

Transport Processes

Transport processes - Irreversible processes:

- System is in a non-equilibrium state and relaxes towards an equilibrium
- Transport occurs due to a potential applied to the system:

Process	Potential	Flow of	Equilibrium State	Experiment:
Electrical conduction	Electrostatic	Electrons	Uniform electrostatic potential	Electrophoresis
Heat Conduction	Temperature	Heat	Uniform temperature	---
Diffusion	Chemical Potential	Molecules	Uniform chemical potential	light scattering, analytical ultracentrifugation
Sedimentation	Total potential (chemical potential + centrifugal potential energy)	Molecules	Uniform total potential	analytical ultracentrifugation

The flow is proportional to the gradient in the potential: $J_i = -L_i \frac{\partial U_i}{\partial x}$

Transport Processes

Diffusion:

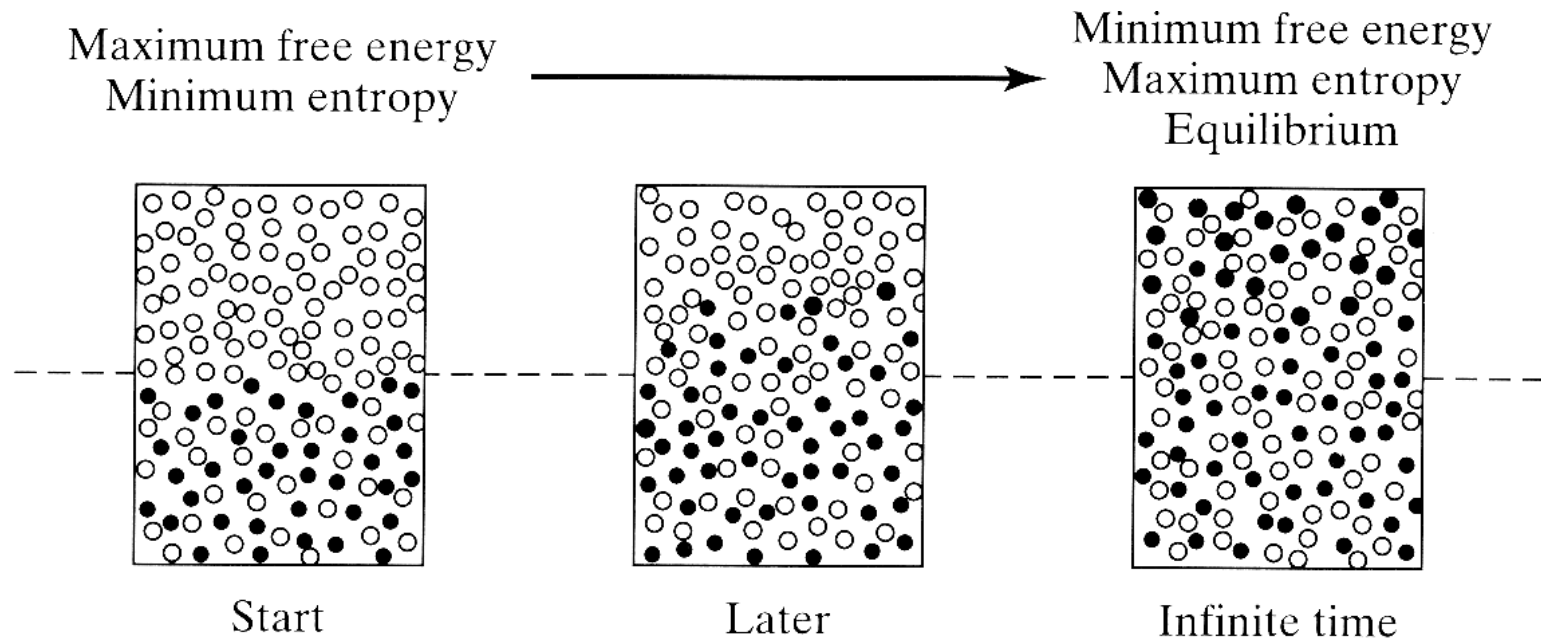


Figure 14.3 Diffusion as a mixing process. If the solvent is layered over solution, diffusion ultimately leads to uniform concentration throughout the vessel.

From: van Holde, Johnson
& Ho (1998)

Transport Processes

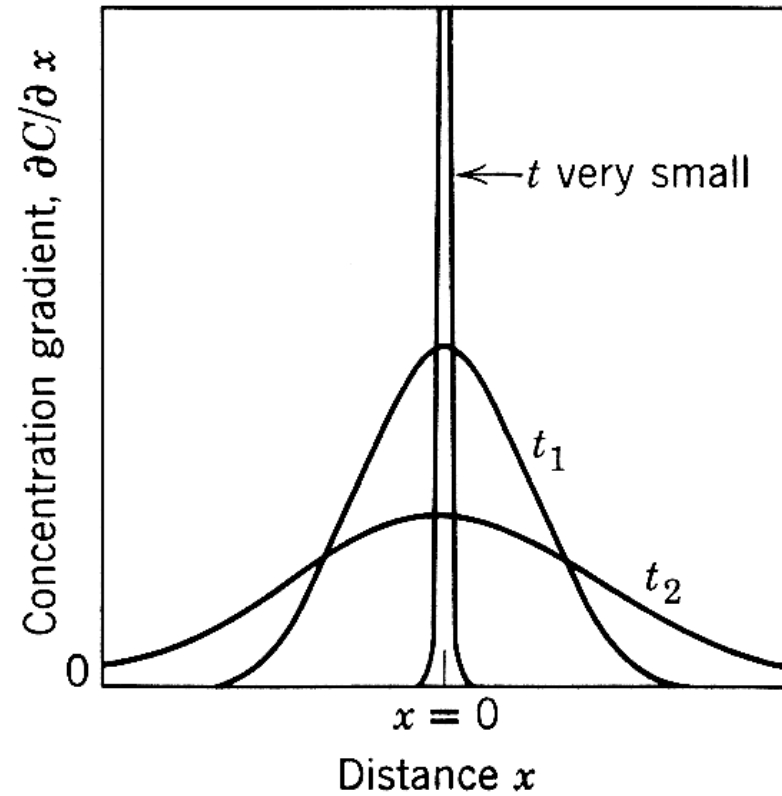
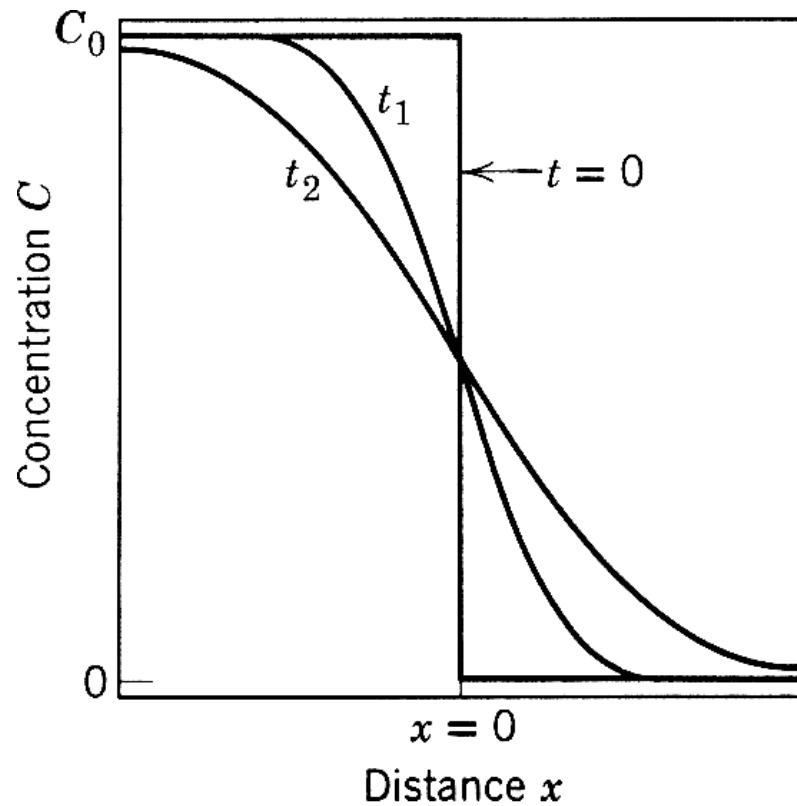


Fig. 21-1. Progress of a diffusion experiment with initially sharp boundary at $x = 0$.

Transport Processes

How do we measure Diffusion?

- 1. Boundary method**
- 2. Dynamic light scattering**
- 3. Sedimentation Velocity**

Diffusion: Boundary Method

Diffusion Equation:

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

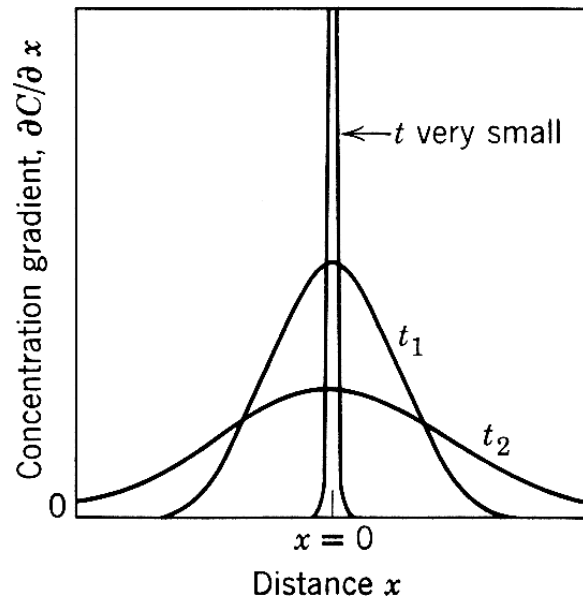
with solution:

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

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

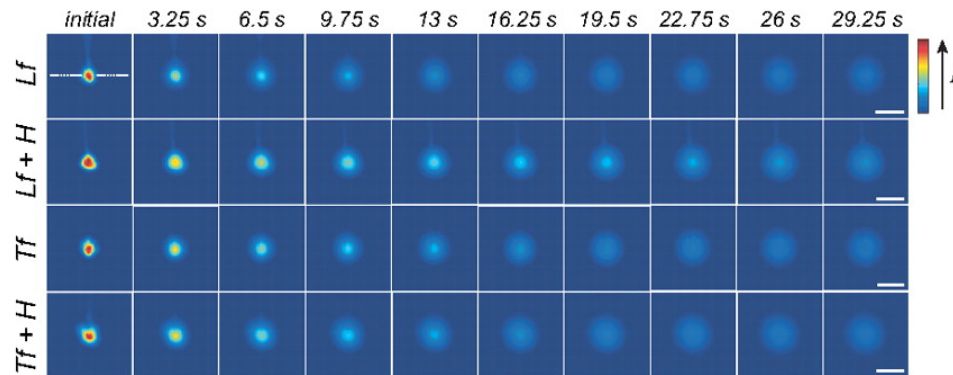
Gaussian Equation:

$$\left(\frac{\partial C}{\partial x} \right)_{T,P} = \frac{1}{\sigma\sqrt{2\pi}} e^{-\frac{(x-\mu)^2}{2\sigma^2}}$$



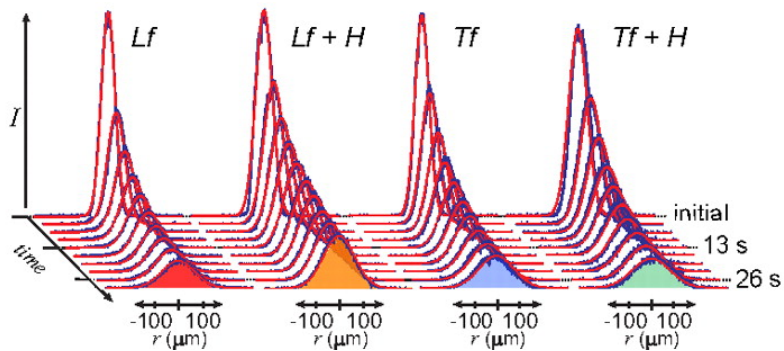
Diffusion: Boundary Method

Gaussian Equation:
$$\left(\frac{\partial C}{\partial x}\right)_{T,P} = \frac{I}{\sigma\sqrt{2\pi}} e^{-\frac{(x-\mu)^2}{2\sigma^2}}$$

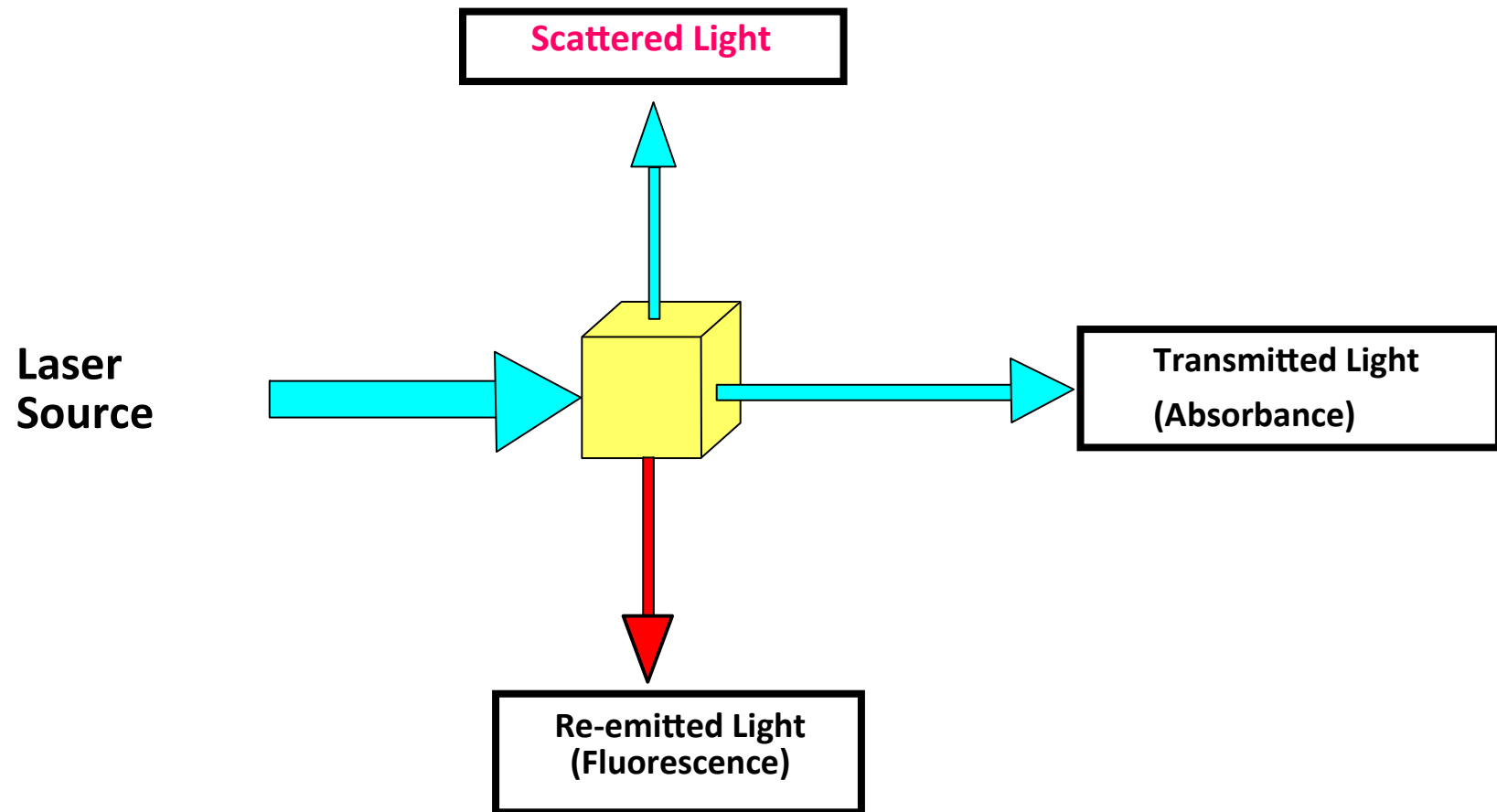


Thorne R G et al. PNAS
2008;105:8416-8421

PNAS

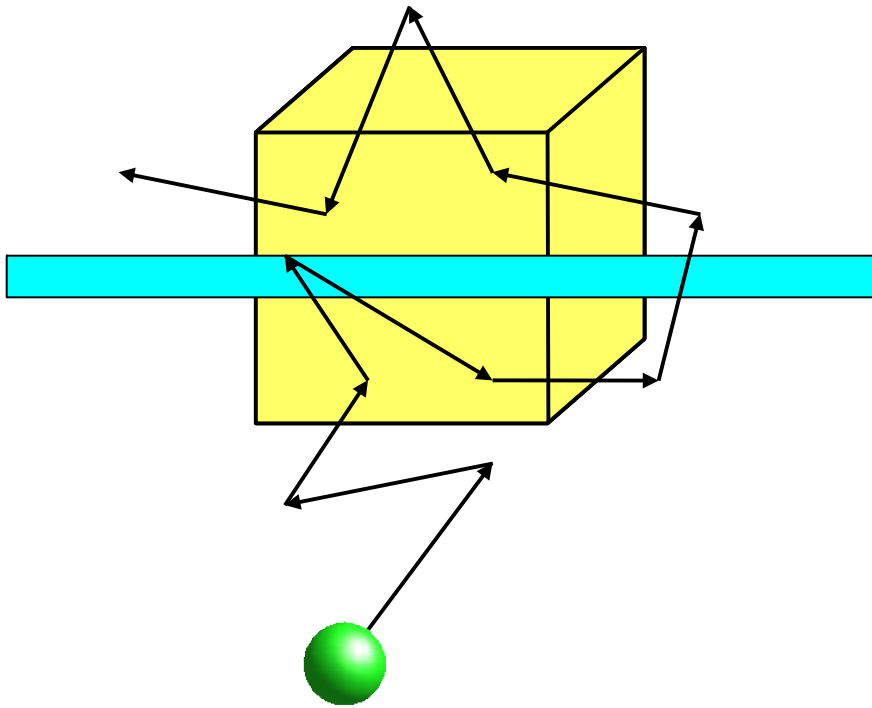


Diffusion Measurement: Light Scattering



Diffusion Measurement: Light Scattering

Consider a small volume element:



**A macromolecule
will diffuse through the
volume element because it
experiences**

Brownian motion

(dependent on temperature)

Light Scattering

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)

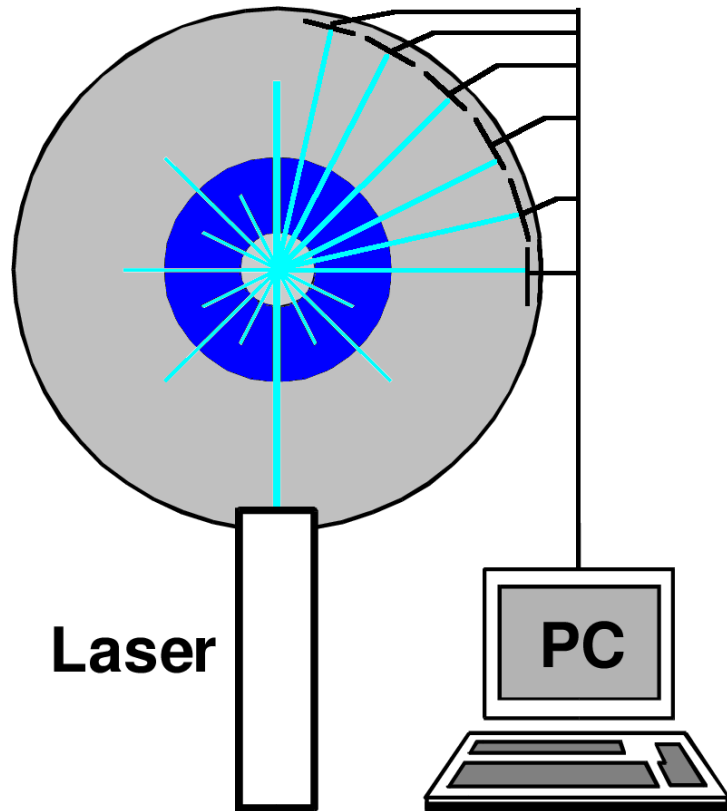
Obtain diffusion coefficients

Small-angle X-ray/neutron scattering

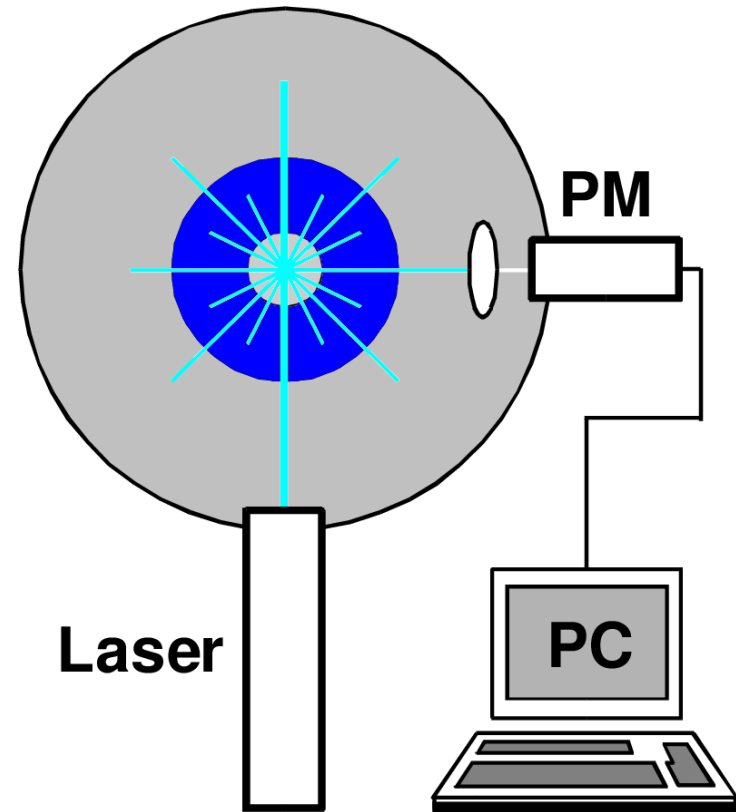
Short wavelengths provide shape information

Light Scattering

Multi-Angle/Static Light Scattering (MALS)



Dynamic Light Scattering (DLS)



Light Scattering

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 ($\emptyset < 1/50^{\text{th}} \lambda$): Rayleigh Scattering

Rayleigh Ratio:

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

Instantaneous Scattering:

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

For a single particle:

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

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 ($\emptyset < 1/50^{\text{th}} \lambda$): Rayleigh Scattering

Combine equations (1) and (2):

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

This can be approximated by:

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

At zero concentration:

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

Rayleigh Scattering

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**.

Light Scattering from Large Particles

Scattering from **large** particles (diameter $> 1/50^{\text{th}} \lambda$):

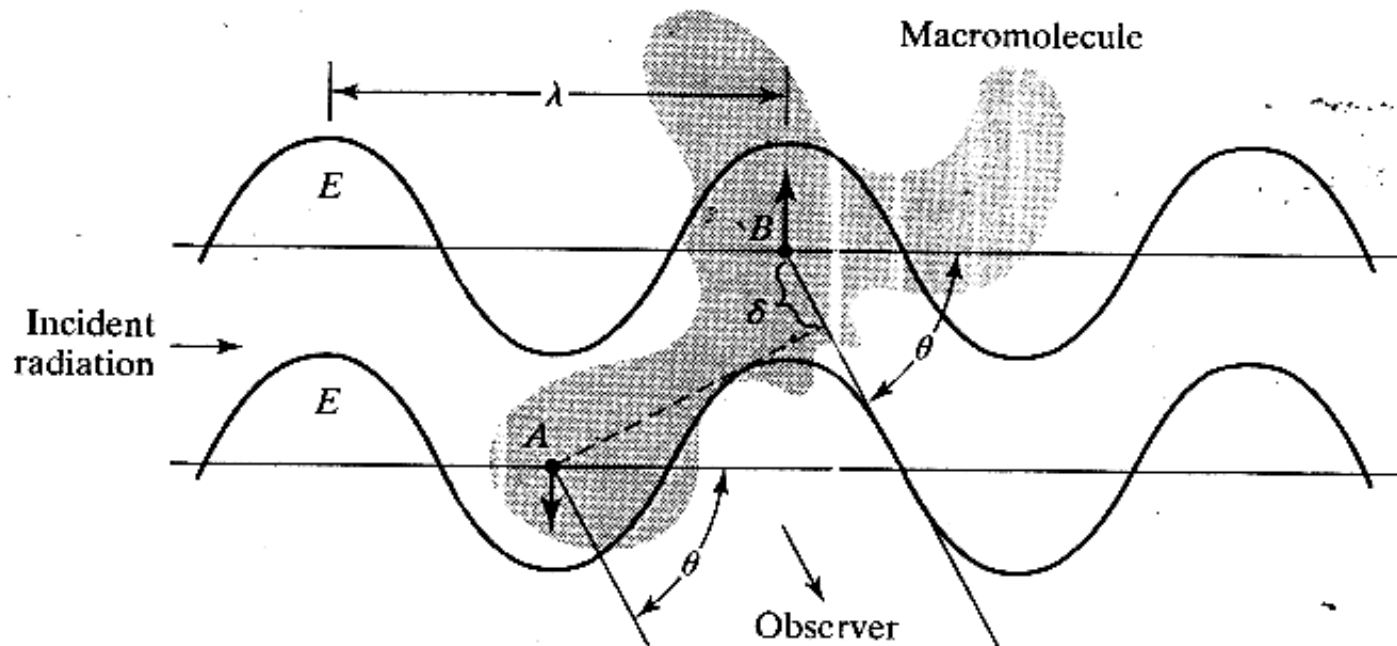


Figure 7.5 Scattering from a macromolecule that is large compared to λ . Two points from which scattering occurs are shown at A and B. The phase of the radiation (and hence of the induced dipoles) is clearly different at the two points. Also, the two points are at different distances from the observer.

Light Scattering

The scattering from all scattering centers in large molecules needs 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, $P(\theta)$. $P(\theta)$ 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 $\lambda = 500$ nm and $R_G = 50$ nm:

$1/P(\theta) = 1.530$ at $\theta = 180^\circ$ (max. angle)

$1/P(\theta) = 1.265$ at $\theta = 90^\circ$

$1/P(\theta) = 1.013$ at $\theta = 9^\circ$

If $\lambda = 500$ nm and $R_G = 5$ nm:

$1/P(\theta) = 1.0053$ at $\theta = 180^\circ$

$1/P(\theta) = 1.0003$ at $\theta = 90^\circ$

$1/P(\theta) = 1.0001$ at $\theta = 9^\circ$ (No correction)

(Rayleigh scattering is observed)

Light Scattering

For large particles, we need to introduce a function $P(\theta)$ 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:

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

For large molecules, we have:

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

Light Scattering

For large molecules, we have:

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

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

$R_{i,j}$ 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 $P(\theta) \rightarrow 1$

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

Light Scattering

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

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

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

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

Light Scattering

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

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

Light Scattering

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:

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

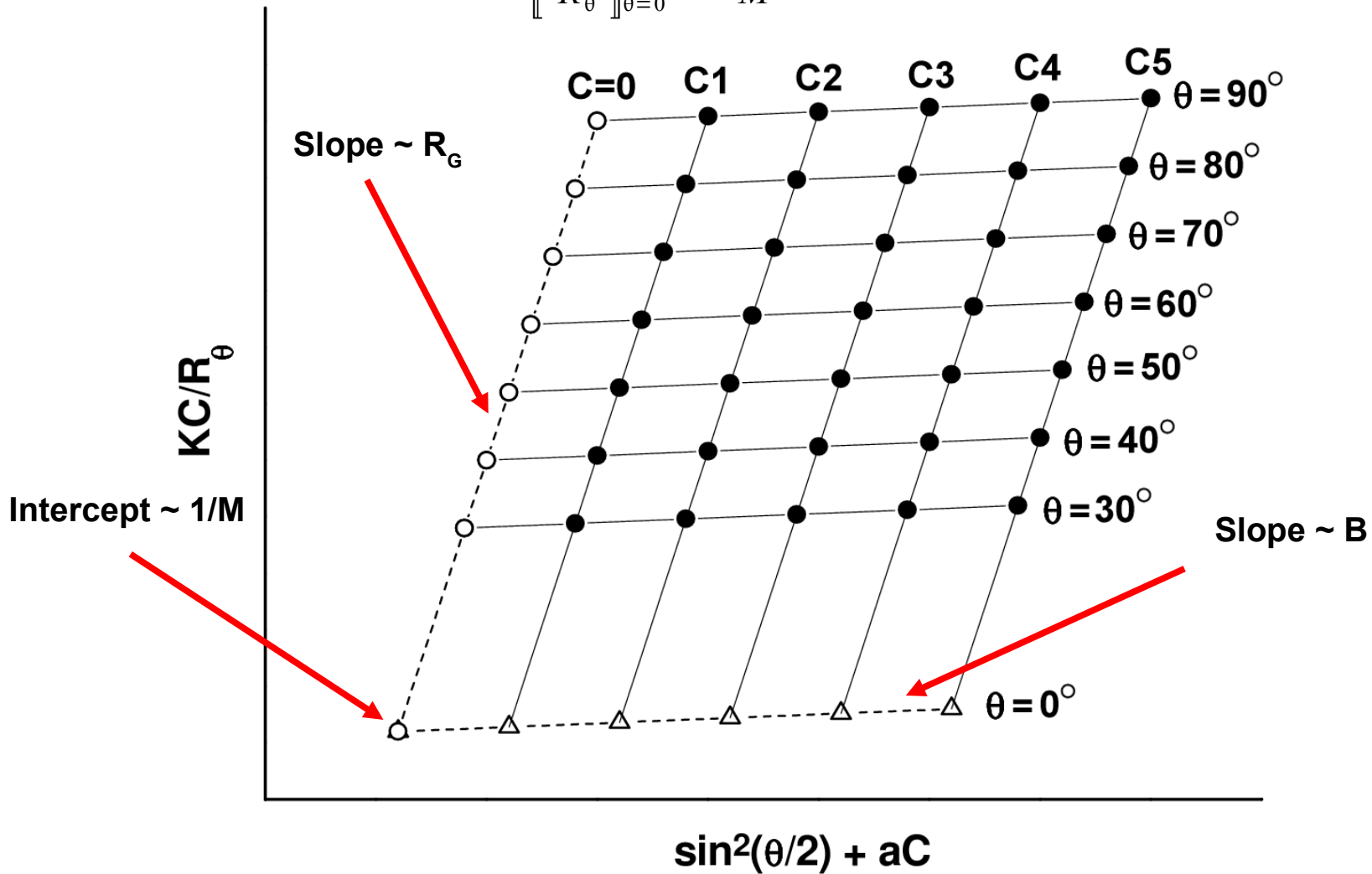
$$\left[\frac{KC}{R_\theta} \right]_{\theta=0} = \frac{1}{M} + 2BC \quad \text{Slope} \sim B, \quad \text{Intercept} \sim M$$

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_θ for multiple angles and multiple concentrations to zero concentration and zero angle:

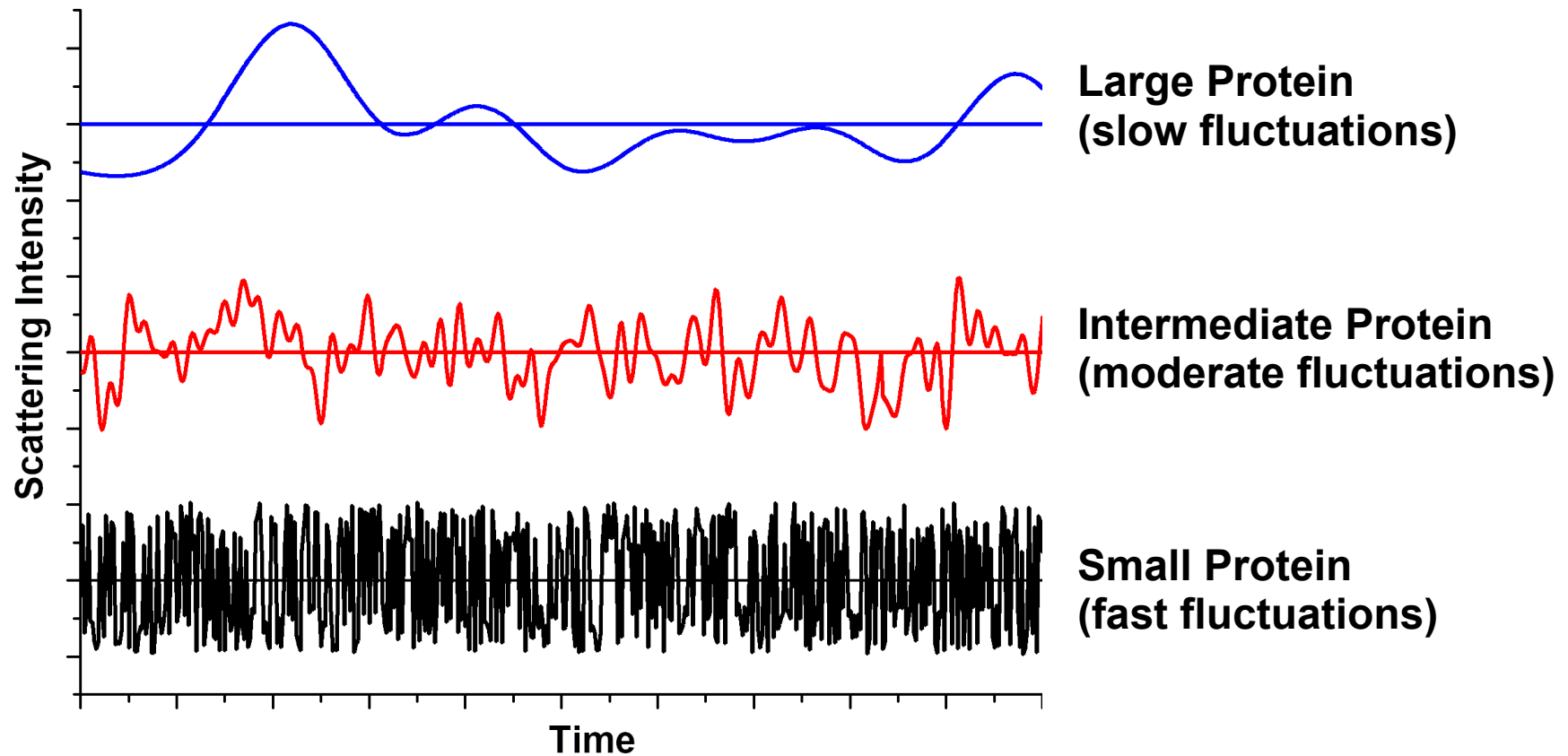
$$\left[\frac{KC}{R_\theta} \right]_{c=0} = \frac{1}{M} \left(1 + \frac{16\pi^2 R_G^2}{3\lambda^2} \sin^2 \frac{\theta}{2} \right), \text{ Slope: } \frac{16\pi^2 R_G^2}{3\lambda^2}, \text{ Intercept: } \frac{1}{M}$$

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



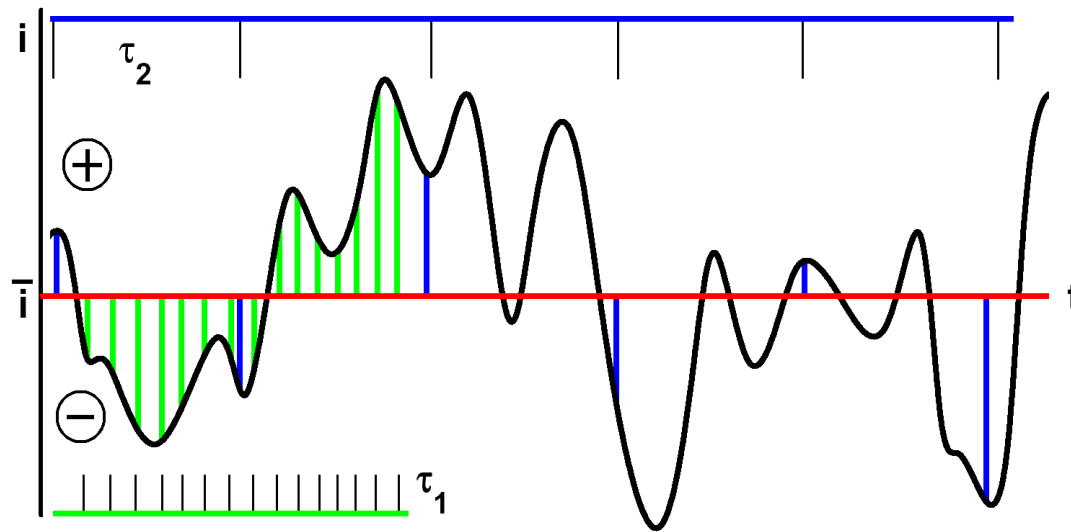
Dynamic Light Scattering

Fluctuations in scattering intensity of three different sized proteins:



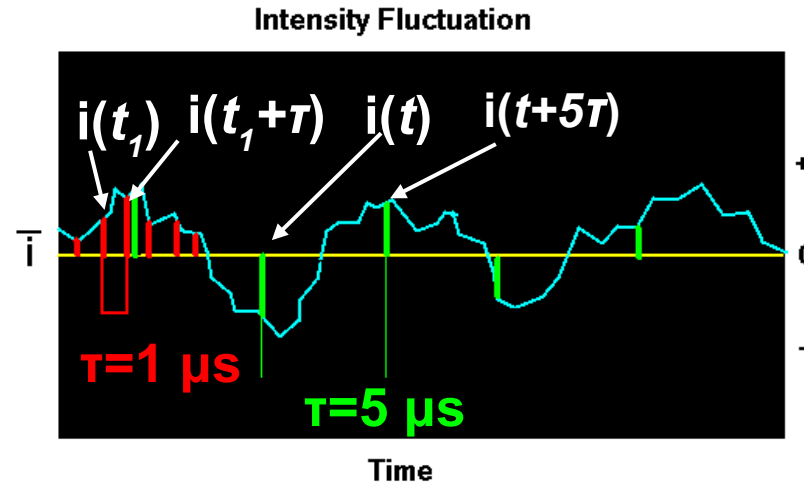
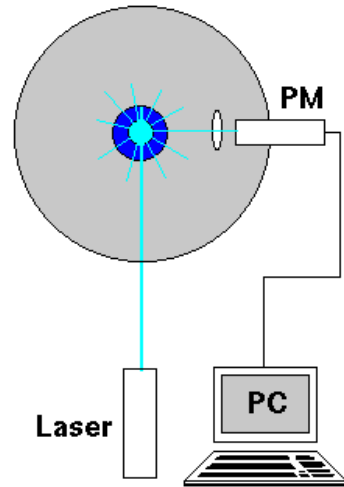
Dynamic Light Scattering - Autocorrelation

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



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

Dynamic 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

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

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

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

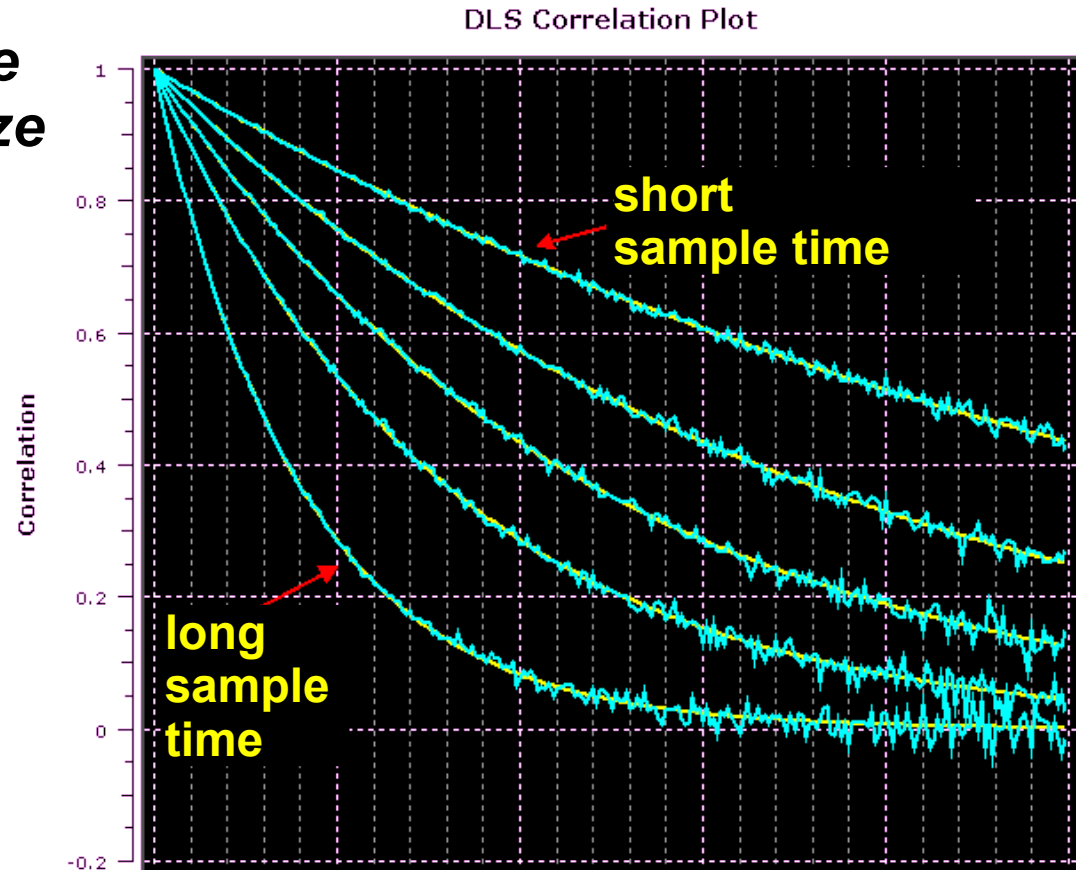
Dynamic Light Scattering - Autocorrelation

Variable time scale
Constant protein size

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

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

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



Dynamic Light Scattering - Autocorrelation

Constant sample time
Variable protein size

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

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

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

