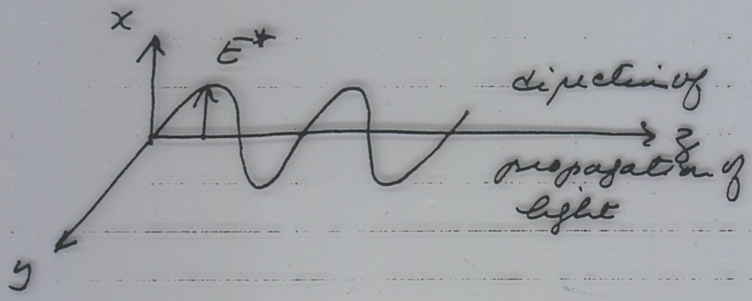
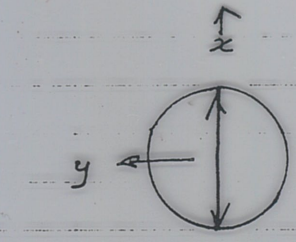
Lecture 18
Physical Chemistry
for Life Scientists
National Tsing-Hua
University
June 2, 2001

Optical Rotation, Optical Rotatory Dispersion, Circular Dichroism (Interaction of molecules with circularly polarized light)

→ Polarization of Light





$\vec{E}$ as viewed along z-direction

wave linearly polarized along x-direction

If all the waves have $\vec{E}$ linearly polarized as above, Light is plane-polarized (plane defined by electric field direction and direction of propagation of light wave)

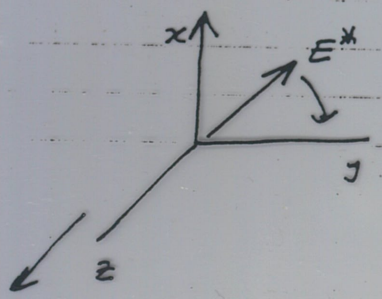
Unpolarized light

$\vec{E}$ of different waves as viewed along direction of propagation

electric fields oscillating in all directions

Plane-polarized light can be produced by passing unpolarized light thru a highly anisotropic material (e.g. Polaroid), which absorbs all light with $\vec{E}$ pointing in one-direction (e.g. horizontal), thereby leaving only rays whose electric field point vertically. Another method is to pass the unpolarized light through a Nicol prism, which removes light polarized in one-direction by <u>reflection</u>

Plane-polarized light may be considered as a superposition of left and right-circularly polarized light in equal proportions



$$E_R = \vec{i}E_0^* \cos\omega t + \vec{j}E_0^* \sin\omega t \quad \text{clockwise}$$

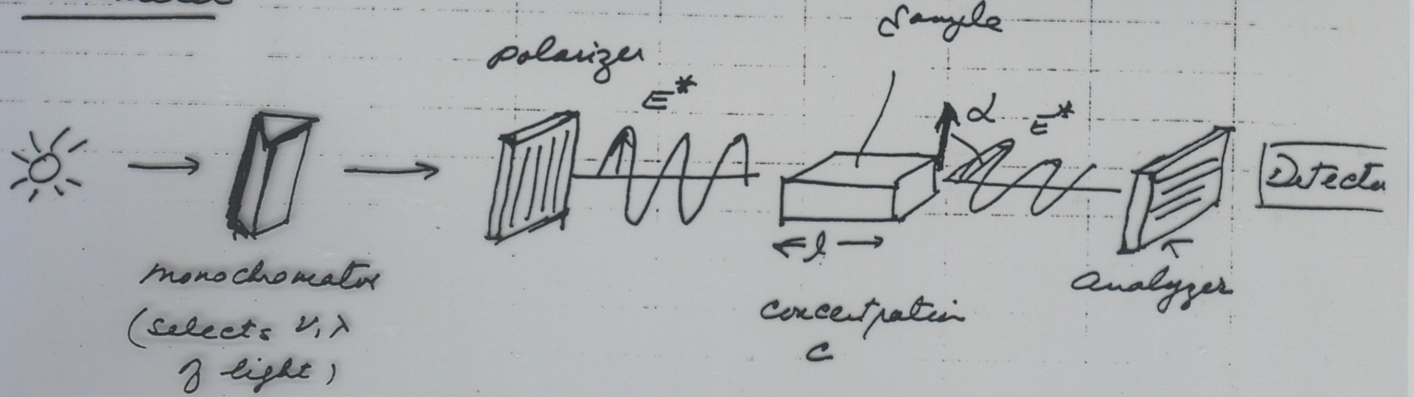
$$E_L = \vec{i}E_0^* \cos\omega t - \vec{j}E_0^* \sin\omega t \quad \text{counterclockwise as one coming light beam is viewed.}$$

$$E_R + E_L = \vec{i}\,2E_0^* \cos\omega t$$

→ <u>Optical Rotation</u>

When plane-polarized light passes thru an optically active substance, the plane of polarization of the light is rotated.

Polarimeter



$\alpha \equiv$ angle of rotation of the plane of polarization

Detector measures intensity of transmitted light.
Null method is usually used; i.e., analyzer is set such that light that is passed by the first polarizer (say vertically polarized) is absorbed by the analyzer.
If the sample rotates the plane of the polarization of the light incident on it, say by angle α, then the analyzer must be rotated by angle α to attain null at detector; i.e., polarizer and analyzer are rotated by $90° + \alpha$ w.r.t. each other.

Specific rotation (in degrees) is defined as follows:

$$[\alpha]_\lambda^t = \frac{100\,\alpha}{l\,c}$$

l = path length of cell in decimeters (1 decimeter = 10 cm)
λ = wavelength of light
c = concentration of solute in grams per 100 ml solution
t = temp in °C

Examples

α-D glucose in water $[\alpha]_D^{20} = +112.2$

Na-D-line
20°C
+ plane of polarization rotated to right as one looks at beam.

L-serine in water $[\alpha]_D^{20} = -6.8$

For macromolecules, the optical rotation is usually expressed as the reduced residue rotation, defined by

$$[m'] = \frac{3}{n^2+2} \frac{m}{100} [\alpha]_\lambda^t$$

where m = mean residue molecular weight
n = refractive index of the solution

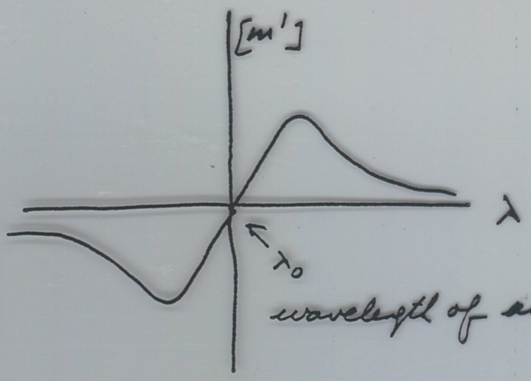
→ Optical Rotatory Dispersion

Optical rotatory dispersion is the dependence of optical rotation on the wavelength of incident light.

α is measured at each wavelength by a spectropolarimeter and [m'] is plotted against λ

Optical Rotation exhibits like & dip similar to dispersion of the refractive index near chromophore absorptions

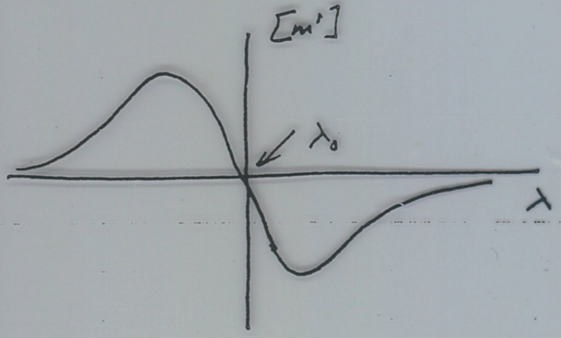
ORD of a single asymmetric optically active chromophore



dip + rise called Cotton effect

wavelength of absorption of chromophore

For the enantiomorph (mirror image) of chromophore,



mirror image of above.

→ Circular Dichroism (CD)

Circular Dichroism is the differential absorption of a sample for left-circularly polarized and right-circularly polarized light.

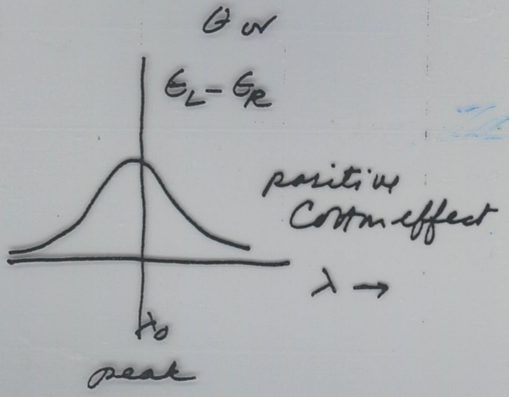
$A_L - A_R$ absorbance difference

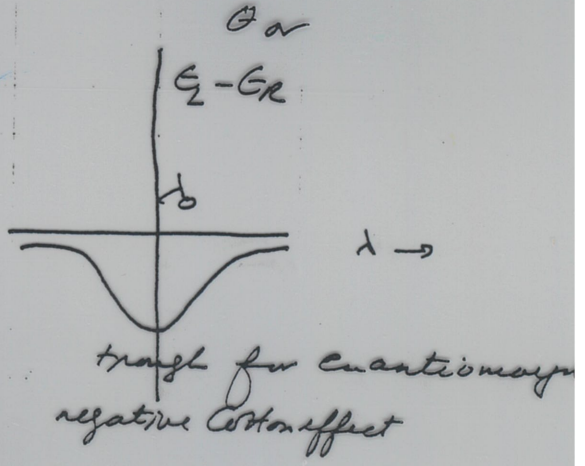
or $\epsilon_L - \epsilon_R$ extinction coefficient difference

Recall $\epsilon = A/$ [redacted] lc

CD

for a single optically active chromophore

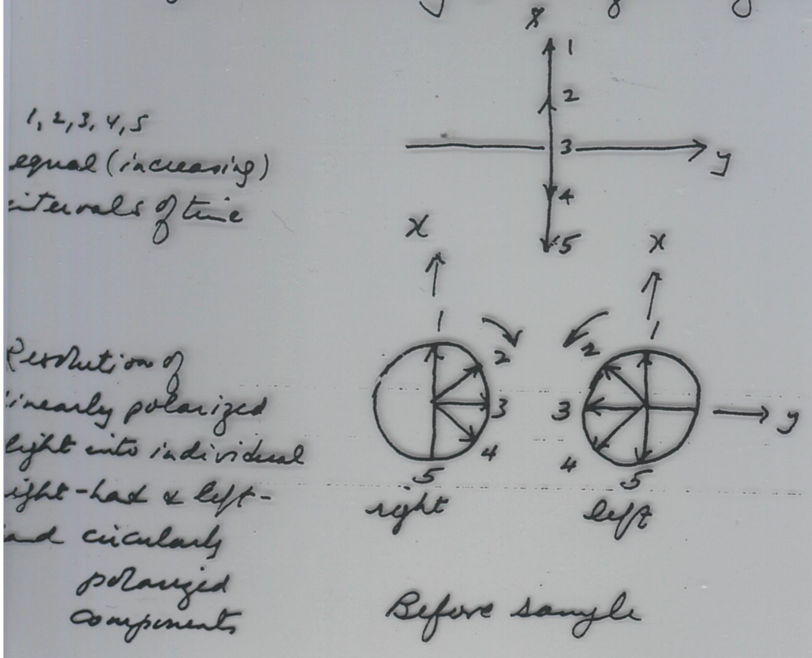


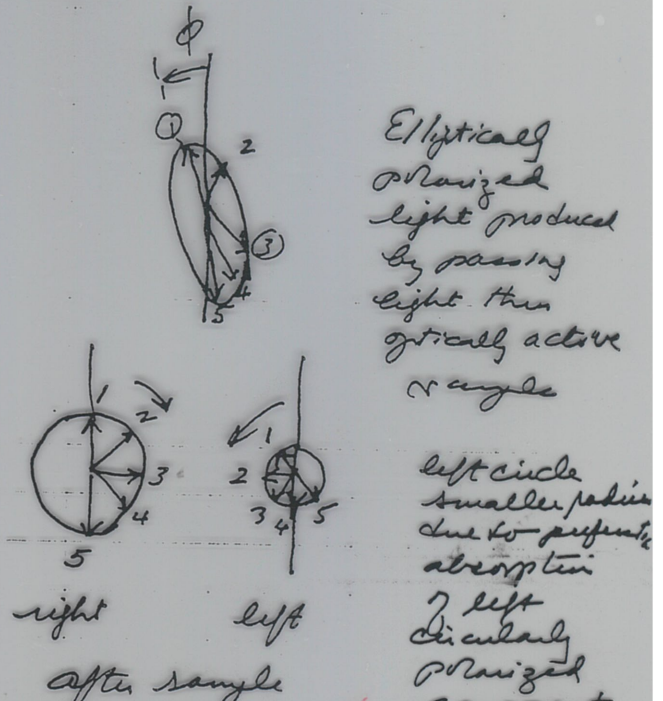


CD or $\epsilon_L - \epsilon_R$ determined by measuring ellipticity following absorption of linear or plane polarized light or by measuring ϵ_L and ϵ_R using left- and right-circularly polarized light respectively. The latter approach is obvious.

Discuss experiment using plane polarized light.

Recall, can decompose plane polarized light into left- and right circularly polarized light.





Effect of an optically active sample on two circularly polarized components

$$\text{Ellipticity} = \tan^{-1}\left(\frac{\text{minor axis}}{\text{major axis}}\right)_{\text{ellipse}} = \theta$$

$$= 2.303\,(A_L - A_R)\,180/4\pi \text{ degrees}$$

$$\text{molar ellipticity} = [\theta] = 100\,\theta/C \cdot l$$

$$= 2.303\,(\epsilon_L - \epsilon_R)\,l \cdot c \;\frac{100}{C \cdot l} \cdot \frac{180}{4\pi} \text{ degrees}$$

$$= 2303 \times \frac{9}{2\pi}\,(\epsilon_L - \epsilon_R)$$

$$= 3{,}300\,(\epsilon_L - \epsilon_R)$$

Circular birefringence

If the two circularly polarized components are absorbed to different extents at any wavelength, then it follows that the sample will also have a different index of refraction (n) for the two components at virtually all wavelengths. One component will propagate more rapidly through the other the medium. The result is a phase shift between the two components, proportional to the refractive index difference, $n_L - n_R$. This effect is called circular birefringence. When the two components are combined, the phase shift results in a permanent rotation of the long axis of the elliptically polarized light.

$$\phi = 180 \cdot l \cdot (n_L - n_R)/\lambda \text{ degrees}$$

or $[\phi] = 100\,\phi / C \cdot l$.

ORD and CD are related

$$[\phi(\lambda)] = \frac{2}{\pi} \int_0^\infty \frac{[\theta(\lambda')]\,\lambda'}{\lambda^2 - \lambda'^2}\, d\lambda' \qquad \text{optical rotation}$$

$$[\theta(\lambda)] = -\frac{2\lambda}{\pi} \int_0^\infty \frac{[\phi(\lambda')]}{\lambda^2 - \lambda'^2}\, d\lambda' \qquad \text{circular dichroism}$$

Order of magnitudes

For typical protein or nucleic acid solutions at 10^{-4} M chromophore concentrations,

$\phi \sim 0.01$ to $0.1°$ for a 1 cm sample

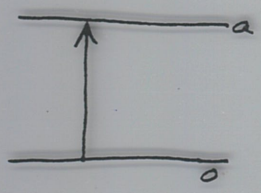
Current instruments can detect rotations as small as 10^{-4} degree.

θ typically 0.03% to 0.3% of total absorption, which can be determined quite accurately with modern instrumentation measuring $\epsilon_L - \epsilon_R$ by left & right circularly polarized light.

θ, ellipticity is small to measured directly.

Physical origin of CD and ORD

absorption spectroscopy



Electric dipole transition

Dipole strength

$$= \left| \int \Psi_0^* \vec{\mu} \Psi_a \, d\tau \cdot \int \Psi_a^* \vec{\mu} \Psi_0 \, d\tau \right|$$

$$= \left| \int \Psi_0^* \vec{\mu} \Psi_a \right|^2$$

Magnetic dipole transition

Dipole strength

$$= \left| \int \Psi_0^* \vec{m} \Psi_a \, d\tau \right|^2$$

where $\vec{m} = \sum_i \frac{e}{2m_e c} (\vec{r}_i \times \vec{p}_i)$

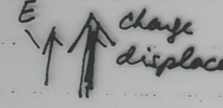
Circular Dichroism

Rotational strength

$$= \mathrm{Im} \left(\int \Psi_0^* \vec{\mu} \Psi_a \, d\tau \cdot \int \Psi_a^* \vec{m} \Psi_0 \, d\tau \right)$$

↑ dot product

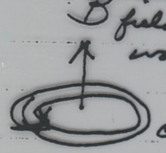
pure electronic absorption



$\left| \int \Psi_0^* \vec{\mu} \Psi_a \, d\tau \right|^2$

Light induced linear displacement

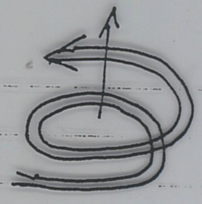
pure magnetic absorption



$\left| \int \Psi_a^* \vec{m} \Psi_0 \, d\tau \right|^2$

Light-induced current loop

optical activity



$\mathrm{Im} \int \Psi_0^* \vec{\mu} \Psi_a \, d\tau \cdot \int \Psi_a^* \vec{m} \Psi_0 \, d\tau$

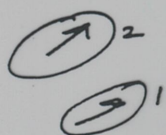
Helical structure facilitate flow of charge

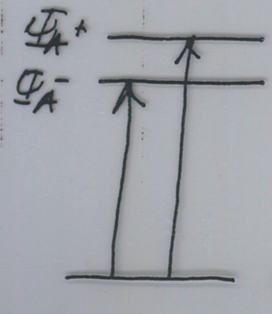
A light wave contains both oscillating electric and magnetic components. The electric component usually dominates pure absorption properties because magnetic effects are small. However, optical activity is a phenomenon that involves combined electric and magnetic interactions. Note dot product — critical feature. This means that for a molecule to be optically active, $\int \Psi_0^* \vec{m} \Psi_a \, d\tau$ must have a component parallel to $\int \Psi_0^* \vec{\mu} \Psi_a \, d\tau$. The molecule must be asymmetric for this to occur, otherwise there could be no preferred helical direction.

CD in Biomacromolecules

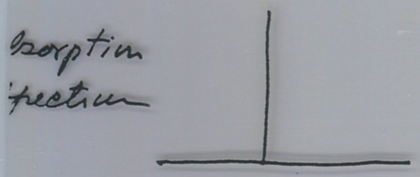
Most important contribution to CD in biomacromolecules arises in change in optical activity when a set of chromophores is assembled into an asymmetric macromolecular structure.

Consider 2 interacting chromophores; assume interaction is electric dipole-dipole interaction

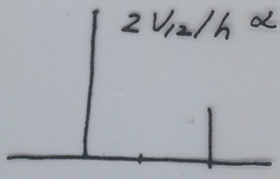




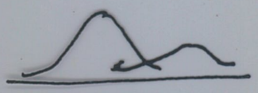
$\phi_0(1)\phi_a(2) \pm \phi_a(1)\phi_0(2)$



monomer



Dimer

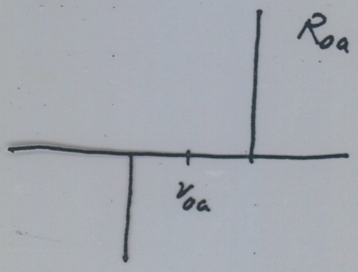


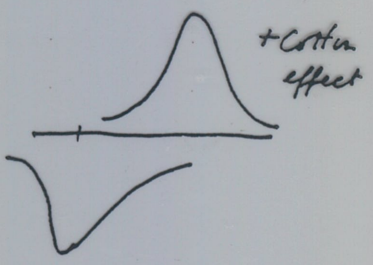
absorption spectrum

if V_{12} small

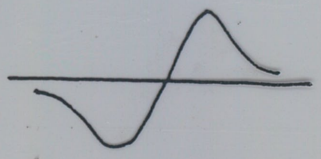
small exciton splitting

CD





Composite for 2 transitions



evidence for 2 interacting chromophores.

exciton interaction contribution to CD depends on distance between chromophores ($\propto R_{12}$), & geometry of molecule. Can be large, even if monomeric chromophores are not optically active.

Excitan contributions to $R_{oa} = 0$ if chromophores are coplanar, or if they are oriented parallel or perpendicular to one another.

Example (1) poly L-alanine

a) No contribution from side chain
b) There is near UV $\pi - \pi^*$ and $n - \pi^*$ electronic transition of peptides

CD spectrum on next page.

CD spectrum sensitive to α-helix, β-sheet, random coil

(2) Membrane Proteins

Transmembrane domains are mainly α-helical.

ORD away from λ_{oa}

Optical Rotation at λ far removed from absorption band

$$[m'] = \frac{96 N \pi}{hc} \left(\frac{n^2+2}{3}\right) \left(\frac{\lambda_{oa}^2 R_{oa}}{\lambda^2 - \lambda_{oa}^2}\right)$$ for 1 absorption band at λ_{oa}

Form of equation explains why molecules such as proteins can demonstrate substantial optical rotation with visible light even though they cannot absorb this light

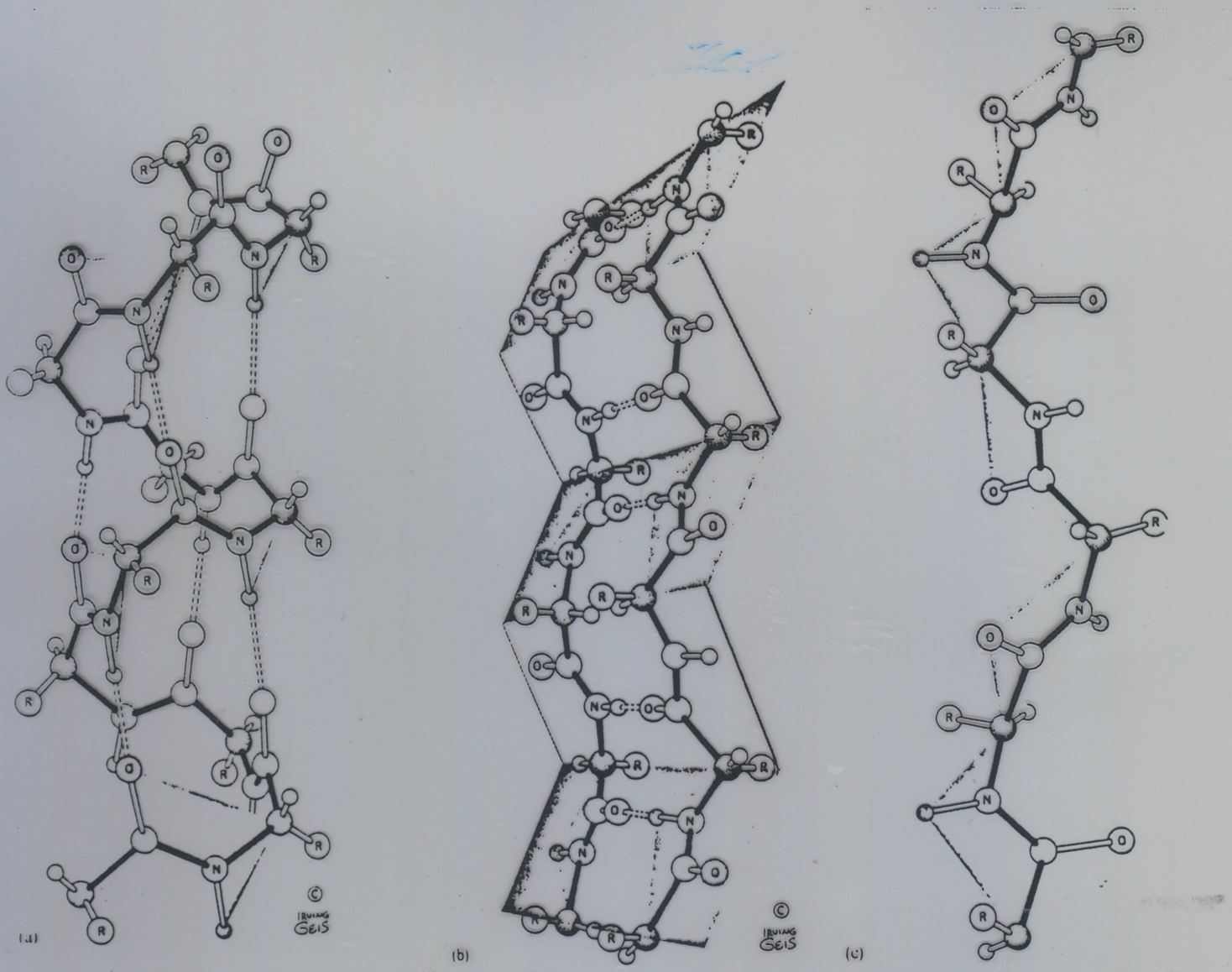


Figure 2-23

Three of the most common polypeptide secondary structures. Only atoms in the peptide-chain backbone are shown. A parallelogram indicates the plane of each peptide bond. (a) The right-handed α helix. (b) Two antiparallel β-sheet strands. Note how alternate side chains will be placed on opposite sides of the sheet. (c) The left-handed polyproline II helix. [Drawings by Irving Geis.]

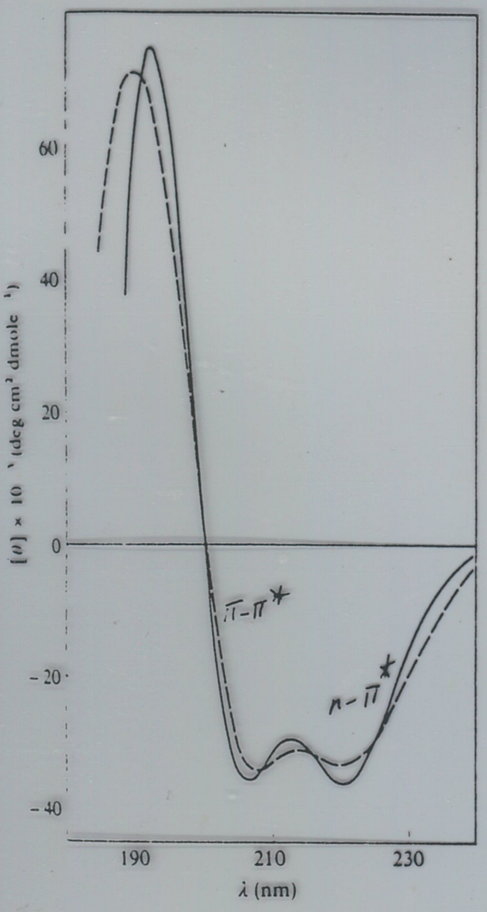


Figure 8-8

CD spectrum of poly-L-alanine in an α-helical conformation. Calculated (*dashed line*) and observed (*solid line*) spectra are shown. [After R. W. Woody, *J. Chem. Phys.* 49:4797 (1968).]

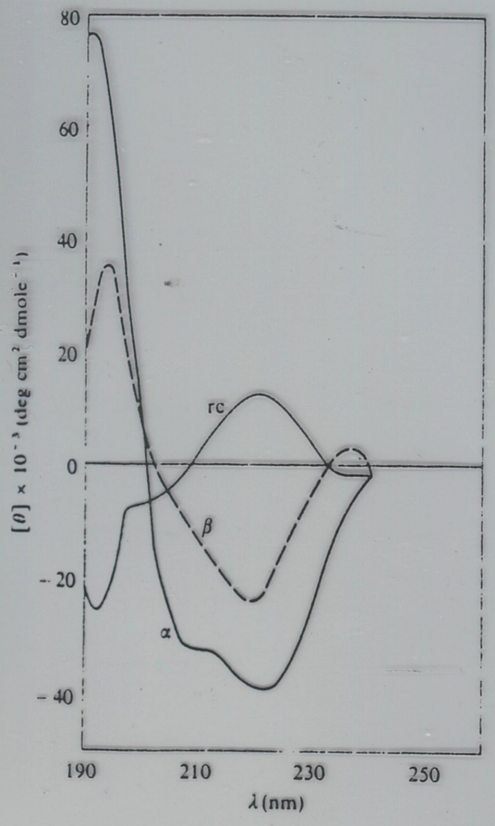


Figure 8-9

CD spectra for α-helix, β-sheet, and random coil conformations, extracted from the spectra of proteins of known three-dimensional structure by the Wetlaufer method. [After V P Saxena and D. B. Wetlaufer, *Proc. Natl. Acad. Sci. USA* 66:969 (1971).]

For more than 1 absorption band,

$$[m'] = \frac{96 N \pi}{hc} \frac{n^2+2}{3} \sum_i \frac{\lambda_{0i}^2 R_i}{\lambda^2 - \lambda_{0i}^2}$$