Transport Processes – Diffusion:

$$D = \frac{RT}{Nf} \quad and \quad f = 6\pi\eta r$$

$$r = \frac{RT}{N 6\pi\eta D}$$

For a spherical particle, we can predict the frictional coefficient with the Stokes - Einstein relationship.

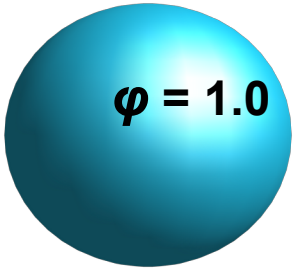
For any molecule, the measured frictional coefficient can then be used to calculate the corresponding radius. This is called the Stokes radius. This is the radius of a hypothetical sphere that has the same frictional coefficient as the molecule. The Stokes radius has a volume that is larger or equal to the volume of the actual molecule. Most macromolecules are NOT spherical.

If the volume is known, the radius r_0 of a hypothetical minimal sphere can be calculated, as well as its frictional coefficient, f_0.

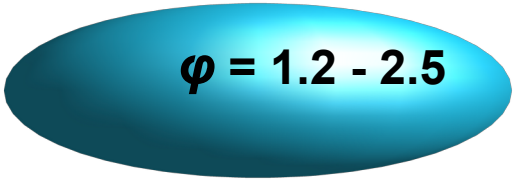
The ratio of $\varphi = f/f_0$ is called the frictional ratio, and defines the anisotropy of the molecule.

Frictional Ratio

The frictional ratio $f/f_0 = \varphi$ is a convenient way to parameterize the diffusion coefficient and the shape of a molecule .



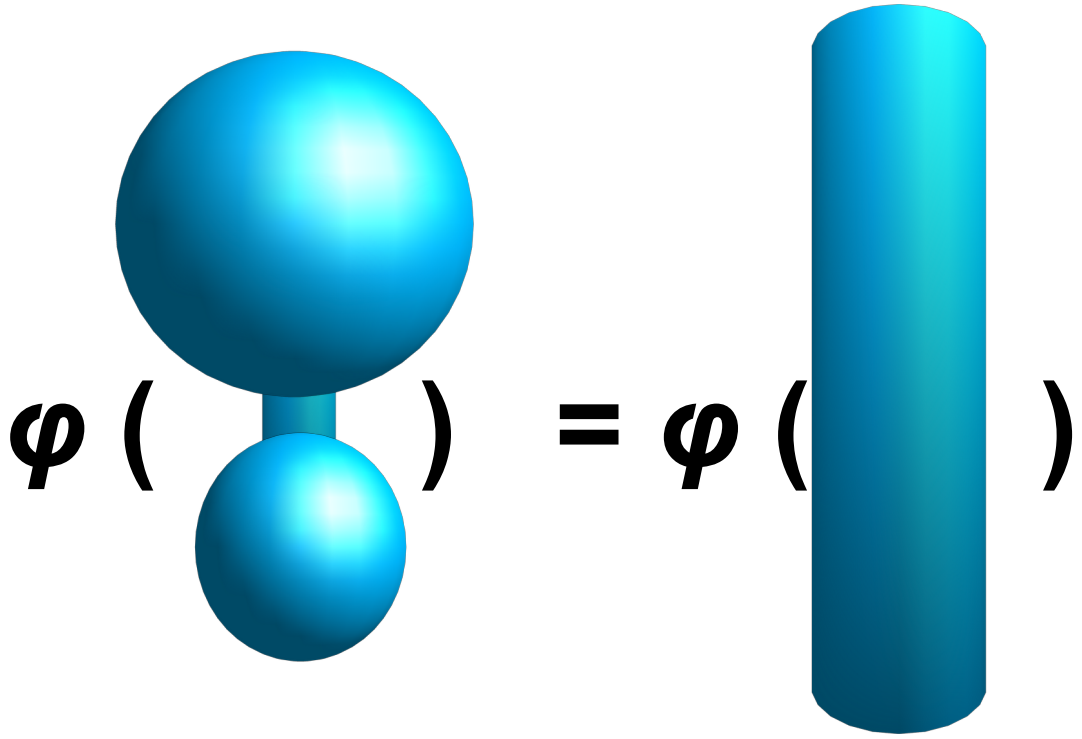
The frictional ratio φ is 1.0 for a sphere since $f = f_0$ and hence φ has a convenient lower limit



$1.0 \leq \varphi \leq 4.0$ for most proteins, higher for rod-shaped, disordered and unfolded proteins, DNA, fibrils and aggregates or linear molecules

$\varphi > 3$

Frictional Ratio



φ may be the same for different shapes, we cannot distinguish them by AUC, we can only measure the anisotropy!

Transport Processes – Sedimentation

Fb (buoyancy) = $\omega^2 r\, m_s$

Fd (viscous drag) = $f\, v$

Fc (centrifugal force) = $\omega^2 r\, m$

Substitute the mass of the solvent, m_s, with the mass of the solute, m

$$m_s = m\bar{v}\rho, \quad Fb = \omega^2 r m \bar{v}\rho$$

Rearrange the force equation:
Fc - Fb - Fd = 0 **and substitute**

$$\omega^2 r\, m - \omega^2 r\, m \bar{v}\rho = fv$$

Place molecular parameter on one side and experimental parameters on the other

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

Put into molar units by multiplying with Avogadro's number, N

$$\frac{M\left(1-\bar{v}\rho\right)}{Nf} = \frac{v}{\omega^2 r} = s$$

Transport Processes – Fundamental Equations:

We have:

$$s = \frac{M\left(1 - \bar{v}\ \rho\right)}{N\ f} \qquad and \qquad D = \frac{RT}{N\ f}$$

Svedberg equation:

$$\frac{s}{D} = \frac{M\left(1 - \bar{v}\,\rho\right)}{RT}$$

Stokes-Einstein:

$$f = 6\,\pi\,\eta\,r$$

s depends on $\bar{v}$ and ρ

s and D are inversely proportional to f

f depends on the viscosity

Transport Processes – Viscosity and Density Corrections

The density and also the viscosity of the solvent affect the sedimentation and diffusion of the particle in solution, so the measured values need to be corrected to standard conditions. Moreover, temperature and buffer composition affect the solvent density and viscosity, so they need to be considered. To correct for density and viscosity, use these formulas:

$$s_{20,W} = s_{T,B} \frac{\left(1-\bar{v}\rho\right)_{20,W} \eta_{T,B}}{\left(1-\bar{v}\rho\right)_{T,B} \eta_{20,W}}$$

$$D_{20,W} = D_{T,B} \frac{293.15\ \eta_{T,B}}{T\ \eta_{20,W}}$$

Transport Processes – Partial Specific Volume and Buoyancy

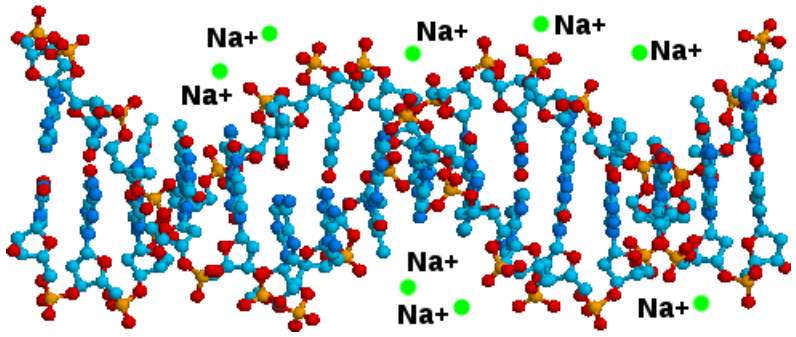
$$s = \frac{M(1 - \bar{v}\rho)}{N f}$$

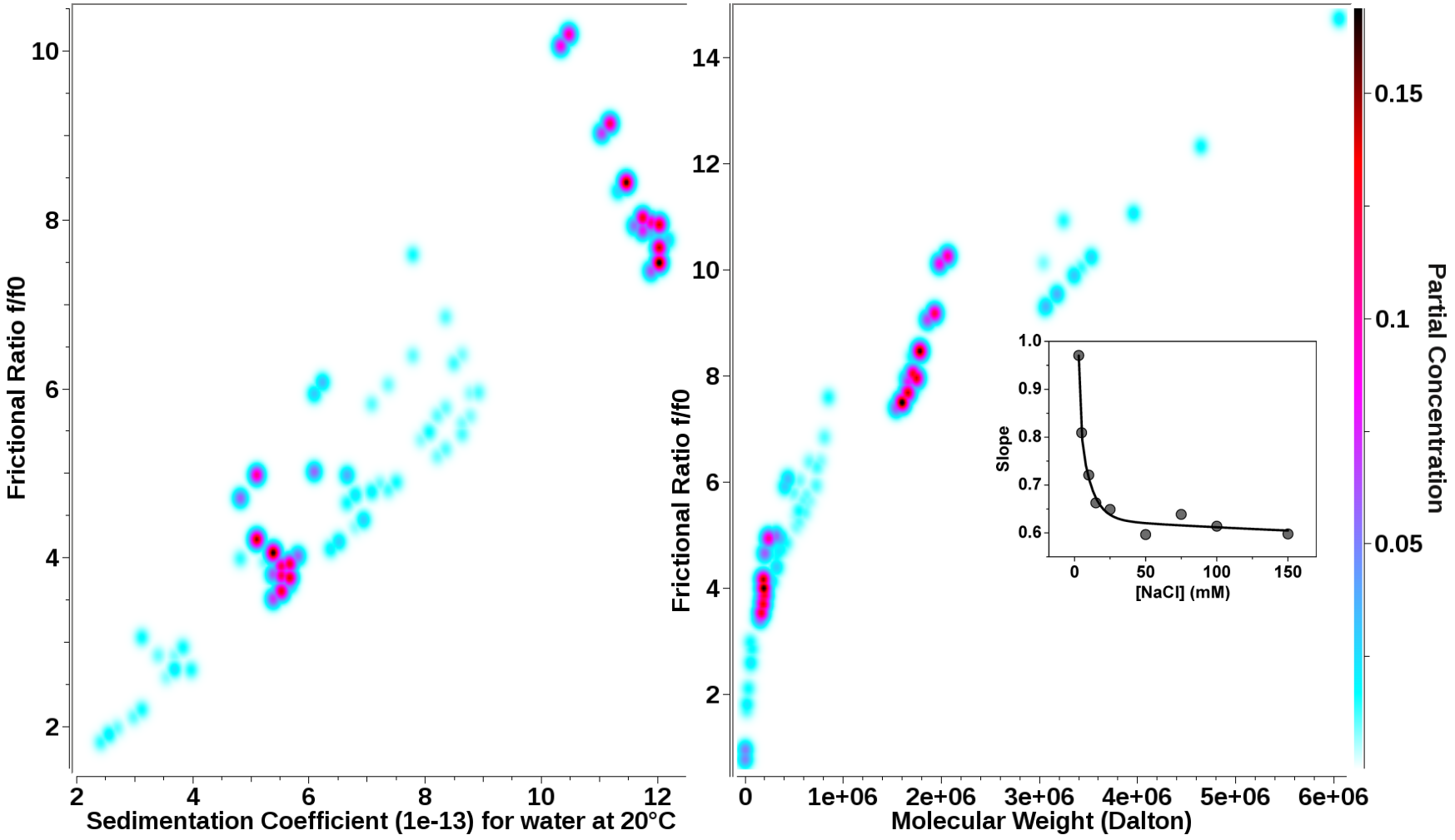
What effect does the *partial specific volume have on sedimentation*?

The partial specific volume is the volume that includes the hydration of the sedimenting particle, plus any ions bound. If the hydration shell is large (e.g., a charged nucleic acid or protein in low salt), the vbar will increase compared to the anhydrous molecule, while its density decreases. However, water molecules are only bound transiently, so they are not considered in the molecular weight of the macromolecule. Hence, given a measurement for *s*, *f* and *ρ*, the vbar value is the value that makes the molecular weight come out "correctly", i.e., for the value expected from sequence or mass spectrometry.

The value of vbar can be very sensitive to solution conditions.

Transport Processes – Partial Specific Volume and Buoyancy





Transport Processes – Sedimentation and Diffusion

$$\left(\frac{\partial C}{\partial t}\right)_r = \frac{-1}{r}\frac{\partial}{\partial r}\left[s\omega^2 r^2 C - D r \frac{\partial C}{\partial r}\right]_t$$

Concentration **Sedimentation** **Diffusion**

$$f = \frac{RT}{N D}$$

$$M = \frac{s N f}{1-\bar{\nu}\rho}$$

$$V = \frac{M \bar{\nu}}{N}$$

$$r_0 = \left(\frac{3V}{4\pi}\right)^{1/3}$$

$$f_0 = 6\pi\eta r_0$$

$$\phi = \frac{f}{f_0}$$

Transport Processes – Homework

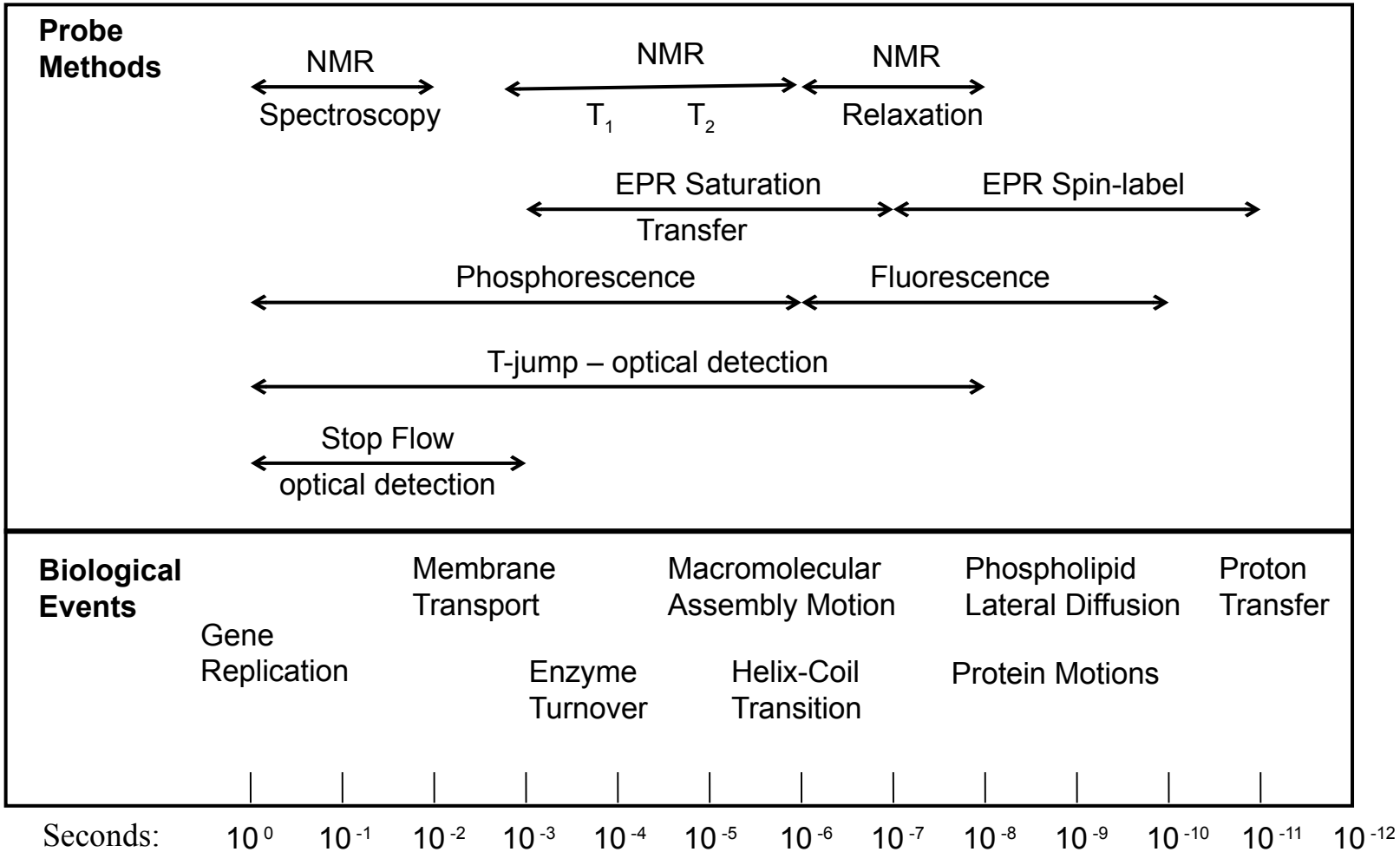
1. A molecule has a sedimentation coefficient of 4.2e-13 S and a diffusion coefficient of 7.5e-7 cm^2/sec, and a partial specific volume of 0.72 ml/g. Find the molar mass and the frictional ratio (anisotropy), and the Stokes radius. Assume a buffer density of 1 g/ml, and a viscosity of 0.01 poise. The gas constant is 8.314e7 erg/(mol*K). Show all work and all units.

2. What is the value of the molar mass and the value of the f/f_0 if the partial specific volume is 0.55?

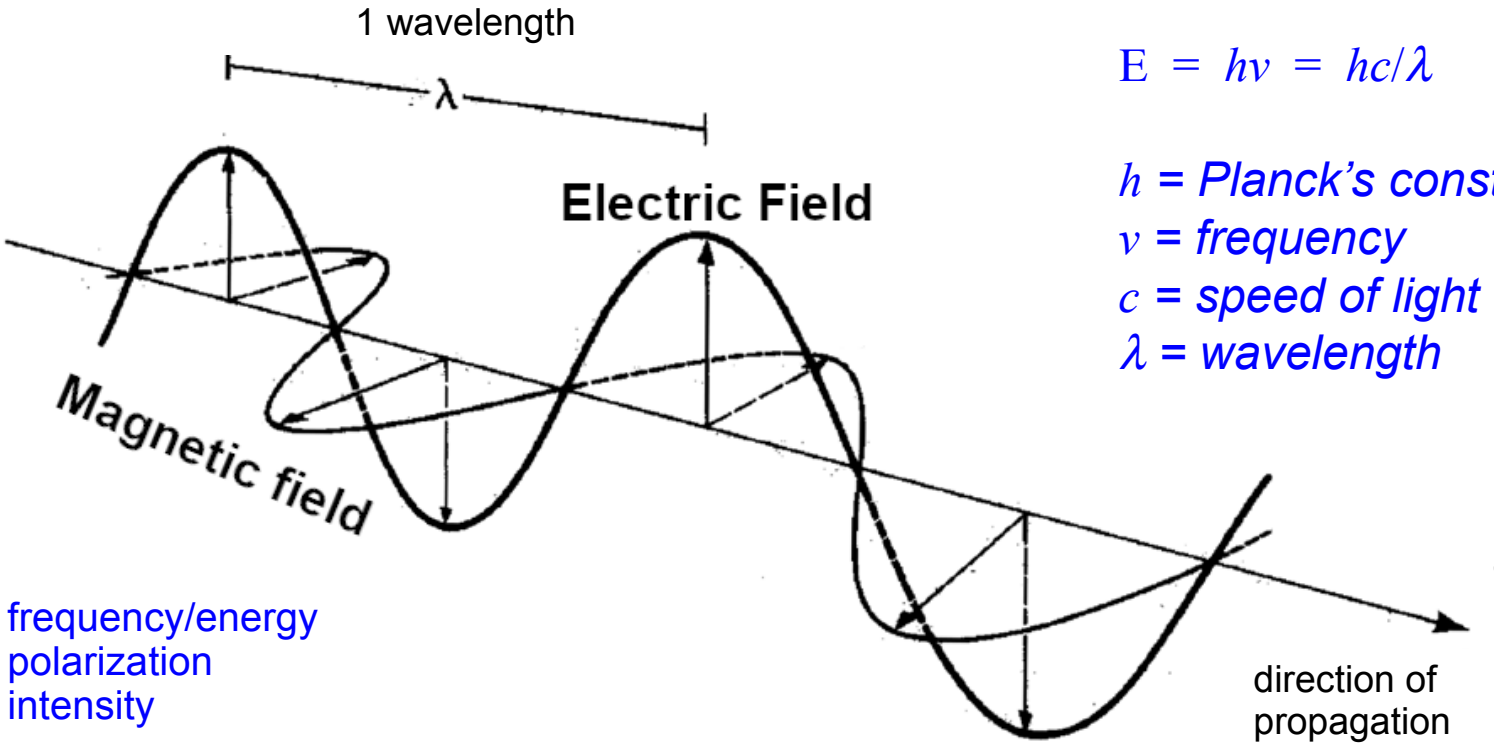
3. Explain for each of these cases:
a) a molecule changes conformation and unfolds from globular to extended.
b) the density of the solvent increases
c) the viscosity of the solvent decreases

Does the sedimentation coefficient increase, decrease or stay the same? Does the diffusion coefficient increase, decrease or stay the same? Does the frictional coefficient increase, decrease or stay the same? Does the molar mass increase, decrease or stay the same? Explain your answer.

Spectroscopy



Electromagnetic Radiation



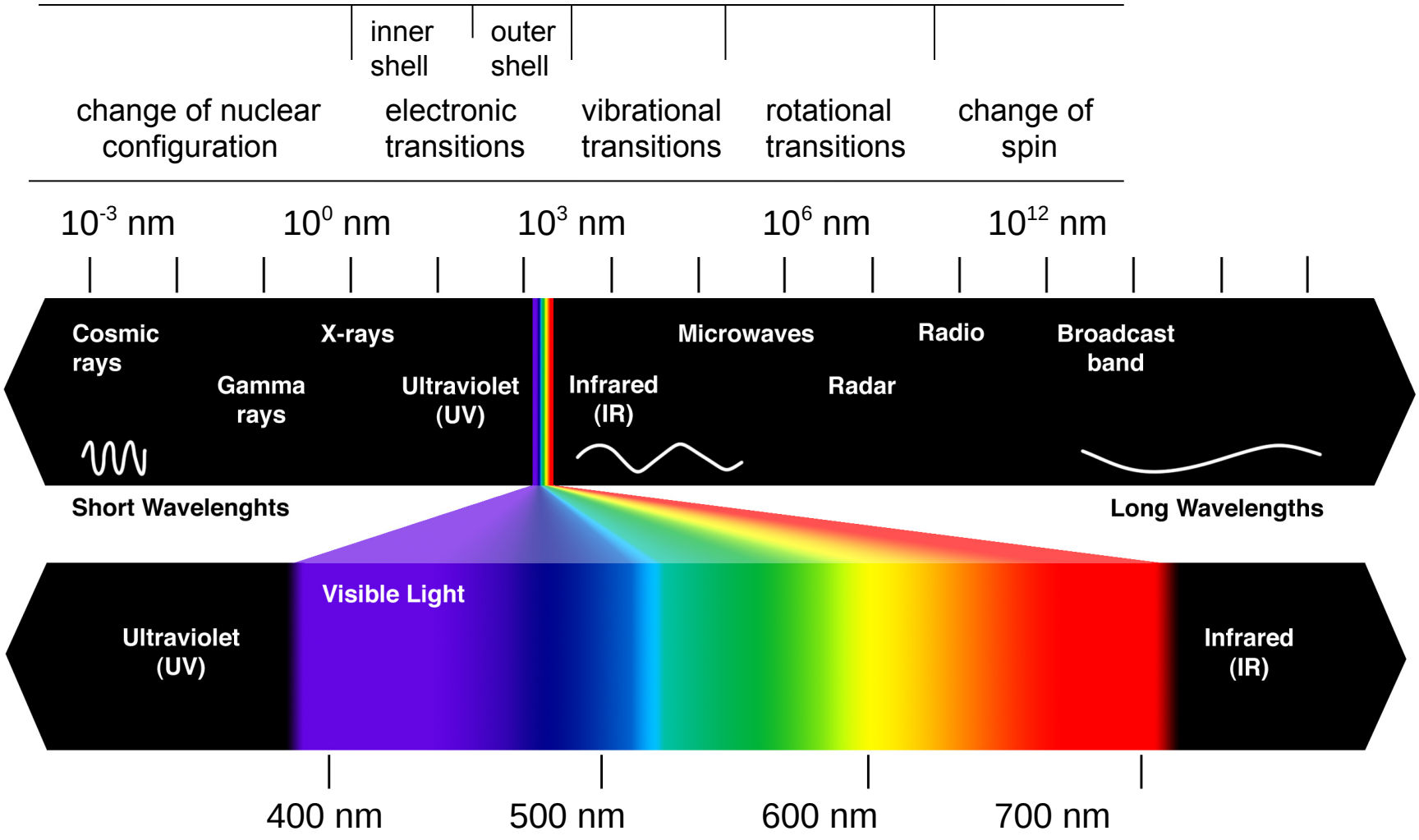
Planck's Law:

$$E = h\nu = hc/\lambda$$

h = Planck's constant
ν = frequency
c = speed of light
λ = wavelength

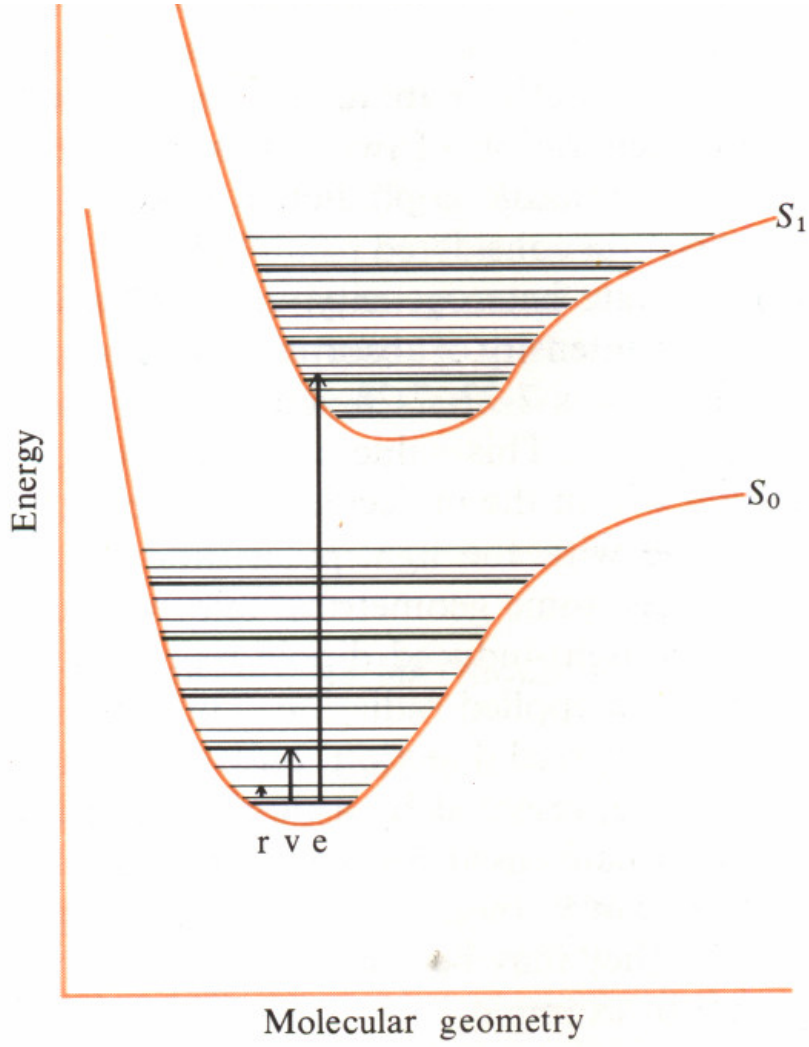
- frequency/energy
- polarization
- intensity

Spectroscopy



Spectroscopy

- Energy levels of a small molecule. Transitions occur between electronic states (e), vibrational (v) and rotational (r) levels. There can be many energy levels making absorbance bands broad.
- Energy spacing between electronic states is around 80 kcal/mol → higher than room temperature, only possible by absorbing light
- Energy spacing between vibrational leves is about 10 kcal/mole, higher than thermal energies, so only the ground states are normally populated
- Energy spacing between rotational levels is about 1 kcal/mol, sufficiently small for multiple levels to be populated at room temperature.
- The energy spacing between vibrational and electronic states is large enough that molecules are mostly in their ground states at room temperature.

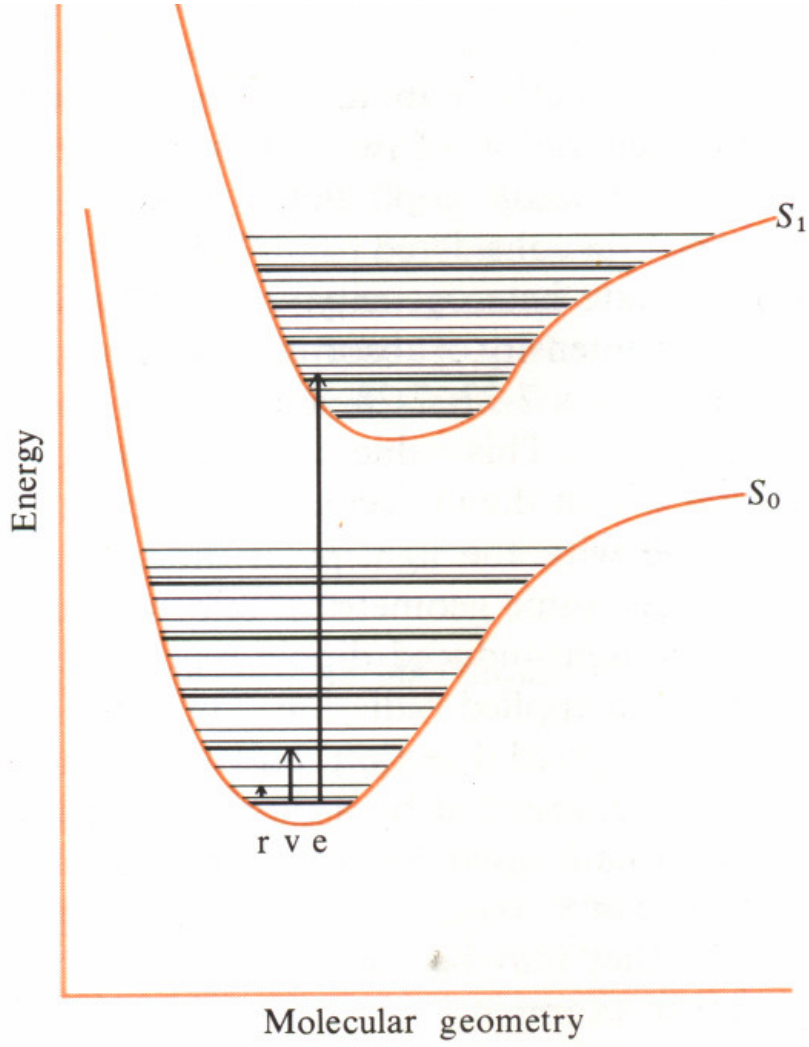


Spectroscopy

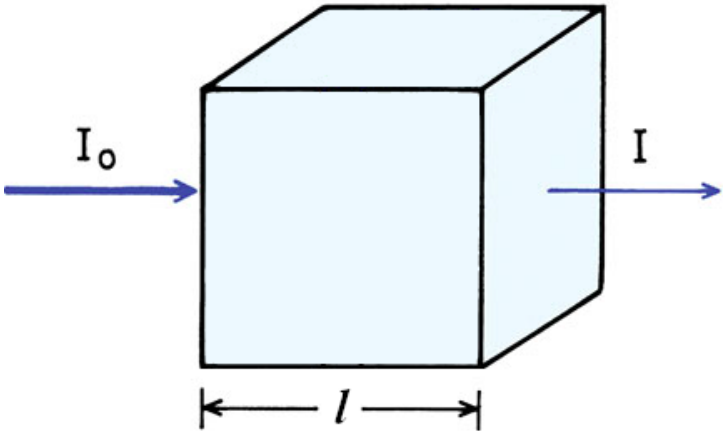
- The Franck-Condon principle states that electronic transitions that involve different vibrational levels have the highest probability of occurring when the vibrational levels overlap in the momentum and nuclear positions, i.e., no displacement of nuclei.

Energies required for various transitions:

- Electronic transitions: UV/visible light
- Vibrational transitions: Infrared light
- Rotational transitions: microwave radiation, not suitable for absorbance spectroscopy, they will be considered with nuclear magnetic resonance.



Beer-Lambert Law (of absorbance)



Intensity: $I_A = I_0 - I$

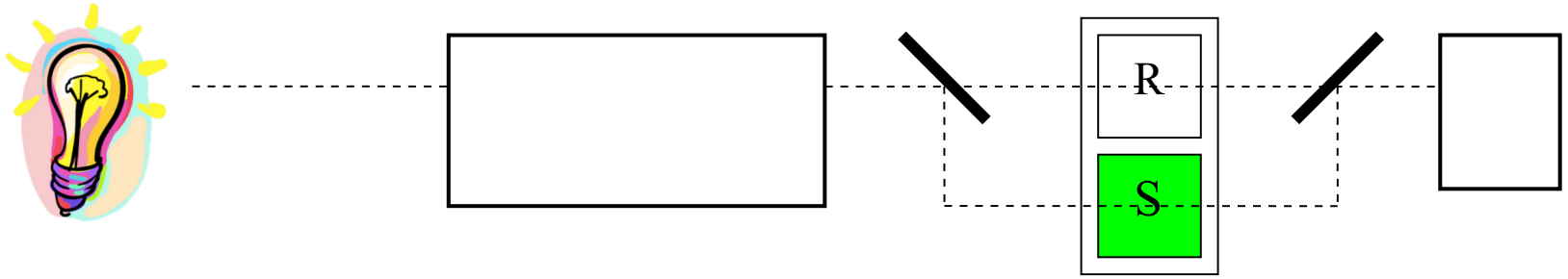
Transmittance: $T = I/I_0$

Absorbance $A = - \log T$

Incoming intensity, I_0, outgoing intensity, I, and absorbed intensity, I_A, in a cell of length l. Consider the light intensity absorbed (I_A) in a volume of thickness l and cross-sectional area *A*. Each molecule, randomly oriented in solution, contributes to the observed absorption of light based on its cross-sectional area, and the probability of absorbing a photon of the energy from the incident light over a given time period.

Absorption

Measured by: single or double beam spectrophotometer



light source (xenon or deuterium)

monochromator

cuvette holder

detector

Absorption Spectroscopy

BEER LAMBERT LAW:

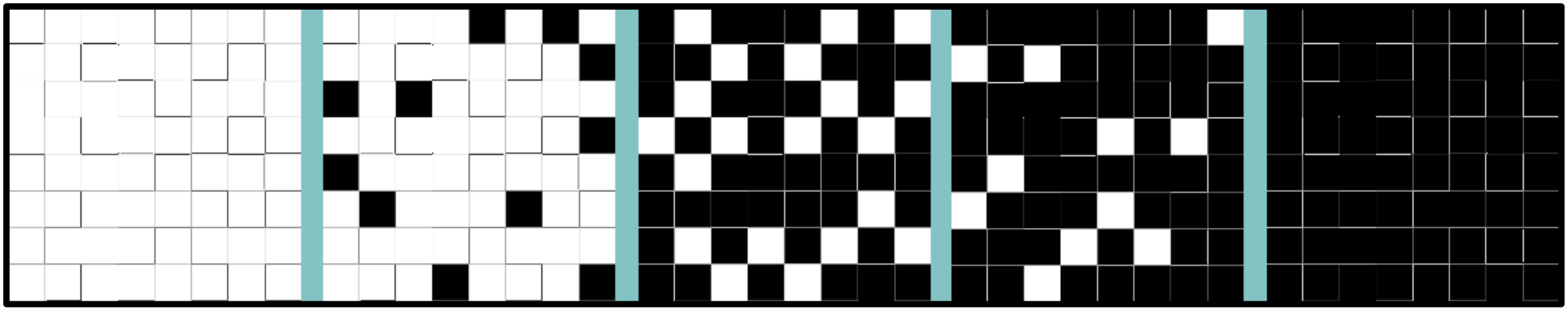
$$\frac{dI}{dl} = I C \epsilon' \qquad \ln\left(\frac{I_0}{I}\right) = C \epsilon' l$$

$$A(\lambda) = log_{10}\left(\frac{I_0}{I}\right) = C \epsilon(\lambda) l$$

A **is the absorbance or optical density (OD) of the sample at a particular wavelength. This measure can be used to determine the concentration of sample**

The extinction coefficient ε is a function of wavelength and independent of concentration, where ε = ε'/2.303. ε is typically expressed in OD/mol for a 1 cm pathlength.

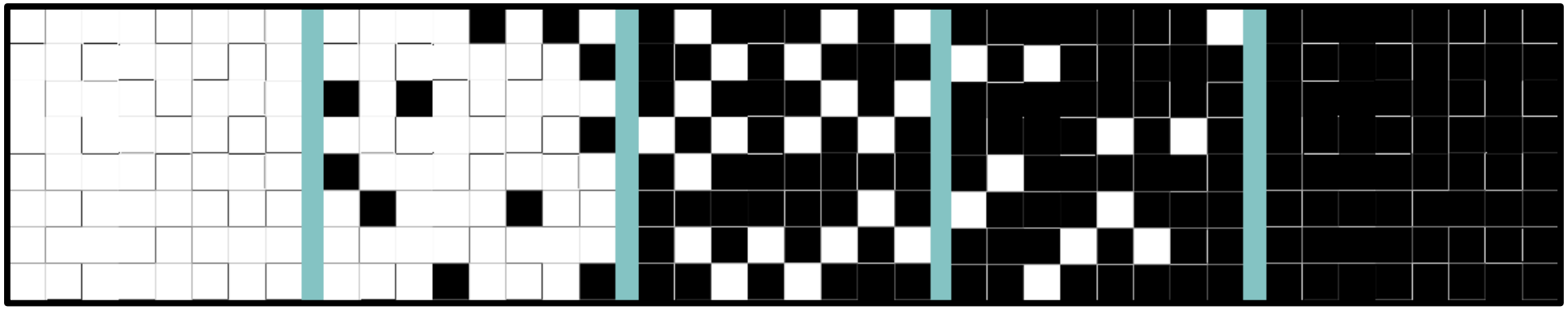
Spectroscopy

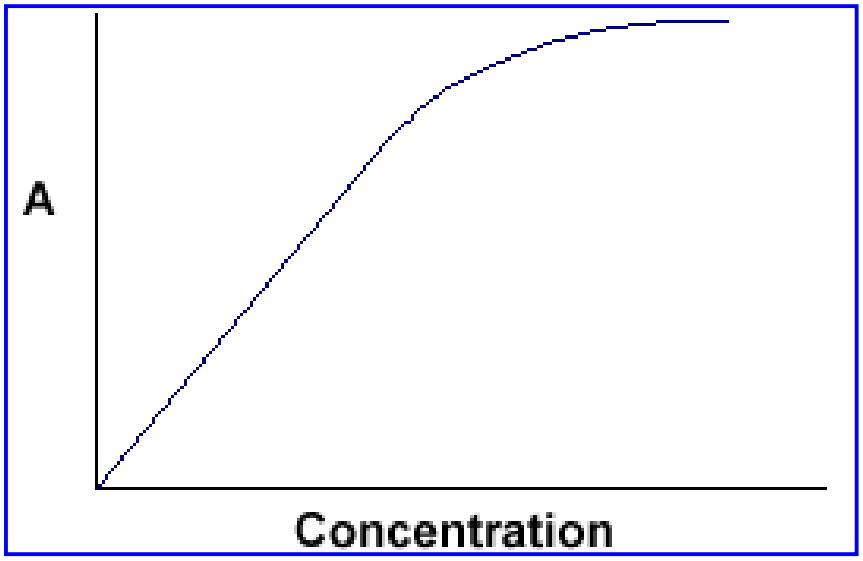


The absorbance is proportional to the number of the molecules (concentration C) and the "darkness" (molar absorptivity ε) of the molecules. The number of the molecules interfering with the light beam increases with the thickness of the volume element l

$$\mathrm{A} = -\log\left(I/I_0\right) = \varepsilon\, C\, l = \mathrm{OD}$$

C is the concentration, [M]; ε is the molar extinction coefficient, [M^{-1} cm^{-1}]; l is the pathlength (in cm), and OD is the optical density, also called absorbance.



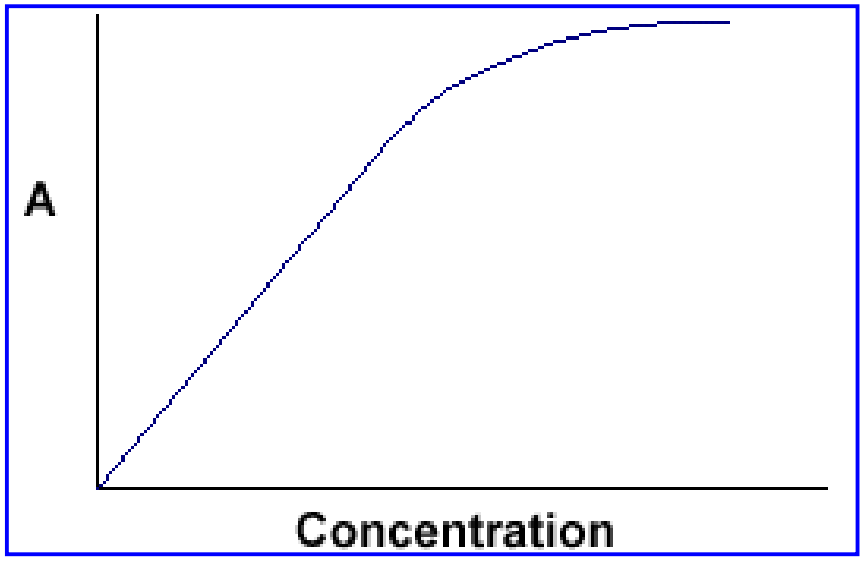


Relationship between Absorbance and Concentration

Why is this plot not linear for the entire range?

At some point the absorbance is so high that not sufficient light passes through to the detector, and linearity is no longer satisfied.

Relationship between Absorbance and Concentration



The absorbance at which UV-visible spectrophotometer becomes non-linear depends on the following factors:

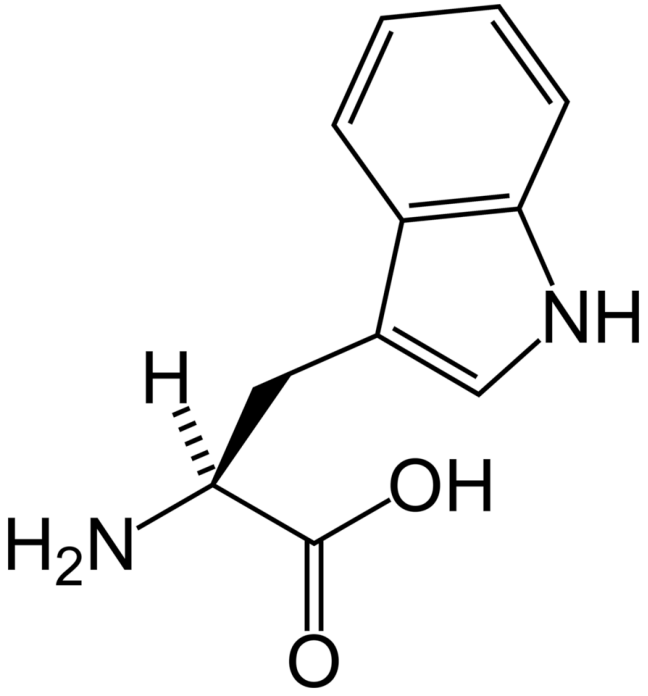
1. Concentration of the analyte
2. Lamp intensity at the measured wavelength
3. Extinction coefficient of the analyte at measured wavelength
4. Sensitivity of detector at the measured wavelength

To measure C accurately, always measure around 0.2-0.8 OD, which corresponds to a transmission of ~63% - 16% T

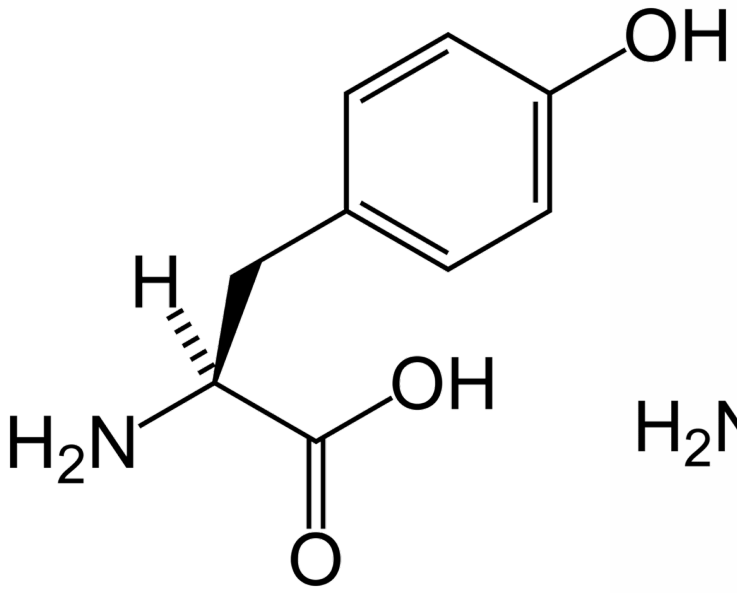
Deviations from the Beer-Lambert Law – watch out for these:

- **Exceeding the dynamic range of the detector**
- **Chromatic shifts due to macromolecular interaction**
- **Chromatic shifts due to solvent variation**
- **Chromatic shifts due to conformational changes in analyte**
- **Improper blanking of the reference absorbance**
- **Dirty cuvette in the reference beam**
- **Dilutions with an absorbing buffer different from the solvent**
- **Analyte is contaminated with an absorbing molecule**

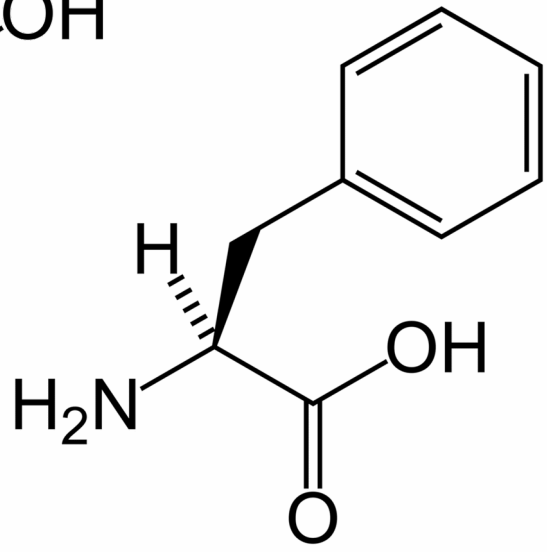
Aromatic Amino Acids



Trp (W)



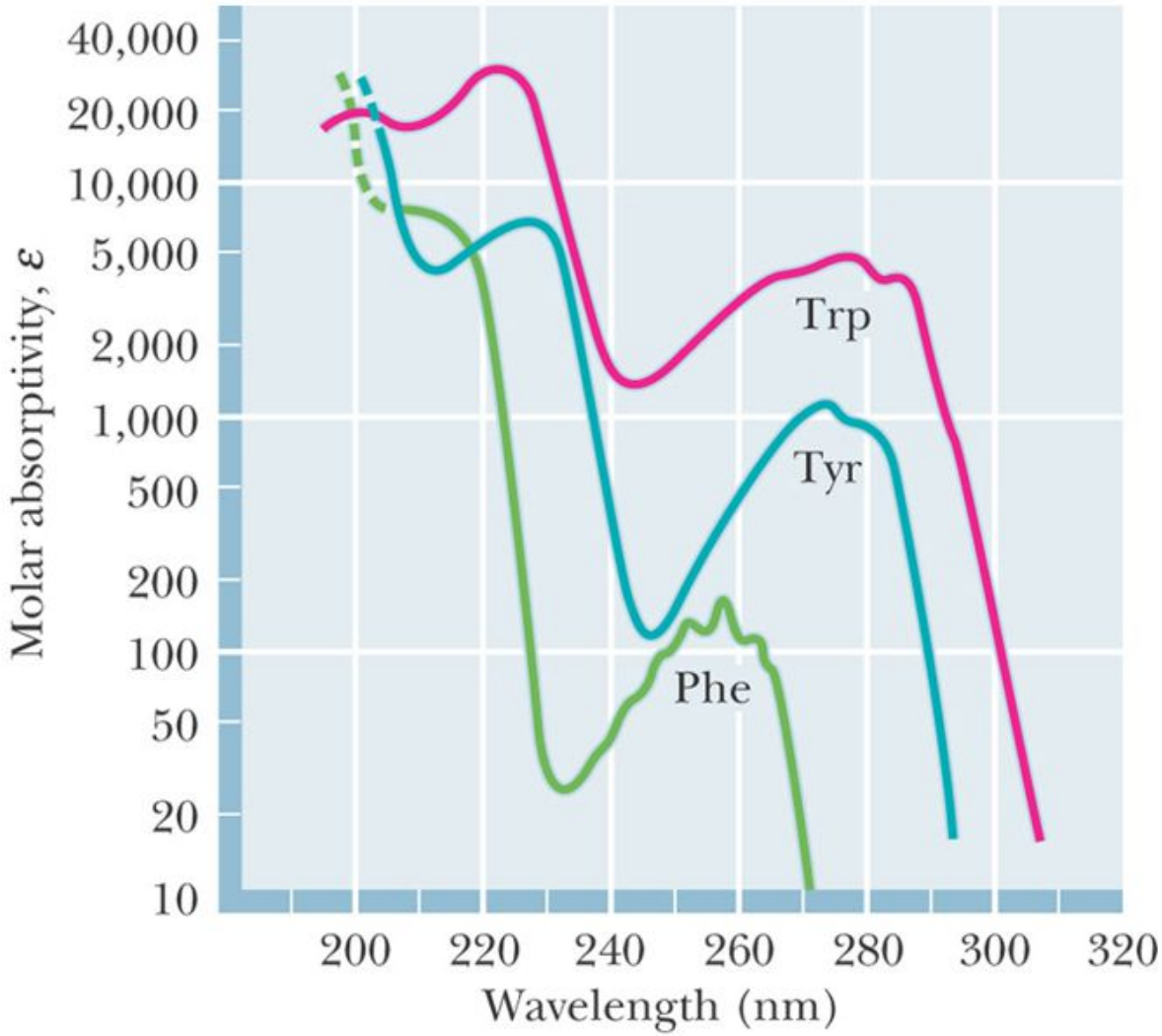
Tyr (Y)



Phe (F)

Spectroscopy

The ultraviolet absorption spectra of the aromatic amino acids at pH 6. (*From Wetlaufer, D.B., 1962. Ultraviolet spectra of proteins and amino acids.* Advances in Protein Chemistry **17**:*303–390.*)



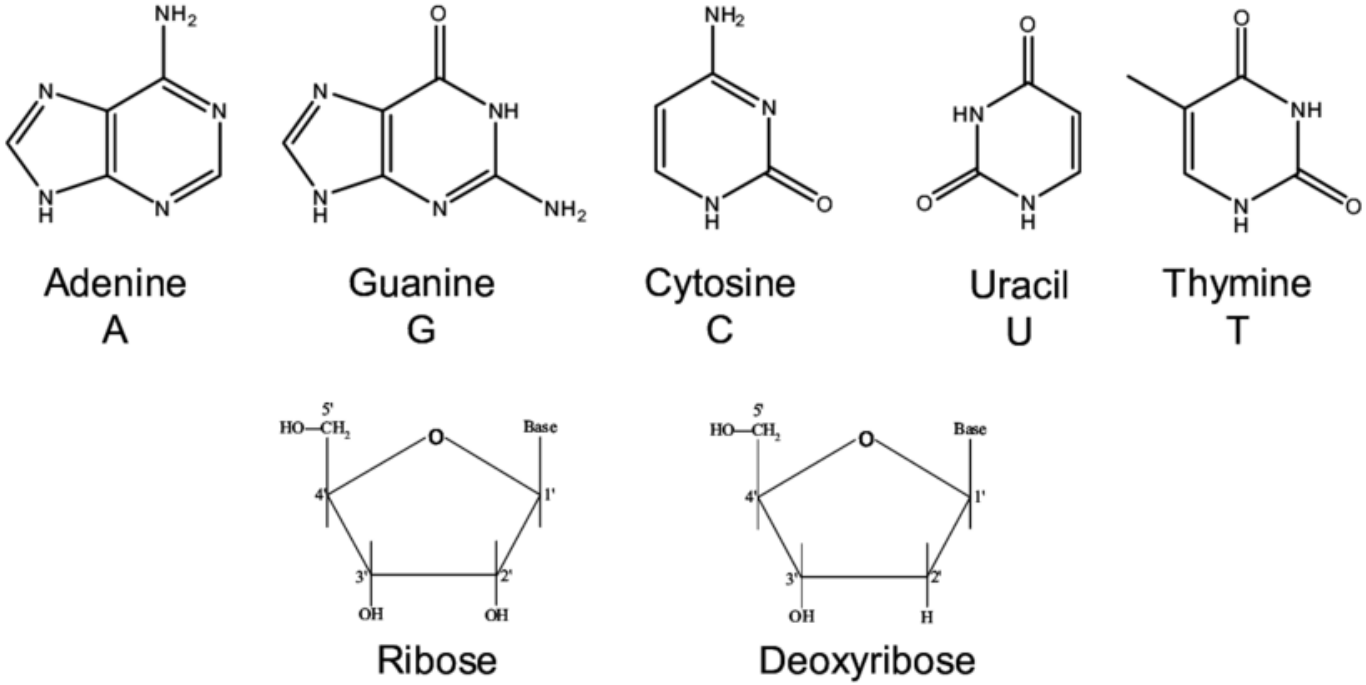


Figure 1.1. Structures of nucleic acid constituents

Absorption Spectra of the Nucleic Acids