

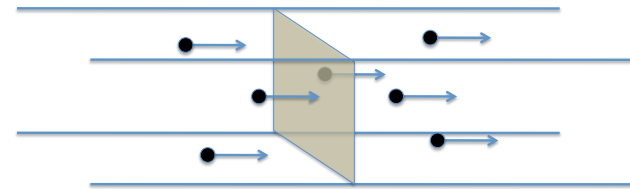
Size and Shape of Macromolecules: Diffusion and Light Scattering

Main References: 1. Chapters 5 & 7 of van Holde
2. Chapters 6 & 7 of Tinoco

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Random Walk, Diffusion and Transport

- Random Brownian motion of molecules: diffusion
 - Driven by thermal energy
 - A fundamental process that underlies virtually all biomolecule separation techniques
 - Intimately related to mass, size and shape
- Transport process: diffusion under influence
 - Measured by (mass) flow J = mass (or mole) per unit area per second

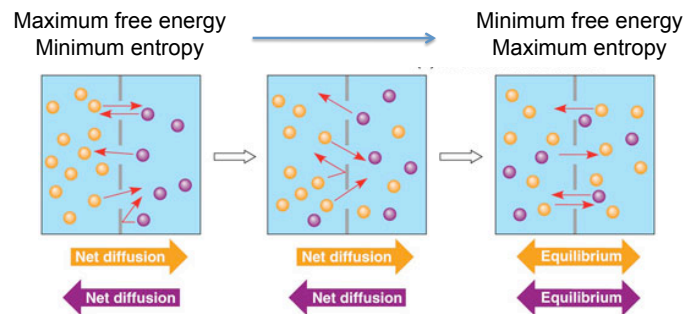


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Thermodynamic Equilibrium

- Without external influence, minimum free energy achieved with uniform concentration distributions
 - Separation require external work!



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Fick's First and Second Laws

- Transport by diffusion under a concentration gradient, $C=C(x)$

$$J = -D \frac{\partial C}{\partial x}$$

Fick's 1st Law

- D : diffusion constant (how fast a substance diffuses)
- $J = 0$ if C is uniform (i.e. $\delta C / \delta x = 0$)

- In most cases, concentration evolves with time as a result of diffusion, $C=C(x,t)$

$$\frac{\partial C}{\partial t} = - \frac{\partial J}{\partial x}$$

coupled with Fick's first law, we have

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

Fick's 2nd Law

- Solving this "differential equation" requires so-called initial and boundary conditions, $C(t=0)$ and $C(x=\infty)$.

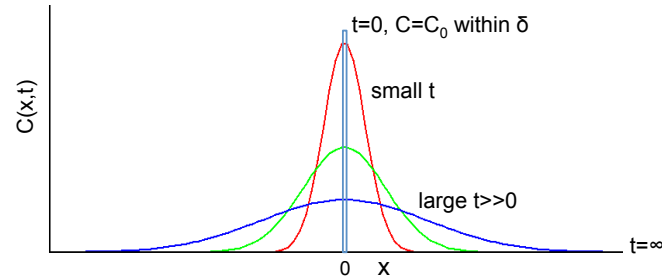
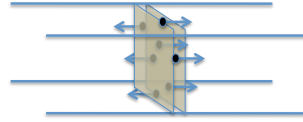
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1D Diffusion with Concentration Impulse

- $C=C_0$ within an thin slab of thickness δ
- $C=0$ at $x=\pm\infty$ at all time
- Solution:

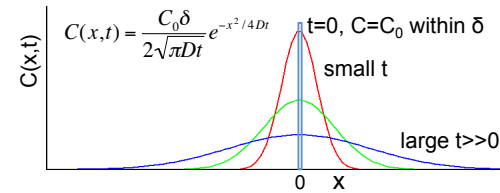
$$C(x,t) = \frac{C_0\delta}{2\sqrt{\pi Dt}} e^{-x^2/4Dt}$$



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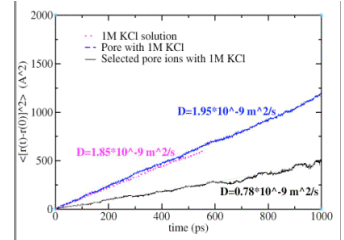
Mean Square Displacement



- Average position: $\langle x \rangle = 0$
- Mean square displacement: $\langle x^2 \rangle \neq 0$

$$\langle x^2 \rangle = \frac{\int x^2 c(x,t) dt}{\int c(x,t) dt} = \frac{\int x^2 e^{-x^2/4Dt} dt}{\int e^{-x^2/4Dt} dt} = 2Dt$$

- Indeed, D measures the diffusion speed!
- Relation to random walks: available from simulation
- Dynamic light scattering measurement of D (see later slides)



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Diffusion and Molecular Size/Shape

- Einstein's relation: $D = kT/f$
 - f : frictional coefficient (resistance to movement through solvent);
 - f is determined by the size and shape of the molecule as well as the viscosity of the solvent
- For spherical particles of radius r : $f = 6\pi\eta r$ (Stokes's law)
 - η : solvent viscosity
- Combine Stokes's law and Einstein's relation

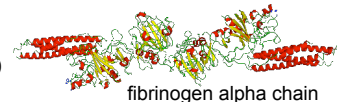
$$D = \frac{kT}{6\pi\eta r}$$

- Deviation from spherical shape (with the same volume) always leads to larger friction (and smaller diffusion constant)
 - "effective" hydrodynamic radius often reported as: $r = kT/6\pi\eta D$
 - Can be misleading for irregularly shaped molecules

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Friction Ratio

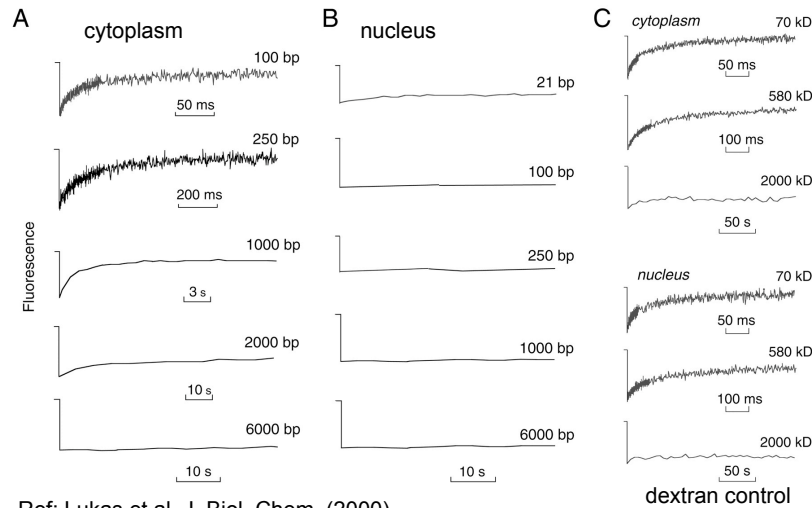


- The minimal friction coefficient is given by mass and partial specific volume:

$$f_0 = 6\pi\eta r_0 = 6\pi\eta \left(\frac{3m\bar{V}}{4\pi} \right)^{1/3}$$
- Then the ratio of measured friction coefficient (f) with f_0 is indicative of the molecular shape (and solvation)
 - if f/f_0 is much greater than unity, the particle is significantly elongated and/or highly solvated (the later leads to larger effective size)

Protein	Molecular Mass (kD)	Partial Specific Volume, $\bar{V}_{20,w}$ ($\text{cm}^3 \cdot \text{g}^{-1}$)	Sedimentation Coefficient, $s_{20,w}$ (S)	Frictional Ratio, f/f_0
Lipase (milk)	6.7	0.714	1.14	1.190
Ribonuclease A (bovine pancreas)	12.6	0.707	2.00	1.066
Cytochrome c (bovine heart)	13.4	0.728	1.71	1.190
Myoglobin (horse heart)	16.9	0.741	2.04	1.105
α -Chymotrypsin (bovine pancreas)	21.6	0.736	2.40	1.130
Fibrinogen (human)	340	0.725	7.63	2.336
Hemocyanin (squid)	612	0.724	19.50	1.358
Glutamate dehydrogenase (bovine liver)	1015	0.750	26.60	1.250
Turnip yellow mosaic virus protein	3013	0.740	48.80	1.470

Example: diffusion of DNA in Cells



Ref: Lukas et al, J. Biol. Chem. (2000)

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Sedimentation

- Concerns the motion of molecules in solutions or particles in suspensions in response to an external force such as gravity, centrifugal force or electric force

- Balance of forces

- $F_c = mr\omega^2$ (centrifugal force)
- $F_b = -m_0r\omega^2$ (buoyancy)
- $F_d = -f v$ (friction)

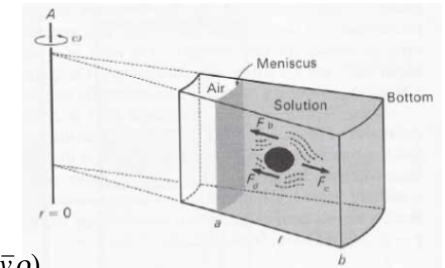
$$F_c + F_b + F_d = 0$$

$$mr\omega^2 - m_0r\omega^2 - fv = 0$$

$$s = \frac{v}{r\omega^2} = \frac{m - m_0}{f} = \frac{m(1 - \bar{v}\rho)}{f}$$

\bar{v} : partial specific volume

- s : sedimentation coefficient (has the unit of time)
- If $m = m_0$, $s = 0$ (no sedimentation)

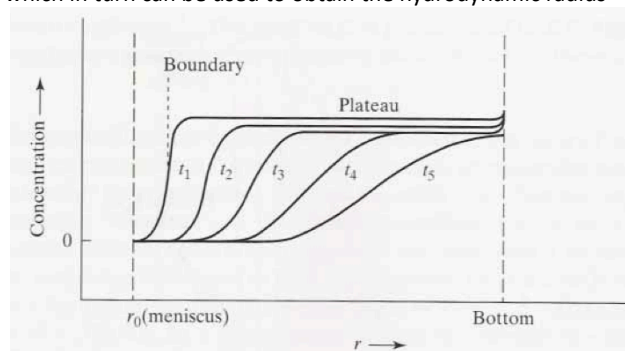


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Moving Boundary Sedimentation

- Analytical ultracentrifuge coupled with scanning absorption optical system
 - Speed of moving boundary: $v = dr/dt = s\omega^2$
 - From measurement of s , diffusion constant (or friction) is calculated, which in turn can be used to obtain the hydrodynamic radius

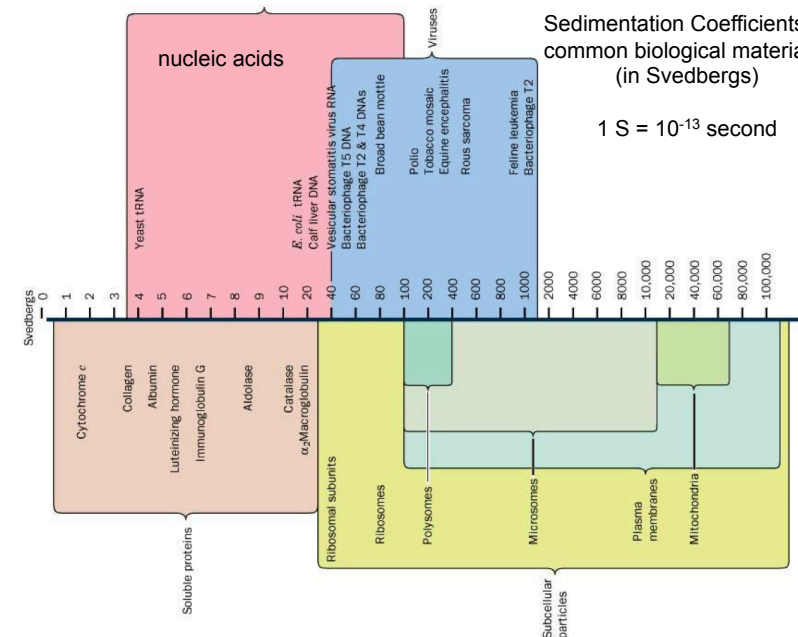


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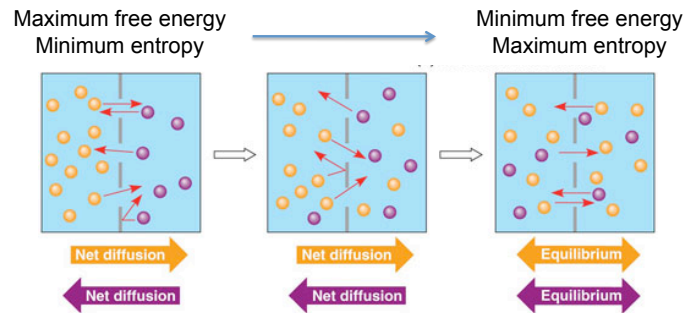
Sedimentation Coefficients of common biological materials (in Svedbergs)

1 S = 10^{-13} second



Sedimentation Equilibrium

- External influence such as centrifugal or electric force can shift the equilibrium distribution of concentration and such a shift depends on a range of molecular properties including mass, size/shape, charges etc

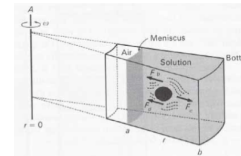


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Sedimentation Equilibrium

- At equilibrium, a steady-state concentration gradient is established: backflow due to gradient (J_D) and outflow by centrifugal sedimentation (J_S) perfectly cancel at every point



$$J_D = -D \frac{dC}{dr}$$

$$J_S = v_s C = s \omega^2 C, \text{ where } s = m(1 - \bar{v}\rho) / f$$

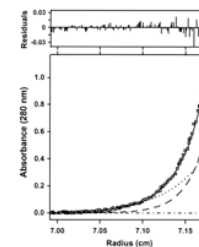
$$J_S + J_D = s \omega^2 C - D \frac{dC}{dr} = 0$$

$$\frac{dC}{dr} = \frac{s \omega^2}{D} C$$

$$\frac{d \ln C}{d(r^2)} = \frac{s \omega^2}{2D} = \frac{m(1 - \bar{v}\rho)}{2kT}$$

Molecular weight determined by fitting the gradient!

$$C(r) = C(r_0) e^{\frac{s \omega^2}{2D} (r^2 - r_0^2)} = C(r_0) e^{\frac{m(1 - \bar{v}\rho)}{2kT} (r^2 - r_0^2)}$$

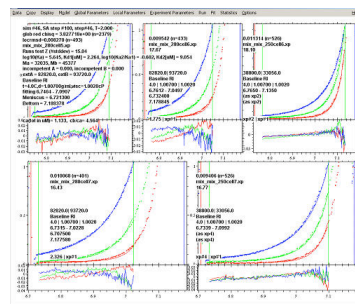
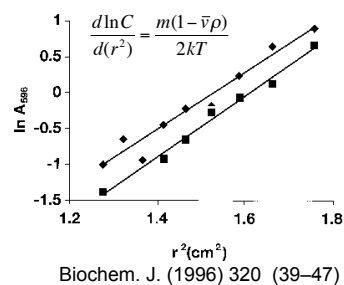


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Mass Determination by Sedimentation Eq.

- Obtained from fitting $C(r)$ $C(r)/C(r_0) = e^{\frac{m(1 - \bar{v}\rho)}{2kT} (r^2 - r_0^2)}$
- Very accurate and works over a large range (ω adjustable!)
- Noninteracting mixtures: multiexponential fitting
- Interacting mixtures: requires more complex functional forms for data fitting
 - Yields binding constants!



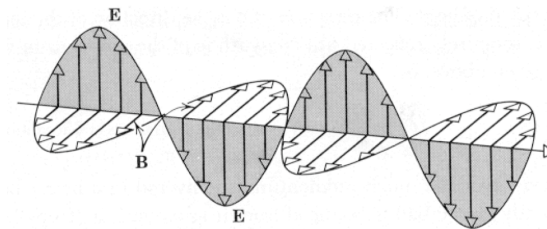
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Screenshot of SEDPHAT fitting analysis of protein-DNA binding

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Light Scattering

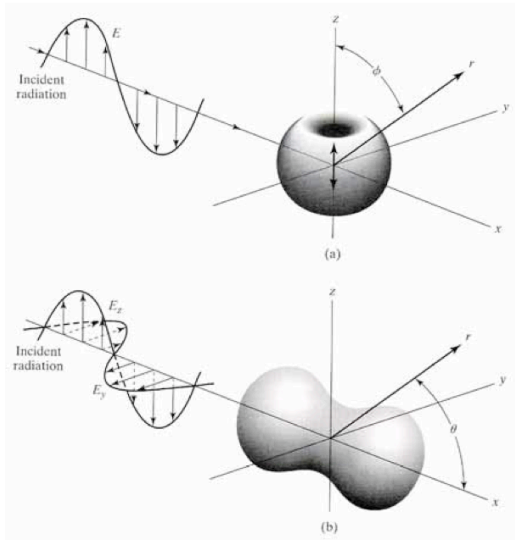
- Wave-particle duality
 - scattering tied to the wave nature, while photoelectric effect and absorption explained by the particle nature
 - Wave nature: oscillating electric and magnetic components (electric magnetic radiation, or EMR)
 - A few basic relations: $c = \lambda \nu$, $E = h \nu$



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Single-Particle Scattering



- Polarized light

$$\frac{i}{I_0} = \frac{16\pi^4\alpha^4}{r^2\lambda^4} \sin^2 \phi$$

- Unpolarized light

$$\frac{i}{I_0} = \frac{8\pi^4\alpha^4}{r^2\lambda^4} (1 + \cos^2 \theta)$$

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Scattering of Multiple Small Particles

- Rayleigh Scattering: if particles are much smaller than the wavelength (visible spectra: 380-750 nm)

- Shorter wavelength (blue light) scattered more!



- In the limit of low concentration:

$$\frac{i}{I_0} = N \frac{8\pi^4\alpha^4}{r^2\lambda^4} (1 + \cos^2 \theta)$$

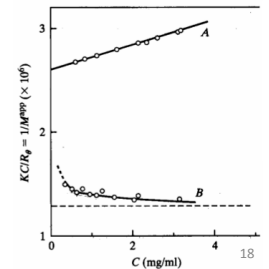
- Polarizability \$\alpha\$ is ultimately linked to molecular weight (M) and concentration (C):

$$R_\theta = \frac{i}{I_0} \frac{1 + \cos^2 \theta}{r^2} = KCM$$

- Non ideal solutions: B = 2nd virial coefficient

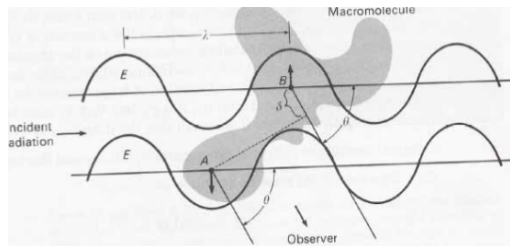
$$\frac{KC}{R_\theta} = \frac{1}{M} + 2BC + \dots$$

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Scattering of Larger Particles

- Mei Scattering: if particle size is greater than the wavelength
 - Such as viral particles; or x-ray and neutron scattering (small \$\lambda\$)
 - Must include inference between light scattered from all scattering points within the molecule
 - Provide information on mass, dimension and even internal structures!
 - Define so-called particle form factor \$P(\theta)\$
 - Estimation of M requires extrapolation to \$C=0\$ and \$\theta=0\$ (a practical limitation due to diffraction effects)



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

$$P(\theta) = 1 - \frac{h^2 R_G^2}{3} + \dots$$

$$R_G^2 = \frac{1}{2n^2} \sum_i \sum_j r_{ij}^2$$

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Dynamic Light Scattering

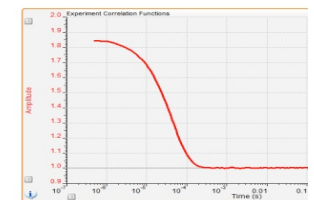
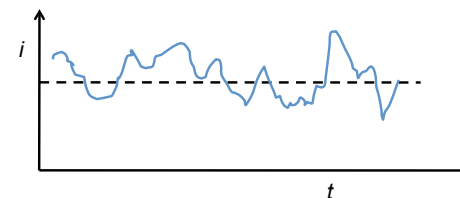
- Measure light scattering intensity as a function of time from a small volume (where local concentration fluctuates substantially due to Brownian motions)

- The larger D is, the faster scattering intensity will fluctuate

- Commonly analyzed with autocorrelation analysis:

$$g^{(2)}(\tau) = \langle i(t)i(t+\tau) \rangle / \langle i(t) \rangle^2$$

$$g^{(2)}(\tau) = 1 + ce^{-h^2 D \tau}, \text{ with } h = 4\pi\lambda^{-1} \sin(\theta/2)$$



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