THE UNIVERSITY of TEXAS

SCHOOL OF HEALTH INFORMATION SCIENCES AT HOUSTON

# Circular Dichroism

For students of HI 6001-125

"Computational Structural Biology"

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Adopted from material by Mathew Baker, Univ. of Bath

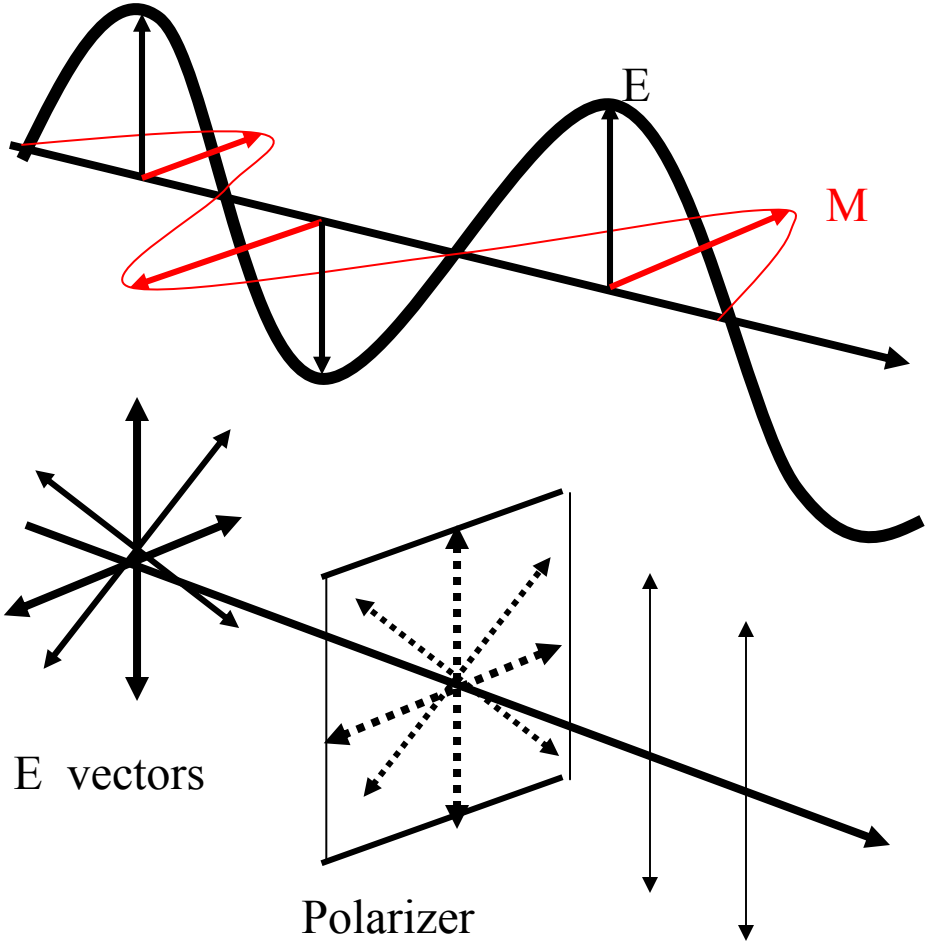
http://staff.bath.ac.uk/bssmdb/

http://biomachina.org/courses/structures/05.html

# 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



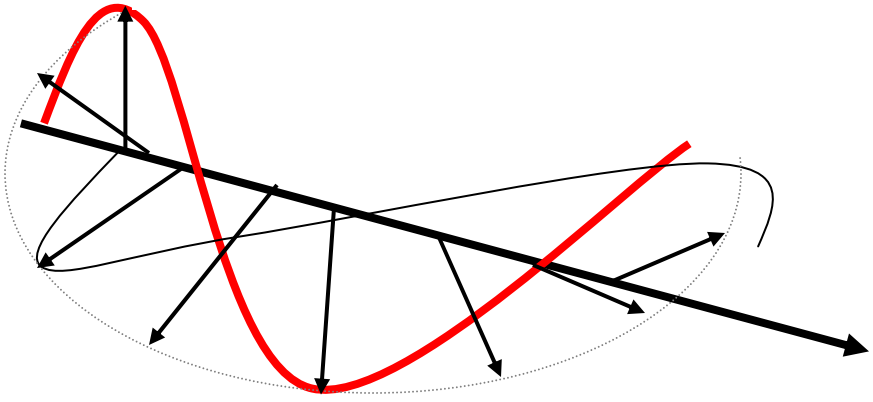

Direction of propagation

Direction of propagation

# Plane & Circularly Polarized Light

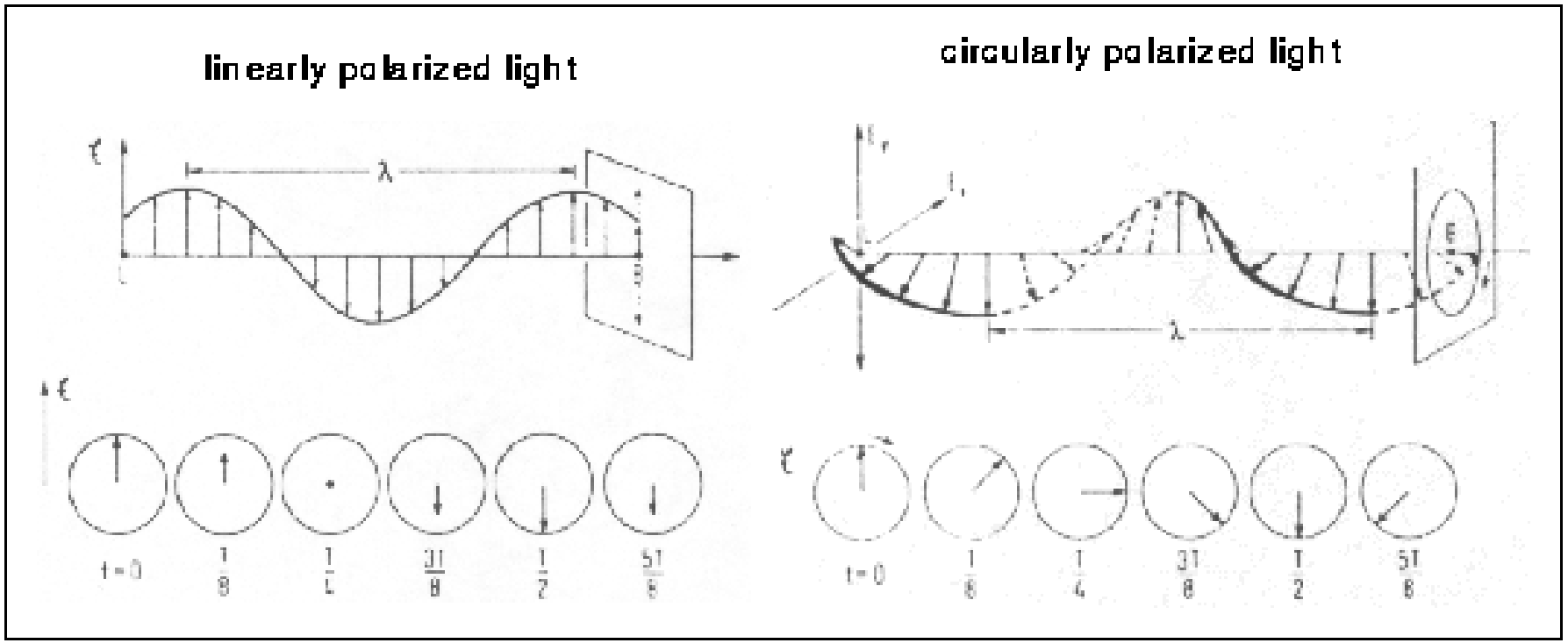
•A light source usually consists of a collection of randomly orientated emitters, the emitted light is a collection of waves with all possible orientations of the E vectors.

•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:

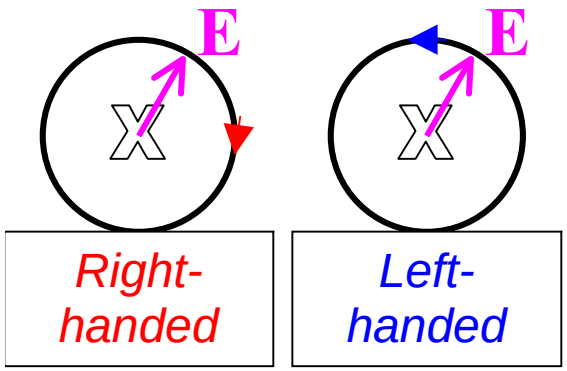
  Electric vector direction varies - magnitude constant

# Circularly Polarized Light

- Circular polarized light:

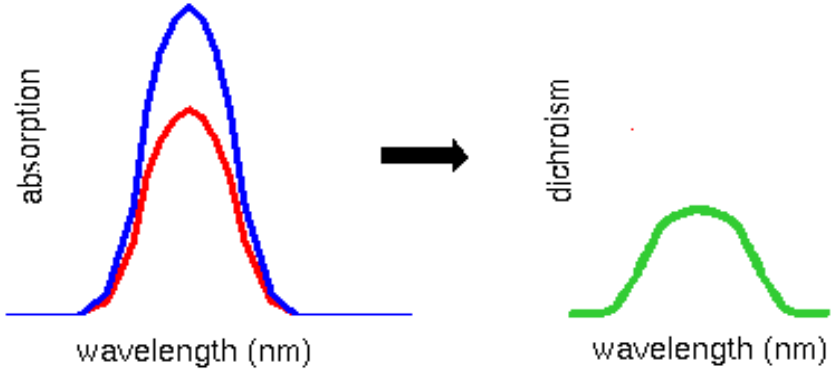
  Electric vector direction varies - magnitude constant

- So its in two forms: left and right handed



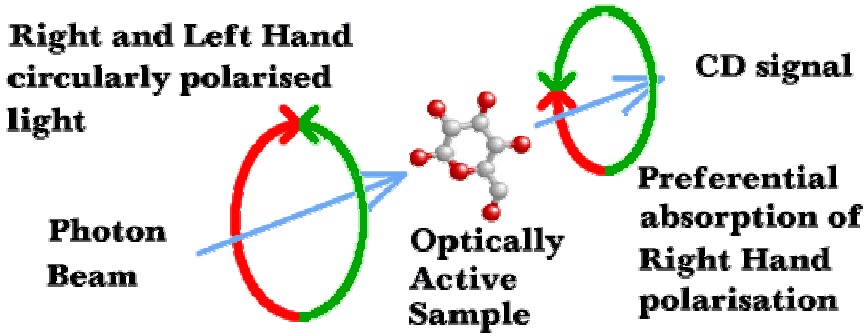
# Circular Dichroism

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









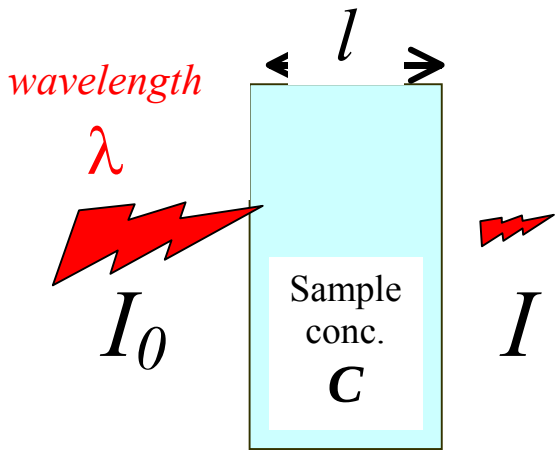

- This is measured as a function of wavelength, & the difference is always very small (<<1/10000 of total). After passing through the sample, the L & R beams have different amplitudes & the combination of the two unequal beams gives elliptically polarized light.Hence, CD measures the ellipicity of the 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:

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

$\varepsilon$: 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 $\varepsilon$ around 20 000 $M^{-1}cm^{-1}$
- So **the CD signal is a very small difference between two large originals**.

# 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.

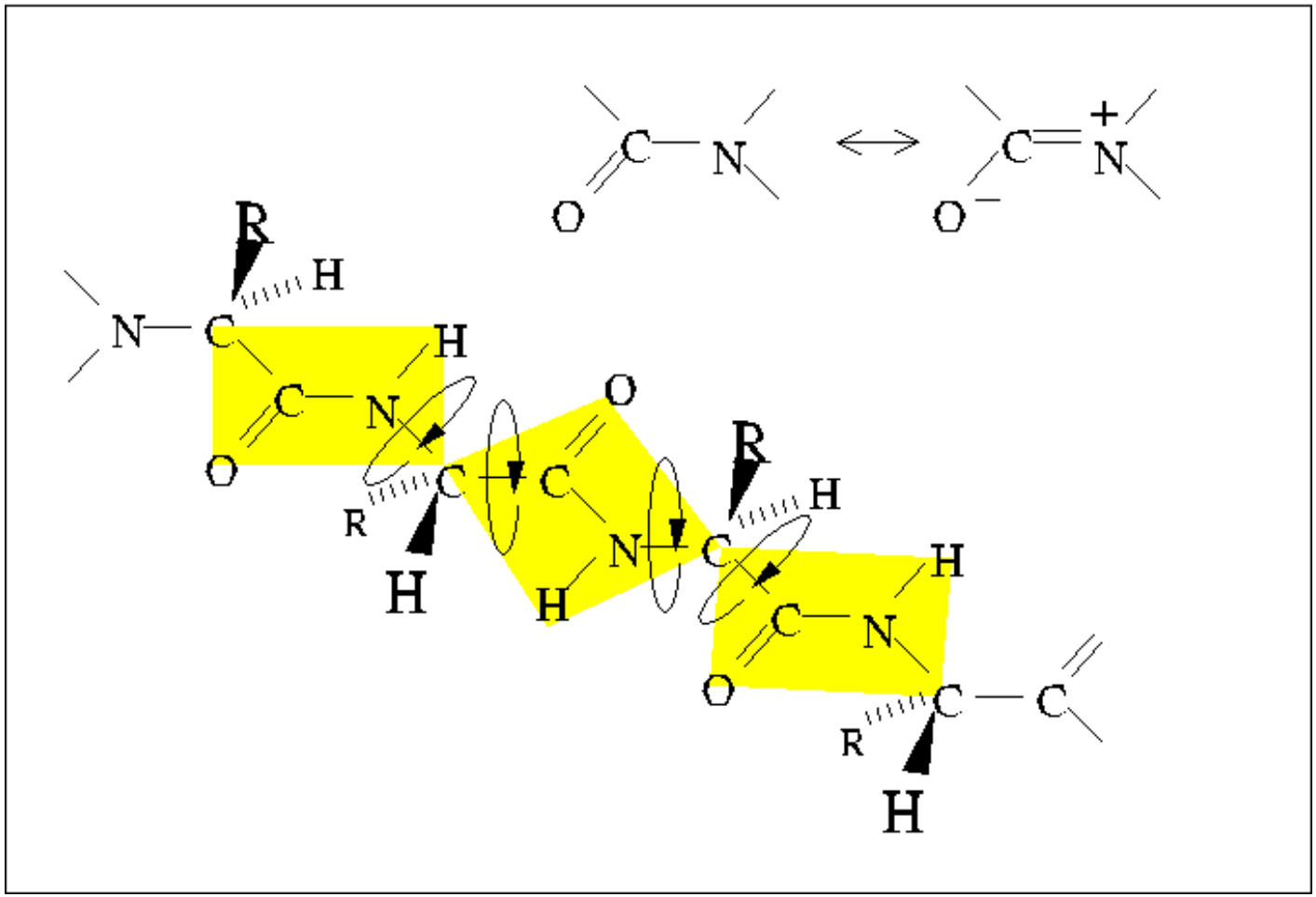
- So in general $\Delta\varepsilon$ is much more conformation dependent that $\varepsilon$.

- 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.

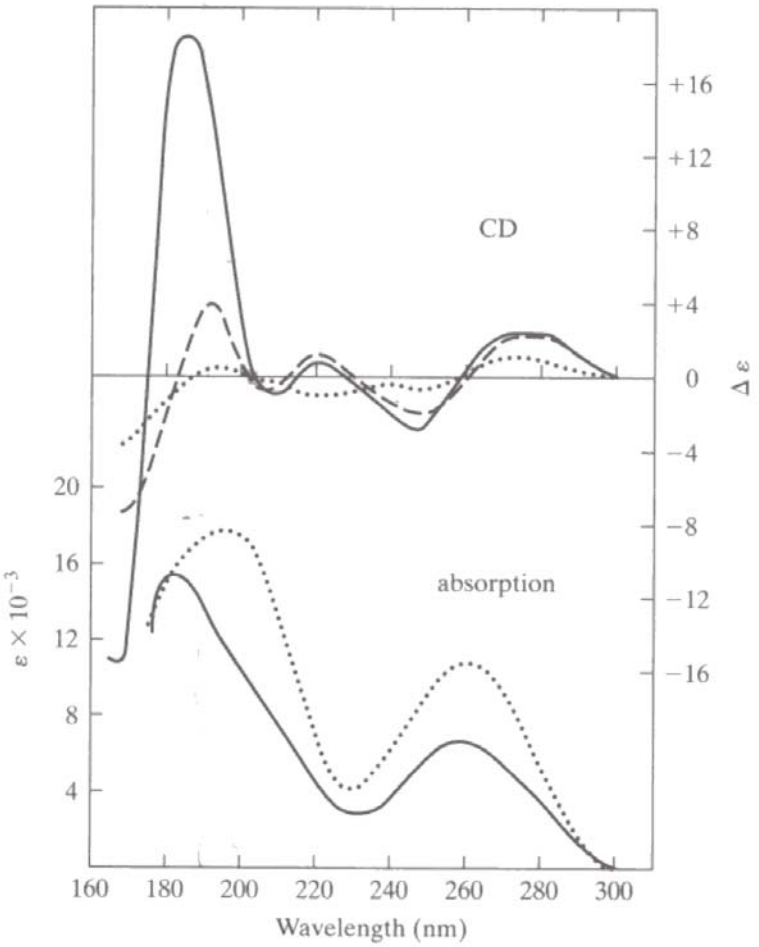
- Can also do CD in near UV (look at trp side chains), visible (cofactors etc.) and IR regions.

•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 Signal is a Small Difference Between Two Large Originals




- CD of *E.Coli* DNA

—— Native

------ Denatured

- *For instance* at 260 nm

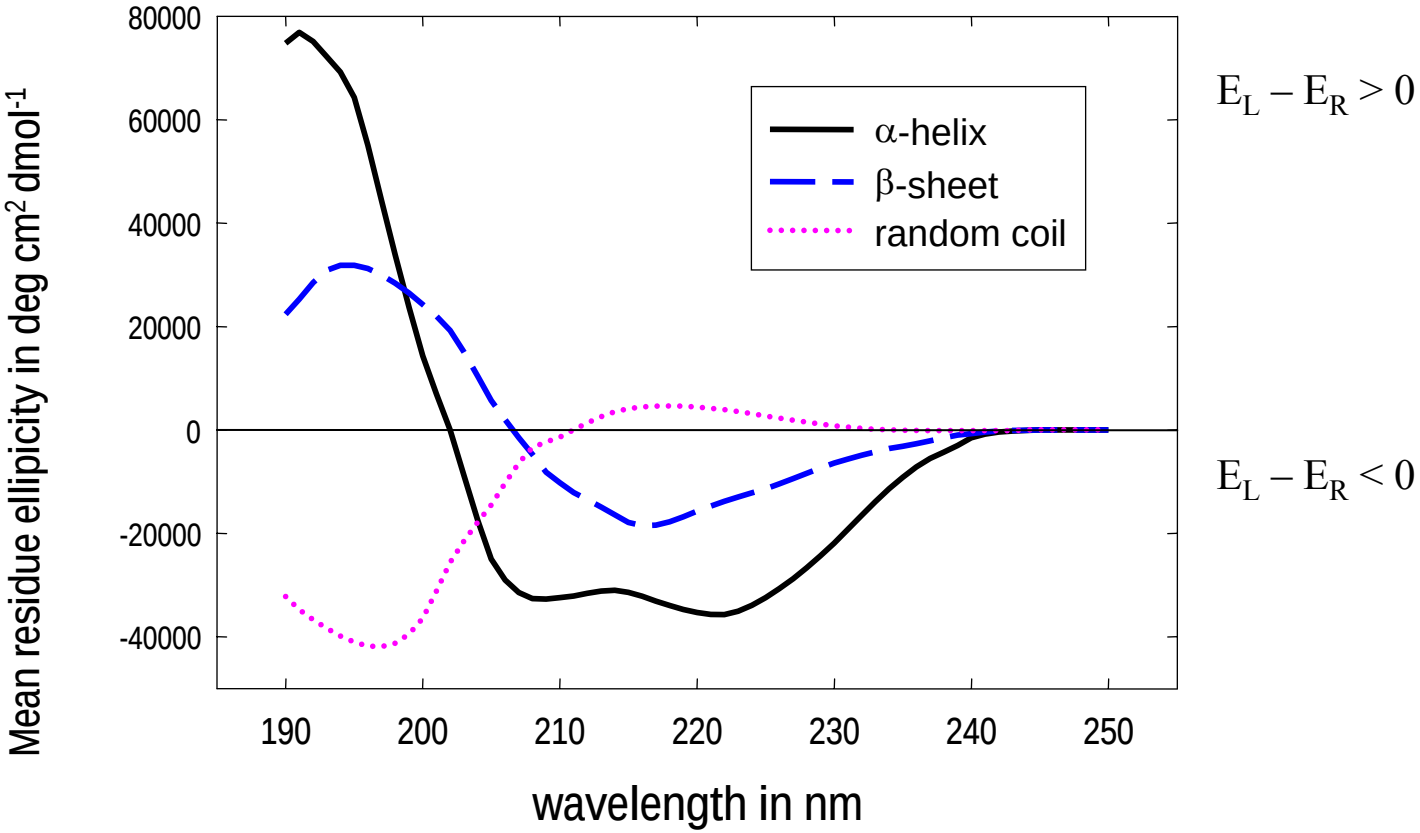
  $\Delta\varepsilon$ = ~3 $M^{-1}cm^{-1}$

  $\varepsilon$ = ~6000 $M^{-1}cm^{-1}$

- *i.e.* CD signal 0.05% of original

- need to measure signals ~1/100 of this!

# CD Signals for Different Secondary Structures



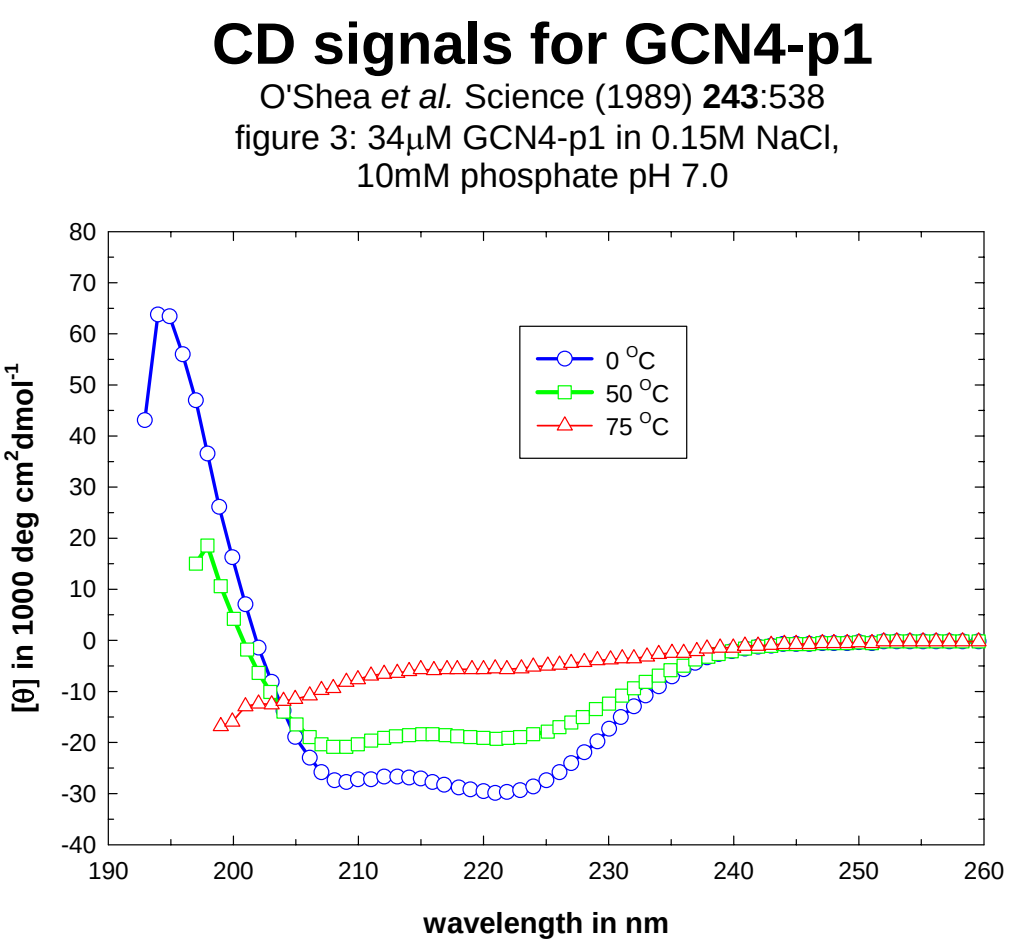

- These are Fasman standard curves for polylysine in different environments

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

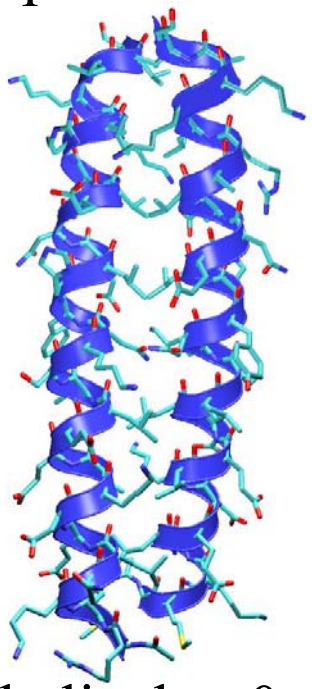
# CD Spectra of Protein 2$^{ndary}$ Structures

| | -ve band (nm) | +ve band (nm) |
|---|---|---|
| **α-helix** | 222<br>208 | 192 |
| **β-sheet** | 216 | 195 |
| **β-turn** | 220-230 (weak)<br>180-190 (strong) | 205 |
| **L.H polypro II helix** | 190 | 210-230 weak |
| **Random coil** | 200 | |

# 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

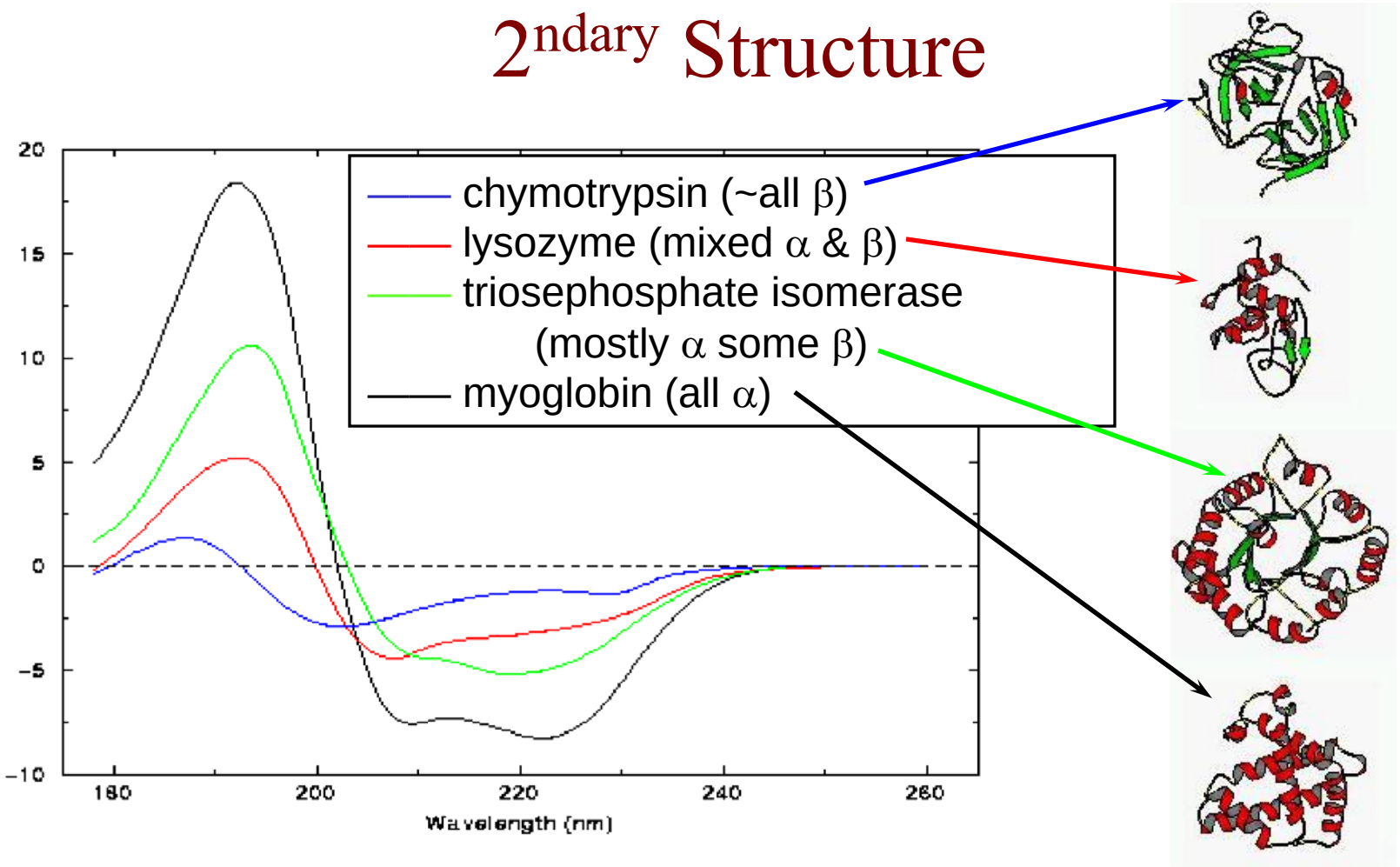
# Applications of CD

- 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

# Total Signal for a Protein Depends on its 2ndary Structure



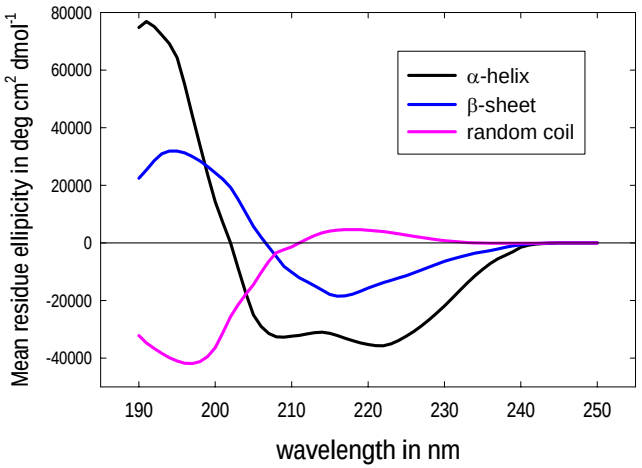

- Notice the progressive change in $\theta_{222}$ as the amount of helix increases from chymotrypsin to myoglobin

# Finding Proportion of 2$^{ndary}$ Structures

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

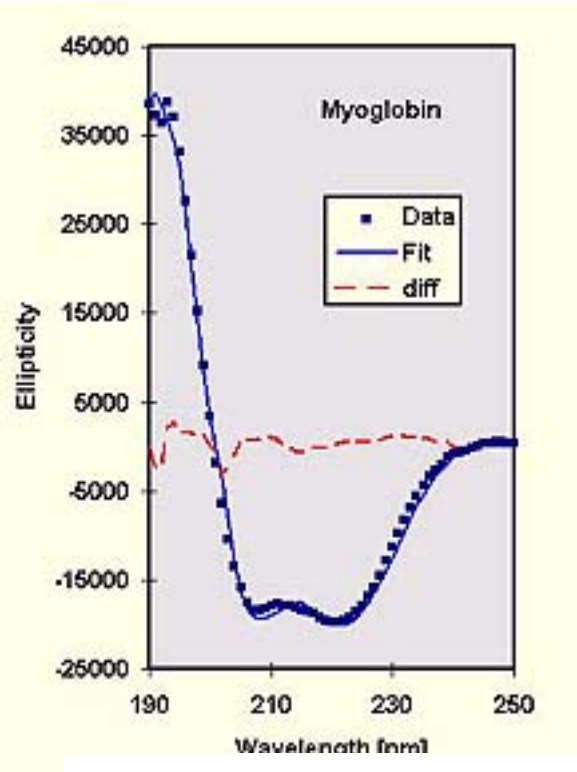
$$\theta_t = x_\alpha\theta_\alpha + x_\beta\theta_\beta + x_c\theta_c$$

- Vary $x_\alpha$, $x_\beta$ and $x_c$ to give the best fit of $\theta_t$ to $\theta_u$ while $x_\alpha + x_\beta + x_c = 1.0$




- Do this by least squares minimization

# Example Fit: Myoglobin

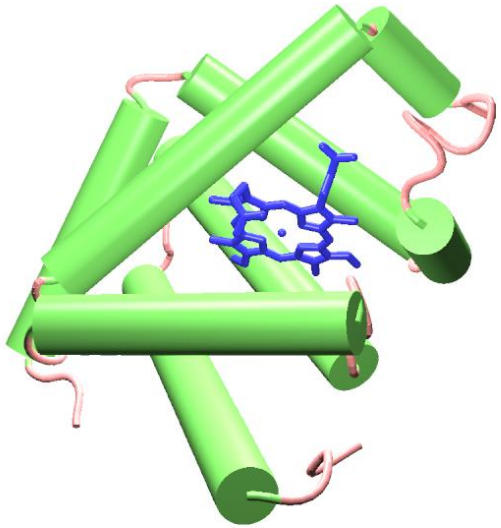


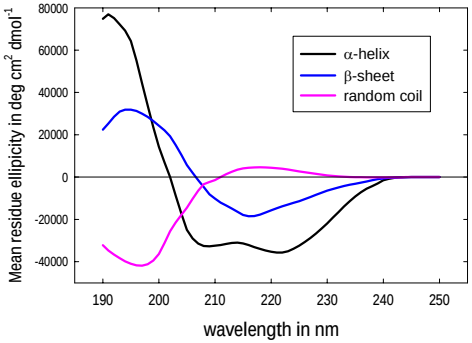

- In this case:

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

- fits best with

$x_\alpha = 80\%$,
$x_\beta = 0\%$
$x_c = 20\%$

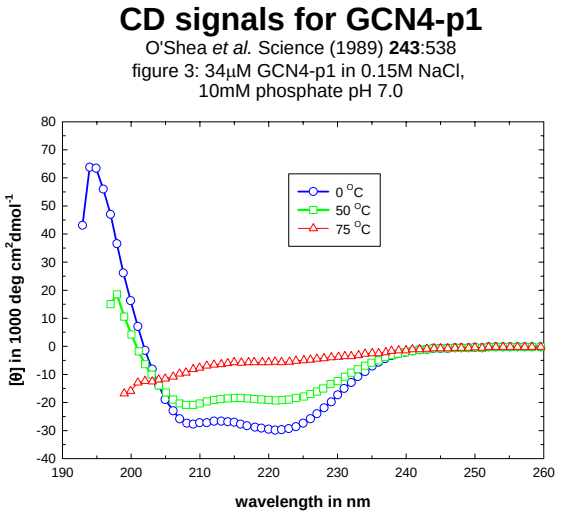


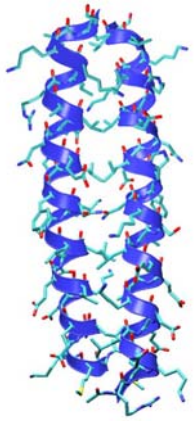


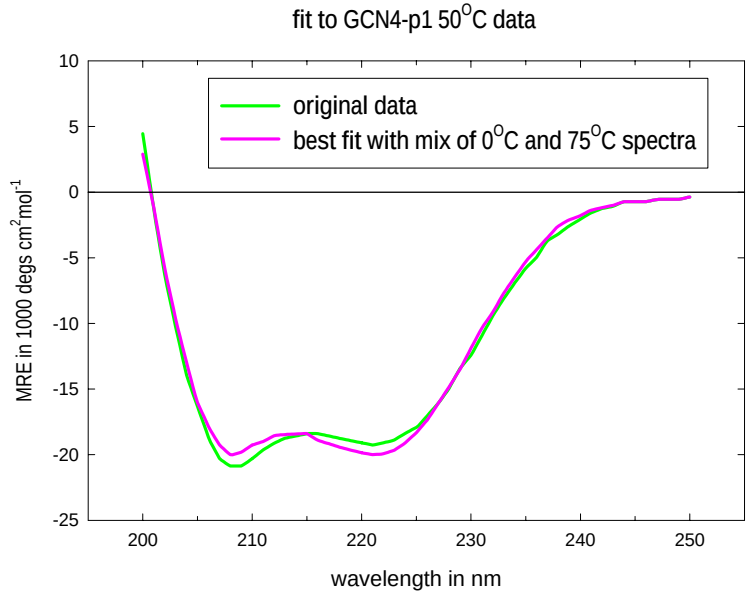

- agrees well with structure

  78% helix, 22% coil

# 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

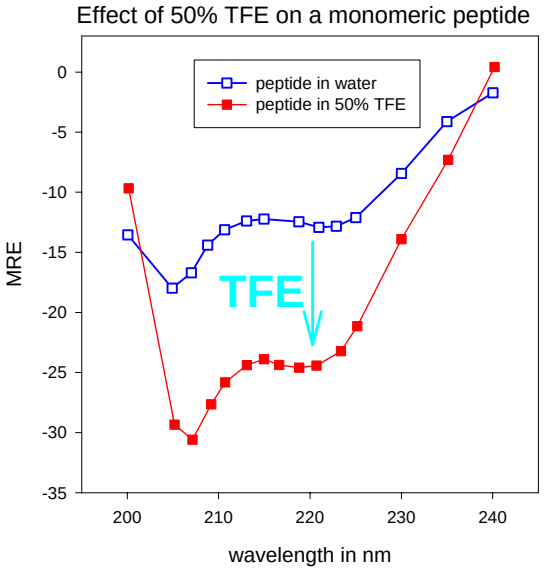
  $x_o = 50\%$,
  $x_{75} = 50\%$
- Shows that at 50°C

  1/2 of peptide α-helix dimer

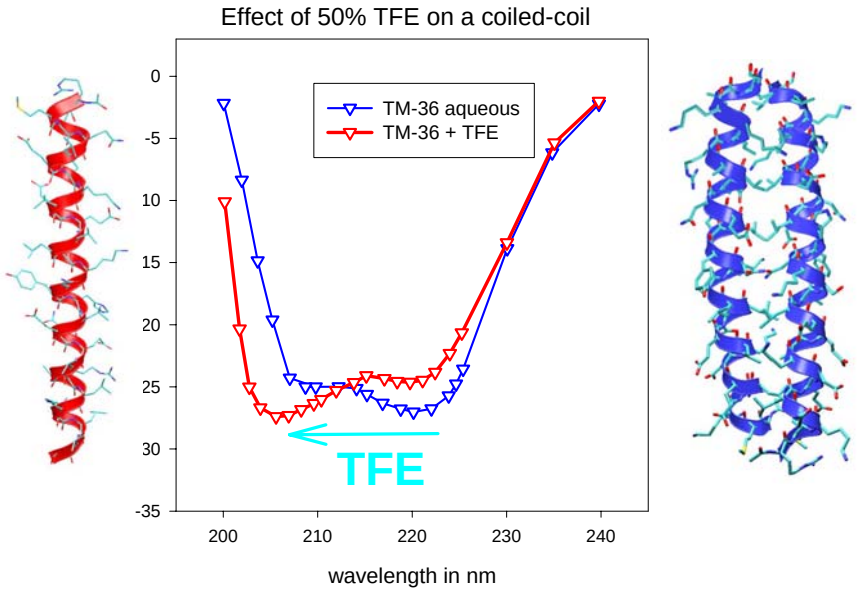
  1/2 of peptide random coil monomer

# However, CD Signal Depends Somewhat on Environment

- Can see this by looking at the effect of trifluoroethanol (TFE) on a coiled-coil similar to GCN4-p1
- TFE induces helicity in all peptides




- But on a coiled-coil breaks down helical dimer to single helices




- Although 2ndry structure same CD changes

Lau, Taneja and Hodges (1984)
J.Biol.Chem. **259**:13253-13261

# Best Fitting Procedures Use Many Different Proteins For Standard Spectra

- There are many different algorithms.
- All rely on using up to 20 CD spectra of proteins of known structure.
- By mixing these together a fit spectra is obtained for an unknown.
- For full details see

  Dichroweb: the online CD analysis tool
  www.cryst.bbk.ac.uk/cdweb/html/

- Can generally get accuracies of

  0.97 for helices,
  0.75 for beta sheet,
  0.50 for turns, and
  0.89 for other structure types

(Manavalan & Johnson, 1987, Anal. Biochem. **167**, 76-85).

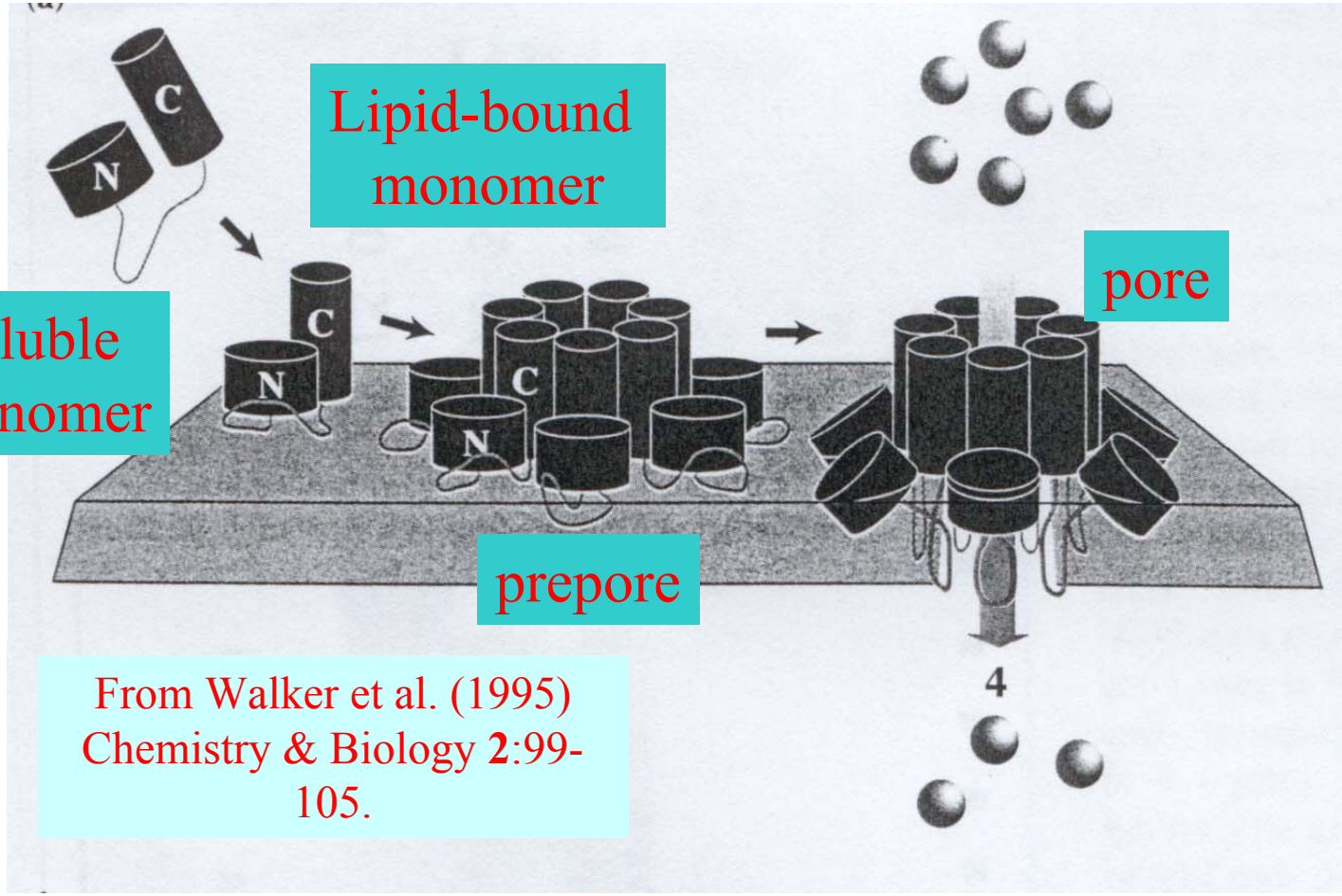
# Limitations of Secondary Structure Analysis

- The simple deconvolution of a CD spectrum into 4 or 5 components which do not vary from one protein to another is a gross over-simplification.
- The reference CD spectra corresponding to 100% helix, sheet, turn etc are not directly applicable to proteins which contain short sections of the various structures e.g. The CD of an α-helix is known to increase with increasing helix length, CD of β-sheets are very sensitive to environment & geometry.
- Far UV curves (>275nm) can contain contributions from aromatic amino-acids, in practice CD is measured at wavelengths below this.
- The shapes of far UV CD curves depend on tertiary as well as secondary structure.

# CD is Very Useful for Looking at Membrane Proteins

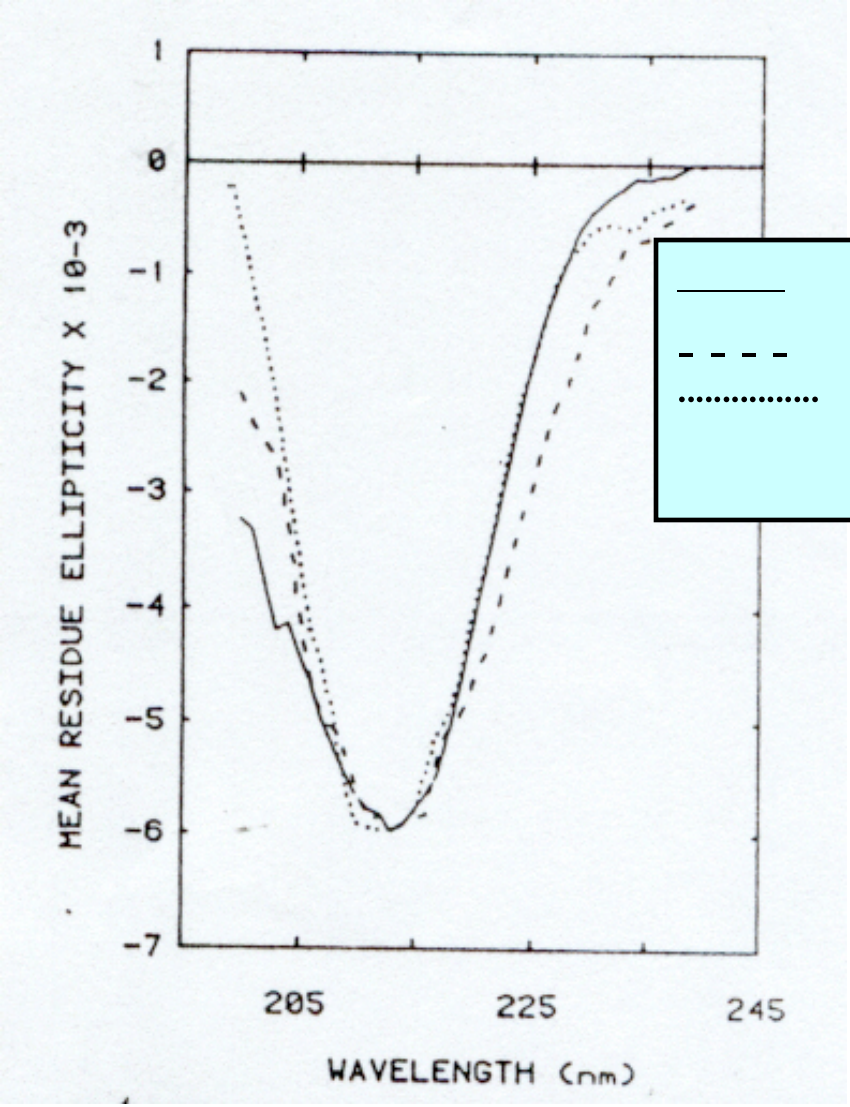
- Membrane proteins are difficult to study.
- Crystallography difficult - need to use detergents
  Even when structure obtained:
  Q- is it the same as in lipid membrane?
- CD ideal can do spectra of protein in lipid vesicles.
- We look at Staphylococcal $\alpha$-hemolysin as an example

# α-Hemolysin Channel Formation




- This model was built by combining CD results with mutagenesis cross-linking, channel measurements

# α-Hemolysin CD Results

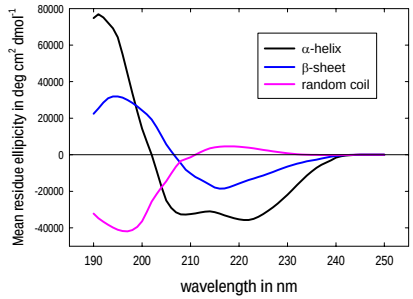



soliid monomer

lipid monomer

assembled hexamer or heptamer

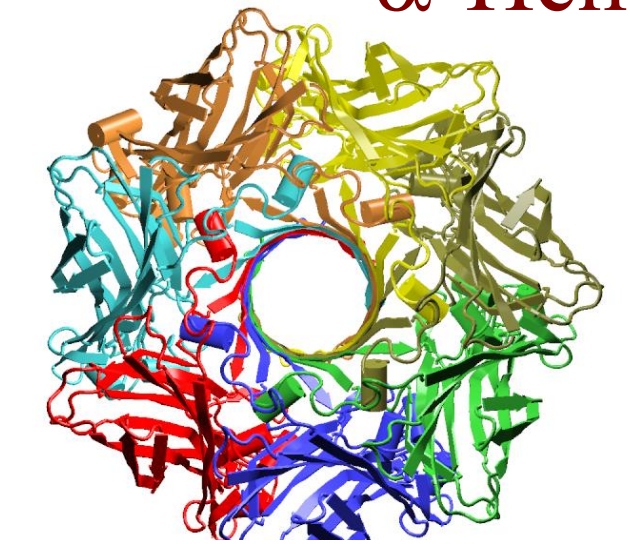
- Tobkes et al. (1985) Biochemistry 24:1915

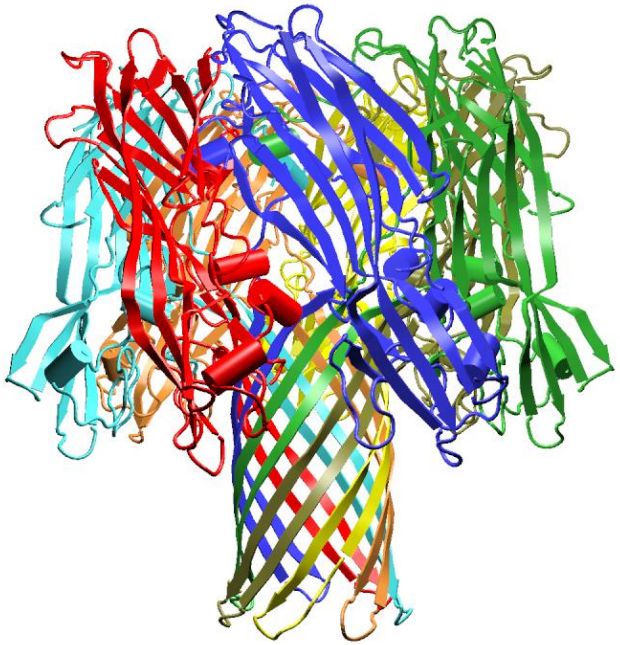



- The 3 spectra are similar

  So the 3 conformations have similar 2ndry structure
- What is the 2ndry structure?
- Is this normal for a membrane protein?

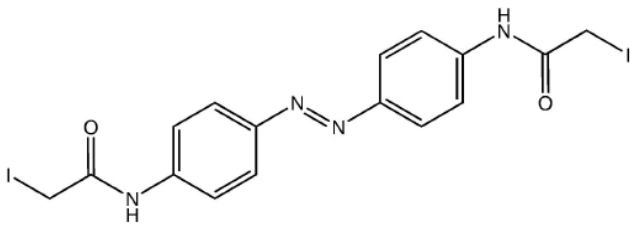
# α-Hemolysin Structures

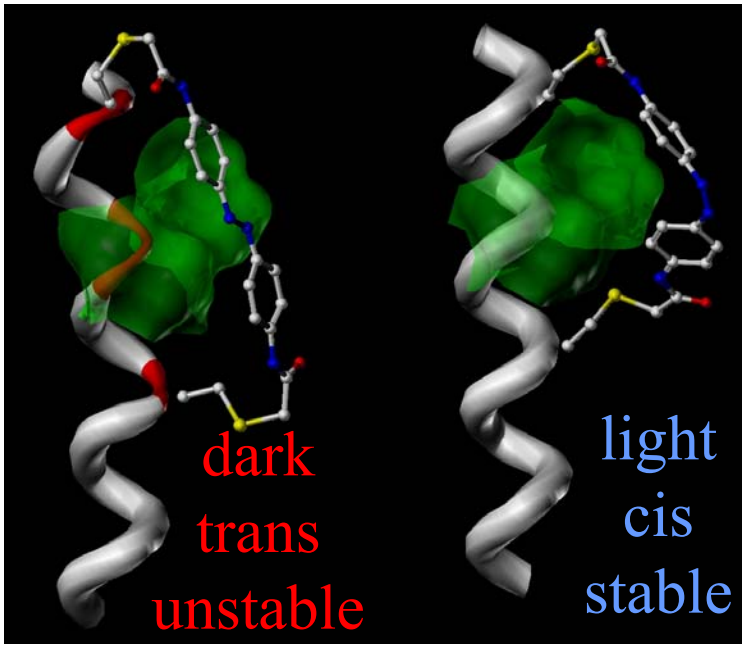




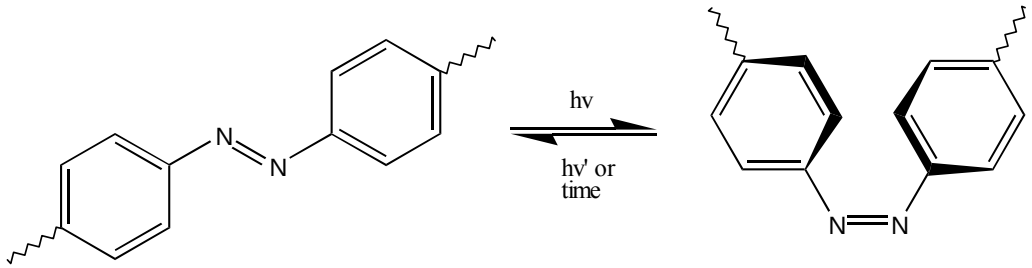
- Crystal structure of pore
  - Song et al. Science 1996 274:1859
- CD used to show that the structure in detergent was the same as that of the pore in lipid.
- Just about all β
- Recently have structure of related soluble monomer

# Using CD to Test a Peptide Designed to be Controlled by Light



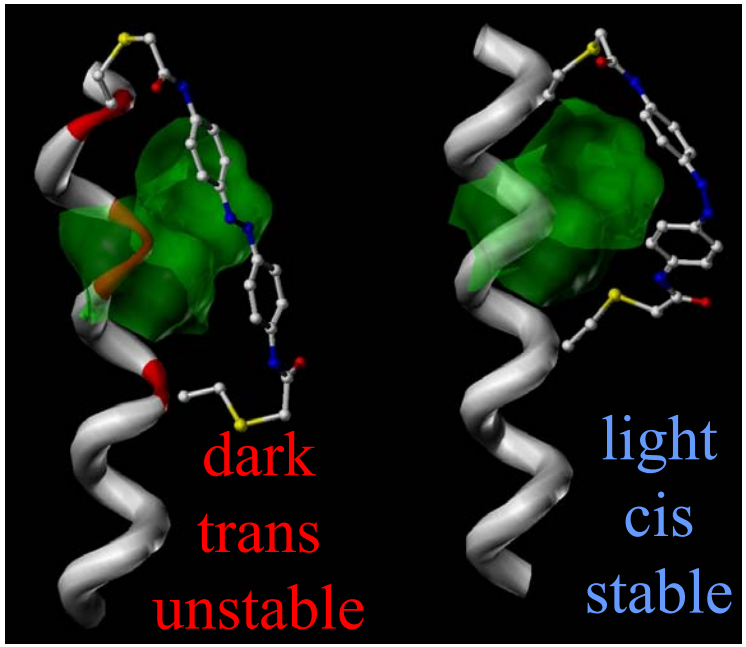


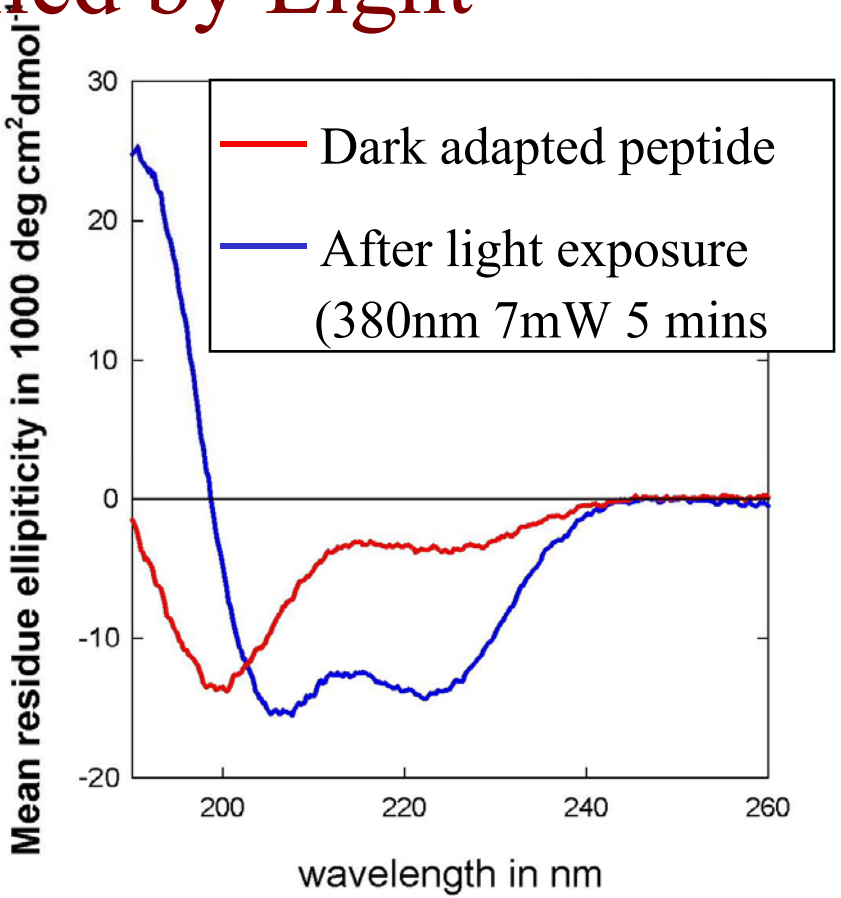

- Uses a bifunctional iodoacetamide derivative of azobenzene that cross links a pair of cys residues.
- The azobenzene group adopts a trans conformation in the dark but can be forced to adopt a cis conformation by exposure to visible light of the appropriate wavelength:




- 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 be 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

# Practicalities

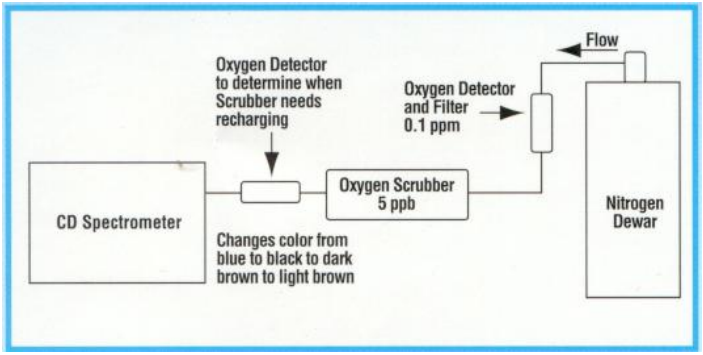
- CD is based on measuring a very small difference between two large signals must be done carefully
- the Abs must be reasonable max between ~0.5 and ~1.5.
- Quartz cells path lengths between 0.0001 cm and 10 cm. 1cm and 0.1 cm common
- have to be careful with buffers TRIS bad - high UV abs
- Measure 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 2ndary structure estimation must know concentration of sample**

# Typical Conditions for CD

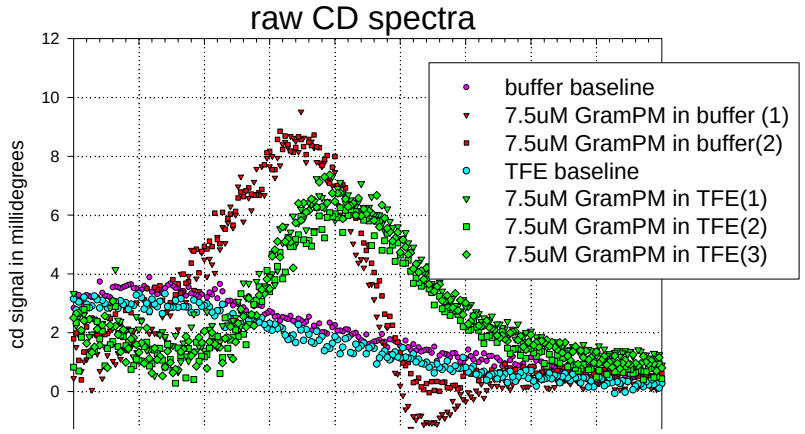
- Protein Concentration: 0.25 mg/ml
- Cell Path Length: 1 mm
- Volume 400 μl
- Need very little sample 0.1 mg
- Concentration reasonable
- Stabilizers (Metal ions, etc.): minimum
- Buffer Concentration : 5 mM or as low as possible while maintaining protein stability
- A structural biology method that can give real answers in a day.

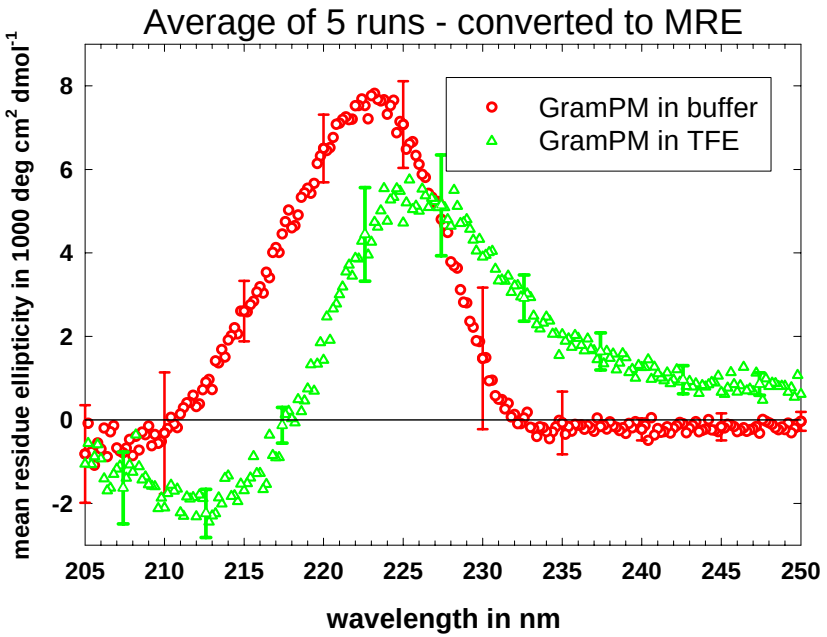
# Instrumentation - Lab-Based Spectropolarimeter


- $120k+
- automatic vs λ, time, temperature, stopped flow…
- down to 190nm (if you are lucky)
- 450W Xe bulb - produces ozone: hazard for health and silver coated optics
- So flush with large amounts of $N_2$ use boil off from liquid $N_2$



# CD Analysis

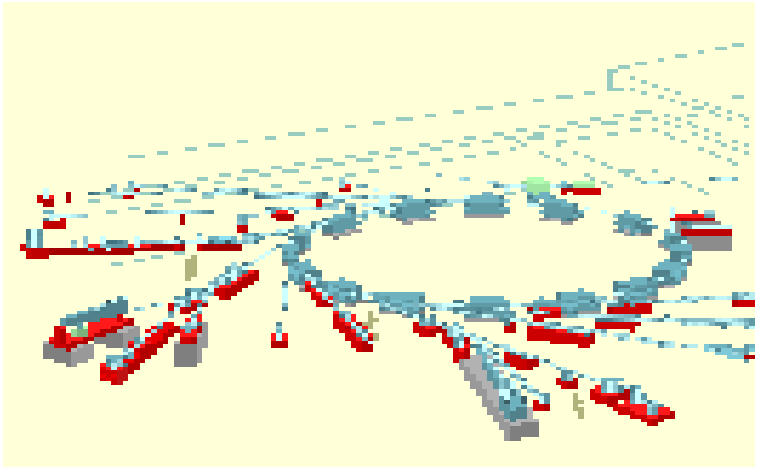


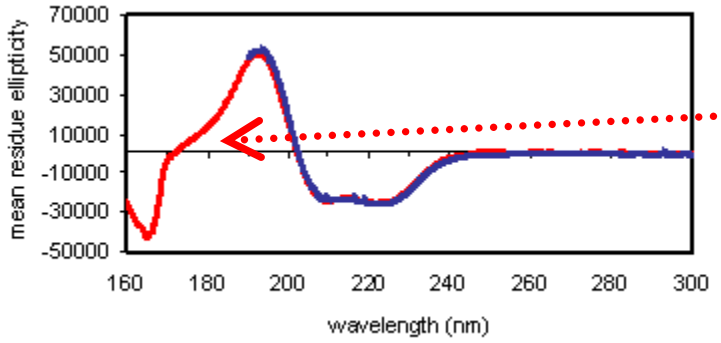




- Looking at the CD of a gramicidin suspension in water
- Raw spectrum - data every 0.2nm from 205nm to 250nm, each data point measured 5 times 1sec avg.
- Note the baselines, these vary from cell to cell or if instrument moved, new bulb, recalibrated….
- Note noise - that's why we measure so many points.
- Final spectra the average of 5 runs (with about 3 baselines).

- Result - gramicidin suspension in buffer has a novel CD spectrum
- But what is the structure??

# Instrumentation: Synchrotron-Based



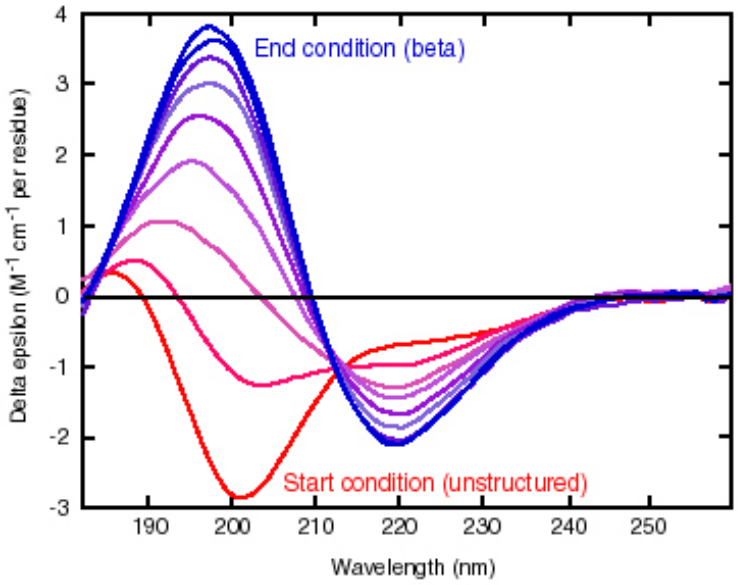


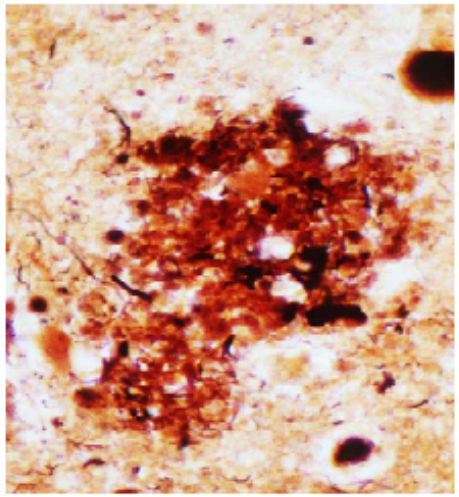

- Synchrotron - whiz electrons around a ring.
- Can be used to produce very intense radiation by wiggling beam.
- Commonly used to produce X–rays ($\lambda$ around 0.1nm)
- But can be used to push signals down to 160nm and below
- Great for fast stopped-flow to see rapid changes

# 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.

# Summary

- CD is a useful method for looking at secondary structures of proteins and peptides.
- It is an adaptation of 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.

# Resources

- Books: van Holde KE, Johnson W & Ho P,
  Principles of Physical Biochemistry
  Prentice Hall 1998 *or*

  Campbell, ID and Dwek, RA
  Biological Spectroscopy
  Benjamin/Cummings Publishing, 1984.

- MIT CD Links
  http://web.mit.edu/speclab/www/cd_links.html
- Birkbeck College CD Tutorial
  www.cryst.bbk.ac.uk/BBS/whatis/cd_website.html
- CD links page at Daresbury Synchrotron
  www.srs.dl.ac.uk/VUV/CD/links.html
- Dichroweb: online CD analysis tool
  www.cryst.bbk.ac.uk/cdweb/html/

# Figure and Text Credits

Text and figures for this lecture were adapted from the following source:

http://staff.bath.ac.uk/bssmdb/cd_lecture.ppt