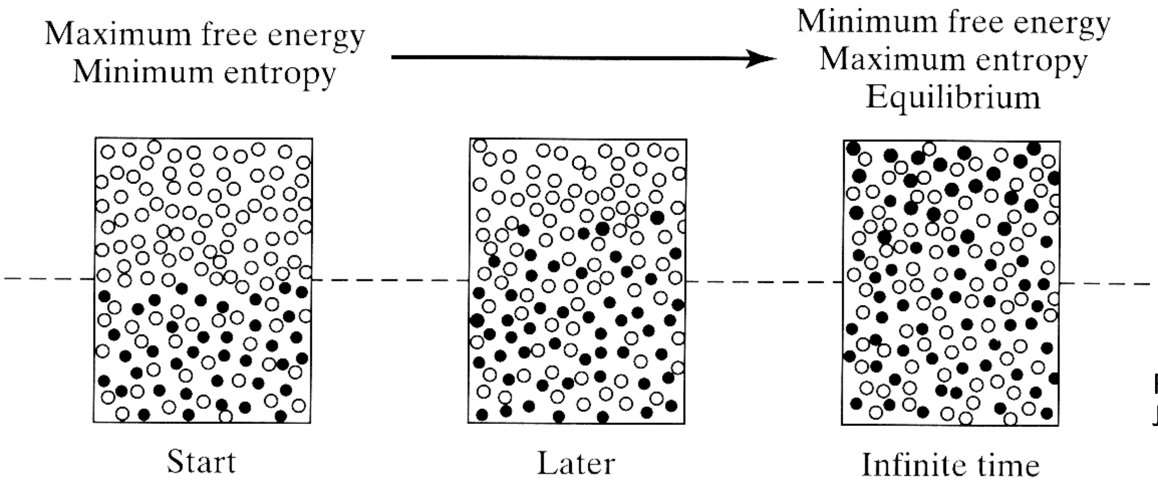
Diffusion:



From: van Holde, Johnson & Ho (1998)

Figure 14.3 Diffusion as a mixing process. If the solvent is layered over solution, diffusion ultimately leads to uniform concentration throughout the vessel.

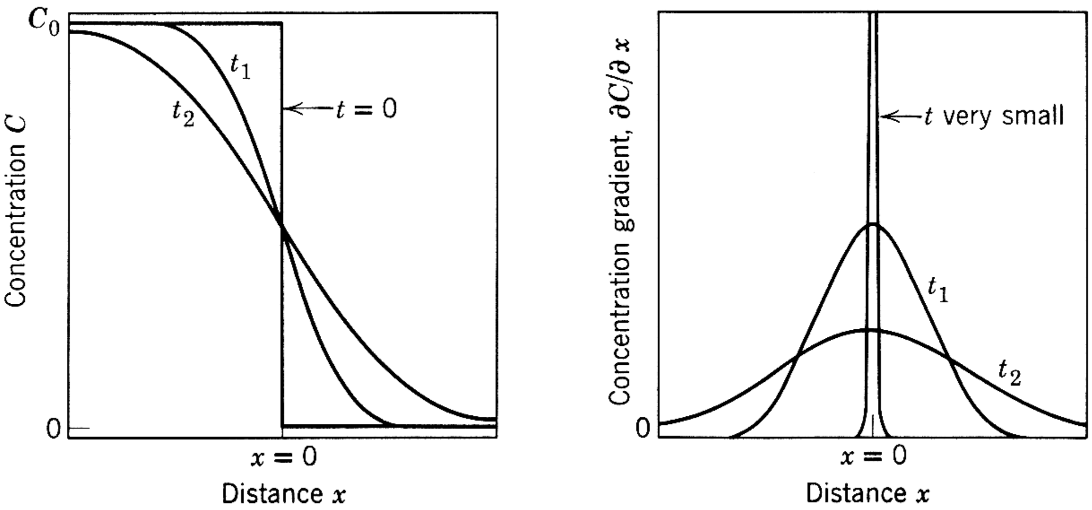
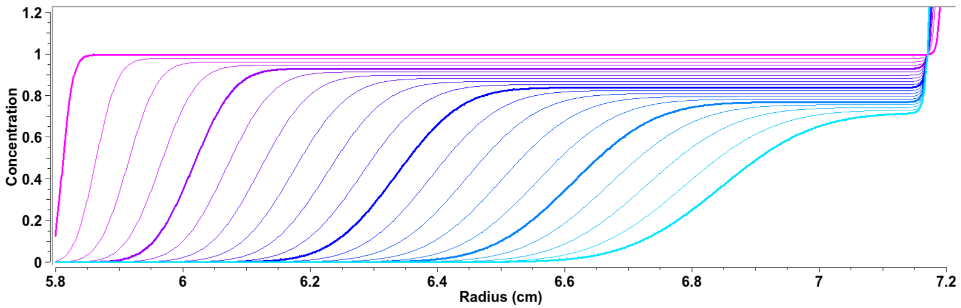
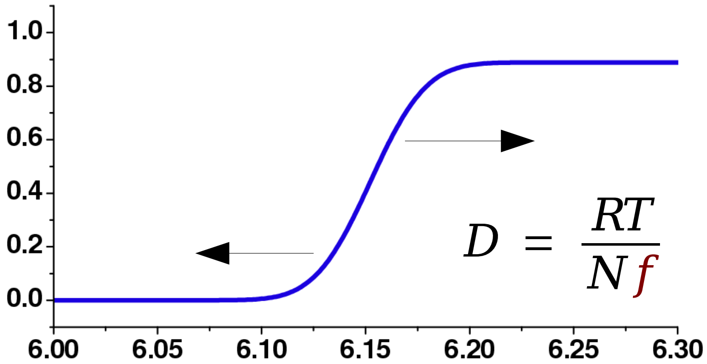


Fig. 21–1. Progress of a diffusion experiment with initially sharp boundary at $x = 0$.

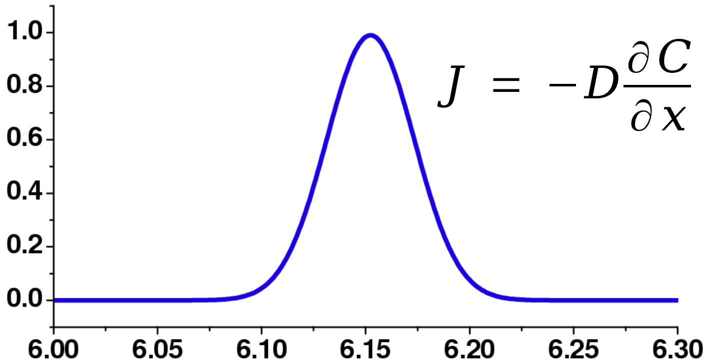


Random translation of a particle due to Brownian motion



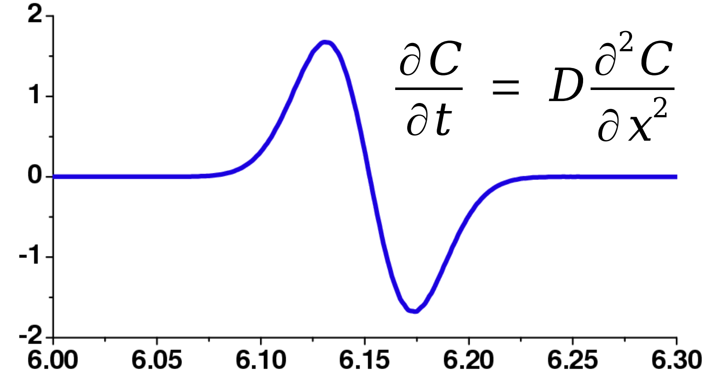
$$D = \frac{RT}{Nf}$$

The flow due to diffusion is proportional to the concentration gradient and the diffusion coefficient (Fick's first law):



$$J = -D\frac{\partial C}{\partial x}$$

The rate of change of concentration is proportional to the change in steepness of the concentration gradient (Fick's second law):

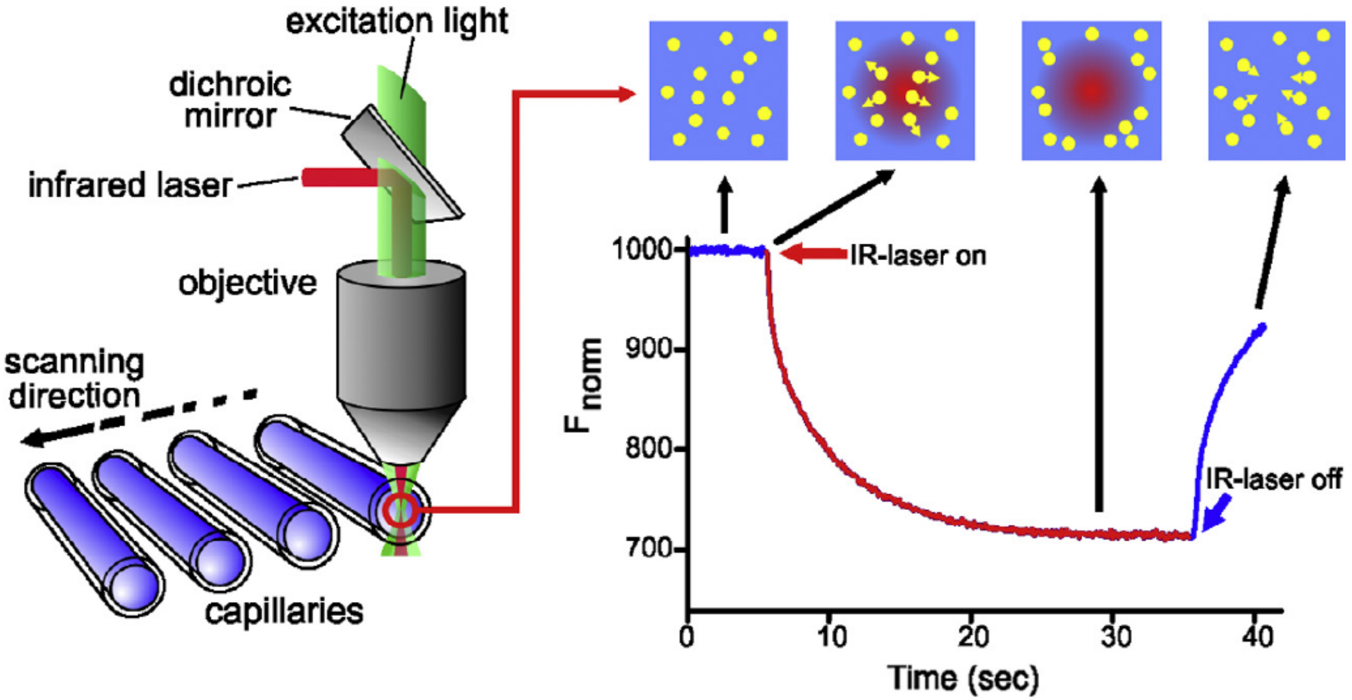


$$\frac{\partial C}{\partial t} = D\frac{\partial^2 C}{\partial x^2}$$

Transport Processes – Thermophoresis:

Two observables are measured in a microscale thermophoresis experiment: 1. decrease of fluorescence from the **T**emperature **R**elated **I**ntensity **C**hange (TRIC), and 2. from the thermophoresis of the fluorescently tagged molecules. The thermophoresis can be described by:

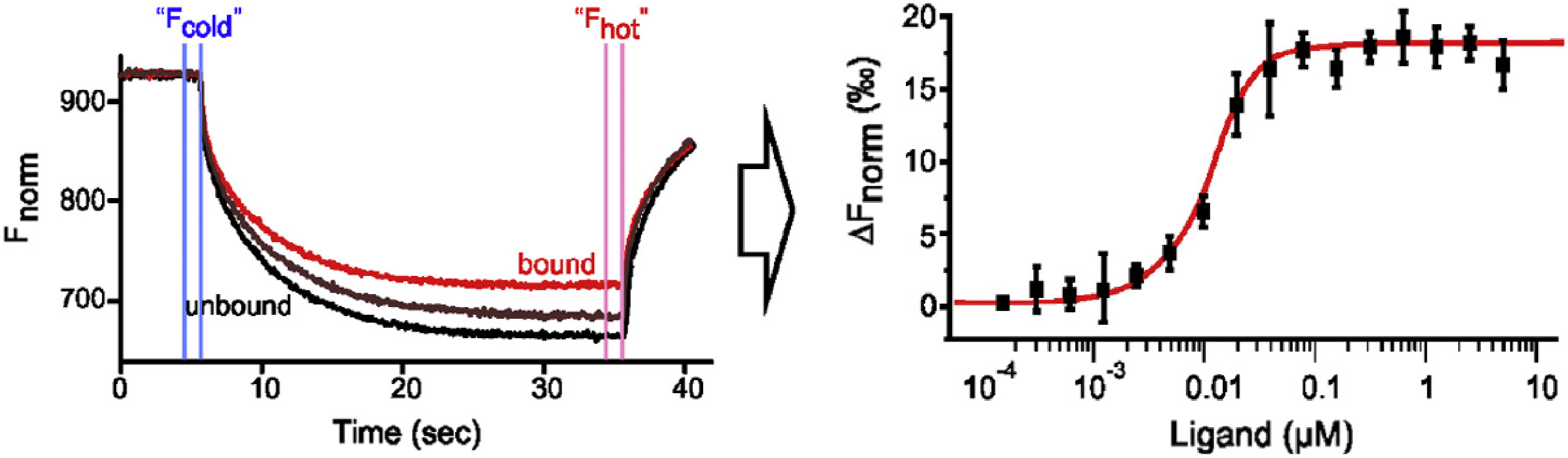
$$\frac{C_{hot}}{C_{cold}} = e^{\left(-S_t \Delta T\right)}$$



Transport Processes – Thermophoresis:

It is important to realize that you can neither measure the local concentration nor the local temperature, so a true S_T is not really available.

Instead, plot the normalized ratio of F_{hot} over F_{cold} for different ligand concentrations to obtain a binding isotherm for the titration:



Credits: M. Jerabek-Willemsen et al., J. Mol. Struct., 2014 (1077), 101-113

Transport Processes – Thermophoresis:

It is important to realize that you can neither measure the local concentration nor the local temperature, so a true S_T is not really available.

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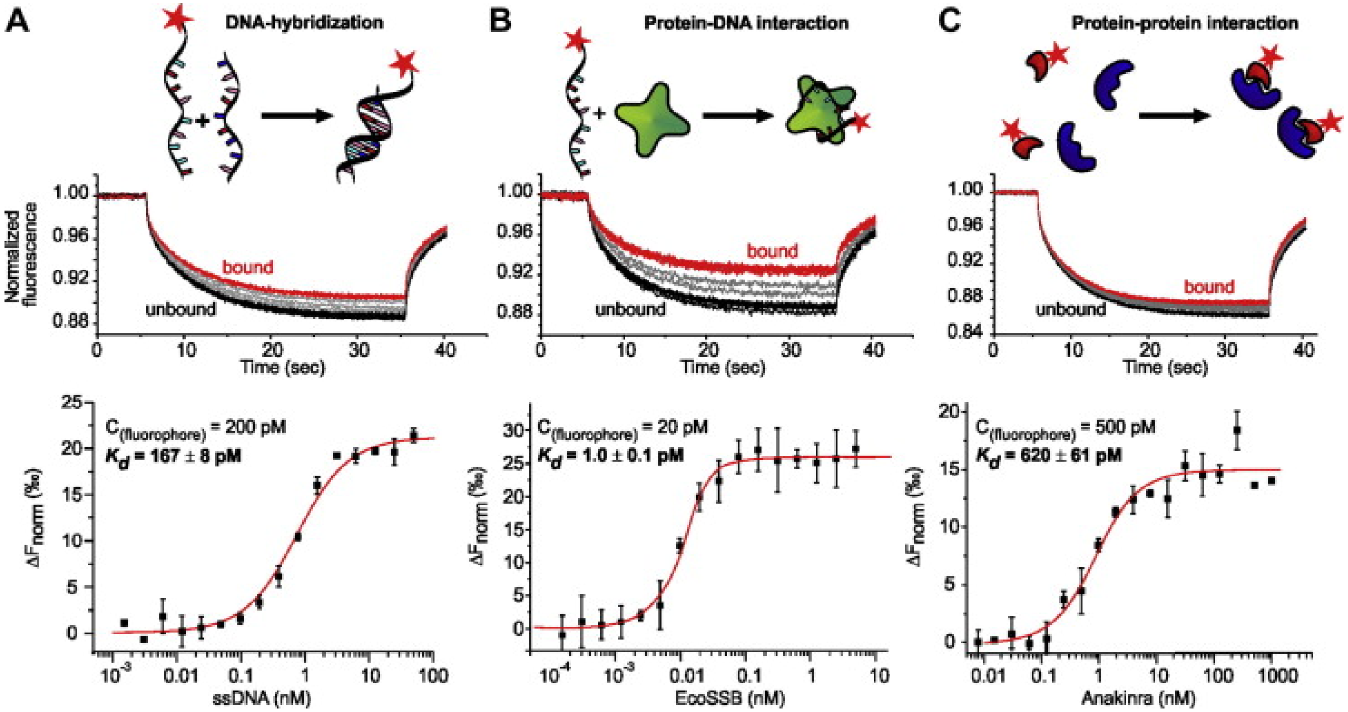


How do we measure Diffusion?

1. **Boundary method**

2. **Dynamic light scattering**

3. **Sedimentation Velocity**

Diffusion:
Boundary Method

Diffusion Equation:

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

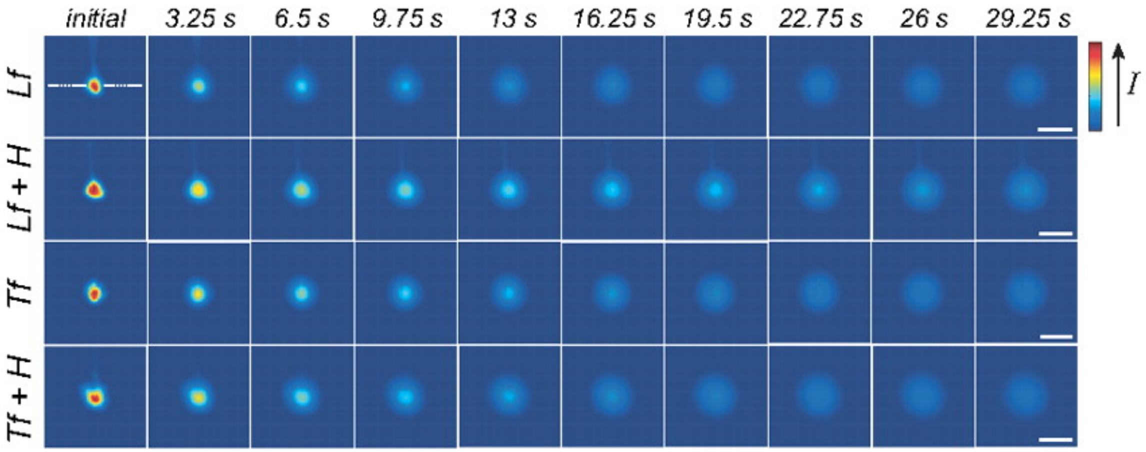
with solution:

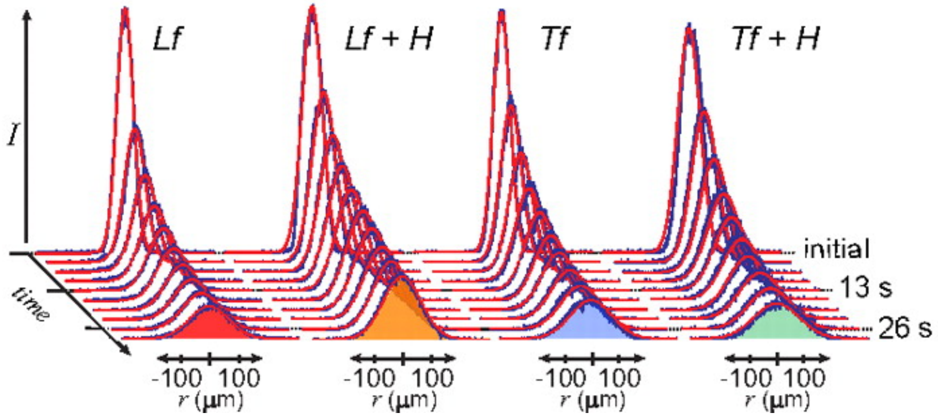
$$\left(\frac{\partial C}{\partial x}\right)_{T,P} = C_0\frac{1}{2\sqrt{\pi D t}}e^{\frac{-(x-x_0)^2}{4Dt}}$$

Substitute: $4Dt = 2\sigma^2$

Gaussian Equation:

$$y = \frac{1}{\sigma\sqrt{2\pi}}e^{\frac{-(x-\mu)^2}{2\sigma^2}}$$

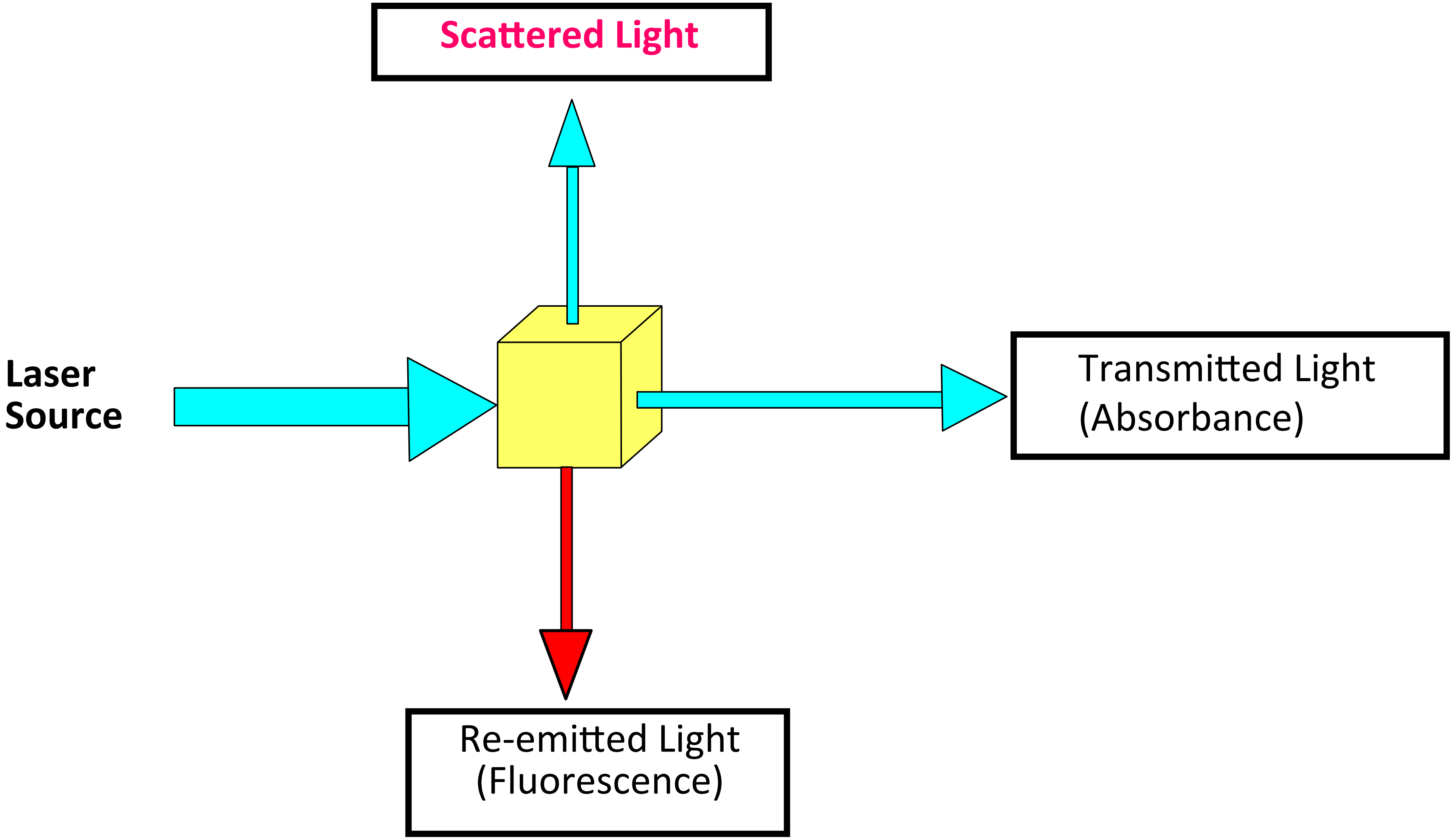




Thorne R G et al. PNAS 2008;105:8416-8421

PNAS

Light Scattering:



Scattered Light

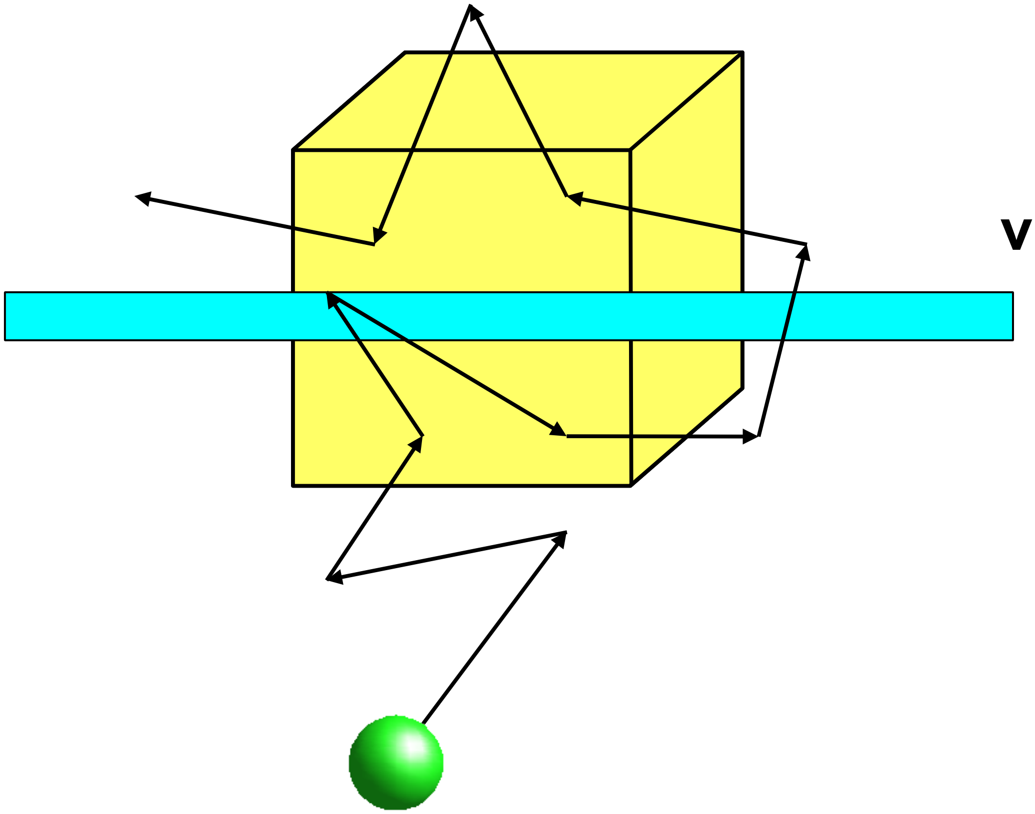
Laser Source

Transmitted Light (Absorbance)

Re-emitted Light (Fluorescence)

Light Scattering:

Consider a small volume element:



A macromolecule will diffuse through the volume element because it experiences

Brownian motion

(dependent on temperature)

There are 3 types of light scattering in use:

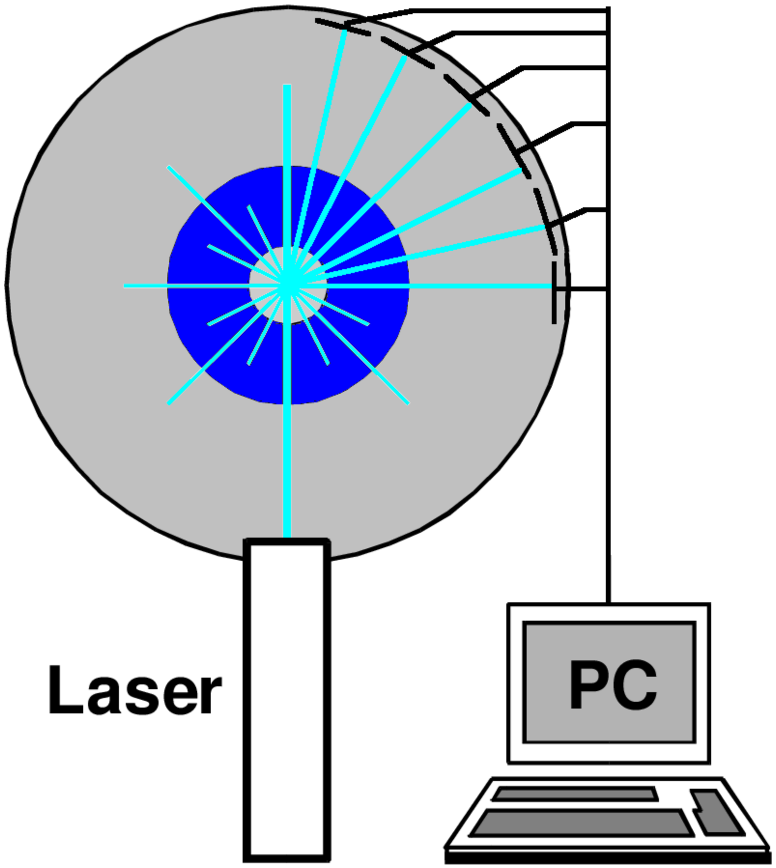
- Static light scattering (observe over long time)
  - Obtain weight-average molecular weight
  - Measure inline with SEC (SEC-MALS)
  - Detect aggregates
  - Obtain radius of gyration (large molecules only)

- Dynamic light scattering (observe over short time)
  - Obtain diffusion coefficients

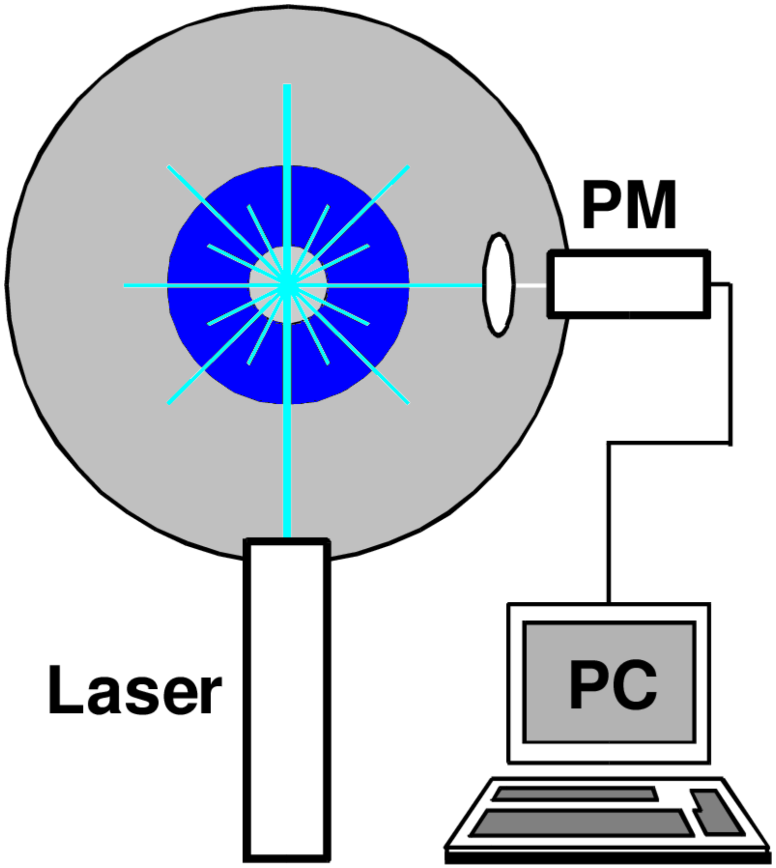
- Small-angle X-ray/neutron scattering
  - Short wavelengths provide shape information

Modern Laser Light Scattering Apparatus

Multi-Angle/Static
Light Scattering (MALS)



Dynamic Light Scattering (DLS)



For small particles (particle size < 1/50 of wavelength) the scattering is essentially independent of angle.
This type of scattering is also called *Rayleigh Scattering*

Larger molecules produce an angular dependency and the *Radius of Gyration* can be measured directly.

The scattering is dependent on the concentration, the angle (for large molecules), and the size of the particle.

STATIC LIGHT SCATTERING

Scattering from SMALL Particles

(Diameter < 1/50th λ): Rayleigh Scattering

Rayleigh Ratio:

$$R_\theta = \frac{\bar{i}}{I_0}\frac{r^2}{1+\cos^2\theta} \qquad (1)$$

Instantaneous Scattering

$$\frac{\bar{i}}{I_0} = K\frac{\left(1+\cos^2\theta\right)\ MC}{r^2\left[1+C\left(\partial \ln y/\partial C\right)\right]} \qquad (2)$$

$$\frac{\bar{I}}{I_0} = \frac{1+\cos^2\theta}{2r^2}\left(\frac{2\pi}{\lambda}\right)^4\left(\frac{n^2-1}{n^2+2}\right)^2\left(\frac{d}{2}\right)^6 \qquad (3) \quad \textit{where} \quad K = \frac{2\pi^2 n_0^2\left(dn/dC\right)^2}{N\lambda^4}$$

θ = scattering angle
R_θ = Rayleigh ratio at angle θ
$\bar{i}$ = average measured scattering intensity
I_0 = incident intensity
N = Avogadro's number
n = refractive index
n_0 = refractive index of solvent

dn/dC = refractive index increment
M = molecular weight
C = concentration of solute
$\ln y$ = chemical potential
B = 2nd virial coefficient
r = distance from scattering center
d = diameter of the scattering particle

Rayleigh Scattering:

Scattering from SMALL Particles (Diameter < 1/50th λ):

Combine equations (1) and (2):

$$\boxed{\frac{KC}{R_\theta} = \frac{1}{M}\left(1 + C\,\frac{\partial \ln(y)}{\partial C}\right)}$$

This can be approximated by:

$$\boxed{\frac{KC}{R_\theta} \approx \frac{1}{M} + 2BC}$$

At zero concentration:

$$\boxed{\frac{KC}{R_\theta} = \frac{1}{M}}$$

For heterogeneous solutions the light scattering method gives a *weight average* molecular weight:

$$R_\theta = \sum_{i=1}^{k} R_{\theta,i} = \sum_{i=1}^{k} K C_i M_i = K C \left[\frac{\sum_{i=1}^{k} C_i M_i}{\sum_{i=1}^{k} C_i} \right] = K \bar{M}_w C$$

The weight average molecular weight is biased towards the large molecules in the mixture. This makes light scattering sensitive for *aggregates*.

Scattering from large particles (diameter > 1/50th λ)

Large particles introduce an angular dependence due to interference patterns

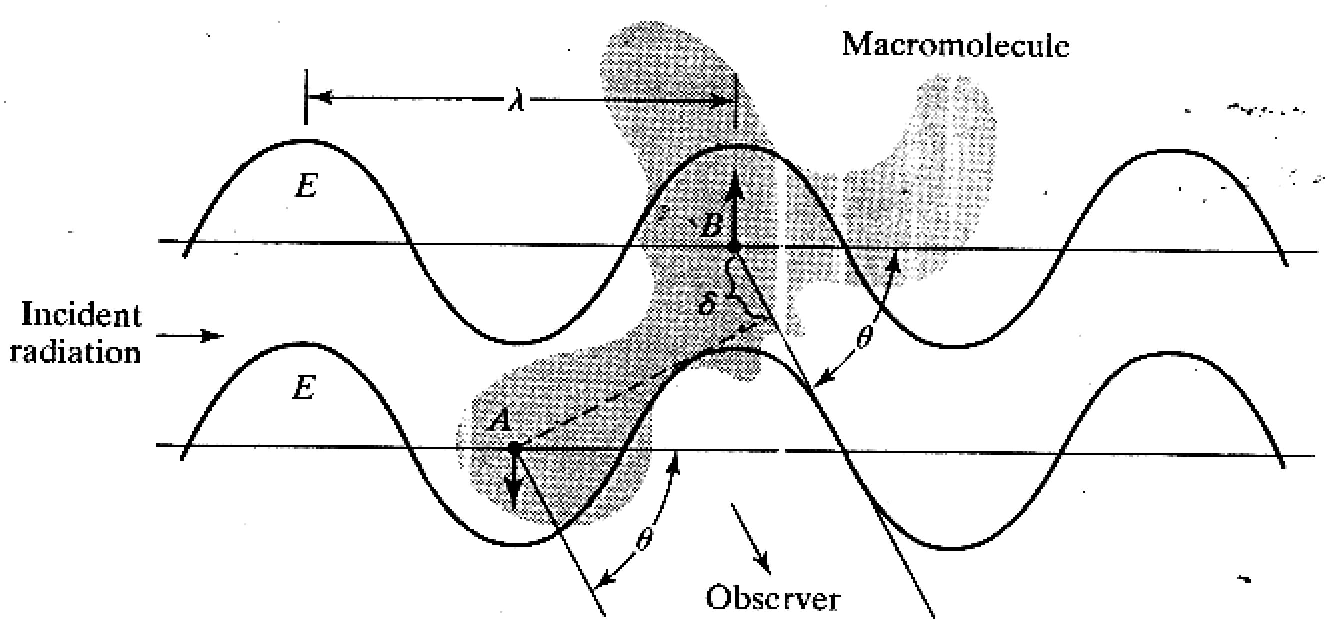


Figure 7.5 Scattering from a macromolecule that is large compared to λ. Two points from which scattering occurs are shown at A and B. The phase of the radiation (and hence of the induced dipoles) is clearly different at the two points. Also, the two points are at different distances from the observer.

$$R_\theta = \frac{\bar{i}}{I_0} \frac{r^2}{1+\cos^2\theta}$$

The scattering from all scattering centers in a large molecules need to be considered. Scattering centers are fixed with respect to each other, but since Brownian motion places the molecule in multiple orientations, the observed scattering is influenced by interference and we need to introduce a new function, P(θ). P(θ) is the ratio of the observed scattered intensity over the theoretical scattering intensity if the molecule were a Rayleigh scatterer with scattering centers infinitesimal in size compared to λ.

$$\frac{1}{P(\theta)} \cong \left(1 + \frac{16\pi^2}{3} \frac{{R_G}^2}{\lambda^2} \sin^2(\theta/2) \right)$$

If λ = 500 nm and R_G = 50 nm, then

1/P(θ) = 1.530 at θ = 180° (max. angle)
1/P(θ) = 1.265 at θ = 90°
1/P(θ) = 1.013 at θ = 9°

If λ = 500 nm and R_G = 5 nm, then
1/P(θ) = 1.0053 at θ = 180°
1/P(θ) = 1.0003 at θ = 90°
1/P(θ) = 1.0001 at θ = 9° (No correction)

(Rayleigh scattering is observed)

For large particles, we need to introduce a function P(θ) to account for the angular dependency. For small particles this function reduces to unity, and the large molecule function reduces to the Rayleigh Scattering function:

For small molecules, we have:

$$\boxed{\frac{KC}{R_\theta} \approx \frac{1}{M} + 2\mathrm{BC}}$$

For large molecules, we have:

$$\boxed{\frac{KC}{R_\theta} \approx \frac{1}{P(\theta)}\left[\frac{1}{M} + 2\mathrm{BC}\right]}$$

For large molecules, we have:

$$\boxed{\frac{KC}{R_\theta} \approx \frac{1}{P(\theta)}\left[\frac{1}{M} + 2\mathrm{BC}\right]}$$

where: $$P(\theta) = \frac{1}{N^2}\left[\sum_{i=1}^{N}\sum_{j=1}^{N}\frac{\sin h R_{i,j}}{h R_{i,j}}\right]$$ **and:** $$h = \frac{4\pi}{\lambda}\sin\frac{\theta}{2}$$

$R_{i,j}$ **are the scattering centers,** N **is the number of centers**

For small molecules, $R_{i,j} \rightarrow 0$, **for small angles,** $h \rightarrow 0$, **and** $P(\theta) \rightarrow 1$

$$P(\theta) = \frac{1}{N^2}\sum_{i=1}^{N}\sum_{j=1}^{N}(1) = \frac{1}{N^2}N^2 = 1$$

Without further derivation – the angular dependency can be expressed in terms of the radius of gyration, R_G:

$$R_G^2 = \frac{1}{2N^2} \sum_{i=1}^{N} \sum_{j=1}^{N} R_{ij}^2$$

$$\frac{1}{P(\theta)} \approx 1 + \frac{16\pi^2 R_G^2}{3\lambda^2} \sin^2 \frac{\theta}{2} \qquad \frac{KC}{R_\theta} \approx \frac{1}{P(\theta)} \left[\frac{1}{M} + 2BC \right]$$

$$\boxed{\frac{KC}{R_\theta} \approx \frac{1}{P(\theta)} \left[\frac{1}{M} + 2BC \right] \approx \left(1 + \frac{16\pi^2 R_G^2}{3\lambda^2} \sin^2 \frac{\theta}{2} \right) \left(\frac{1}{M} + 2BC \right)}$$

At zero angle: $$\left[\left[\frac{KC}{R_\theta} \right]\right]_{\theta=0} = \frac{1}{M} + 2BC$$

At zero concentration: $$\left[\left[\frac{KC}{R_\theta} \right]\right]_{C=0} = \frac{1}{M} \left(1 + \frac{16\pi^2 R_G^2}{3\lambda^2} \sin^2 \frac{\theta}{2} \right)$$

To measure the radius of gyration, the molecular weight and the 2nd virial coefficient, make a *ZIMM* plot:

Extrapolate KC/R_θ for multiple angles and multiple concentrations to zero concentration and zero angle:

$$\left[\!\left[\frac{KC}{R_\theta}\right]\!\right]_{C=0} = \frac{1}{M}\left(1 + \frac{16\pi^2 R_G^2}{3\lambda^2}\sin^2\frac{\theta}{2}\right),\ \ \textit{Slope:}\ \ \frac{16\pi^2 R_G^2}{3\lambda^2},\quad \textit{Intercept:}\ \ \frac{1}{M}$$

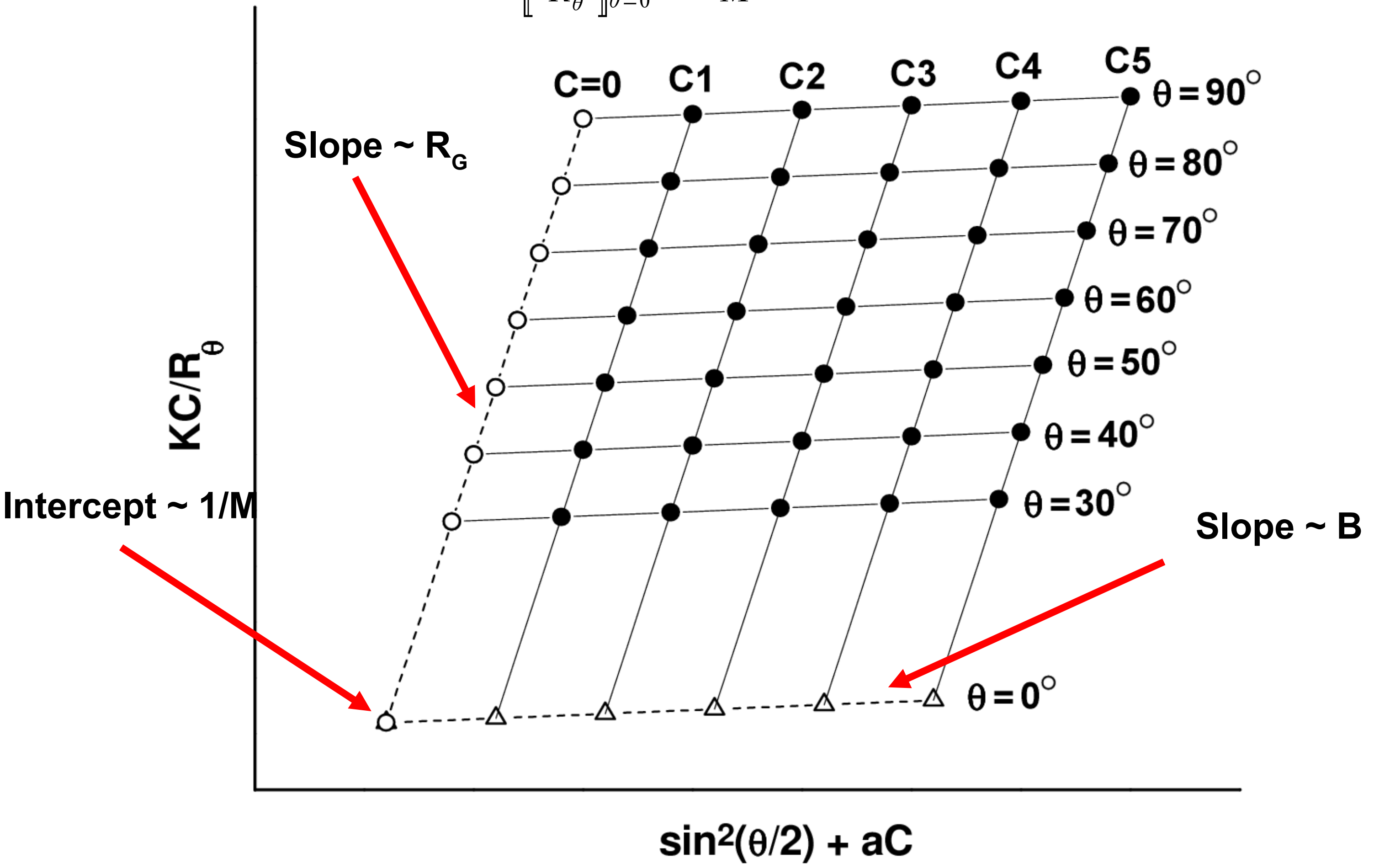
$$\left[\!\left[\frac{KC}{R_\theta}\right]\!\right]_{\theta=0} = \frac{1}{M} + 2\mathrm{BC}\quad \textit{Slope} \sim B,\quad \textit{Intercept} \sim M$$

**For further details and a derivation of these equations, consult:
K. E. van Holde, *Biophysical Chemistry*, chapter 9, 2nd edition, Prentice Hall, 1985**

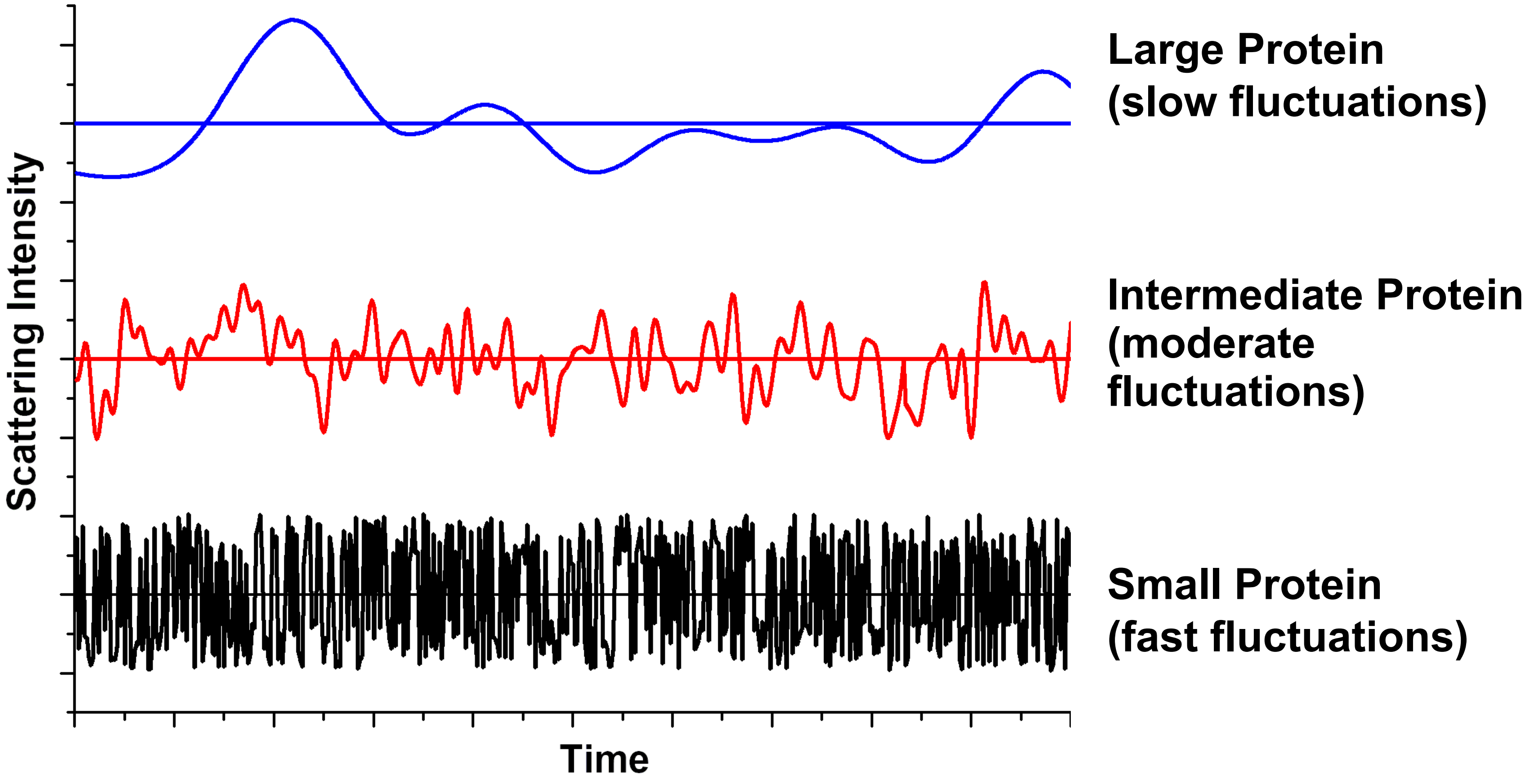
To measure, make a *ZIMM* plot: Extrapolate KC/R$_\theta$ for multiple angles and multiple concentrations to zero concentration and zero angle:

$$\left[\frac{KC}{R_\theta}\right]_{C=0} = \frac{1}{M}\left(1 + \frac{16\pi^2 R_G^2}{3\lambda^2}\sin^2\frac{\theta}{2}\right), \quad Slope{:}\ \frac{16\pi^2 R_G^2}{3\lambda^2}, \quad Intercept{:}\ \frac{1}{M}$$

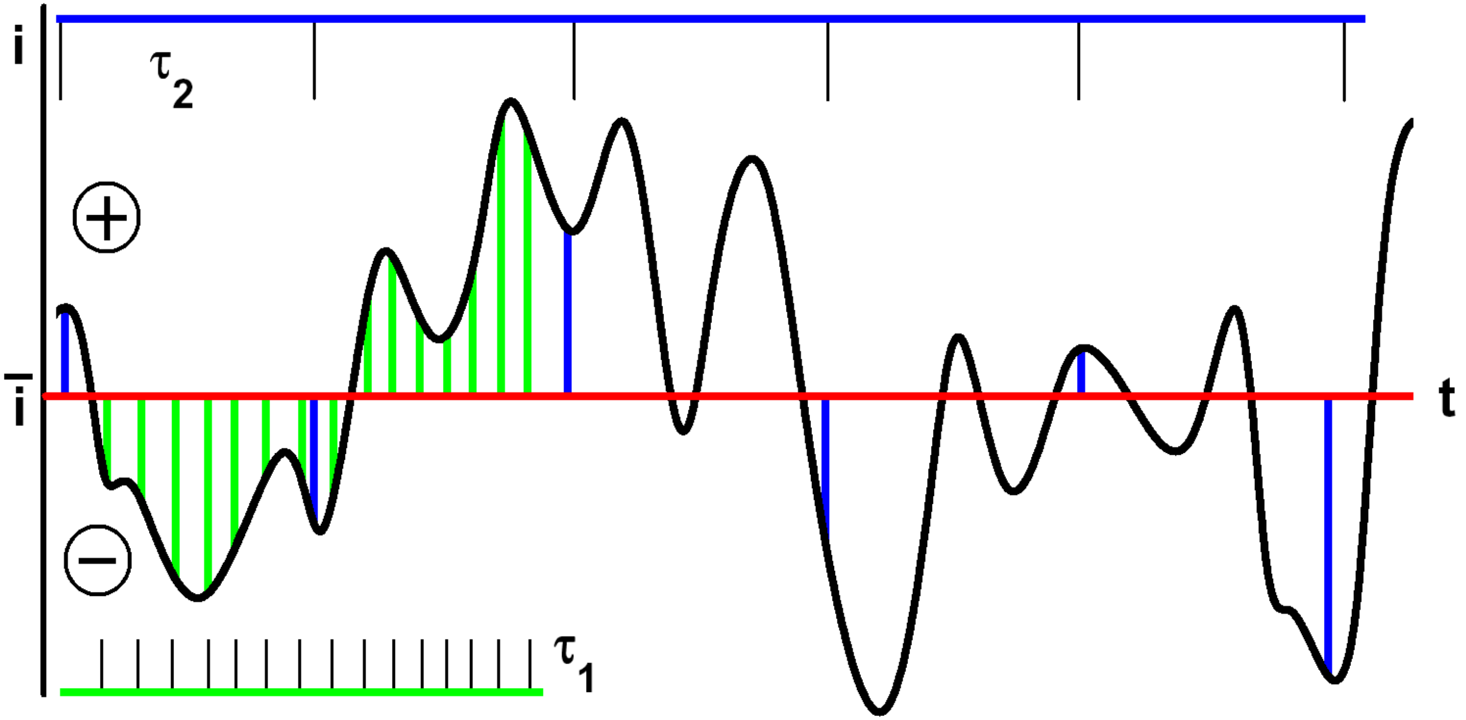
$$\left[\frac{KC}{R_\theta}\right]_{\theta=0} = \frac{1}{M} + 2BC \quad Slope \sim B, \quad Intercept \sim M$$



Fluctuations in Scattering Intensity of Three Proteins having Different Sizes

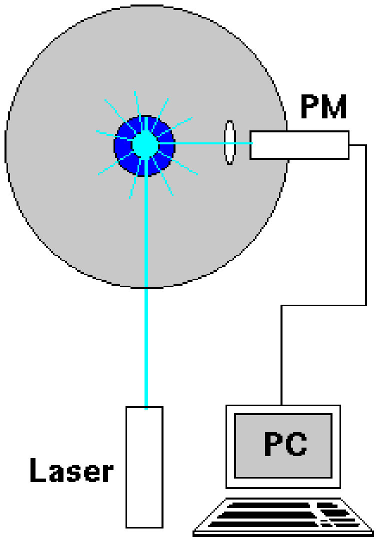


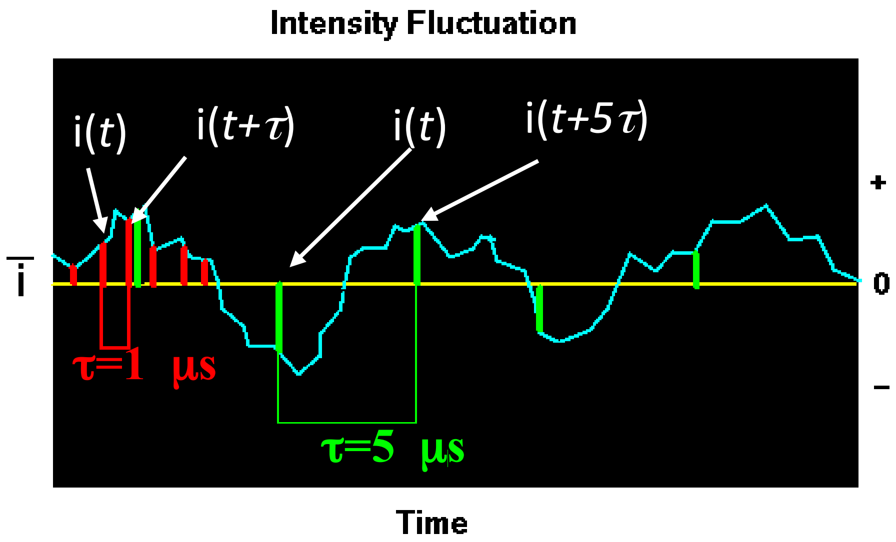
$$\bar{i} = \frac{1}{n}\sum_{j=1}^{n} i_j(t), \quad \Delta i(t) = i(t) - \bar{i}$$



$$A(\tau) = \sum \Delta i(t)\Delta i(t+\tau)$$

Dynamic Laser Light Scattering





$$\bar{i} = \frac{1}{n}\sum_{j=1}^{n} i_j(t), \quad \Delta i(t) = i(t) - \bar{i}$$

$$A(\tau) = \sum \Delta i(t) \Delta i(t+\tau)$$

τ is on the order of microseconds

$$A(\tau) = A(0)e^{-\tau/\tau_0}$$

$$\text{where } \tau_0 = \frac{1}{K_2 D},$$

$$K = \frac{4\pi n}{\lambda_0}\sin\frac{\theta}{2}$$

Dynamic Light Scattering - Autocorrelation

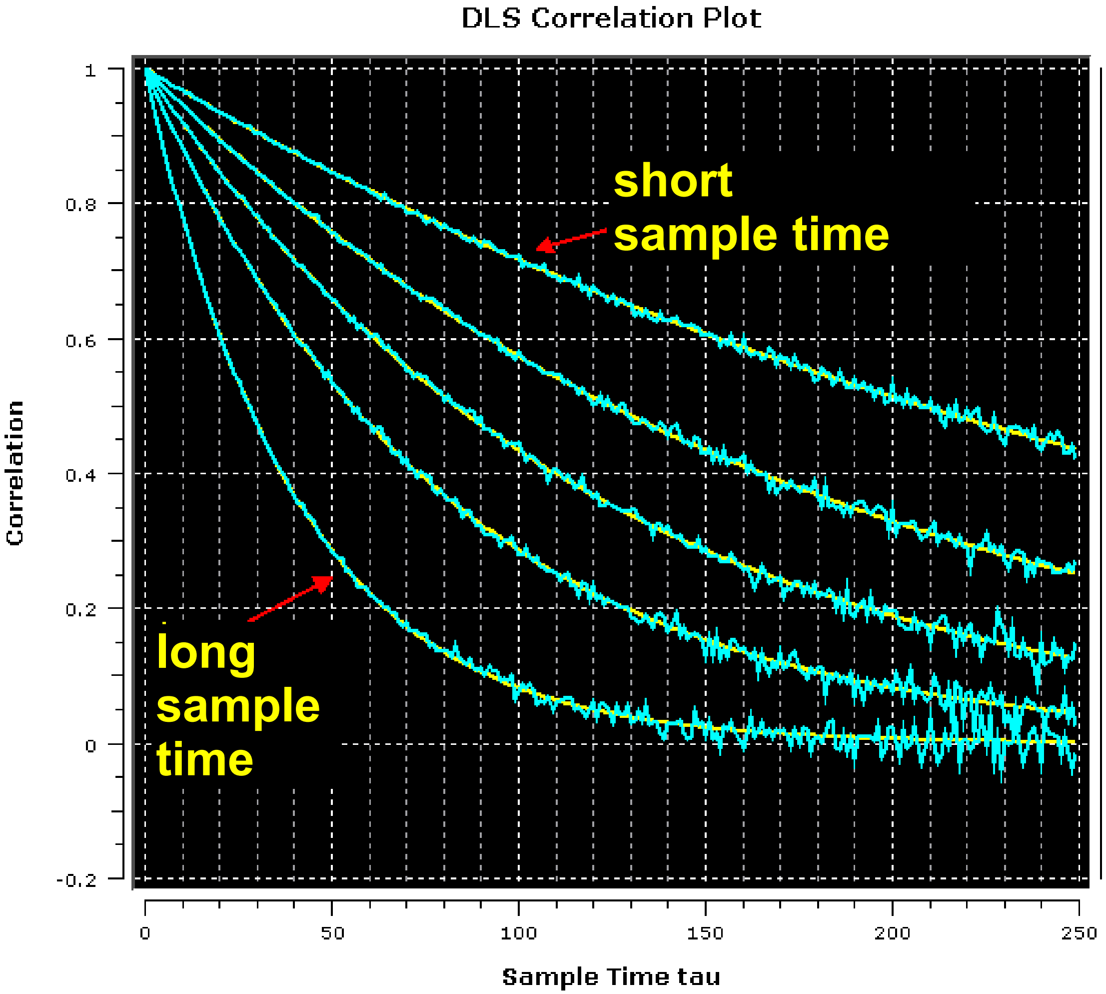
Variable time scale

Constant protein size

$$A(\tau) = A(0)e^{-\tau/\tau_0}$$

$$\text{where} \quad \tau_0 = \frac{1}{K_2 D},$$

$$K = \frac{4\pi n}{\lambda_0}\sin\frac{\theta}{2}$$



Dynamic Light Scattering - Autocorrelation

Constant sample time

Variable protein size

$$A(\tau) = A(0)e^{-\tau/\tau_0}$$

$$\text{where} \quad \tau_0 = \frac{1}{K_2 D},$$

$$K = \frac{4\pi n}{\lambda_0}\sin\frac{\theta}{2}$$