m \cdot g \cdot h = 4 \cdot 10^{-27} \text{ J}$
- Gravity is irrelevant on biological scales

Mass $\hat{=}$ diffusion coefficient

- Macroscopic world:
Immobile = Large mass



WIND
(random force)

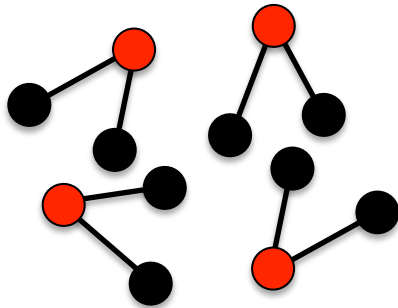


paper

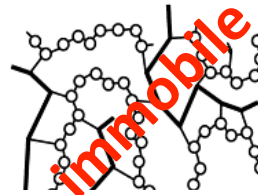
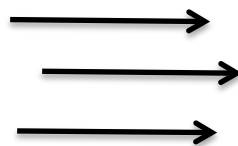


dumbbell

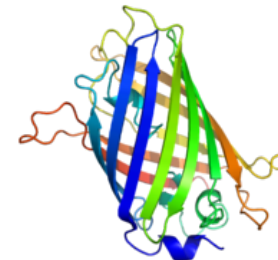
- Microscopic world:
Immobile = Large friction (=diffusion coefficient)



COLLISIONS
(random force)



dextran

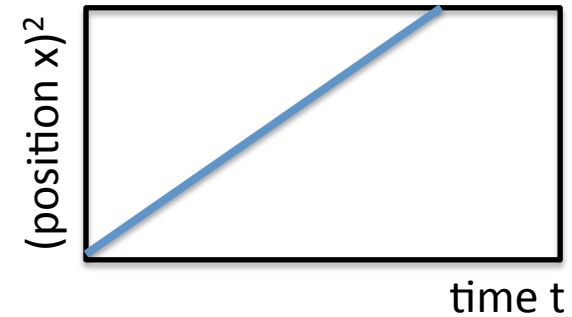
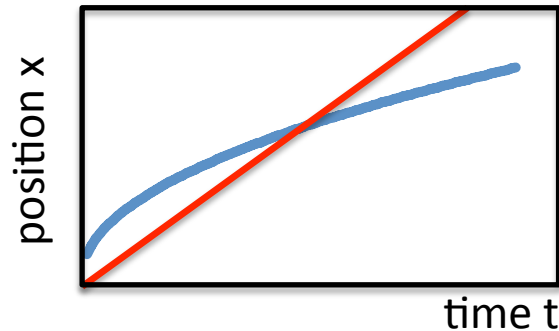


protein

The diffusion coefficient

- The diffusion coefficient describes how far a particle can travel within a given time t :

$$x = \sqrt{6Dt}$$

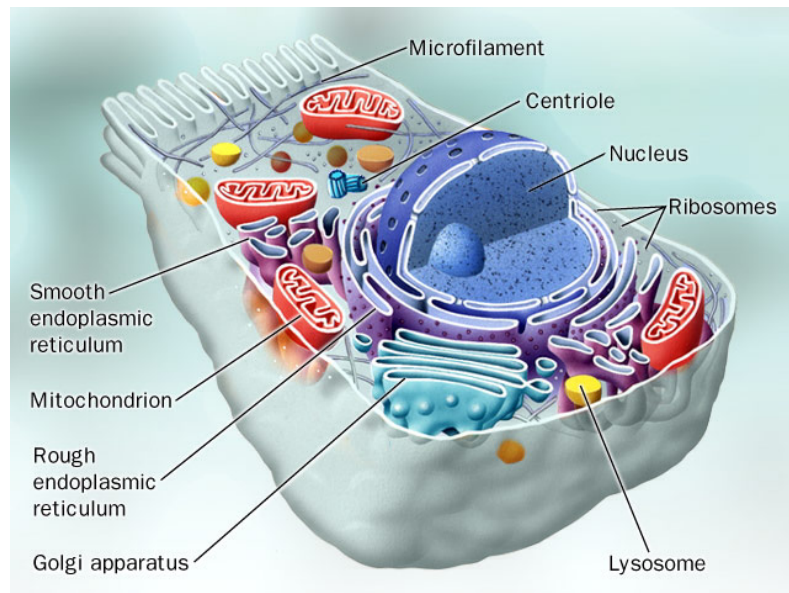


- For a sphere it is given by $D = \frac{kT}{6\pi\eta r}$
- A particle is fast at high temperature T , small radius r and low viscosity η of the liquid

Typical diffusion coefficients

- Proteins: 5-40 $\mu\text{m}^2\text{s}^{-1}$
 - mRNA: 0.04 $\mu\text{m}^2\text{s}^{-1}$
 - Membrane: 0.001 - 1 $\mu\text{m}^2\text{s}^{-1}$
 - Telomere: 0.002 $\mu\text{m}^2\text{s}^{-1}$
 - PML body: 0.0001 $\mu\text{m}^2\text{s}^{-1}$
- Proteins/RNAs need < 2 minutes to diffuse through the nucleus
- Nuclear bodies and chromosome loci are less mobile
- Only binding to chromatin/NBs can slow you down

The cell is well-mixed



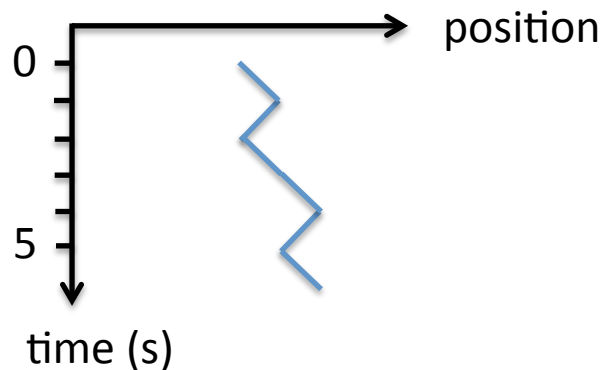
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- The content of all compartments is well-mixed
 - Only membranes represent barriers for diffusion
- Steady-state distribution not super-important:
Everything gets everywhere (example: NoRC, HP1)

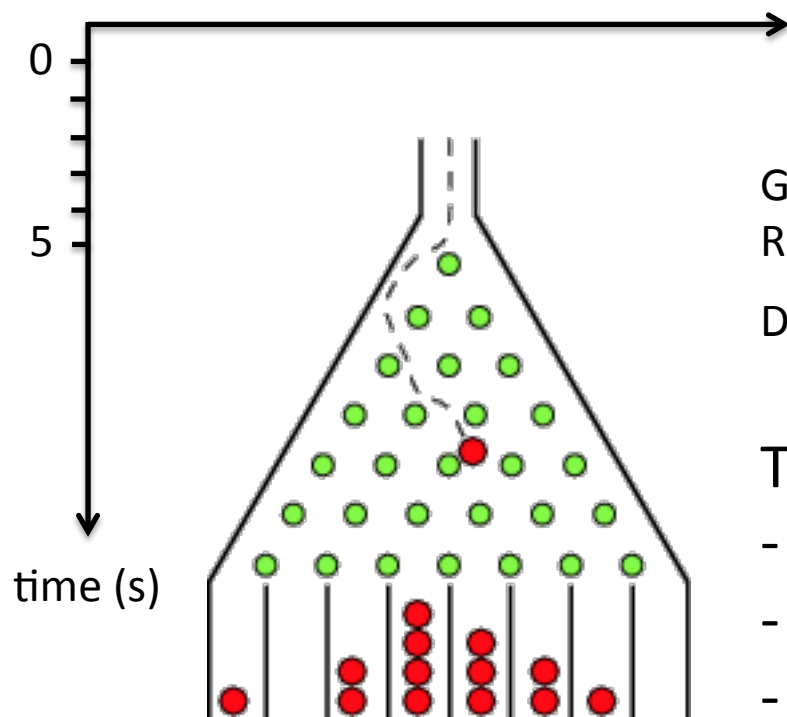
Microscopic world: Random Walk

- Consider a particle (or person) that moves every second randomly one step left or right



- Where is its most probable position?
- What is the most distant point it visited

The Galton board



Green: obstacles (nails)

Red: Spheres

Distance between nails: Step width

The 'Random walk' experiment:

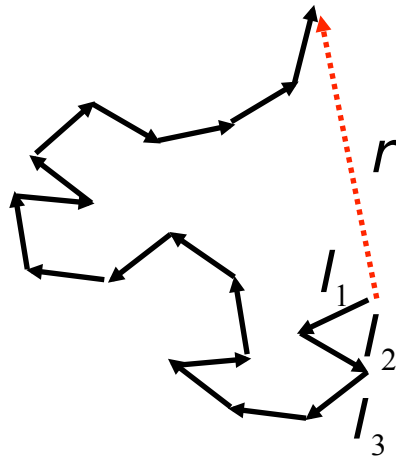
- Take spheres
- Throw them in a Galton board
- Look where they ended up

→ Video

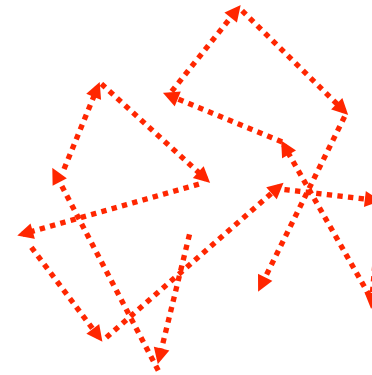
Random Walk: Features

- The average value of x is zero
(no net translocation/directionality)
- The longer the time the broader the final distribution
- Width = Mean squared displacement (MSD) that equals $x = \sqrt{6Dt}$
- Particles explore positions between $x = 0 \dots s \cdot t$
(with s being the step size), but efficiently
positions up to $x = \sqrt{6Dt}$

Diffusion in solution is a 3D random walk



$$r = \sum_{i=1}^n l_i$$



$$\langle r \rangle = 0$$

After n steps the molecule has moved a distance given by the vector r

The average of all vectors r , $\langle r \rangle$, is zero

The average distance the molecule has travelled after a certain number of steps or time is larger than zero

$$\sqrt{\langle r^2 \rangle} > 0$$

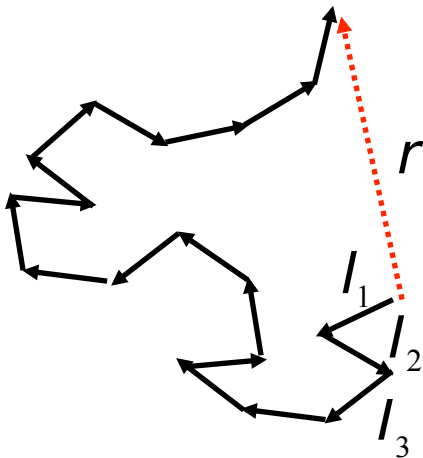
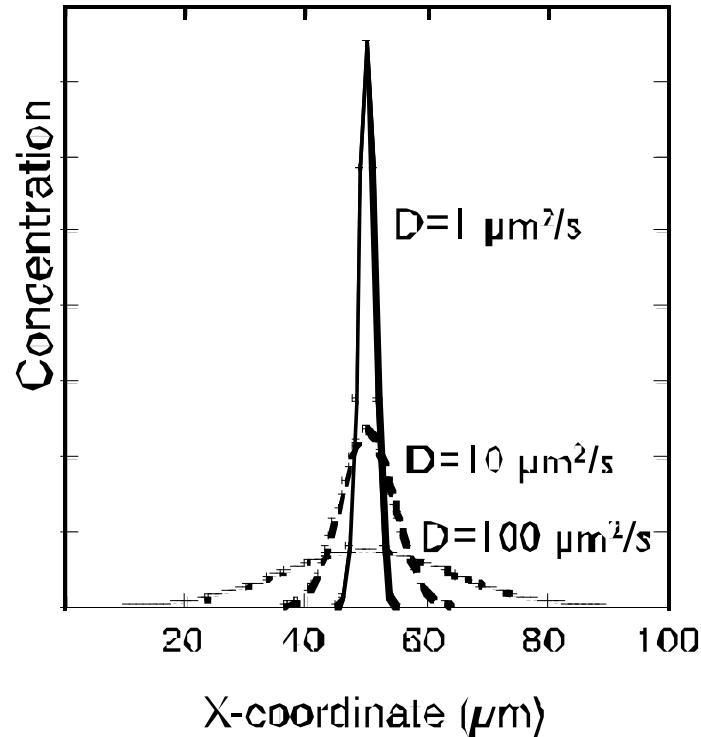
Particle transport by diffusion follows a random walk in time

Gaussian distribution

$$c(x, t) = \frac{1}{\sqrt{2Dt}} e^{-\frac{x^2}{4Dt}}$$

Start at $x=50$

Resulting distribution after 1 second:
equivalent to same D value at different times



After n steps the molecule has moved a distance given by the vector r

mean squared displacement in three dimensions: $\langle r^2 \rangle_{x,y,z} = 6Dt$

Displacement of proteins due to diffusion after a certain time

one dimension: $\langle d^2 \rangle_x = 2 D t$

two dimensions: $\langle d^2 \rangle_{x,y} = 4 D t$

three dimensions: $\langle d^2 \rangle_{x,y,z} = 6 D t$

D (in $\text{cm}^2 \cdot \text{s}^{-1}$) is the diffusion coefficient
(reference state: pure water at 20 °C)

Lysozyme and other proteins (10 - 100 kDa):

$$D = 10^{-6} \text{ cm}^2 \cdot \text{s}^{-1} = 100 \text{ } \mu\text{m}^2 \cdot \text{s}^{-1}$$

RNA polymerase II complex (2000 kDa): $D \approx 10^{-7} \text{ cm}^2 \cdot \text{s}^{-1}$

The value of D depends on the temperature T , the viscosity η of the solution and the hydrodynamic radius

r

$$D = \frac{k_B T}{f} = \frac{k_B T}{6 \pi \eta r} \quad \text{Stokes-Einstein relation}$$

The hydrodynamic radius r is the radius of a sphere that would have the same diffusion coefficient than the particle

$$f = 6 \pi \eta r \quad \text{Stokes relation for friction coefficient } f \text{ of a sphere with radius } r$$

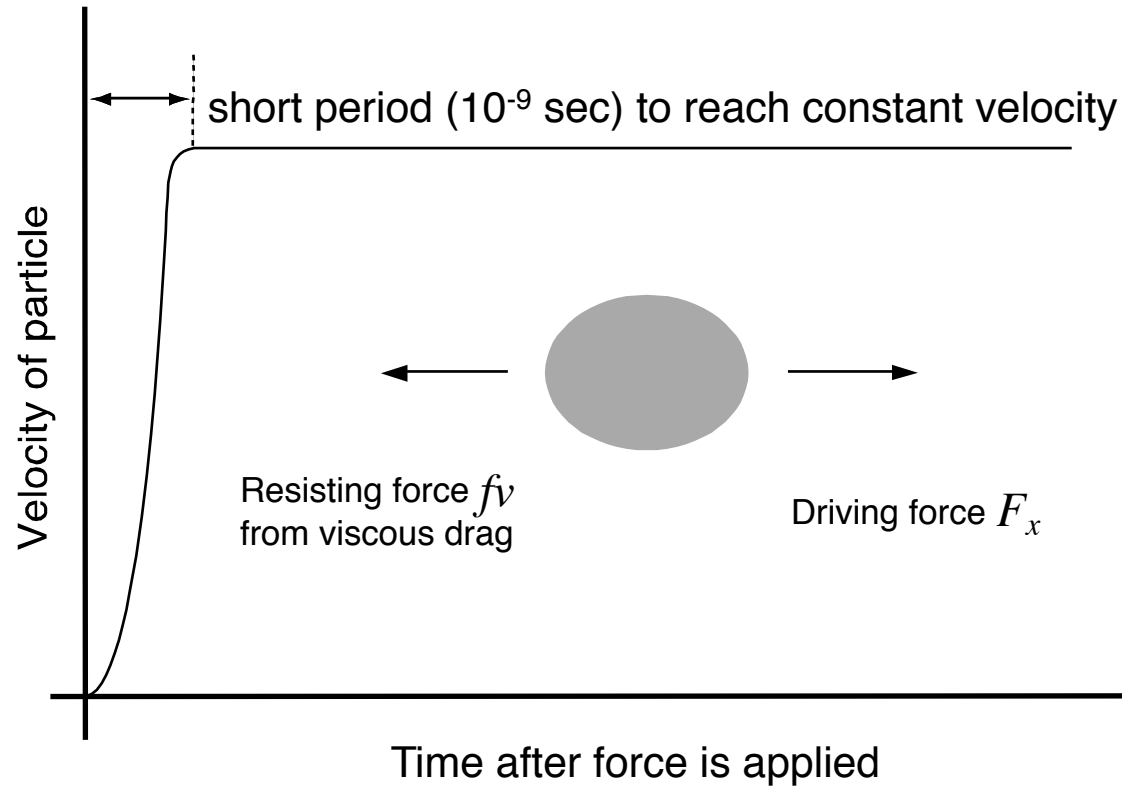
$k_B T$ (Boltzmann constant times temperature) = $4 \cdot 10^{-21}$ J at physiological temperature

viscosity η for water/buffer is $1 \cdot 10^{-3}$ Pa s

Parameters that describe the hydrodynamic properties of macromolecules in solution

- diffusion coefficient D $\rightarrow D = \frac{kT}{f}$
- frictional coefficient f \rightarrow
- sedimentation coefficient s $s = \frac{dr/dt}{\omega^2 r} = \frac{M \cdot (1 - \bar{v}\rho)}{N_A f_t}$
- partial specific volume \bar{v} () $\bar{v} = \frac{\partial v}{\partial m}$
 protein: 0.73 ml g⁻¹, DNA: 0.55 ml g⁻¹
- mass M of the molecules
- density ρ of buffer, 0.9982 g ml⁻¹ for water at 20 °C
- viscosity η of buffer, 1.002 mPa second for water at 20 °C

Directed movement in solution induced by an external force (gravitation, centrifugation, electric field)



$$-f \cdot v + F_x = 0, \text{ or } F_x = f v$$

⇒ if we measure the velocity of motion produced by a known force we can determine the friction coefficient and diffusion coefficient

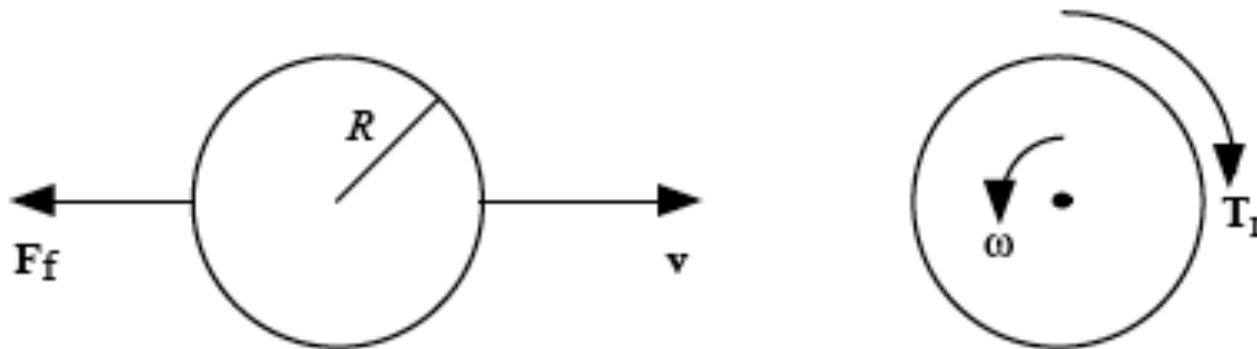
Translational and rotational frictional coefficients

$$F_f = -f_t \cdot v$$

A particle is moved with velocity v . F_f is the resulting frictional force with the frictional coefficient f_t for the translation

$$T_r = -f_r \cdot \omega$$

For the rotation of a particle with angular velocity ω the frictional torque T_r resisting rotation has the frictional coefficient f_r



$$D = \frac{kT}{f}$$

The frictional coefficient f is related to the diffusion coefficient D as derived by Einstein

Frictional coefficient of a spherical protein

$$V_{\text{sphere}} = \frac{4}{3} \cdot \pi \cdot r^3$$

A sphere has radius r and volume V .

$$V_{\text{sphere}} = \frac{M \cdot \bar{v}}{N_A}$$

V is calculated from molecular mass M , the partial specific volume \bar{v} () and Avogadro's number N_A .

$$\bar{v} = \frac{\partial v}{\partial m}$$

\bar{v} is about 0.73 ml/g for proteins and 0.55 ml/g for DNA

$$f = 6 \cdot \pi \cdot \eta \cdot r$$

For the translation the resulting frictional coefficient f in a medium with viscosity η can be calculated according to Stokes law.

$$f_r = 8 \cdot \pi \cdot \eta \cdot r^3$$

The frictional coefficient f_r for the rotation

Parameters that describe the hydrodynamic properties of macromolecules in solution

Diffusion coefficient D depends

- on shape = frictional coefficient f (not on particle mass!)
- absolute temperature T
- Boltzmann constant k

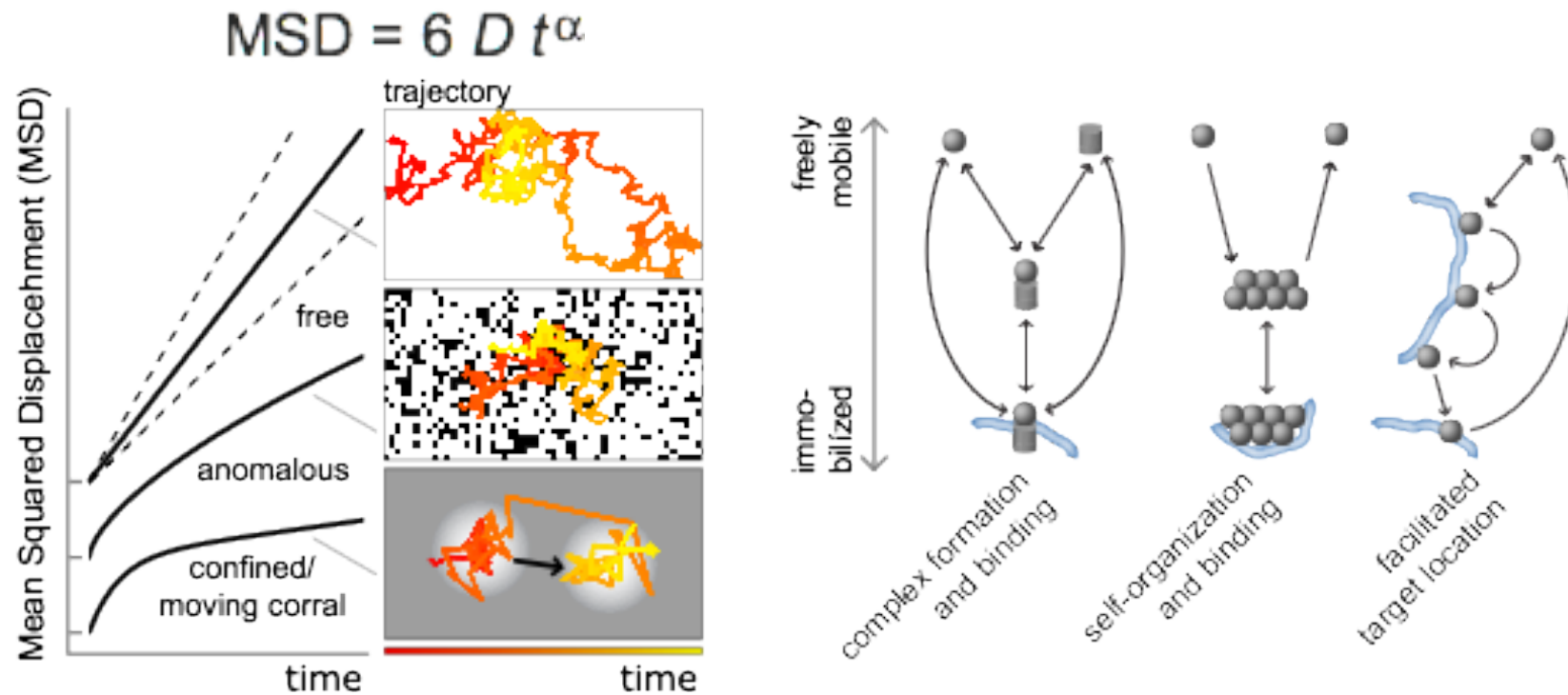
$$D = \frac{kT}{f}$$

Sedimentation coefficient s depends

- on friction coefficient f (= shape)
- on molar mass M (or particle mass m)
- density ρ of buffer, 0.9982 g ml⁻¹ for water at 20 °C
- viscosity η of buffer, 1.002 mPa second for water at 20 °C
- partial specific volume v bar (\bar{v})
protein: 0.73 ml g⁻¹, DNA: 0.55 ml g⁻¹

$$s = \frac{dr/dt}{\omega^2 r} = \frac{M \cdot (1 - \bar{v}\rho)}{N_A f_t}$$

Protein mobility due to diffusion



Dependence of diffusion coefficient D and molecular mass M

protein: $D \propto M^{-\frac{1}{3}}$

DNA: $D \propto M^{-\frac{1}{2}}$

double mass $M \Rightarrow$ 0.8 fold lower D double mass $M \Rightarrow$ 0.7 fold lower D

Why studying diffusion?

Learn about:

- the macromolecules
- maximum reaction rates
- the environment (around the tracer)
- pattern formation
- many other things

Activity and mobility

- Structural protein → immobile
- Active chromatin remodeler → immobile

Examples:

- High mobility of HP1:
Only small part is structural
- High mobility of ISWI remodelers:
Only small part is actively remodeling

Smoluchowski limit

- For chemical reactions both reactants have to collide, which they do during their random walk
- Thus, the maximum reaction rate is determined by the diffusion coefficients:



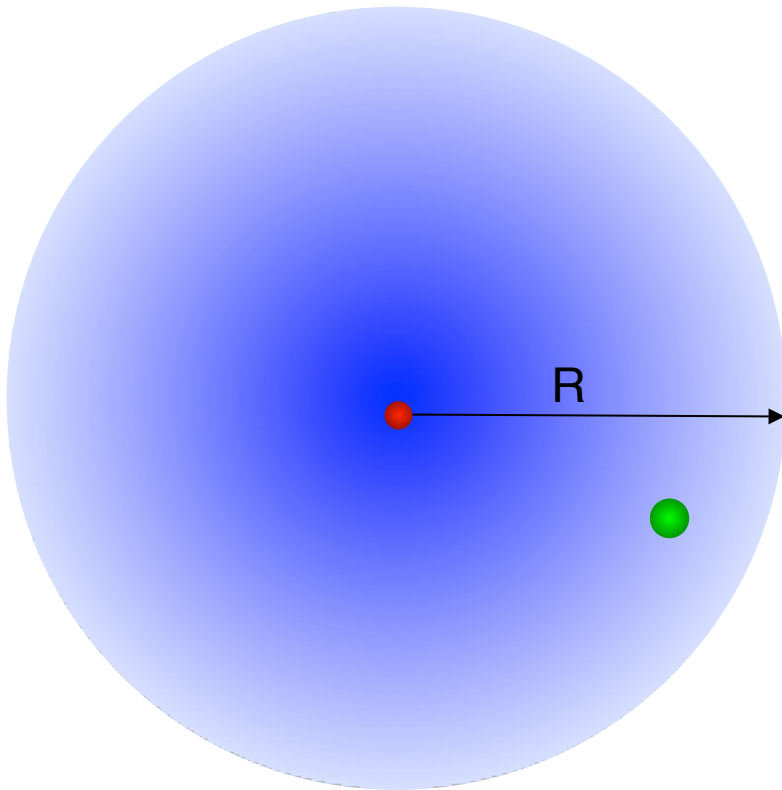
$$k_{\max} = (D_A + D_B)(r_A + r_B)N_0 / 1000$$

Finding home ...

Drunkard: “Will I ever, ever get home again?”

George Pólya (1921): “You cant’ t miss; just keep going and stay out of 3D!”

The mean diffusion time to reach a small target of radius r in the middle of a cell of radius R with $R \gg r$



one

dimension:

$$\tau_1 = \frac{R^2}{3D_1}$$

two dimensions:

$$\tau_2 = \frac{R^2}{2D_2} \cdot \ln\left(\frac{R}{r}\right)$$

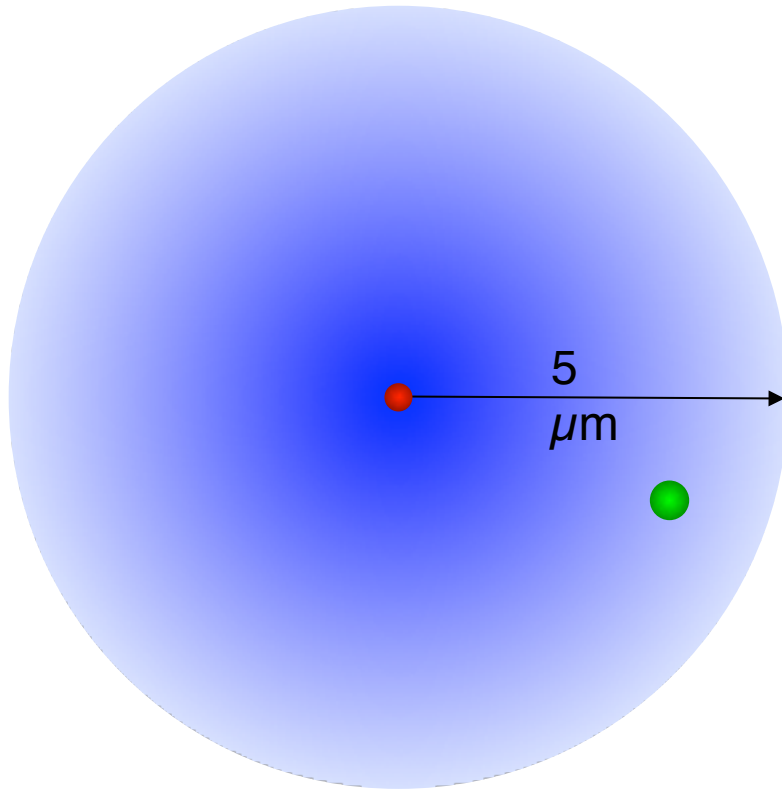
three dimensions:

$$\tau_3 = \frac{R^2}{3D_3} \cdot \frac{R}{r}$$

● Protein with diffusion constant D

● target with radius r

How long would it take on an average for RNA polymerase II to find its target promoter?



one dimension: $t \approx 4$ sec

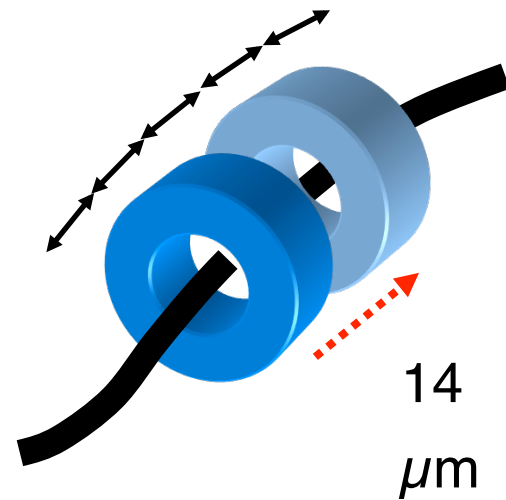
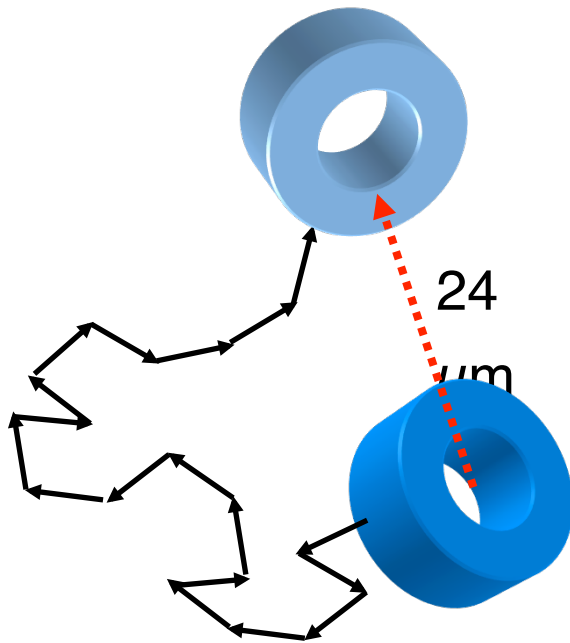
two dimensions: $t \approx 40$ sec

three dimensions: $t \approx 2000$ sec

● Pol II complex ($M \approx 2\,000$ kDa)
 $D_{\text{eff}} \approx 2 \cdot 10^{-8} \text{ cm}^2 \cdot \text{s}^{-1}$

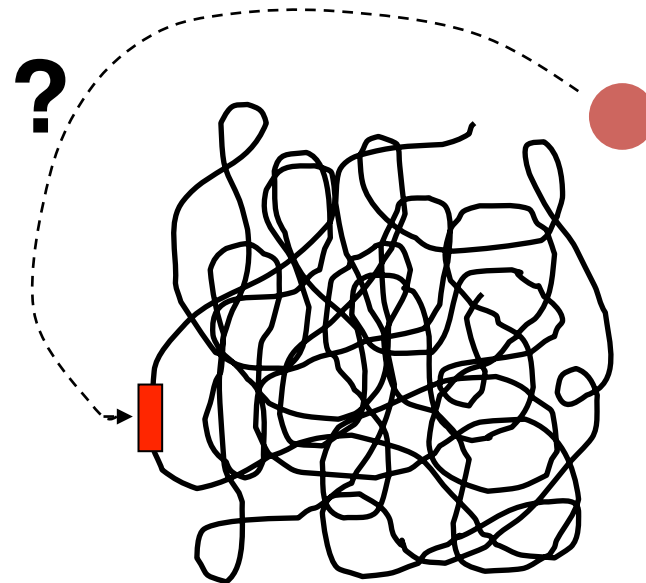
● target promoter ($r = 5$ nm)

For a protein with $D = 10^{-6} \text{ cm}^2 \cdot \text{s}^{-1}$ the displacement after 1 sec would be $24 \mu\text{m}$ in 3 dimensions and $14 \mu\text{m}$ in 1 dimension



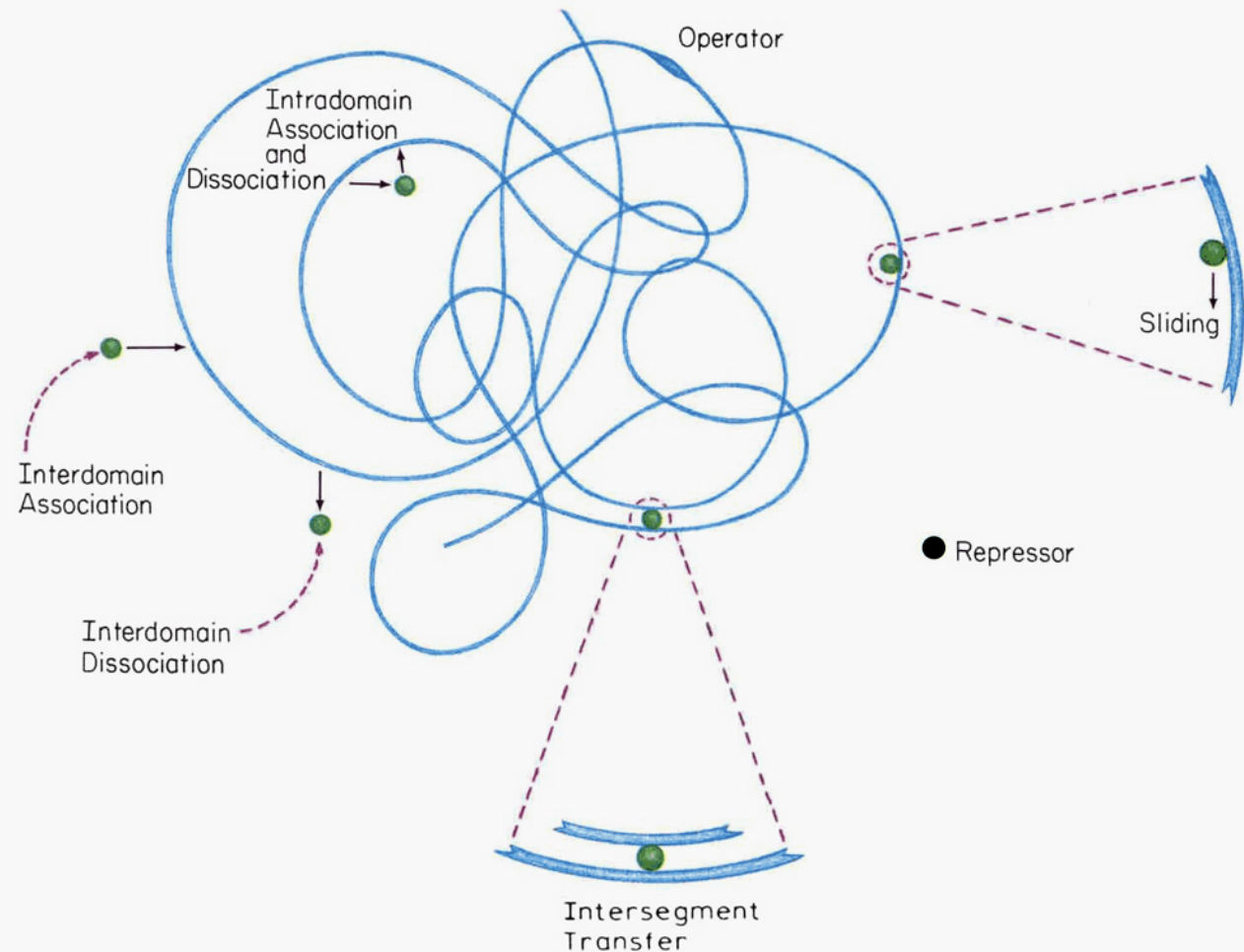
Facilitated diffusion of proteins on DNA

the problem: How will a protein find its binding site on a long DNA?

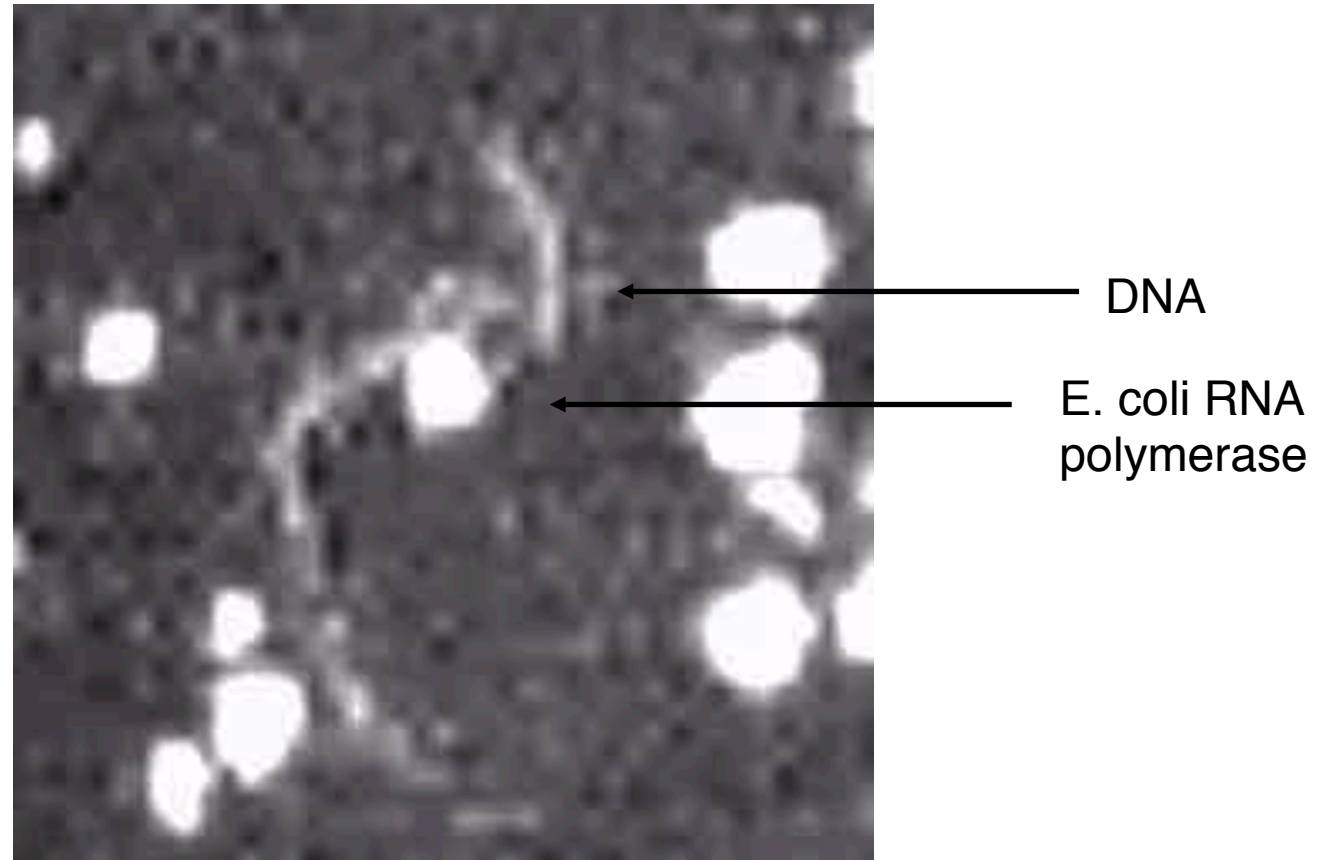


Model for facilitated diffusion of lac repressor

FIG. 1. Schematic view of *lac* repressor interacting with a large operator-containing DNA molecule in dilute solution. (The DNA molecules are well separated into “domains” under these conditions.) The (*upper*) expanded view shows repressor bound to a segment of non-operator DNA, on which it can either “slide” or engage in intradomain dissociation-association processes in seeking its specific (operator) target site. The (*lower*) expanded view shows a repressor molecule double bound to two DNA segments; this corresponds to the intermediate state in the intersegment transfer process.



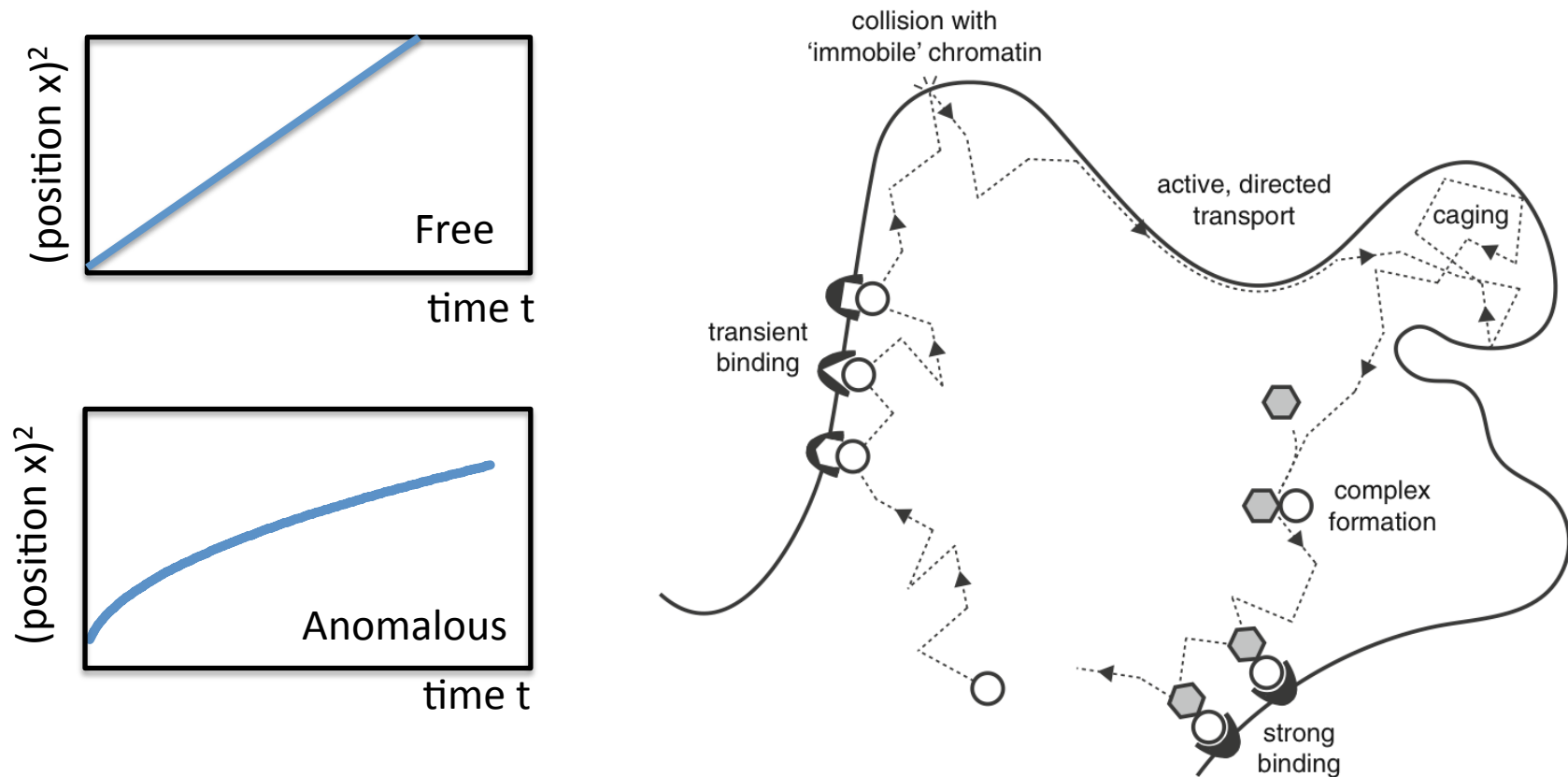
RNA polymerase finds its promoter by “sliding” along the DNA as visualized by SFM



Guthold, M. et al. (1999). Direct observation of one-dimensional diffusion and transcription by escherichia coli RNA polymerase. *Biophys J* 77, 2284-2294.

Anomalous diffusion

Free diffusion vs anomalous/obstructed diffusion

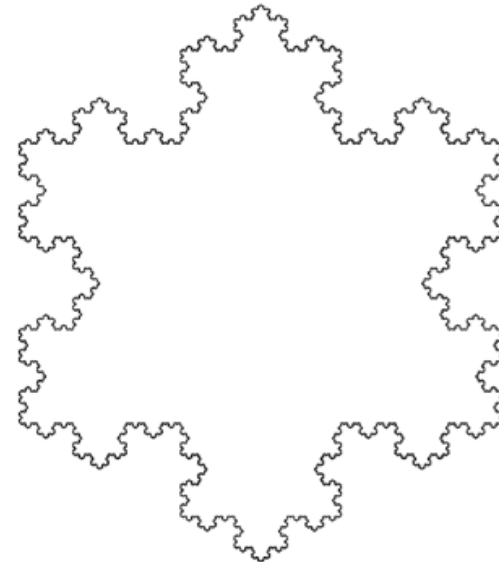


Fractal chromatin?

- From mobility measurements one can infer that chromatin folds back more often than expected
→ maybe it is a fractal globule
- Fractals are self-similar patterns



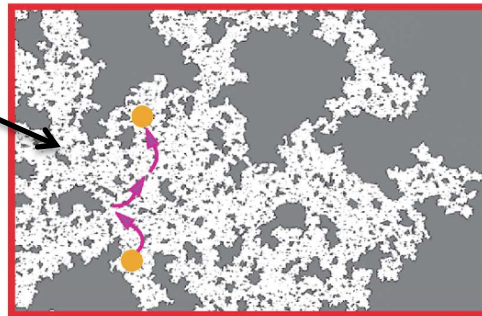
Romanesco broccoli



Koch snowflake

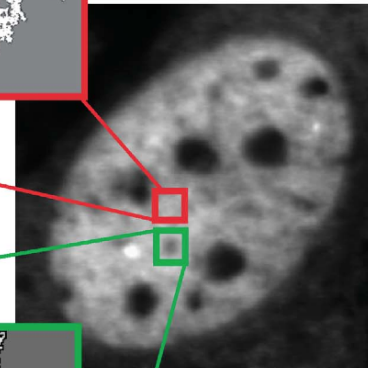
Fractal chromatin?

Euchromatin:
large fractal dimension



Accessible volume

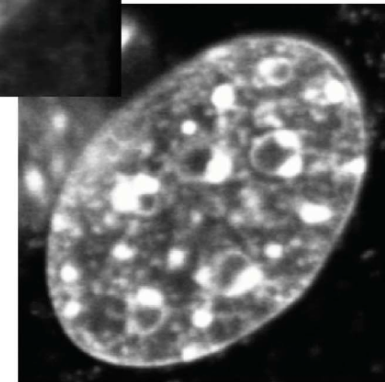
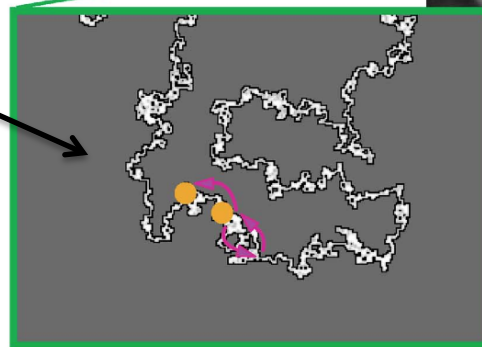
500 kDa dextran



Chromatin density

Hoechst

Heterochromatin:
small fractal dimension



Accessible space: white
Fractal contour: black

Diffusion-driven patterns

- Reaction-diffusion systems can create patterns
- Required:
Activator and inhibitor with different mobility

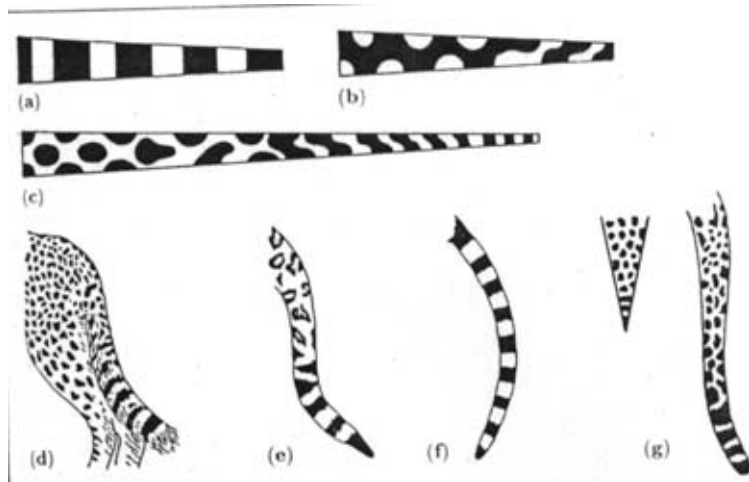
Tiger



Leopard



Diffusion-driven patterns



(a) (b) (c) Numerical simulations

(d) Cheetah tail markings

(e) Jaguar tail markings

(f) Genet tail markings

(g) Leopard tail markings

→ Patterns can be predicted based on mobility parameters and boundary conditions

Summary I

- Thermal energy is very important in biological systems (= small systems)
- All macromolecules diffuse quickly, otherwise they bind very strongly to immobile things like chromatin
- Friction instead of mass determines particle mobility
- Without mediating transport in a particular direction diffusion allows for exploring the environment
- Studying diffusion can tell you something about the diffusing particle and about its environment

Summary II

- Free diffusion is fast (seconds) on the length scale of the cell (μm).
- The average displacement from the start after some time is smaller in one dimension than in 3 dimensions. *However:*
- Finding a target site is much faster if the space that has to be searched by the protein is reduced.