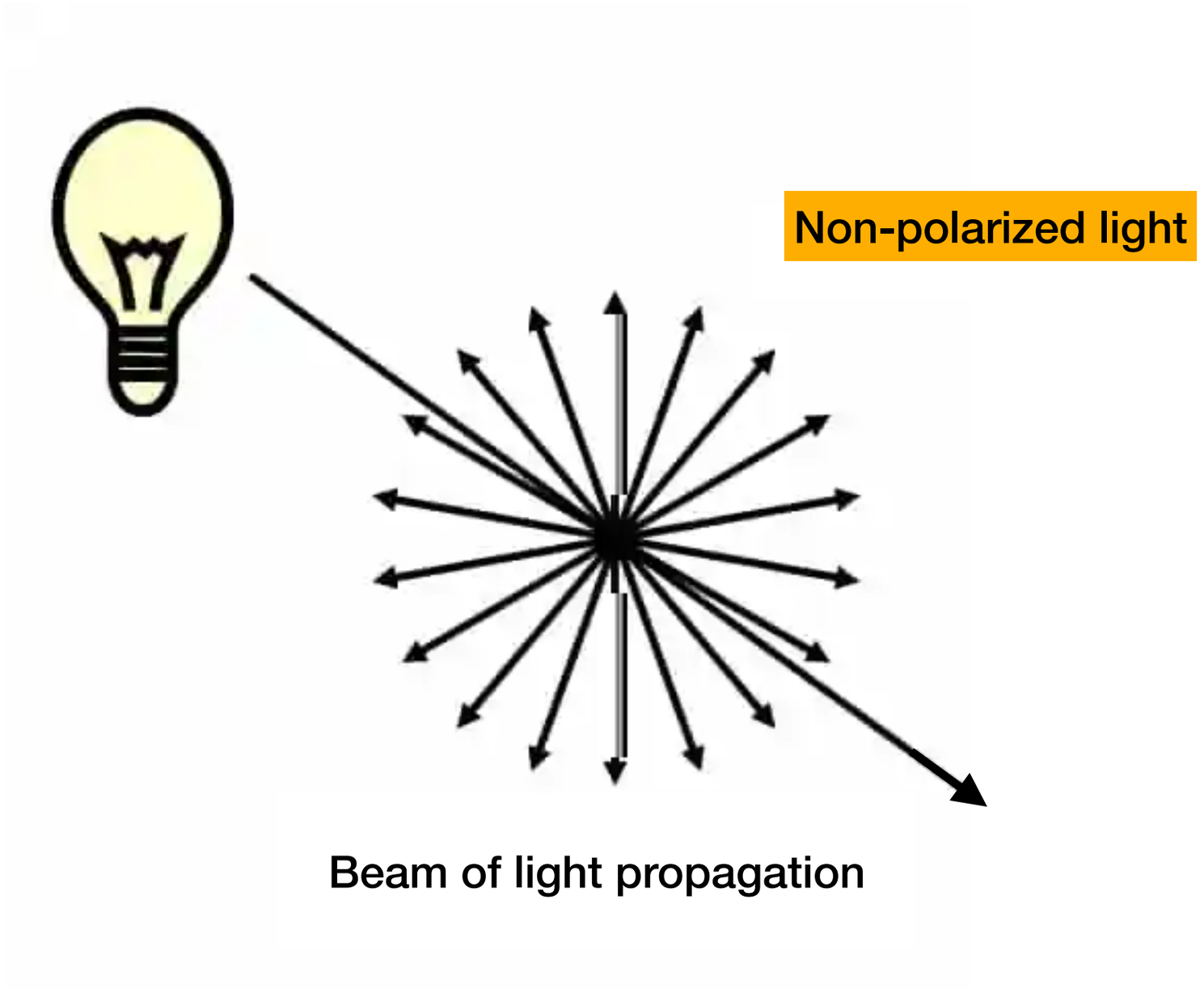
Today’s class:

Absorption Spectroscopy part 3

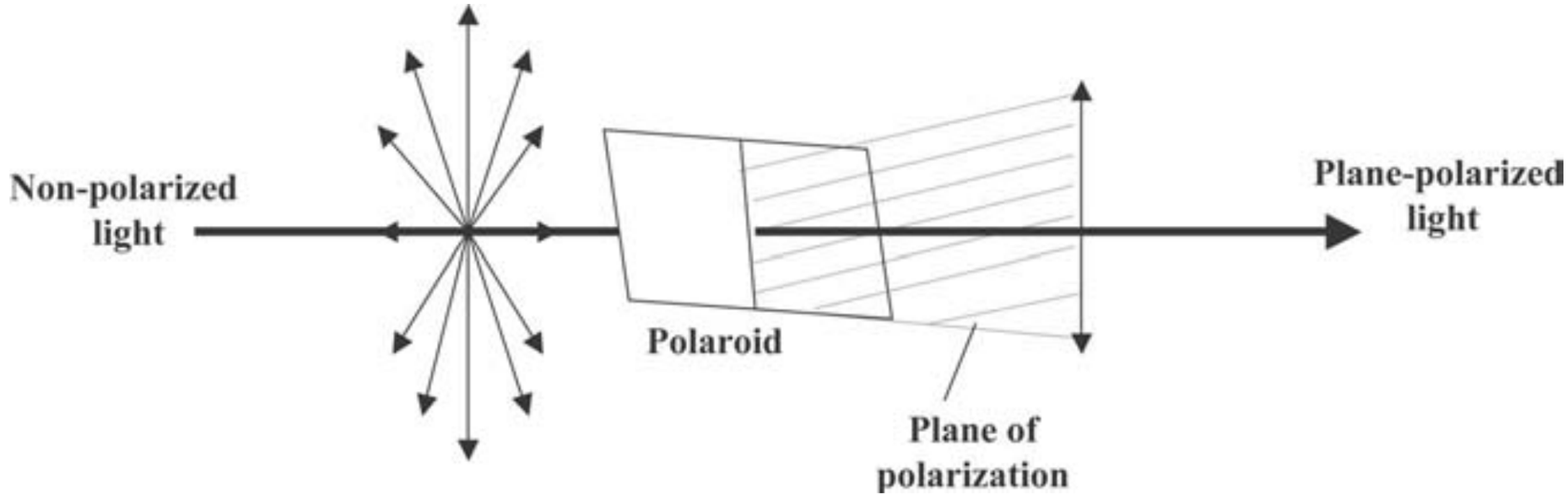
This lecture follows the materials from the following book

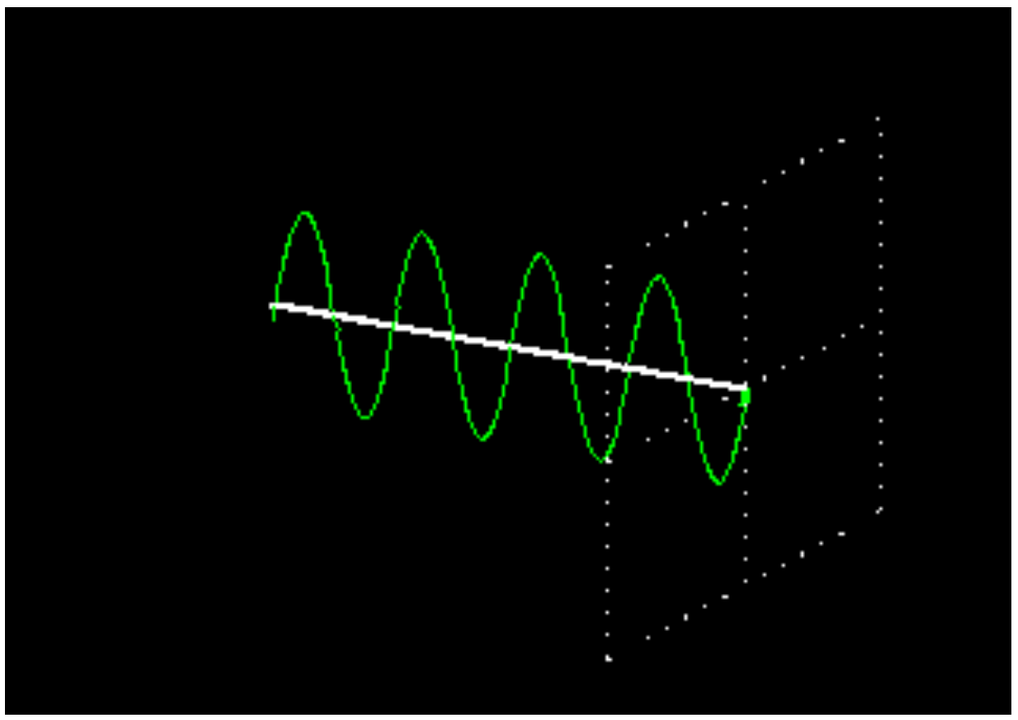
- *Physical Biochemistry by David Sheehan, 2nd Ed, Wiley, 2009*

Light for normal sources vibrate in all directions



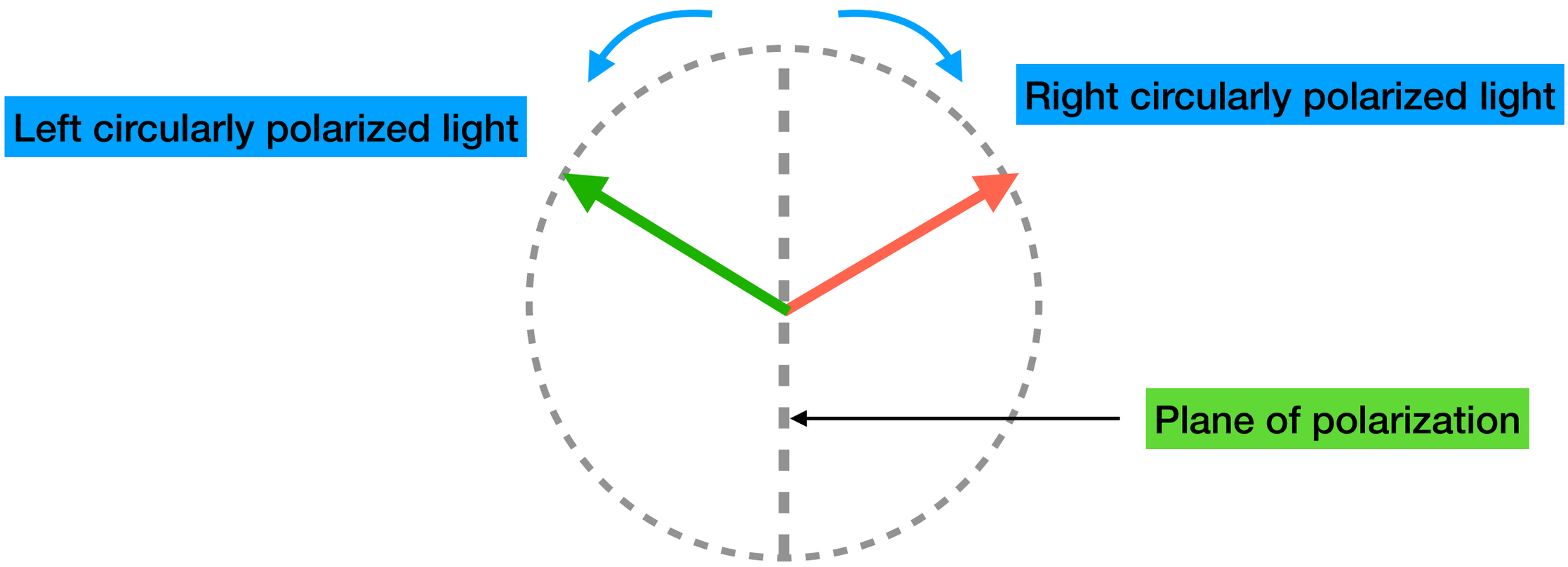
Light for normal sources can be forced to vibrate in one direction





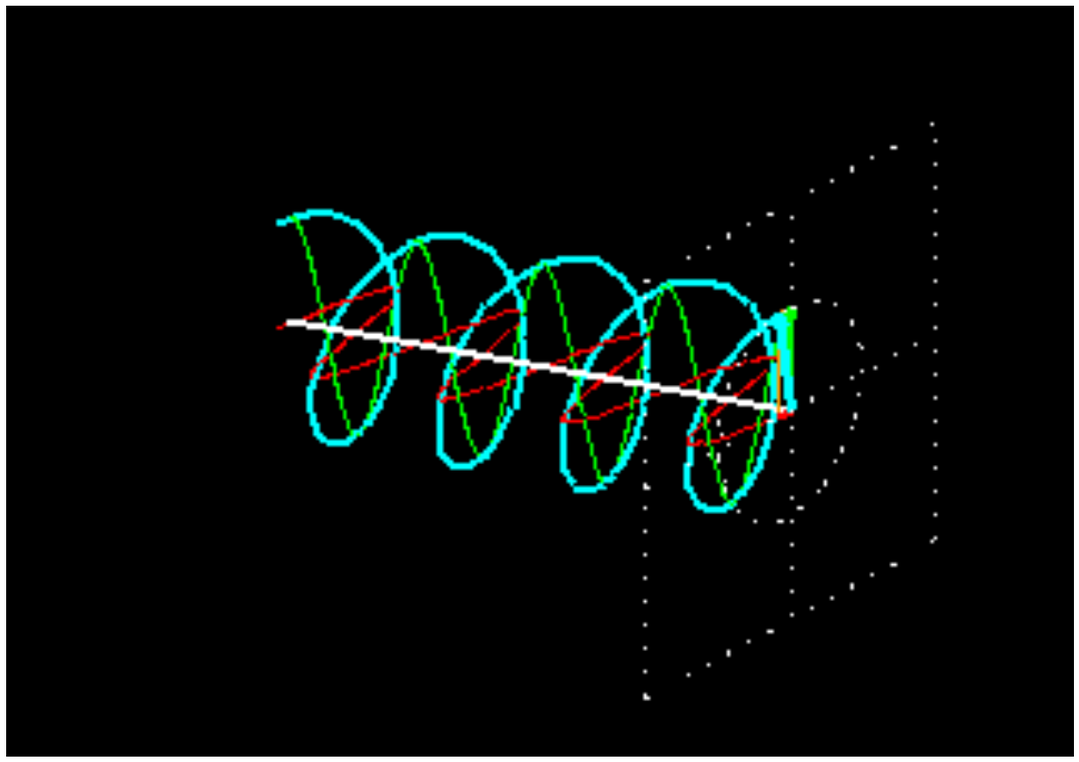
Linearly polarized light
or
Vertically polarized light

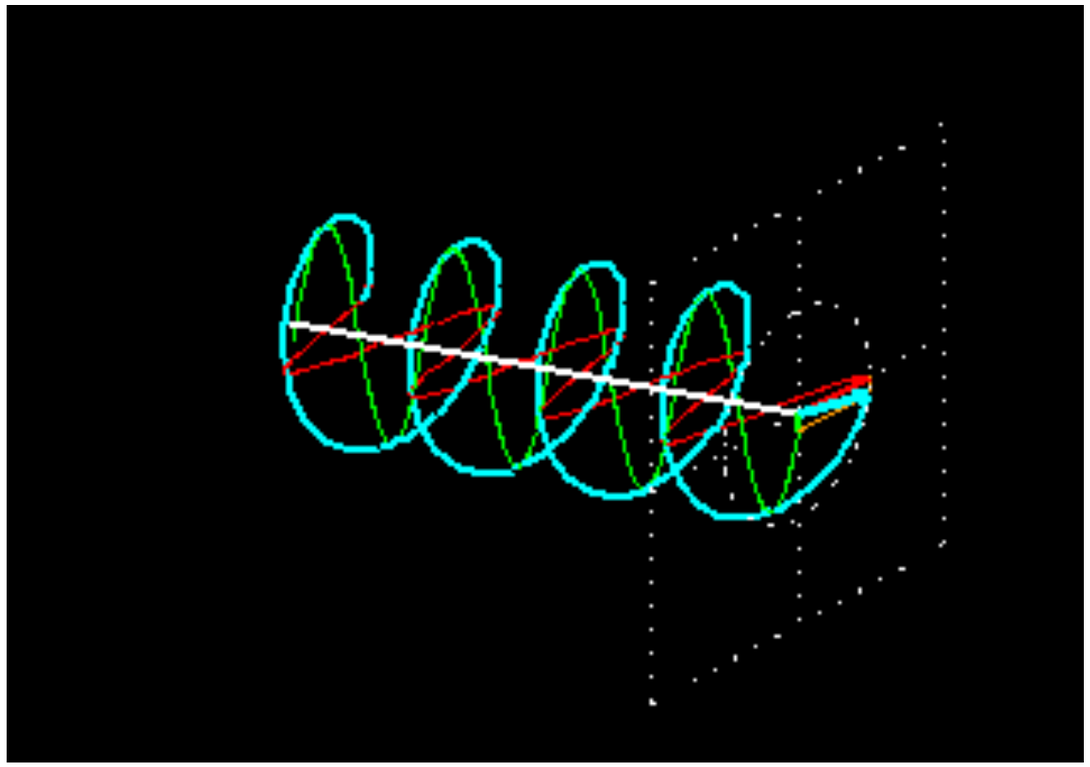
Plane polarized light is made of two components

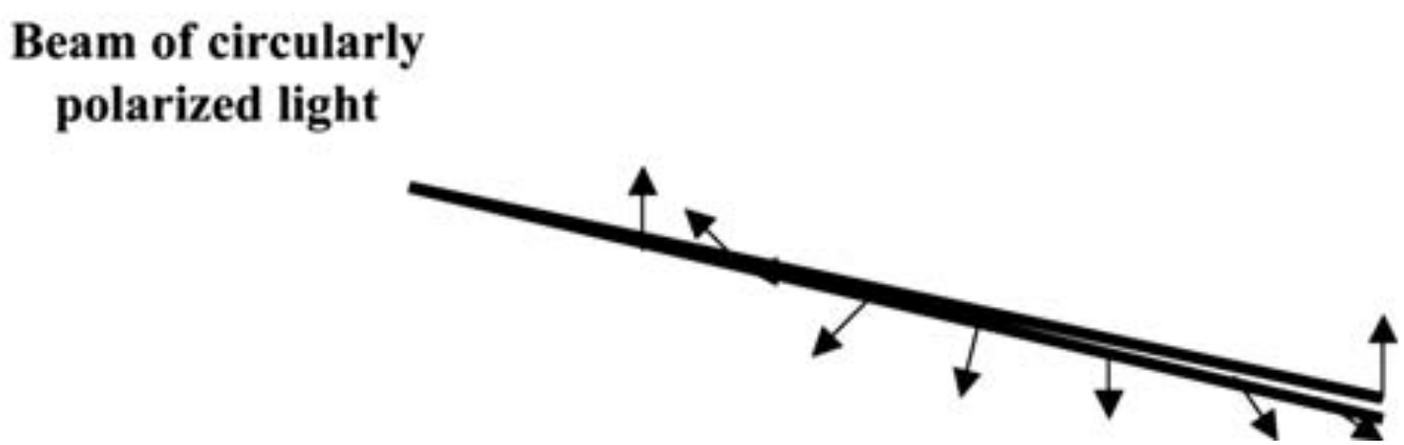


The two components are equal in strength but opposite in rotation

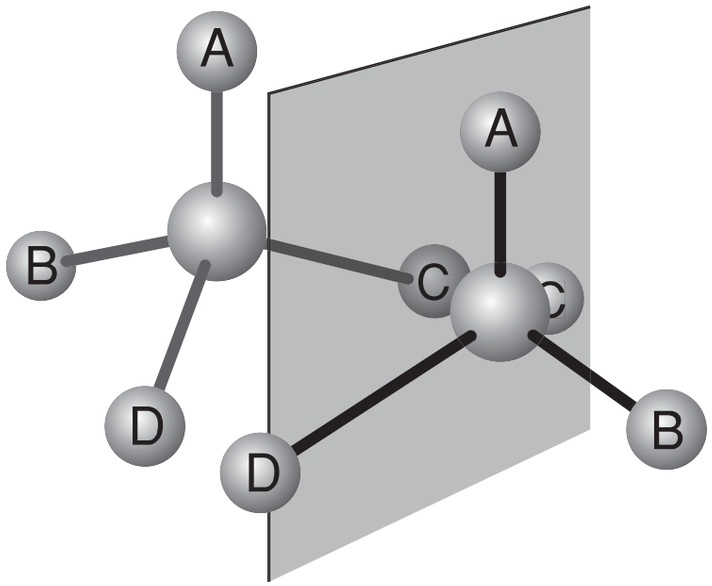
Beam of circularly polarized light traces a helical path







Chirality leads to differential interaction with polarized light

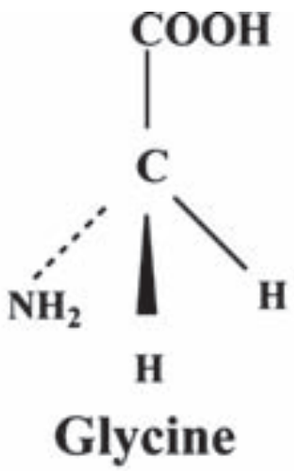


Chiral molecule = not superimposable with its mirror image

Chiral center = C-atom with four different substituents

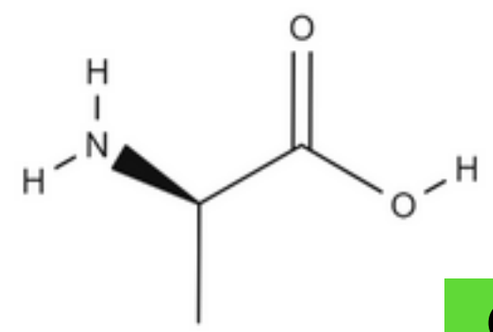
For any chiral center there are two enantiomers possible - D & L

D and L-enantiomers interact differently with polarized light



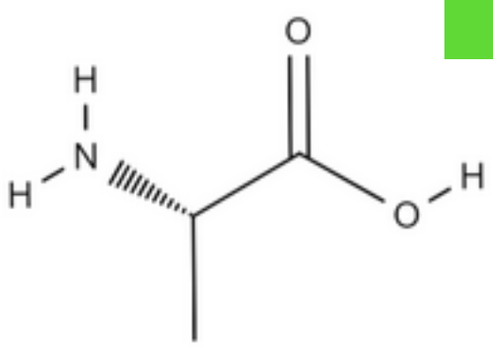
Glycine

Not chiral



D-Alanine

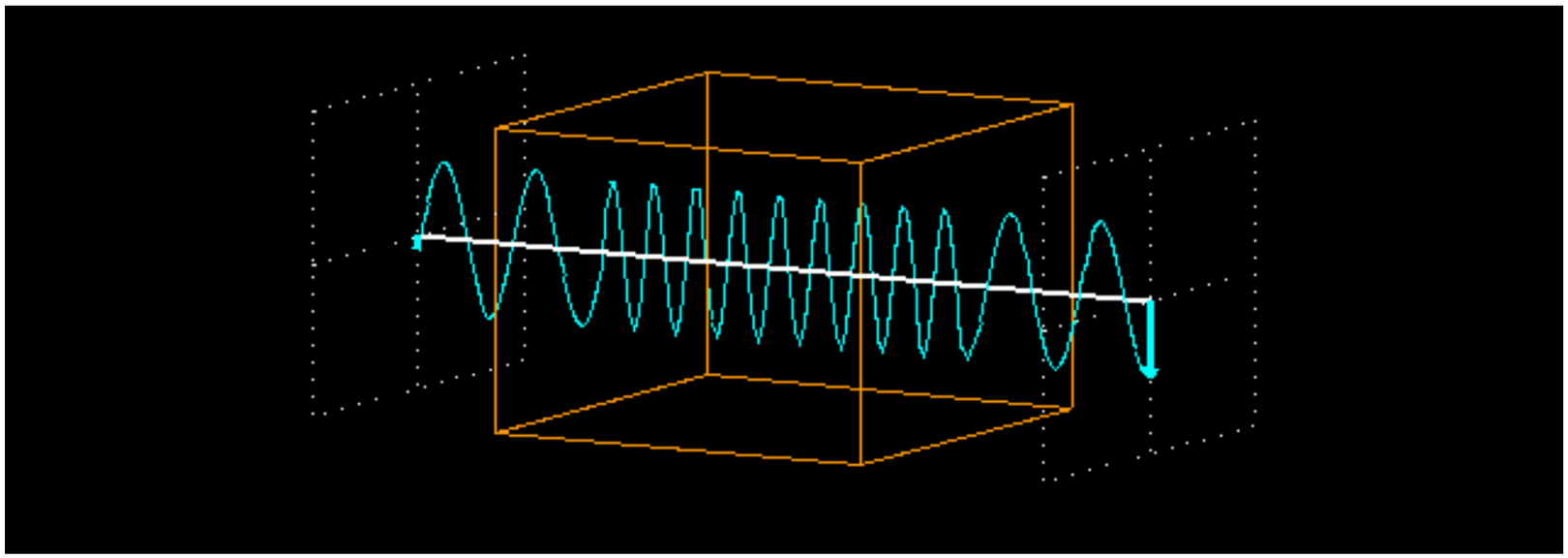
Chiral but only L-form is found in proteins



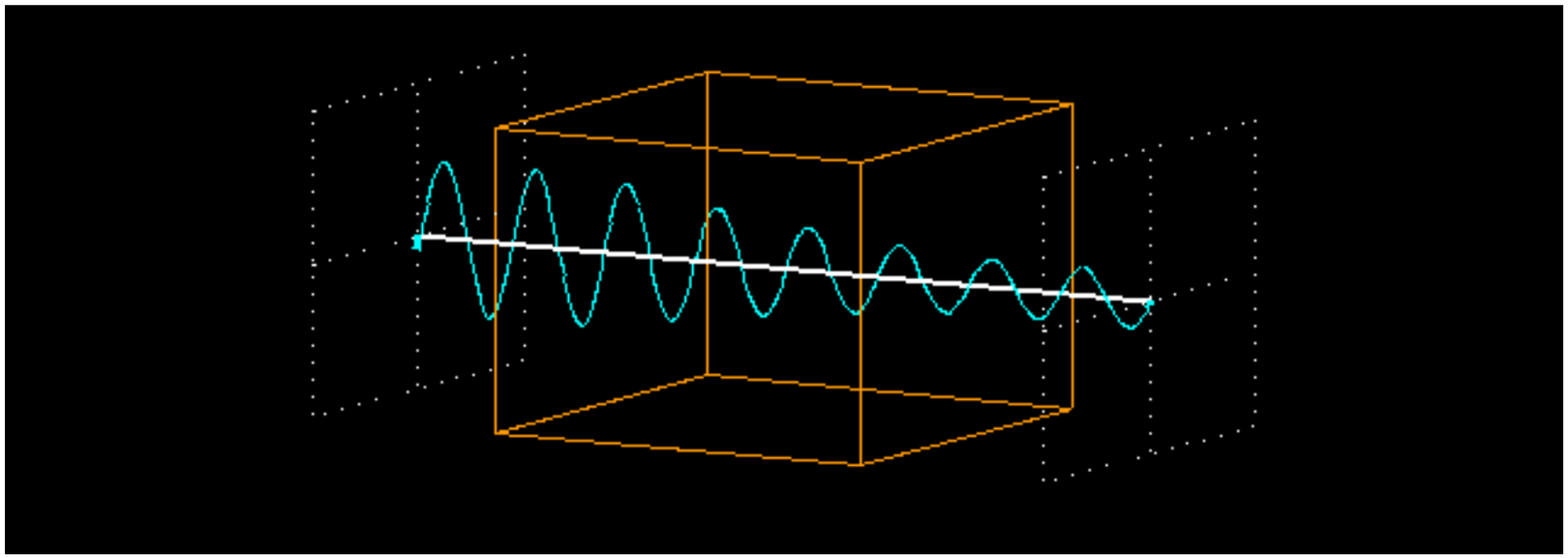
L-Alanine

Chiral samples are called optically active

Differential interaction of chiral samples with light



Refraction

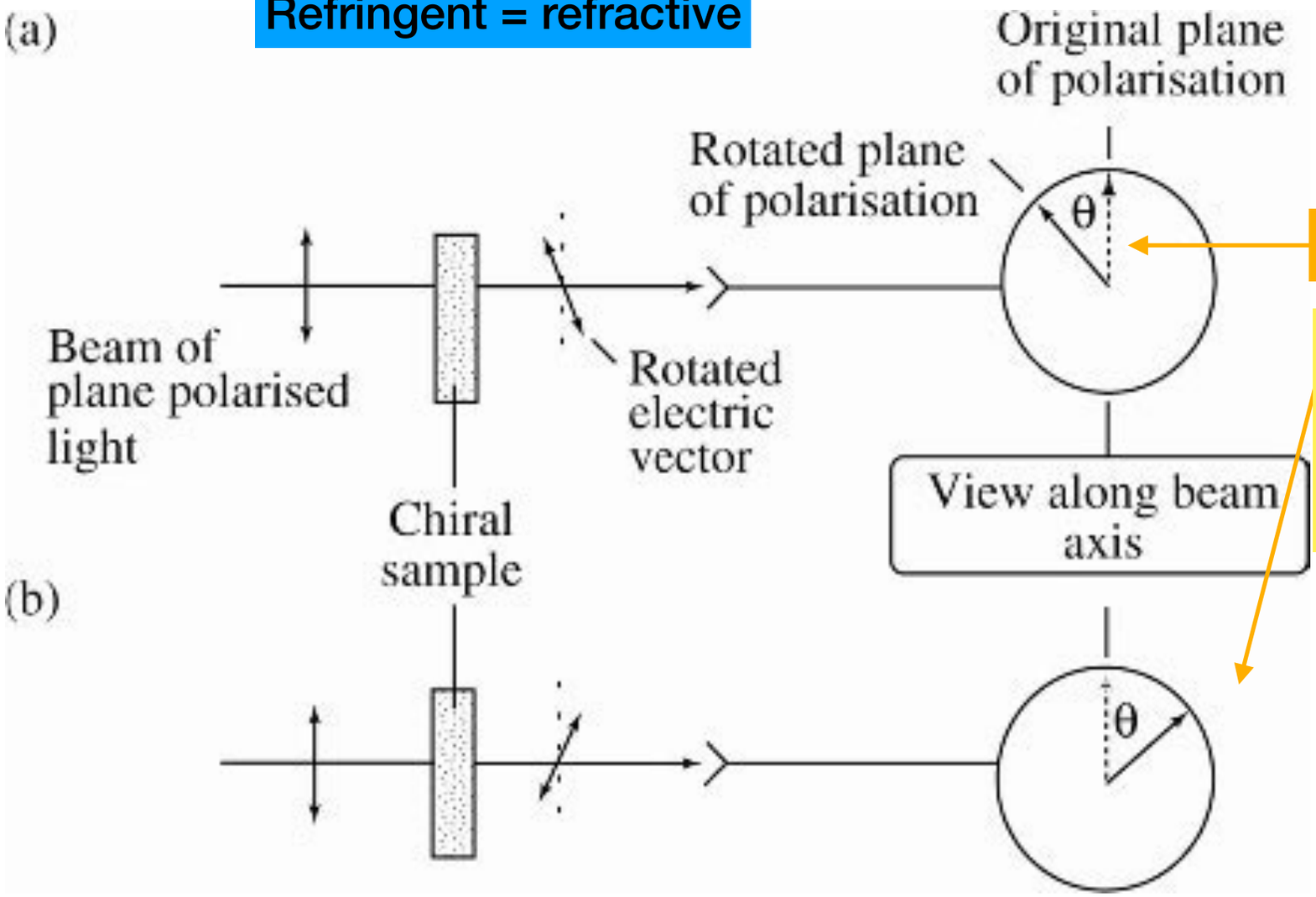


Absorption

These two phenomena can be used to characterized the structure of the chiral molecules

Solutions of chiral molecules exhibit circular birefringence

Refringent = refractive



Optical rotation

This phenomenon is also called optical rotatory dispersion or ORD

Origin of this rotation

Different refractive index for LCP and RCP

$$n_L \neq n_R$$

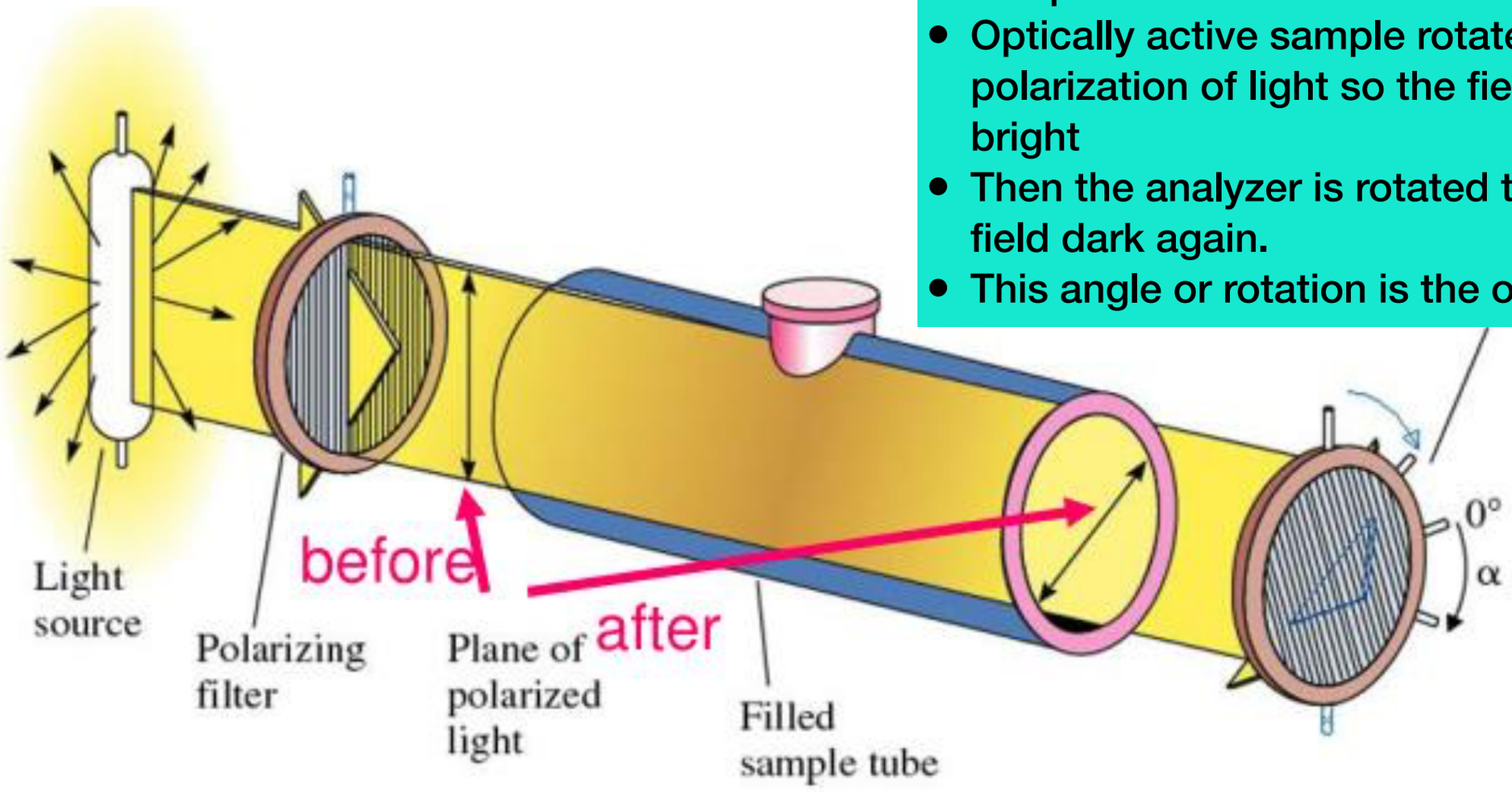
Optical rotation

$$\alpha = \frac{n_L - n_R}{\lambda}$$

Unit of α: Radians per unit length

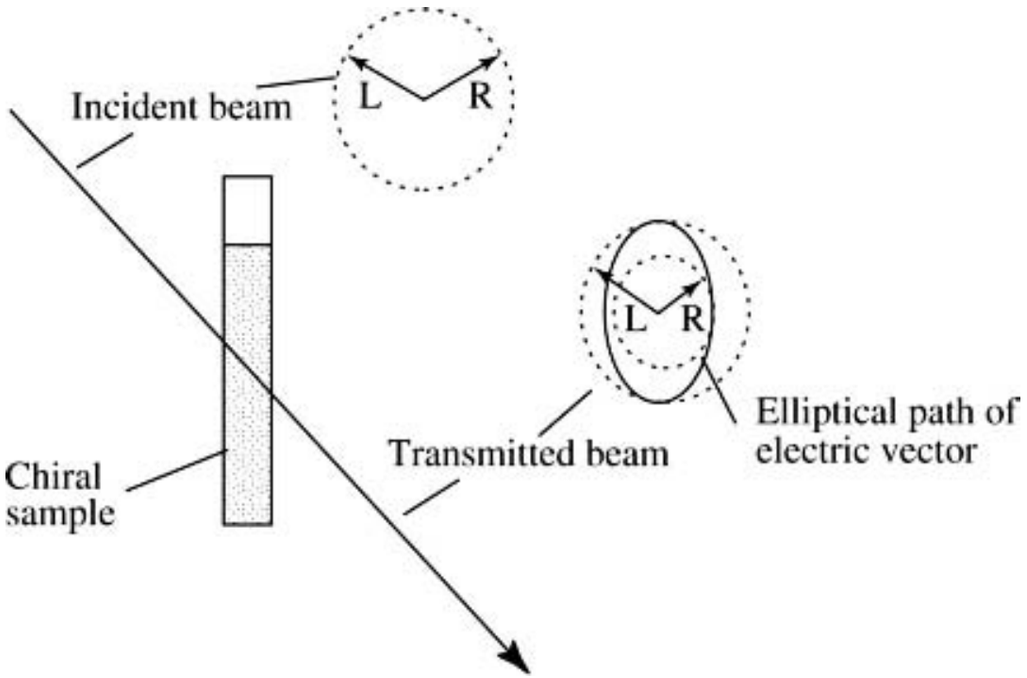
Optical rotations are measured in polarimeter

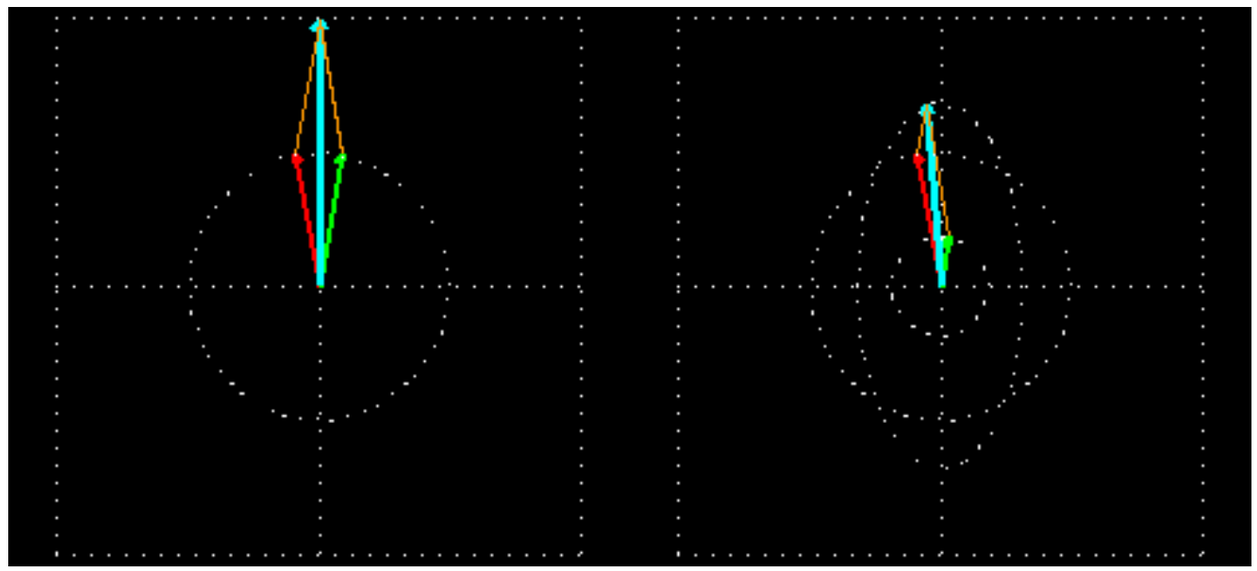
- Field of view is adjusted so its dark when no sample
- Optically active sample rotate the plane of polarization of light so the field become bright
- Then the analyzer is rotated to make the field dark again.
- This angle or rotation is the optical rotation



Optical rotation as a function of wavelength $\alpha(\lambda)$ is called the ORD spectrum

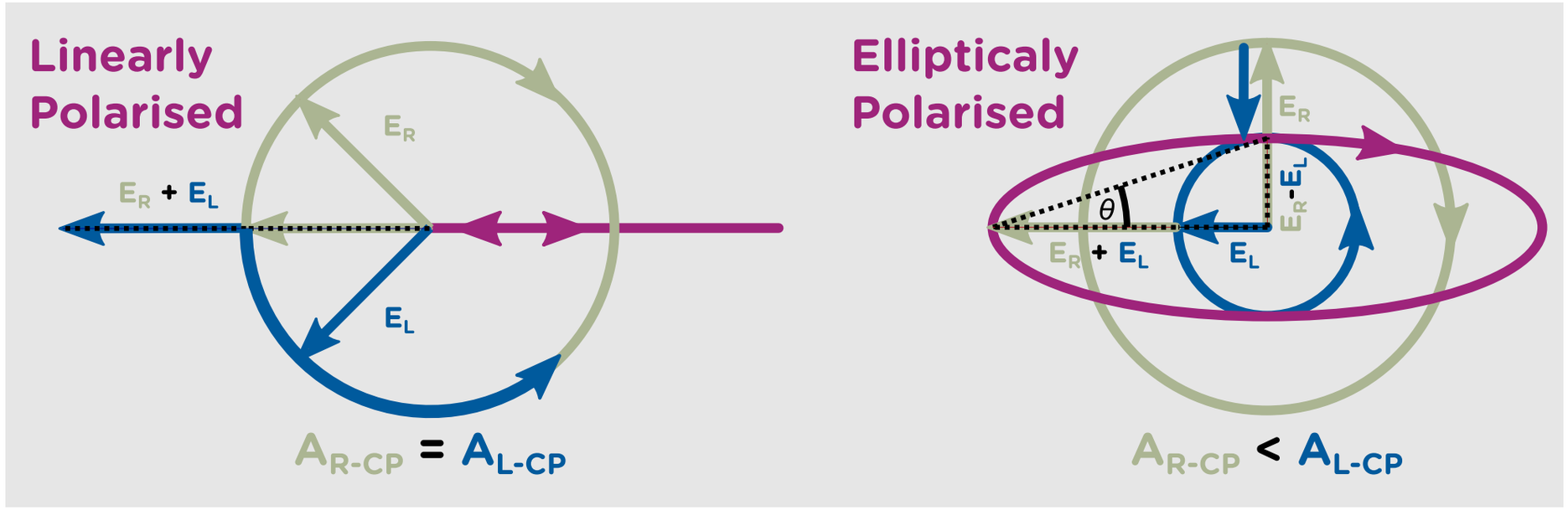
Solutions of chiral molecules absorb LCP and RCP differently





- Different molar extinction coefficient for LCP and RCP: $\epsilon_L \neq \epsilon_R$
- LCP and RCP are absorbed by different amount: $\Delta A = \Delta \epsilon c l$
- The combined beam of light becomes elliptically polarized
- This phenomenon is called circular dichroism measured by $\Delta \epsilon$

Circular dichroism is reported as ellipticity



- Ellipse of the elliptically polarized light has a major axis given by $E_R + E_L$ and a minor axis given by $E_R - E_L$
- The ellipticity is given by the angle θ defined by: $\tan\theta = \dfrac{E_R - E_L}{E_R + E_L}$
- For linearly polarized light, $E_R = E_L \implies \tan\theta = 0$
- As the typical $E_R - E_L$ is very small we can express $\tan\theta \approx \theta$ (radians)
- θ (radians) can be converted to degrees by: $\theta = \dfrac{180 \times ln10 \times \Delta A}{4\pi}$ (degrees)

$$\implies \theta \approx 32.982\ \Delta A$$

Molar ellipticity

To compare results from different experiments θ is often expressed as molar ellipticity

$$[\theta] = \frac{100\theta}{cl}$$

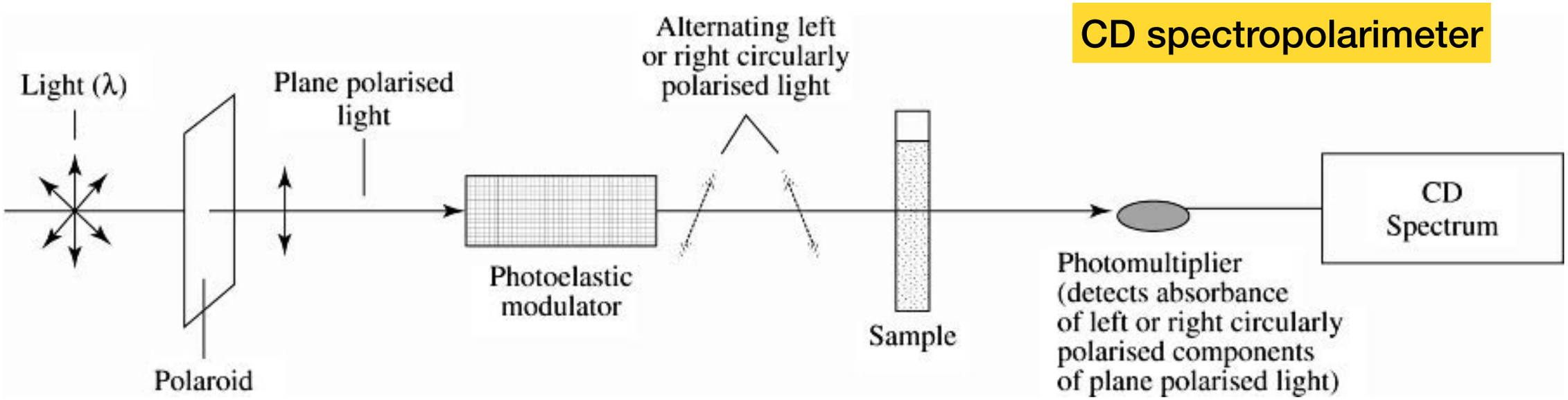
Here, $[\theta]$ is in $\mathrm{deg\ M^{-1}\ m^{-1}}$,
c in M and l in cm

This convention introduces the factor 100

Molar ellipticity can also be written in terms of the circular dichroism $\Delta\epsilon$

$$[\theta] = \frac{100\theta}{cl} = \frac{3298.2\ \Delta A}{cl} = 3298.2\ \Delta\epsilon$$

Circular dichroism spectroscopy



- Here LCP and RCP beams are not combined
- The photomultiplier detector converts the light into two different signals
- One signal is DC that gives the regular absorption spectra
- One signal is AC that gives the CD spectra in terms of ellipticity

Applications of CD spectroscopy

- CD has an important role in the structural determinants of proteins or DNA or RNA, etc.

- Comparing with characteristic CD spectrum of known homo-polymers of a specific secondary structure one can estimate the % of helix, sheet or coil in an unknown protein or GC content in an unknown nucleic acid.

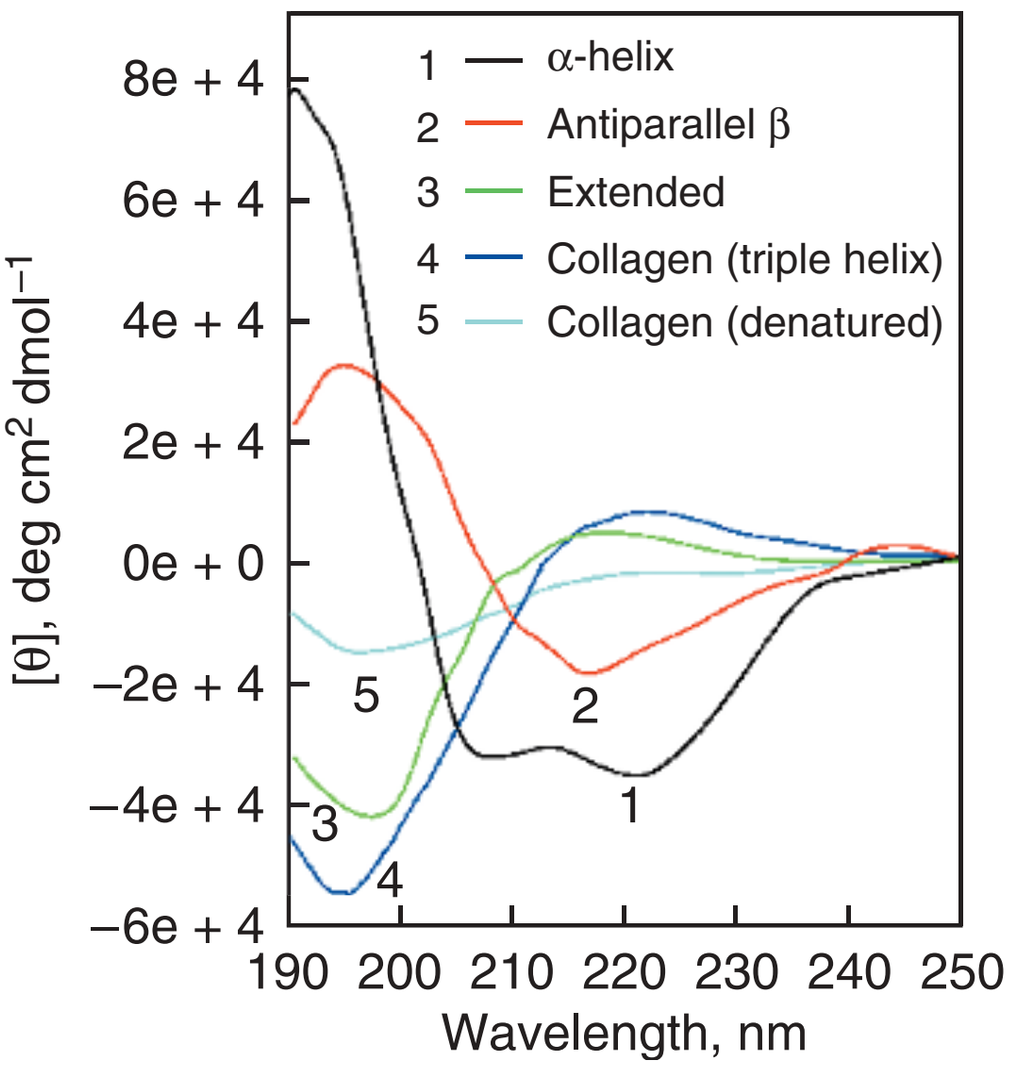
- The real power of CD is in the analysis of structural changes in a protein or DNA or RNA upon some perturbation, or in comparison of the structure of an engineered protein or DNA or RNA to the parent protein or DNA or RNA.

- CD spectra can be used to follow protein folding.

- CD is rapid and requires a very low concentration of sample. So it can be used to analyze a large number of candidate proteins in quick time from which interesting candidates can be selected for more detailed structural analysis like NMR or X-ray crystallography.

CD spectra of polypeptides and proteins

NJ Greenfield, Nature Protocols 2006



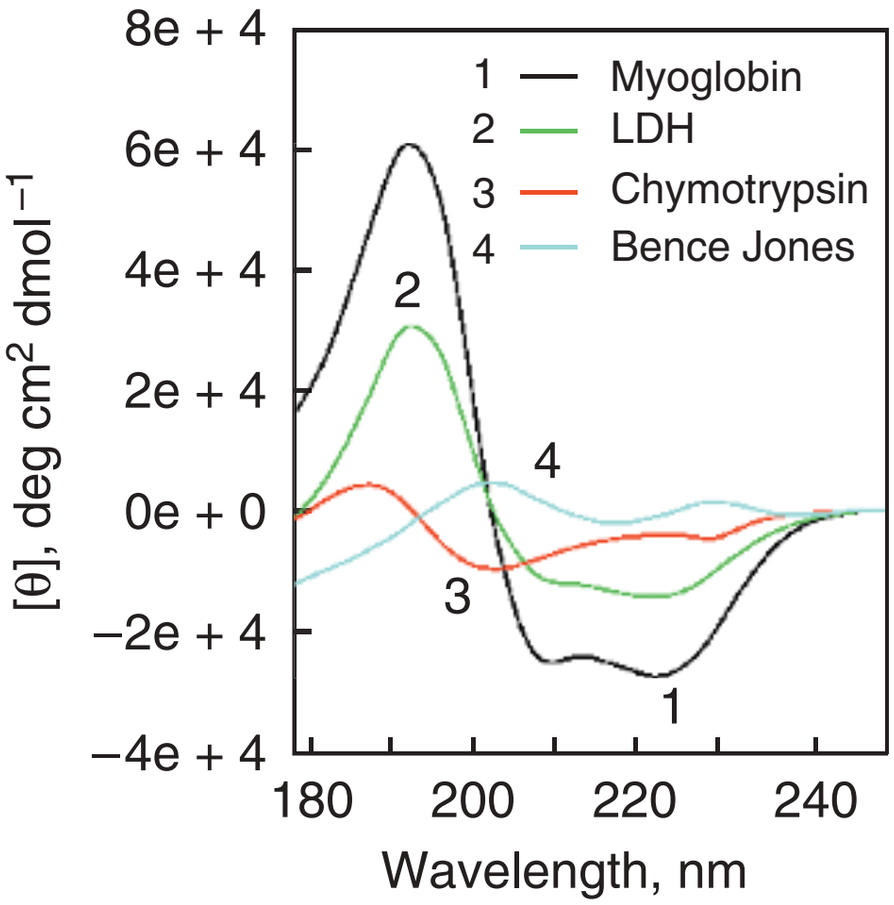
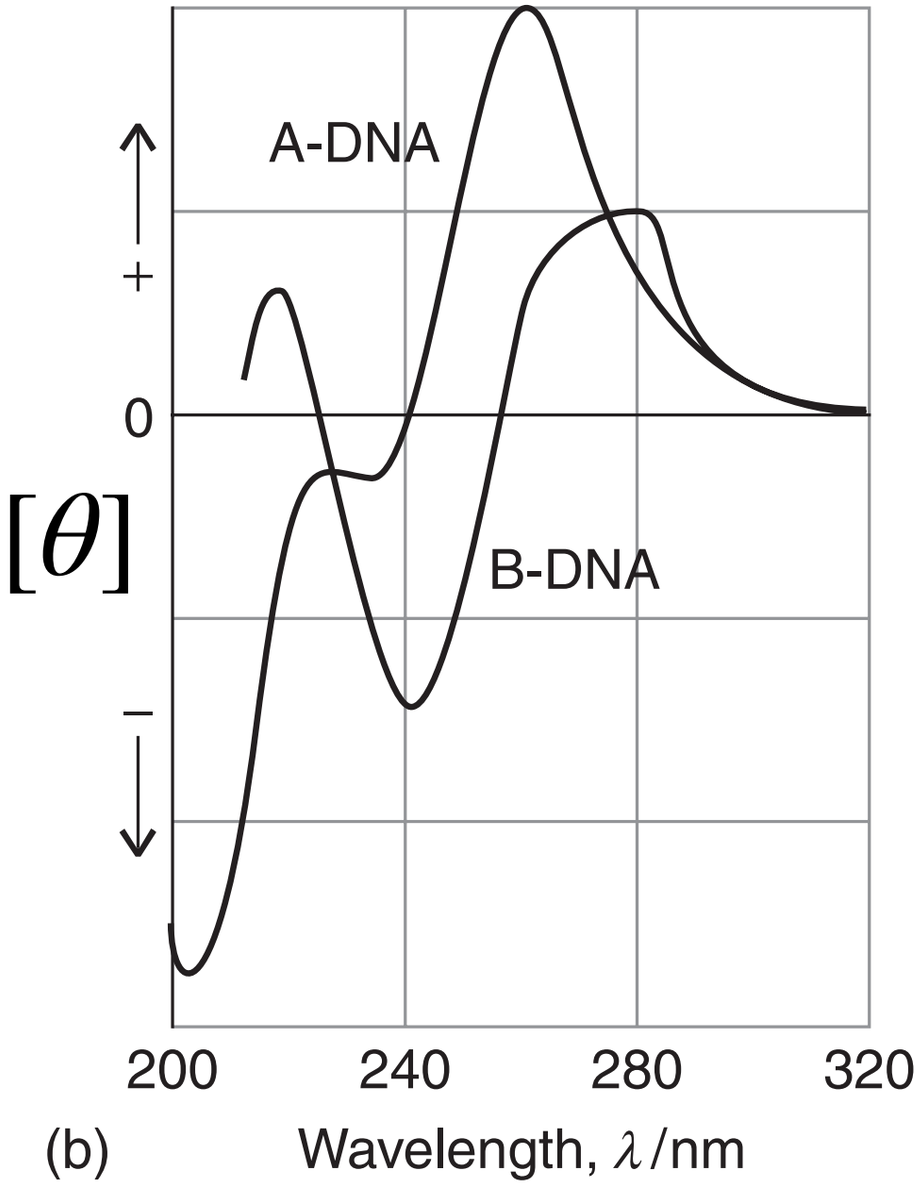


Figure 1 | CD spectra of polypeptides and proteins with representative secondary structures. (**a**) CD spectra of poly-L-lysine at pH 11.1 in the (1, black) α-helical and (2, red) antiparallel β-sheet conformations and at pH 5.7 in the (3, green) extended conformations[5] and placental collagen in its (4, blue) native triple-helical and (5, cyan) denatured forms[64]. (**b**) CD spectra of representative proteins with varying conformations: 1 (black), sperm whale myoglobin; 2 (green), chicken heart lactate dehydrogenase; 3 (red), bovine α-chymotrypsin and 4 (cyan), human Bence Jones protein REI light chain, which is a human immunoglobulin light chain of κ type. Spectra are from data sets supplied by Dr. W.C. Johnson (Oregon State University, Corvallis, Oregon, USA).

CD spectra of nucleic acids

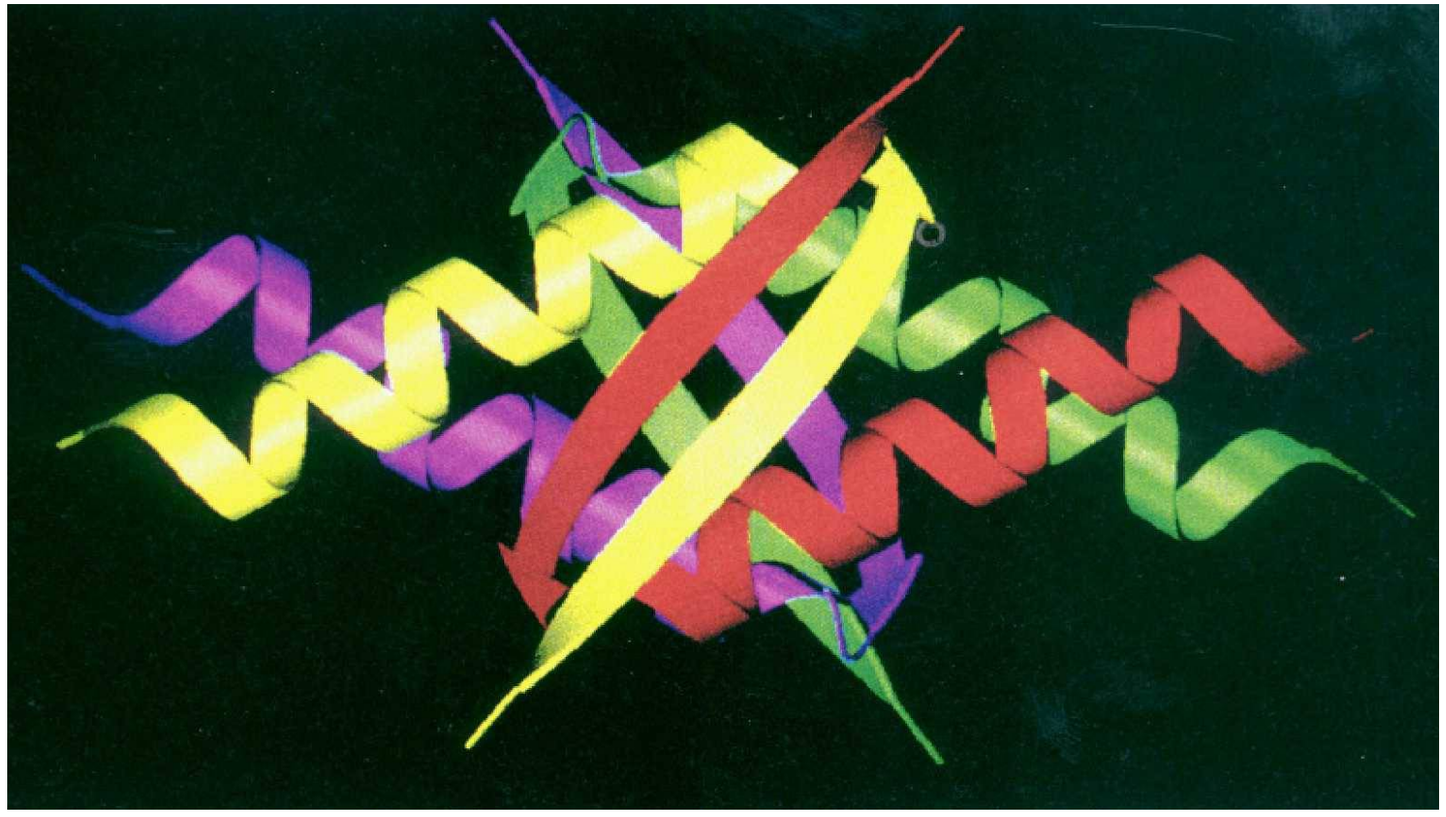


- Chirality in nucleic acids arises due to presence of pentose sugar
- For small DNA or RNA the signal is very weak
- As length of chain increases and higher order structures, like double helix form the signal strength becomes higher
- CD spectra is sensitive to different factors listed below:
  - variation in sequences,
  - GC composition,
  - base-stacking in different forms of DNA (A-DNA, B-DNA or Z-DNA),
  - ligand binding,
  - formation of macromolecular assemblies, like nucleosomes.

Case study: CD of protein structural stability

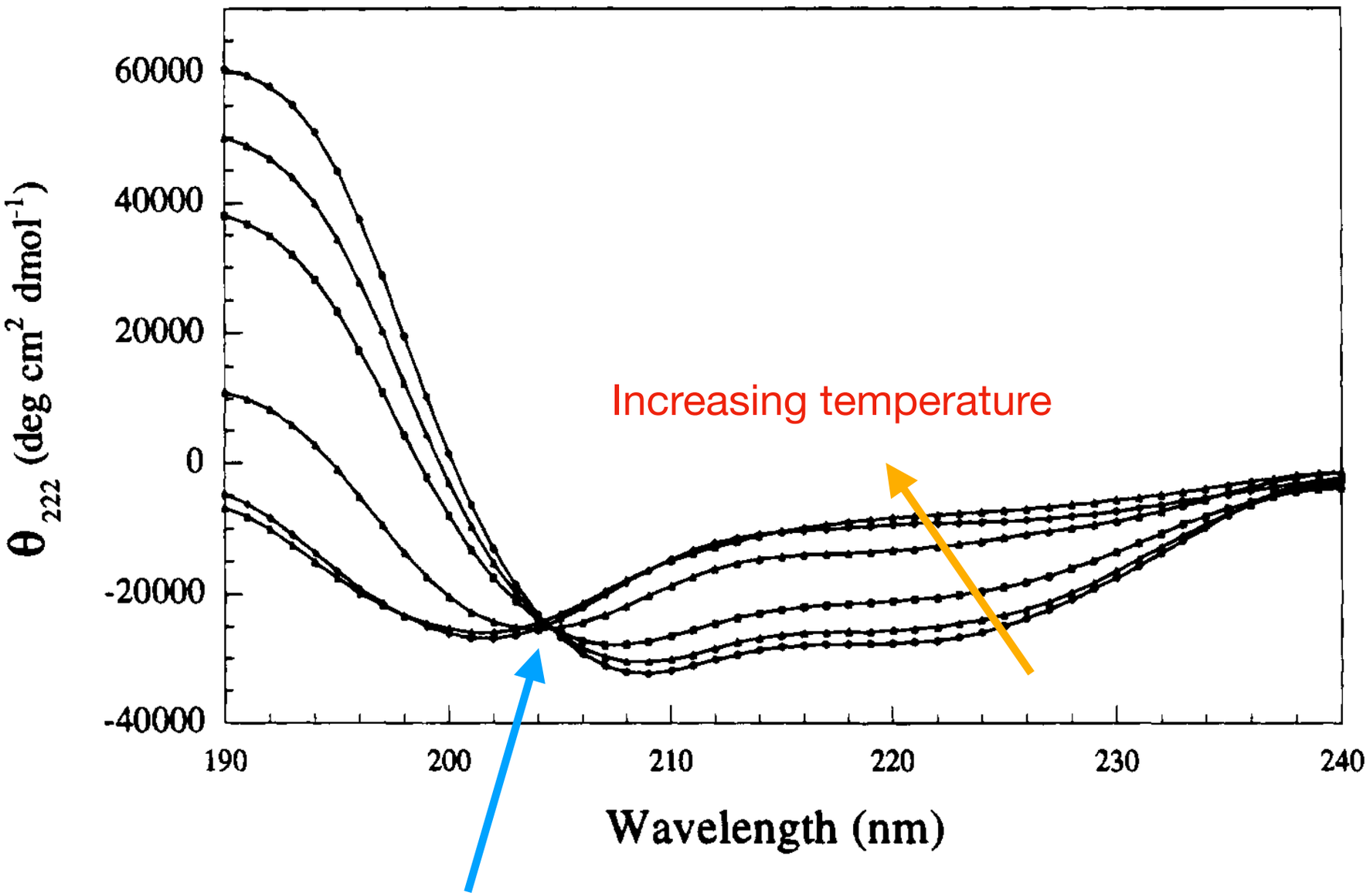
- p53 is a tumor supressor protein in humans.
- It is one of the most commonly mutated protein in human tumors.
- Functionally important part of the structure of p53 is the tetramerization domain which includes residues 325–355.
- **p53tet** (res. 325-355) is the smallest known protein tetramer.

Johnson et al, Biochemistry 1995



Case study: CD of protein structural stability …*contd*

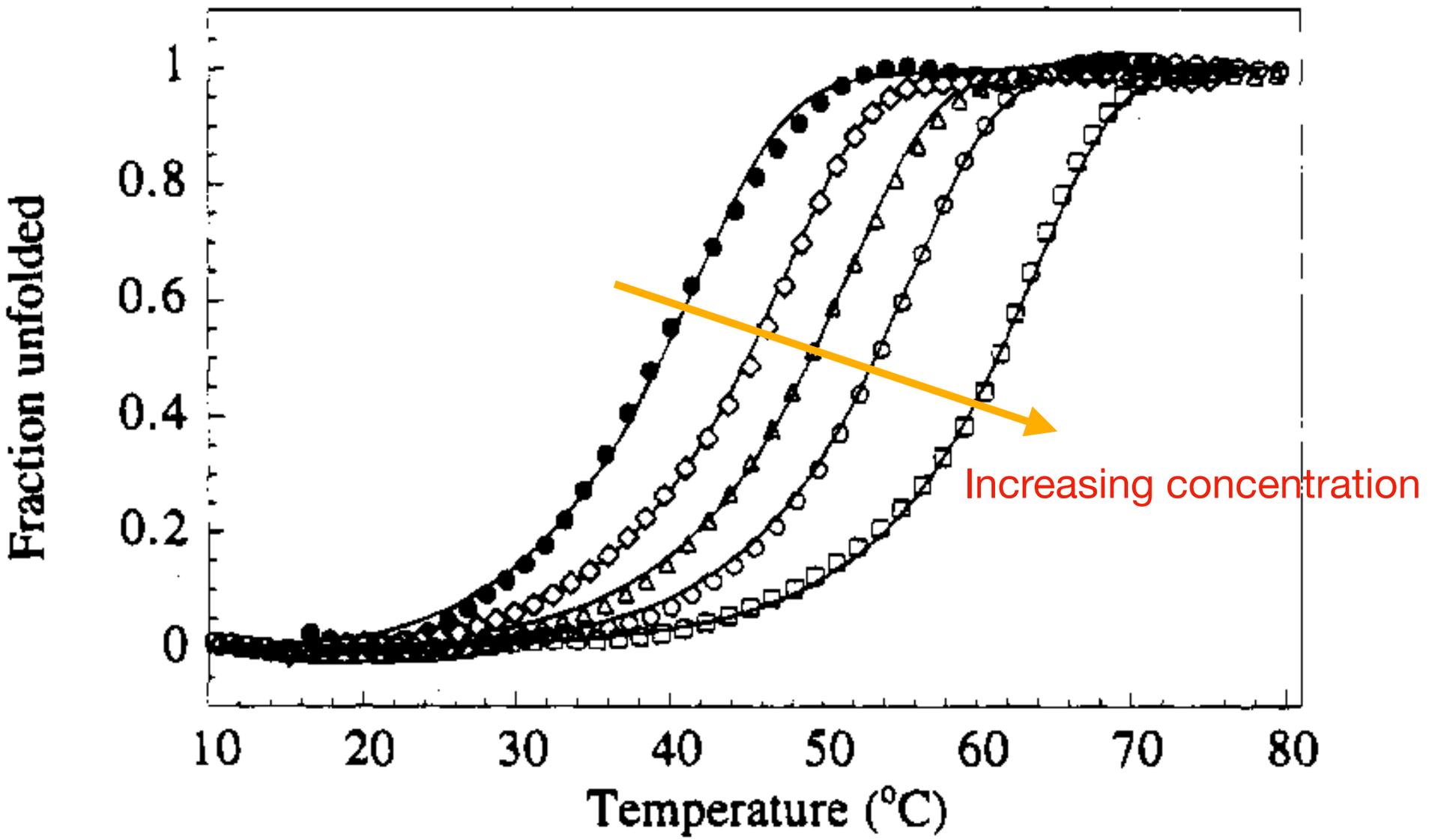
Johnson et al, Biochemistry 1995



The intersection of all the CD traces at a single point called the ***isodichroic* point** is characteristic for a two-state system such as a protein only existing in either a folded or unfolded state

Case study: CD of protein structural stability …*contd*

Ellipticity readings at 222 nm expressed as a fraction unfolded p53tet



Johnson et al, Biochemistry 1995