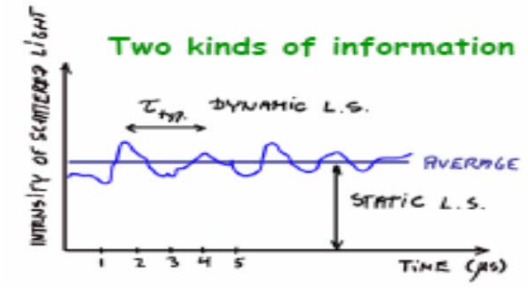
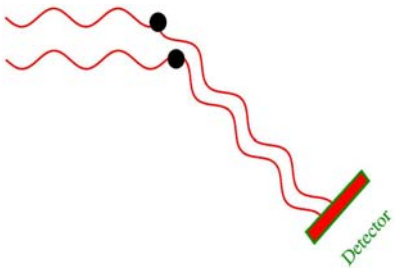
Static light scattering vs. Dynamic light scattering

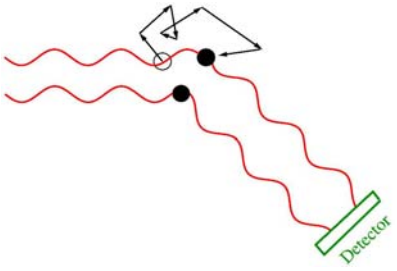
- Static light scattering measures time-average intensities (mean square fluctuations)
  - molecular weight
  - radius of gyration
  - second virial coefficient
- Dynamic light scattering measures real-time intensities i(t), and thus dynamic properties
  - diffusion coefficient
  - (hydrodynamic radius)
  - size distributions



How DLS Works: Interference of Light



Constructive interference



Destructive interference

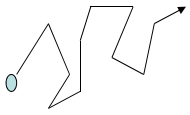
Brownian motion results in light intensities which fluctuate in time.

The timescale of those intensity changes is a measure of the diffusion constant.

Brownian motion is a phenomenon that is fundamental to this experiment.

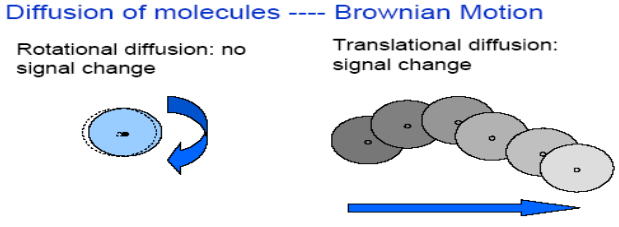
It describes the way in which very small particles move in fluid suspension, where the fluid consists of molecules much smaller than the suspended particles.

The motion of suspended particles is random in nature, and arises from the cumulative effect of bombardment by the suspending medium's molecules.

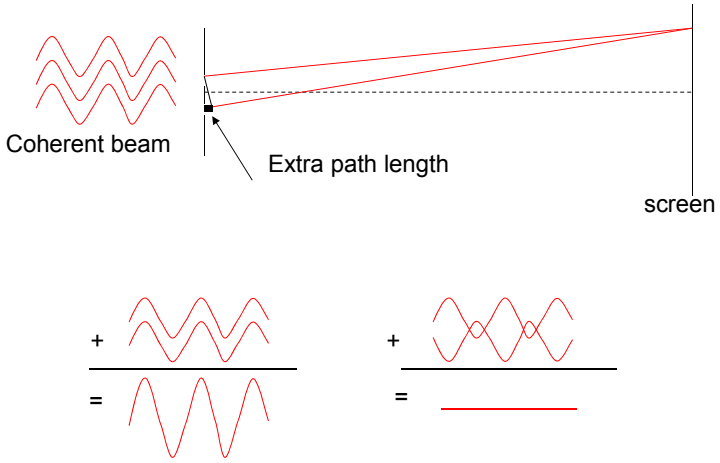


Molecules in a liquid are constantly in motion, randomly bouncing off one another. As these molecules move around in the liquid, they are also bouncing off any suspended particles in a random manner, imparting a momentum to the suspended particles, the magnitude and direction of which fluctuate in time.

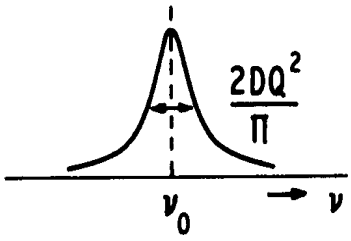
It is the resulting 'random walk' behavior of the suspended particles that is called Brownian motion, and that randomness makes this experiment possible.



Double Slit Experiment



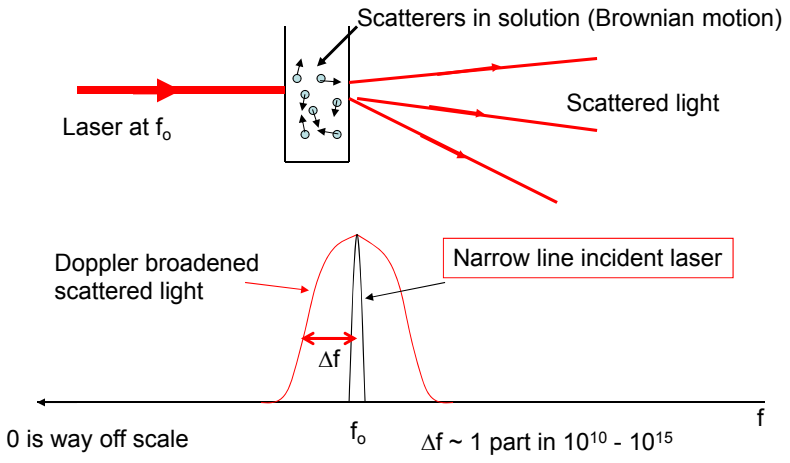
- The incident laser light is highly monochromatic (ν_o)
- The scattered radiation will display a range of frequencies, due to the Doppler shift caused by Brownian motion.
- For particles moving away from the detector, $\nu < \nu_o$
- For particles moving toward the detector, $\nu > \nu_o$
- The technique is also called “quasi-elastic light scattering”, reflecting the apparent change in frequency of the scattered radiation.
- The spread of frequencies is dependent on the diffusion coefficient *D*.



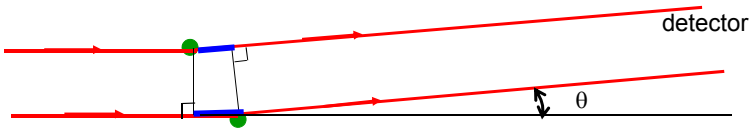
$\Delta\nu_{1/2} = \frac{2Dq^2}{\pi}$ where $\Delta\nu_{1/2}$ is the frequency spread at half - height and $q = \frac{4\pi}{\lambda}\sin\left(\frac{\theta}{2}\right)$

The line width at half height of the spectrum of frequencies observed is related to the diffusion coefficient of the scatterer

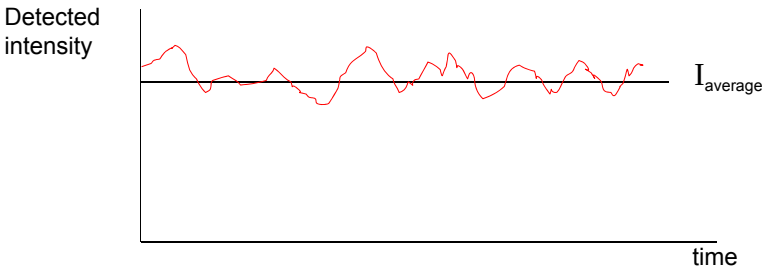
Light Scattering Experiment



More Detailed Picture



Inter-particle interference

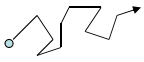


How can we analyze the fluctuations in intensity?

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

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)



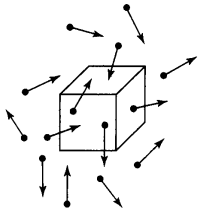
- The correlation time τ_c is a measure of the time needed to diffuse a characteristic distance in solution – this distance is defined by the wavelength of light, the scattering angle and the optical properties of the solvent – ranges from 40 to 400 nm in typical systems

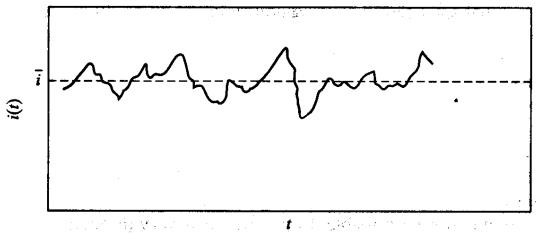
- Values of τ_c can range from
  0.1 μs (small proteins) to days (glasses, gels)

Dynamic light scattering experiment:

- Light intensity is I(t) at time t
- At the time t + τ, which is a very small time later than t, the diffusing particles will have new positions and the intensity at the detector will have a value I(t+τ)
- The detector saves the values for I(t + τ) at numerous times
- The autocorrelator automatically calculates the function instead of the discrete intensities

Correlation spectroscopy





$\Delta i(t) = i(t) - \bar{i}$ where $\bar{i}$ is the average scattering intensity.

Measure $\Delta i(t)$ and $\Delta i(t+\tau)$

where τ is an arbitrary delay time.

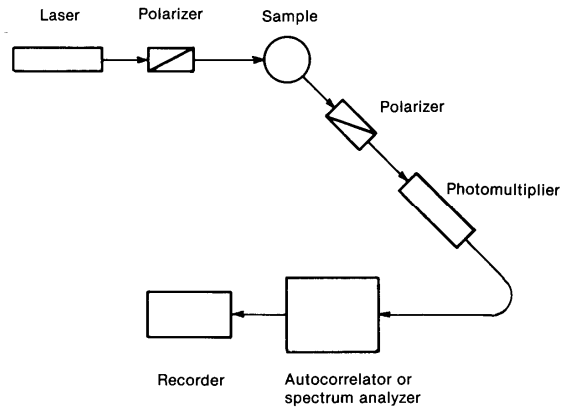
Define an autocorrelation function :

$A(\tau) = \overline{\Delta i(t) * \Delta i(t+\tau)}$ where the

bar denotes an average over many observations.

Correlation spectroscopy

The number of macromolecules present in the volume element illuminated by the laser fluctuates with time. Thus, the scattering intensity also fluctuates.



In a single detector setup, autocorrelation takes the place of cross-correlation.

Autocorrelation is mathematically identical to cross-correlation, except that rather than comparing two signals with one another, one signal is compared with a time-delayed version of itself.

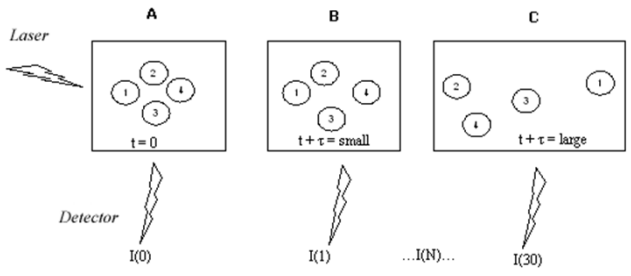
I(t+τ) correlates with I(t), the closer the measurement is to time zero, the more similar I(t+τ) is to I(t) since the particles have not had much time to move:

$$\lim_{\tau \longrightarrow 0} I(t+\tau) = I(t)$$

As time goes on there is no more similarity between the starting state and the current state - the measured intensities do no correlate anymore to the beginning one.

This happens faster if the particles are smaller since smaller particles move faster. One needs a method for quantifying how fast the correlation takes to break down between the starting measurement and one a short time later.

- Light intensity is I(t) at time t
- At t + τ new positions of particles - the intensity at the detector will have a value I(t+τ)
- The detector saves the values for I(t + τ) at numerous times
- The autocorrelator automatically calculates the function instead of the discrete intensities



The function used to calculate this correlation is the *autocorrelation function* it describes how a given measurement relates to itself in a time dependent manner.

At time zero there is a 100% autocorrelation.

As time progresses, the autocorrelation diminishes reaching zero as there is no more similarity between starting and ending states.

The decay of the autocorrelation is described by an exponential decay function g(τ) which relates the autocorrelation to the diffusion coefficient D and the measurement vector q:

$$g(\tau) = g_o \exp[-Dq^2\tau]$$

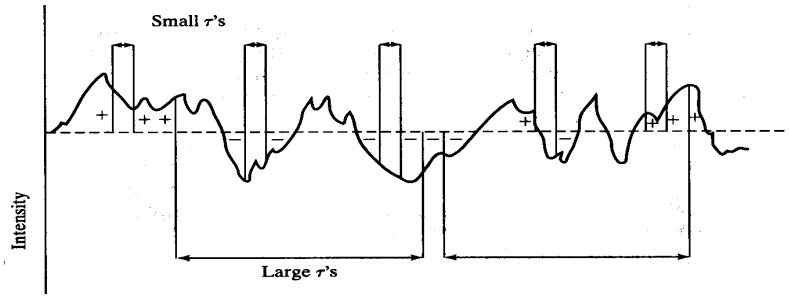
$$q = \frac{4\pi n}{\lambda}\sin(\frac{\theta}{2})$$

n = refractive index of the solution (1.33 for water)
λ = wavelength of the laser (e.g 632.8 nm)
θ = angle of scattering measurement

The intensity varies in the time scale of the molecules moving the distance of a wavelength:

ovalbumin (45 kDa, D = $8.10^{-11}m^2/s$) moves moves 828 nm (laser wavelength) in 4 ms

As indicated in the figure, $g(\tau)$ will tend to be >0 for short τ and will be ≈0 for long τ. Thus, if we measure $g(\tau)$ as a function of τ, we observe an exponential decay to zero.



$$g(\tau) = g_o \exp[-Dq^2\tau]$$

Thus, a plot of ln[$g(\tau)$] vs τ will have a slope of $-Dq^2$.

Brownian motion

Considering the 1-D movement of a suspended particle in a solvent:

In the steady state: $\langle V(t)\rangle = 0$

The statistical average of the product of the velocities at two different times depends only on the time difference:

$$\langle V(t_1)V(t_2)\rangle = C_v(t_1-t_2) = 2D\delta(t_1-t_2)$$

$$\langle V(t_1)V(t_2)\rangle = \langle V(t)V(0)\rangle = C_v(t) = 2D\delta(t)$$

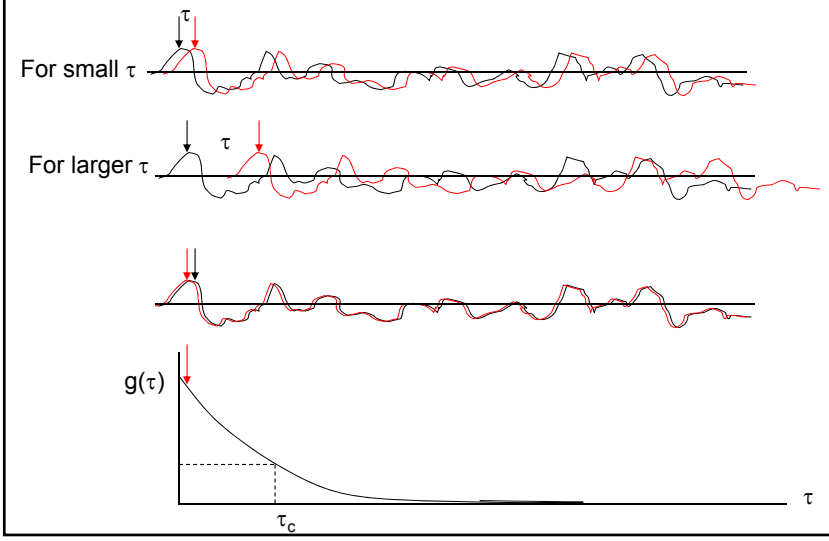
Where, **D** is *the diffusion coefficient* and $\mathbf{C_v(t)}$ is *the velocity correlation function* that is equivalent to the thermal motion for short time difference

Autocorrelation Function

- The average of the time varying portion of the intensity at some initial time, *t*, with the time varying portion of the intensity at some later time, $t+\Delta t$.
- This calculation is done for many values of Δt.
- For small values of Δt, the signal is still correlated with the signal at $t = 0$; positive average intensity.
- For large values of Δt, this is not true, and the autocorrelation function will eventually average out to zero.
- By measuring how long it takes the function to go to zero, we can tell how fast the particles are moving

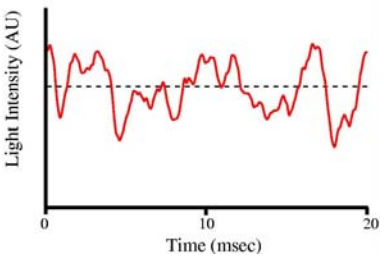
Intensity autocorrelation

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

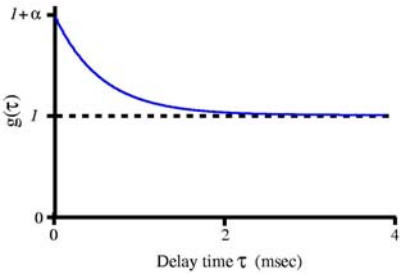


Analysis of Light Intensity Fluctuations

Light Intensity Fluctuations

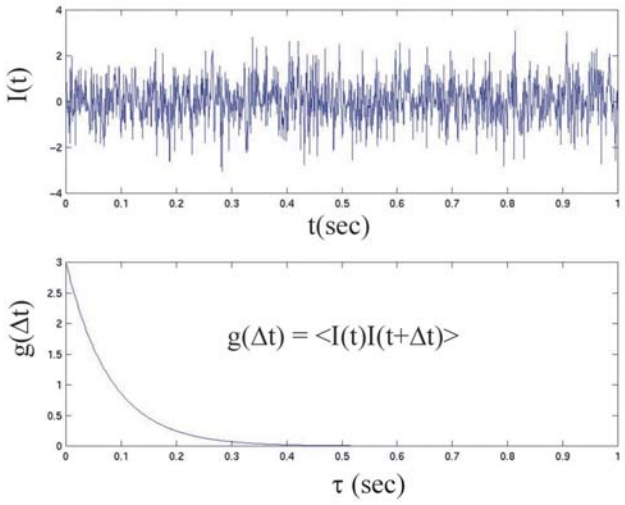


Autocorrelation Function



Particles with a large physical dimension (radius) diffuse more slowly through a solvent, while small particles diffuse more quickly. Intensity fluctuations seen through time are therefore slower for large particles.

Autocorrelation functions



Diffusion

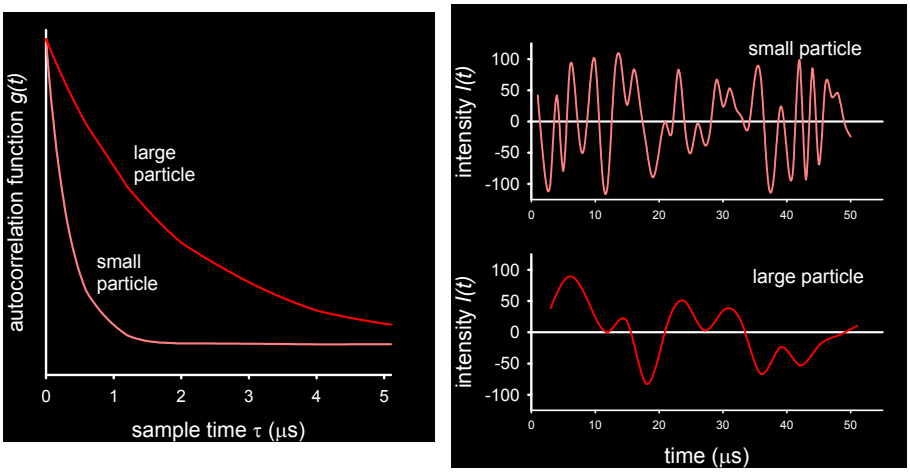
- What can we learn from the correlation time?
- Knowing the characteristic distance and correlation time, we can find the diffusion coefficient D
- According to the Stokes-Einstein equation

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

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

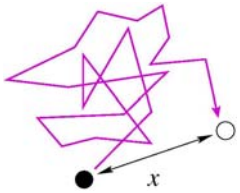
- So, if η is known we can find R (or if R is known we can find η)

Smaller particles diffuse faster



Timescale of Motion

How does one get from a diffusion coefficient to R_h?

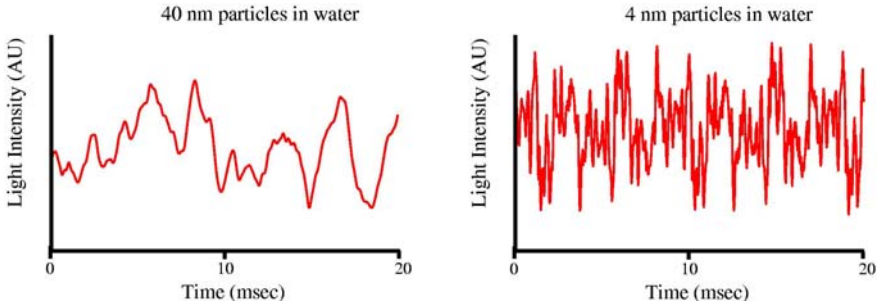


Stokes - Einstein Relation

$$R_h = \frac{k_B T}{6\pi\eta D_t}$$

k_B – Boltzmann's constant
T – temperature (Kelvin)
η – viscosity of solvent
R_h – hydrodynamic radius

Intensity Fluctuations



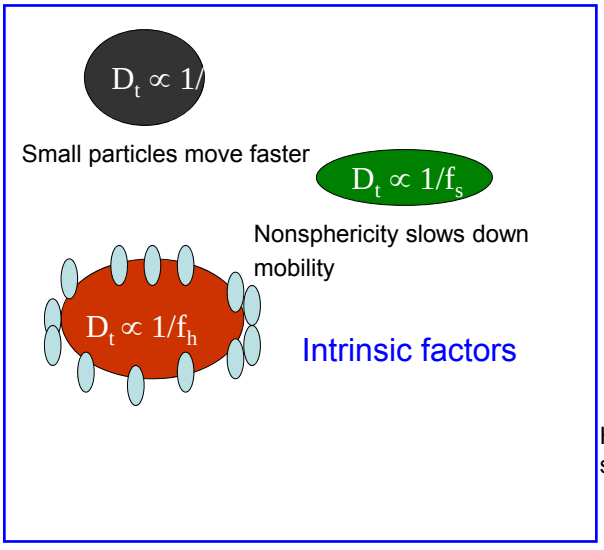
The rate at which particles diffuse is related to their size, provided all other parameters are constant.

By fitting the points of autocorrelation to the function g(τ), the diffusion coefficient can be measured and related to the equivalent sphere of diameter d using the Stokes - Einstein equation:

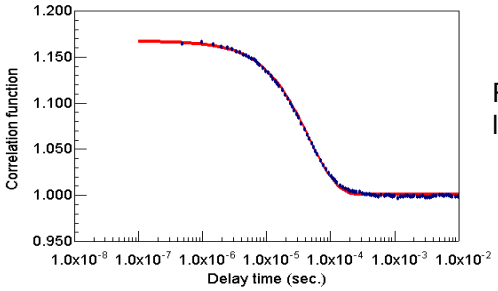
$$D = \frac{k_B T}{3\pi\eta d}$$

η = diluent viscosity (water = $8.94*10^{-4}$ kg/(ms)
T = Temperature (K) (room temp = 298 K)
D = diffusion coefficient (in m^2/s)
k_B = Boltzmann constant ($1.3807*10^{-23}$ J/K)
d = sphere diameter (m)

What Affects Translational Diffusion?

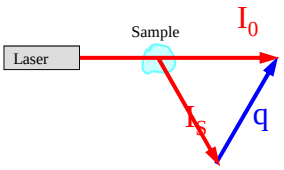


Autocorrelation Function

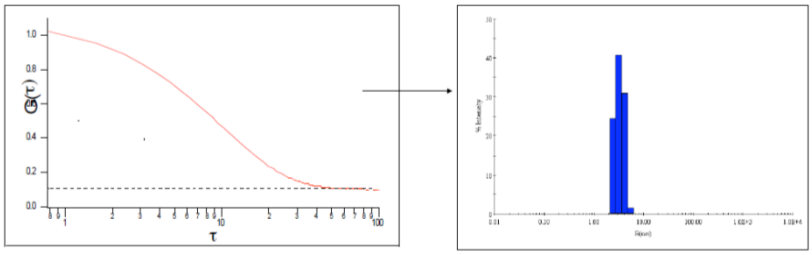


R_h = 9 nm
latex spheres

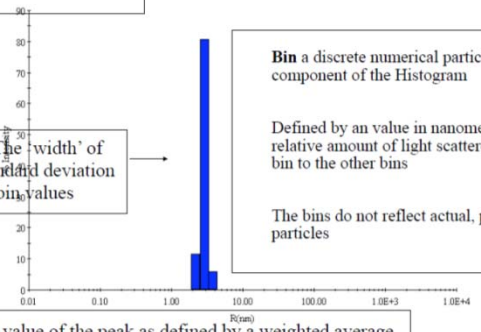
Autocorrelation function: $g^2(\tau) = \langle I \rangle^2 \left(1 + \alpha \exp\left(-2D_t q^2 \tau\right)\right)$



$$q = \frac{4\pi n_0}{\lambda_0} \sin(\theta/2)$$



Y-Axis: Relative amount of light scattered by each bin



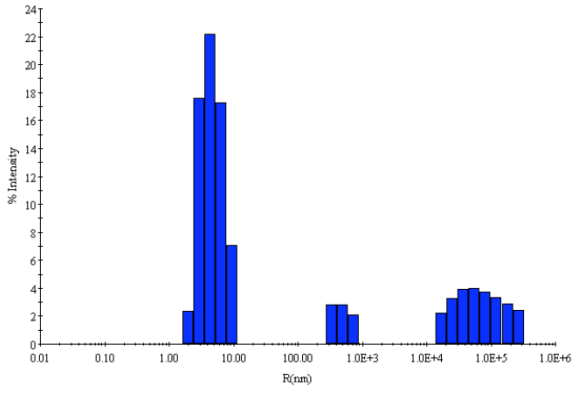
Polydispersity The 'width' of the peak, the standard deviation of the weighted bin values

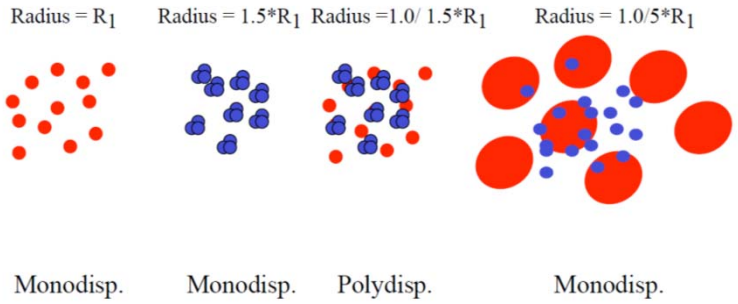
Bin a discrete numerical particle size component of the Histogram

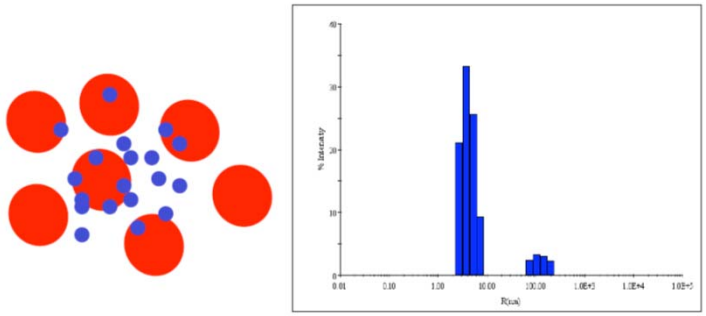
Defined by an value in nanometers (size), relative amount of light scattered by the bin to the other bins

The bins do not reflect actual, physical particles

Rh. The mean value of the peak as defined by a weighted average of the number of bins comprising the histogram

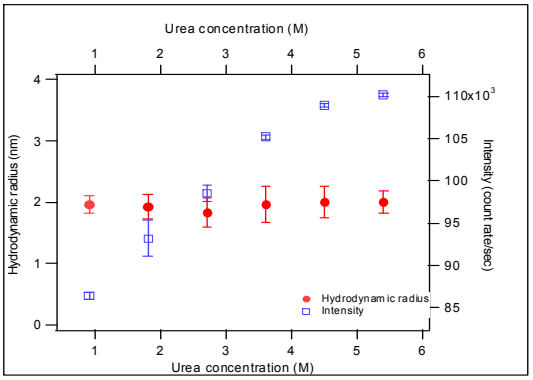



Radius = R_1
Radius = 1.5*R_1
Radius =1.0/ 1.5*R_1
Radius = 1.0/5*R_1
Monodisp.
Monodisp.
Polydisp.
Monodisp.


% Intensity
0.01
0.10
1.00
10.00
100.00
1.0E+3
1.0E+4
1.0E+5
R(nm)

Hydrodynamic radius of lysozyme as a function of urea concentration

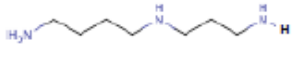
Concentration of urea (M)	Hydrodynamic radius (nm)
0.9	1.96 ±0.14
1.8	1.93 ±0.20
2.7	1.83 ±0.23
3.6	1.96 ±0.29
4.5	1.89 ±0.25
5.4	1.99 ±0.18



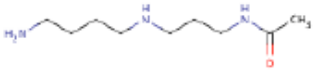
- The hydrodynamic radius of lysozyme (1mg/ml) is independent of the urea concentration

Spermine/spermidine acetyl transferase is similar in a.a. sequence and structure to serotonin acetyl transferase, but it is a dimer, with two subunits of about 170 a.a. each.

protein dimer

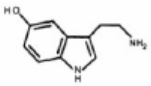


spermidine

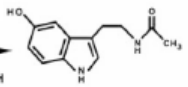


acetylated spermidine

protein monomer



serotonin

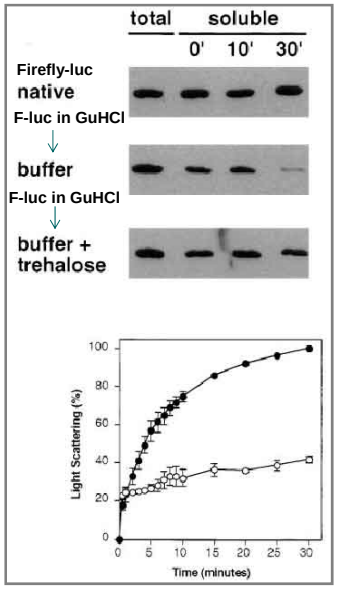


N-acetylserotonin

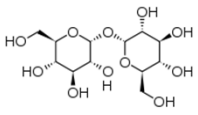
The data given below describe the variation of sedimentation coefficient, s, and diffusion coefficient, D for a protein as a function of pH.

pH	2	3	4	5	6	7	8	9	10
s (S)	2.93	3.02	3.89	4.41	4.40	4.15	3.60	2.25	2.20
D (x 10^7) cm^2s^{-1}	7.91	8.00	8.00	5.90	5.92	5.61	4.86	3.08	2.97

Explain what happens to the protein at low and high pH, assuming that the protein adopts its native structural state (i.e. structural conformation) between pH 5 and pH 6.



light scattering monitored at 360 nm



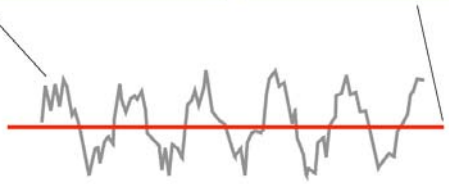
Closed circles: buffer
Open circles: buffer + trehalose

Singer and Lindquist (1998) *Mol. Cell* **1**, 639.

Dynamic Signal **Static Signal**

Time dependent fluctuations induced by Brownian motion.

Angle and concentration dependent time average scattering intensity.



Dynamic light scattering
real-time intensities

- **Diffusion coefficient D_T**
- **Modality**
- **Hydrodynamic radius R_h**
- **Size Distribution**
- **Estimation of MW**

Static light scattering
time-average intensities

- **Molecular weight**
- **Radius of gyration**
- **Second virial coefficient**