Diffusion in Cells

BIO311

Motivation

- Intracellular compartments, protein sorting and vesicle trafficking
- Genetics regulates biological form and function
- Template and Rule based
- Regulation of DNA-RNA-Protein
- Role of physical laws- self organization
- Interplay of effects- chemical template and physical laws

Outline

- Thermal motion
- Free diffusion
- Diffusion in cells
- Viscosity
- Macromolecular crowding
- Measuring diffusion

Molecular Mobility in Cells

- Molecules in cell
  - surface immobilized
  - free
- Free molecules in constant thermal motion
- Gene encoded targeting sequences: NLS, golgi, ER
- Budding, fusion, movement

Active or Passive

- Active
  - Requires ATP
  - Directional
- Passive
  - Random
  - Thermal energy: $k_BT/2$ along each axis

  (T=temp, k_B=boltzmann's constant)

  Mean 1D velocity $\langle v_x \rangle$

  $E_{kin} = m.\langle v_x^2 \rangle/2 = k_BT/2$

  Mean sq. velocity: $\langle v_x^2 \rangle = k_BT/m$

  Root mean square velocity: $\langle v_x^2 \rangle^{1/2} = (k_BT/m)^{1/2}$

How Fast is Thermal Motion?

G-Actin M.W.=4.3×10^4 Da

Mass of one molecule of G-Actin =7×10^{-20} g

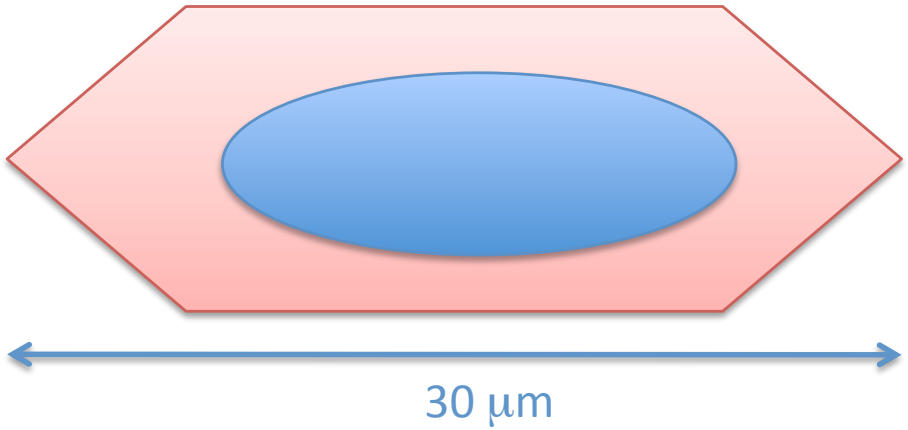
@300 K, $k_B T = 4.14 \times 10^{-14}$ g-cm^2/s^2

RMS Velocity $\langle v_x^2 \rangle^{1/2} = 7.7 \times 10^2$ cm/s

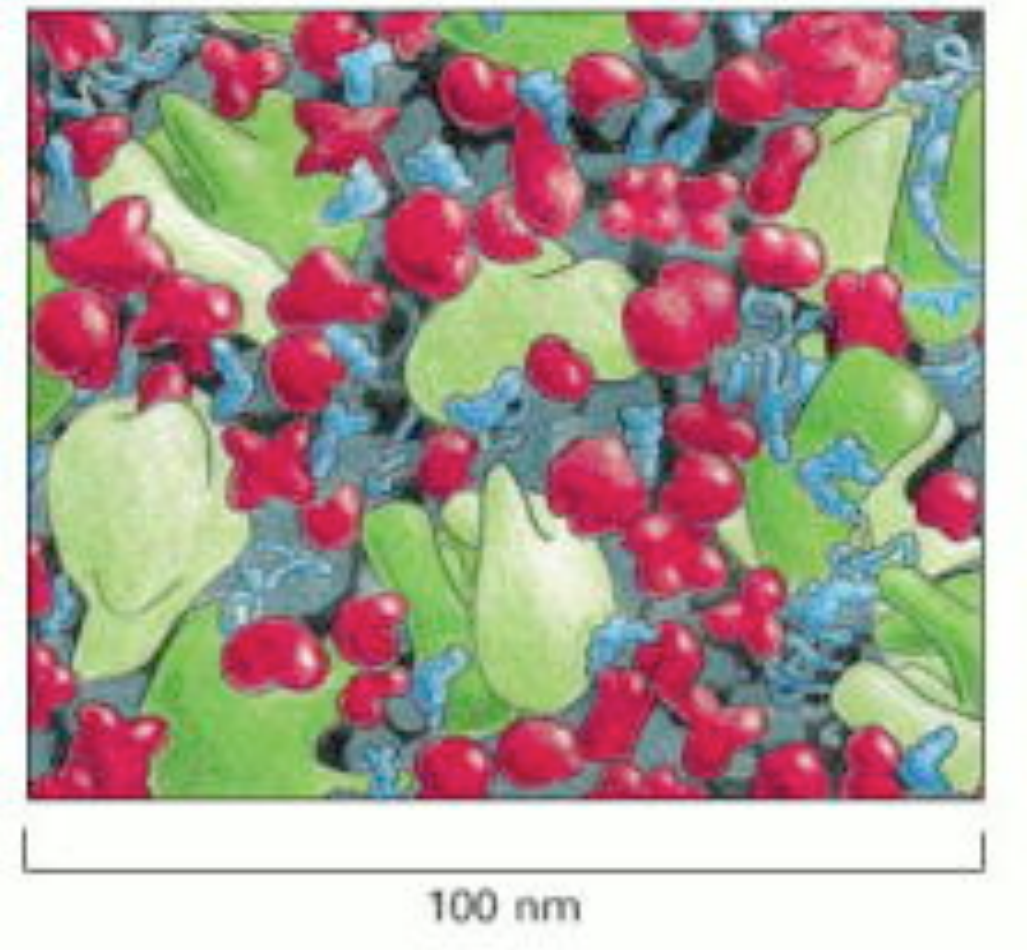
Time to traverse cell of

10 μm, ~1.3×10^{-6} s

30 μm, ~3.9×10^{-6} s



Environment of a Cell



Macromolecules

RNAs *blue,*

Ribosomes *green,*

Proteins *red.*

Collisions!

Goodsell D.S. (1991) *Trends Biochem. Sci.* 16:203.

Random Walk

Pseudo Code

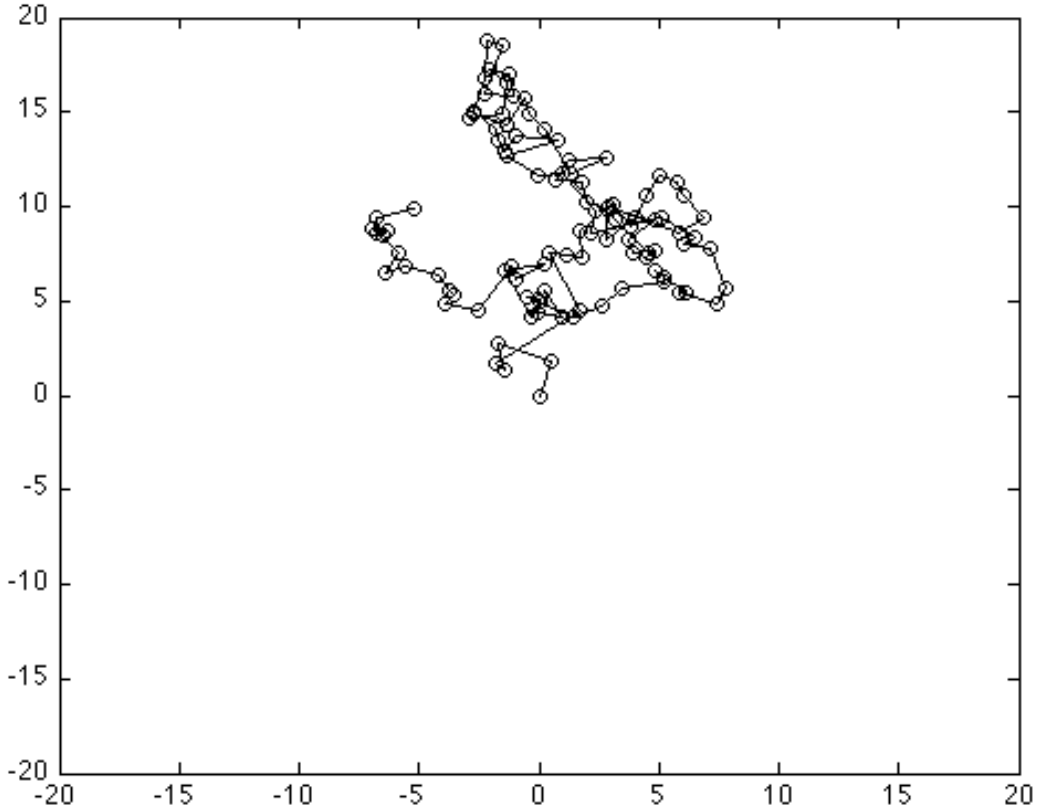
Initially:
X0=0,Y0=0

For 100 steps

Xt=X0+random
Yt=Y0+random

X0=Xt
Y0=Yt

Display



Free diffusion

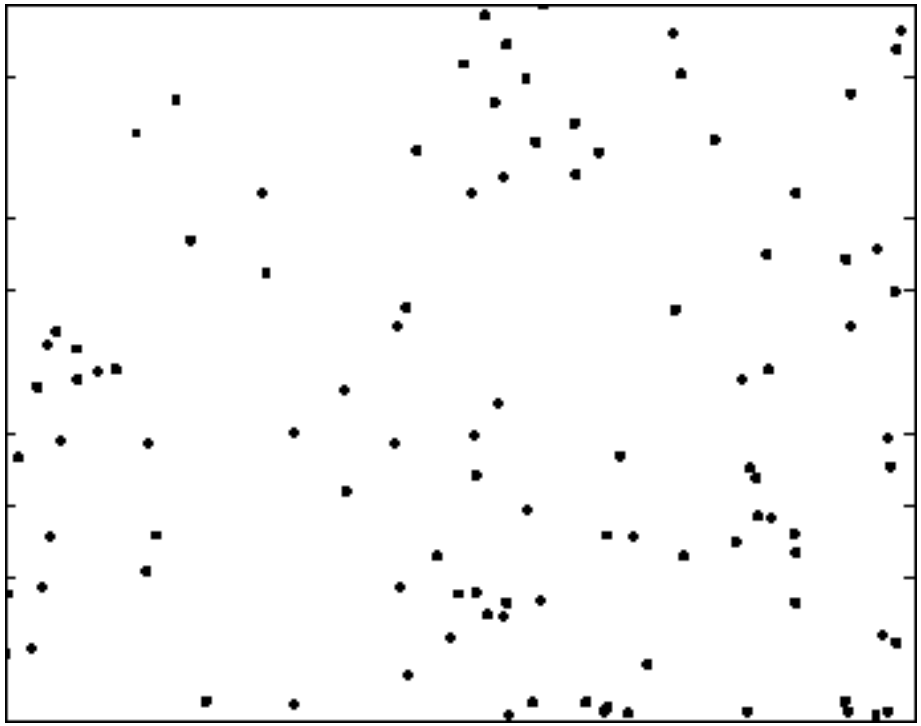
Displacement (x) in 1D

$\langle x^2 \rangle = 2Dt$

$\langle x^2 \rangle^{1/2} = (2Dt)^{1/2}$

(t=time, D=diffusion coefficient)

For small molecule in water at room temperature D ~ 10^{-5} cm^2/s



How Fast is Diffusion?

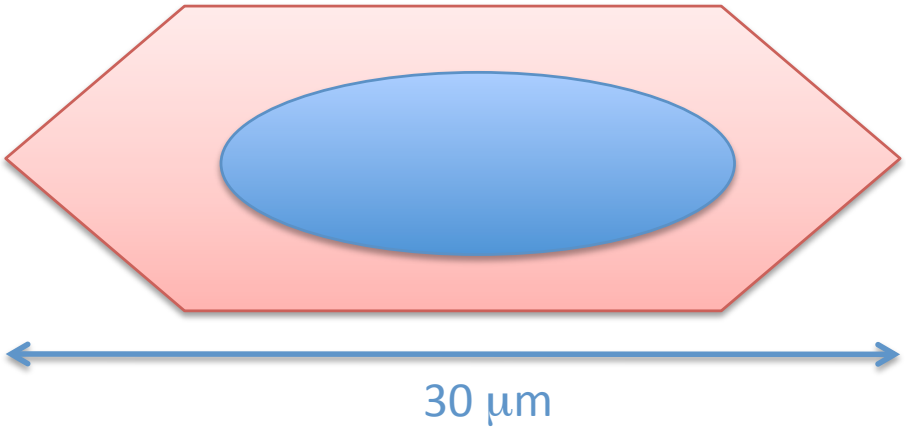
G-actin in water at 300 K, $D=10^2\ \mu m^2/s$

Diffusion velocity $v_D=\langle x^2\rangle^{1/2}/t$

Time to traverse cell of diameter

10 μm, ~0.7s

30 μm, ~ 2s



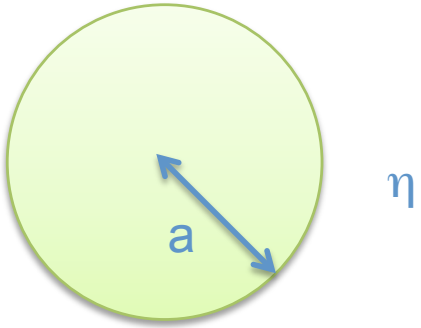
Diffusion

- 1D: $\langle x^2 \rangle = 2Dt$
- 2D: $\langle x^2 \rangle = 4Dt$
- 3D: $\langle x^2 \rangle = 6Dt$

Stoke-Einstein's Relation

Diffusion coefficient of a sphere in a liquid

$$D_{sphere} = k_B T/6\pi\eta a$$



k_B=Boltzmann's constant
T=temp
a=radius of particle
η=viscosity of liquid

For a molecule of a= 10^{-4} cm

$6\pi\eta a=1.9 \times 10^{-5}$ g/s,, T=300 K, $k_B T=4.14 \times 10^{-14}$ g-cm^2/s^2,
$D_{sphere} \sim 2 \times 10^{-9}$ cm^2/s

Diffusion Inside Cells

- Crowding
  - Collisions
  - Obstruction
  - Trapping
  - Corrals
- Anomalous diffusion
  - Sub diffusion: corralled
  - Super-diffusion: transported by ATP dependent process

Viscosity of cytoplasm

- Effective viscosity
- Gel like nature of cytoplasm
- η

Medium	Temperature (°C)	Viscosity (cP)
Water	27	~0.8
Cytoplasm	37	2-3
Nucleoplasm	37	1.5-2

Viscosity of the nucleus

- More crowded
- DNA content
- Size sieving observed

Measuring Diffusion

- collective for proteins:
- FRAP
- FLIP
- photoactivation

Measuring Diffusion

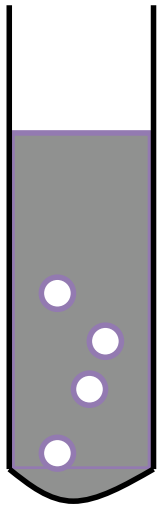
- Single molecule
- FCS
- Single particle tracking

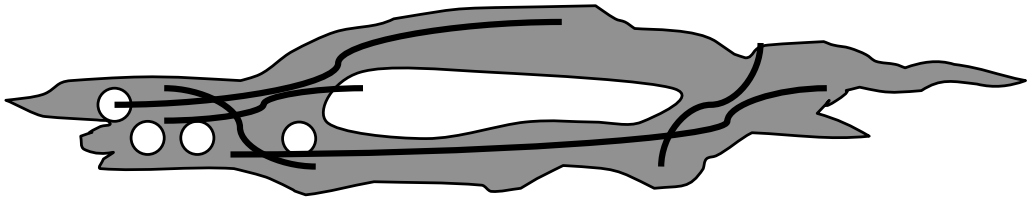
Conclusions

- Diffusion as a means of transport
- Fast over short distances, slow over long
- Cellular diffusion anomalous
- Measurements inside cells

- NEXT:
  - Role of diffusion in cells
    - Transport
    - Pattern formation
    - Development

Biochemistry in Cells





time