Size and Shape of Macromolecules: Diffusion and Light Scattering

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

Copyright: Jianhan Chen

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 \( J = \text{mass (or mole) per unit area per second} \)

Thermodynamic Equilibrium

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

Maximum free energy
Minimum entropy

Minimum free energy
Maximum entropy

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 \( C(x=\infty) \).
1D Diffusion with Concentration Impulse

- \( C = C_0 \) within a thin slab of thickness \( \delta \)
- \( C = 0 \) at \( x = \pm \infty \) at all time
- Solution:
  \[
  C(x,t) = \frac{C_0 \delta}{2\sqrt{\pi Dt}} e^{-x^2/4Dt}
  \]

Diffusion and Molecular Size/Shape

- Einstein’s relation: \( D = \frac{kT}{f} \)
  - \( f \): 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)
  - \( \eta \): 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: \( r = \frac{kT}{6\pi\eta D} \)
  - Can be misleading for irregularly shaped molecules

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)

Friction Ratio

- The minimal friction coefficient is given by mass and partial specific volume:
  \[
  f_0 = 6\pi\eta V_p = 6\pi\eta \left( \frac{3mV_p^{1/3}}{4\pi} \right)
  \]
- 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)
Example: diffusion of DNA in Cells

A cytoplasm

Fluorescence

B nucleus

C cytoplasm

nucleus

Fluorescence


Moving Boundary Sedimentation

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

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)
  - \( F_b = -m_0 r\omega^2 \) (buoyancy)
  - \( F_d = -f \nu \) (friction)

\[
F_c + F_b + F_d = 0
\]

\[
mr\omega^2 - m_0 r\omega^2 - fv = 0
\]

\[
s = \frac{v}{r\omega^2} = \frac{m - m_0}{f} = \frac{m(1 - \nu \rho)}{f}
\]

\( \nu \): partial specific volume

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

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.

![Diagram of Sedimentation Equilibrium](image)

**Mass Determination by Sedimentation Eq.**

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

![Graph of Mass Determination by Sedimentation Eq.](image)

**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: $c = \lambda u \mathbf{E} = h \mathbf{u}$

![Diagram of Light Scattering](image)
Single-Particle Scattering

- Polarized light
  \[ \frac{i}{I_0} = \frac{16\pi^4 \alpha^4}{r^2 \lambda^4} \sin^2 \phi \]

- Unpolarized light
  \[ \frac{i}{I_0} = \frac{8\pi^4 \alpha^4}{r^2 \lambda^4} (1 + \cos^2 \theta) \]

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).
  \[ R_0 = \frac{i}{I_0} \frac{1 + \cos^2 \theta}{r^2} = KC \]
  - Non ideal solutions: \( B = 2nd \) viral coefficient
  \[ \frac{KC}{R_0} = \frac{1}{M} + 2BC + ... \]

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 pints 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)
  \[ \frac{KC}{R_0} = \frac{1}{P(\theta)} \left( \frac{1}{M} + 2BC \right) \]
  \[ P(\theta) = 1 - \frac{h^2 R_0^2}{3} + ... \]
  \[ R_0^2 = \frac{1}{2n^2} \sum_i \sum_j r_{ij}^2 \]

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:
  \[ g^{(2)}(\tau) = \langle (i(t)) (i(t + \tau)) \rangle / \langle (i(t))^2 \rangle \]
  \[ g^{(2)}(\tau) = 1 + ce^{-2\lambda h\tau}, \text{ with } h = 4\pi n\lambda^2 \sin(\theta/2) \]

\[ \text{g}^{(2)}(\text{tau}) \text{ vs. tau} \]

\[ \text{Dynamic light scattering intensity} \]