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DIFFUSION

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Diffusion is the movement of molecules within a liquid driven by thermal fluctuations. It is important for most biological processes; indeed, the diffusion together of two reactants can be the limiting factor for many processes, when the reaction is said to be **diffusion-controlled**.

There are two types of diffusional motion: (*1*) *translational diffusion*, where a molecule moves its relative position within a three-dimensional system; and (*2*) *rotational diffusion*, where a molecule spins or rotates about one or more of its axes. The rates at which molecules undergo both types of diffusion are measured by their respective diffusion coefficients, D_t and D_r. The SI unit for D_t is the "Fick," or m^2/s, but for historical and other reasons, biologists tend to use the cgs (Centigrade–gram–second) system unit of cm^2/s. These measurements can give important information about the sizes, structures, and physical properties of the molecules. At room temperature and in dilute solution, a small protein of molecular weight approximately 20,000 will have D_t of about 10^{-6} cm^2/s; a large virus between 10^{-7} to 10^{-8} cm^2/s; a bacterial spore about 10^{-9} cm^2/s. Some representative values are given in Table 1.

TRANSLATIONAL DIFFUSION

The translational diffusion coefficient, D_t, describes the tendency of a molecule to move (translational motion) under the influence of either (*1*) a concentration gradient or (*2*) Brownian motion.

The movement of molecules in a gradient in which their concentration varies, dc/dx, where c is the concentration (in grams per milliliter) at each point x, is given by Fick's first law:

$$J = -D_t(dc/dx) \tag{1}$$

where J is the mass of particles crossing a 1-cm^2 cross section per second.

The same D_t characterizes the Brownian diffusion of the molecule:

$$\langle x^2 \rangle = 2D_t t \tag{2}$$

Table 1. Translational Diffusion Coefficients and Derived Parameters of Some Molecules, Macromolecules and Biomolecular Assemblies

Substance	Molecular Weight	$10^7 \times D^{\circ}_{20,w}$, cm^2/s	r_H, Å	$10^8 \times f$, g/s
Water	18	230	—	—
Sucrose	342	46.0	4.7	0.88
Ribonuclease	13,700	11.1	19.3	3.64
Ovalbumin	45,000	7.8	27.5	5.18
Fibrinogen	330,000	2.0	107	20.2
Dynein[a]	2.5×10^6	1.1*	195	36.7
Turnip yellow mosaic virus	5.7×10^6	1.4	152	28.9

[a] Although the molecular weight of dynein is smaller than that of turnip yellow mosaic virus, its diffusion coefficient is smaller because it is more asymmetric. Values of $D^{\circ}_{20,w}$ and molecular weight M_r for dynein are also strongly dependent on salt concentration.

where t is the time and $\langle x^2 \rangle$ is the average of the square of the distance the particle has moved.

The value of D_t depends not only on the intrinsic size and shape of the molecule but also on the viscosity and temperature of the medium in which it is suspended. The value of D_t must therefore be normalized to standard conditions; the standard conditions normally used are those of water at 20.0°C. The D_t corresponding to these conditions is normally designated $D_{20,w}$. It can be calculated from the value actually measured at absolute temperature T and in buffer "b," $D_{T,b}$, with the equation

$$D_{20,w} = \left(\frac{293.15}{T}\right)\left(\frac{\eta_{T,b}}{0.01}\right)D_{T,b} \quad (3)$$

where $\eta_{T,b}$ is the viscosity (in cgs units) of the buffer at temperature T and 0.01 is the viscosity of water at 20.0°C.

The value of $D_{20,w}$ can also depend on the concentration of the molecule in the solution, due to thermodynamic nonideality resulting primarily from the finite size of the molecule and its electric charge. This nonideality is represented (1) by the parameter k_d (in units of milliliters per gram) in the equation

$$D_{20,w} = D^{\circ}_{20,w}(1 + k_d c) \quad (4)$$

$D^{\circ}_{20,w}$ is the value of $D_{20,w}$ at zero concentration of the molecule; its value can be estimated with equation 4 by measuring $D_{20,w}$ at several different concentrations. For diffusion measurements, the non-ideality term is often negligible and can be neglected.

The value of D_t can be measured either by following the rate of disappearance of a concentration gradient or by dynamic light scattering. The former is the traditional method of measuring D_t (2,3). The principle is to determine how the concentration changes with time at the boundary between two solutions, one containing the molecule of interest and the other, just the buffer or dialyzate. A special diffusion cell can be used with an appropriate optical system for recording either the concentration, c, or the concentration gradient, dc/dx, as a function of distance, x, from the center of the boundary. Alternatively, the optical system of an analytical **ultracentrifuge** can be used to measure the boundary spreading in a synthetic boundary cell. A speed is chosen to be sufficiently small that sedimentation of the molecule is negligible. The Schlieren optical system is very useful, as it gives the value of dc/dx as a function of x. The value of D_t can be determined by measuring the variation in the height of the boundary, specifically, the maximum value of dc/dx, with time, t:

$$\left(\frac{A}{H}\right)^2 = 4\pi D_t t \quad (5)$$

where A is the area under the curve of dc/dx versus x. A plot of $(A/H)^2$ versus time will yield D_t from the slope; an example is given in Figure 1.

Equation 5 assumes that there is no loss of material from the boundary, that is, that the area A remains constant. This assumption is reasonable for homogeneous protein preparations but may not be valid for polydisperse materials such as mucus **glycoproteins** and polysaccharides.

These methods have also generally been superseded by **dynamic light scattering**. Nevertheless, the classical measurements are preferable with nonglobular macromolecules, with asymmetric shapes.

INTERPRETATION OF THE TRANSLATIONAL DIFFUSION COEFFICIENT

The value of $D_{20,w}$ or $D^{\circ}_{20,w}$ obtained by either dynamic light scattering or boundary spreading can be used to provide a number of useful characteristics about a macromolecule.

Hydrodynamic Radius

The simplest deduction that can be made from the $D^{\circ}_{20,w}$ is the size of the molecule as represented by its equivalent hydrodynamic radius, r_H, which is also known as the *Stokes radius*. The r_H is the radius of the equivalent sphere that would have the same $D^{\circ}_{20,w}$. The two parameters are related by the *Stokes–Einstein equation*:

$$r_H = \frac{k_B T}{6\pi\, \eta_{20,w} D^{\circ}_{20,w}} \quad (6)$$

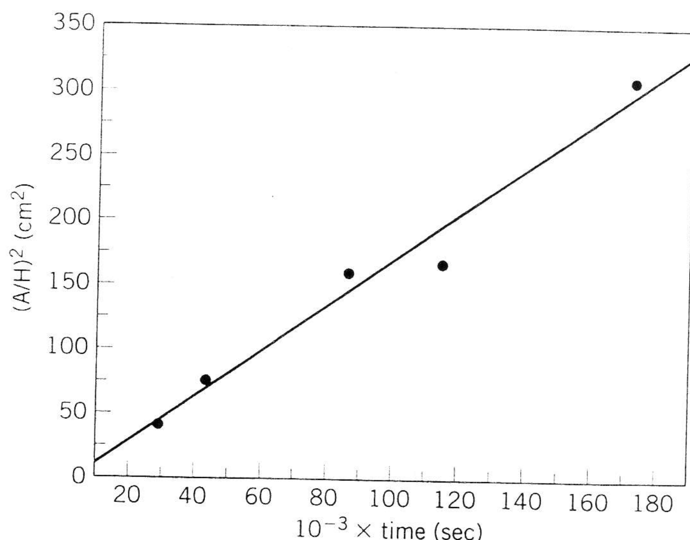

Figure 1. Measurement of the translational diffusion coefficient, D_t, for **ovalbumin** by the boundary spreading technique (4). As in Equation 5 of the text, the square of the ratio of area (A) to the height (H) of the boundary is plotted as a function of time.

where k_B is the Boltzmann constant (1.379×10^{-16} erg/K), T is the temperature (293.15 K at 20.0°C), and $\eta_{20,w}$ is the viscosity of water (0.01 P at 20.0°C). If the value of D_t is not corrected to standard conditions, the appropriate values for T and η must be used. Table 1 gives the values of r_H for several macromolecules.

Frictional Coefficient, *f*

The **frictional coefficient** is inversely related to the diffusion coefficient, giving a measure of the resistance to movement due to both its size and shape. It can be calculated directly from $D^{\circ}_{20,w}$:

$$f = \frac{RT}{N_A D^{\circ}_{20,w}} = \frac{k_B T}{D^{\circ}_{20,w}} \qquad (7)$$

where R is the gas constant (8.314×10^{-7} erg $\text{mol}^{-1}\text{K}^{-1}$) and N_A is Avogadro's number (6.02×10^{23}). Values of f calculated from $D^{\circ}_{20,w}$ are given in Table 1.

It is often more informative to use the **frictional ratio,** f/f_o, which is the dimensionless ratio of the observed frictional coefficient to that of an equivalent spherical molecule of the same anhydrous mass and density.

Molecular Weight (M_r)

The molecular weight of a molecule can be calculated from the combination of its diffusion coefficient and its **sedimentation coefficient**, $s^{\circ}_{20,w}$ when corrected to standard conditions. The equation analogous to (7) for the sedimentation coefficient is

$$f = \frac{M_r(1 - \bar{v}\rho_{20,w})}{N_A s^{\circ}_{20,w}} \qquad (8)$$

where $\bar{v}$ is the **partial specific volume** of the macromolecule and $\rho_{20,w}$ is the density of the standard solvent, water at 20°C. Elimination of f between equations 7 and 8 yields the well-known Svedberg equation:

$$M_r = \frac{s^{\circ}_{20,w}}{D^{\circ}_{20,w}} \frac{RT}{(1 - \bar{v}\rho_{20,w})} \qquad (9)$$

Equation 9, of course, makes it possible to calculate $D^{\circ}_{20,w}$ if both $s^{\circ}_{20,w}$ and M_r are known.

It is possible, in principle, to measure both $D^{\circ}_{20,w}$ and $s^{\circ}_{20,w}$ simultaneously from analysis of the shape of the boundary in **sedimentation velocity** analytical ultracentrifugation, although in practice this requires data of high quality and a totally homogeneous sample.

If the general shape of the macromolecule is known to a first approximation, it is possible to estimate its molecular weight from only its diffusion coefficient, just as in the case of the **sedimentation coefficient**. The power-law relation between M_r and D_t is also known as a *Mark–Houwink–Kuhn–Sakurada relation*:

$$D_t = KM_r^{-\varepsilon} \qquad (10)$$

where $\varepsilon = 0.333$ for a sphere, 0.85 for a rod, and 0.5–0.6 for **random coil** polymers. The appropriate value of the constant K is obtained from a collection of standard molecules of known D_t and M_r (5). Of course, the shape of the macromolecule will normally be known only approximately, so any molecular weight calculated in this way must be considered only an estimate.

Shape

The translational frictional coefficient, f, or the frictional ratio, f/f_0, can be used directly to provide information about the shape of the molecule. The function defining the shape and flexibility of a macromolecule is the *Perrin translational frictional function*, P:

$$P = \frac{f}{f_0}\left(\frac{\bar{v}}{\bar{v} + (\delta/\rho_0)}\right)^{1/3} \qquad (11)$$

where δ is the amount of bound solvent, relative to the mass of the molecule, and ρ_o is the density of the bound solvent. For a molecule that is fairly rigid over a time-averaged period, the gross shape can be specified using P in terms of the axial ratio of the equivalent hydrodynamic ellipsoid or in terms of an arrangement of spheres, in hydrodynamic bead models (Figure 2). Computer programs are available for both types of modeling (6,7). Especially with the latter approach, the diffusion coefficient should be used in conjunction with other hydrodynamic measurements, such as $s_{20,w}$, to obtain a unique solution to the modeling.

ROTATIONAL DIFFUSION

Rotational diffusion coefficients, which result from the tumbling motion of a macromolecule about an axis or axes, are very sensitive functions of the shape of the molecule and permit the inference of some parameters of the overall shape. Unfortunately, this sensitivity comes at a severe price: measurement of rotational diffusion is usually considerably more difficult than translational diffusion.

There are two principal methods for measuring rotational diffusion. One is based on fluorescence measurements and is called *fluorescence anisotropy* decay. The other is based on electrooptical measurements and is termed *electric birefringence* (or the related *electric dichroism*) decay.

Fluorescence Anisotropy Decay

Tryptophan residues provide intrinsic fluorescent chromophores in many proteins (ie, the ability to reradiate electromagnetic energy at a wavelength longer than that absorbed).

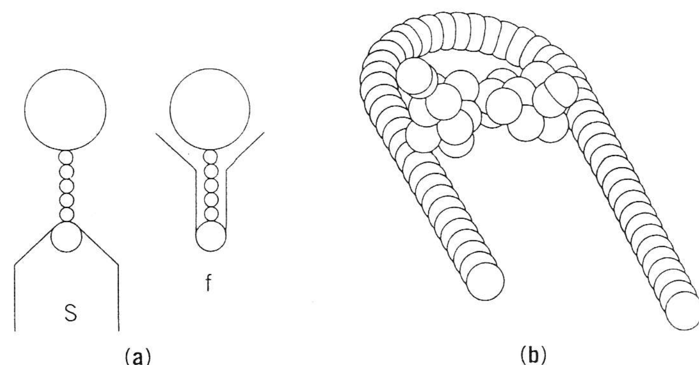

Figure 2. Use of diffusion measurements to represent the shapes of biomolecular assemblies using the hydrodynamic bead model approach: (**a**) from translational diffusion measurements: bead models of the slow "s" (tails out) and fast "f" (tails retracted) forms of a T-even **bacteriophage** (19); (**b**) from rotational (electric dichroism) relaxation measurements: bead models of the complex formed from a DNA fragment of 203 bp with two specific sites and two catabolite activator protein dimers (12).

If the incident monochromatic light is plane-polarized, the reradiated radiation will not only be at a longer wavelength but will also be wholly or partially depolarized depending (among other factors) on the extent of rotational Brownian motion of the macromolecule. If the macromolecule is not a protein and does not have an intrinsic fluorescent chromophore, or is a protein-containing insufficient tryptophan residues, a chromophore can be attached synthetically.

The emitted polarization is measured by two detectors, one normal to, and the other perpendicular to, the plane of polarization; the extent or "anisotropy" of polarization, $r(t)$, is recorded as a function of time, t:

$$r(t) = \frac{I_{\parallel} - I_{\perp}}{I_{\parallel} + 2I_{\perp}} \quad (12)$$

where $I_{\parallel}$ is the emitted intensity in a plane parallel to the polarization of the incident light and $I_{\perp}$ is in a plane perpendicular. In time-resolved fluorescence depolarization anisotropy, this rapid decay in anisotropy of polarization in response to a pulse of incident radiation is recorded and averaged over many pulses (in some ways, the situation is analogous to the measurement of translational diffusion using **dynamic light scattering**). Allowance has to be made for the decay in intensity of the chromophore itself, specifically, the decay of the intrinsic fluorescence intensity has to be deconvoluted from the anisotropy decay function. The decay in $r(t)$ with time can then be analyzed in terms of the rotational relaxation times of the molecule. There will be one relaxation time for a spherical particle, three for a particle with an axis of symmetry. For a general asymmetric molecule, there will be five relaxation times that need resolving:

$$r(t) = c_1 \exp(-t/\tau_1) + c_2 \exp(-t/\tau_2) + c_3 \exp(-t/\tau_3) + c_4 \exp(-t/\tau_4) + c_5 \exp(-t/\tau_5) \quad (13)$$

or, more simply $r(t) = \sum_{i=1}^{5} c_i \exp(-t/\tau_i)$ where $i = 1–5$. In practice, at least two pairs of relaxation times are similar; hence the problem is one of resolving three decay constants (this will be particularly true for macromolecules with an axis of symmetry). Once resolved, these can be related to macromolecular shape and hydration (6,7) using relations similar to Equation (11). However, extraction of decay constants from a "multiexponential decay"—of which equation (13) is an example—is what the mathematicians call "an ill-conditioned problem" and is not easy, especially if the relaxation times are relatively similar. A further problem is that the chromophore most not relative to the rest of the macromolecule.

A much simpler procedure is to measure fluorescence depolarization or anisotropy decay in the steady state, where the light source is continuous rather than pulsed (8). It can be used to obtain the harmonic mean rotational relaxation time, τ_h. τ_h and $1/\tau_{1-5}$ can all be related to the shape and hydration of the macromolecule (7,9). A study of **fibrinogen** provides a good example of the application of both time-resolved and steady-state fluorescence measurements (10,11).

Electric Birefringence Decay

Solutions of macromolecules oriented in an electric field will be *birefringent*, having different refractive indices for light polarized parallel to and perpendicular to the electric field. This is known as *electric birefringence*. A related phenomenon, for macromolecules with absorbing chromophore, is *electric dichroism*, where a solution of macromolecules oriented in an electric field exhibits different extinction coefficients parallel to and perpendicular to the electric field.

When the electric field is switched off, the birefringence (or difference in refractive indices) Δn will decay because of rotational motions of the macromolecule:

$$\Delta n(t) = \sum_{i=1}^{5} c_i \exp(-t/\tau_i) \quad (14)$$

where $i = 1–5$. However, there will be just two relaxation times for molecules that can be approximated by homogeneous ellipsoidal shapes, and just one for a homogeneous ellipsoid with an axis of symmetry. An electrically homogenous spherical particle exhibits no birefringence.

Like fluorescence anisotropy decay measurements, the relaxation times τ_i can be related to molecular shape and hydration (Fig. 2) (12), but there are also practical problems to be overcome. The main problem has been that of local overheating in the solution caused by the large orienting electric fields. This has meant in the past that experiments have been limited to solutions of low ionic strength. With significant advances in charge shielding in modern instrumentation, however, physiological ionic strengths are now a reality (13).

Diffusion of Small Molecules through Biomolecular Systems

Although most attention is given to diffusion phenomena in macromolecules, the importance of the diffusion through biopolymer matrices of small molecules and ions, even water molecules themselves, cannot be ignored; indeed, many physiological processes involve passive or active transport of water and other low-molecular-weight species through cellular and other matrices. As a direct comparison with macromolecular diffusion, Table 1 also gives the self-diffusion coefficient at 20.0°C of water (2.3×10^{-5} cm²/s) and a small sugar molecule, sucrose ($\sim 4.6 \times 10^{-6}$ cm²/s); the diffusion rates are orders of magnitude greater than those for more bulky macromolecules.

Arguably the best method for measuring diffusion of water and other small molecules is *pulsed field gradient spin echo* **NMR** (14). Spinning charged particles, such as atomic nuclei, will have an associated magnetic dipole moment, which generates a magnetic energy when placed within a magnetic field. Quantum-mechanical considerations restrict the energies to a limited number of discrete values. Transitions between levels—whose spacing depends on the external magnetic field—correspond to radiofrequency (RF) radiation, so the nucleus will interact or "resonate" with certain radiofrequencies if the external magnetic field is varied; this is known as nuclear magnetic resonance. These frequencies, and the strength and breadth of the resonances, depend on the particular atomic nuclei that are being examined and on the environment in which they find themselves. It is therefore possible to "home in" on a particular nuclear species; for example, such nuclei could be the hydrogen atoms in a water molecule.

With the pulsed field gradient technique, an excitation pulse of RF radiation is applied across a sample—in this case a biomolecular matrix—causing alignment of spins and the generation of an NMR signal; this signal subsequently decays as a result of diffusion. If a second RF pulse is applied after an interval τ, the decay processes are reversed and a refocused "echo" signal is obtained at a time 2τ after application of the first pulse. This recovery process can be interrupted as follows. A pair of matched magnetic field gradient pulses that

vary linearly across the sample are also applied; the first is applied between the RF pulses and the second, after the second RF pulse. The echo only has no net effect if there has been no diffusive movement of the molecules; any movement will result in an attenuation of the echo. Analysis of this attenuation can be used to determine the diffusion coefficient.

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Suggestions for Further Reading

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K. E. Van Holde (1985) *Physical Biochemistry*, Prentice-Hall, Englewood Cliffs, N. J. [Chapters 4 (general diffusion), 6 (electro-optics) and 8 (fluorescence depolarisation) give an excellent introduction.]