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PHOTON CORRELATION SPECTROSCOPY

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Dynamic light scattering is a method for measuring the **diffusion** of macromolecules within a solution due to Brownian motion. It differs from "classical" or "static" **light scattering**,

which permits the measurement of the molecular weight and radius of gyration of macromolecules from the light they scatter at varying scattering angles. Dynamic light scattering measures instead the intensity fluctuations of the scattered light on the timescale of 10^{-3} to 10^{-9} s. The frequencies of these fluctuations depend on how rapidly the molecules are moving by diffusion. These measurements require lasers, which provide light of high intensity, collimation, monochromaticity, and, most importantly, spatial and time coherence; the last attributes mean that the light is emitted from the laser as a continuous wave rather than as short bursts.

The physics underlying dynamic light scattering is complicated (1). In simple terms, the moving macromolecules will *Doppler-broaden* the otherwise monochromatic incident radiation. The scattered intensity will fluctuate because of *beating interference* of scattered waves of different, but similar, wavelengths; the situation is analogous to the fluctuations in intensity of a radio channel caused by interference from another radio channel of a very close wavelength. This broadening of the originally monochromatic light is why the technique is often referred to as *quasi-elastic light scattering* (QLS). The detector sends the intensity signal to a special computer, called an *autocorrelator*, which compares or correlates the intensity at different times; for this reason, the technique has another term, *photon correlation spectroscopy* (PCS). How rapidly the intensity fluctuates over short periods of time, or delay times, τ, is represented by how a parameter known as the *normalized intensity autocorrelation function*, $g^{(2)}(\tau)$, decays as a function of τ. The superscript "(2)" is used to indicate that it is an intensity, as opposed to an electric field "(1)," autocorrelation function. Many data sets of $g^{(2)}(\tau)$ as a function of τ are accumulated and averaged. The degree of averaging necessary depends on the incident laser intensity and the size and concentration of the scattering macromolecule (at a given concentration; larger molecules scatter more). For globular proteins, sufficient data can usually be acquired within one to several minutes.

Analysis of how the normalized intensity autocorrelation function $g^{(2)}(\tau)$ decays as a function of τ can be used to evaluate the translational diffusion coefficient, D_t. For dilute solutions of spherical or near-spherical (ie, globular) macromolecules, the variation of $g^{(2)}(\tau)$ with τ can be represented by the simple logarithmic equation

$$\ln[g^{(2)}(\tau) - 1] = -2D_t k^2 \tau \qquad (1)$$

where the parameter k is known as the *Bragg wave vector*, whose magnitude is defined by

$$k = (4\pi n/\lambda)\sin(\theta/2) \qquad (2)$$

where n is the refractive index of the medium, θ is the scattering angle, and λ is the wavelength of the incident light. Consequently, the value of D_t can be found from a plot of $\ln[g^{(2)}(\tau) - 1]$ versus τ. Figure 1 illustrates an example for the motility protein **dynein**.

Two important practical conditions need to be noted: (*1*) the value of D_t is sensitive to the temperature, which needs to be accurately controlled or, at the very least, monitored during the measurement; and (*2*) the scattering signal is very sensitive to the presence of trace amounts of dust or aggregation products; solutions and the scattering cell or cuvette need to be scrupulously clean and free of particulates. The samples must be filtered and centrifuged and the vessels washed. Special filling devices have been constructed to minimize this dust problem (2).

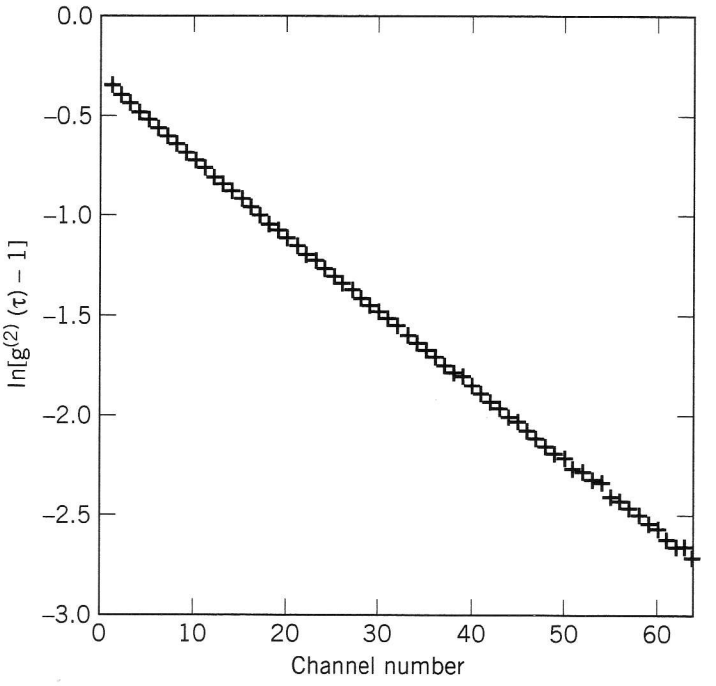

Figure 1. Dynamic light scattering of a solution of dynein. The channel number is a measure of the delay time, τ (see Eq. 1 of the text).

The value of D_t can be converted to the standard conditions of water at 20.0°C and extrapolated to zero sample concentration (see **Diffusion**). An additional extrapolation is necessary if the macromolecule is not globular. There is an extra term on the right-hand side of equation 1 that arises from rotational diffusion at finite angles θ. This term approaches zero as θ does. Therefore, it is necessary to make the measurements of D_t at a number of angles θ and to extrapolate to zero angle (or Bragg vector k). The extrapolations to zero sample concentration and to zero angle θ (or k) can be performed simultaneously on a biaxial extrapolation plot known as a *dynamic Zimm plot* (3). If

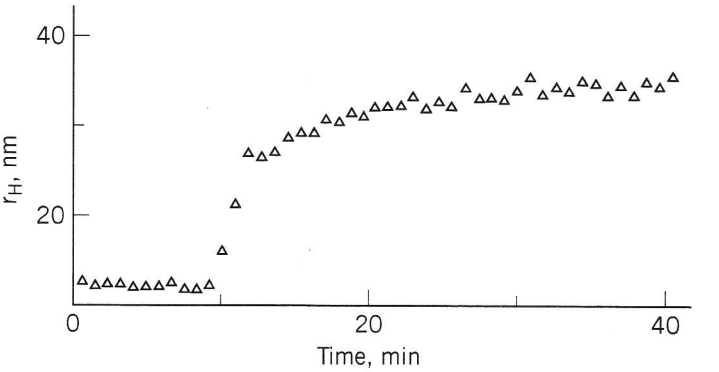

Figure 2. Following the dynamics of a process using dynamic light scattering: effect of removal of calcium ions (by adding **EGTA** at 10 min) on the hydrodynamic radius (r_H) of southern bean mosaic virus (adapted from Ref. 6).

an angular extrapolation is not necessary, a scattering angle of 90° is usually chosen, and fixed-angle instruments are usually set at this angle. At lower angles, the problems due to contamination with large particles (see (*2*) above) are accentuated.

If a sample is heterogeneous, it is possible, at least in principle, to obtain a distribution of diffusion coefficients after various assumptions and mathematical manipulation of the autocorrelation data; these methods have been reviewed (4), and several commercially available computer programs are available. A more simple way of representing heterogeneity is with the *polydispersity factor* (PF), which is obtained by comparing linear with quadratic or more complex fits of the normalized autocorrelation function decay data (5).

Measurements can be made very rapidly with dynamic light scattering, and it is possible to follow biomolecular assembly–disassembly processes that occur on a timescale of minutes. An example of the swelling of southern bean mosaic virus in response to the removal of bound Ca^{2+} ions is given in Fig. 2 (6).

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Suggestions for Further Reading

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