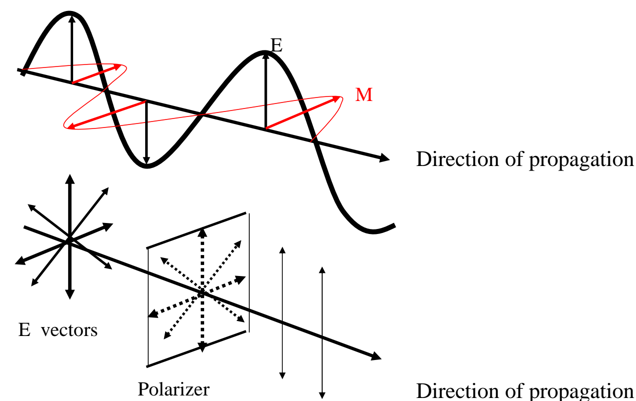
Circular Dichroism

Goals for this unit:

- Terms: Linearly polarized / Circularly polarized / Dichroism (ε) / Birefringence (n)
- How do we make Circularly Polarized Light?
- What is Circular Dichroism? Why use CD?
- CD Instrument (λ used) / Practical Considerations
- Applications:
  - Protein secondary structure determination
  - Protein folding/unfolding – dynamic process
    - Induced structural changes, i.e. pH, heat & solvent
  - Ligand binding
  - Structural aspects of nucleic acids, polysaccharides, peptides, hormones & other small molecules

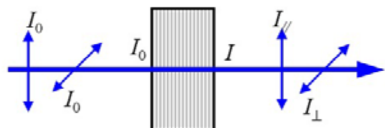
Note: Some of the material in these slides was modified from that presented by Smart - University of Birmingham

Plane polarized light



Dichroism

Linear dichroism (LD)



$$I(\lambda) = I_0 \exp(-\varepsilon(\lambda)cl)$$

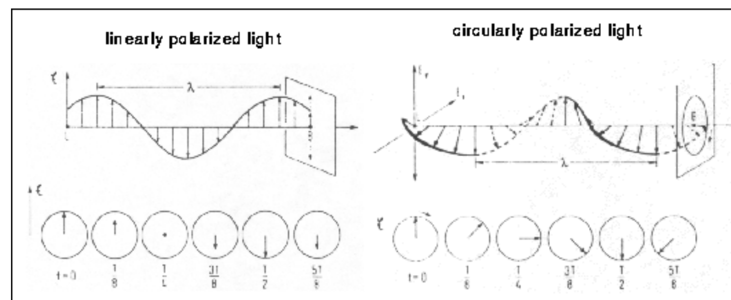
$$I_{//}(\lambda) = I_0 \exp(-\varepsilon_{//}(\lambda)cl)$$

$$I_{\perp}(\lambda) = I_0 \exp(-\varepsilon_{\perp}(\lambda)cl)$$

Linear Dichroism $LD(\lambda) = (\varepsilon_{//}(\lambda) - \varepsilon_{\perp}(\lambda))cl$

Only orientationally aligned sample give linear dichroism.

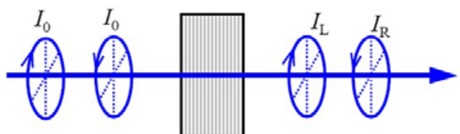
Circularly polarized light



- **Linearly polarized** light:
  Electric vector direction constant - magnitude varies.
- **Circular polarized** light:
  Electric vector direction varies - magnitude constant*

Circular dichroism

Circular dichroism (CD)



$$I_R(\lambda) = I_0 \exp(-\varepsilon_R(\lambda)cl)$$

$$I_L(\lambda) = I_0 \exp(-\varepsilon_L(\lambda)cl)$$

Circular Dichroism $CD(\lambda) = (\varepsilon_L(\lambda) - \varepsilon_R(\lambda))cl$

$$\theta = 3298(\varepsilon_L(\lambda) - \varepsilon_R(\lambda))$$

Chiral molecules show CD even if they are not orientationally aligned.

Circular Dichroism

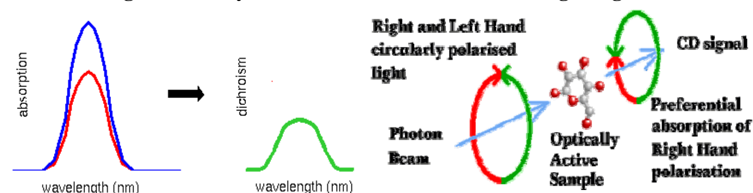
- **CD** measures the **difference** between the absorption of **left** and **right** handed circularly-polarized light. polarized light:

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

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 > 20,000 $M^{-1}cm^{-1}$
- **CD signal is a very small difference between two large originals**.



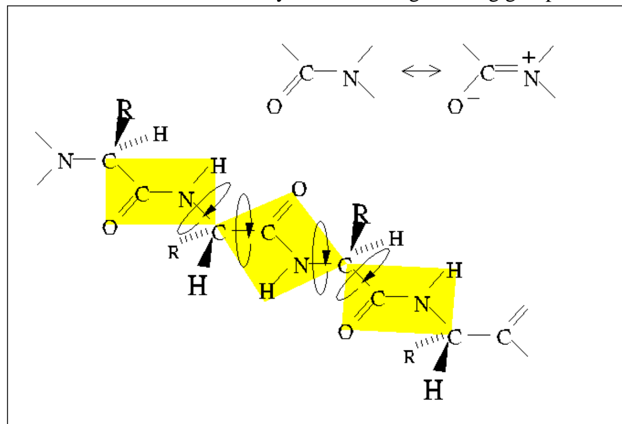
Why use CD?

- Simple and quick experiments - A structural biology method that can give real answers in a day.
- No extensive preparation
- Measurements on solution phase
- Relatively low concentrations/amounts of sample
- Microsecond time resolution
- Any size of macromolecule

CD Spectra – General Considerations

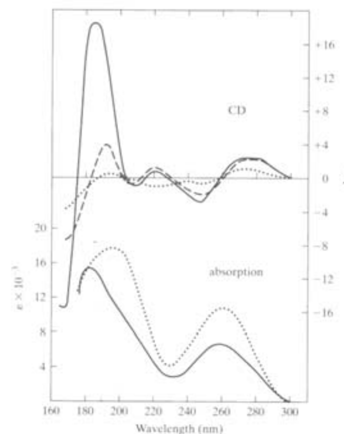
- CD is only observed at wavelengths where absorbances of R & L components of circularly polarized light are not zero i.e. in absorption bands.
- The CD arises because of the interaction between different transition dipoles doing the absorption. As this depends on the relative orientation of different groups in space the signal is very sensitive to conformation. So in general $\Delta\varepsilon$ is much more conformation dependent that ε.
- Most CD work with proteins uses **"electronic CD" of peptides and proteins below 240nm (180-230 nm)**. 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.



Note: Some of the material in these slides was modified from that presented by Smart - University of Birmingham

CD signal is a small difference between two large originals



- CD of *E.Coli* DNA

—— Native
------ Denatured

- *For instance* at 260 nm
  $\Delta\varepsilon = \sim 3\ M^{-1}cm^{-1}$
  $\varepsilon = \sim 6000\ M^{-1}cm^{-1}$
- *i.e.* CD signal 0.05% of original
- need to measure signals ~1/100 of this!

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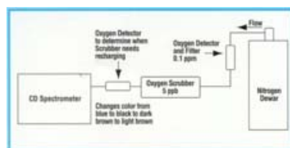
Instrumentation - lab-based spectropolarimeter

- £80k+
- automatic vs λ, time, temperature, stopped flow…
- down to 190nm (if you are lucky)
- 450W Xe bulb - produces ozones
- ozone kills (a) you (b) silver coated optics (as important)
- So flush large amounts of N_2 use boil off from liquid N_2



- www.avivinst.com

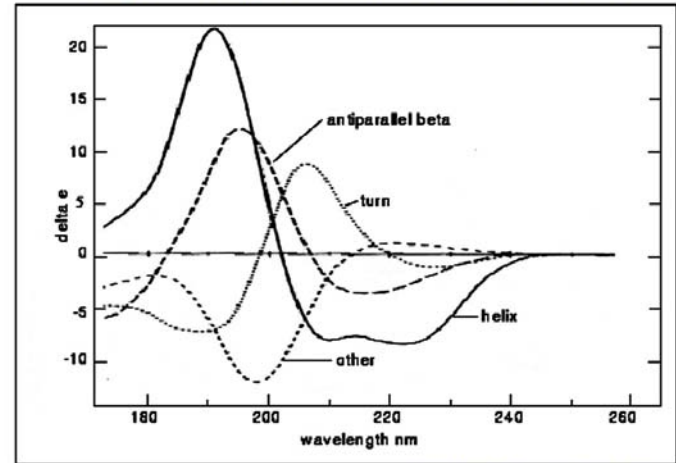
CD in Practice

- CD is based on measuring a very small difference between two large signals must be done carefully
- the Abs must be reasonable max between ~0.5 and ~1.5.
- Quartz cells path lengths between 0.0001 cm and 10 cm. 1cm and 0.1 cm common
- have to be careful with buffers TRIS bad - high UV abs
- Measure cell base line with solvent
- Then sample with same cell **inserted same way around**
- Turbidity kills - filter solutions
- Everything has to be **clean**
- **For accurate secondary structure estimation - must know concentration of sample**

Typical Conditions for CD

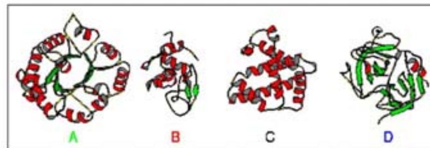
- Protein Concentration: 0.25 mg/ml
- Cell Path Length: 1 mm
- Volume 400 µl
- Need very little sample 0.1 mg
- Stabilizers (Metal ions, etc.): minimum
- Buffer Concentration : 5 mM or as low as possible while maintaining protein stability

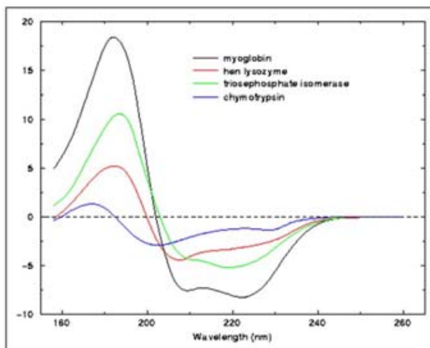
CD Spectra of "pure" Protein Secondary Structures



Adapted from Brahms and Brahms, 1980

CD Spectrum of Representative Protein Structures





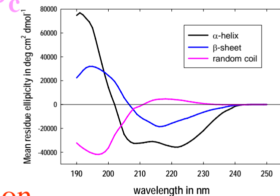
After tutorials by Kurt D. Berndt, Karolinska Institute, Stockholm, Sweden
http://broccoli.mbb.ki.se/pps_course_96/ss_960723_1.html

If we measure the CD signal for a protein of unknown structure we can find its proportions of secondary structures

- Fit the unknown curve θ_u to a combination of standard curves.
- In the simplest case use the Fasman standards

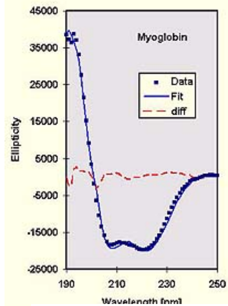
$$\theta_t = x_\alpha\theta_\alpha + x_\beta\theta_\beta + x_c\theta_c$$

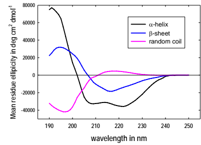
- Vary x_α, x_β and x_c to give the best fit of θ_t to θ_u while $x_\alpha + x_\beta + x_c = 1.0$



- Do this by least squares minimization

Example fit: myoglobin



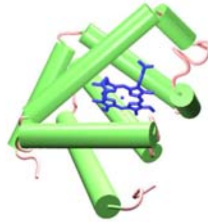


- In this case:

$\theta_t = x_\alpha\theta_\alpha + x_\beta\theta_\beta + x_c\theta_c$

- fits best with

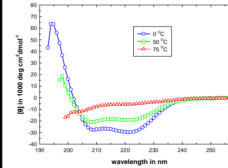
x_α = 80%,
x_β = 0%,
x_c = 20%

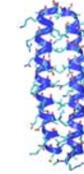


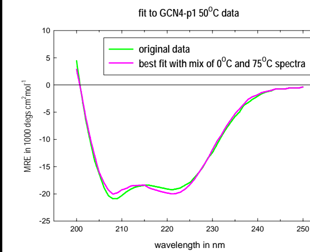
- agrees well with structure
  78% helix, 22% coil

For further details:
www-structure.llnl.gov/cd/cdtutorial.htm

Example fit (2): GCN4-p1







- At 0°C 100% helix
  75°C 0% helix
- Q: what about 50°C?

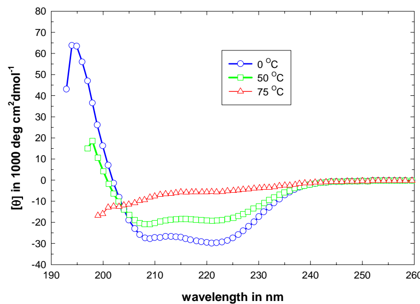
$\theta_t = x_o\theta_o + x_{75}\theta_{75}$

- fits best with

x_o = 50%,
x_{75} = 50%

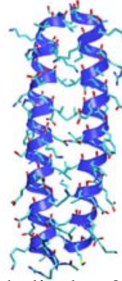
- Shows that at 50°C
  1/2 of peptide α-helix dimer
  1/2 of peptide random coil monomer

CD signals are sensitive to secondary structure



This is the CD spectrum for an α-helix

- GCN4-p1 is a coiled–coil:



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

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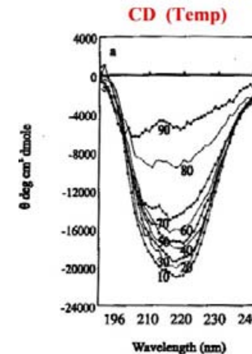
Circular Dichroism

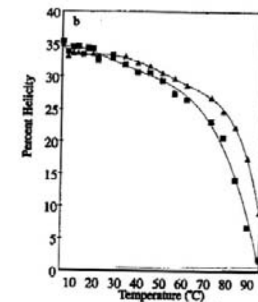
CD can be used to monitor protein or nucleic acid denaturation.

Ribsomal protein L9

CD (Temp)



% Helix Content vs. Temp



Nucleotides have very small CD spectra, but
the helical DNA structures have strong CD signals.

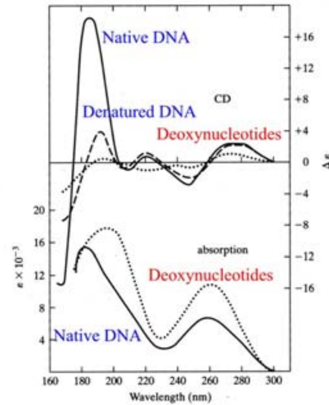
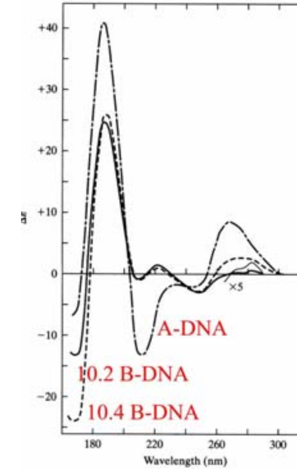


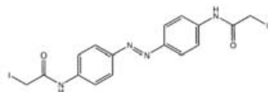
Figure 10.7 The CD of native *E. coli* DNA (——), denatured *E. coli* DNA (– – – –), and the average CD for the four corresponding deoxynucleotides (· · ·) in aqueous solution. The absorption spectrum of native *E. coli* DNA (——) and the average spectrum for the four component deoxynucleotides (· · ·) illustrates the point that there is only CD where there is normal absorption. [Reprinted from C. A. Sprecher and W. C. Johnson, Jr. (1977) *Biopolymers* **16**, 2243–2264, copyright 1977 by John Wiley & Sons.]

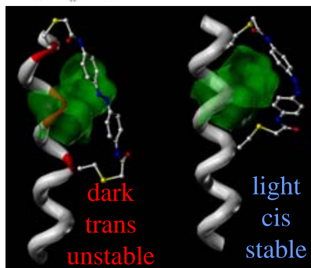


E. coli DNA - CD of different secondary structures.

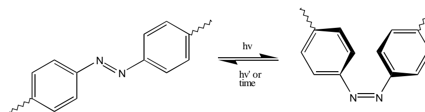
Figure 10.11 The CD of *E. coli* DNA in various secondary structures: 10.4 B-form in aqueous buffer at pH 7.0 (– – – – –); the 10.2 B-form in 6M NH_4F, aqueous buffer at pH 7 (——); the A-form in 80% 2,2,2-trifluoroethanol (- · - · - ·). [Adapted from data in C. A. Sprecher, W. A. Baase, and W. C. Johnson (1979) *Biopolymers* **17**, 1009–1019. Reprinted from W. C. Johnson, "Circular Dichroism and Its Empirical Application to Biopolymers," *Methods of Biochemical Analysis*, vol. 31, ed. D. Glick, copyright 1985 by John Wiley & Sons.]

Using CD to test a peptide designed to have its conformation controlled by light



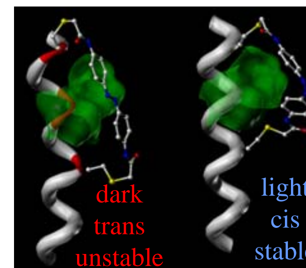


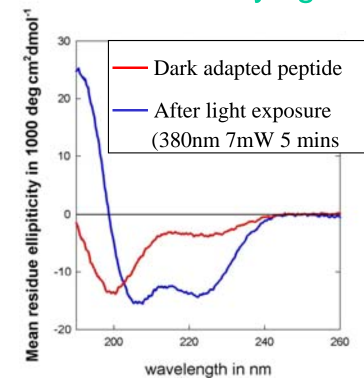
- Uses a bifunctional iodoacetamide derivative of azobenzene that cross links a pair of cys residues.
- The azobenzene group adopts a trans conformation in the dark but can be forced to adopt a cis conformation by exposure to visible light of the appropriate wavelength:



- Designed peptide to be helical in the cis (light) but helix to be unstable in the dark

Using CD to test a peptide designed to have its conformation controlled by light





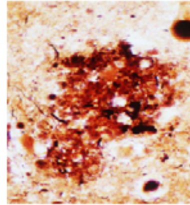
- Can roughly gauge helicity
  helicity = $[\theta]_{222}/32000$
- In this case
  dark 11% helix, Light 48%

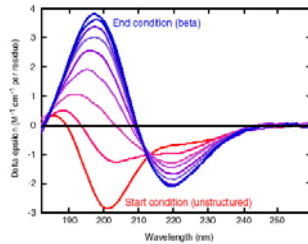
Kumita, Smart & Woolley
PNAS (2000) **97**:3803-3808

Amyloid diseases

A number of diseases (e.g. Alzheimer's, CJD, BSE) involve the folding of proteins and peptides into beta-sheet structures which can polymerise, forming insoluble plaques in nerve tissue (below right).

A model for the Alzheimer's peptide is LRRN, which forms spontaneously into gels with a β-sheet structure.





SRCD spectra* (left) taken during the polymerisation of LRRN peptide show that the rate of polymerisation varies with substitution of a single amino acid residue.

*Collaboration with N.Gay and M. Symmons, Cambridge University

The SRCD data provide important information about the processes involved in polymerisation, and may lead to the development of drugs to treat these diseases.

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Summary

- CD is a useful method for looking at secondary structures of proteins and peptides.
- It is an adaptation standard absorption spectroscopy in which the difference in the abs between left and right hand circularly polarized light is measured.
- CD can be measured under a wide range of conditions - e.g., good for membrane proteins.
- CD can be used to measure change.
- CD compliments other more detailed techniques such as crystallography.

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