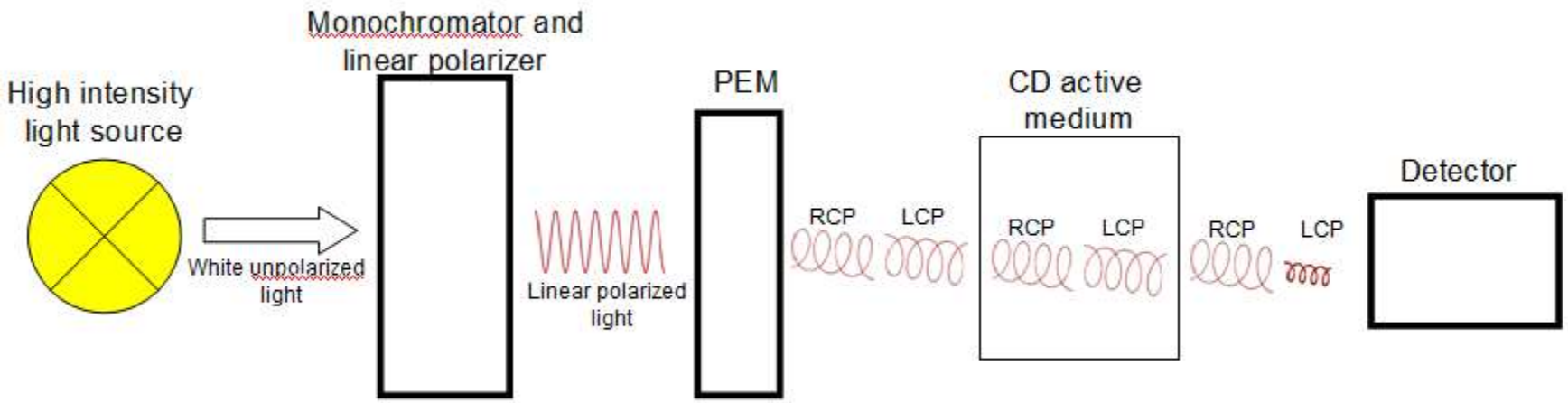
Circular Dichroism and Absorption Spectroscopy

Lecture of ZOOL 5002



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What is Circular Dichroism?

• Circular Dichroism (CD) is a type of absorption spectroscopy that can provide information on the structures of many types of biological macromolecules

• It measures the difference between the absorption of left and right handed circularly-polarized light by proteins. CD is used for;

• Protein structure determination.

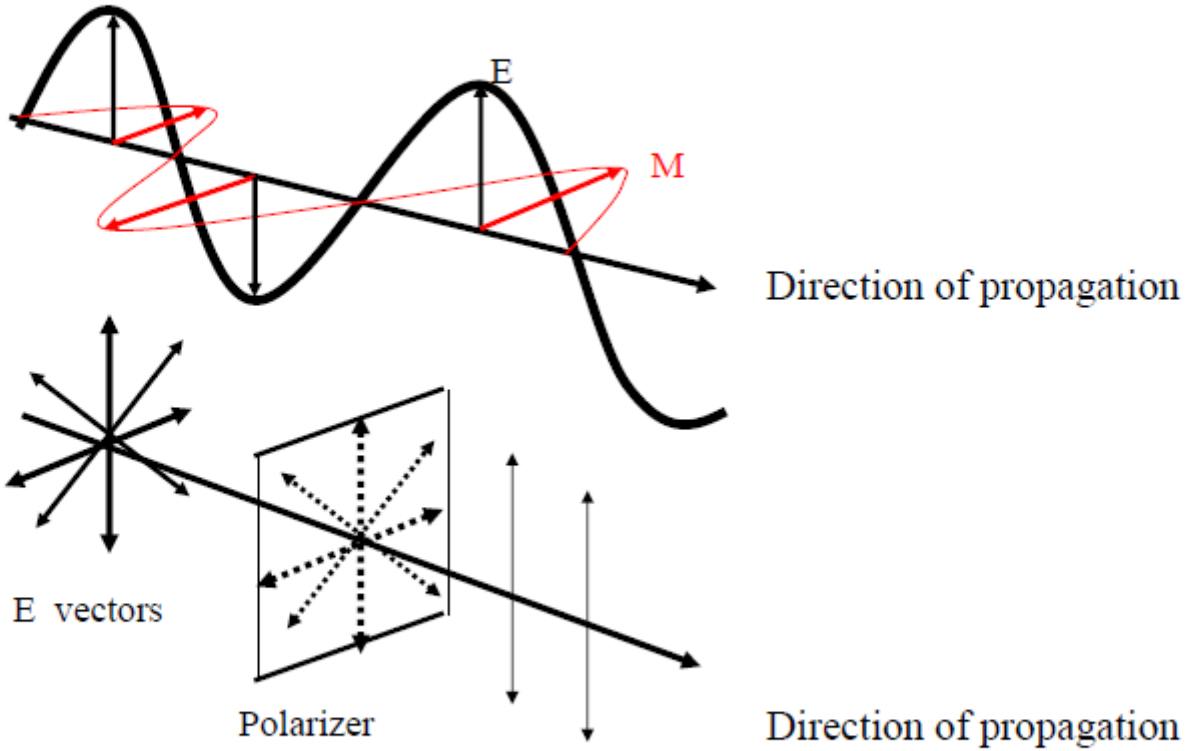
• Induced structural changes, i.e. pH, heat & solvent.

• Protein folding/unfolding.

• Ligand binding

• Structural aspects of nucleic acids, polysaccharides, peptides, hormones & other small molecules.

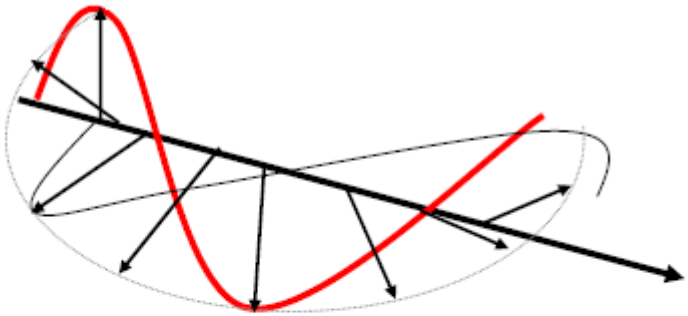
Plane Polarized Light



Plane & Circularly Polarized Light

•A light source usually consists of a collection of randomly orientated emitters, the emitted light is a collection of waves with all possible orientations of the E vectors.

•Plane polarized light is is obtained by passing light through a polarizer that transmits light with only a single plane of polarization. i.e. it passes only those components of the E vector that are parallel to the axis of the polarizer.



•Circular polarized light; The E vectors of two electromagnetic waves are ¼ wavelength out of phase & are perpendicular. The vector that is the sum of the E vectors of the two components rotates so that its tip follows a helical path (dotted line).

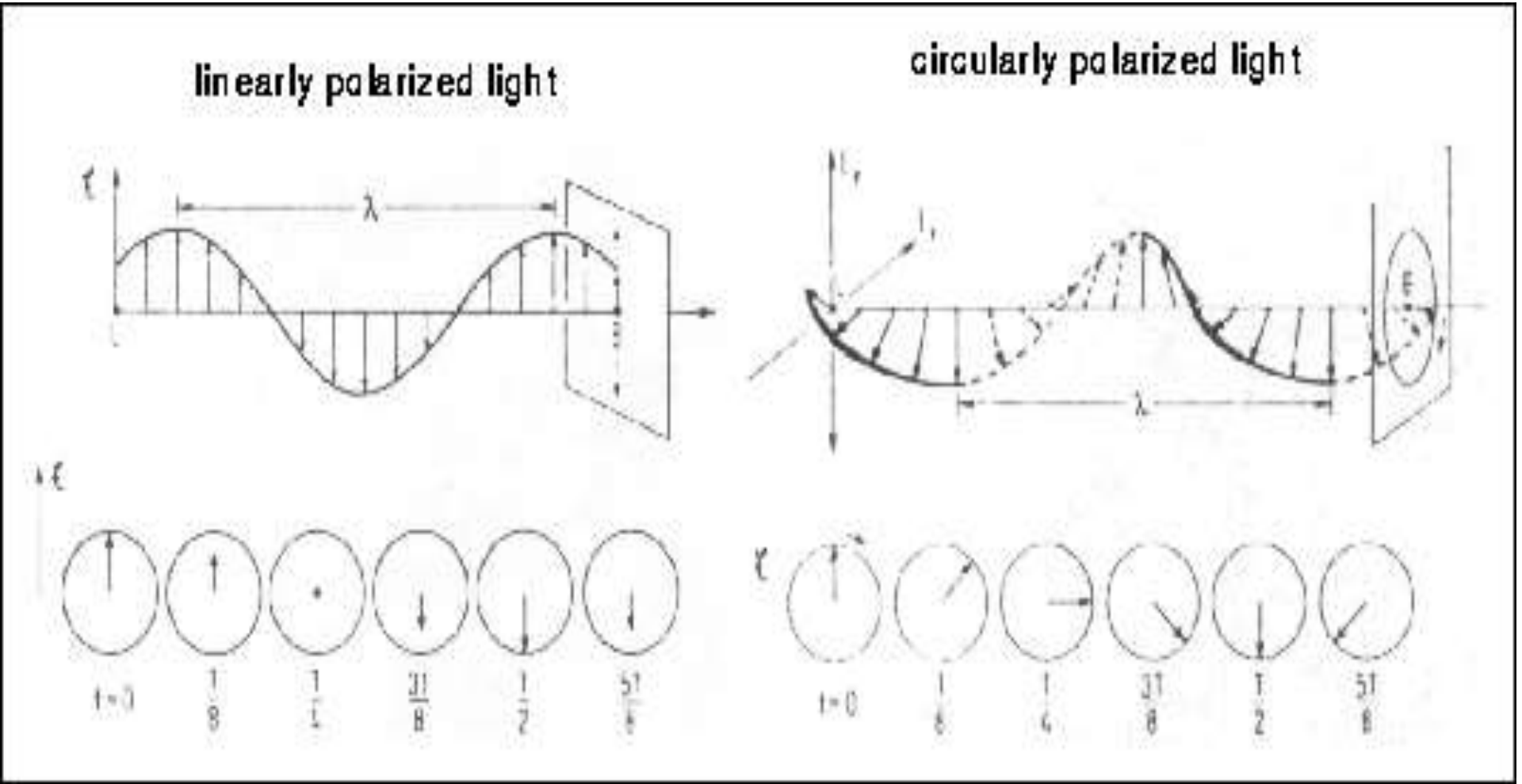
Circularly Polarized Light

- Linearly polarized light:

Electric vector direction constant - magnitude varies.

- Circularly polarized light:

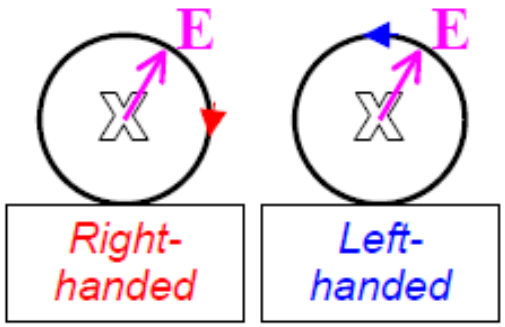
Electric vector direction varies - magnitude constant



Circularly Polarized Light

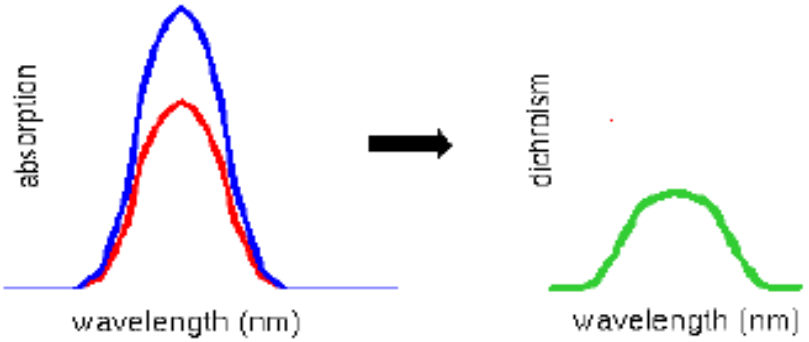
- Circular polarized light:

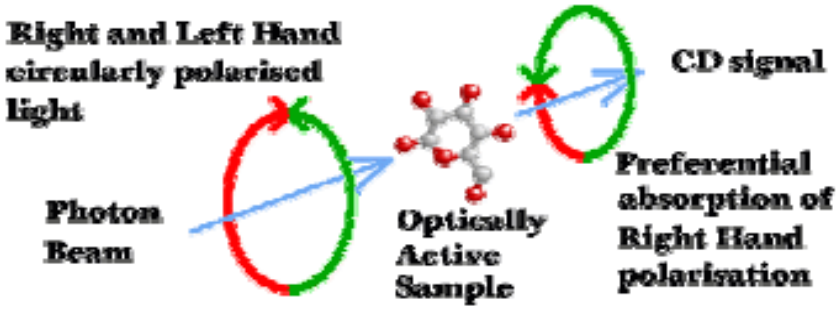
  Electric vector direction varies - magnitude constant
- So its in two forms: left and right handed



Circular Dichroism

- CD measures the difference between the absorption of left and right handed circularly-polarized light. polarized light:





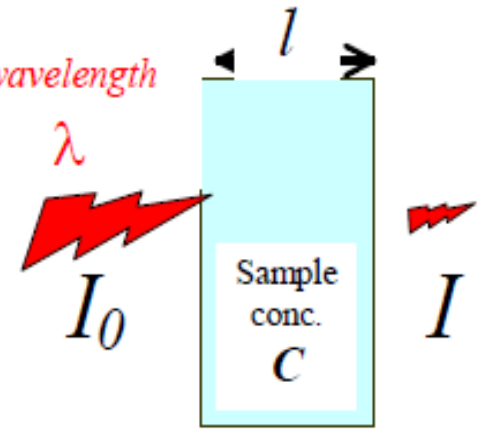
- This is measured as a function of wavelength, & the difference is always very small (<<1/10000 of total). After passing through the sample, the L & R beams have different amplitudes & the combination of the two unequal beams gives elliptically polarized light.Hence, CD measures the ellipicity of the transmitted light (the light that remains that is not absorbed):

Absorption Spectroscopy

- Shine light through a sample and measure the proportion absorbed as a function of wavelength.
- Absorbance $A = \log(I_0/I)$
- Beer-Lambert law:

  $A(\lambda) = \varepsilon(\lambda)lc$

  ε: extinction coefficient



- The longer the path or the more concentrated the sample, the higher the absorbance

Absorption Spectroscopy

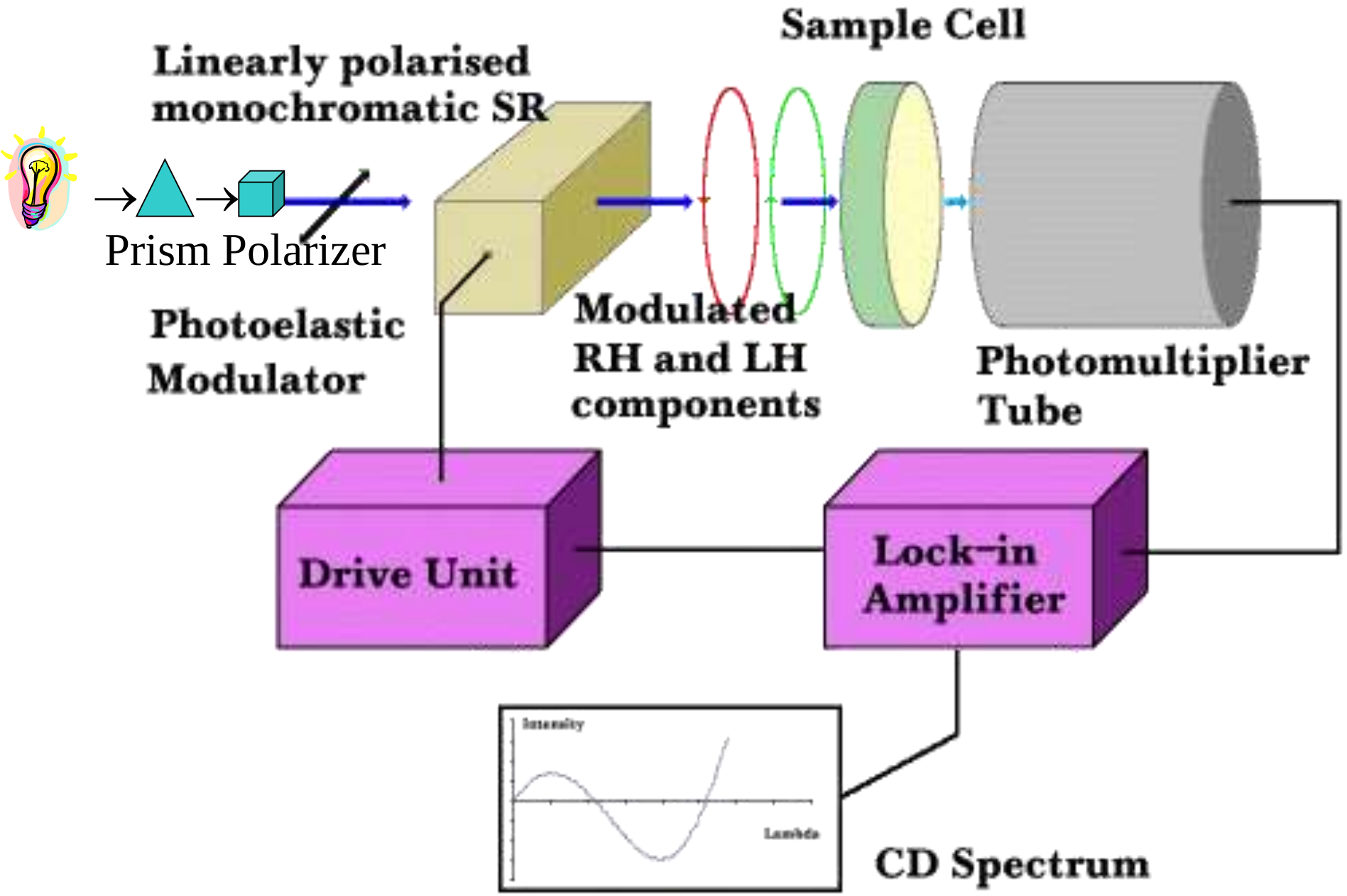
- CD measures the difference between the absorption of left and right handed circularly-polarized light:

$$\Delta A(\lambda) = A_R(\lambda) - A_L(\lambda) = [\varepsilon_R(\lambda) - \varepsilon_L(\lambda)]lc$$

or

$$\Delta A(\lambda) = \Delta\varepsilon(\lambda)lc$$

- $\Delta\varepsilon$ is the difference in the extinction coefficients
- typically $< 10\ M^{-1}cm^{-1}$
- typical ε around $20\,000\ M^{-1}cm^{-1}$
- So **the CD signal is a very small difference between two large originals**.


Sample Cell
Linearly polarised monochromatic SR
Prism Polarizer
Photoelastic Modulator
Modulated RH and LH components
Photomultiplier Tube
Drive Unit
Lock-in Amplifier
Intensity
Lambda
CD Spectrum

Absorption Spectroscopy

CD is only observed at wavelengths where absorption occurs, in absorption bands.

CD arises because of the interaction between different transition dipoles doing the absorption. As this depends on the relative orientation of different groups in space, the signal is very sensitive to conformation.

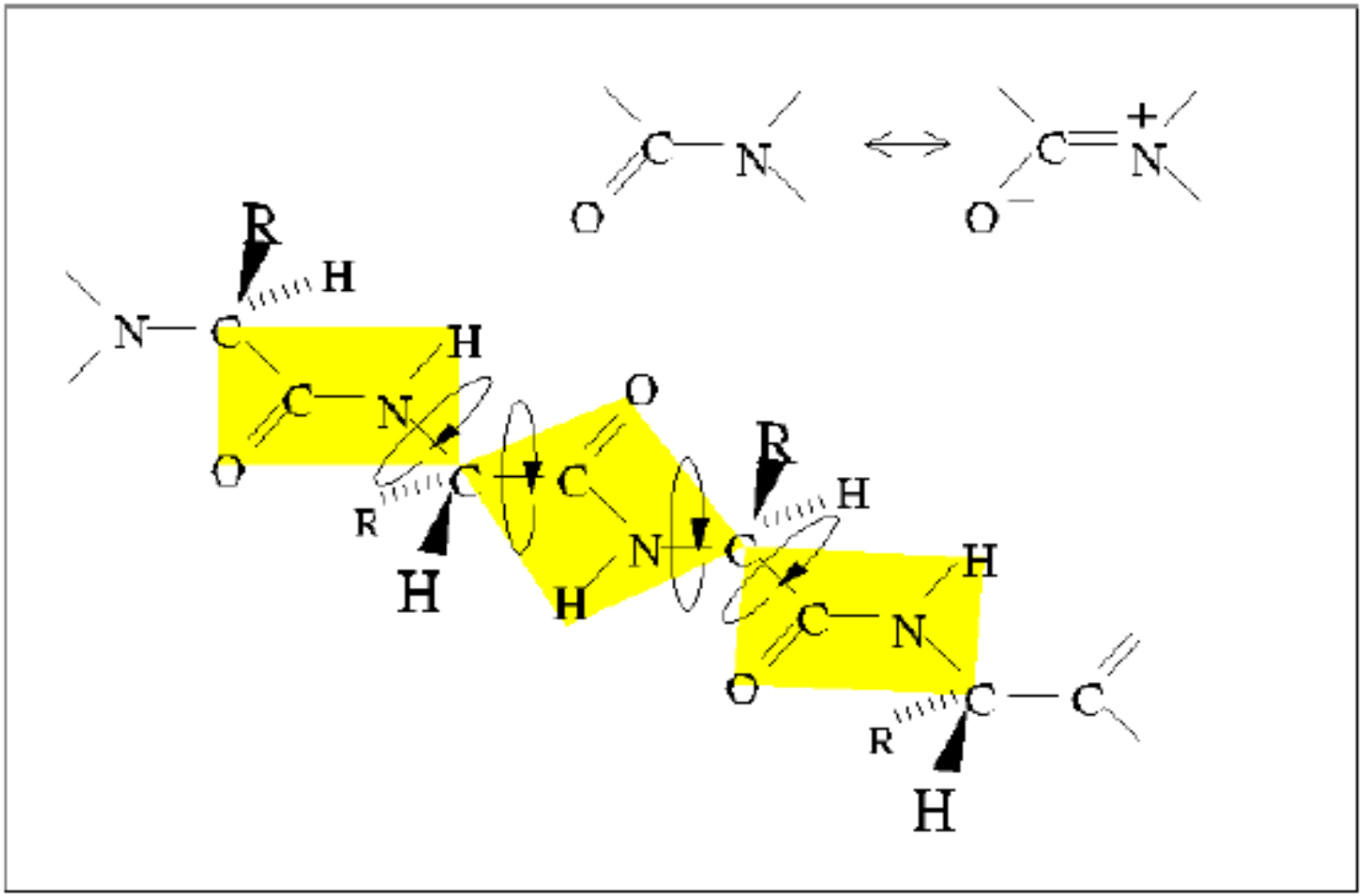
So in general $\Delta\varepsilon$ is much more conformation dependent that ε.

We will concentrate on the "electronic CD" of peptides and proteins below 240nm. This region is dominated by the absorption of peptide bond and is sensitive to changes in secondary structure.

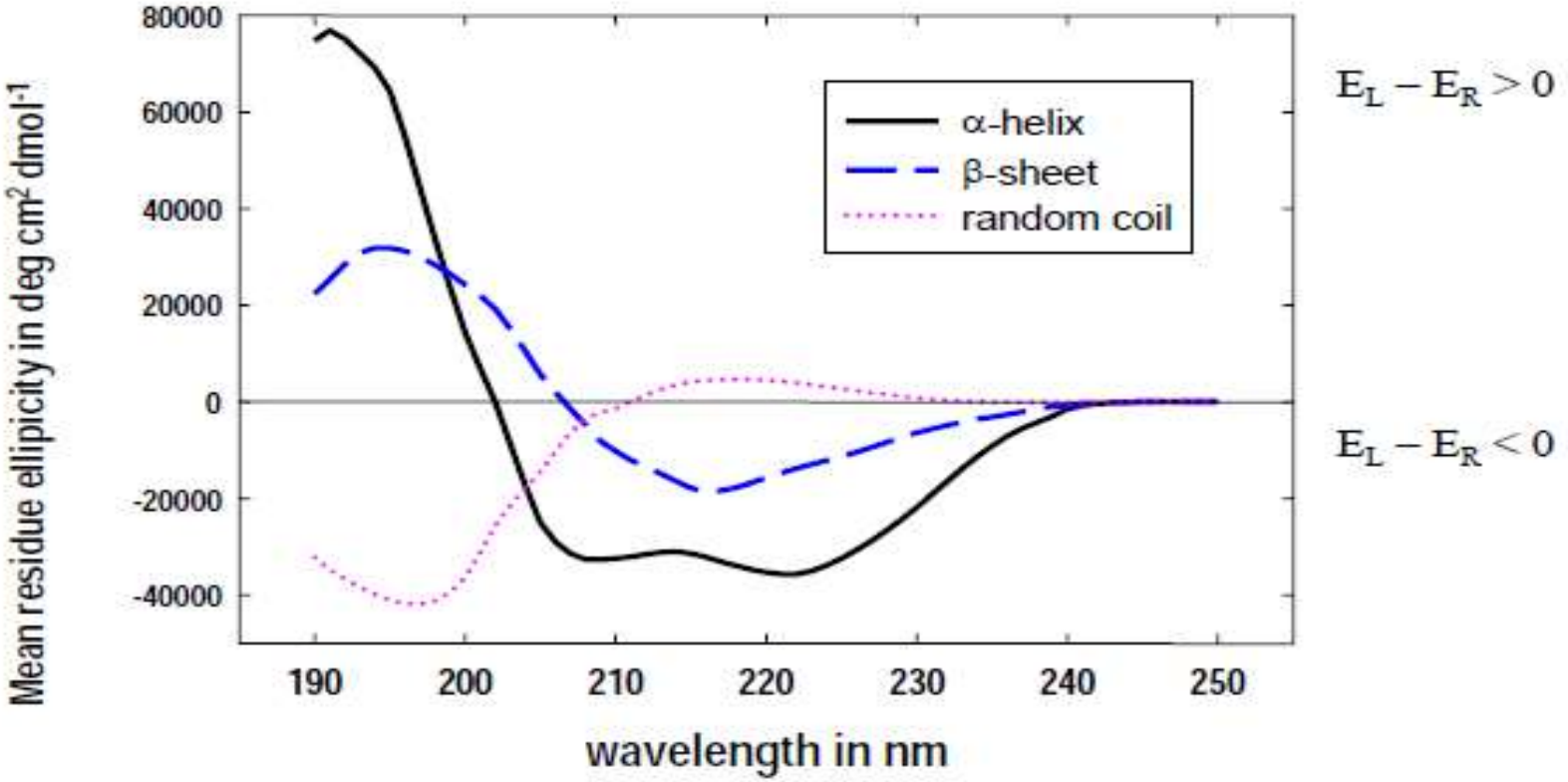
Can also do CD in near UV (look at trp side chains), visible (cofactors etc.) and IR regions.

•The peptide bond is inherently asymmetric & is always optically active.

• Any optical activity from side-chain chromophores is induced & results from interactions with asymmetrical neighbouring groups.



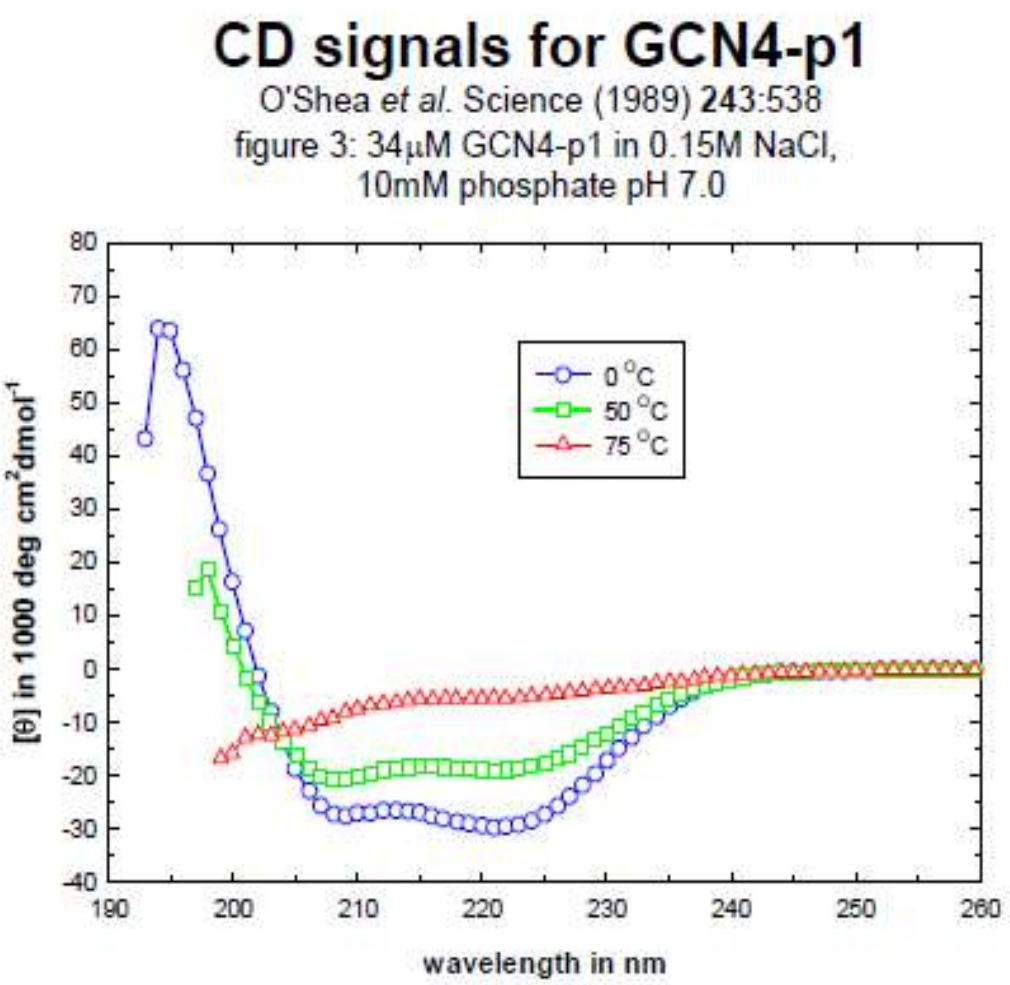
CD Signals for Different Secondary Structures



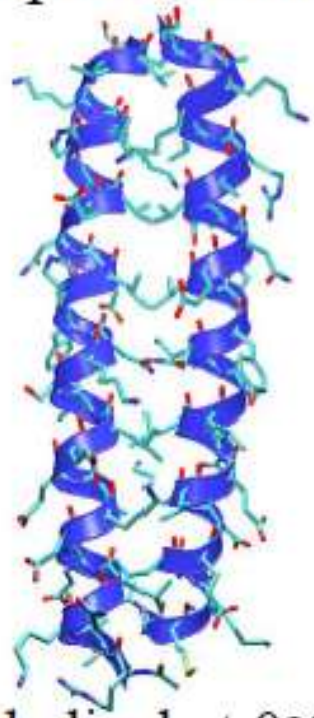
- These are Fasman standard curves for polylysine in different environments

(data from ftp://jgiqc.llnl.gov)

CD Signals are Sensitive to 2ndary Structure



- GCN4-p1 is a coiled–coil:



- 100% helical at 0°C
- It melts to a random coil at high temperature

Applications of CD

- 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

ADVANTAGES

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


Thank
You