Circular Dichroism (CD) and Optical Rotatory Dispersion (ORD)

Techniques and Applications with Plane and Circularly polarized light

Animations taken from:
(http://www.enzim.hu/~szia/cddemo/edemo0.htm)

"Dichroism" is used to denote direction-dependent light absorption.

Linear dichroism refers to the differential absorption of light polarized parallel or perpendicular to the some reference axis.

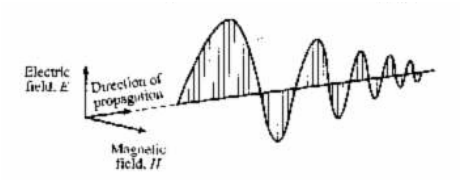
"Birefringence" refers to the direction-dependent index of refraction

Polarized Light

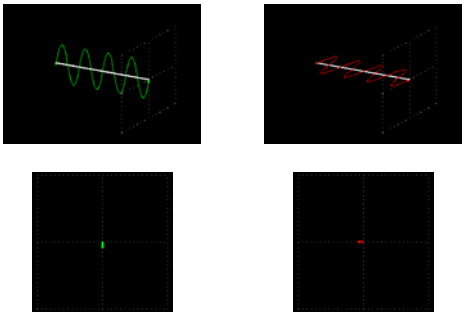
Linear Polarized Light

$$E_x(z,t) = E_0 \sin 2\pi\nu\left(\frac{z}{c} - t\right) = E_0 \sin\frac{2\pi}{\lambda}(z - ct)$$

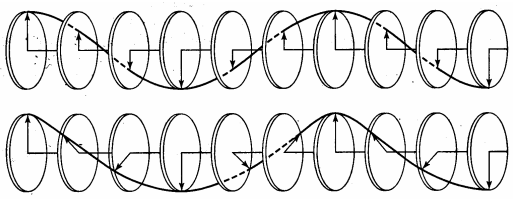
$$E_y(z,t) = 0$$



Plane polarized light

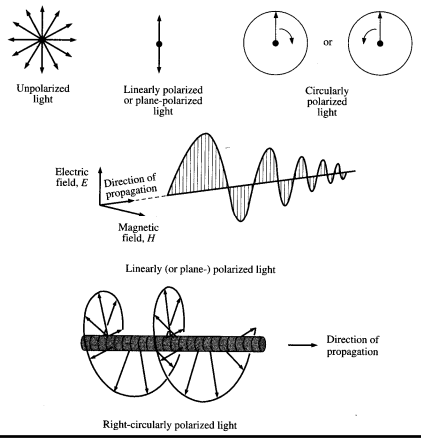


The **E** vector of linearly polarized light (also called plane-polarized light has a constant direction and a modulated amplitude. By contrast, the **E** vector of circularly polarized light has a constant amplitude but a modulated direction.

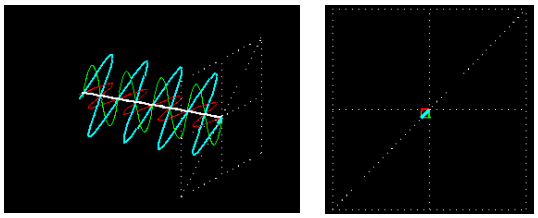


Types of polarized light

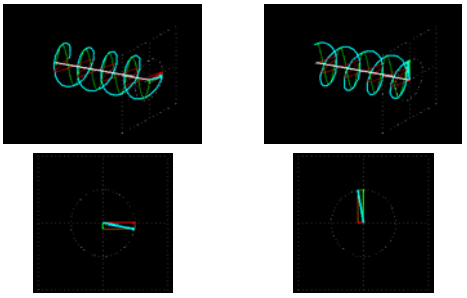
- Plane polarized light consists two circularly polarized components of equal intensity
- Two circularly polarized components are like left- and right-handed springs/helices
- As observed by looking at the source, right-handed circularly polarized light rotates clockwise
- Frequency of rotation is related to the frequency of the light
- Can be resolved into its two circularly polarized components
- When added together after passing through an optically isotropic medium, plane polarized light results



Superposition of two plane polarized waves, with orthogonal polarizations, but in phase with each other

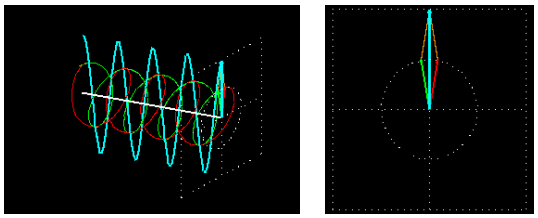


Superposition of two plane polarized waves, with orthogonal polarizations, but with a phase shift of ±90° between them



R L

Superposition of R + L circularly polarized waves of equal amplitudes results in linearly polarized light

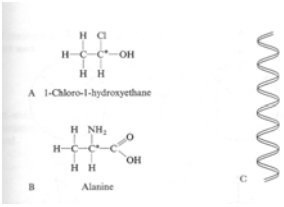


Polarized Light

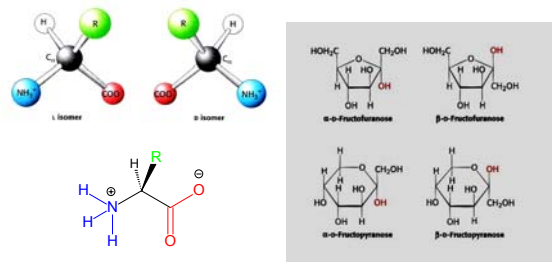
- Have seen how **transition dipoles** have *direction*
  - May probe this with linearly polarized light
- May also probe secondary structure:
  - **Linear Dichroism (LD)**
- Difference in absorption of ‖ versus □ polarized light
  - **Optical Rotary Dispersion (ORD)**
- **Rotation** of linearly polarized light by sample
  - **Circular Dichroism (CD)**
- Difference in absorption of **left** versus **right** circularly polarized light

Optical activity

- Enantiomers are optically active
- An asymmetric environment can also confer optical activity to a molecule
- Optically active molecules have different refractive indices, and different extinction coefficients for L and R circularly polarised light

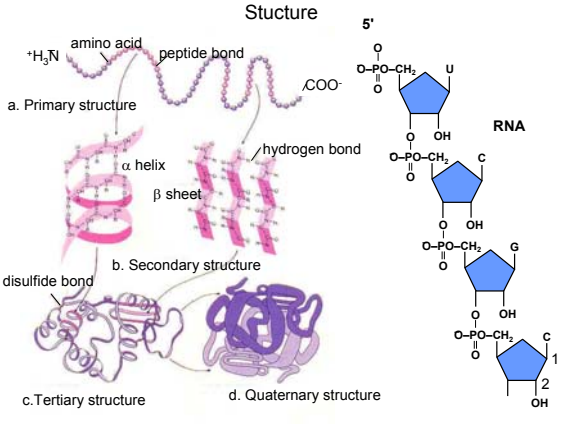


Aminoacids and sugars are chiral

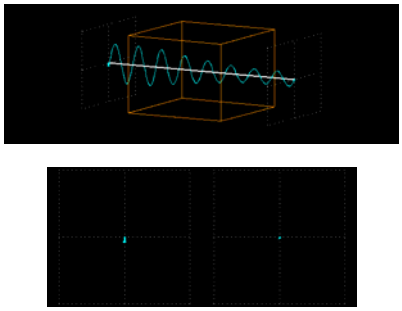


Peptides, proteins, nucleotides, nucleic acids, sugars, polysaccarides and glycerophospholipid (from glycerol, which is pro-chiral) are intrinsically chiral due to their primary structure.

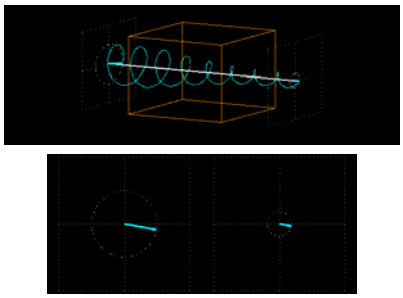
Stucture



Absorption of linearly polarized light

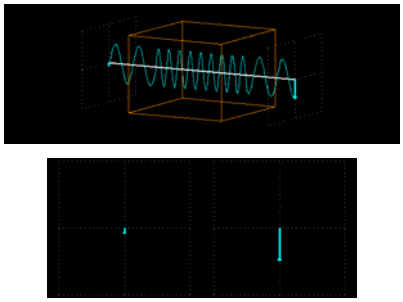


Absorption of circularly polarized light



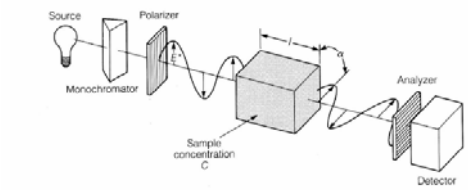
Effect of the refractive index of the medium on linearly polarized light

- v = c / n; n = refractive index

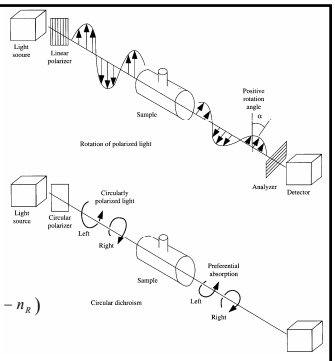


Optical Rotatory Dispersion (ORD) Spectroscopy

- The refractive indices for R and L varies as a function of wavelength, as does the difference between them
- One can measure Δn by measuring the rotation of linearly polarized light



Optical Rotation



$$\text{rotation}\left(\text{rad cm}^{-1}\right) = \alpha = \frac{1}{\lambda}\left(n_L - n_R\right)$$

n refractive index

λ wavelength of light

α angle of rotation

Optical rotary dispersion

- If the refractive indices of the sample for the left and right handed polarized light are different, when the components are recombined, the plane-polarized radiation will be rotated through an angle α
- n_l, n_r are the indices of the refraction for left-handed and right-handed polarized light
- α is in radians per unit length (from λ) $\alpha = \frac{n_l - n_r}{\lambda}$

- Usually reported as a specific rotation [α], measured at a particular T, concentration and λ (normally 589nm; the Na D line)
- Molar rotation [Φ] = $[\alpha] \times MW \times 10^{-2}$

$$[\alpha] = \frac{10^2 \alpha}{lc}$$

l = pathlength in decimeters

$$c = \frac{g}{100\ \text{mL}}$$

Optical rotary dispersion

- ORD curve is a plot of molar rotation [α] or [*M*] vs λ
- Clockwise rotation is plotted positively; counterclockwise rotation is plotted negatively
- ORD is based solely on the index of refraction
- A so-called plain curve is the ORD for a chiral compound that lacks a chromophore
- Chiral compounds containing a chromophore can give anomalous, or Cotton effect, curves

Cotton effect

- Positive Cotton effect is where the peak is at a higher wavelength than the trough
- Negative Cotton effect is the opposite
- Optically pure enantiomers always display opposite Cotton effect ORD curves of identical magnitude
- Zero crossover point between the peak and the trough closely corresponds to the normal UV λ_{max}

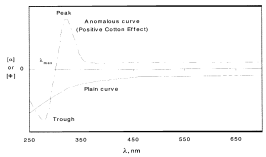
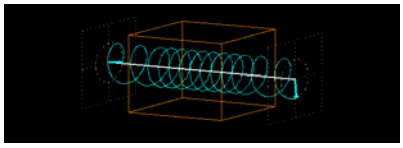
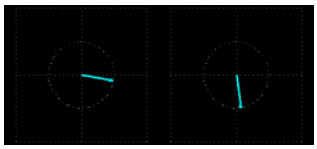


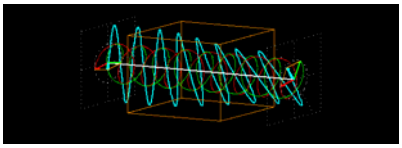
FIGURE 9.5 ORD curves.

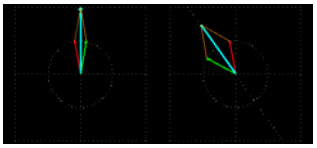
Effect of the refractive index of the medium on circularly polarized





Effect of different refractive indices: circular bifringence

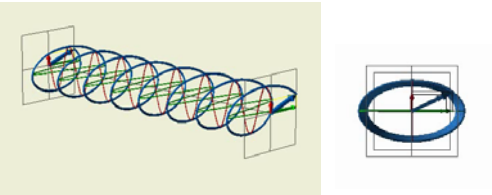




Elliptically polarized light

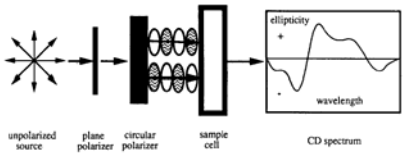
Is what you get when you combine:

- 2 linearly polarized waves (⊥) of equal amplitudes that are phase shifted by any angle other than 0° or 90°
- 2 linearly polarized waves (⊥) of unequal amplitudes that are phase shifted by any angle other than 0°
- R + L circularly polarized waves of unequal amplitudes



Unequal amplitudes, 90° phase shifted

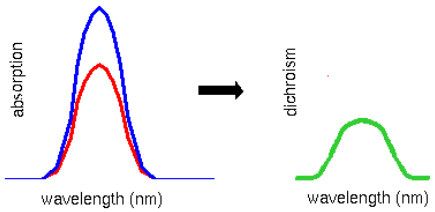
Physical principles of CD

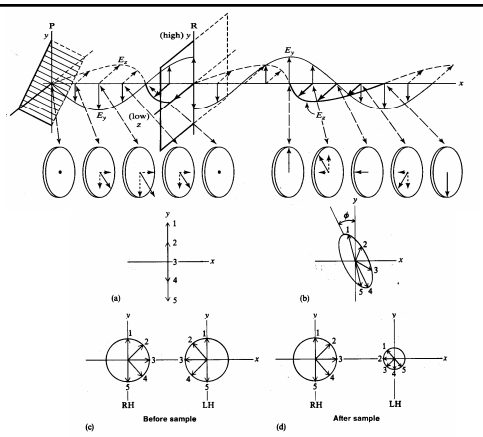


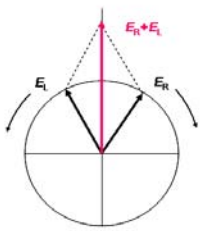
- Chiral or asymmetric molecules produce a CD spectrum because they absorb left and right handed polarized light to different extents and thus are considered to be "optically active"

Circular Dichroism

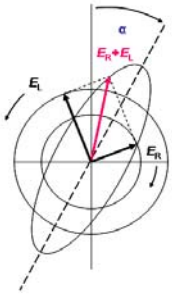
The difference between the absorption of left and right handed circularly-polarised light and is measured as a function of wavelength. CD is measured as a quantity called **mean residue ellipticity**, whose units are *degrees-cm²/dmol.*





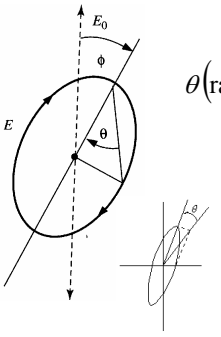


Linear polarized light can be viewed as a superposition of opposite circular polarized light of equal amplitude and phase



Different absorption of the left- and right hand polarized component leads to ellipticity (CD) and optical rotation (OR).

Circular Dichroism



$$\theta\left(\text{rad cm}^{-1}\right) = \frac{2.303\left(A_L - A_R\right)}{4l}$$

θ	ellipticity
l	path length through the sample
A	absorption

Circular dichroism

- Measurement of how an optically active compound *absorbs* right- and left-handed circularly polarized light
- All optically active compounds exhibit CD in the region of the appropriate absorption band
- CD is plotted as ε_l-ε_r vs λ
- For CD, the resulting transmitted radiation is not plane-polarized but elliptically polarized

- Optically active absorbing chromophores present different extinction coefficients for R and L circularly polarized waves
- CD spectroscopy exploits this phenomena to probe the handedness of the environment of the chromophores
- The technique is good at estimating alpha helical content, and at studying dynamic changes is secondary structure

Circular dichroism

- α is therefore the angle between the initial plane of polarization and the major axis of the ellipse of the resultant transmitted light
- A quantity φ is defined such that tan φ is the ratio of the major and minor axis of the ellipse of the transmitted light
- φ' approximates the ellipticity
- When expressed in degrees, φ' can be converted to a specific ellipticity [φ] or a molar ellipticity [θ]
- CD is usually plotted as [θ]

$$\text{specific ellipticity} = [\varphi] = \frac{\varphi'}{c'd'}$$

$$\text{molar ellipticity} = [\theta] = M[\varphi] \times 10^{-2}$$

$$\varepsilon_l - \varepsilon_r = 0.3032 \times 10^{-3}[\theta]$$

Consider a linearly polarised wave as a superposition of R + L circularly polarized waves

Initially these waves are in phase

The superposition of the two circularly polarized components is still a linearly polarized wave – the phase shift manifests itself as a rotation of the polarization plane

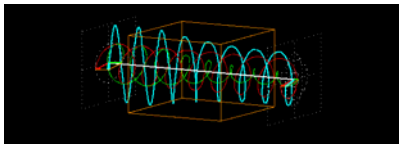
But as they travel through the medium at different speeds, they will be phase shifted when they exit the medium

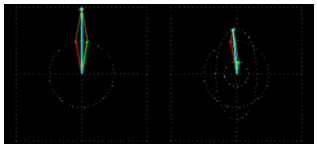
The wave exiting the specimen is the superposition of L and R waves with different amplitudes, i.e. elliptically polarised

The two circularly polarized waves are attenuated by different amounts by the specimen

Effect of different ε: circular dichroism

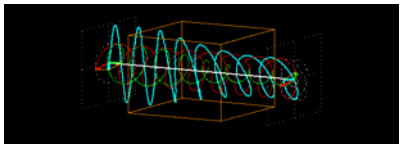
Consider a linearly polarised wave as a superposition of R + L circularly polarized waves

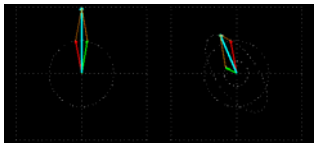




Simultaneous effect of Δn and $\Delta\varepsilon$ on linearly polarized light

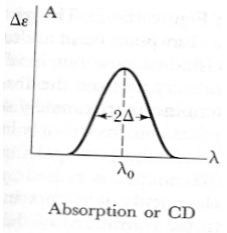
- $\Delta\varepsilon$ leads to ellipticity and Δn to a rotation of the axes of ellipticity





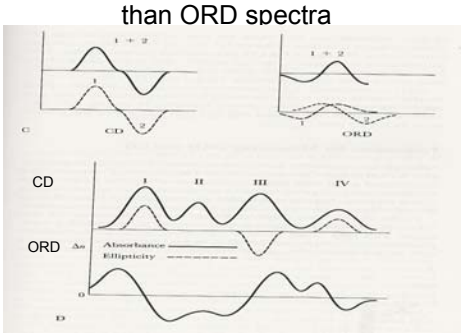
Circular Dichroism (CD) Spectroscopy

To examine the circular dichroism of an optically active specimen, illuminate it directly with circularly polarized light of alternating polarizations



The spectrum obtained is almost identical to an absorption spectrum, except that the peaks can be both positive and negative!

CD spectra are generally easier to interpret than ORD spectra



ORD and CD

- CD plots are Gaussian rather than S-shaped.
- Positive or negative deflections depend on the sign of $\Delta\varepsilon$ or [θ] and corresponds to the sign of the Cotton effect
- Maximum of the CD occurs at the absorption λ_{max}
- Where more than one overlapping Cotton effect, the CD may be easier to interpret than the ORD with overlapping S-shaped bands

Origin of ORD-CD peaks

- Chromophores in inherently asymmetrical environments such as helical molecules where chromophore is part of the helix
- Chromophore near a chiral center (common)
- Chromophore adjacent to a chiral plane or axis (less common)

ORD, CD and UV of Camphor

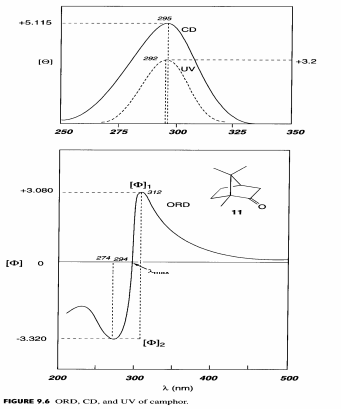
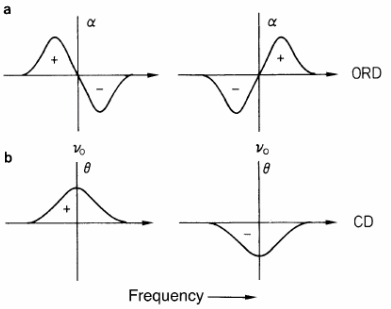


FIGURE 9.6 ORD, CD, and UV of camphor.



ORD spectra are dispersive (*Cotton effect* for a single band) whereas circular dichroism spectra are absorptive. The two phenomena are related by the so-called Krönig-Kramers transforms.

$$CD(\lambda) = A_L(\lambda) - A_R(\lambda) = \left[\varepsilon_L(\lambda) - \varepsilon_R(\lambda)\right]dc = \Delta\varepsilon dc$$

For historical reasons, commercial instruments express CD as "ellipticity" (θ).

$$[\theta]_\lambda = 3300\Delta\varepsilon(\lambda)$$

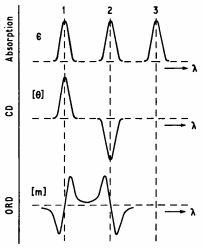
Kronig-Kramers Transforms.

$$[M]_{\lambda_o} = \frac{2}{\pi}\int \frac{[\theta]_{\lambda_o}\,\lambda}{{\lambda_o}^2 - \lambda^2}d\lambda$$

$$[\theta]_{\lambda_o} = \frac{2\lambda}{\pi}\int \frac{[M]_{\lambda_o}}{{\lambda_o}^2 - \lambda^2}d\lambda$$

CD is more commonly used than ORD to study biomolecules.

- Better resolution (the ORD signal is spread over a wider range).
- Better sensitivity.
- Easier to assign (1:1 correspondence between absorption peaks and CD signals).



Optical rotation

Specific rotation = $[\alpha] = \frac{\alpha}{dc}$

Molar rotation = $[\phi] = \frac{100\alpha}{lM}$

Circular dichroism

Molar ellipticity $[\theta] = \frac{100\psi}{lM}$

Circular dichroism = $\Delta\varepsilon = \varepsilon_L - \varepsilon_R = \frac{A_L - A_R}{lM}$

$[\theta] = 3298(\varepsilon_L - \varepsilon_R)$

* α = rotation angle, degrees; ψ = ellipticity, degrees; ε = molar absorptivity; d = path length, dm; l = path length, cm; c = concentration, g cm^{-3}; M = concentration, mol L^{-1}

CD Data Analysis

- The difference in absorption to be measured is very small. The differential absorption is usually a few 1/100ths to a few 1/10th of a percent, but it can be determined quite accurately. The raw data plotted on the chart recorder represent the ellipticity of the sample in radians, which can be easily converted into degrees

$$\theta_r = \frac{2.303}{4}\cdot(A_L - A_R)\cdot[rad]$$

$$\theta_d = \frac{2.303}{4}\cdot(A_L - A_R)\cdot\frac{180}{\pi}\cdot[\deg]$$

CD Data Analysis

- To be able to compare these ellipticity values a normalized value has to be used. The unit most commonly used in protein and peptide work is the mean molar ellipticity per residue. We need to consider path length l, concentration c, molecular weight M and the number of residues.

$$\theta_{mr} = \theta_d \cdot \frac{M}{c\cdot l\cdot n_r}$$ in proper units (use decimol)

$$\theta_{mrd} = \frac{\theta_{mr}}{10}\left[\deg\cdot\frac{g}{dmol}\cdot\frac{cm^3}{g}\cdot\frac{1}{cm}\cdot\frac{1}{residue}\right]$$ which finally reduces to

$$\theta_{mrd} = \frac{\theta_{mr}}{10}\left[\frac{\deg\cdot cm^2}{dmol\cdot residue}\right]$$ The values for mean molar ellipticity per residue are usually in the 10,000's

CD Data Analysis

- The molar ellipticity $[\theta]$ is related to the difference in extinction coefficients

$$\Delta\varepsilon\,[\theta] = 3298\,\Delta\varepsilon.$$

- Here $[\theta]$ has the standard units of degrees cm^2 dmol $^{-1}$
- The molar ellipticity has the units degrees deciliters mol^{-1} decimeter^{-1}.

-Circular dichroism is an absorptive quantity and ORD is dispersive.

-CD is a higher resolution method since we measure (relatively narrow) absorption bands.

-In ORD, the dispersive peak is quite spread out – if two bands are close to each other then it is difficult to distinguish them.

- ORD measurements can be performed at wavelengths where the substance being investigated does not necessarily absorb light.

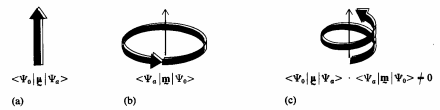
Selection Rule for CD

- Charge displacements (**μ**) that accompany absorption events are *linear*.
- The circular component (if present) generates a magnetic dipole (**m**).

Transitions that generate a magnetic dipole are *magnetically allowed.*

An example is the nπ* transition of the peptide bond.

- Optical activity requires both a finite **μ** and a finite **m**. The product of these two vectors corresponds to a helical displacement of charge.



By analogy with the dipole strength associated with normal absorption, we can define a *rotational strength* that indicates the intensity, or probability, of a CD transition.

$$R = \frac{(2.303)(3000)hc}{32\pi^3 N_A}\int\frac{\Delta\varepsilon}{\lambda}d\lambda$$

An absorption band can be characterized by its rotational strength, which is given by

$$R = \frac{(2.303)(3000)\hbar c}{32\pi^3 N_A}\int \frac{\Delta\varepsilon}{\lambda} d\lambda$$

where h is Planck's constant and c is the speed of light.

Since the left- and right-handed light will be absorbed differently, we will have ellipticity. The occurrence of ellipticity is called circular dichroism.

It can be shown (quantum mechanically) that R. the rotational strength is

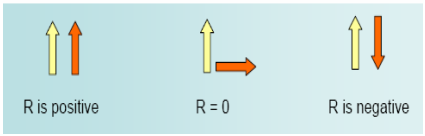
$$R_{fi} = \mathrm{Im}(\boldsymbol{\mu}_{fi} \cdot \mathbf{m}_{fi}) \text{ where}$$

$$\mathbf{m}_{\mathrm{fi}} = \int \Psi_f{}^*(q)\hat{\mathbf{m}}\Psi_i(q)d\tau \text{ and}$$

$$\boldsymbol{\mu}_{\mathrm{fi}} = \int \Psi_f{}^*(q)\hat{\boldsymbol{\mu}}\Psi_i(q)d\tau$$

i.e. the imaginary component of the scalar product between the electric and magnetic moments.

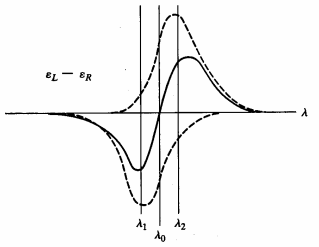
For most purposes, we can say that R = μm cos θ
where θ is the angle between the two dipoles.



The rotational-strength-rule:
The rotational strength generated in chromophore A by the interaction is exactly balanced by a rotational strength of opposite sign generated in chromophore B. Thus, *the sum of all rotational strengths in a CD spectrum must be zero.*

When the interacting transitions are degenerate, then the resulting rotational strengths of opposite sign cancel giving rise to the characteristic sigmoidal CD curve

This transition is called *conservative*, because it obeys the rotational strength rule.



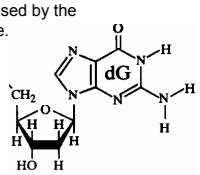
Applications of CD in structural biology
Determination of secondary structure of proteins that cannot be crystallised
Investigation of the effect of e.g. drug binding on protein secondary structure
Dynamic processes, e.g. protein folding
Studies of the effects of environment on protein structure
Secondary structure and super-secondary structure of membrane proteins
Study of ligand-induced conformational changes
Carbohydrate conformation
Investigations of protein-protein and protein-nucleic acid interactions
Fold recognition

Why use CD?
Simple and quick experiments
No extensive preparation
Measurements on solution phase
Relatively low concentrations/amounts of sample
Microsecond time resolution
Any size of macromolecule

In symmetric molecules, magnetic transition dipoles are always perpendicular to electric transition dipoles. Thus, the rotational strength in symmetric molecules is zero, and they exhibit no CD.

Most of the chromophores in biopolymers are symmetric. (Exceptions include the peptide bond and the disulfide bond.) We observe CD bands because of interactions between the transition dipoles of the chromophore and the asymmetric transition dipoles in other parts of the molecule.

Asymmetric disposition of electric and magnetic dipoles may arise because of an intrinsic asymmetry (e.g., that of an α carbon of a sugar) or because of a super-asymmetry imposed by the macromolecular secondary or tertiary structure.



•An electrical transition dipole acting at a distance has the properties of a magnetic transition dipole. Thus, an oriented array of strongly allowed transition dipoles (e.g., the ππ* transition dipoles of the bases in double helical DNA or of the peptide bonds in an α helix) can function as a strongly allowed magnetic dipole. Thus, interactions between the repetitive ππ* chromophores in biopolymers can produce CD bands.

•An electrically allowed transition in a chromophore (e.g., the ππ* transition in a DNA base) can exhibit rotational strength due to its interaction with a magnetically allowed transition in some other part of the molecule (i.e., the deoxyribose).

•Also, a magnetically allowed transition (e.g., the peptide nπ* transition) can exhibit rotational strength because of its interaction with adjacent electronically allowed transitions in another part of the molecule.

•However, the magnitude of the interaction decreases with the distance between the transition dipoles and with the energy difference between the transitions. Since the electronic transitions of the deoxyribose fall at substantially higher energy, the CD of individual nucleosides/nucleotides is weak.

Optical rotation or circular birefringence results from different indices of refraction for right- and left-circularly polarized light.

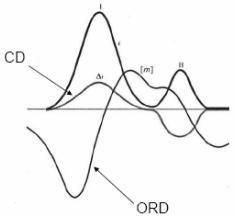
The angle of rotation depends on the nature of the substance, the thickness of the sample d, and the concentration C, such that α = [α] dC where [α] is the specific rotation.

Optical rotatory dispersion (ORD) is the change in the optical rotation with wavelength.

The quantity used to describe optical rotatory dispersion is not the specific rotation [α] but the molar rotation

$$[m'] = [\alpha]\frac{3}{n^2+2}\frac{M}{100}$$

where n is the refractive index of the medium and M is the molecular weight of the solute.
The term $(3/n^2 +2)$ is a correction factor which accounts for the slight polarizability of the medium and its effect on the solute.

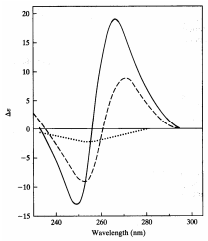


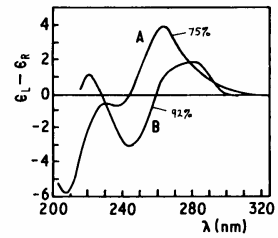
Electronic CD of nucleic acids.

rA (dotted), $(rA)_2$ (dashed), and oligo(rA)

The spectrum of the isolated nucleotide is weak

The spectrum of oligo(rA) is much stronger and conservative.
- CD of oligo(rA) is primarily due to interaction between neighboring electrical transition dipoles.
- hydrophobic interactions alone are strong enough to induce helical structure in single-stranded polynucleotides.
- dimer spectrum shows evidence of base stacking.





The CD of nucleic acids is largely a result of higher-order structure. Correspondingly, the spectra are sensitive to variations in secondary/tertiary structure, as indicated by the CD spectra of DNA in the A and B form (10.4 bp/turn).

CD can monitor very subtle alterations in structure, such as the conversion of (B form) DNA having 10.4 bp/turn to that containing 10.2 bp/turn that occurs upon addition of methanol (0-95%).

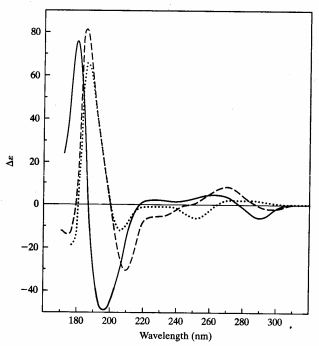
The first observation of Z-form (left-handed) DNA was made by CD in 1972.

The CD spectrum of poly d(GC)-poly d(GC) shown under conditions that produce either the

B-form DNA (dotted, aqueous buffer, pH 7)

A-form DNA (dashed, 80% trifluoroethanol, pH 7)

Z-DNA (solid, 2M sodium perchlorate, pH 7)

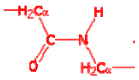


Circular Dichroism of Proteins

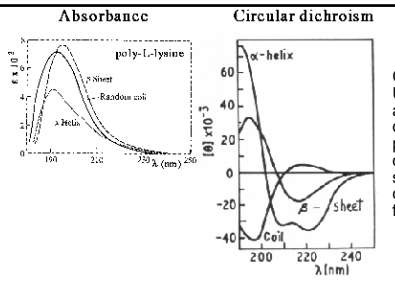
- It has been shown that CD spectra between 260 and approximately 180 nm can be analyzed for the different secondary structural types: alpha helix, parallel and anti-parallel beta sheets, turns, and other.
- Modern secondary structure determination by CD are reported to achieve accuracies of 0.97 for helices, 0.75 for beta sheet, 0.50 for turns, and 0.89 for other structure types

CD Signal of Proteins

- For proteins we will be mainly concerned with absorption in the ultraviolet region of the spectrum from the peptide bonds (symmetric chromophores) and amino acid sidechains in proteins.
- Protein chromophores can be divided into three classes: the peptide bond, the amino acid sidechains, and any prosthetic groups.
- The lowest energy transition in the peptide chromophore is an n → π* transition observed at 210 - 220 nm with very weak intensity (ε_{max}~100).



----π* π → π* ~ 190 nm ε_{max}~7000
----n n → π 208–210, 191–193 nm ε_{max}~100
----π



Comparison of the UV absorbance (left) and the circular dichroism (right) of poly-L-lysine in different secondary structure conformations as a function of pH.

- The n → π* transition appears in the α-helical form of the polymer as a small shoulder near 220 nm on the tail of a much stronger absorption band centered at 190 nm. This intense band, responsible for the majority of the peptide bond absorbance, is a π → π* transition (ε_{max} ~ 7000).
- Using CD, these different transitions are more clearly evident. Exciton splitting of the π → π* transition results in the negative band at 208 and positive band at 192 nm.

CD Spectra of Proteins

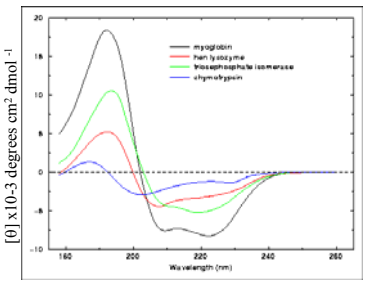
- Different secondary structures of peptide bonds have different relative intensity of n → π* transitions, resulting in different CD spectra at far UV region (180 - 260 nm).
- CD is very sensitive to the change in secondary structures of proteins. CD is commonly used in monitoring the conformational change of proteins.
- The CD spectrum is additive. The amplitude of CD curve is a measure of the degree of asymmetry.
- The helical content in peptides and proteins can be estimated using CD signal at 222 nm

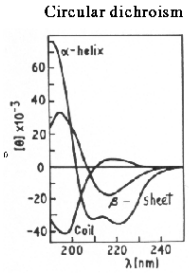
  ε_{222}= 33,000 degrees cm^2 dmol $^{-1}$ res^{-1}
- Several curve fitting algorithms can be used to deconvolute relative secondary structures of proteins using the CD spectra of proteins with known structures.

Protein CD Signal

- The three aromatic side chains that occur in proteins (phenyl group of Phe, phenolic group of Tyr, and indole group of Trp) also have absorption bands in the ultraviolet spectrum. However, in proteins, the contributions to the CD spectra in the far UV (where secondary structural information is located) is usually negligible. Aromatic residues, if unusually abundant, can have significant effects on the CD spectra in the region < 230 nm, complicating analysis.
- The disulfide group is an inherently asymmetric chromophore as it prefers a gauche conformation with a broad CD absorption around 250 nm.

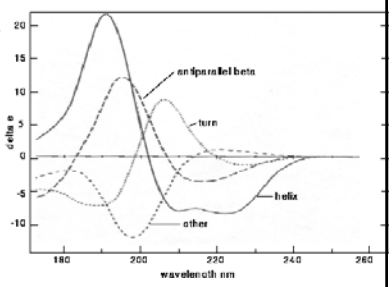
Far UV CD Spectra of Proteins





CD Spectra of Proteins

- Each of the three basic secondary structures of a polypeptide chain (helix, sheet, coil) show a characteristic CD spectrum. A protein consisting of these elements should therefore display a spectrum that can be deconvoluted into the three individual contributions.



CD Spectra Fit

- In a first approximation, a CD spectrum of a protein or polypeptide can be treated as a sum of three components: α-helical, β-sheet, and random coil contributions to the spectrum.
- At each wavelength, the ellipticity (θ) of the spectrum will contain a linear combination of these components:

$$\theta_T = x_1 \cdot \theta_h + x_2 \cdot \theta_s + x_3 \cdot \theta_c \quad (1)$$

- θ_T is the total measured susceptibility, θ_h the contribution from helix, θs for sheet, θc for coil, and the corresponding χ the fraction of this contribution.

CD Spectra Fit

- As we have three unknowns in this equation, a measurement at 3 points (different wavelengths) would suffice to solve the problem for χ, the fraction of each contribution to the total measured signal.
- We usually have many more data points available from our measurement (e.g., a whole CD spectrum, sampled at 1 nm intervals from 190 to 250 nm). In this case, we can try to minimize the total deviation between all data points and calculated model values. This is done by a minimization of the sum of residuals squared (s.r.s.) :

$$s.r.s. = \sum_{i=1}^{ndata} (x_1 \cdot \theta_{h(i)} + x_2 \cdot \theta_{s(i)} + x_3 \cdot \theta_{c(i)} - \theta_{T(i)})^2$$

Using CD to Monitor 3º Structure of Proteins

- CD bands in the near UV region (260 – 350 nm) are observed in a folded protein where aromatic sidechains are immobilized in an asymmetric environment.
- The CD of aromatic residues is very small in the absence of ordered structure (e.g. short peptides).
- The signs, magnitudes, and wavelengths of aromatic CD bands cannot be calculated; they depend on the immediate structural and electronic environment of the immobilized chromophores.
- The near-UV CD spectrum has very high sensitivity for the native state of a protein. It can be used as a finger-print of the correctly folded conformation.

The CD response obtained was expressed in terms of mean residue ellipticity (MRE) in deg cm^2 dmol^{-1} using the following equation

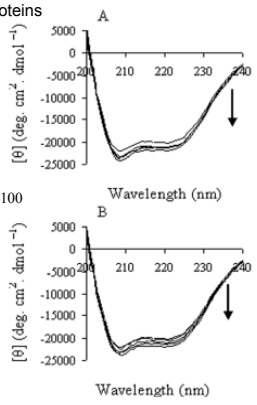
$$MRE = \frac{observedCD\ (m\deg)}{C_P \times n \times l \times 10}$$

where C_p is the molar concentration of the protein, n is the number of amino acid residues in the protein and l is the path length (0.1 cm). The α-helical content can be calculated from the *MRE* values at 208 nm by using the equation

$$\alpha - Helix(\%) = \frac{(-MRE_{208} - 4000)}{33{,}000 - 4000} \times 100 = \frac{(-MRE_{208} - 4000)}{29{,}000} \times 100$$

where MRE_{208} corresponds to the observed *MRE* values, 33,000 and 4000 are the *MRE* values of a pure α-helix and of the β-form and random coil conformation at 208 nm respectively.

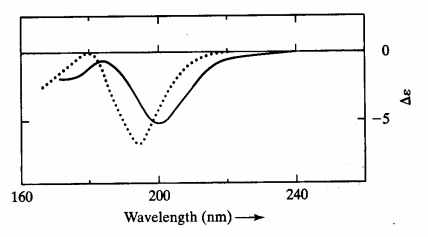
Interactions of ligands with proteins



$$\alpha - Helix(\%) = \frac{(-MRE_{208} - 4000)}{29{,}000} \times 100$$

Electronic CD of proteins and polypeptides.

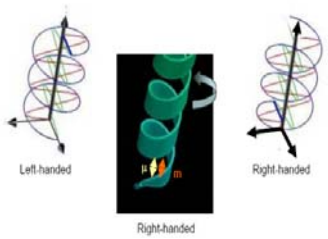
As noted for isolated nucleotides, the CD of isolated peptide chromophores is weak and nonconservative.



CD spectrum of N-acetyl-L-alanine-N′-methylamide (dotted) is shown along with the corresponding spectrum of denatured collagen (pH 3.5, 45° C).

Secondary structure from CD spectra

- We can assume that a spectrum is a linear combination of CD spectra of each contributing secondary structure type (e.g., "pure" alpha helix, "pure" beta strand etc.) weighted by its abundance in the polypeptide conformation to extract secondary structure content from CD data.
- Several methods have been developed which analyze the experimental CD spectra using a database of reference protein CD spectra containing known amounts of secondary structure.



-The electric field will induce an oscillating dipole μ which will have a component along the helix axis
- Electric field is always accompanied by a magnetic field.
- An oscillating magnetic field parallel m to the helix axis will induce current in the helix.

Both the electric field *and* the magnetic field will contribute to an electron displacement along a helical path. So we will have an electric and a magnetic dipole moment.

Now if the circularly polarized light is right-handed, the phase of the light and that of the electron are the same.

In other words, both the electric field and the magnetic field act in concert on the electron.

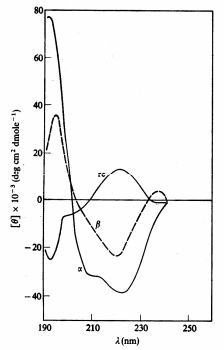
If the circularly polarized light is left-handed, then there is a phase difference of 180 degrees. In this case, the electric and magnetic fields acting on the electron are in opposite directions.

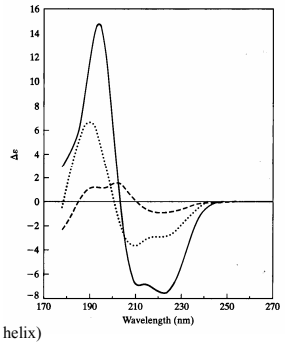
CD Spectra

The adoption of secondary structure by a polypeptide imposes a super-asymmetry that gives rise to characteristic CD bands as a result of degenerate interactions between adjacent electric transition dipoles.

These spectra can be analyzed both qualitatively and quantitatively to analyze the secondary structure of a protein.

- The presence of minima at 222 nm and 208 nm (or a minimum and a shoulder, respectively) is diagnostic for significant α-helical content.
- A broad minimum at 217 nm is indicative of substantial β-sheet structure.
- A weak positive CD band at 220-230 nm is indicative of substantial disordered structure.



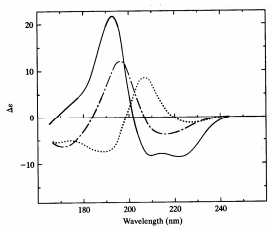


CD spectra of

hemoglobin (largely α helix)

tumor necrosis factor (primarily β-sheet) and

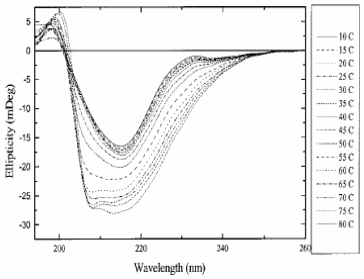
Eco RI restriction enzyme (mix of α and β structure)



It is also possible to estimate the content of β-turn structure in a protein. The spectrum of poly (L-alanine$_2$-glycine$_2$) – believed to adopt a series of β turns in aqueous solution at pH 7 – is shown above (dotted). It is also possible to estimate the parallel- and antiparallel contributions to the overall β-sheet structure.

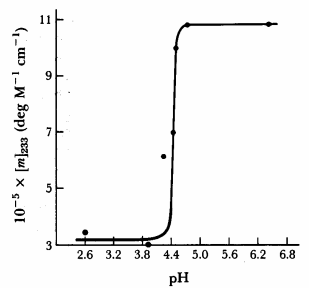
Public-domain computer programs are available for estimating the content of helix, parallel β sheet, antiparallel β sheet, β turn, and "other" secondary structure from the CD spectrum of a protein. These programs use a combination of *pattern recognition* and *singular value decomposition.* In general, they are quite accurate.

Secondary structure from CD spectra



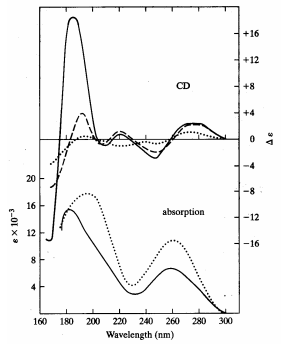
CD is particularly useful for measuring the temperature dependence of protein secondary structure.

Denaturation of a helical protein can be readily monitored at 222 or 208 nm (e.g., acid denaturation of myoglobin) and of a β-sheet protein at 217 nm.



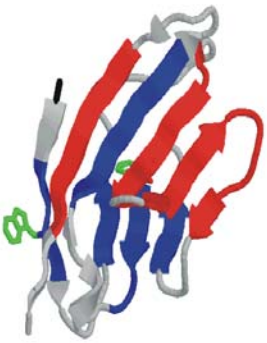
Empirical uses of CD

Melting of the DNA double helix can be monitored at 190 nm



(native DNA, solid line; denatured DNA, dashed; constituent nucleotides, dotted)

Domain 1of CD2

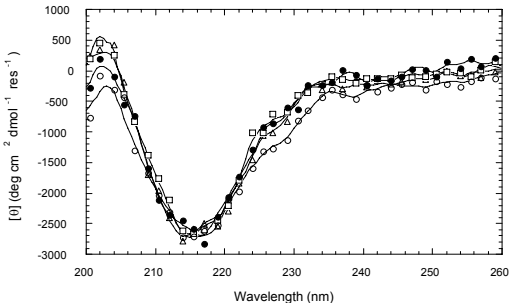


CD2 is a cell adhesion molecules.

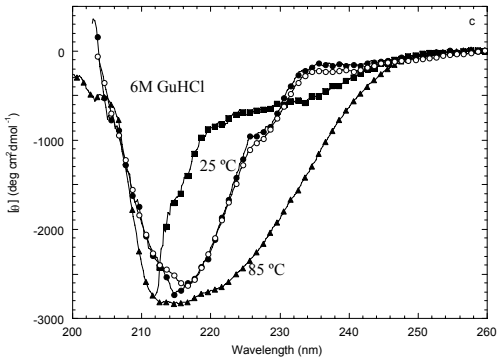
Domain 1 of CD2 has a IgG fold. Nine β-strands form a beta-sandwich structure.

Two Trp residues, W-7 and W-32 (green) are located at the exposed and buried region of the protein, respectively.

CD2 is Stable from pH 1 to 10



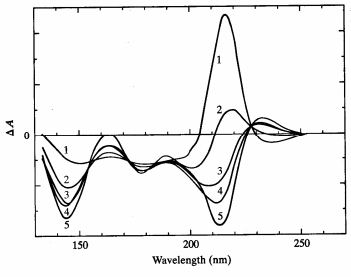
Conformational Change of CD2



Kinetics

The progress of a reaction can be monitored by CD.

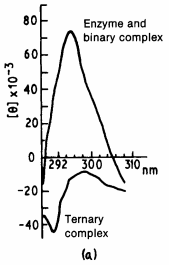
e.g., the slow conversion of all *cis* poly-proline to all *trans* poly-proline

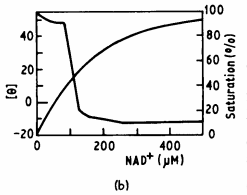


Ligand/substrate binding

Binding of NAD^+ to glutamate dehydrogenase in the presence of glutarate.

The enzyme-glutarate complex [top line, (a)] is comparable to the free enzyme. When excess NAD^+ is added, the near UV CD spectrum changes [bottom line].





The progress of an NAD^+ titration of the GDH-glutarate complex is shown in (b). The CD signal is largely unchanged until three of the six subunits have bound NAD^+, whereupon there is a large conformational change that is known to reduce affinity of the remaining sites for NAD^+

(negatively cooperative binding).

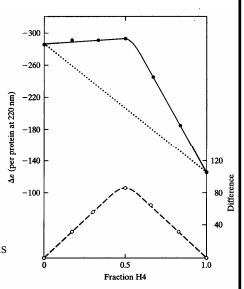
Macromolecular interactions. Complex formation

Interaction between histones H3 and H4.

The ellipticities at 222 nm for 0% H4 and 100% H4 represent the CD signals of pure H3 and pure H4, respectively. If there were no interaction between the two, a linear change in signal would be observed, proportional to the amount of H4 (dotted line).

Pattern obtained (indicated by solid data points)

When a *continuous variation* curve is plotted (the difference between the observed values and those predicted in the absence of interaction), a maximum is observed at a 1:1 molar ratio, indicative of dimer formation.



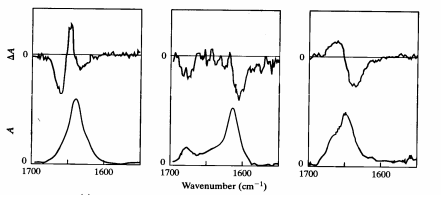
Sample Preparation

- Additives, buffers and stabilizing compounds: Any compound which absorbs in the region of interest (250 - 190 nm) should be avoided.
- A buffer or detergent or other chemical should not be used unless it can be shown that the compound in question will not mask the protein signal.
- Protein solution: From the above follows that the protein solution should contain only those chemicals necessary to maintain protein stability, and at the lowest concentrations possible. Avoid any chemical that is unnecessary for protein stability/solubility. The protein itself should be as pure as possible, any additional protein or peptide will contribute to the CD signal.

Vibrational CD spectroscopy

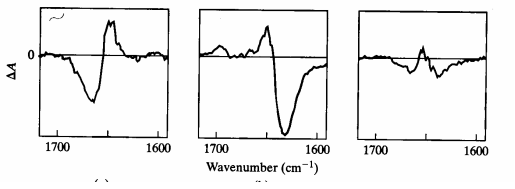
CD signals can be measured for vibrational transitions, as well as electronic transitions. Vibrational CD (VCD) spectra have the advantage that the bands correspond to specific functional groups.

The appearance of the CD signals for proteins in the Amide I region are sensitive to secondary structure. The fraction of each type of secondary structure can be deduced from VCD spectra, as well as from electronic CD spectra.



From left to right, we have the VCD and IR absorption spectra for poly-L-lysine in the α-helical, β-sheet, and random coil forms.

From left to right, the vibrational CD spectra in the Amide I region for myoglobin, chymotrypsin, and lysozyme.



The vibrational CD spectra of nucleic acids are not as sensitive to conformation as the electronic CD spectra. Left-handed DNA (Z-DNA) can be readily distinguished from right-handed, the VCD spectra of A- and B-form DNA are fairly similar, differing primarily in intensity.