Circular dichroism

Background

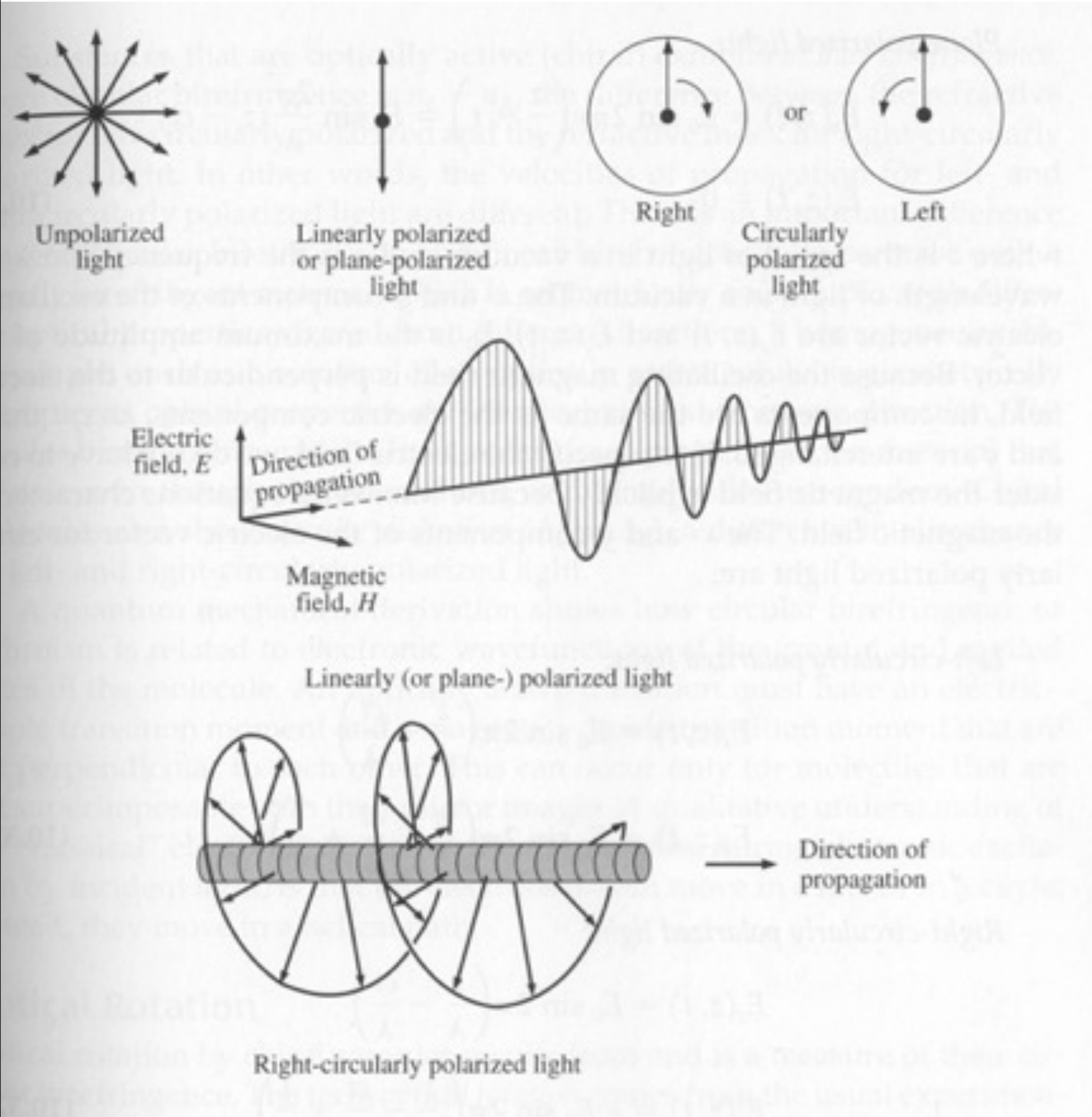
- most biological molecules are asymmetrical or chiral
- Therefore molecules have a “handedness”
- higher order structures have a “hand” associated with it
- most DNA and protein helices are right handed
- there are exceptions due to the composition of bases or amino acids
- Chiral structures can be distinguished and characterized by polarized light

Optical Activity

- *optical rotation dispersion,* ORD - change the angle of polarization of a beam of light that is shone through them as a function of wavelength
- *circular dichroism,* CD - absorb light differently according to its wavelength and polarization
- Louis Pasteur observed that *optically inactive* crystals of sodium ammonium tartrate were in fact mixtures of two classes.
- The molecules making up the crystals were themselves in one of two asymmetric forms, *enantiomorphs (*mirror image of the other).
- The form that turns the axis of polarised light to the right when viewed towards the light source in a *polarimeter* is called *dextro-rotatory* or the *D-form*; the form that turns the axis to the left is called the *L-form* or *laevo-rotatory* (from the Latin for right and left). The same terms apply to *circularly polarized* light
- Linus Pauling and Robert Corey discovered *α*-helices and *β*-sheets.
- Demonstrated that these are optically active structures that could be studied by ORD and CD

Types of Polarized Light

- Light has a electronic and magnetic components that are perpendicular to each other
- Three types of polarized light
  - Plane-polarized light
  - Circularly polarized light
  - Elliptically polarized light - intermediate between plane and circular
- Plane and circularly polarized light can be produced from unpolarized light by passing it through an appropriate film or crystal
- Plane polarized light use a film that is geometrically anisotropic
  - refractive index is different for polarized planes. It allows only one plane of light through



◀ FIGURE 10.21

Different types of polarized light. At the top, the arrows represent the electric vector of the light as seen by an observer moving with the light. The light is moving into the page. At the middle and bottom, the light is seen by a stationary observer.

Left and Right Circularly Polarized Light

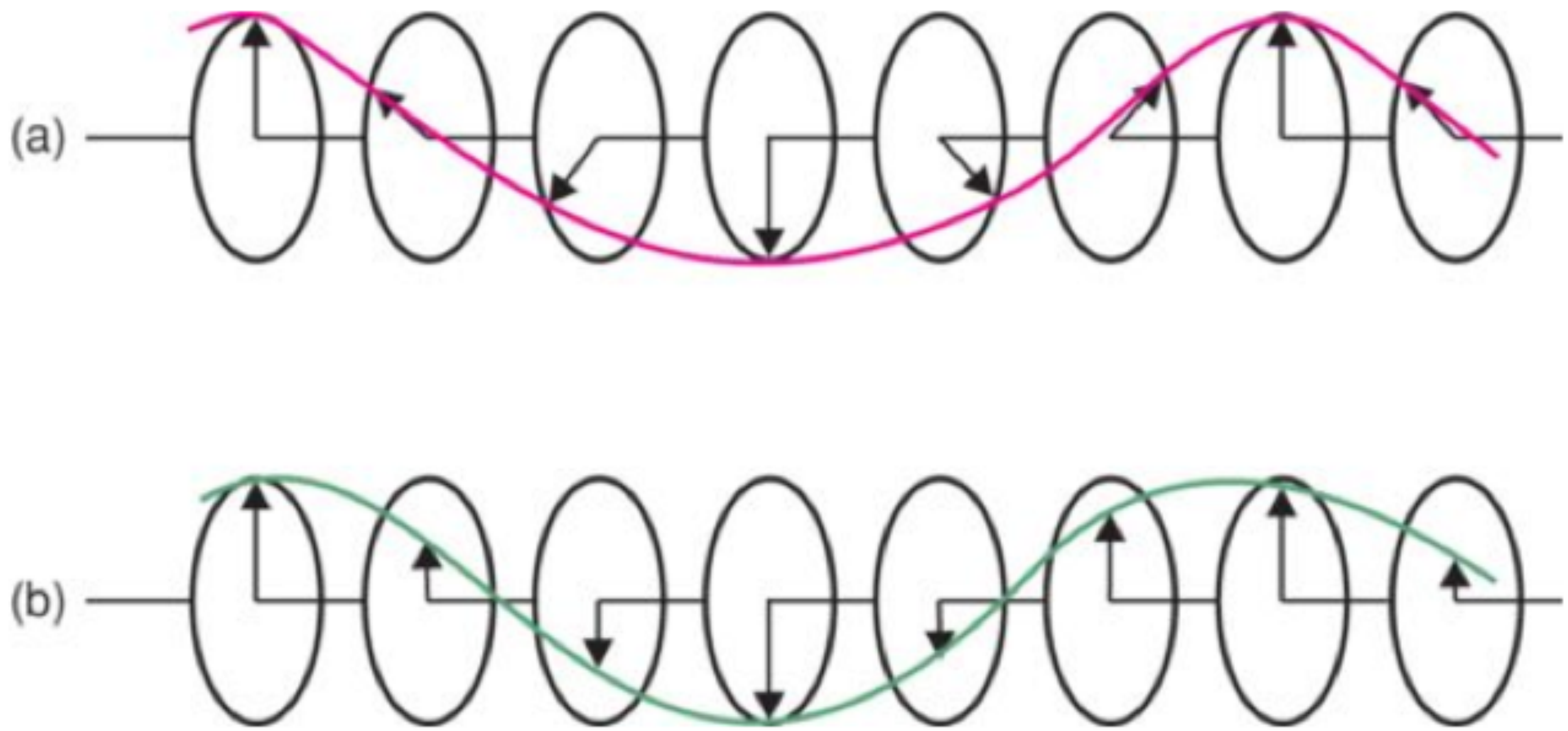
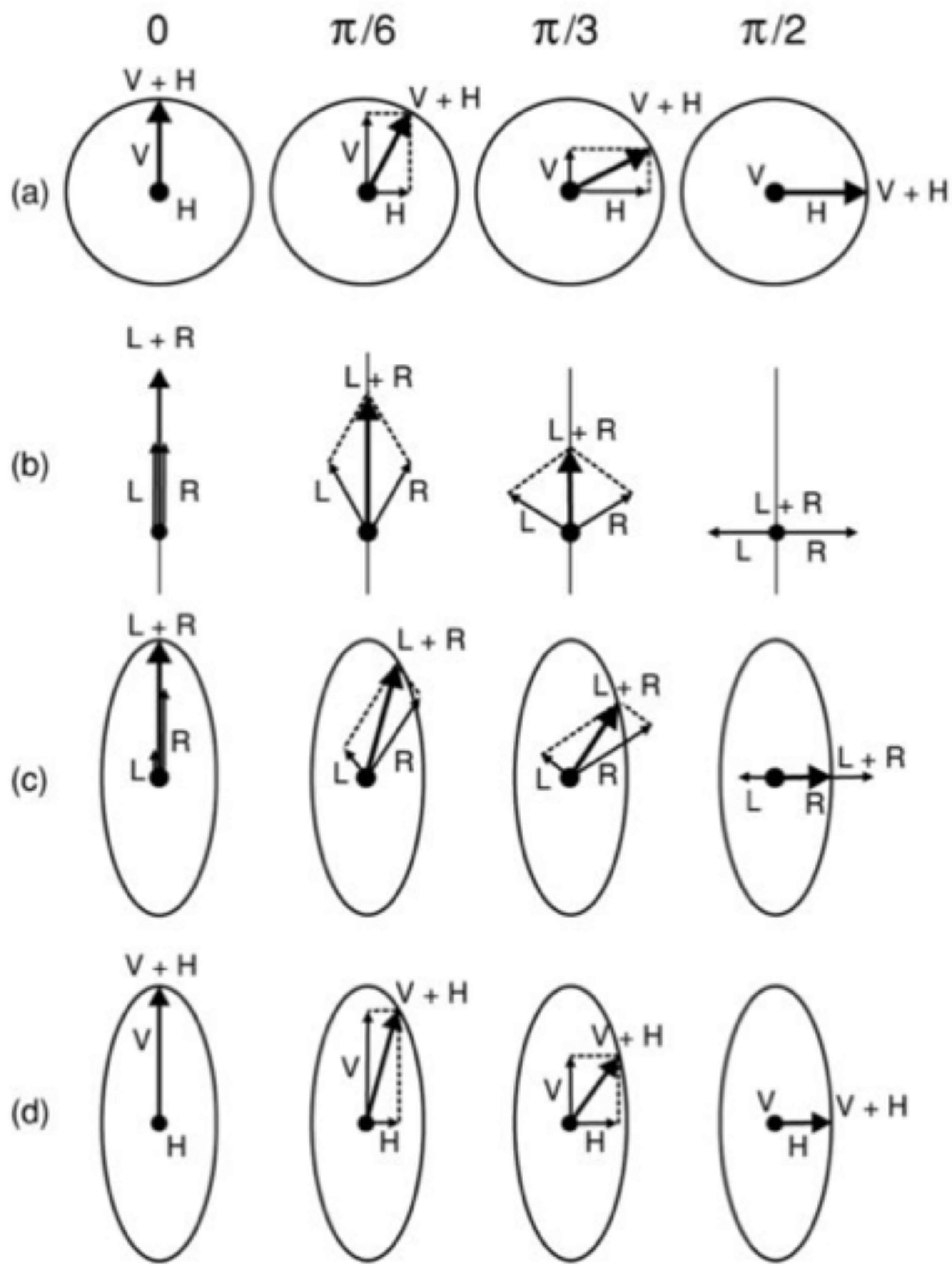


Fig. E4.1. (a) The path traced out by the tip of the electric vector (the arrow) during the propagation of right circularly polarised light traces a right-handed helix. The vector in left circularly polarised light traces a left-handed helix. Note that in both cases the length of the vector remains constant. (b) In linearly polarised light the electric vector stays in the same plane but its length is modulated by the wavelength. (Johnson, 1985.) (Figure reproduced with permission from John Wiley & Sons.)

Projections



right circularly polarized light shown as made up of two plane-polarized components, V and H, of equal magnitude out of phase by 90◦;

plane-polarized light shown as made up of left (L) and right (R) circularly polarized light of equal magnitude;

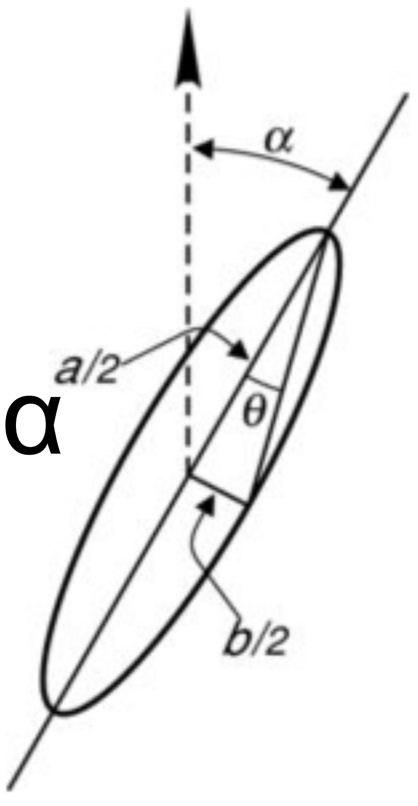
elliptically polarized light shown as made up of left (L) and right (R) circularly polarized light of unequal magnitude

Elliptically polarized light shown as made up of two plane-polarized components, V and H, of unequal magnitude out of phase by 90◦.

Optical Rotation

- Plane-polarized light propagated through a chiral sample will emerge plane polarized but at different rotational angle.

- ellipticity is defined as the angle θ
- given in radians as the value of its tangent of minor axis/major axis
- optical rotation is defined by the angle α



Circular Dichroism

- Circular dichroism (CD) is the differential absorption of right- and left-circularly polarized light

- $A_p = \log_{10} (I^0_p/I_p) = \varepsilon_p C l$

- Expressed as

  $\Delta A = A_L - A_R = \varepsilon C l$

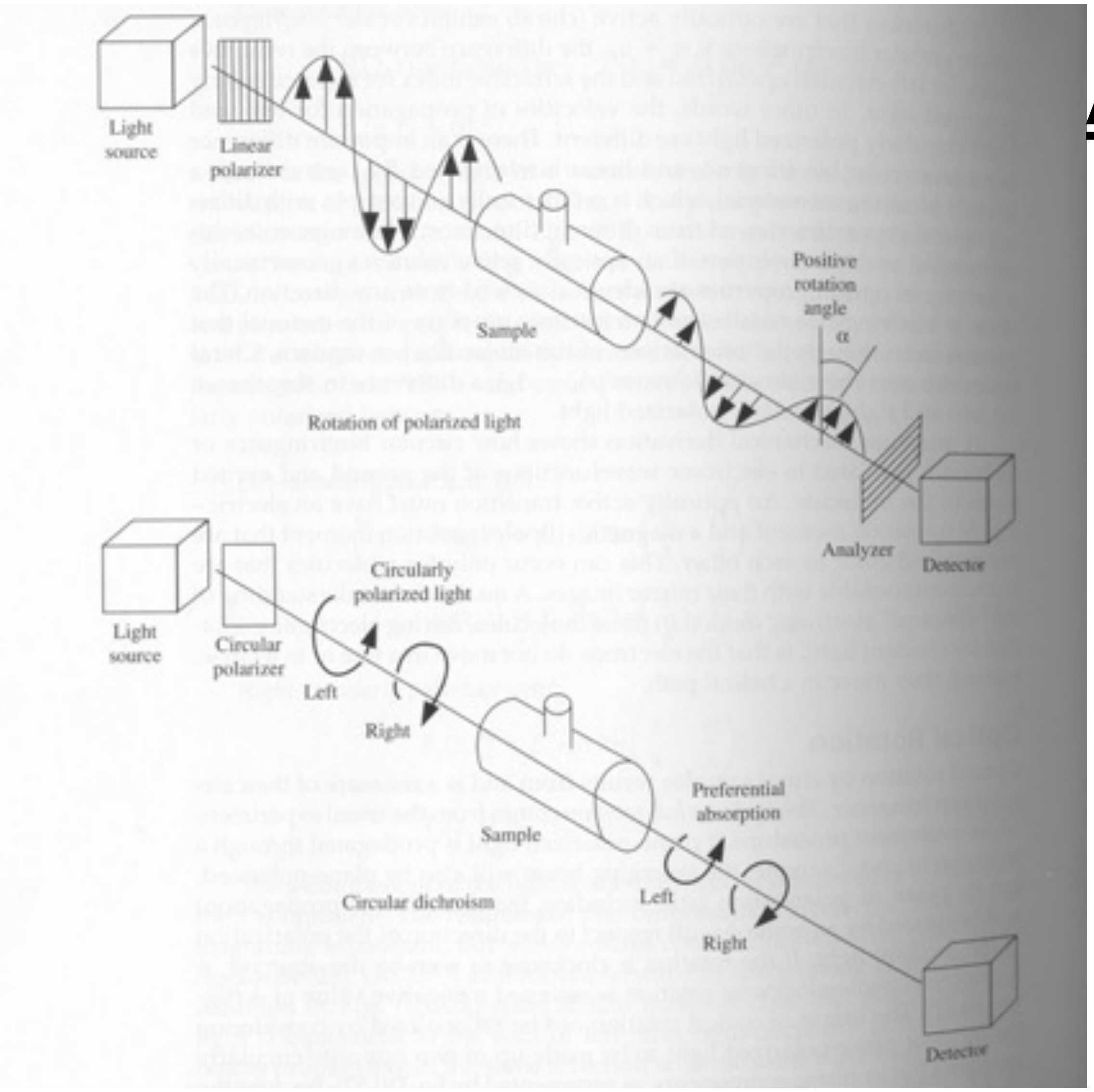
- A_L and A_R is the absorbances for left and right circular polarized light

- CD is defined in terms of the *molar ellipticity* [θ]

- [θ] needs to be normalized for residue concentration, protein concentration or even subunit concentration in the case of oligomeric structures

- units per decimole of amino acid residue

Measurement of optical Rotation and Circular Dichroism



<u>Advantages</u>

- Sensitive
- little sample required (250uL of .3mM)
- not destructive
- no labeling required
- can be performed at various temp

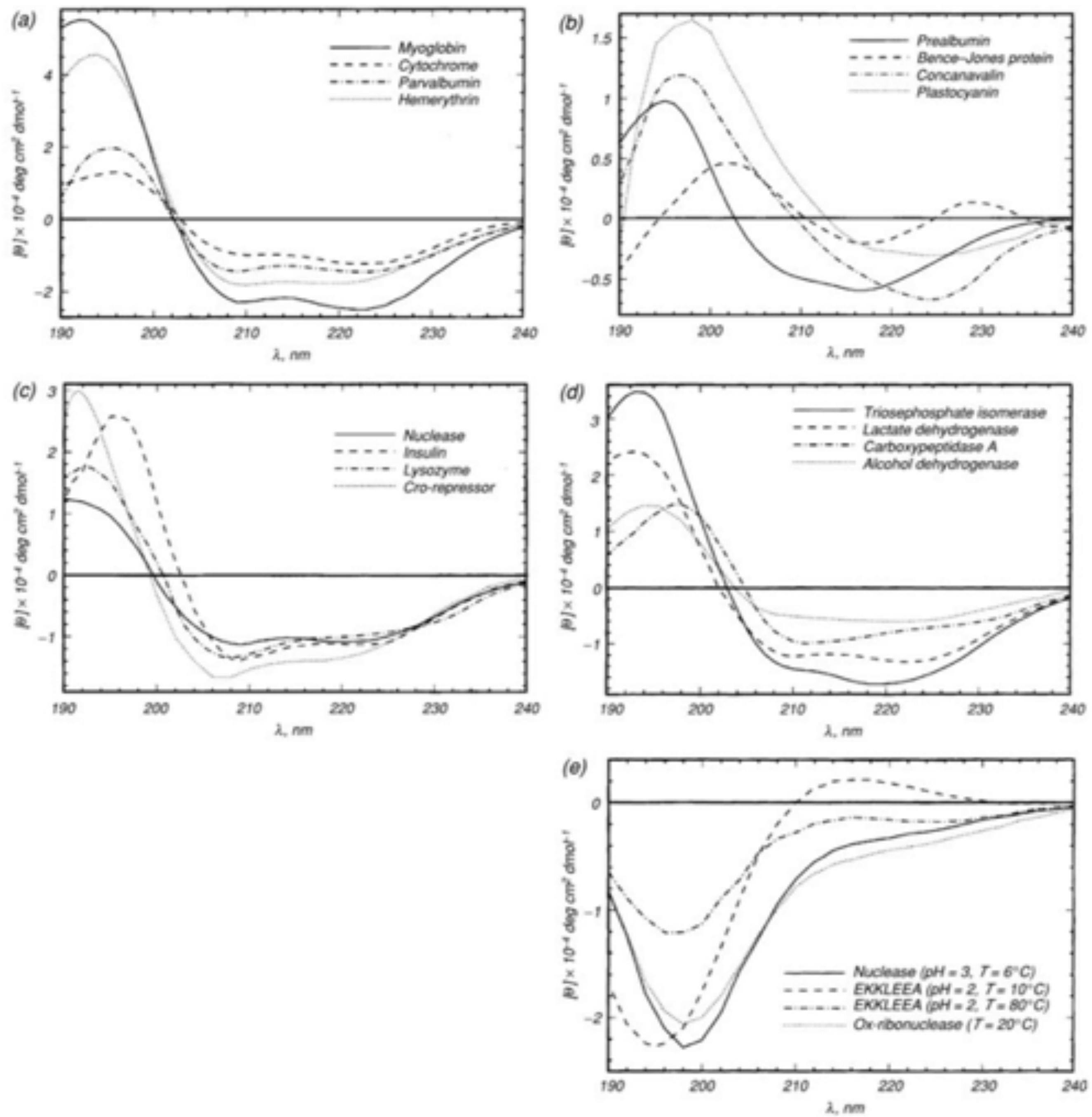
CD of Proteins

- CD is the most appropriate method for assessing the secondary structure of a protein in solution.
- Monitor structural and conformational changes
- the interpretation in terms of structure is necessarily based on empirical criteria based on known structures from other methods (X-ray crystallography and NMR
- CD due to aromatic groups, such as tryptophan and tyrosine, in the near-UV is sensitive to tertiary structure.
- the CD signal of an unstructured polypeptide is distinctively different from the signals arising from secondary structures

CD Spectra of Proteins

Mostly α

Mostly β



α + β

α/β

Disordered
Random coil

Characteristics of Secondary structure

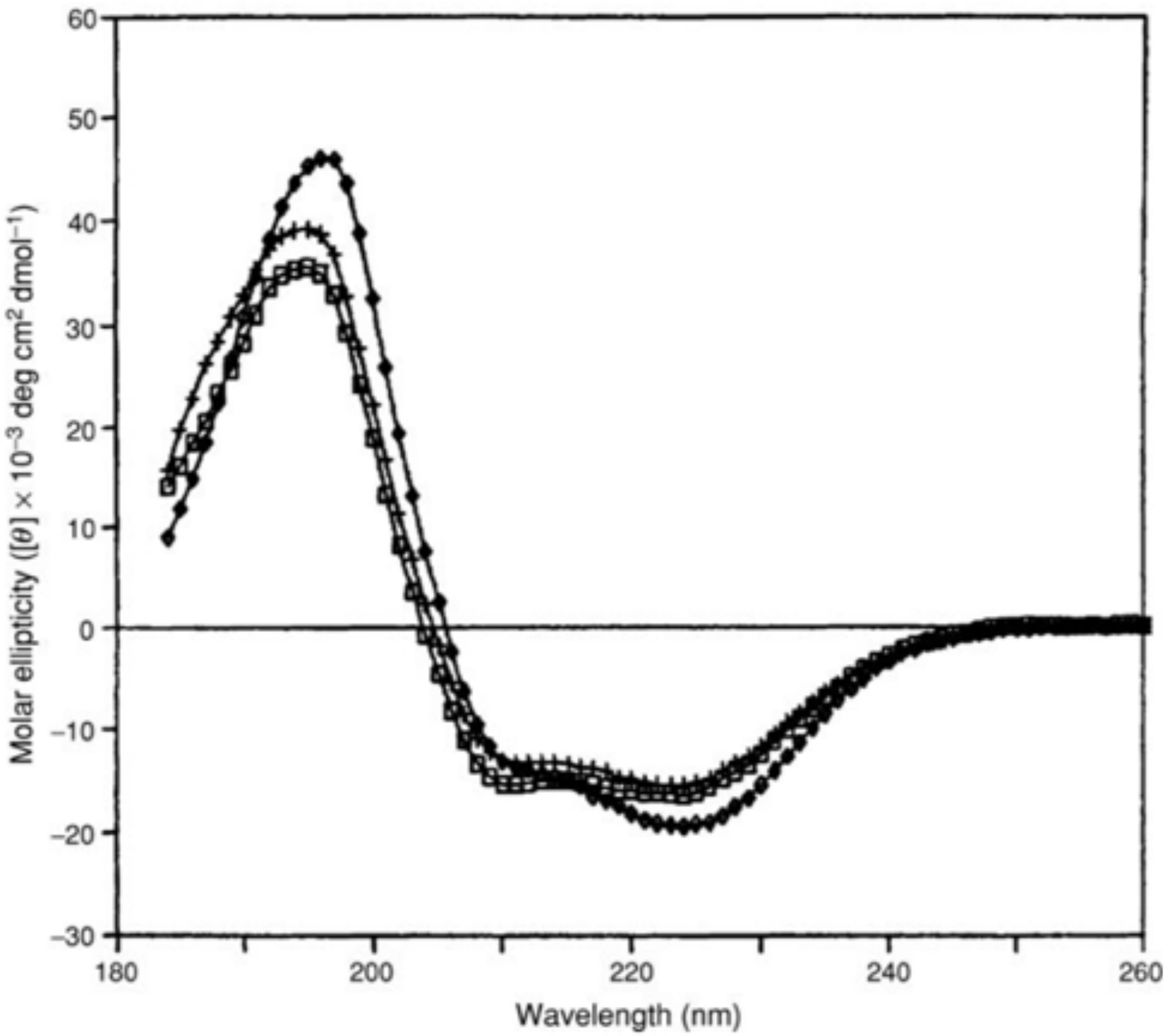
- Mostly α - minima at about 209 and 222 nm
- Mostly β - minimum between 210 and 225 nm. greater diversity and weaker signal
- random coil/denatured A strong negative band near 200 nm, and weak positive or negative bands above 210 nm

Fitting Spectra

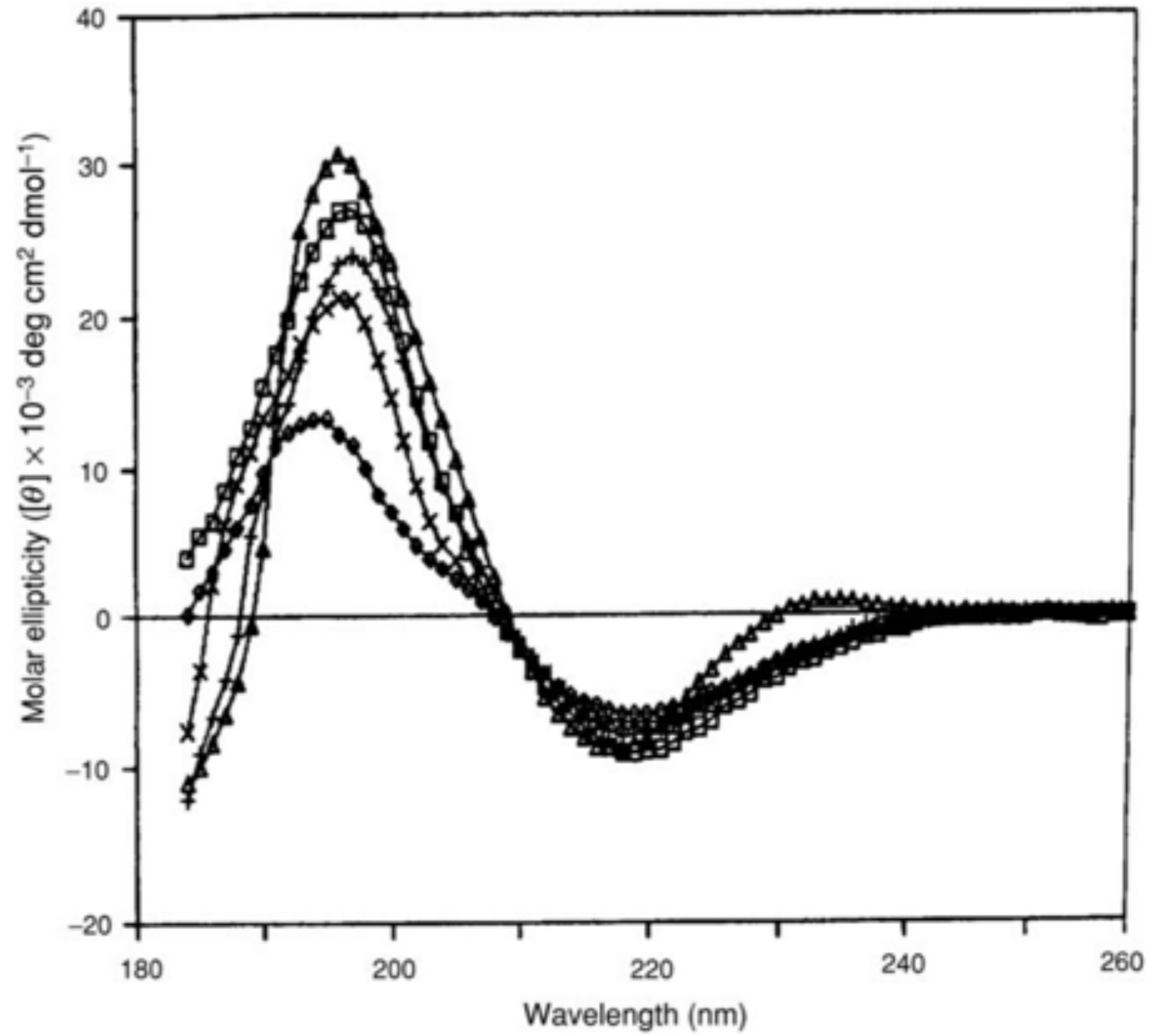
- spectrum could be considered as a simple sum of component spectral bands corresponding to pure secondary structures. Problem with this???
- develop a databases of CD spectra corresponding to different protein secondary structure families, which could be used as basis sets for the fitting of spectra of ‘unknown’ proteins.

Membrane Proteins

- Inherently challenging to study protein in membranes. Why??



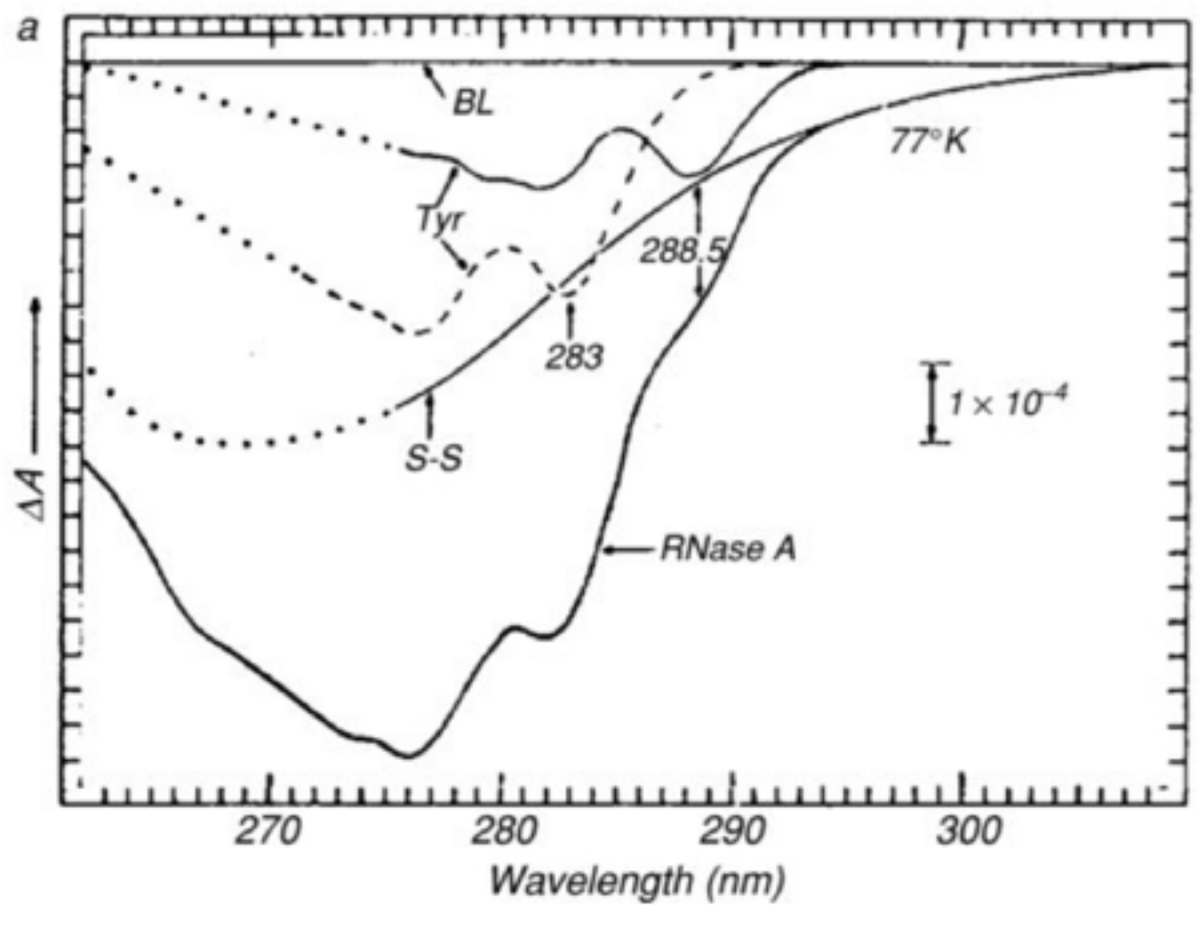
CD spectra of bacteriorhodopsin and rhodopsin in detergent laurylmaltoside



maltoporin (triangles); *E. coli* porin (squares); *E. coli* porin in slightly different solvent conditions (crosses)

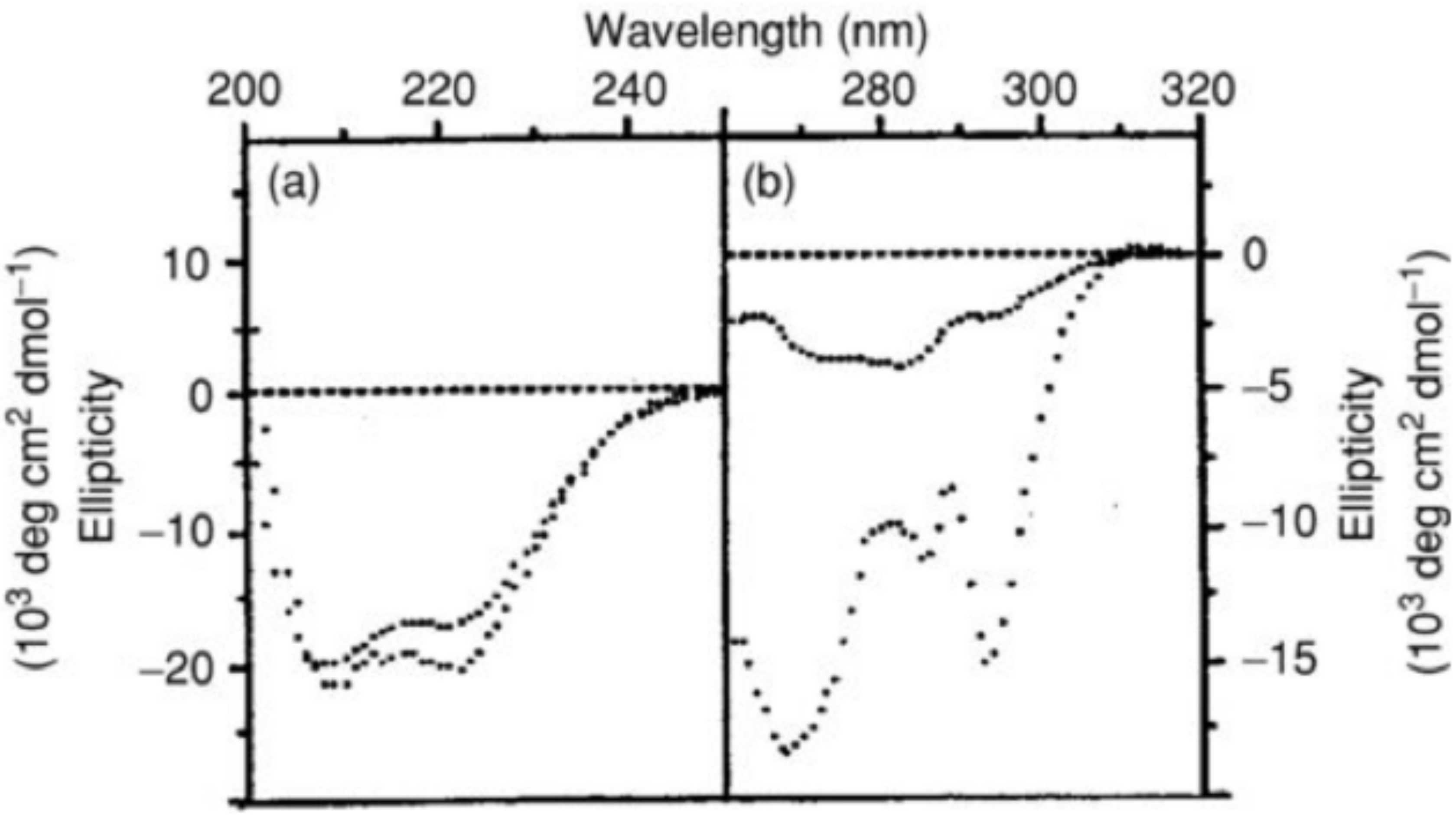
Near-UV CD and protein tertiary structure

- aromatic residues absorb around 280nm. Determine whether aromatics are buried or solvent exposed



Near UV CD of RNase A. ribonuclease A (RNase A) has six Tyr, three Phe and four disulfides.

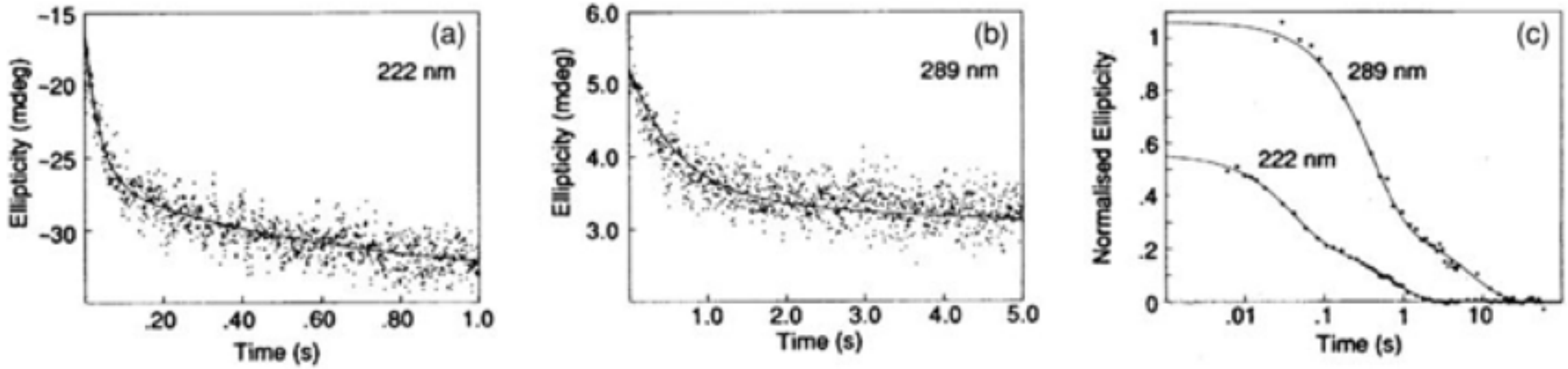
Far- and near-UV CD of colicin A



colicin A inserts into membranes at low pH

CD Measurements of Protein Folding

kinetic CD measurements to look at protein unfolding and refolding

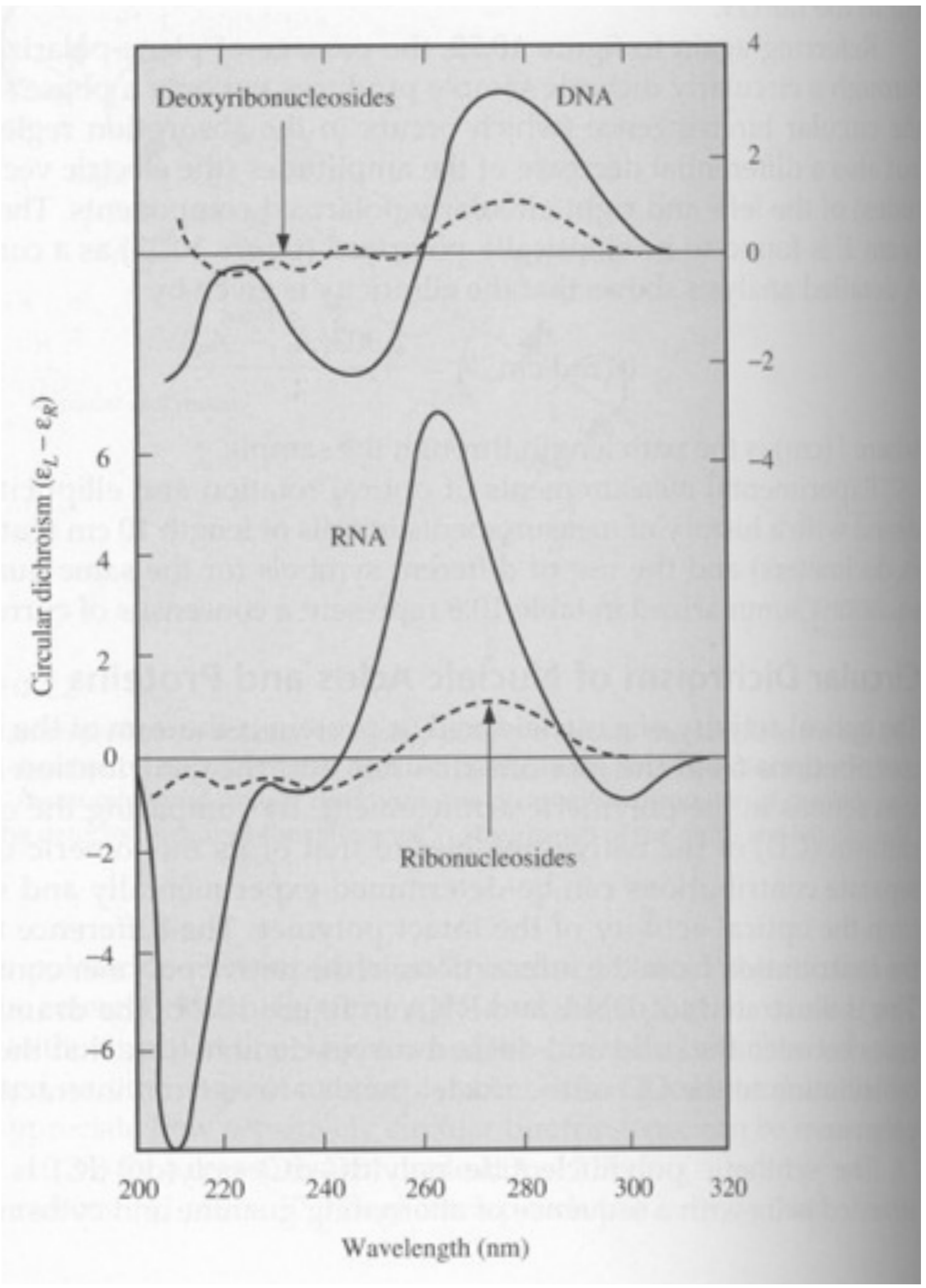


CD stopped-flow refolding kinetics for oxidised cytochrome *c*. 4.3 M to 0.7 M guanidine hydrochloride

CD of Nucleic Acids

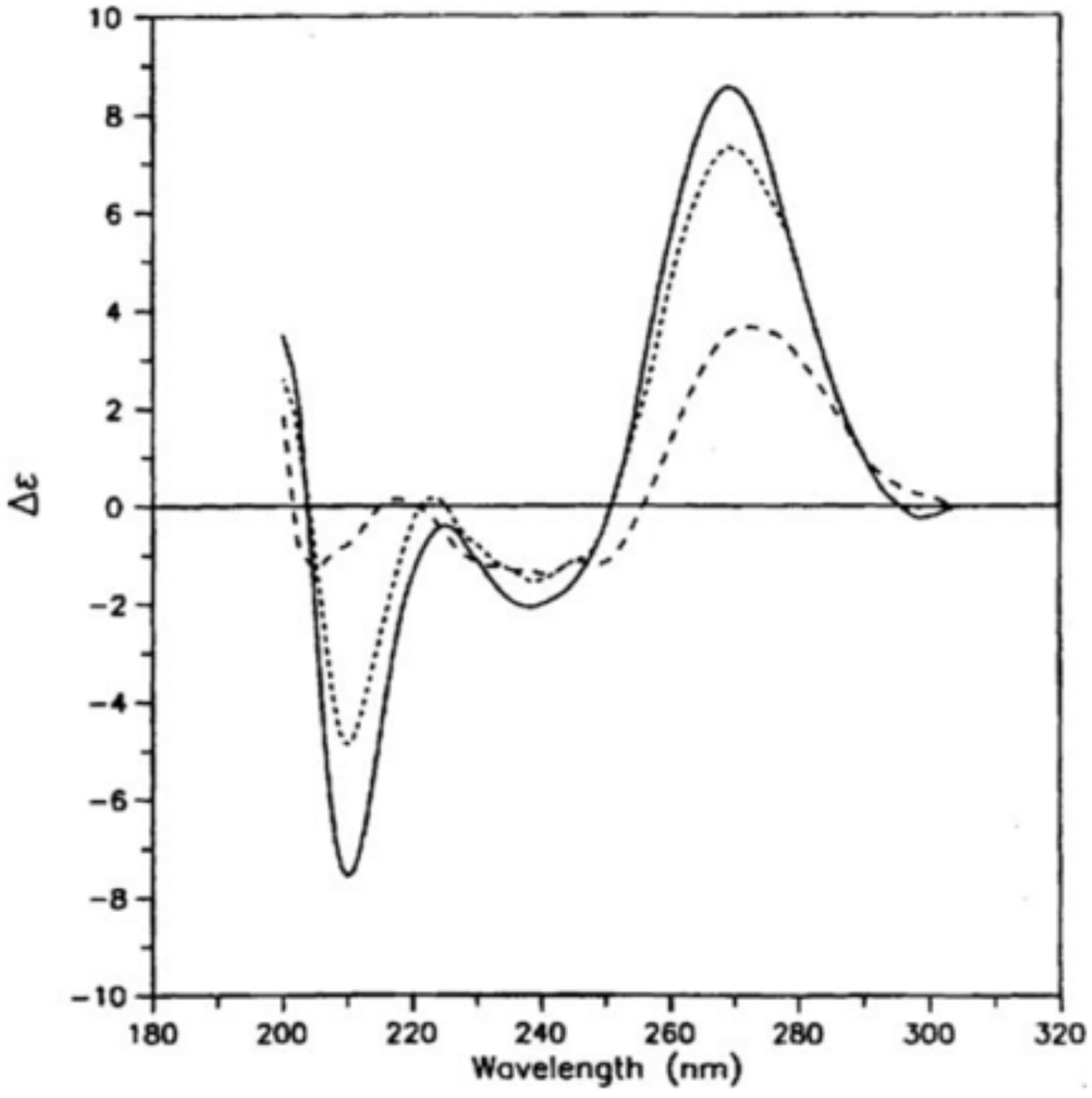
- The CD signal of the bases in nucleic acid comes to a small extent from the asymmetry of the sugar, but is dominated by the hydrophobic stacking of the base-pairs in the helical structures.
- sensitive to the secondary structure induced by different solvent environments and base compositions,

CD of Nucleic Acids



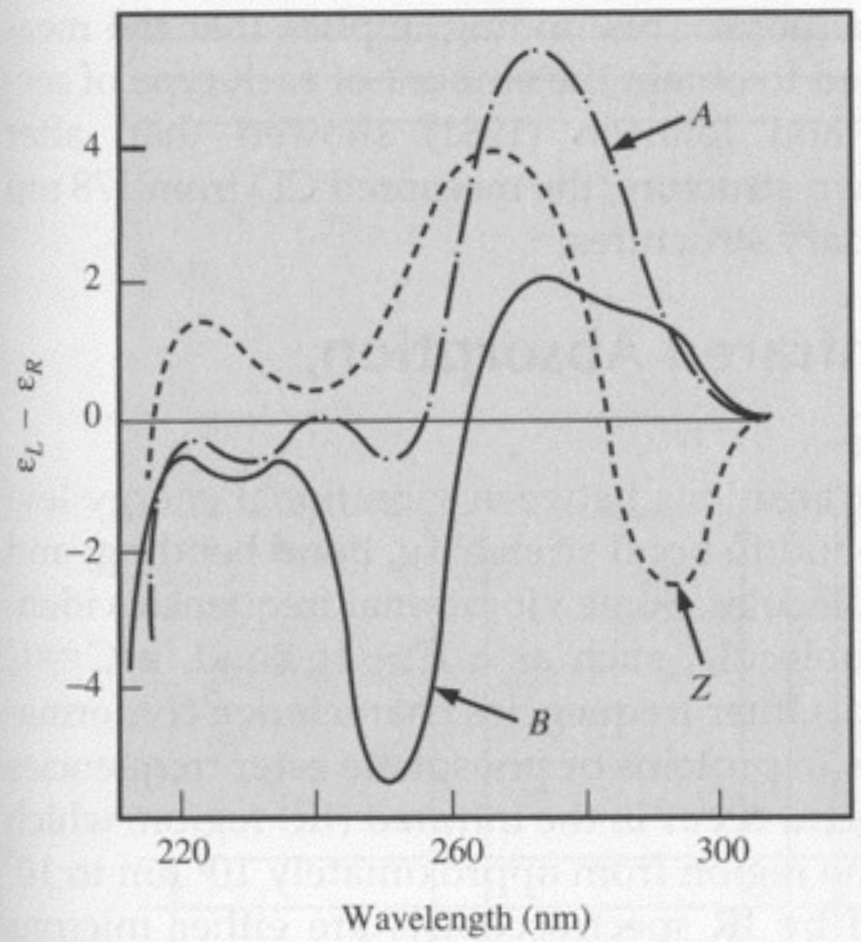
- CD of RNA and DNA compared to their component mononucleosides.
- The difference between the mononucleosides and polymer show the contribution from interactions within the polymer

CD of RNA



CD RNA PK5 at different temperatures: 0 ◦C (line); 30 ◦C (dots); 70 ◦C(dashes)

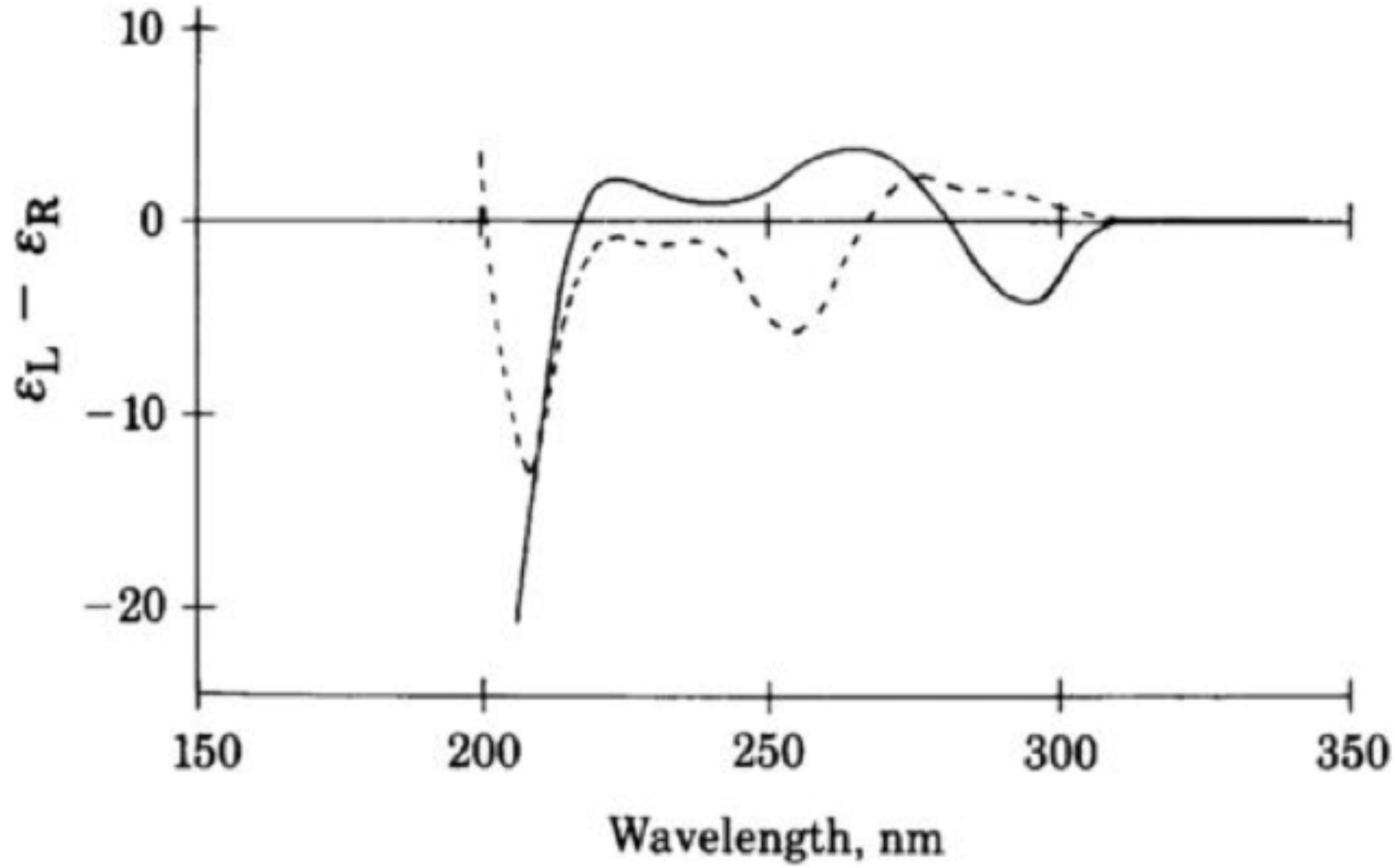
Different Conformations of Nucleic Acids



◀ FIGURE 10.25

Circular dichroism of the synthetic polynucleotide poly(dG-dC)·poly(dG-dC) in different conformations. The polynucleotide is a double-stranded helix; each strand has a sequence of alternating deoxyguanylic acid (dG) and deoxycytidilic acid (dC). Different conformations are obtained by changing the solvent. The B form is obtained in 0 to 40% ethanol or 10^{-3} *M* to 2 *M* NaCl; it is a right-handed helix with about 10 base pairs per turn of the double helix. The Z form is obtained in 56% ethanol or 3.9 *M* NaCl; it is a left-handed helix. The A form is obtained in 80% ethanol; it is a right-handed helix with about 11 base pairs per turn. (From F. M. Pohl, 1976, *Nature* 260:365. Copyright © 1976 Macmillan Journals Limited.)

Change in DNA conformation



CD spectra of poly d(GC): 0.1M NaCl (full line); and 4M NaCl or in 60% ethanol (dashed line)