Lecture 8: Diffusion in the cell

Goal: Role of Brownian motion in living systems. Compute the time to travel a distance, model diffusion in gradient.

- Brownian motion
- Concentration fields and diffusive dynamics

macro/micro models

PBOC Chapter 13.1, 13.2.1-13.2.3

Diffusion in the cell

Active vs. passive transport

↓ ↓

directed motion

Something does work

energy is consumed

next lecture

passive random walk

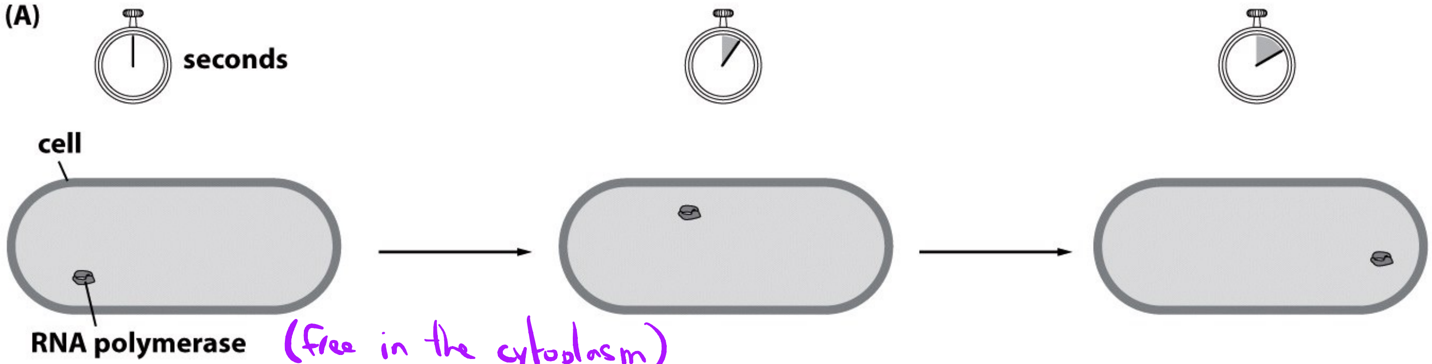
Brownian motion = diffusion (thermal)

this week

Diffusion in the cell

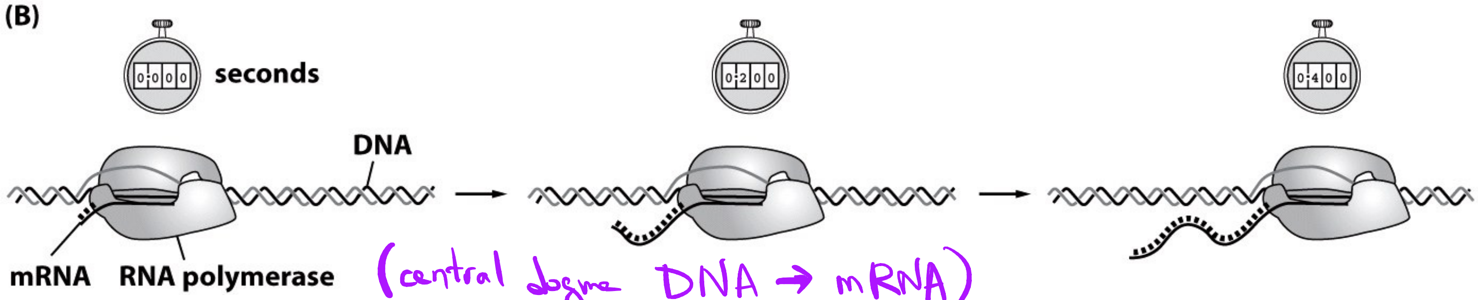
Active vs. passive transport

passive, diffusive



(free in the cytoplasm)

active, directed



(central dogma DNA → mRNA)

How long to get from one place to another?

$\langle x^2 \rangle = C D \tau_{diff}$

where C depends on dimension

time $\tau_{diff} \propto \dfrac{\langle x^2 \rangle}{D}$ mean squared displacement / diffusion coefficient

$D = \dfrac{k_B T}{6\pi \eta a}$ thermal energy $\approx 100\ \mu m^2/s$ for a 5 nm protein in H_2O

η viscosity, a size

Numerical estimates: $\tau_{diff}^{E.coli} \approx$? $\qquad L_{E.coli} = 1\ \mu m$

$\tau_{diff}^{squid\ axon} \approx$? $\qquad L_{squid} = 50$ cm

How long to get from one place to another?

$\langle x^2 \rangle \propto D\,\tau_{diff}$

time $\tau_{diff} = \dfrac{\langle x^2 \rangle}{D}$ mean squared displacement

diffusion coefficient

$D = \dfrac{k_B T}{6\pi \eta a}$ thermal energy $\approx 100\ \mu m^2/s$ for a 5 nm protein in H_2O

size

viscosity

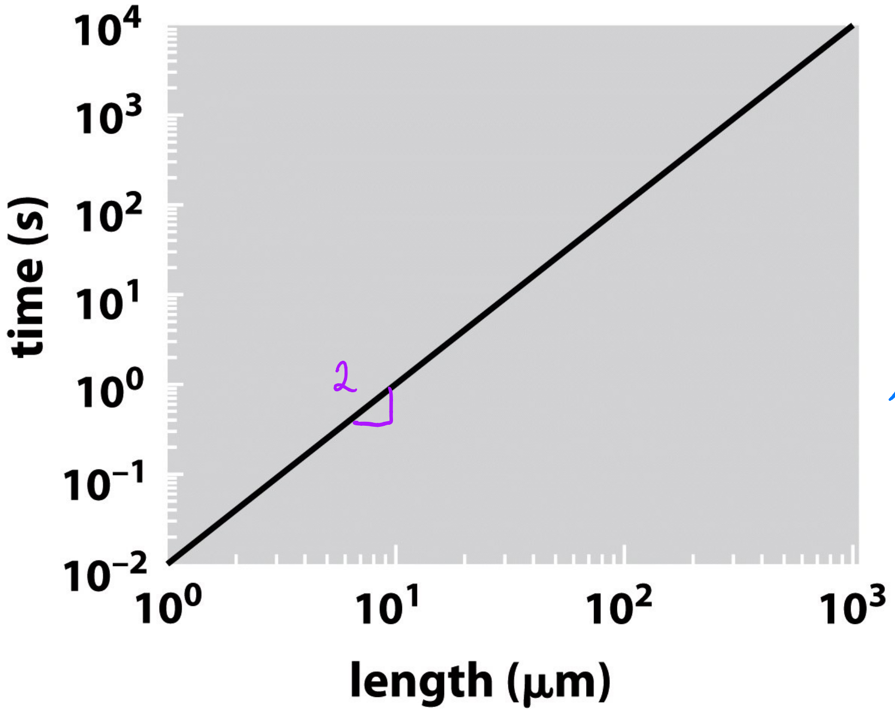
Numerical estimates: $\tau_{diff}^{E.coli} \approx \dfrac{L_{E.coli}^2}{D} \approx \dfrac{1\ \mu m^2}{100\ \mu m^2/s} = 0.01\ s$

$\tau_{diff}^{squid\ axon} \approx \dfrac{5^2 \cdot (10\ cm)^2}{100\ \mu m^2/s} = \dfrac{25 \cdot 10^{10}\ \mu m^2}{100\ \mu m^2/s} = 3 \times 10^9\ s \approx 100$ years

$1\ cm = 10^4\ \mu m$

Diffusion in the cell

Time to diffuse biological distances



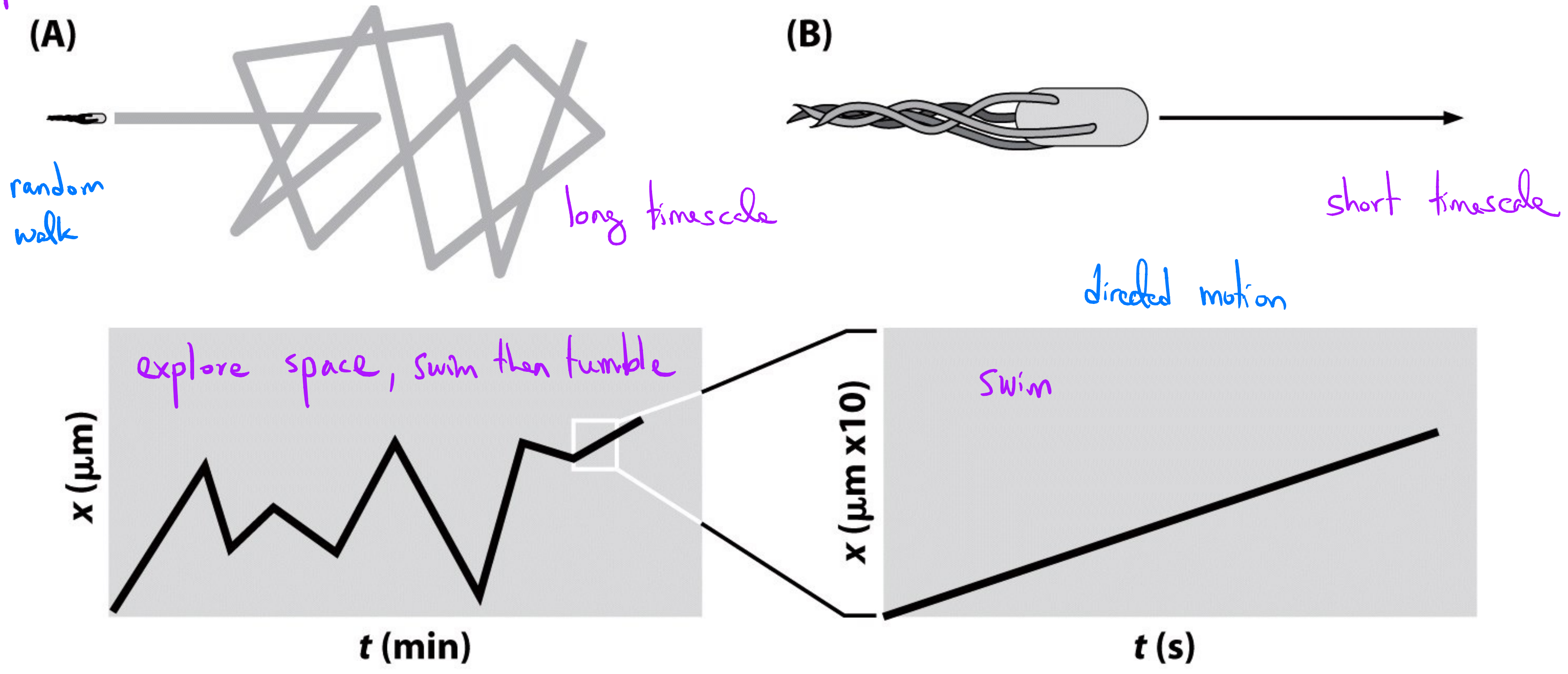
$$\tau_{diff} = \frac{\langle x^2 \rangle}{D}$$

$$\log(\tau) = 2\log\left(\langle x^2 \rangle^{1/2}\right) - \log D$$

Diffusion in the cell

Example: Bacterial motion

Active vs. passive transport



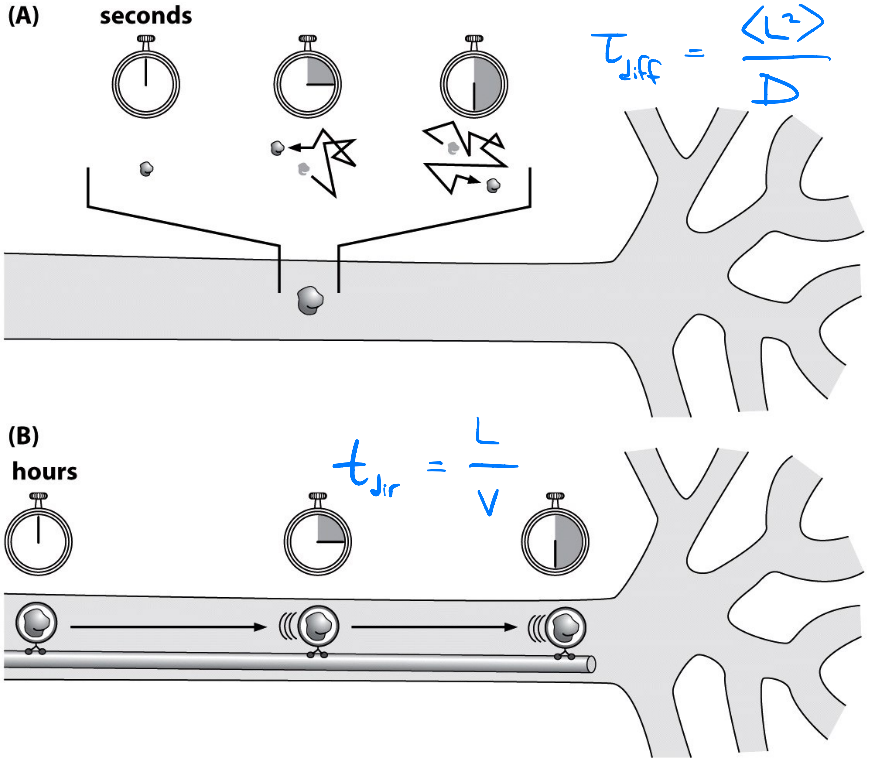
Diffusion in the cell

Example: protein cargo in neuron

Time to diffuse biological distances

diffusion is efficient over short distances.

$$\tau_{diff} = \frac{\langle L^2 \rangle}{D}$$



$$t_{dir} = \frac{L}{V}$$

directed transport is efficient over long distances.

$$\tau^*_{diff} = \tau^*_{dir}$$

crossover distance:

$$\frac{{L^*}^2}{D} = \frac{L^*}{V} \Rightarrow L^* = \frac{D}{V}$$

Diffusion in the cell

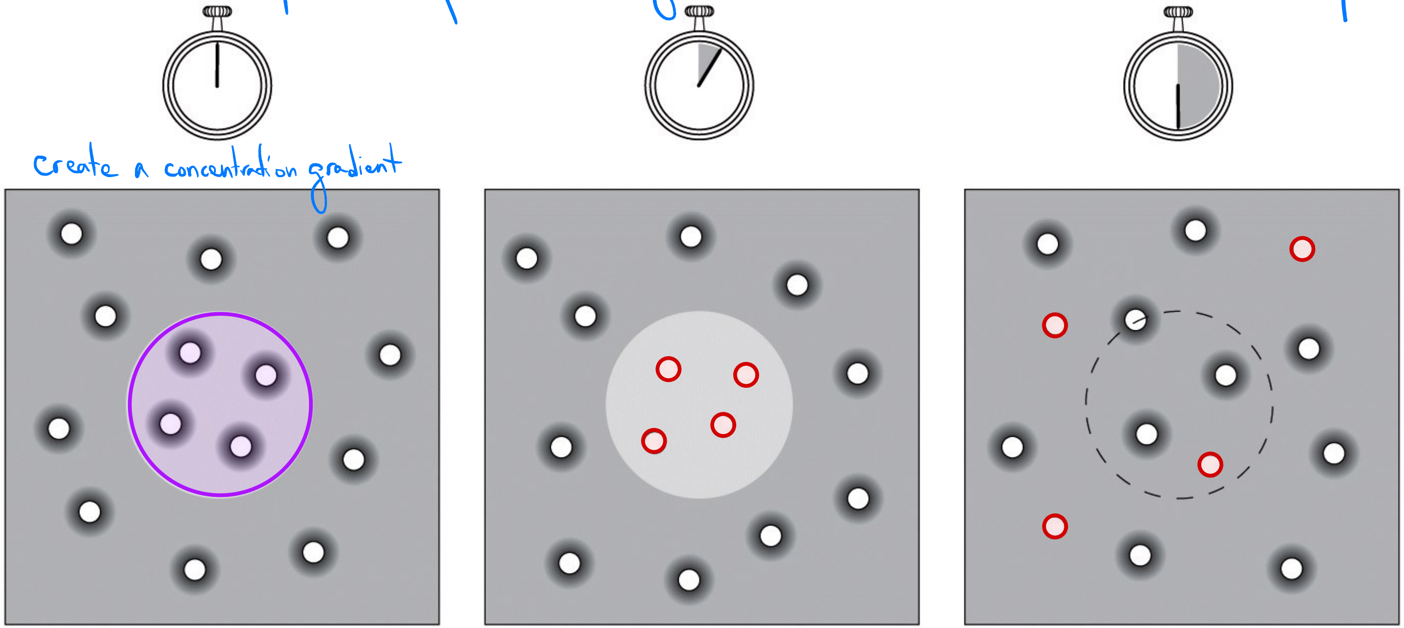
How to measure ?

Time to diffuse biological distances

Fluorescence recovery after photobleaching (FRAP) can measure diffusive dynamics

create a concentration gradient

proteins in a cell, fluorescently tagged



shine strong excitation light

photobleached

diffusion, "recovery"

Diffusion in the cell

How to model?

Concentration fields and diffusion

Models that govern diffusive dynamics

- macroscopic:

  concentration field, $c(\vec{r},t)$

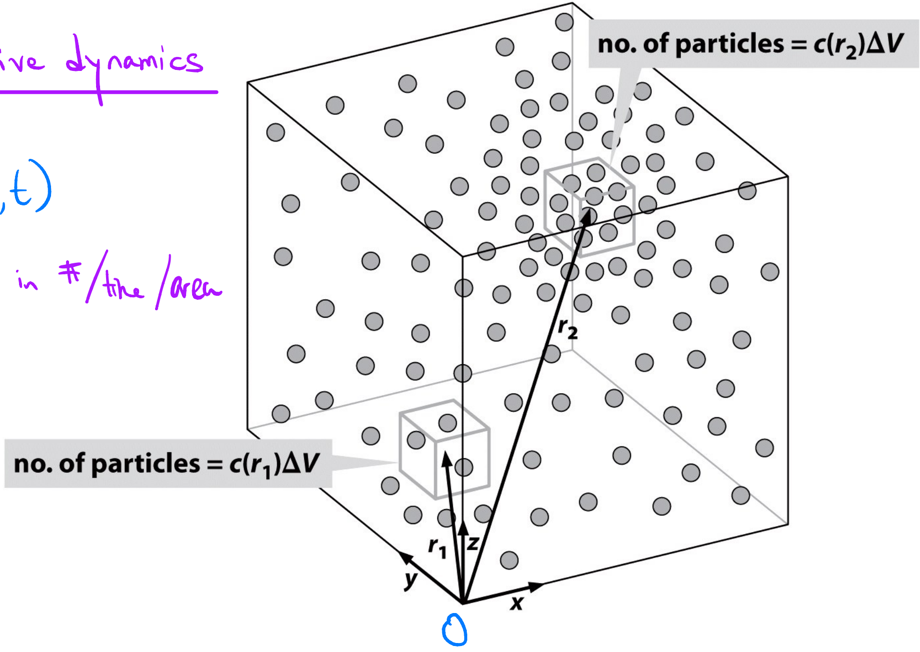
  flux $j(\vec{r},t)$ change in #/time/area

- microscopic:

  particle trajectories,

probability of hopping

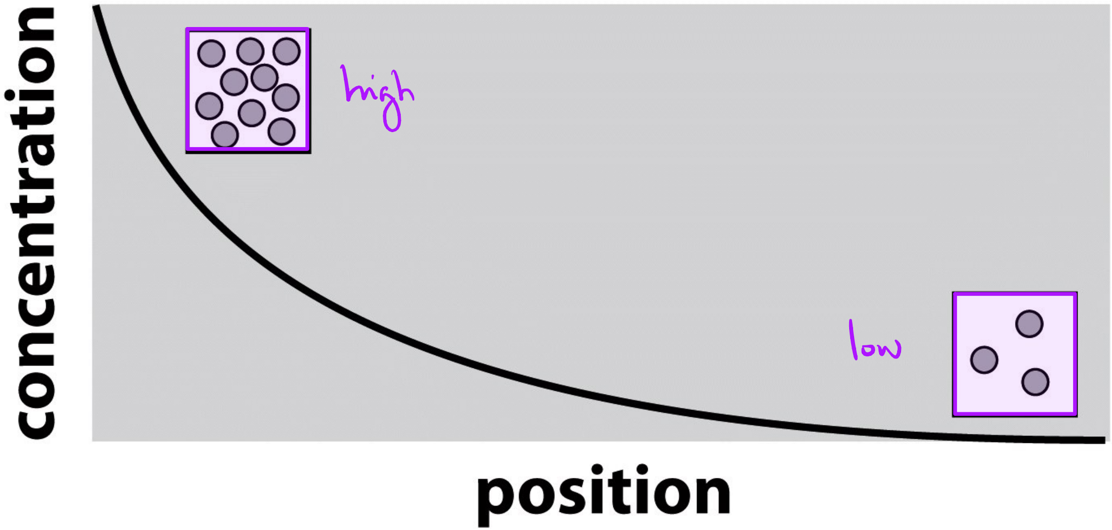
Two models, one process



Diffusion in the cell

Concentration fields and diffusion

macroscopic
diffusion is
a consequence
of concentration
gradients.
+
microscopic
random walks



concentration gradient

Diffusion in the cell

How to model?

Concentration fields and diffusion

Models that govern diffusive dynamics

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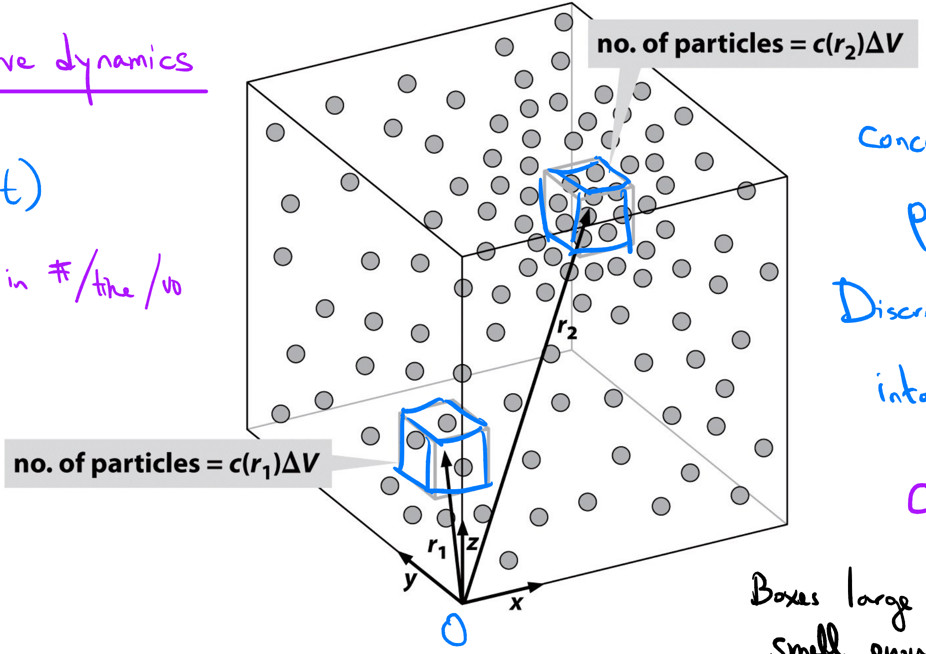
Two models, one process



MACROSCOPIC

Concentration varies with position, $c(\vec{r})$

Discretize: divide system into small boxes, volume ΔV

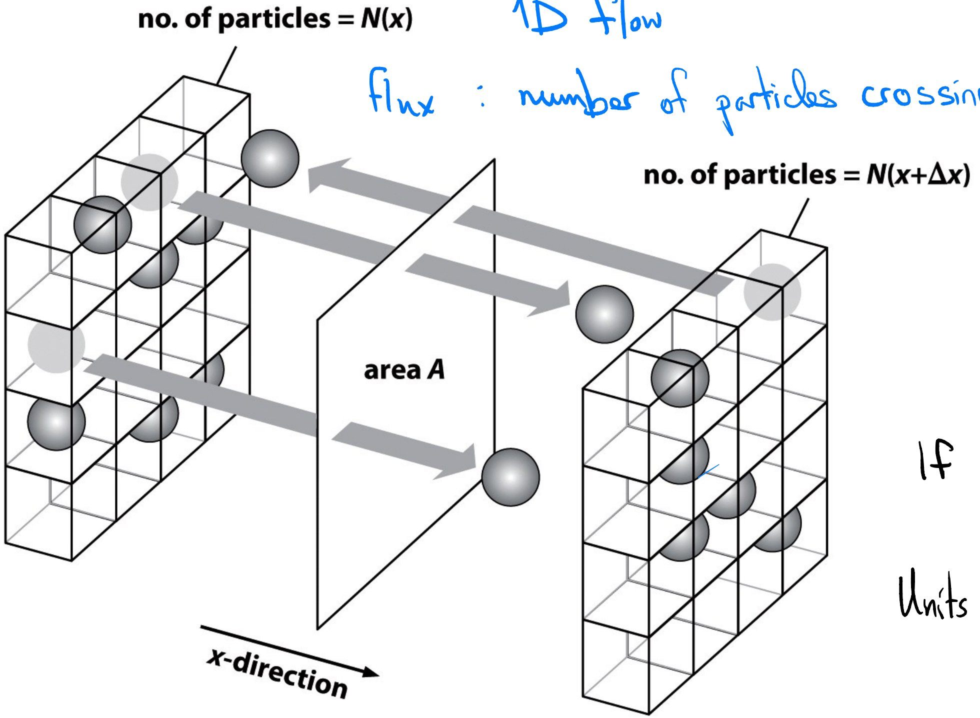
$$c(\vec{r}_i) = \frac{N_i}{\Delta V} \qquad \frac{\#\text{ particles}}{\text{volume}}$$

Boxes large enough to contain many particles, small enough that concentration is ~ constant

Diffusion in the cell

MACROSCOPIC

Concentration fields and diffusion: Fick's Law



1D flow

flux : number of particles crossing per area per unit time

Units: $\frac{1}{[\text{length}^2][\text{time}]}$

Assertion for diffusing particles:

$$\boxed{j = -D\frac{\partial c}{\partial x} \quad \text{Fick's Law}}$$

If $\frac{\partial c}{\partial x}$ is negative, particles will flow toward $+x$.

Units of D: $\frac{[\text{length}^2]}{[\text{time}]}$

MACROSCOPIC

Diffusion equation, change in concentration with time:

change in # of particles in a box of volume ΔV per time:

$$\frac{\partial N_i}{\partial t} = \frac{\partial c(r_i)}{\partial t} \Delta V$$

Conservation of mass: change = number entering − number leaving

$$\frac{\partial c}{\partial t} \Delta V = j(x)\Delta A - j(x+\Delta x)\Delta A$$

Taylor expansion:

$$\frac{\partial c}{\partial t} \Delta V = j(x)\Delta A - \left[j(x) + \frac{\partial j}{\partial x}\Delta x\right]\Delta A$$

$$\Rightarrow \quad \frac{\partial c}{\partial t} = -\frac{\partial j}{\partial x}$$

universal as long as mass conserved

Previously:

$$\boxed{j = -D\frac{\partial c}{\partial x} \quad \text{Fick's Law}}$$

may differ if other constraints hold

substitute $\Rightarrow$

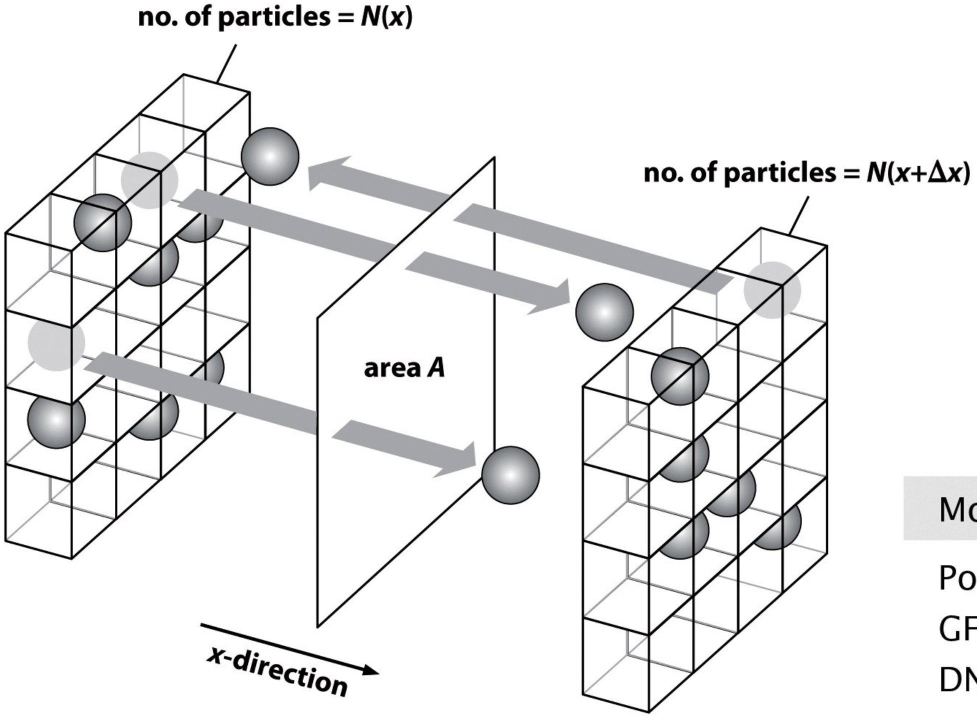
$$\boxed{\frac{\partial c}{\partial t} = D\frac{\partial^2 c}{\partial x^2}}$$

1 dimensional diffusion equation
constant D

Diffusion in the cell

MACROSCOPIC

Concentration fields and diffusion: Fick's Law



$$\boxed{\frac{\partial c}{\partial t} = D\frac{\partial^2 c}{\partial x^2} \quad \text{1D diffusion equation}}$$

$$\frac{\partial c}{\partial t} = D\nabla^2 c \quad \text{3D}$$

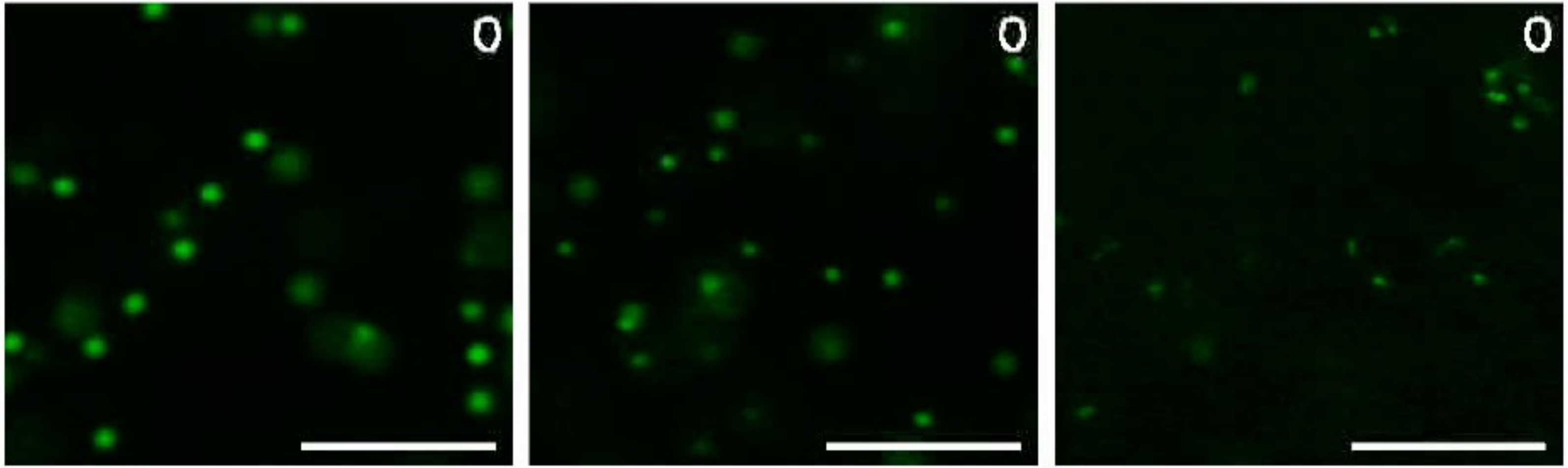
Typical values of $D \sim \frac{1}{a}$

Molecule	Diffusion coefficient
Potassium ion in water	$\approx 2000\,\mu m^2/s$
GFP in *E.coli*	$\approx 7\,\mu m^2/s$
DNA in yeast	$5 \times 10^{-4}\,\mu m^2/s$

Diffusion in the cell

Microscopic model:

Particles in a fluid



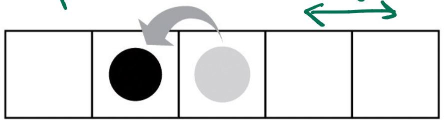
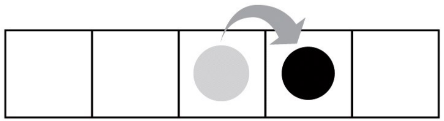
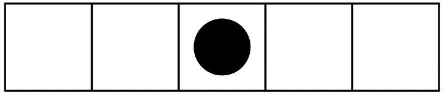
random trajectories

Sum over microtrajectories → macroscopic response

Diffusion in the cell

Microscopic

Summing over microtrajectories

(micro) TRAJECTORY	WEIGHT	DISPLACEMENT
step size a	probability of trajectory in time Δt	
$\xleftrightarrow{a}$ 	$\underbrace{k}_{\text{rate of steps}}\Delta t$	$-a$
	$k\Delta t$	$+a$
	$\underbrace{1-2k\Delta t}_{\text{ensures total probability is 1}}$	0

Diffusion in the cell

Microscopic

Summing over microtrajectories

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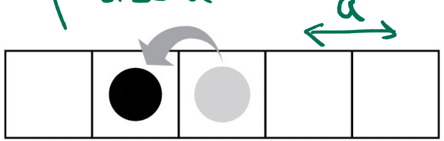
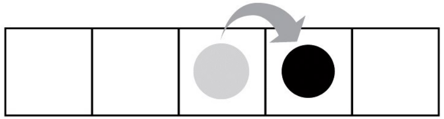
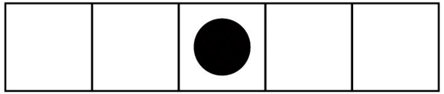
	$\underbrace{k}_{\text{rate of steps}}\Delta t$	$-a$
	$k\Delta t$	$+a$
	$\underbrace{1-2k\Delta t}_{\text{ensures total probability is 1}}$	0

Observables:

$$\langle \Delta x \rangle = a \cdot k\Delta t + (-a)\cdot k\Delta t + (0)\cdot(1-2k\Delta t) = 0$$

$$\langle \Delta x^2 \rangle = a^2 \cdot k\Delta t + (-a)^2 \cdot k\Delta t + 0 = 2a^2 k\Delta t$$

Over time interval t: $\langle \Delta x^2 \rangle = 2\underbrace{a^2 k}_{D}\, t$

Microscopic

MACRO $c(x,t) \leftrightarrow p(x,t)$ MICRO

What is the governing equation for $p(x,t)$, probability density that the particle is at position x at time t?

Given $p(x,t)$, (Markov process, probabilities are history independent)

$$p(x, t+\Delta t) = \underbrace{(1-2k\Delta t)\cdot p(x,t)}_{\text{stays at } x} + \underbrace{k\Delta t \cdot p(x-a,t)}_{\text{was at } x-a \text{, moves right}} + \underbrace{k\Delta t \cdot p(x+a,t)}_{\text{was at } x+a \text{, moves left}}$$

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Taylor expansion:

$$p(x, t+\Delta t) \approx p(x,t) + \Delta t \frac{\partial p(x,t)}{\partial t}$$

$$p(x \pm a, t) \approx p(x,t) \pm a \frac{\partial p(x,t)}{\partial x} + \frac{a^2}{2} \frac{\partial^2 p(x,t)}{\partial x^2}$$

Microscopic

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Substitute into ☆

$$p(x,t) + \Delta t \frac{\partial p(x,t)}{\partial t} = (1-2k\Delta t)\cdot p(x,t) + k\Delta t\cdot\left[p(x,t) - a\frac{\partial p(x,t)}{\partial x} + \frac{a^2}{2}\frac{\partial^2 p(x,t)}{\partial x^2}\right]$$

$$+ k\Delta t\cdot\left[p(x,t) + a\frac{\partial p(x,t)}{\partial x} + \frac{a^2}{2}\frac{\partial^2 p(x,t)}{\partial x^2}\right]$$

$$= p(x,t) + a^2 k\Delta t \frac{\partial^2 p(x,t)}{\partial x^2}$$

Microscopic

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Substitute into ✡

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$$+ k\Delta t \cdot \left[p(x,t) + a\frac{\partial p(x,t)}{\partial x} + \frac{a^2}{2}\frac{\partial^2 p(x,t)}{\partial x^2}\right]$$

$$= p(x,t) + a^2 k \Delta t \frac{\partial^2 p(x,t)}{\partial x^2}$$

$$\Rightarrow \boxed{\frac{\partial p(x,t)}{\partial t} = a^2 k \frac{\partial^2 p(x,t)}{\partial x^2}}$$

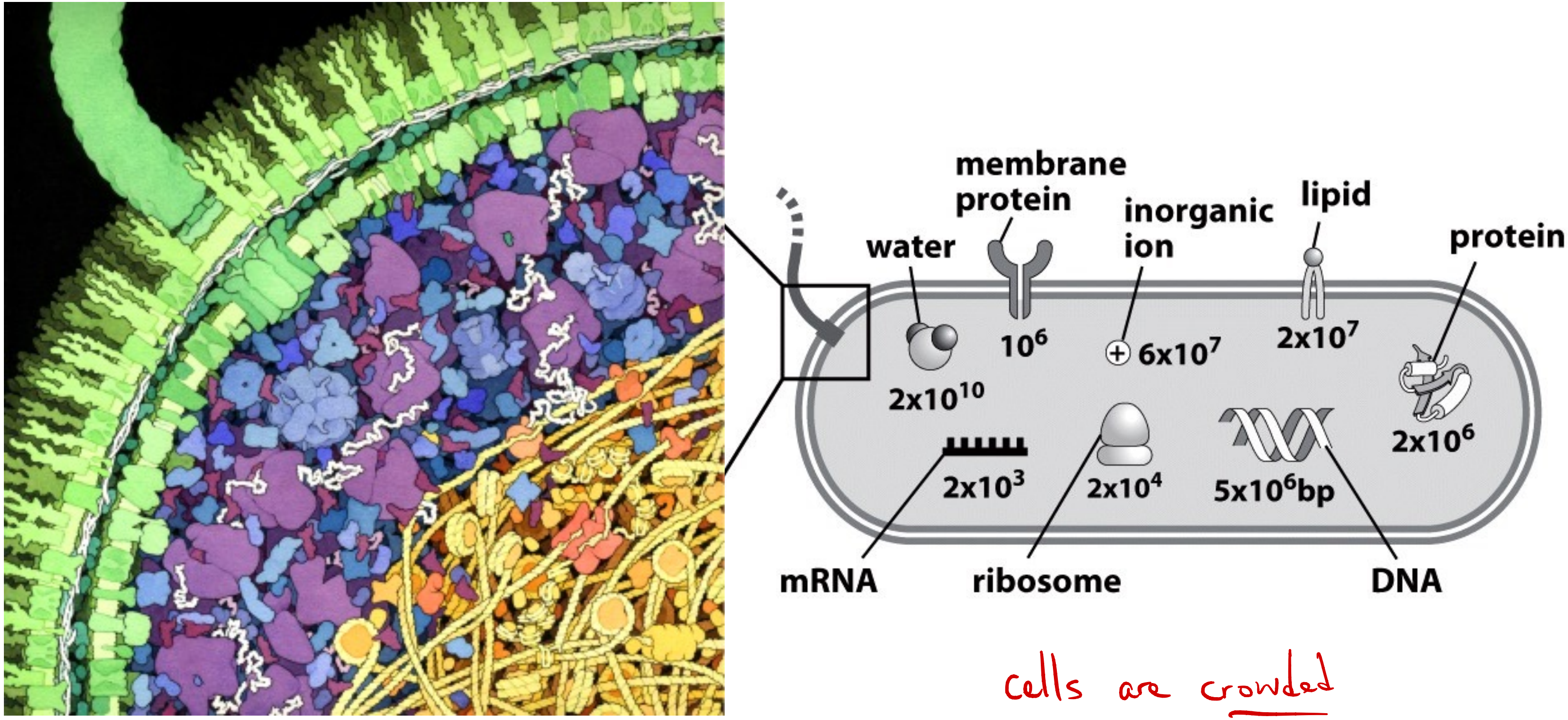
1 dimensional diffusion eqn, constant $D = a^2 k$

Also, (13.15) – (13.22), develop by summing over all microtrajectories

An ode to E. coli

Previously:

Molecular census



cells are crowded

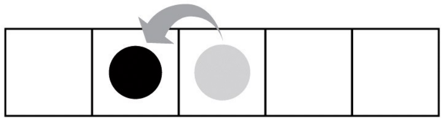
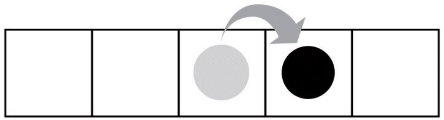
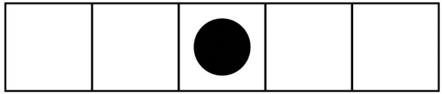
Diffusion in the cell

Diffusion in crowded environments

What is the diffusion coefficient associated with "crowded" random walk?
Assume fraction of occupied lattice sites φ (only one molecule can occupy lattice site)

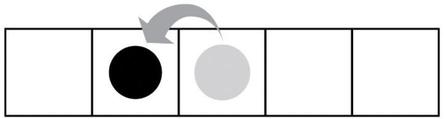
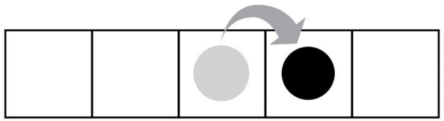
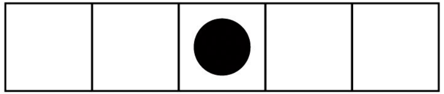
Diffusion in the cell

Summing over microtrajectories: Crowding (14.3.2)

TRAJECTORY	WEIGHT	DISPLACEMENT
	$k\Delta t\underbrace{(1-\varphi)}_{\text{probability site is unoccupied}}$	$-a$
	$k\Delta t(1-\varphi)$	$+a$
	$\underbrace{1-2k\Delta t(1-\varphi)}_{\text{ensures total probability is 1}}$	0

Diffusion in the cell

Summing over microtrajectories: Crowding (14.3.2)

TRAJECTORY	WEIGHT	DISPLACEMENT
	$k\Delta t\,\underbrace{(1-\varphi)}_{\text{probability site is unoccupied}}$	$-a$
	$k\Delta t\,(1-\varphi)$	$+a$
	$\underbrace{1-2k\Delta t(1-\varphi)}_{\text{ensures total probability is 1}}$	0

Observables:

$$\langle \Delta x \rangle = a \cdot k\Delta t\,(1-\varphi) + (-a)\cdot k\Delta t\,(1-\varphi) + 0 = 0$$

$$\langle \Delta x^2 \rangle = a^2 \cdot k\Delta t(1-\varphi) + (-a)^2 \cdot k\Delta t(1-\varphi) + 0$$

$$= 2a^2 k\,\Delta t\,(1-\varphi)$$

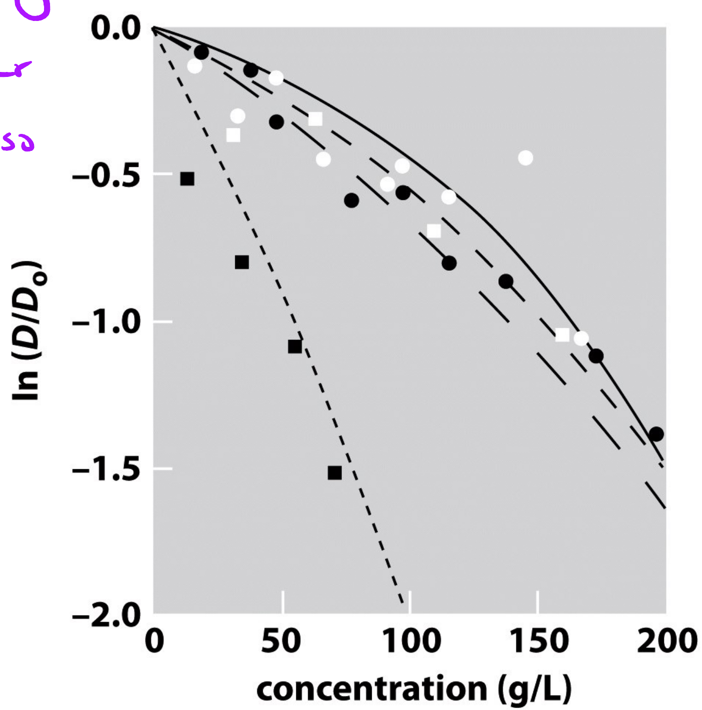
Over time interval t: $\langle \Delta x^2 \rangle = \underbrace{2a^2 k(1-\varphi)}_{D^*}\,t$

$$= D_0 \cdot (1-\varphi)$$

Transport in cellular systems

Diffusion in crowded environments

φ is a good start, next order model also considers excluded volume



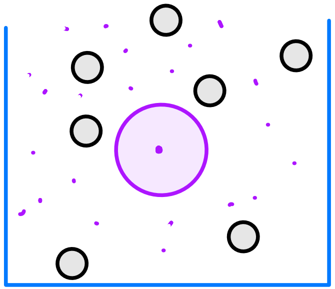
aldolase
↑
tracer diffusion in protein solution

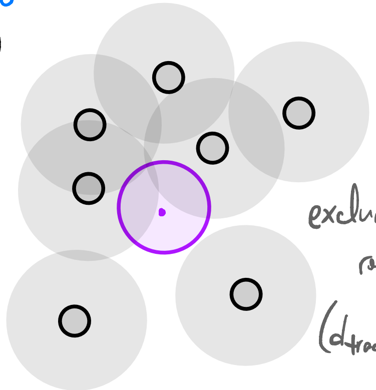
	Molecular species	M_W [Da]	r
■	ribonuclease	12,400	2.3
□	ovalbumin	43,500	1.5
●	BSA	70,000	1.3
○	aldolase	150,000	1

size ratio $= d_{tracer} / d_{protein}$

Good agreement when taking into account excluded volume:

tracer (d_{tracer}) protein ($d_{protein}$)





excluded region ($d_{tracer} + d_{protein}$)

Diffusion in the cell

Solutions to the diffusion equation

Suppose $c(x, t=0)$ is a spike at $x = 0$.

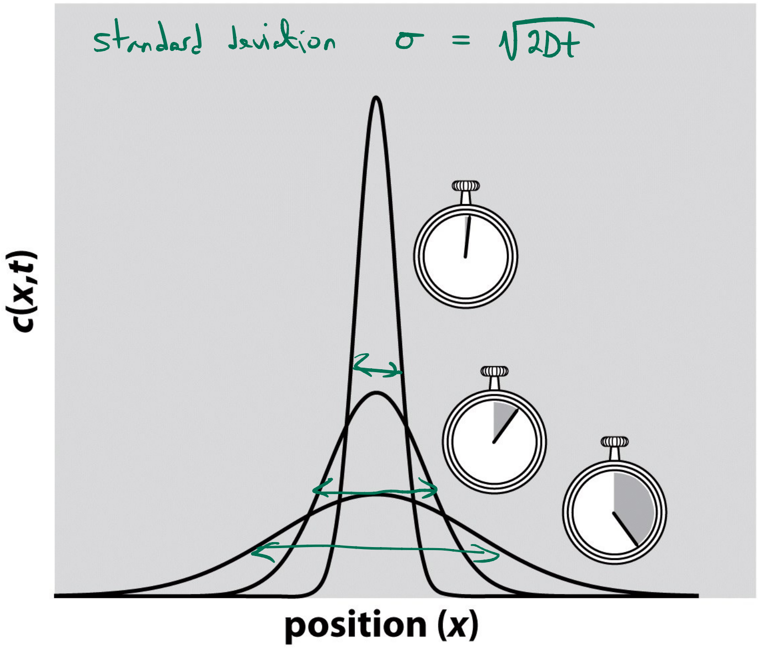
$$c(x, t=0) = \delta(x)$$

Solution to the diffusion equation:

$$c(x,t) = \frac{N}{\sqrt{4\pi Dt}} e^{-x^2/4Dt}$$ (Green's function)

Note:

$$\langle x^2 \rangle = \int_{-\infty}^{\infty} x^2 \underbrace{P(x)}_{c(x,t)/N} dx = 2Dt$$ (13.33-34)

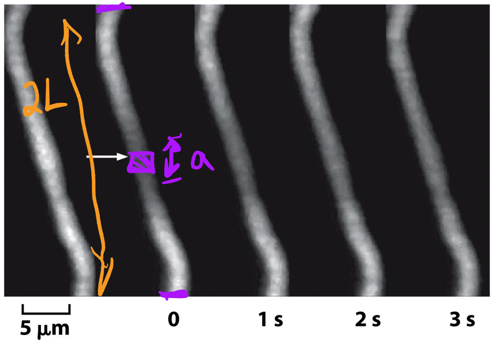


Diffusion in the cell

Macroscopic

Solutions to the diffusion equation

photobleach



time

FRAP of elongated bacterium

1D diffusion, box of length 2L

$$\frac{\partial c}{\partial t} = D\frac{\partial^2 c}{\partial x^2}$$

initial condition $c(x,t=0)\begin{cases} c_0 & -L<x<-a \\ 0 & -a<x<a \\ c_0 & a<x<L \end{cases}$

boundary condition $\left.\frac{\partial c}{\partial x}\right|_{x=\pm L} = 0$

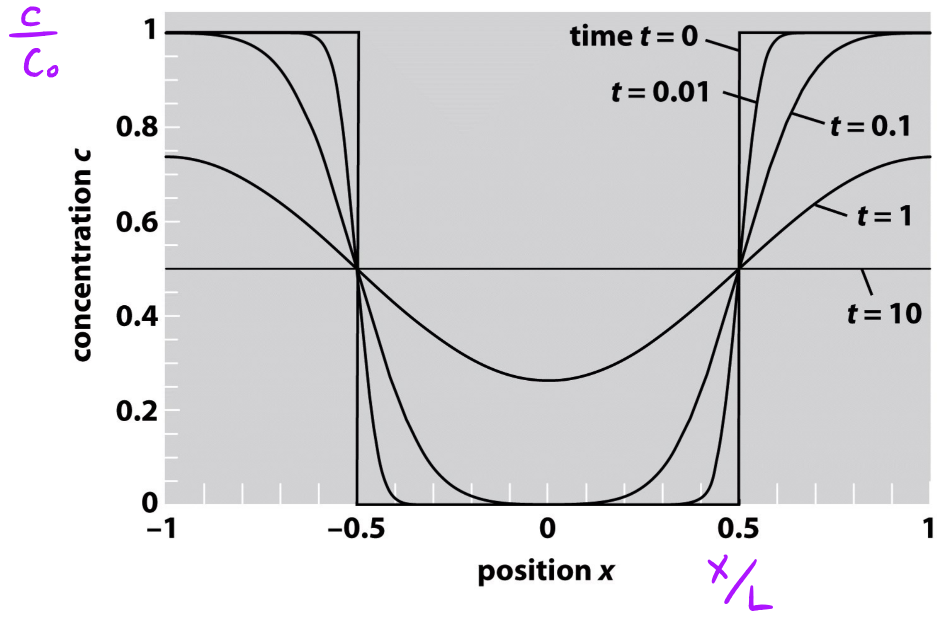
Considering symmetry and B.C., sum of cosine functions. (13.39)

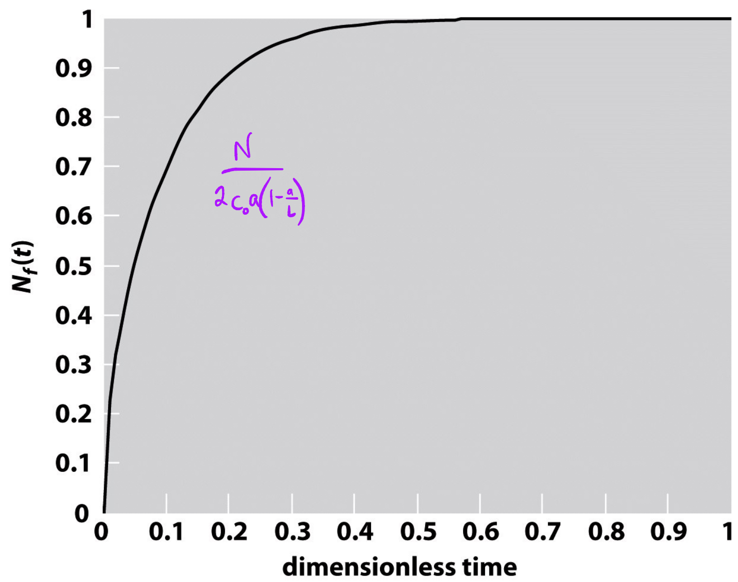
Find weights by applying constraints (13.40 - 13.46) $\Rightarrow c(x,t)$

Also, $N_f = \int_{-a}^{a} c(x,t)\,dx$ number of fluorescent molecules in the bleached region.

Diffusion in the cell

Solutions to the diffusion equation

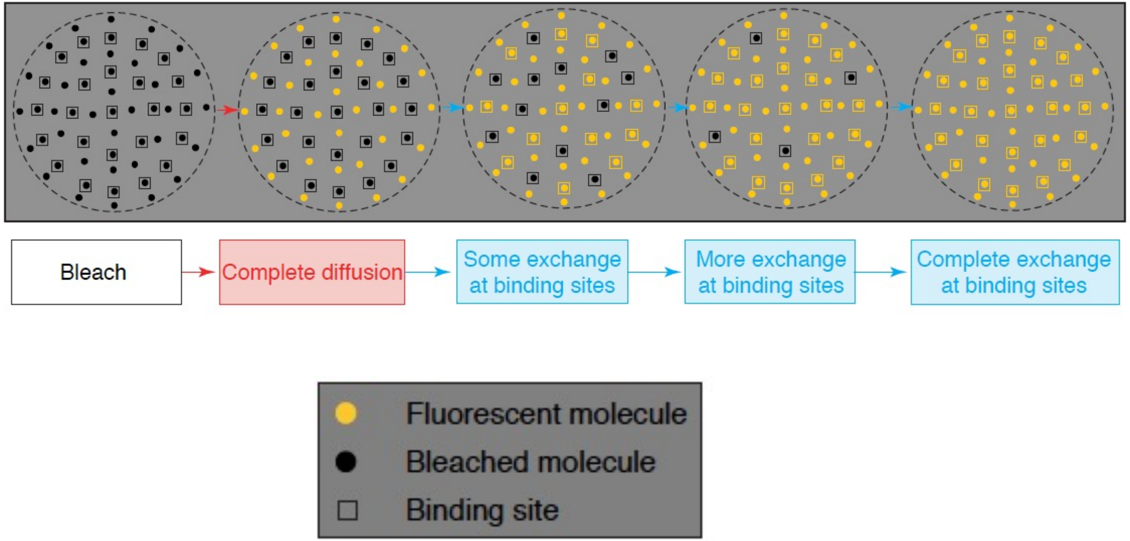


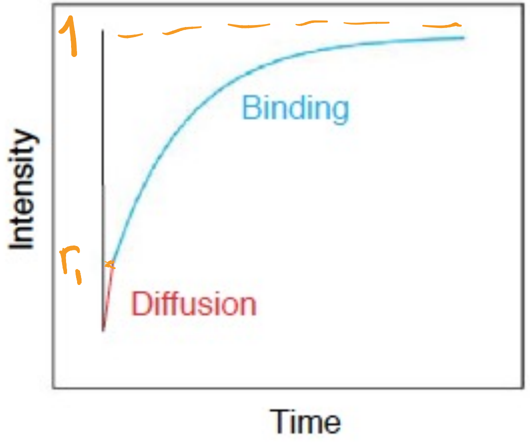


Diffusion in the cell

Complexity: diffusion + binding

Limit case ($t_{diff} << t_{binding}$): Two separable timescales



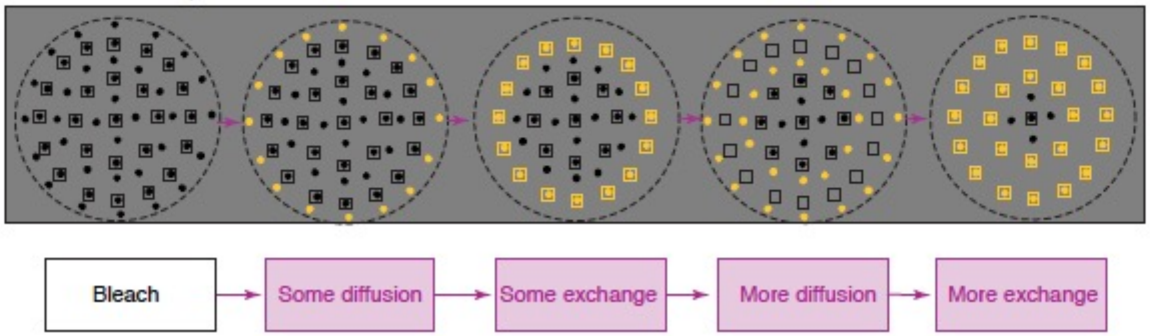


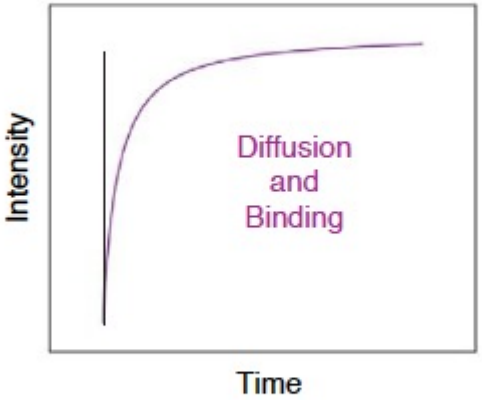
binding delays FRAP recovery, compare with "inert" molecule!

Diffusion in the cell

Complexity: diffusion + binding

Case ($t_{diff} \sim t_{binding}$): Mixing of dynamic modes





Anomalous diffusion:

$$\langle r^2(\tau) \rangle = 6D\tau$$

$$\langle r^2(\tau) \rangle = 6D\tau^{\alpha} = 6D(\tau)\tau$$

Lecture 8: Diffusion in the cell

Summary:

- Diffusion can be modeled based on macroscopic or microscopic considerations.
- In either case, the fundamental physical basis is thermal agitation driving a random walk.
- Transport in a cell may be modified by binding, crowding, barriers.
- More complex physical constraints can be accounted for by modifying micro-trajectories. Deviation from Fick's Law.
- The effective diffusion coefficient reflects physical constraints on particle motion.