Static & Dynamic Light Scattering

- First quantitative experiments in 1869 by Tyndall (scattering of small particles in the air – Tyndall effect)
- 1871 – Lord Rayleigh started a quantitative study and theory
- Basic idea: incident monochromatic linearly polarized light beam incident on a sample. Assume
  - No absorption
  - Randomly oriented and positioned scatterers
  - Isotropic scatterers
  - Independently scattering particles (dilute)
  - Particles small compared to wavelength of light

  We'll remove some of these restrictions later

Classical Wave description

- The incident electric field is

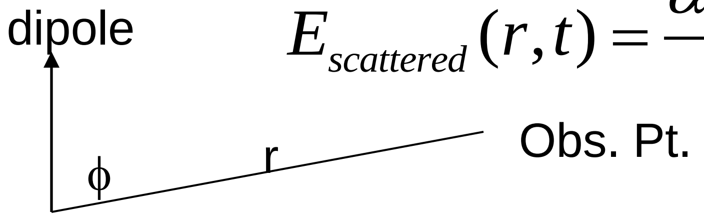
  $E = E_o \cos(2\pi x/\lambda - 2\pi t/T)$

- Interaction with molecules drives their electrons at the same f to induce an oscillating dipole

  $p_{induced} = \alpha\ E_o \cos(2\pi x/\lambda - 2\pi t/T)$ - α = polarizability

- This dipole will radiate producing a scattered E field from the single molecule

$$E_{scattered}(r,t) = \frac{\alpha E_o 4\pi^2 \sin\phi}{r\lambda^2}\cos\left(2\pi x/\lambda - 2\pi t/T\right)$$



Static (or time-average)Rayleigh scattering

1. E ~ 1/r so I ~ 1/r^2 - necessary since I ~energy/time/area and A ~ r^2
2. E ~ 1/λ^2 dependence so I ~ 1/λ^4 – blue skies and red sunsets (sunrises)
3. Elastic scattering – same f
4. sin ϕ dependence – when ϕ = 0 or π – at poles of dipole – no scattering – max in horizontal plane
5. α related to n , but how?

Polarizability and index of refraction

- Note that if n ~ 1 $$n = 1 + \frac{dn}{dc}c$$
  where c is the weight concentration
- Then $$n^2 = 1 + 2\frac{dn}{dc}c + \ldots \quad so \quad n^2 - 1 = 2\frac{dn}{dc}c = 4\pi N\alpha$$
  where N = number concentration
- So,
$$\alpha = \frac{\frac{dn}{dc}c}{2\pi N} \quad or \quad \alpha = \frac{M\frac{dn}{dc}}{2\pi N_A}$$
- For a particle in a solvent with n_{solv}, we have $n^2 - n^2_{solv} = 4\pi N\alpha$ so

$$\alpha = (n + n_{solv})(n - n_{solv})/4\pi N \sim \frac{2n_{solv}}{4\pi}\frac{dn}{dc}\frac{c}{N} = \alpha = \frac{n_{solv}}{2\pi N_A}\frac{dn}{dc}M$$

Scattered Intensity

- Detect intensity, not E, where

$$\left.\frac{I_{scatt}}{I_o}\right]_{1\,particle} = \frac{\left(\dfrac{\alpha E_o 4\pi^2 \sin\phi}{r\lambda^2}\right)^2}{E_o^2} = \frac{16\pi^4\alpha^2\sin^2\phi}{r^2\lambda^4}$$

- Substituting for α, we have

$$\left.\frac{I_{scatt}}{I_o}\right]_{1\,particle} = \frac{4\pi^2 M^2 n_{solv}^2 \left(\dfrac{dn}{dc}\right)^2 \sin^2\phi}{r^2\lambda^4 N_A^2}$$

Scattered Intensity II

- If there are N scatterers/unit volume and all are independent with $N = N_A c/M$, then

$$\left.\frac{I_{scatt}}{I_o}\right]_{per\ unit\ volume} = N \left.\frac{I_{scatt}}{I_o}\right]_{1\,particle} = \frac{4\pi^2 \sin^2\phi\, n_{solv}^2 \left(\frac{dn}{dc}\right)^2 Mc}{N_A r^2 \lambda^4}$$

- We define the Rayleigh ratio R_θ:

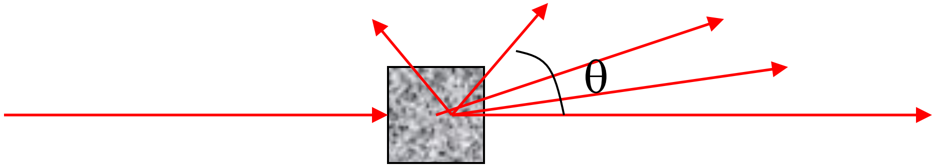
$$R_\theta = \frac{I_{scatt,\theta} r^2}{I_o \sin^2\phi} = \frac{4\pi^2 n_{solv}^2 \left(\frac{dn}{dc}\right)^2 Mc}{N_A \lambda^4} = KMc$$

Basic Measurement

- If the intensity ratio I_θ/I_o, n_{solv}, dn/dc, λ, c, ϕ, and r are all known, you can find M.
- Usually write $Kc/R_\theta = 1/M$
- Measurements are usually made as a function of concentration c and scattering angle θ
- The concentration dependence is given by

$$\frac{Kc}{R_\theta} = \frac{1}{M} + 2Bc$$

where B is called the thermodynamic virial – same as we saw before for c dependence of D (but called A)

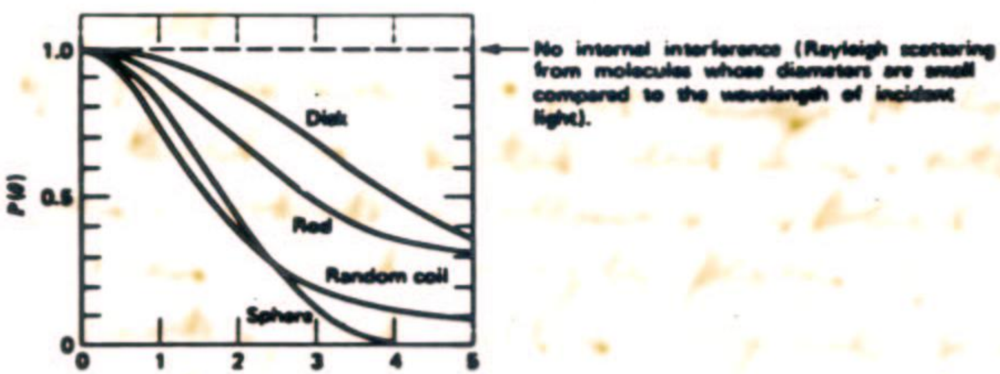


Angle Dependence

- If the scatterers are small (d < λ/20), they are called Rayleigh scatterers and the above is correct – the scattering intensity is independent of scattering angle
- If not, then there is interference from the light scattered from different parts of the single scatterer
- Different shapes give different particle scattering factors P(θ)

Table 15-1 Scattering Function, $P(\theta_s)$ for Various Macromolecular Structures

Structure	$P(\theta_s)$	Dimensions	Radius of Gyration, R_G
Sphere	$[(3/a^3)[\sin(a) - a\cos(a)]]^2; a = \frac{\mu D}{2}$	D = diameter of sphere	$(3/20)^{1/2}D$
Thin rod	$\frac{1}{a}\int_0^{2a}\frac{\sin x}{x}dx - \left(\frac{\sin a}{a}\right)^2; a = \frac{\mu D}{2}$	D = length of the rod	$D/\sqrt{12}$
Random coil	$\frac{2}{a^2}[\exp(-a) + a - 1]; a = \frac{\mu^2 <D^2>}{6}$	$<D^2>$ = root-mean-square end-to-end distance	$\sqrt{<D^2>/6}$
Thin disk	$\frac{2}{a^2}[1 - (1/a)J_1(2a)]; a = (\mu D/2)$	D = diameter of disk; J_1 = Bessel function order 1	$(D/2\sqrt{2})$



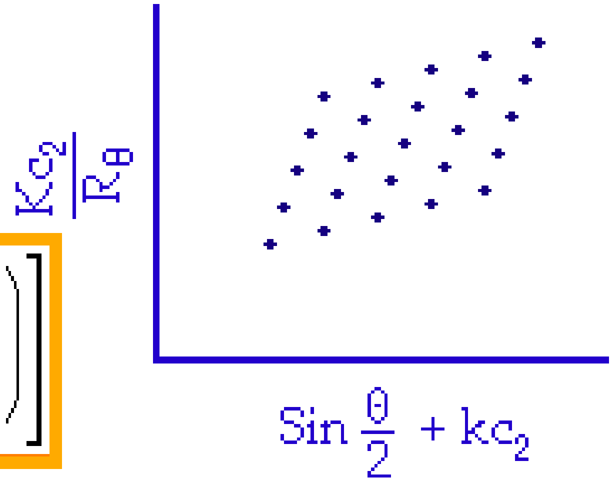
From P(q), we can get a Radius of Gyration for the scatterer

Analysis of LS Data

- Measure I(θ, c) and plot

  Kc/R_θ vs $\sin^2(\theta/2)$ + (const)c

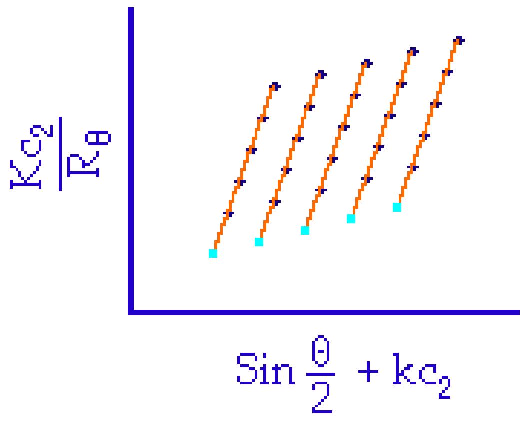
$$\frac{Kc_2}{R_\theta} = \left(\frac{1}{M} + 2Bc_2\right)\left[1 + \frac{16\pi^2\,\overline{r_g^2}}{3\lambda^2}\sin^2\left(\frac{\theta}{2}\right)\right]$$



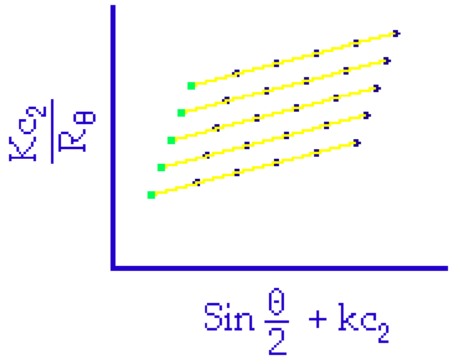
  – Extrapolations: c ⟶ 0

  θ ⟶ 0

Extrapolation to zero concentration

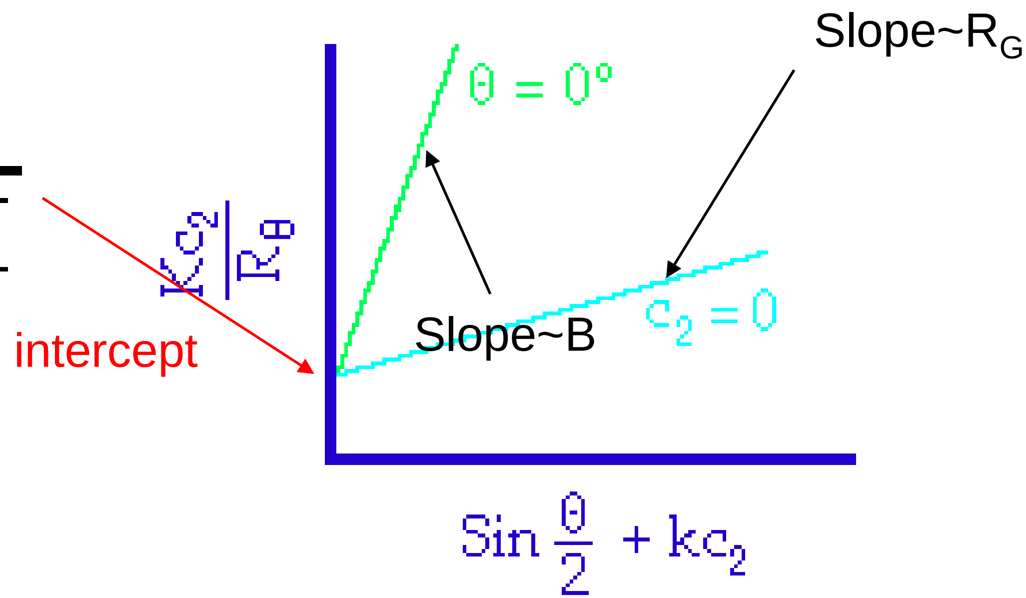


Extrapolation to zero scattering angle



Final result

$$\frac{Kc_2}{R_\theta} = \frac{1}{M}$$



Problems: Dust, Standard to measure I_o, low angle measurement flare

Polydispersity

- If the solution is polydisperse – has a mixture of different scatterers with different M's - then we measure an average M – but which average?

$$R_\theta = K\frac{\Sigma c_i M_i}{\Sigma c_i}c = K\overline{M}_w c$$

- So the weight-averaged M is measured!

Possible averages:

Number-average $$M_N = \frac{\Sigma M_i N_i}{\Sigma N_i}$$

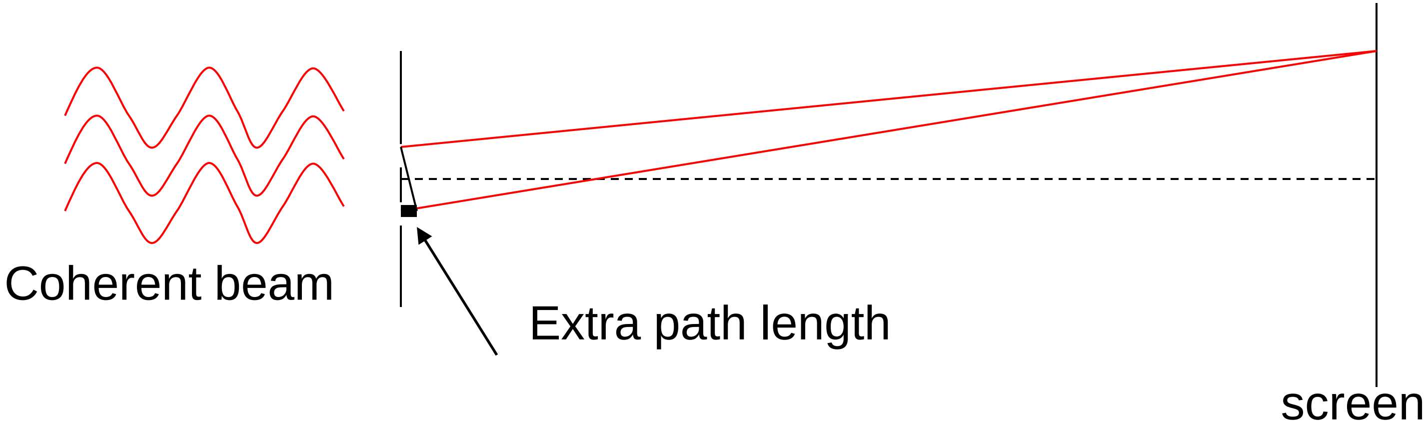
Weight-average $$M_w = \frac{\Sigma M_i c_i}{\Sigma c_i} = \frac{\Sigma M^2{}_i N_i}{\Sigma N_i M_i}$$

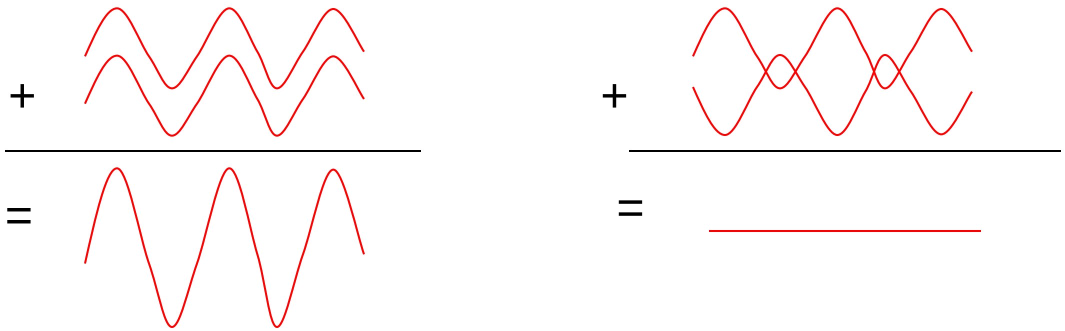
Z-average $$M_z = \frac{\Sigma M_i{}^2 c_i}{\Sigma M_i c_i} = \frac{\Sigma M^3{}_i N_i}{\Sigma N_i M_i{}^2}$$

Dynamic Light Scattering

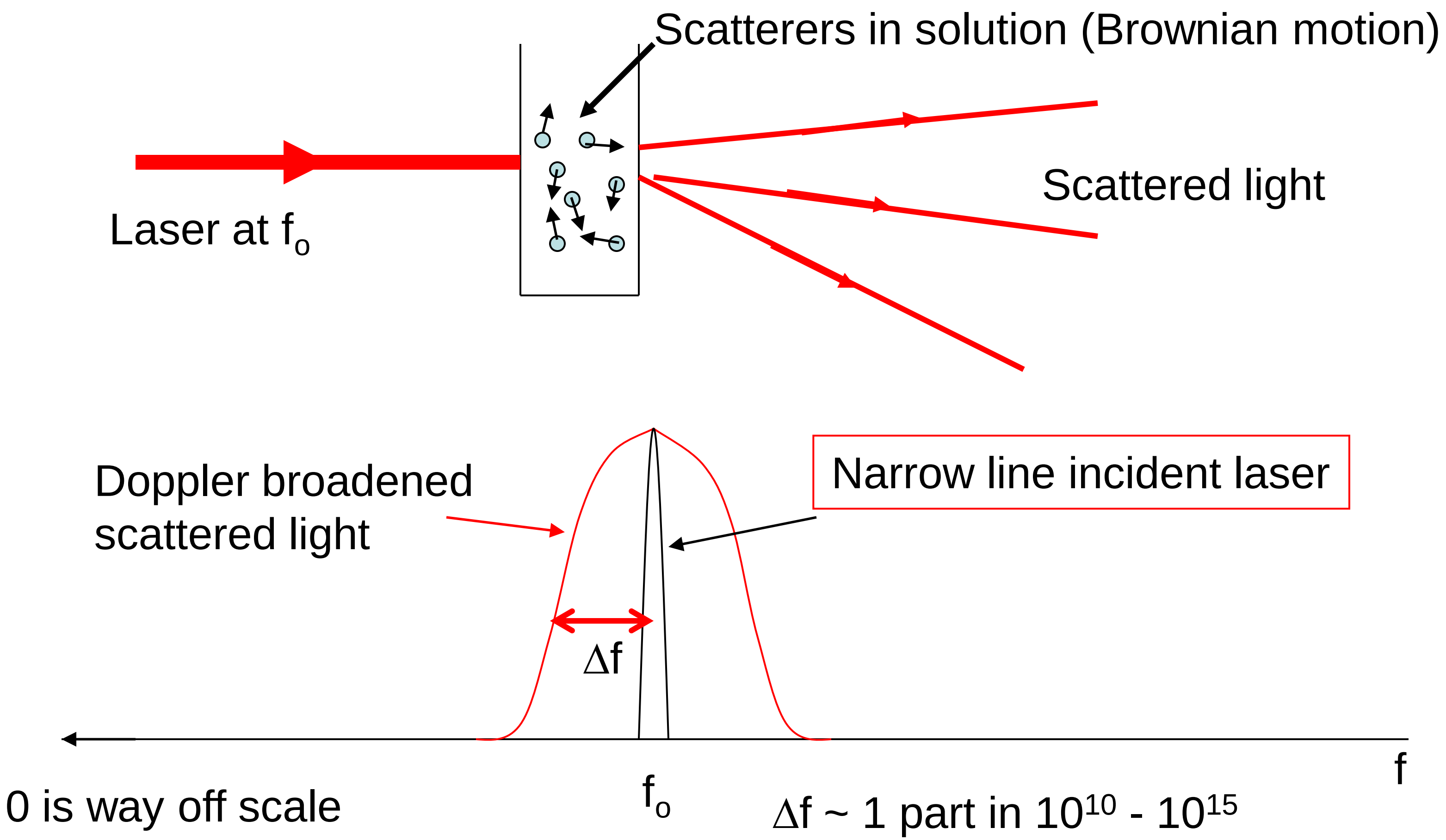
- Basic ideas – what is it?
- The experiment – how do you do it?
- Some examples systems – why do it?

Double Slit Experiment

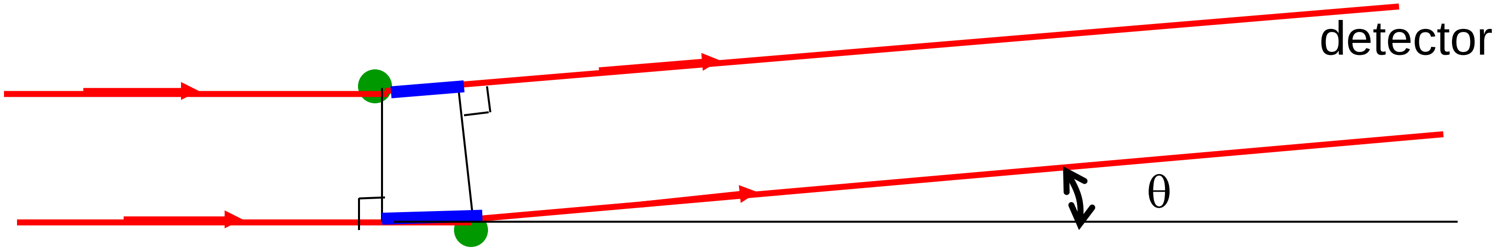




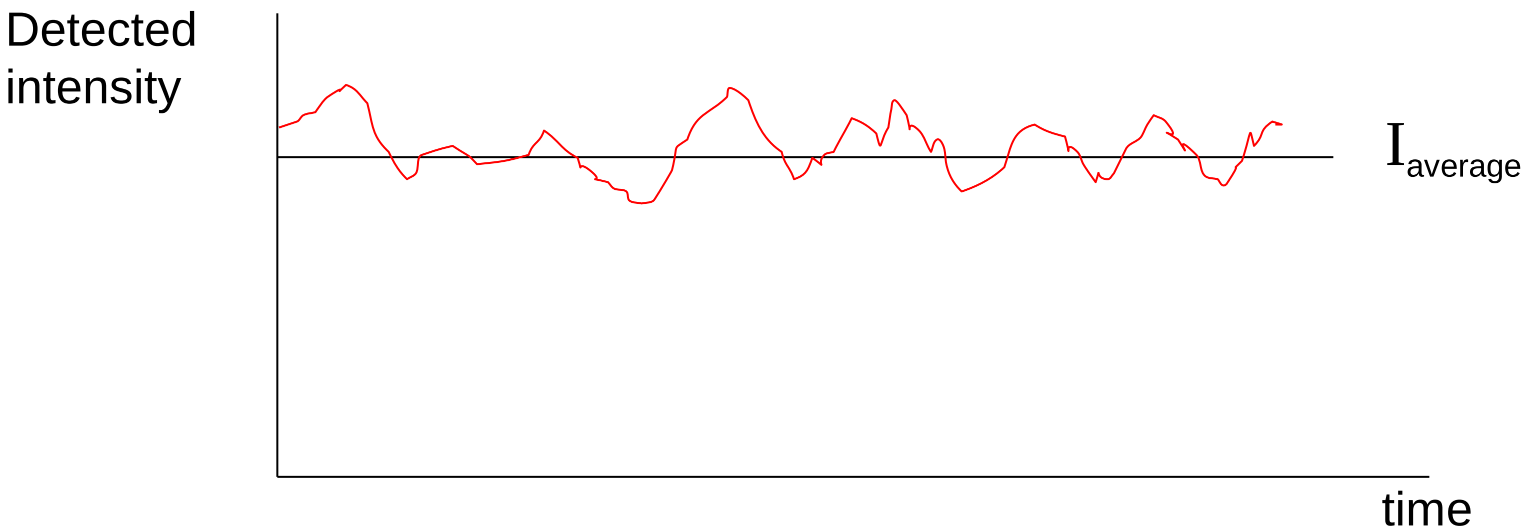
Light Scattering Experiment



More Detailed Picture



Inter-particle interference

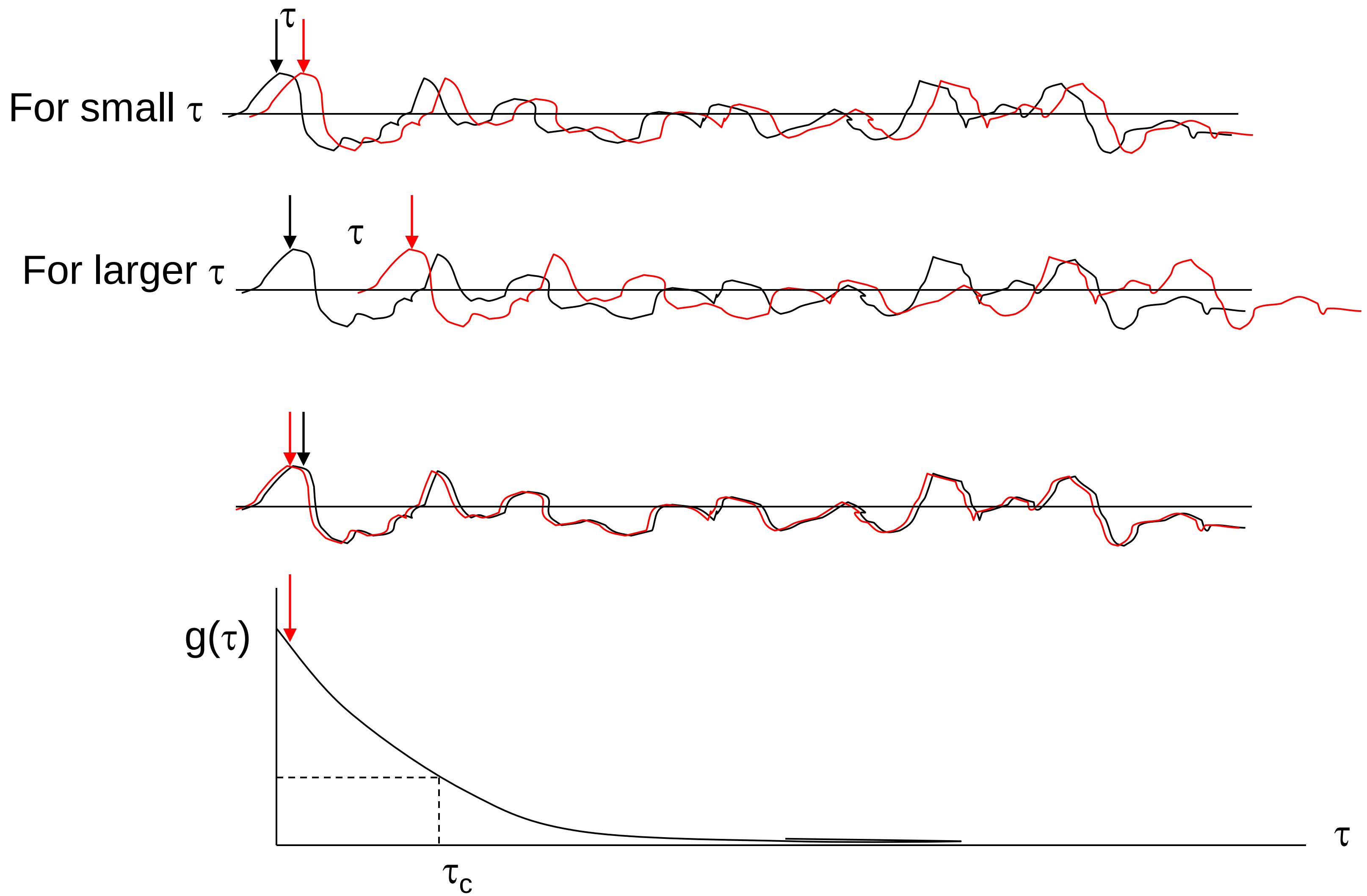


How can we analyze the fluctuations in intensity?

Data = $g(\tau) = \langle I(t)\, I(t+\tau) \rangle_t$ = intensity autocorrelation function

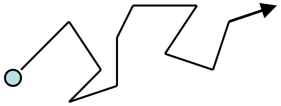
Intensity autocorrelation

- $g(\tau) = \langle I(t)\, I(t+\tau) \rangle_t$



What determines correlation time?

- Scatterers are diffusing – undergoing Brownian motion – with a mean square displacement given by $\langle r^2 \rangle = 6D\tau_c$ (Einstein)



- The correlation time τ_c is a measure of the time needed to diffuse a characteristic distance in solution – this distance is defined by the wavelength of light, the scattering angle and the optical properties of the solvent – ranges from 40 to 400 nm in typical systems
- Values of τ_c can range from 0.1 μs (small proteins) to days (glasses, gels)

Diffusion

- What can we learn from the correlation time?
- Knowing the characteristic distance and correlation time, we can find the diffusion coefficient D
- According to the Stokes-Einstein equation

$$D = \frac{k_B T}{6\pi\eta R}$$

where R is the radius of the equivalent hydrodynamic sphere and η is the viscosity of the solvent

- So, if η is known we can find R (or if R is known we can find η)

Why Laser Light Scattering?

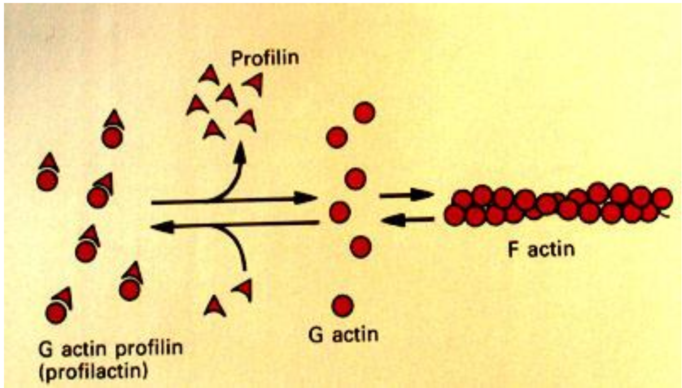
1. Probes all motion
2. Non-perturbing
3. Fast
4. Study complex systems
5. Little sample needed

Problems: Dust and

best with monodisperse samples

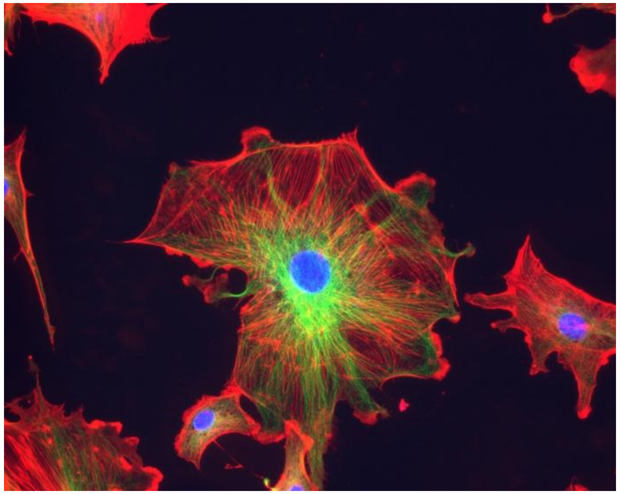
Aggregating/Gelling Systems Studied at Union College

- Proteins:
  - Actin – monomers to polymers and networks



Study monomer size/shape, polymerization kinetics, gel/network structures formed, interactions with other actin-binding proteins

Why?



Epithelial cell under fluorescent microscope

Actin = red, microtubules = green, nucleus = blue

Aggregating systems, con't

- BSA (bovine serum albumin)
- beta-amyloid +/- chaperones
- insulin

what factors cause or promote aggregation?

how can proteins be protected from aggregating?

what are the kinetics?

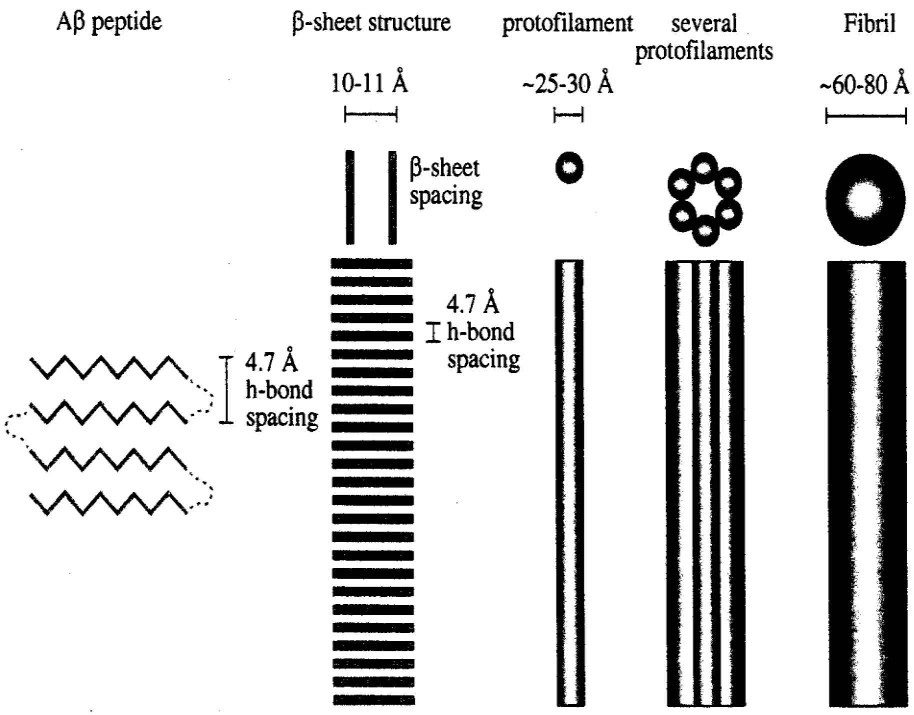
- Polysaccharides:
  - Agarose
  - Carageenan

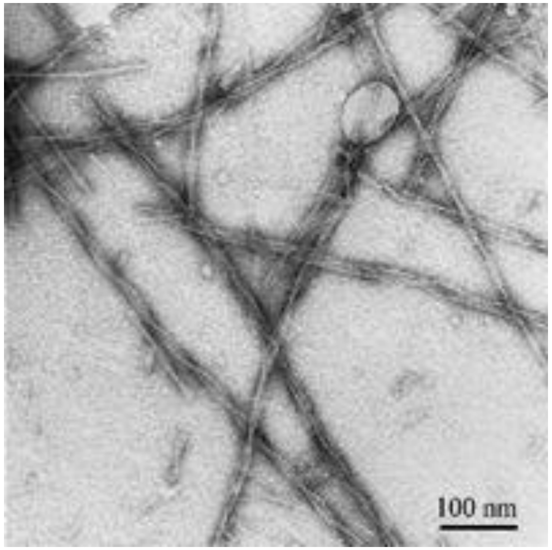
Focus on the onset of gelation –

what are the mechanisms causing gelation?
how can we control them?
what leads to the irreversibility of gelation?

Current Projects

1. β-amyloid – small peptide that aggregates in the brain – believed to cause Alzheimer's disease-





Current Projects

2. Insulin aggregation

- EFFECTS OF ARGININE ON THE KINETICS OF BOVINE INSULIN AGGREGATION STUDIED BY DYNAMIC LIGHT SCATTERING
- By
- Michael M. Varughese
- *********
- Submitted in partial fulfillment
- of the requirements for
- Honors in the Department of Biological Sciences
- and done in conjunction with the Department of Physics and Astronomy
- UNION COLLEGE
- June, 2011