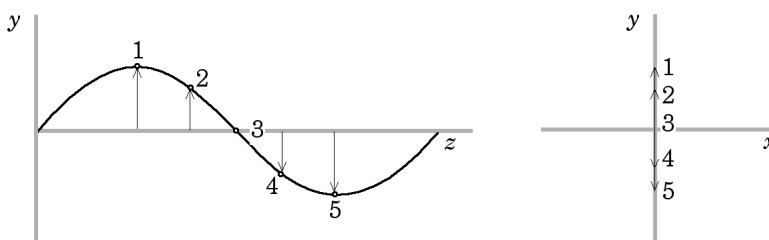


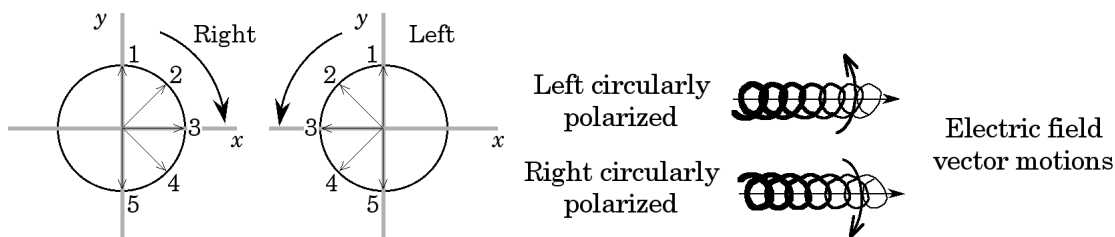
Optical Activity and Circular Dichroism

Absorbance phenomena are the result of the interaction of light with molecules. These phenomena depend on the structure of the molecule and its electronic configuration. If the electromagnetic radiation is polarized, asymmetrical molecules frequently interact with light in ways that symmetrical molecules do not.

Consider plane-polarized light moving in the z direction. The electric field vector of the radiation oscillates in the y direction. Looking at the light down the z -axis reveals that the electric field vectors are all aligned in the yz plane, where the numbers correspond to positions along the z -axis.



Another way of looking at the light is to consider the plane-polarized light as being comprised of equal amounts of left and right circularly polarized light. The magnitude of the electric field vector is constant; instead, for each type of polarized light, the direction of the vector changes. Both the left and right circularly polarized light have electric field vectors that move in a circular screw fashion along the z -axis. If the amount of left and right circularly polarized light is the same, the electric field vector sum will appear to be plane-polarized.



If the amount of left and right polarized light is not the same, the light will be elliptically polarized. Ellipticity is the arc tangent of the ratio between the minor axis and the major axis. In most cases, the ellipse major axis is at an angle to the original plane of the light. If the absorbance creating the ellipticity is very small, the effect will be a rotation of the plane of the light. Optical rotation can be measured at a fixed wavelength (e.g. the sodium D line, 589.6 nm). Alternatively, the optical rotation can be measured as a function of wavelength: optical rotatory dispersion (ORD).

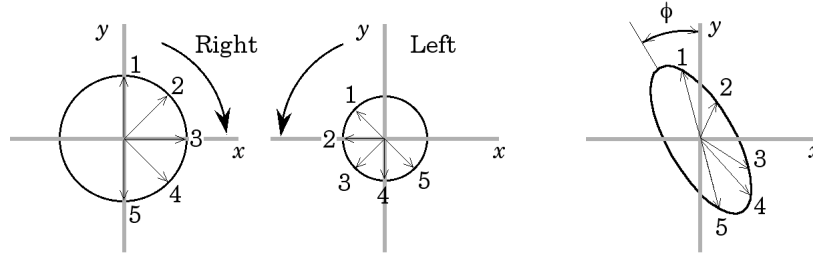
The electric field vector is actually composed of two vectors:

$$\mathbf{E}_R = \frac{1}{2} (\hat{\mathbf{i}}E_0 \sin \omega t + \hat{\mathbf{j}}E_0 \cos \omega t)$$

$$\mathbf{E}_L = \frac{1}{2}(\hat{\mathbf{i}}E_0 \sin \omega t - \hat{\mathbf{j}}E_0 \cos \omega t)$$

where $\hat{\mathbf{i}}$ is the unit vector in the x direction, and $\hat{\mathbf{j}}$ is the unit vector in the y direction.

After interacting with a chiral molecule, the amount of left and right polarized light can be different. The result of the interaction is a differential absorbance of the left and right circularly polarized light.



$$\text{Optical rotation} = [\alpha] = \frac{180l(n_L - n_R)}{d} \text{ degrees}$$

where n is the index of refraction. The difference in index of refraction for left and right polarized light is called **circular birefringence**; technical difficulties in the measurement of circular birefringence mean that optical rotation is more frequently measured.

$$\text{Ellipticity} = [\theta] = \frac{2.303(A_L - A_R)180}{4d} \text{ degrees}$$

Circular dichroism (CD) is measured by detecting the differential absorption of left and right circularly polarized light. This is typically performed by sequentially comparing the absorbance of the left and right polarized light. The data are usually converted to ellipticity using the above equation.

$$\begin{aligned} \text{Molar ellipticity} = [\theta] &= \frac{100\theta}{Cl} \\ \Delta\epsilon &= \epsilon_L - \epsilon_R \\ [\theta] &= 3300\Delta\epsilon \end{aligned}$$

CD and ORD can be interconverted using a complex set of integrals called the Kronig-Kramers transforms, one of which is shown below.

$$[\theta(\omega)] = -\frac{2\epsilon}{\omega} \int_0^{\infty} \frac{[\epsilon(\omega')]}{\omega'^2 - \omega^2} d\omega'$$

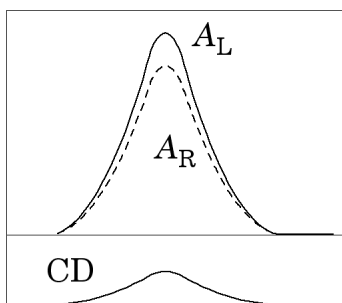
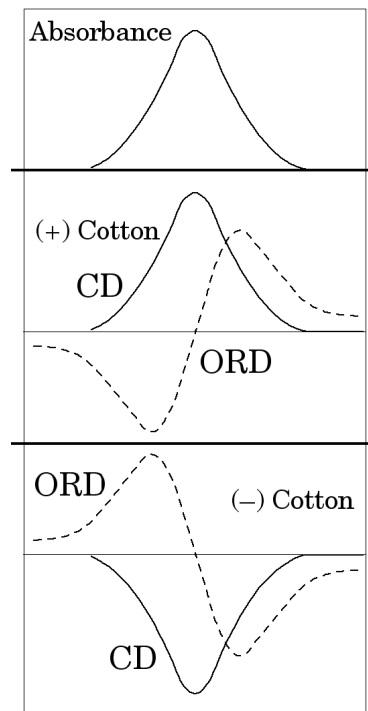
Because the two spectra can be mathematically interconverted, only one spectrum is usually measured. CD is usually measured directly, because CD spectra are less complex and more easily interpreted.

For a single allowed transition, the CD and absorbance spectra have the same shape. In contrast, the ORD spectrum is more complex. In addition, the spectrum has non-zero values at wavelengths distant from the center of the spectrum. The Drude equation shows that the ORD signal approaches zero asymptotically:

$$[\alpha(\lambda)] = \frac{A_0}{(\lambda^2 - \lambda_0^2)}$$

where A_0 is a constant related to the CD spectrum, and λ_0 is the crossover wavelength. Thus, many organic molecules with no absorbance bands in the visible still have optical rotation signals at 589.6 nm (the sodium D line used in most polarimeters).

The diagram to the right does not show the CD spectrum on the same scale as the absorbance spectrum. The diagram below shows the relative signal magnitudes more clearly.



For CD, three conditions must hold:

- 1) the molecule must contain a standard absorbance chromophore;
- 2) the molecule must contain a source of asymmetry (the chromophore may not be asymmetrical, but the overall molecule must be);
- 3) the chromophore and the asymmetry source must be in physical proximity.

Theory

Rotational strength for the $0 \rightarrow a$ transition is given by:

$$R_{0a} = \frac{3hc}{8\pi^3 N_0} \int \frac{[\alpha(\lambda)]}{\lambda} d\lambda$$

Rotational strength is a property equivalent to dipole strength for an absorbance transition.

Rotational strength, like dipole strength, is the product of wavefunction overlap integrals. In the case of rotational strength, however, two operators are necessary:

$$R_{0a} = \text{imaginary}(\langle \Psi_0 | \underline{\mu} | \Psi_a \rangle \cdot \langle \Psi_a | \underline{m} | \Psi_0 \rangle)$$

where $\underline{\mu}$ is the electric dipole operator and \underline{m} is the magnetic dipole operator. R_{0a} corresponds to the imaginary part of the expression. If $\langle \Psi_0 | \underline{\mu} | \Psi_a \rangle = 0$ (in other words, if absorbance is zero), R is also zero. Thus, absorbance is necessary for a CD signal.

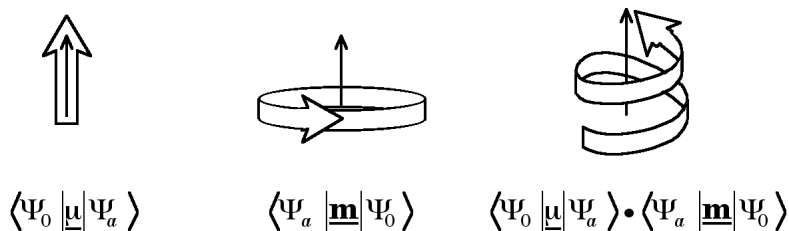
For each electron:

$$\underline{m} = \frac{e}{2mc} (\mathbf{r} \times \mathbf{p})$$

where e is the charge on the electron, m is the mass of the electron, and \mathbf{r} and \mathbf{p} are the momentum and position operators. $(\mathbf{r} \times \mathbf{p})$ is orbital angular momentum. (Recall that dot products of vectors give scalar values, while cross products give vector quantities.)

Because $\mathbf{p} = \frac{h}{i} \nabla$, the magnetic dipole operator is imaginary.

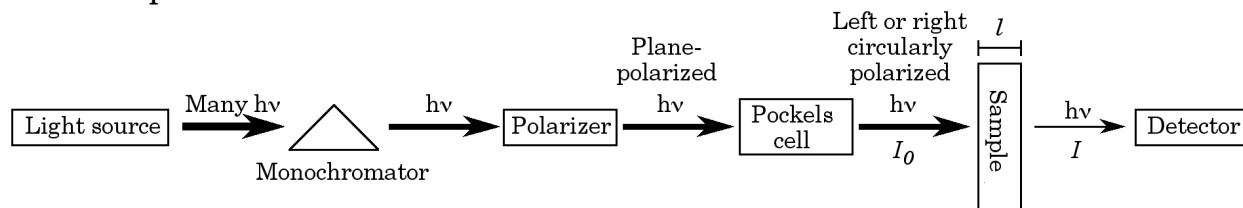
While the electric dipole operator results in a charge separation, the magnetic dipole operator results in charge circulation. Combining the two results in both a charge separation and circulation along a screw axis. If the molecule is symmetrical, there will be no preferred direction for the charge circulation, and R will be zero. In addition, this explains the strong CD signal observed for helices, in which the helical structure acts as a guide for the charge circulation. If $\underline{\mu}$ and \underline{m} are orthogonal, R will also be zero.



Although the CD spectrum cannot be stronger than the absorbance spectrum (if, in the equation $\Delta\epsilon = \epsilon_L - \epsilon_R$, one of the extinction coefficients is zero), in some cases, the apparent transition strength increases, due to larger CD signals than those of nearby bands. Thus, in some conditions, the $n \rightarrow \pi^*$ transition of the peptide bond can have a strong CD signal, in spite of being a weak absorption signal. This actually illustrates the fact that CD signals are normally quite weak. In nearly all cases, $\epsilon < 1^\circ$.

Instruments

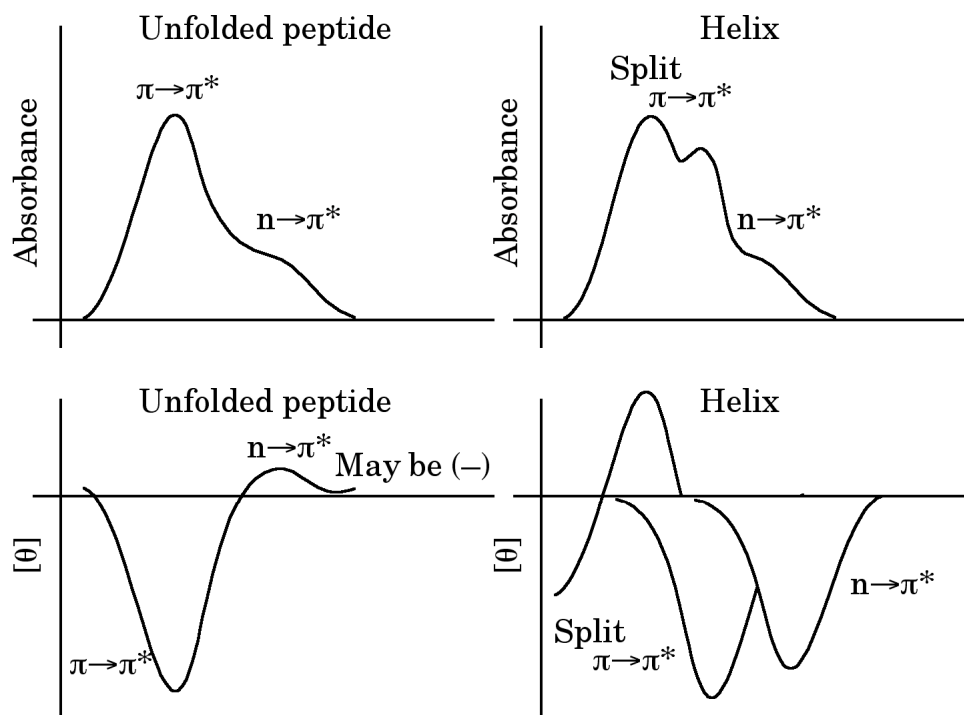
A circular dichroism spectropolarimeter looks fairly similar to a spectrophotometer, with two additions. One addition is a polarizer that creates plane-polarized light. The second addition is a Pockels cell, which allows the separation of plane-polarized light into circularly polarized light of a single direction. It consists of a KD_2PO_4 crystal aligned such that the application of a potential gradient will result in complete absorption of light of one circular polarity, while a potential gradient of the opposite sign will result in absorption of light of the other polarity. The Pockels cell is exposed to alternating current, and the absorbance signals are measured in the same phase as the current.



Experiments

Proteins exhibit three types of CD signals: far ultraviolet signals, which are largely due to the peptide backbone, near ultraviolet signals, largely due to aromatic side-chains, and the signals resulting from prosthetic groups, if these are present.

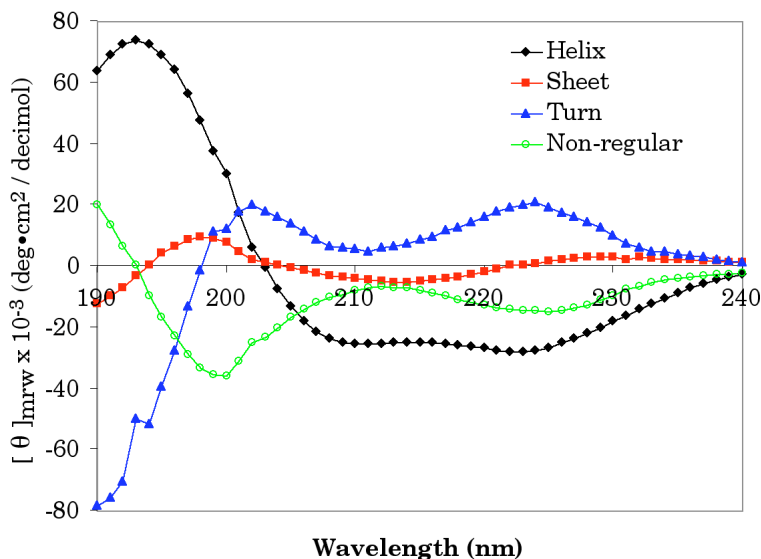
The **far ultraviolet** signal (180-250 nm) is due to the peptide backbone, although some non-peptide chromophores can also contribute. This signal can be fairly strong. Technical issues due to the large absorbance of most molecules in the far UV mean that relatively dilute protein solutions (usually 5 to 20 μM), dilute buffers that do not absorb strongly, and short pathlength cuvettes (0.05 to 0.1 cm) are used.



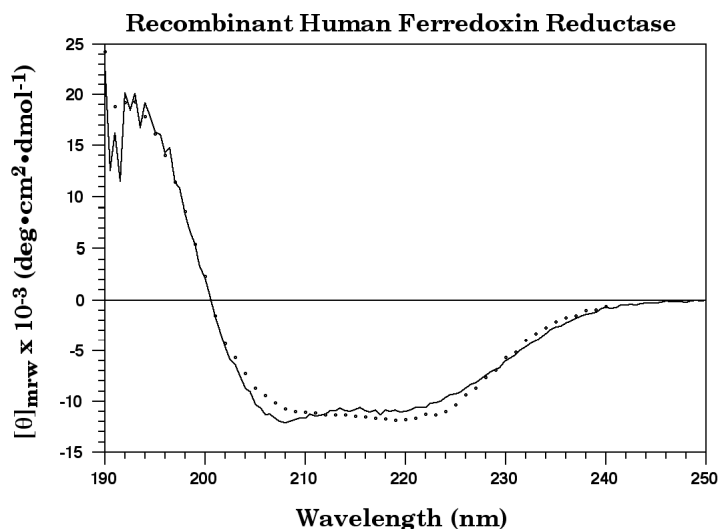
The peptide signal reflects secondary structure. In theory, the CD spectrum in the far ultraviolet is not affected by the side-chains. Assuming that the structural elements do not interact, the overall spectrum should then be a linear sum of the component spectra.

$$[\theta(\lambda)] = f_H[\theta_H(\lambda)] + f_S[\theta_S(\lambda)] + f_C[\theta_C(\lambda)]$$

where $f_H[\theta_H(\lambda)]$ is the fraction in an α -helix multiplied by the helical spectrum. Spectra for each of the major secondary structural types are shown below.⁴



For proteins, examination of the secondary structural spectra is the most common use of CD. An example of a protein far ultraviolet spectrum is shown below. The dotted line shows the theoretical fit ($\sim 20\%$ α -helix and $\sim 50\%$ β -structure) using the reference spectra.



⁴ From Yang, J.T. *et al.* (1986) in *Methods in Enzymology*, Vol. 130, 208-269.

Note the units on the y-axis of the far ultraviolet CD spectra. Most instruments convert the differential absorption values into θ in millidegrees. The θ values need to be corrected for the concentration and the pathlength. For historical reasons, the concentration is converted to decimol. The peptide backbone signals are usually converted to $[\theta]_{mrw}$ where “mrw” is “mean residue weight” by dividing by the number of amino acids, to compensate for the fact that longer sequences have larger signals.

$$[\theta]_{mrw} = \frac{\theta \text{ (millidegrees)}/1000 \frac{\text{millidegrees}}{\text{degree}}}{\text{Conc.} \left(\frac{\text{mol}}{1000 \text{ cm}^3}\right) \cdot \left(10 \frac{\text{decimol}}{\text{mol}}\right) \cdot \text{Pathlength (cm)} \cdot \text{Peptide length (residues)}}$$

Because the two “1000” conversion factors cancel, the actual calculation is:

$$[\theta]_{mrw} = \frac{\theta}{\text{Conc. (M)} \cdot \left(10 \frac{\text{decimol}}{\text{mol}}\right) \cdot \text{Pathlength (cm)} \cdot \text{Peptide length (residues)}}$$

The **near ultraviolet** signal (240 to 300 nm) is due to aromatic side-chains. These signals are generally weak, and require concentrated samples. Because protein and buffer molecules have smaller absorbance in this region, longer pathlengths are frequently used. The near ultraviolet signals are difficult to correlate with structure, although their sensitivity to local environment changes can make them useful for looking at unfolding or conformational changes.

3) Signals from any **prosthetic groups** (the range of wavelengths vary, depending on the group). The CD spectra for the prosthetic group are useful for assessing changes in the local environment. In addition, the spectra are often helpful in distinguishing electronic transition bands that may be difficult to separate using absorbance. Note that in the flavoprotein spectra below, some transitions have positive and some have negative Cotton effects in the CD spectrum. In the case of this flavoprotein, a comparison of the absorption and CD spectra for the flavin chromophore reveal similar intensities for the different transitions in the two types of measurements. As mentioned earlier in the discussion of peptide backbone transitions, this similarity in relative absorption and CD intensity is not observed for all transitions.

Ferredoxin Reductase Visible CD

