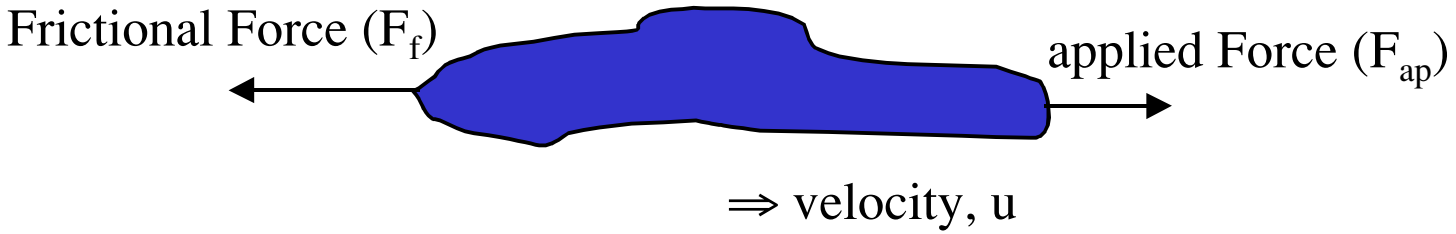
Diffusion

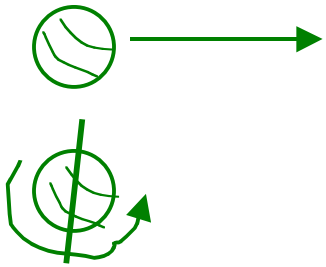
Frictional Resistance to Macromolecule Motion



$F_f = f \bullet u$ f ≡ frictional coefficient

steady state - terminal velocity is reached $u = (F_{ap} / f)$

For a sphere:



$f_{trans} = 6\pi\eta R_S$ translational motion

$f_{rot} = 8\pi\eta R_S^3$ rotational motion

Measure molecular motion ⇒ **f** ⇒ $\mathbf{R_s}$ ⇒ molecular size and shape information

Translational diffusion

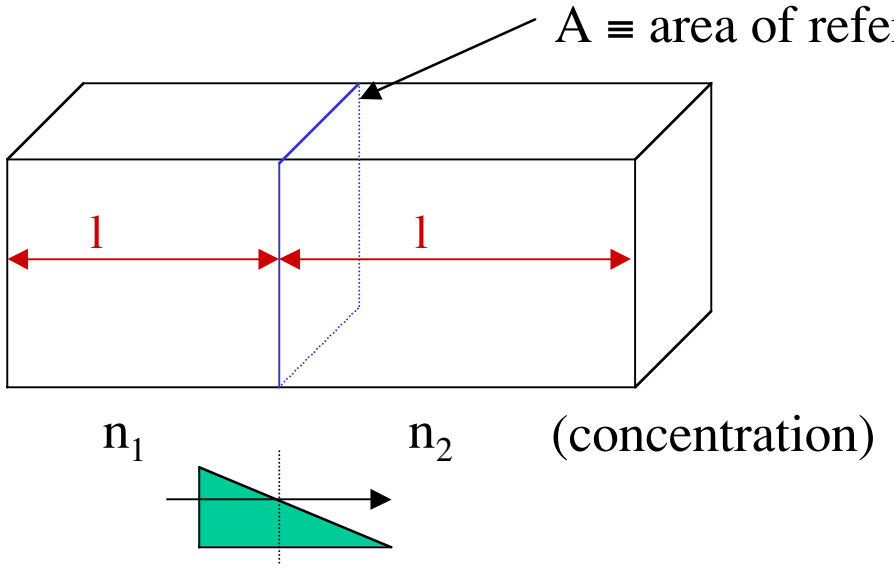
Measured classically by observing the rate of "spreading" of the material: flux across a boundary

Two equations relate the rate of change of concentration of particles as a function of position and time:

Fick's first law

Fick's second law

Flux of particles depends on the concentration gradient
Fick's first law



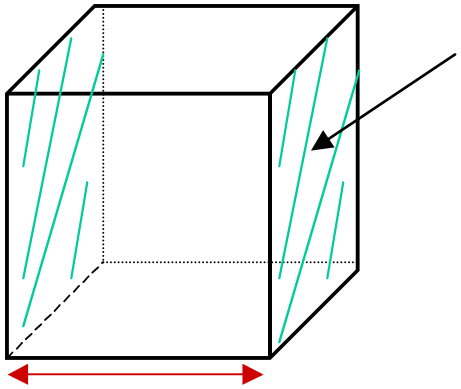
Define the diffusion coefficient: D (cm^2/sec)

$$J = \text{flux} \equiv \frac{\text{moles (net)}}{\text{area} \bullet \text{time}}$$

Fick's 1st law:

$$J = -D\left(\frac{dn}{dx}\right)$$

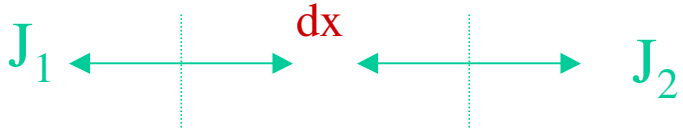
Change in concentration requires a difference in concentration gradient
Fick's second law

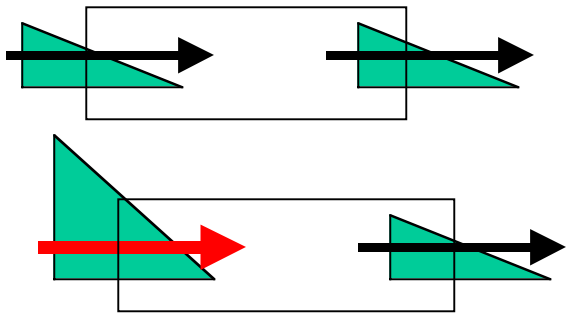


change in # particles per unit time

$$\frac{dn}{dt} = \frac{(J_1 - J_2)\,A}{dx\,A}$$

volume





Fick's 2nd law:

$$\frac{dn}{dt} = D\left(\frac{d^2n}{dx^2}\right)$$

Constant gradient: same amount leaves as enters the box

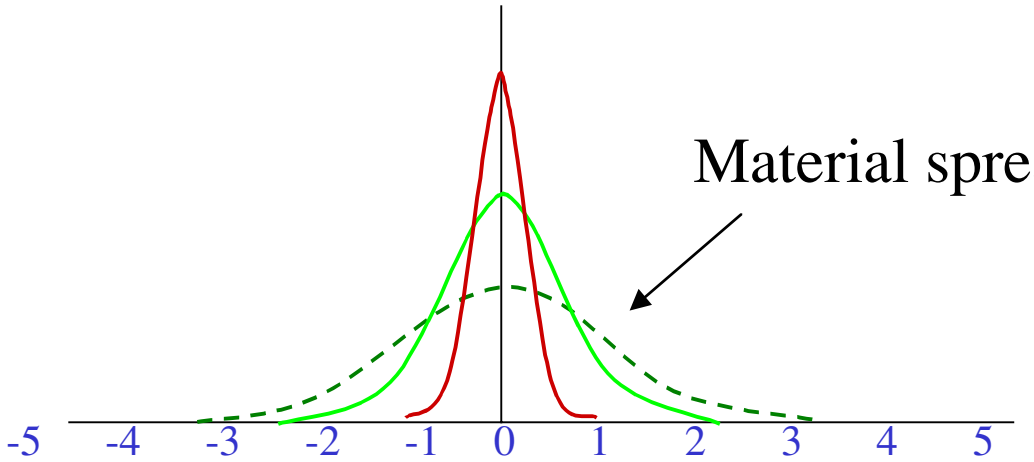
Gradient higher on left: more enters the box than leaves

Translational Diffusion

Solve Fick's Laws - differential equations (relate concentration, position, time)
(define boundary and initial conditions)

diffusion in 1 - dimension

All (N) particles are at x = 0 at t = 0



$$c(x,t) = \frac{N}{2}(\pi D t)^{1/2} \bullet e^{-x^2 / 4 D t}$$ ← solution (Gaussian)

Note: $\langle x^2 \rangle$ = average value of x^2

$$\langle x^2 \rangle = \int_{-\infty}^{+\infty} p(x)\, dx \bullet (x^2)$$ where p(x) is the probability of the molecules being at position x

$\langle x^2 \rangle = 2 D t$ **mean square displacement**

Mean square displacement - in 3-dimensions

isotropic diffusion $D_x = D_y = D_z$

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

$$\boxed{l^2 = 6Dt}$$

where $l^2 = \langle x^2 \rangle + \langle y^2 \rangle + \langle z^2 \rangle$

$$D = \frac{l^2}{6t} \quad cm^2/sec$$

average displacement from the starting point = $(6Dt)^{1/2}$

NOTE: Mean displacement $\propto \sqrt{\text{time}}$

Values of Diffusion Coefficients

For a gas **D** ≈ 1 cm^2/sec

For a solid **D** ~ 10^{-8} - 10^{-10} cm^2/sec or smaller

For a small molecule in solution:

D ~ 10^{-5} cm^2/sec

(takes 3 days to go ~ 4 cm)

For a protein (eg BSA, 70,000 mol weight)

D ≈ 10^{-6} - 10^{-7} cm^2/sec

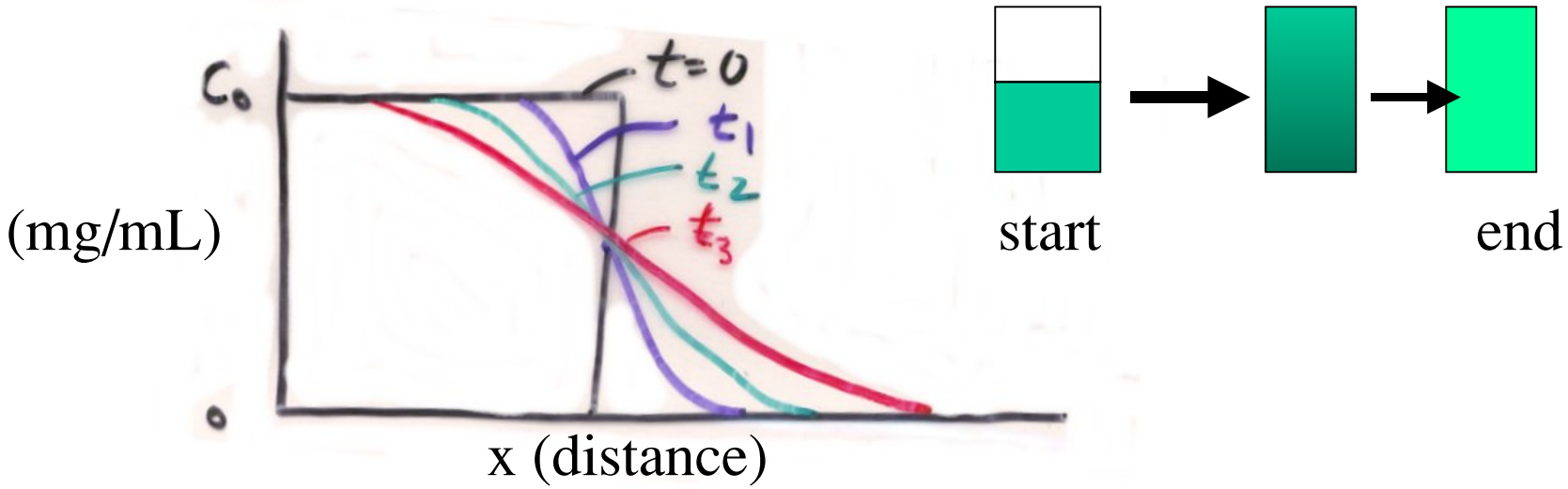
(3 days → 1 cm)

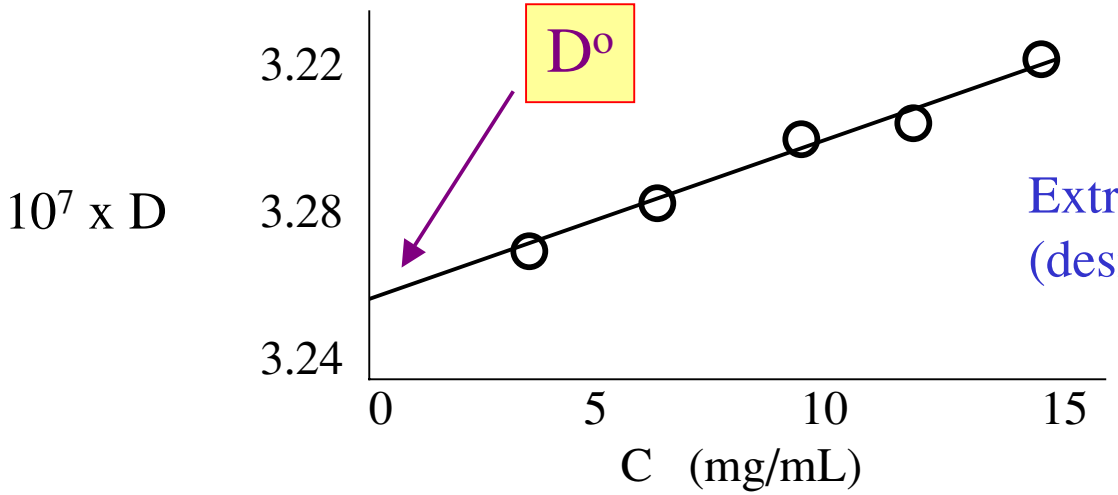
Large Macromolecules (R_s) - e.g DNA

very slow diffusion, NOT a useful technique

Translational Diffusion

Classical technique ≡ boundary spread





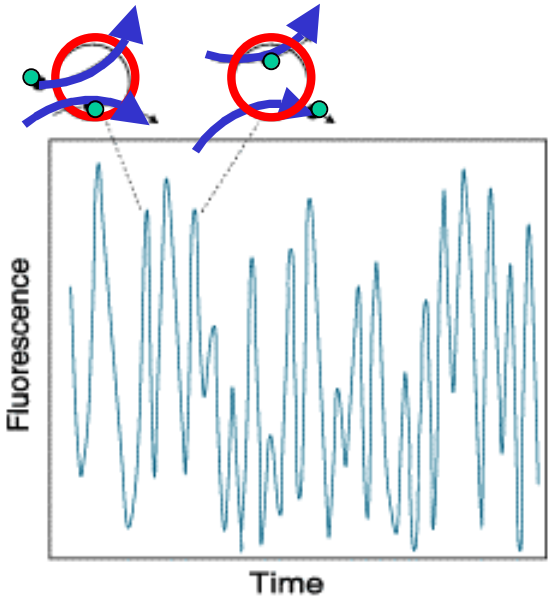
BSA

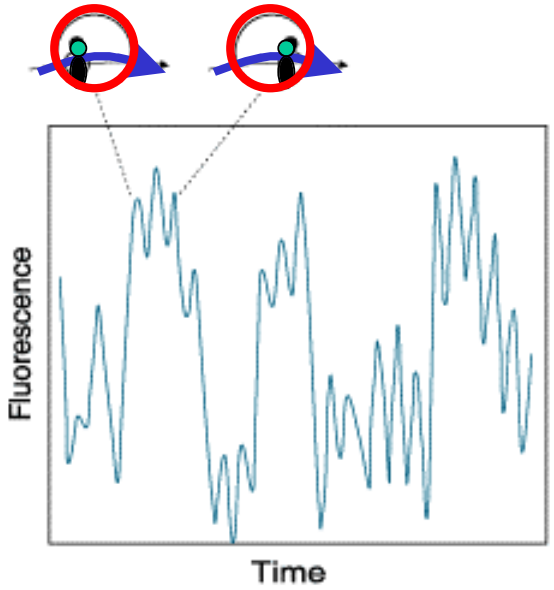
Extrapolate to infinite dilution (designated by D^o)

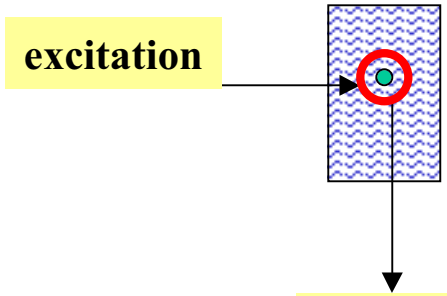
* values of D have NOT been corrected for pure water at 20°C

Another way to measure the value of the Diffusion Coefficient is by Fluorescence Correlation Spectroscopy (FCS)

By measuring fluctuations in fluorescence, the residence time of a fluorescent molecule within a very small measuring volume (1 femtoliter, 10^{-15} L) is determined.
This is related to the **Diffusion Coefficient**







http://www.probes.com/handbook/boxes/1571.html

molecules moving into and out of the measuring volume:
fast (left) vs slow (right)

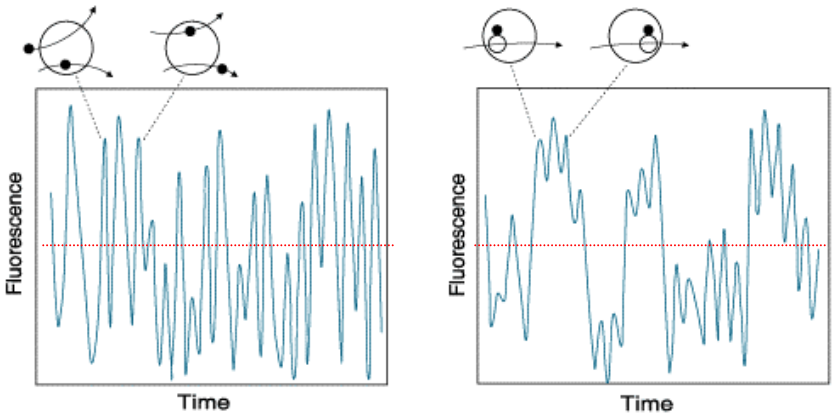
Fluorescence Correlation Spectroscopy: FCS

the time-dependence of the fluorescence is expressed as an autocorrelation function, $G(\tau)$,

the is the average value of the product of the fluorescence intensity at time t versus the intensity at a short time, τ, later. If the values fluctuate faster than time τ then the product will be zero.

deviation from the average intensity

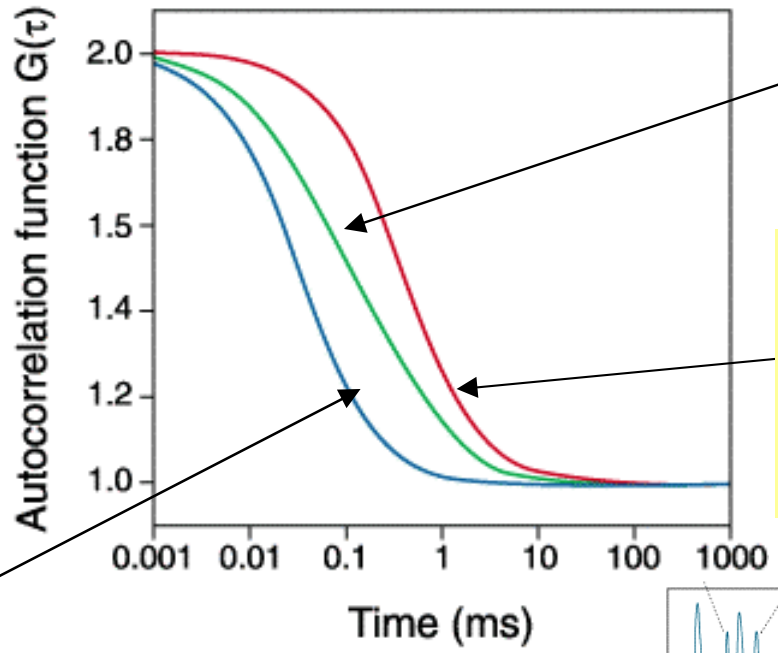
$$G(\tau) = \frac{\langle \delta F(t) \bullet \delta F(t+\tau) \rangle}{\langle F(t) \rangle^2}$$



An example of FCS: simulated autocorrelation functions of a free fluorescence ligand and the same ligand bound to a protein

$$G(\tau) = \frac{\langle \delta F(t) \bullet \delta F(t+\tau) \rangle}{\langle F(t) \rangle^2}$$

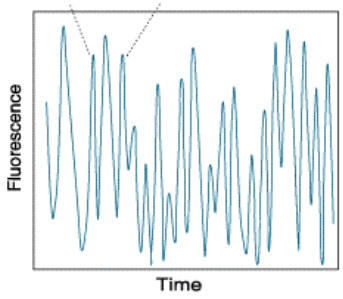
$G(\tau)$ goes to zero at long times

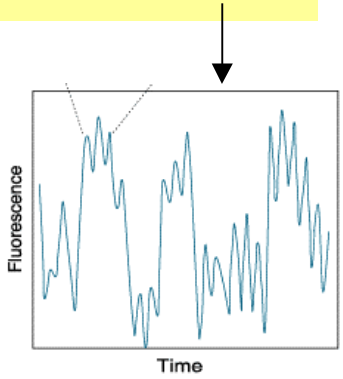


1:1 mix of free/bound ligand

bound ligand on slow moving protein

free ligand (small, fast diffusion)





Relating D to molecular properties

Rate of mass flux is inversely proportional to the frictional drag on the diffusing particle

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

k = Boltzman constant
f = frictional coefficient

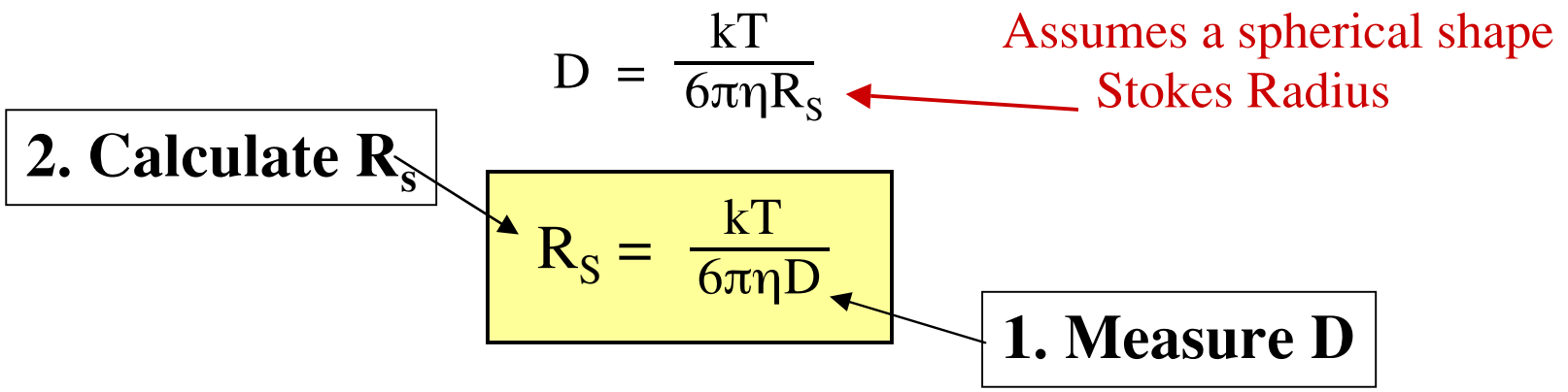
But $f = 6\pi\eta R_S$

for a sphere or radius R_s

η = viscosity of solution

Stokes-Einstein Equation $D = \left(\frac{kT}{6\pi\eta R_S}\right)$

Stokes Radius obtained from Diffusion



1 You need additional information to judge whether the particle is really spherical

-A highly asymmetric particle behaves like a larger sphere - higher frictional coefficient (f)

2 Deviations from the assumption of an anhydrous sphere (R_{min}) are due to either

a) hydration
b) asymmetry

3 Stokes radius from different techniques need not be identical

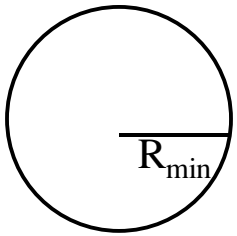
Interpreting the meaning of the Stokes Radius

$$D = \frac{kT}{f} = \frac{kT}{6\pi\eta R_S}$$

1. Measure D and get R_s
2. Compare R_s to R_{min}

Experimental: $f = 6\pi\eta R_s$

Theoretical: Define: $f_{min} = 6\pi\eta R_{min}$

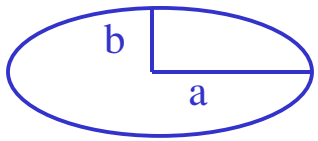


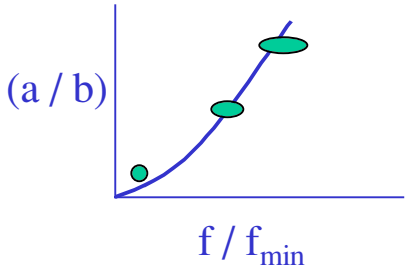
$$vol = [\overline{V_2} \bullet \frac{M}{N}]$$

$$= \frac{4}{3}\pi R^3_{min}$$

so: $\left(\frac{R_s}{R_{min}}\right) = \left(\frac{f}{f_{min}}\right)$

Hydrodynamic theory defines the shape dependence of f , frictional coefficient



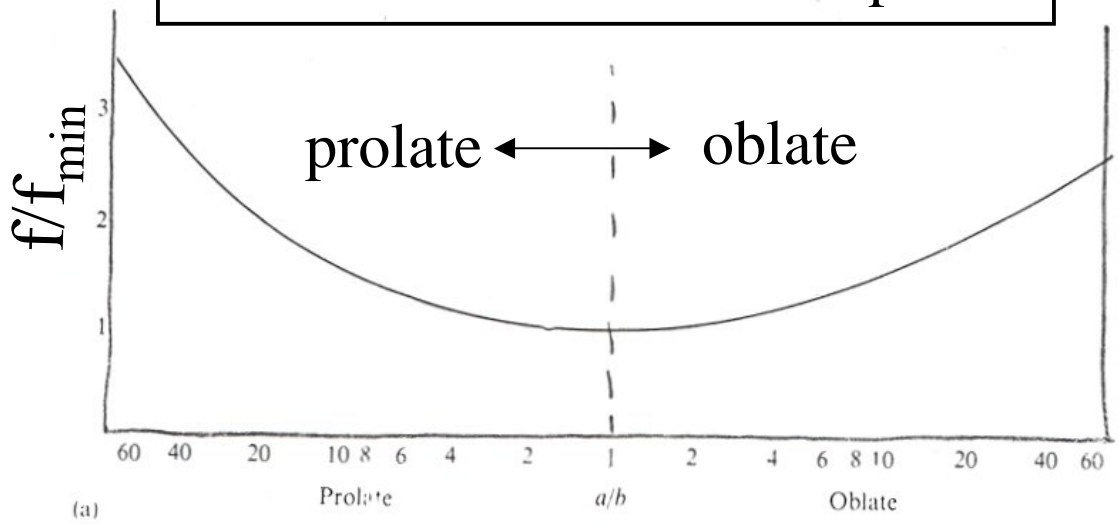


this allows one to estimate
effects due to molecular asymmetry

Shape factor for translational diffusion

for a prolate ellipsoid

frictional coefficient of ellipsoids



Axial ratio	Prolate ν	Prolate f/f_min
1	2.500	1.000
2	2.908	1.044
3	3.685	1.112
4	4.663	1.182
5	5.806	1.250
6	7.098	1.314
8	10.103	1.433
10	13.634	1.543
15	24.65	1.784
20	38.53	1.996
30	74.51	2.356
40	120.76	2.668
50	176.81	2.946
60	242.28	3.201
80	400.5	3.658
100	593.7	4.067
200	2,052.9	5.708

much larger effect of shape on viscosity than on diffusion

viscosity shape factor

frictional coefficient shape factor

(Cantor + Schimmel)

Interpreting Diffusion Experiments:

does a reasonable amount of hydration explain the measured value of D?

Protein	M	$D^{o}_{20,W}$ x10^{-7} cm^2/s	R_S(Å) (diffusion)
RNAse	13,683	11.9	18 (R_{min}=17Å)
Collagen	345,000	0.695	310 (R_{min}=59Å)

protein	Maximum solvation	Maximum asymmetry
RNAse	**δ_{H2O} = 0.35**	a/b = 3.4
Collagen	δ_{H2O} = 218	**a/b = 300**

Volume per gram of anhydrous protein

$$\left[\frac{R_S}{R_{min}}\right] = \left[\frac{(\bar{V}_p + \delta_{H2O})}{\bar{V}_p}\right]^{1/3}$$

solve for δ_{H2O}

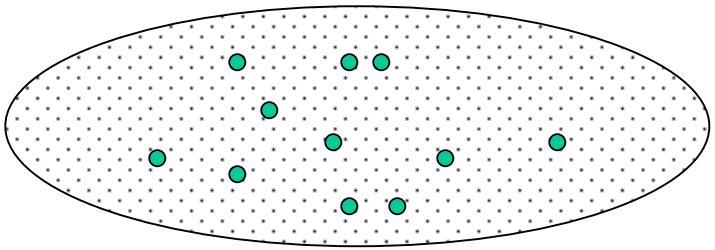
What is the Diffusion Coefficient of a Protein in the bacterial cytoplasm?

Are proteins freely mobile?

Some proteins will be tethered

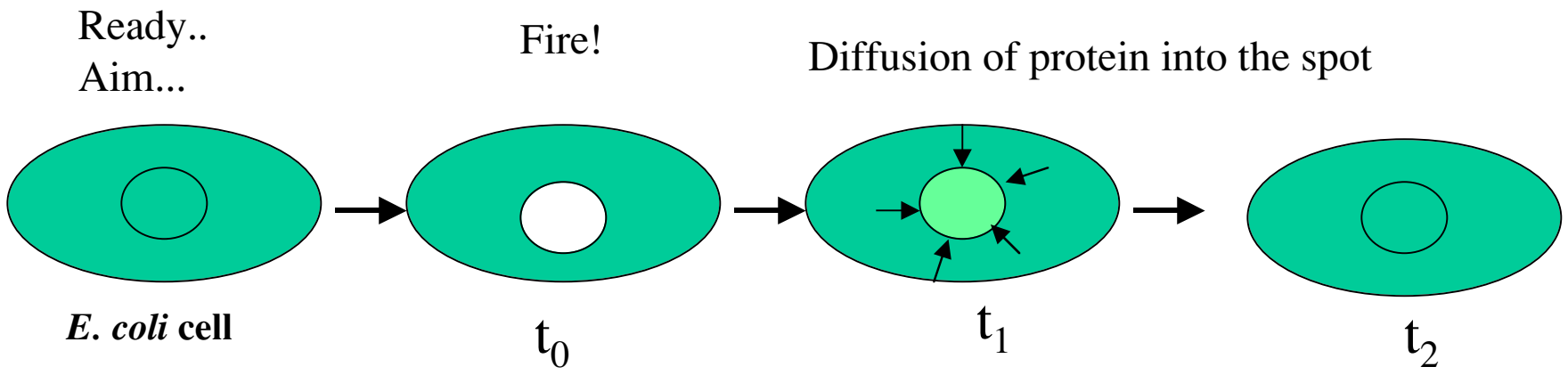
Some proteins will interact transiently with others
and appear to move slowly

Free diffusion will be slower due to “crowding” effect
excluded volume effect at high concentration of protein



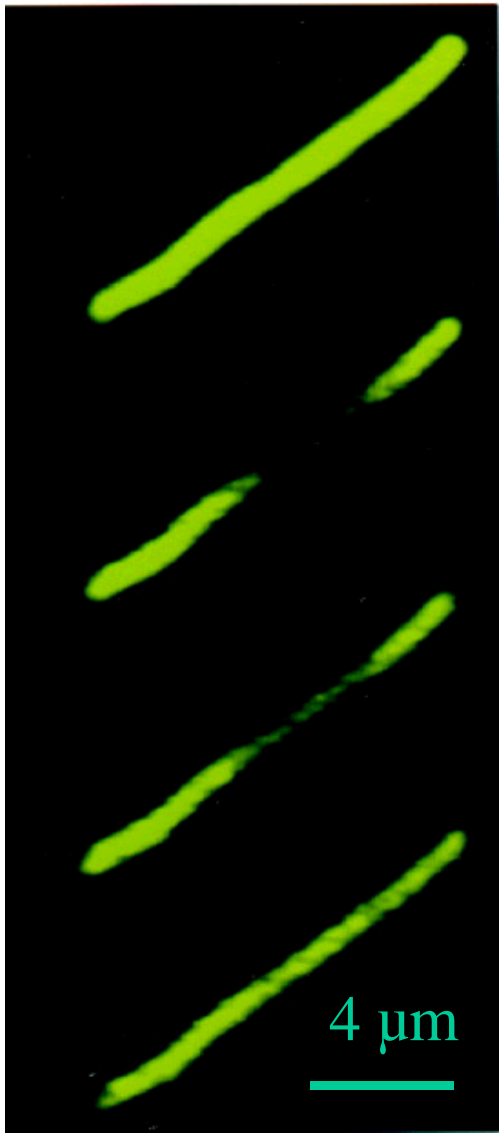
Measuring the Diffusion of Proteins in the Cytoplasm of *E. coli*

Fluorescence Recovery After Photobleaching (FRAP)



1. Express a protein that is fluorescent: green fluorescent protein, GFP.
2. Use a laser to "photo-bleach" the fluorescent protein in part of a single bacterial cell. This permanently destroys the fluorescence from proteins in the target area.
3. Measure the intensity of fluorescence as the protein diffuses into the region which was photo-bleached.

Diffusion of the Green Fluorescent Protein inside *E. coli*



Single cell, expressing GFP

Bleach cell center with a laser, t_0

t = 0.37 sec after flash

t = 1.8 sec after flash

one can observe the molecules diffusing back into the bleached area

J. Bacteriology (1999) 181, 197-203

Diffusion of the Green Fluorescent Protein inside *E. coli*

Results: D = 7.7 μm^2/sec (7.7 x 10^{-8} cm^2/sec)

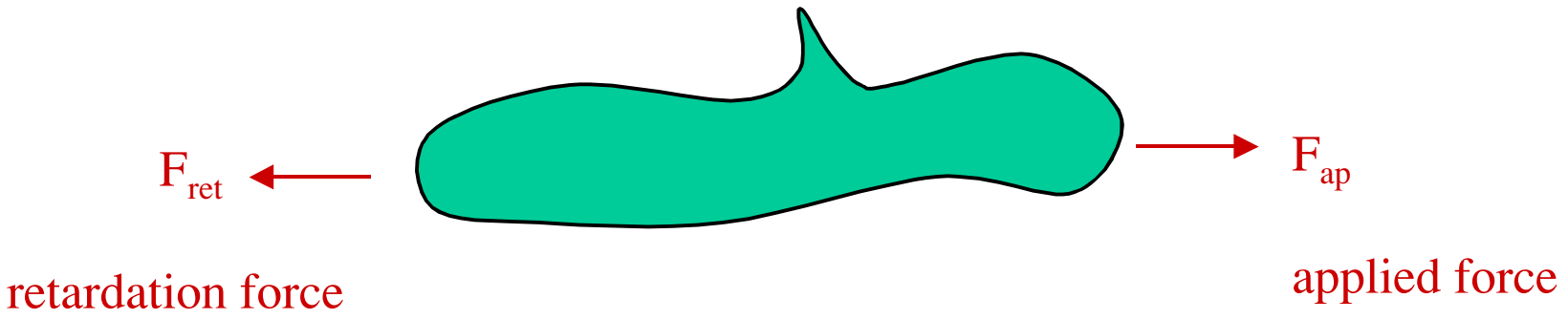
this is 11-fold less than the diffusion coefficient in water = 87 μm^2/sec

Slow translational diffusion is due to the crowding resulting from the very high protein concentration in the bacterial cytoplasm (200 -300 mg/ml)

J. Bacteriology (1999) 181, 197-203

Mass Transport Techniques

Measure the steady state velocity of hydrodynamic particles under the influence of an applied force



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1 Sedimentation velocity

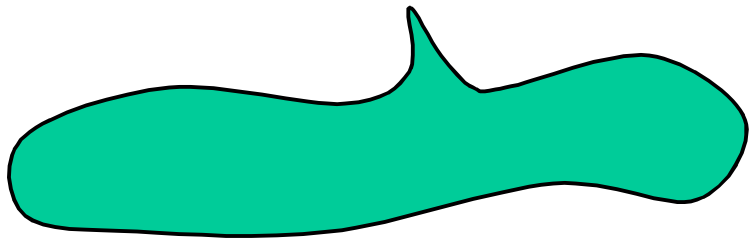
2 Electrophoresis

3 Gel filtration chromatography

Sedimentation velocity

F_{ap} = centrifugal force
F_{ret} = frictional drag

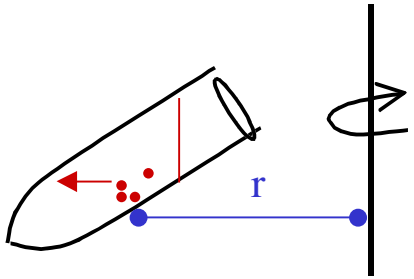
measure: $\frac{\text{velocity}}{\text{centrifugal acceleration}}$ = sedimentation coefficient S



F_{ret} ← retardation force

→ F_{ap} applied force

Sedimentation Velocity



ω = circular velocity
radians / sec

centrifuge

$$F_{ap} = \omega^2 r\,(m_h - \upsilon_h \rho)$$

mass of particle corrected for buoyancy

$$\mathbf{m_h} = \frac{M}{N}(1 + \delta_{H2O})\ ;\ \mathbf{\upsilon_h} = \frac{M}{N}(\overline{V}_2 + \overline{V}_{H2O}\,\delta_{H2O})$$

Substitute:

$$F_{ap} = \frac{\omega^2 r\, M}{N}\ (1 - \overline{V}_2 \rho)$$

Note: terms for bound water drop out of equation

in steady state: $\mathbf{F_{ap} = F_{ret} = f \bullet (velocity)}$

measure →

$$S = \frac{velocity}{\omega^2 r} = \frac{M\,(1 - \overline{V}_2\,\rho)}{Nf}$$

Sedimentation Coefficient:
depends on three molecular variables: M, $\overline{V}_2$, and f

mol. wt — inverse density of particle

$$S = \frac{M\,(1 - \overline{V}_2\rho)}{Nf}$$

shape dependence

$$\mathbf{f = 6\pi\eta R_s}$$

units : seconds

1 Svedberg = 10^{-13} seconds

Sedimentation value depends on solution conditions: η and ρ

$$S = \frac{M(1 - \bar{V}_2\rho)}{Nf}$$

$$f = 6\pi\eta R_s$$

infinite dilution

$S^o_{20,w}$

20°C

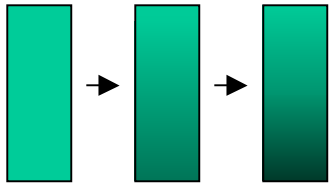
in water (correct for viscosity and temperature from conditions of actual measurement)

S-values are usually reported for "standard conditions"

Types of Centrifuges used to measure the S-value

1 **Analytical Ultracentrifuge** (monitor the distribution of material by absorption or dispersion) as a function of time

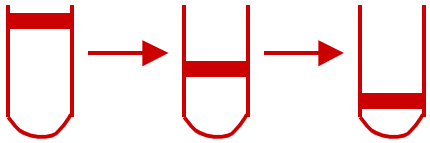
- Method of choice, but requires specialized equipment
- Beckman "Optima" centrifuge
- small sample, but must be pure - optical detection



used to determine sedimentation velocity ⇒ S

(frontal analysis (**moving boundrary** method) - not zonal method)

2 **Preparative Ultracentrifuge**



- common instrumentation
- sedimentation coefficient obtained by a "zonal method"

⇒ **requires a density gradient to stabilize against turbulence / convection**

⇒ obtaining S usually requires comparison to a set of standards of known S value