

Circular Dichroism

- Most protein secondary structure studies use CD
- Method is bandshape dependent. Need a different analysis
- Transitions fully overlap, peptide models are similar but not quantitative
- Length effects left out, also solvent shifts
- Comparison revert to libraries of proteins
- None are pure, all mixed

Circular Dichroism

CD is polarized differential absorption

$$\Delta A = A_L - A_R$$

only non-zero for chiral molecules

Biopolymers are Chiral (L-amino acid, sugars, etc.)

Peptide/ Protein -

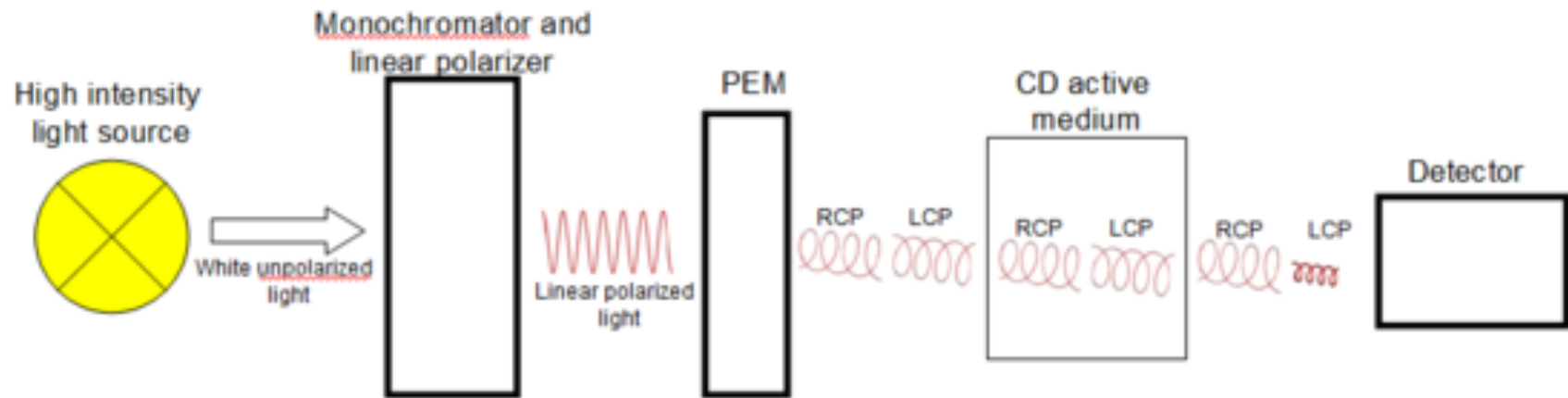
in uv - for amide: $n\text{-}\pi^*$ or $\pi\text{-}\pi^*$ in -HN-C=O-

partially delocalized π -system senses structure

in IR - amide centered vibrations most important

Nucleic Acids – base $\pi\text{-}\pi^*$ *in uv*, PO^{2-} , C=O *in IR*

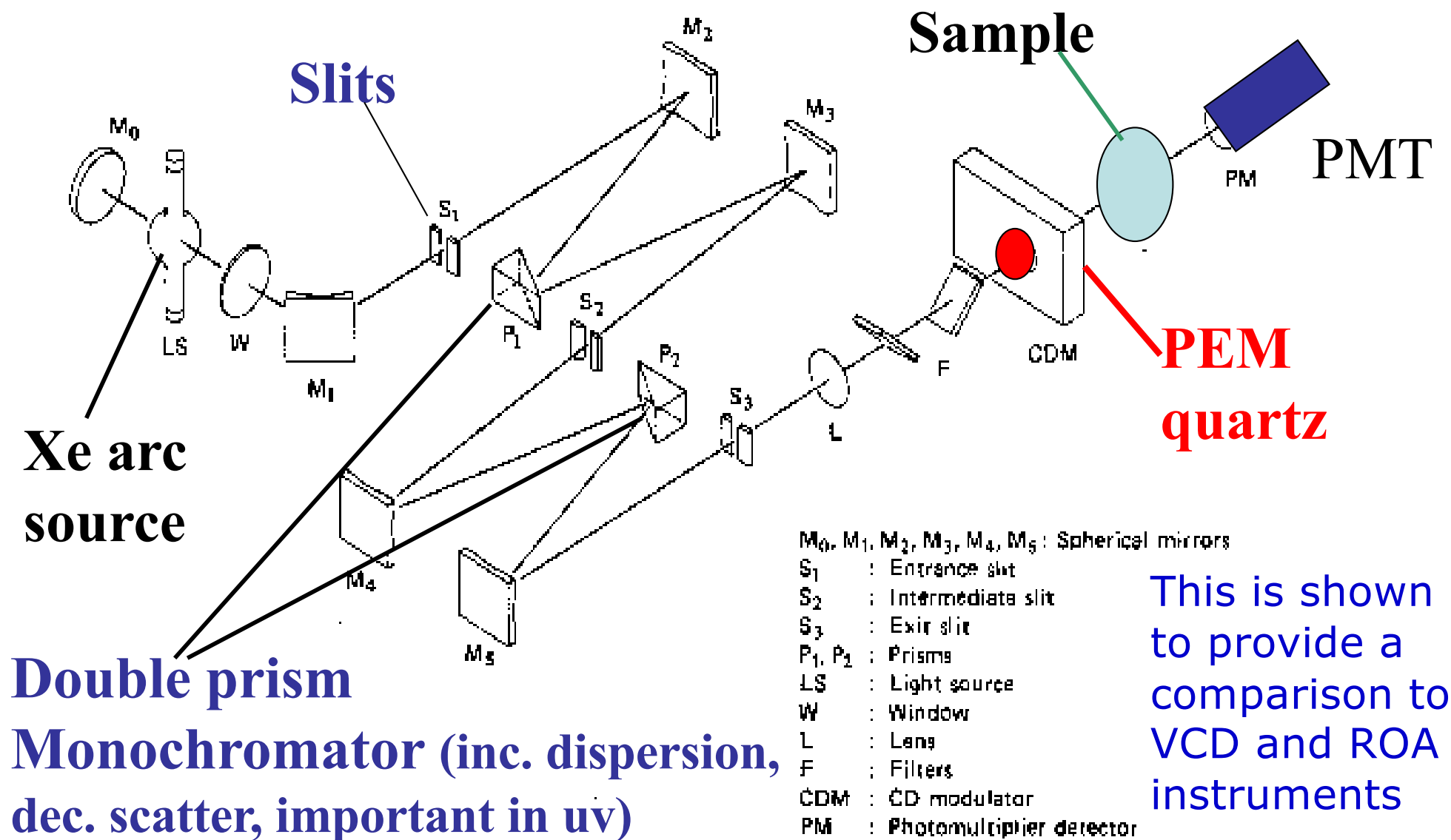
Coupled transitions between amides along chain lead to distinctive bandshapes



Photoelastic Modulator

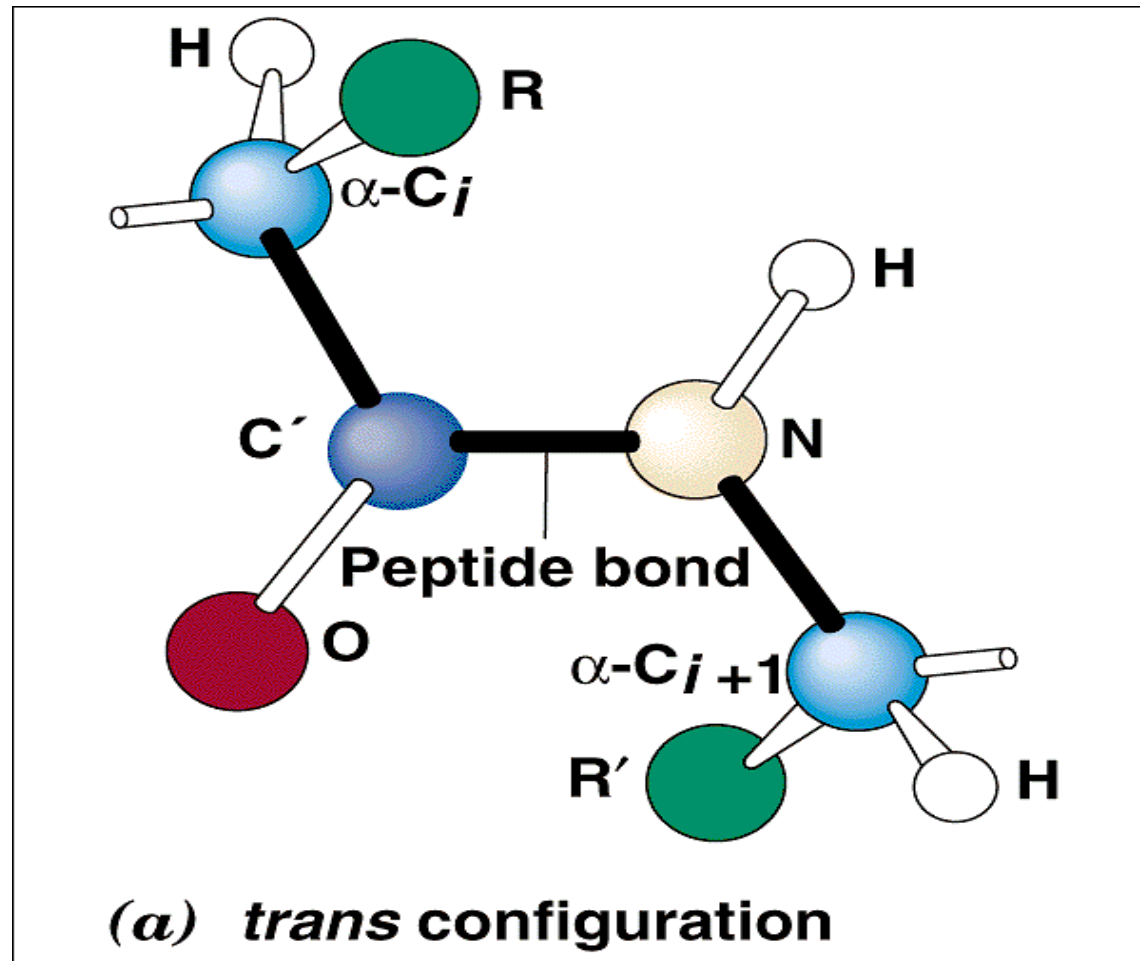


UV-vis Circular Dichroism Spectrometer



JASCO—quartz prisms disperse and *linearly polarize* light

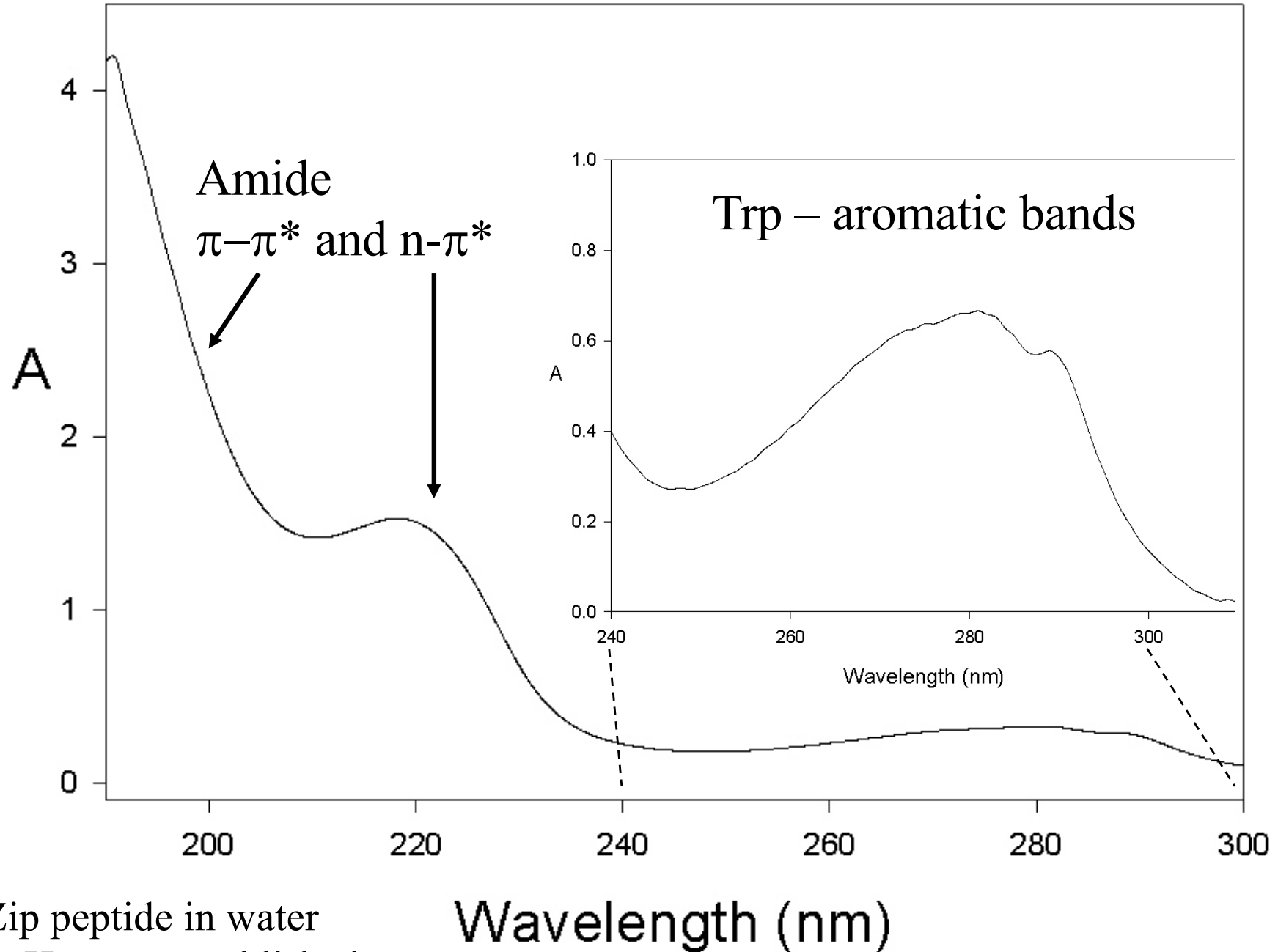
Amino Acids - linked by Peptide bonds → coupling yields structure sensitivity



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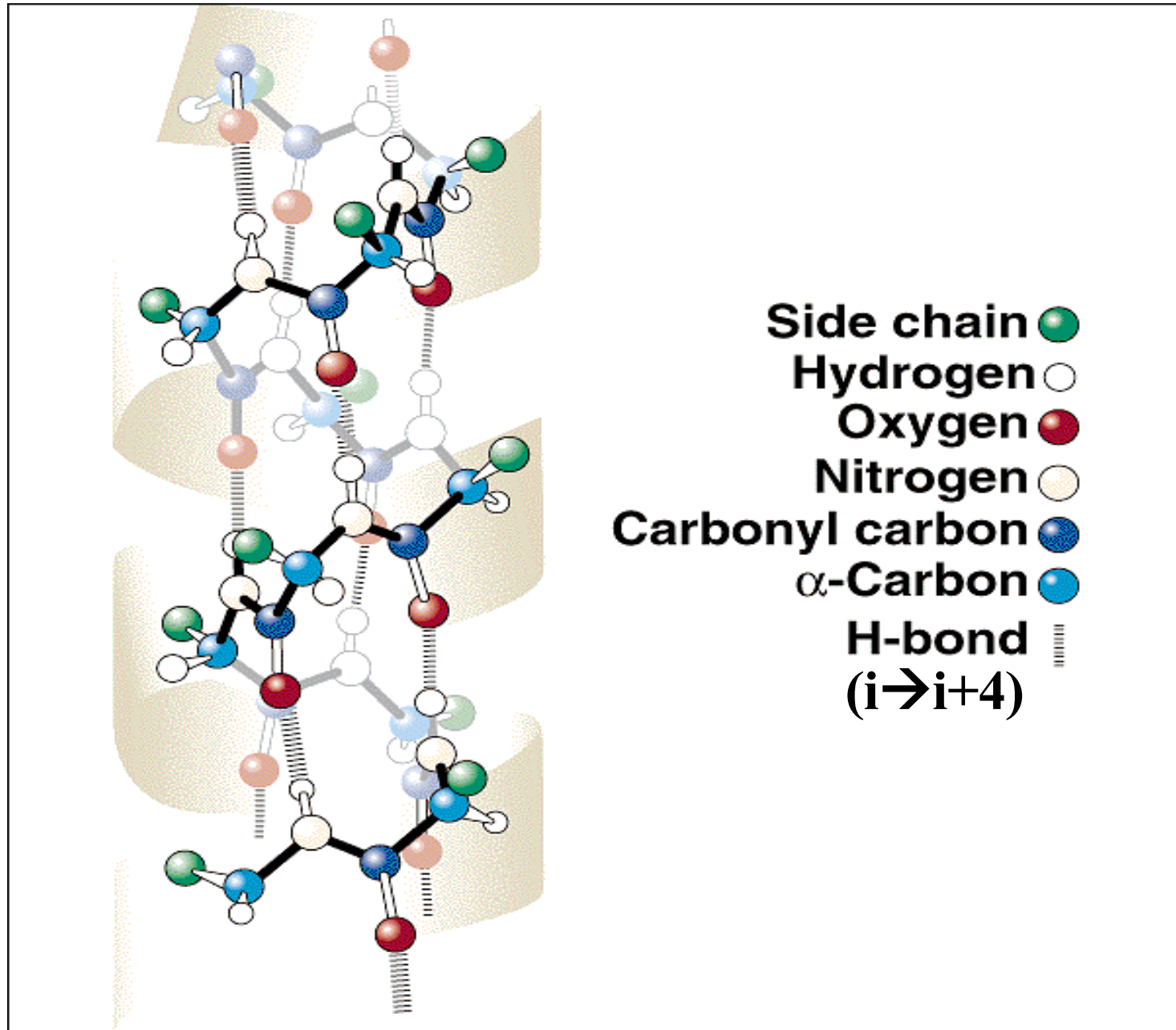
Link is mostly planar and *trans*, except for Xxx-Pro

UV absorption of peptides is featureless --except aromatics

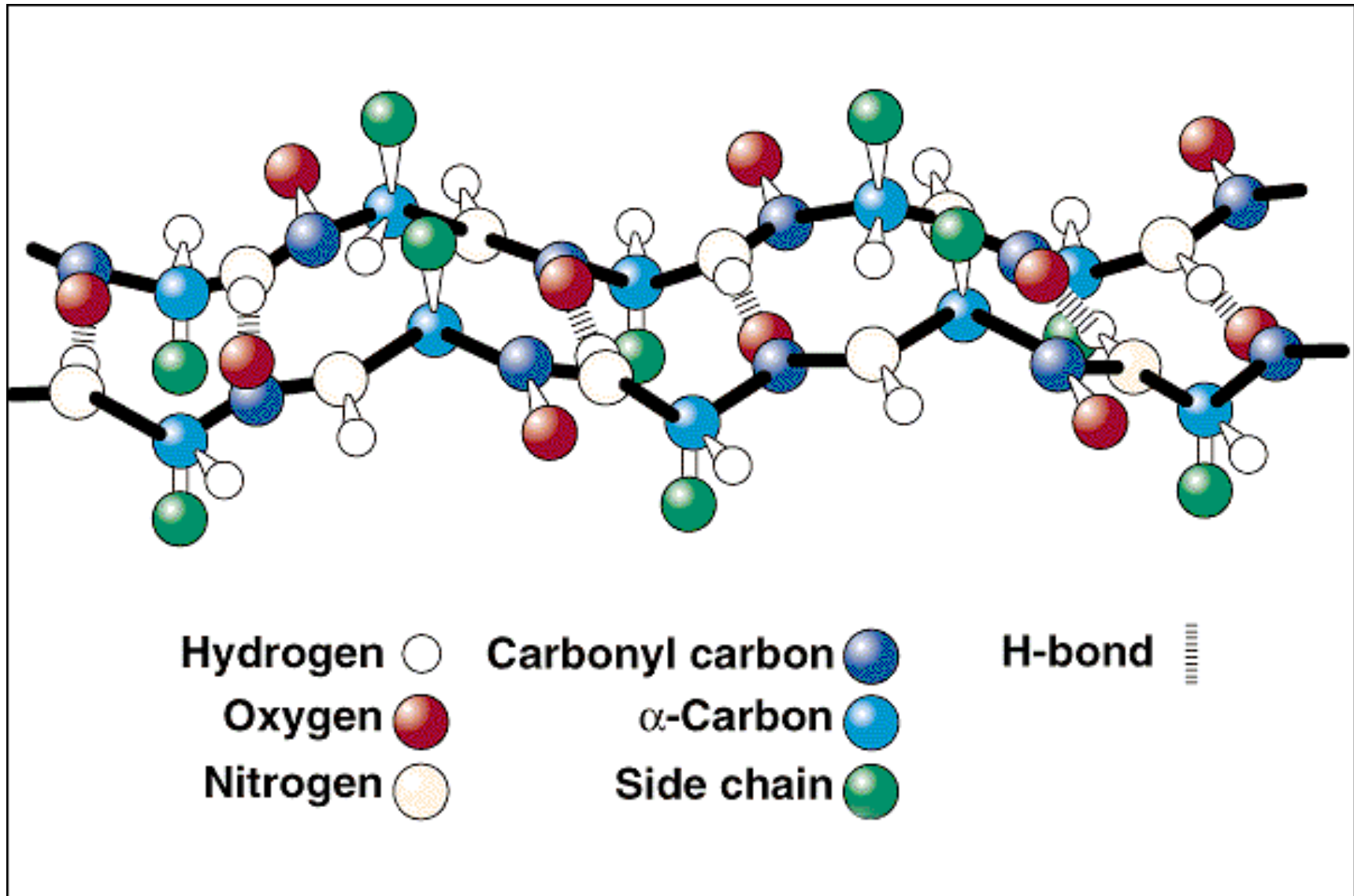


TrpZip peptide in water
Rong Huang, unpublished

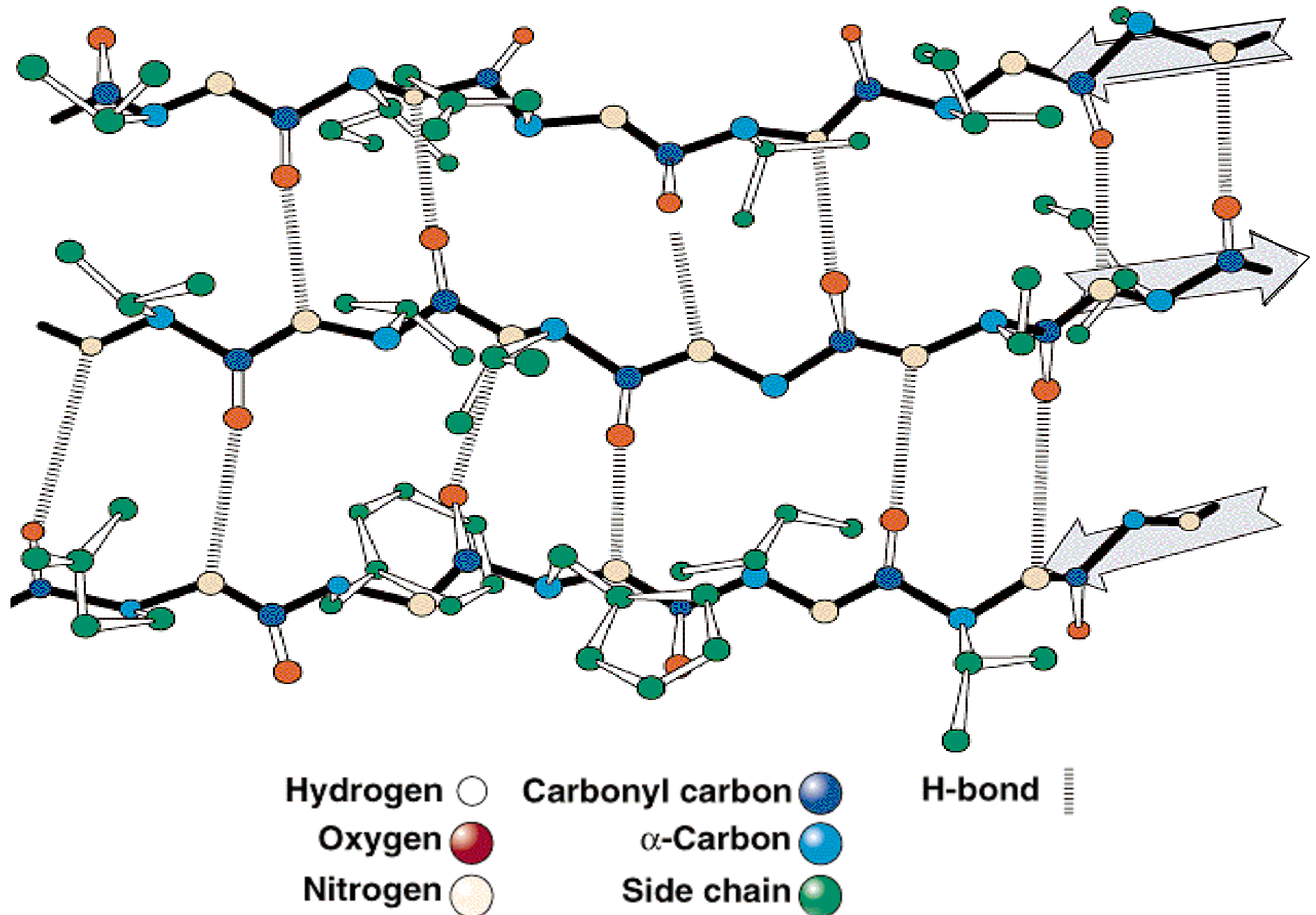
α -helix - common peptide secondary structure



β -sheet cross-strand H-bonding

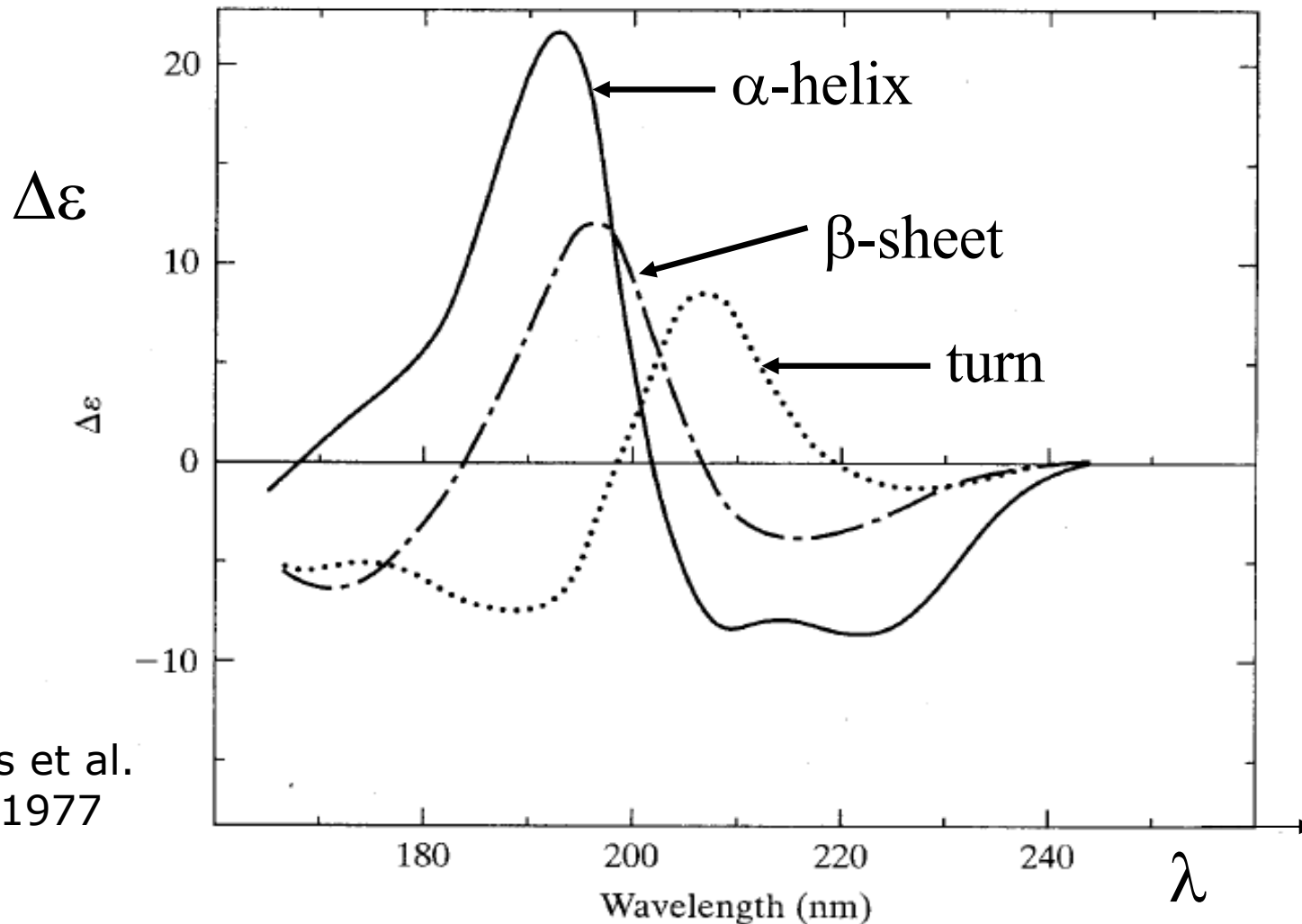


Anti-parallel β -sheet (extended strands)



Polypeptide Circular Dichroism

ordered secondary structure types



Brahms et al.
PNAS, 1977

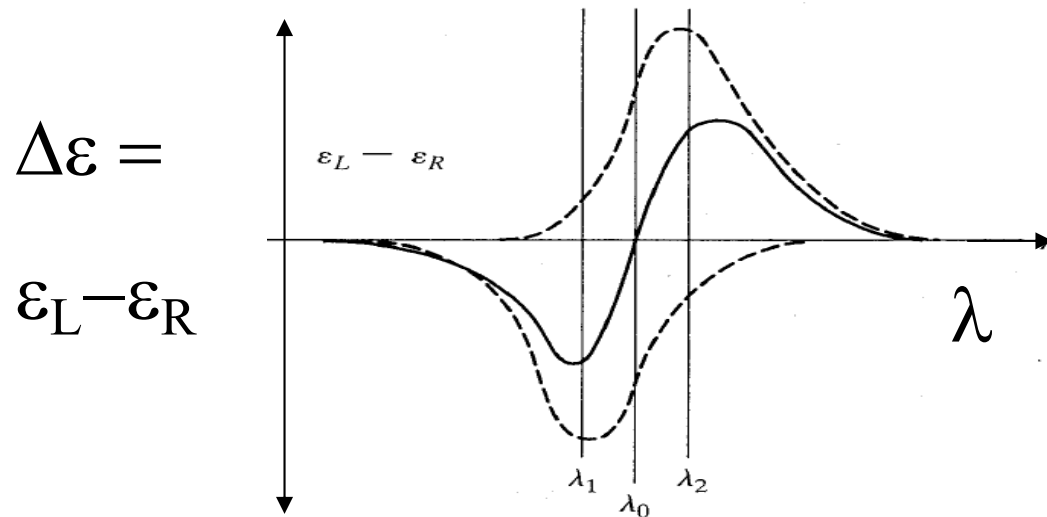
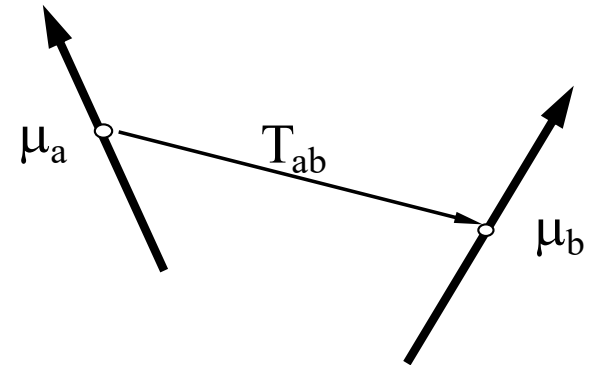
poly-L-glu(α ,—), poly-L-(lys-leu)(β ,- - - -), L-ala₂-gly₂(turn, ····)

Critical issue in CD structure studies is SHAPE of the $\Delta\epsilon$ pattern

Large electric dipole transitions can couple over longer ranges to sense extended conformation

Simplest representation is *coupled oscillator*

$$R^{\pm} = \mp \left(\frac{\pi \nu}{2c} \right) \vec{T}_{ab} \cdot (\vec{\mu}_a \times \vec{\mu}_b)$$

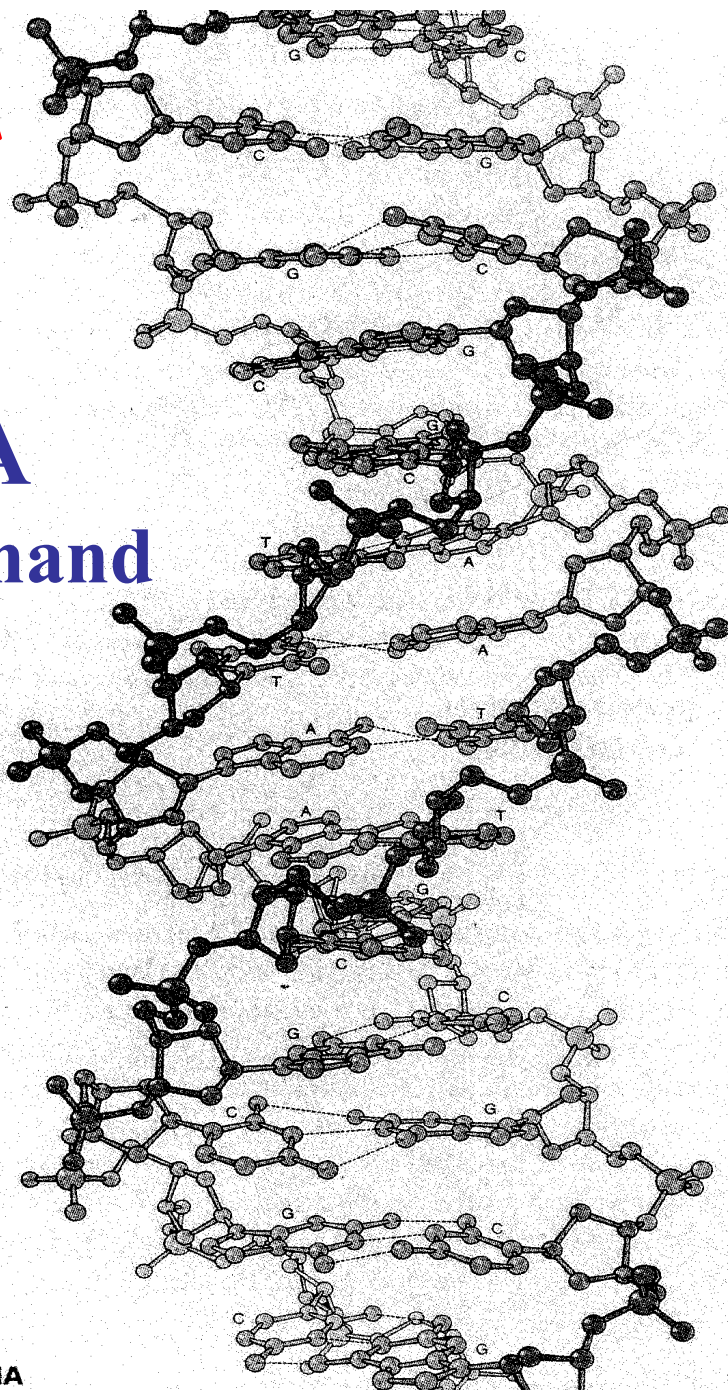


Dipole coupling results in a derivative shaped circular dichroism

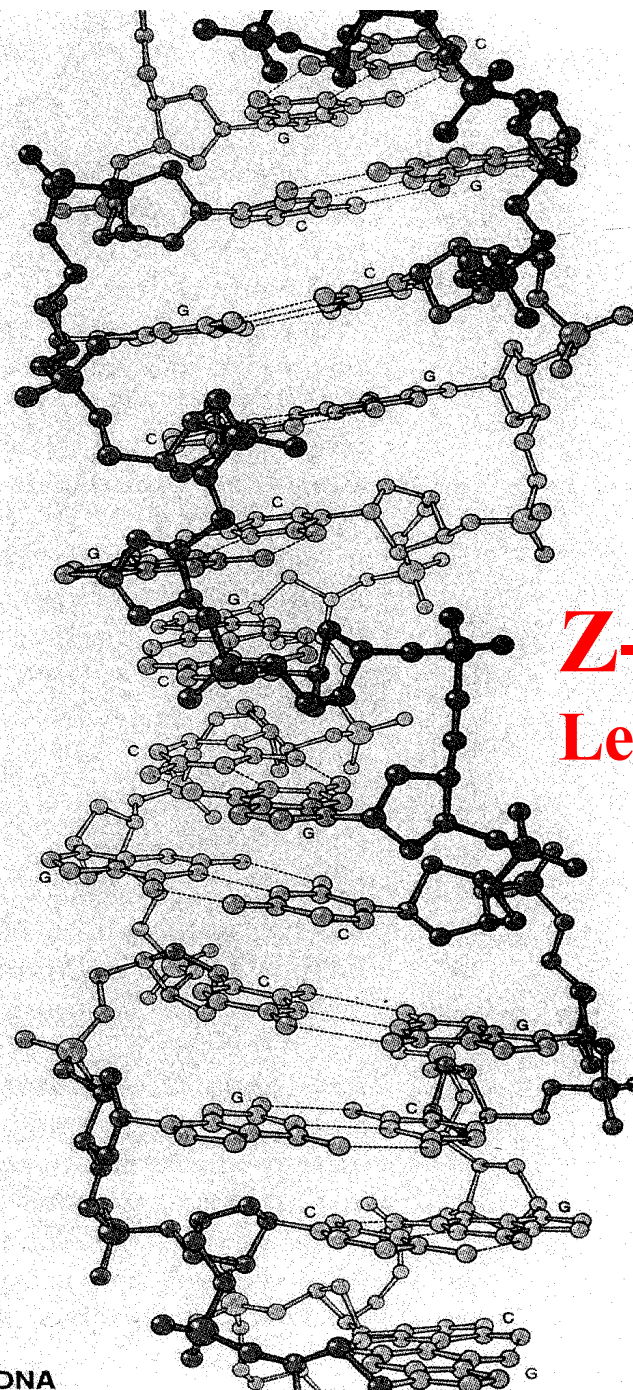
Real systems - more complex interactions
- but pattern is often consistent

DNA

B-DNA
Right -hand



B-DNA



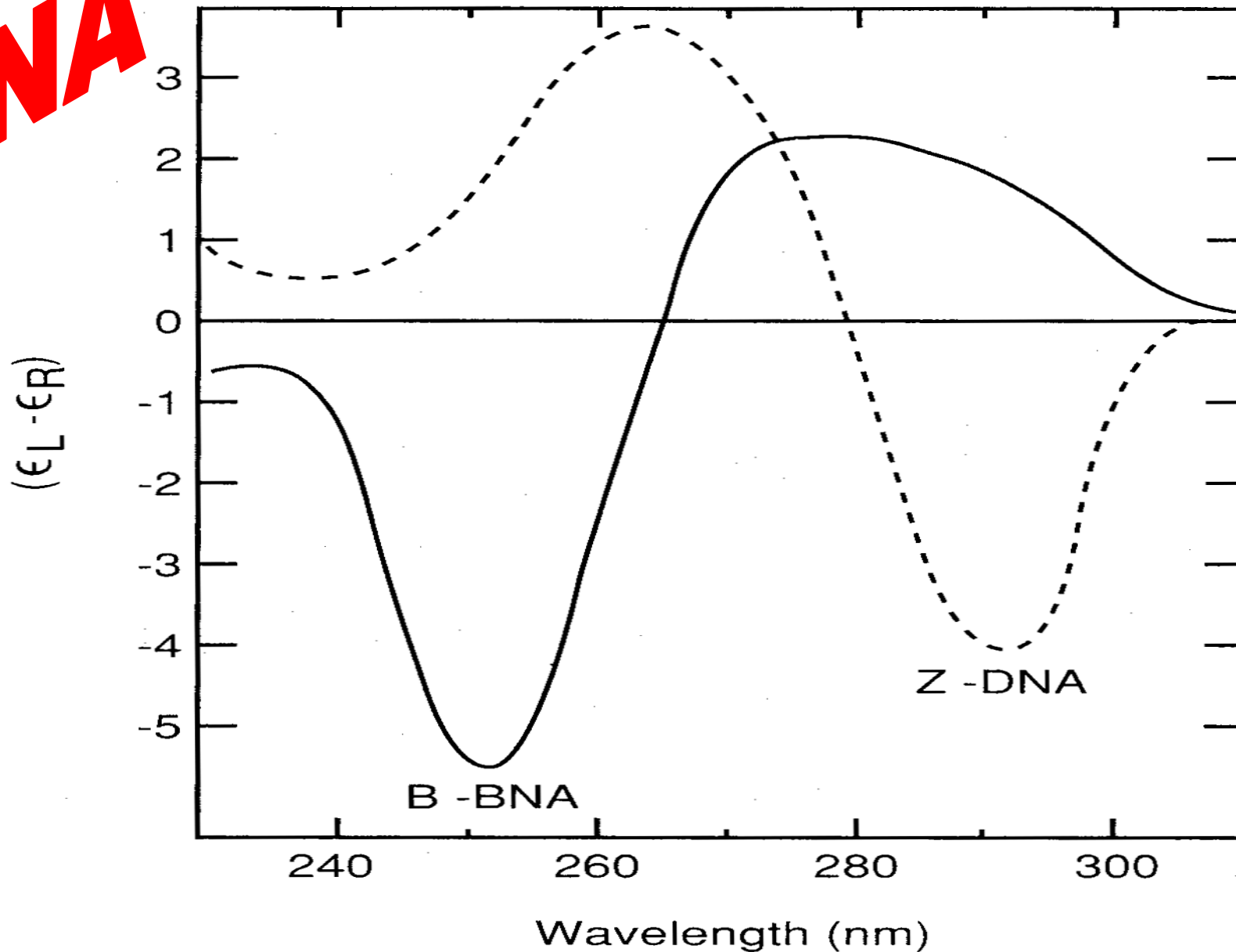
Z-DNA
Left-hand

Z-DNA

Figure 5.2 The B-DNA and Z-DNA helices. The B-DNA helix of GCGCGAATTCGCGC and the Z-DNA helix of (GC)₆ are shown. Copyright by Irving Geis. Used with permission.

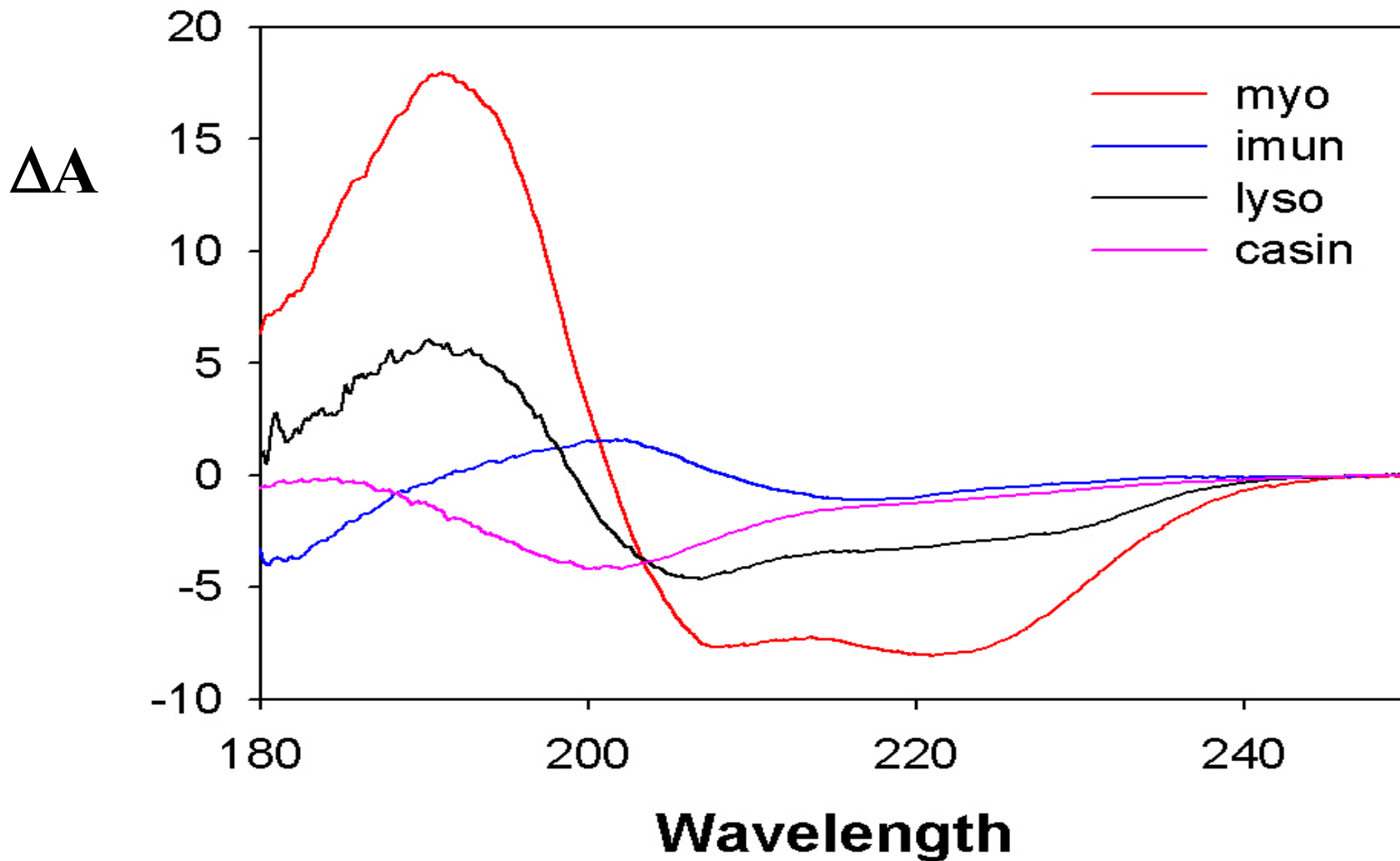
B- vs. Z-DNA, major success of CD

DNA



Sign change in near-UV CD suggested the helix changed handedness

Protein Circular Dichroism



Myoglobin-high helix (—), Immunoglobulin high sheet (—)
Lysozyme, a+b (—), Casein, “unordered” (—),

Coupling → shapes, but not isolated & modeling tough

Simplest Analyses – Single Frequency Response

Basis in analytical chemistry → Beer's law response if isolated

Protein treated as a solution → % helix, etc. is the unknown

Standard in IR and Raman,

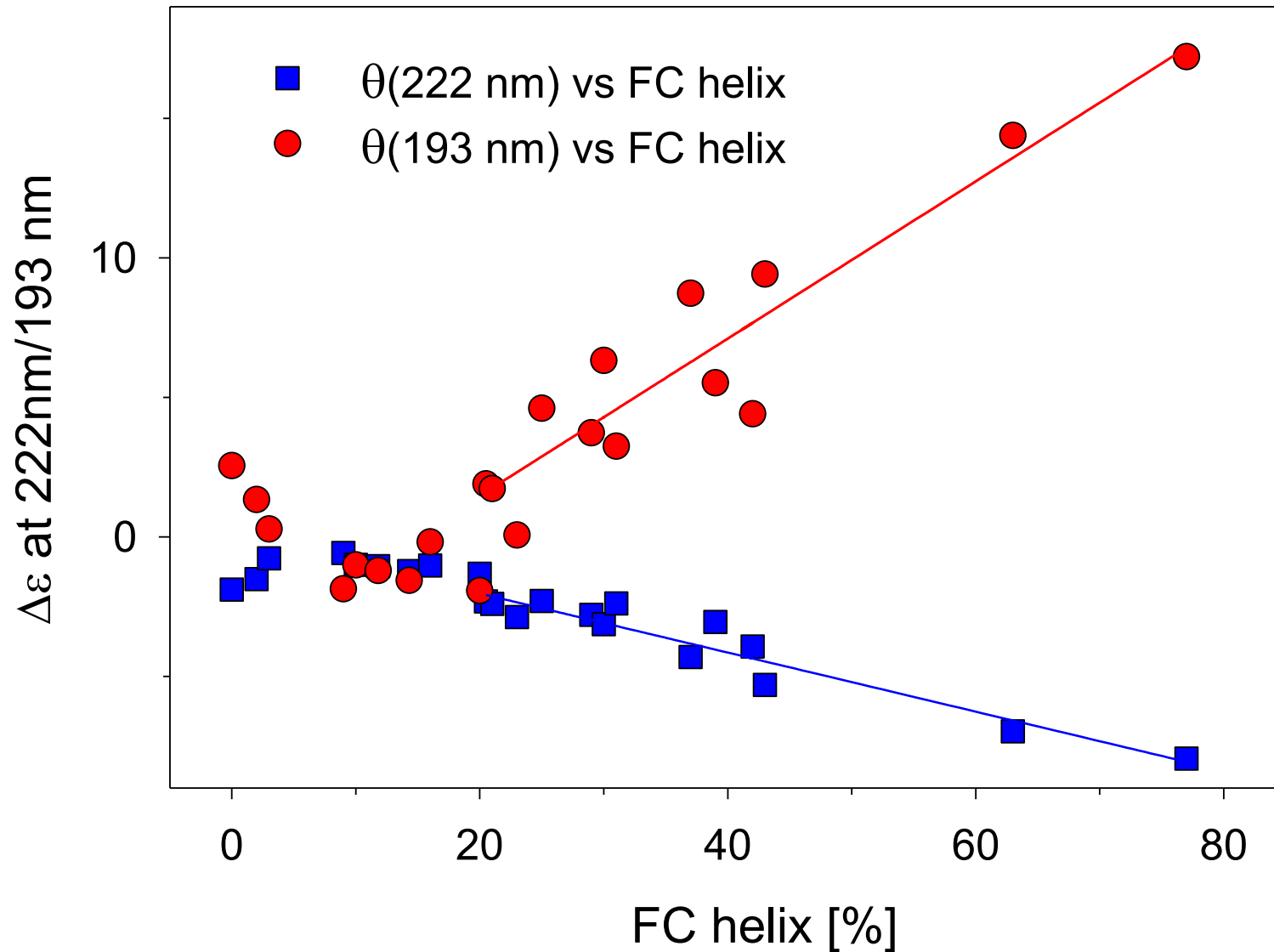
Method: deconvolve to get components

Problem – must assign component transitions, overlap

-secondary structure components disperse freq.

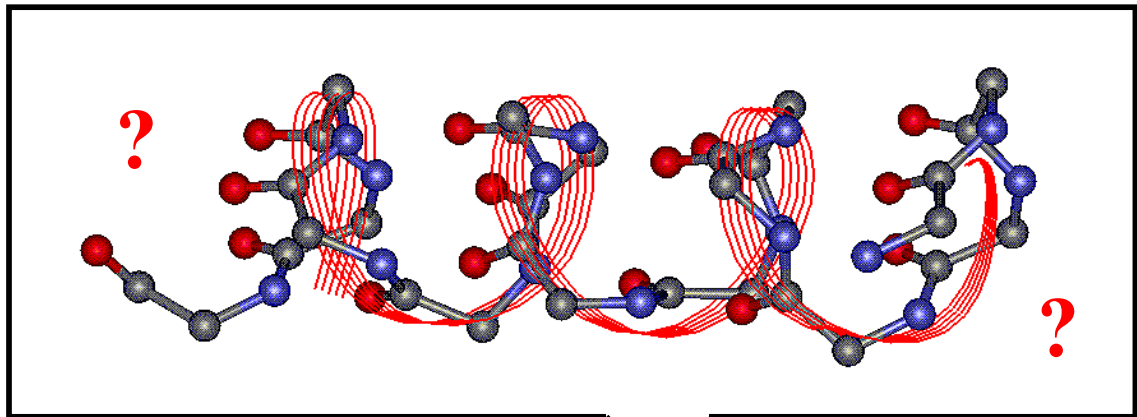
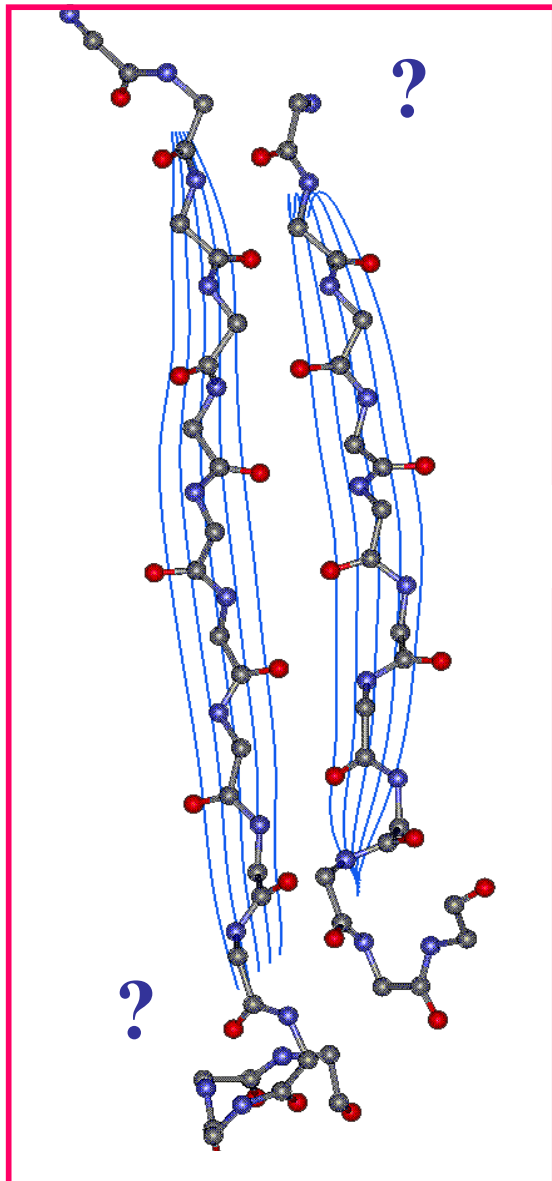
Alternate: uv CD - helix correlate to negative intensity

Single frequency correlation of $\Delta\varepsilon$ with FC helix



Problem of secondary structure definition

No pure states for calibration purposes

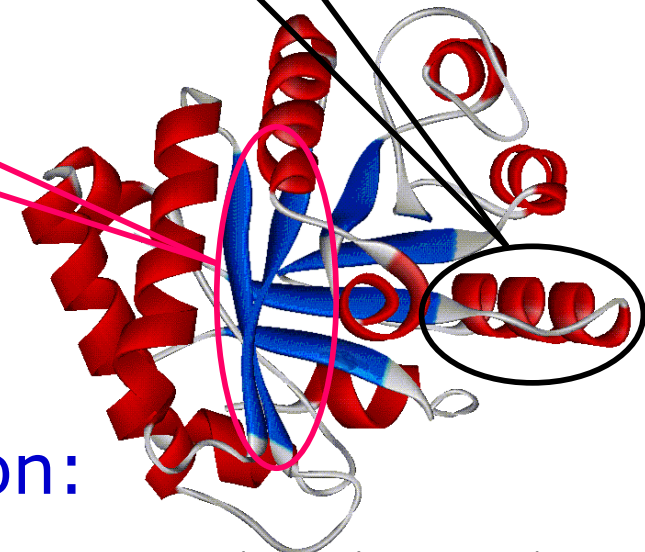


helix

sheet

Need definition:

Where do segments begin and end?



Next step - project onto model spectra

-Band shape analysis

Peptides as models

- fine for α -helix,
- problematic for β -sheet or turns - solubility and stability
- old method:Greenfield - Fasman --poly-L-lysine, vary pH

$$\theta_i = a_i \phi_\alpha + b_i \phi_\beta + c_i \phi_c$$

--Modelled on multivariate analyses

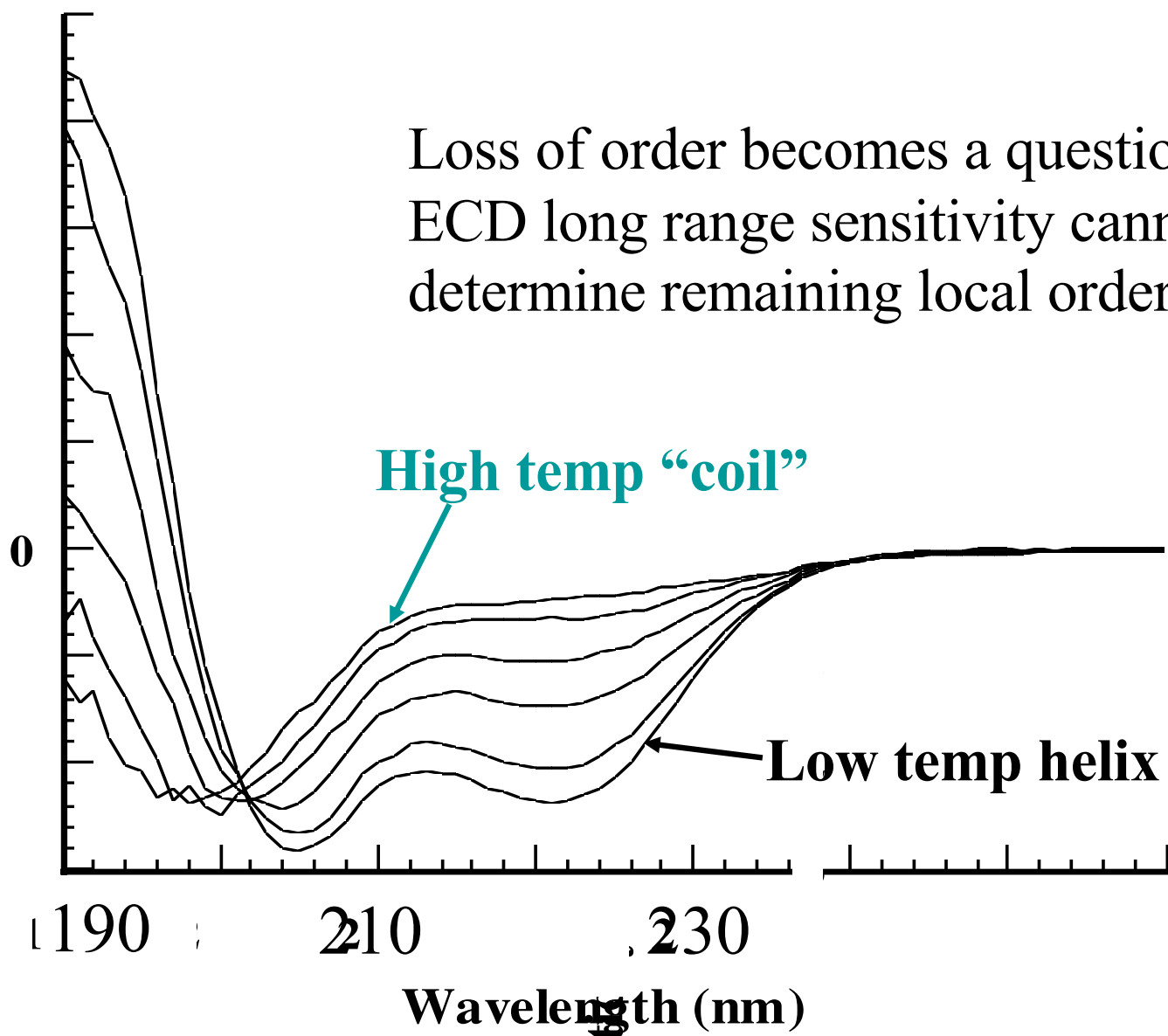
Proteins as models - need to decompose spectra

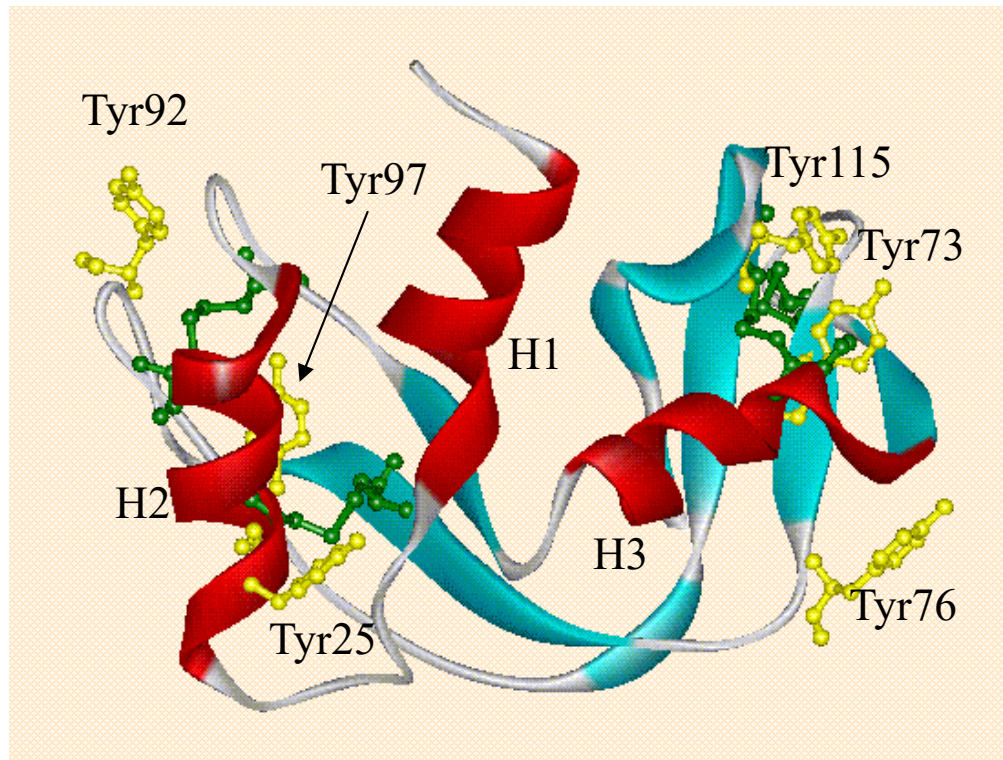
- structures reflect environment of protein

protein structures used as models

Electronic CD for helix to coil change in a peptide

Note helical bands, coil has residual at 222 nm, growth of 200 nm band

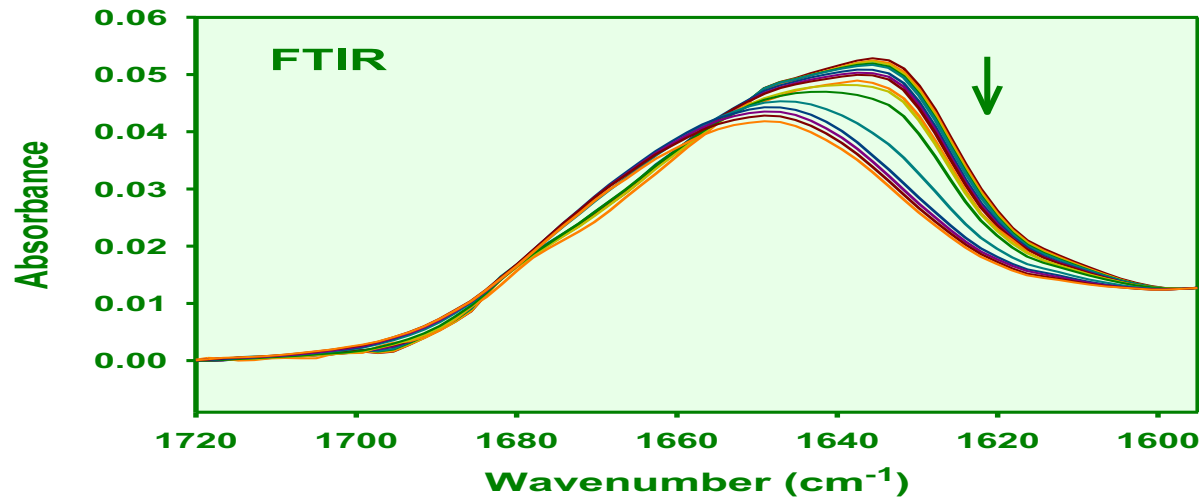




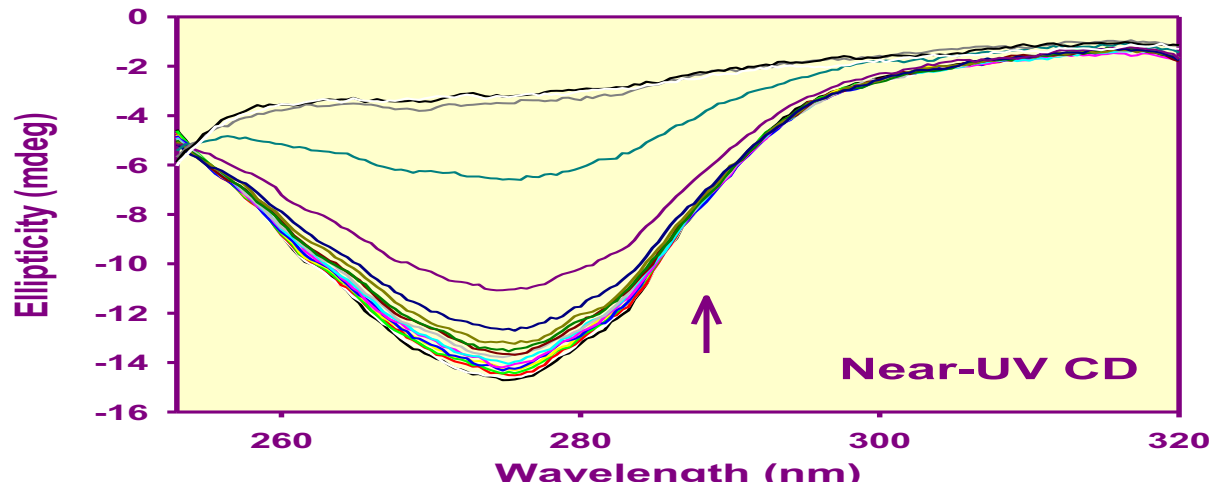
Ribonuclease A combined uv-CD and FTIR study

- **124 amino acid residues, 1 domain, MW= 13.7 KDa**
- **3 α -helices**
- **6 β -strands in an AP β -sheet**
- **6 Tyr residues (no Trp), 4 Pro residues (2 cis, 2 trans)**

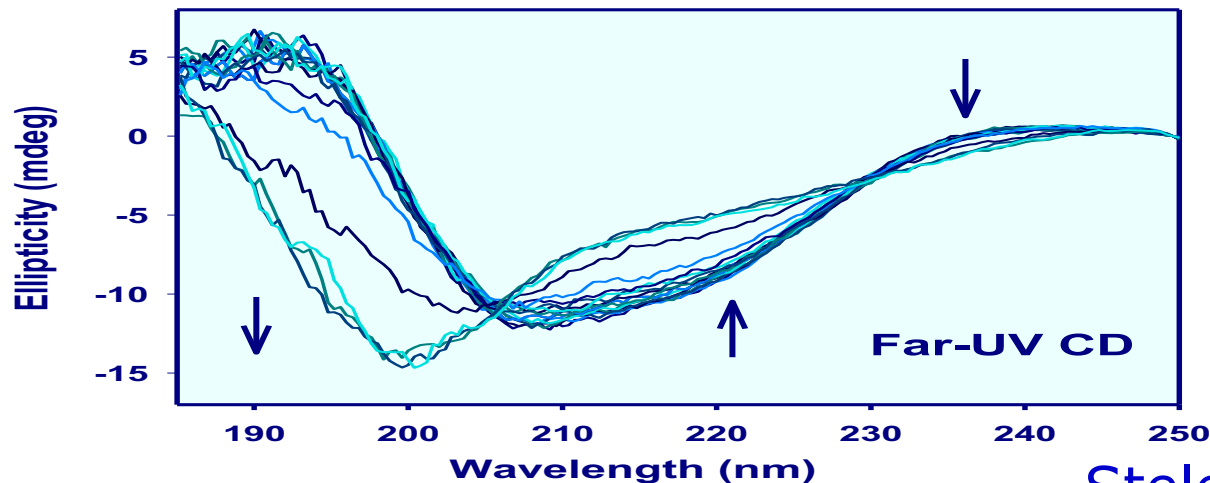
RibonucleaseA



FTIR—amide I
Loss of β -sheet



Near -uv CD
Loss of tertiary
structure



Far-uv CD
Loss of α -helix

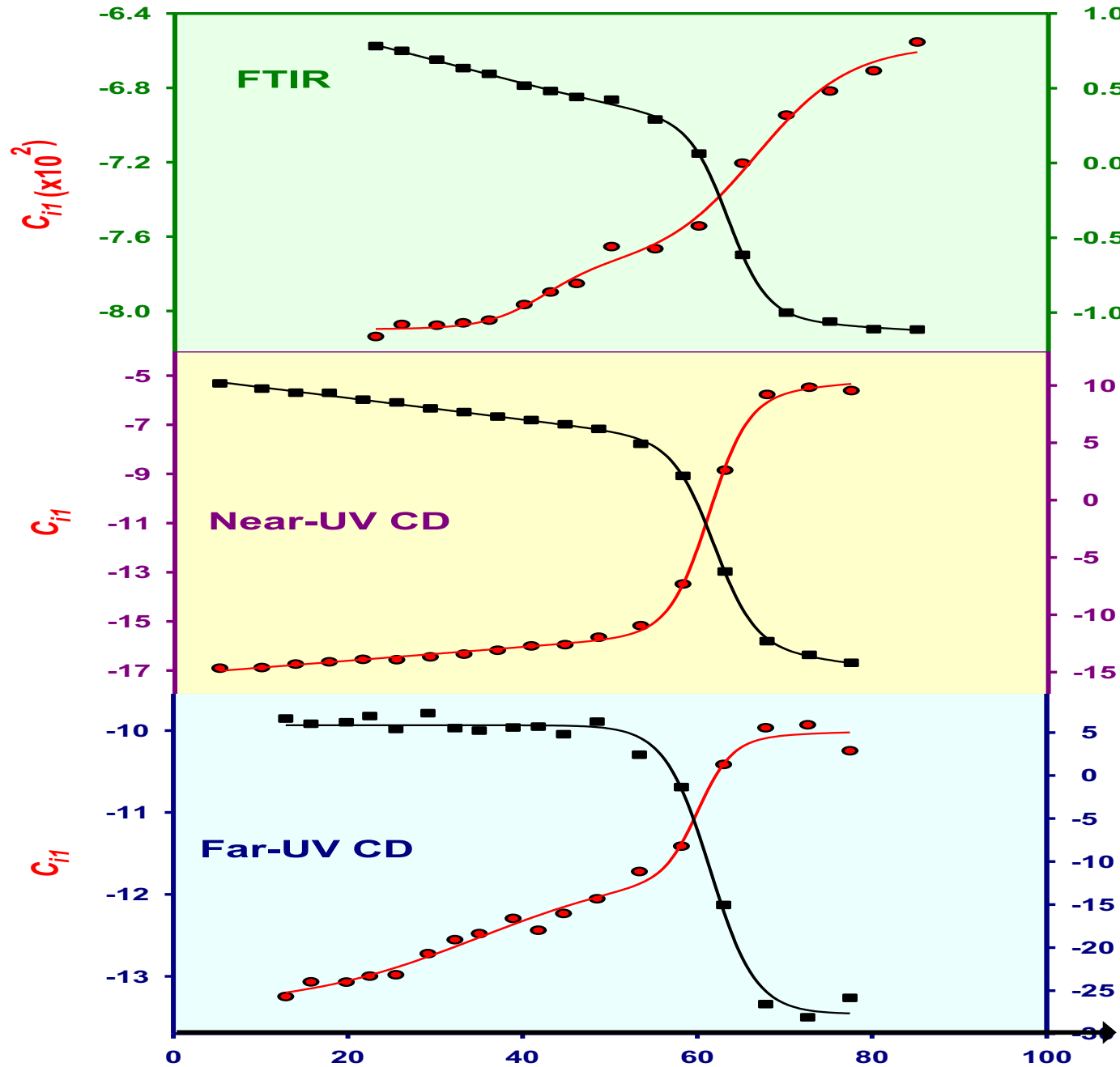
Spectral Change
Temperature 10-70°C

Stelea, et al. Prot. Sci. 2001

Ribonuclease A

PC/FA loadings

Temp. variation



FTIR (α, β)

Near-uv CD
(tertiary)

Far-uv CD
(α -helix)

Temperature

Pre-transition - far-uv CD and FTIR, not near-uv

Stelea, et al.

Prot. Sci. 2001

Changing protein conformational order by organic solvent

TFE and MeOH often used to induce helix formation

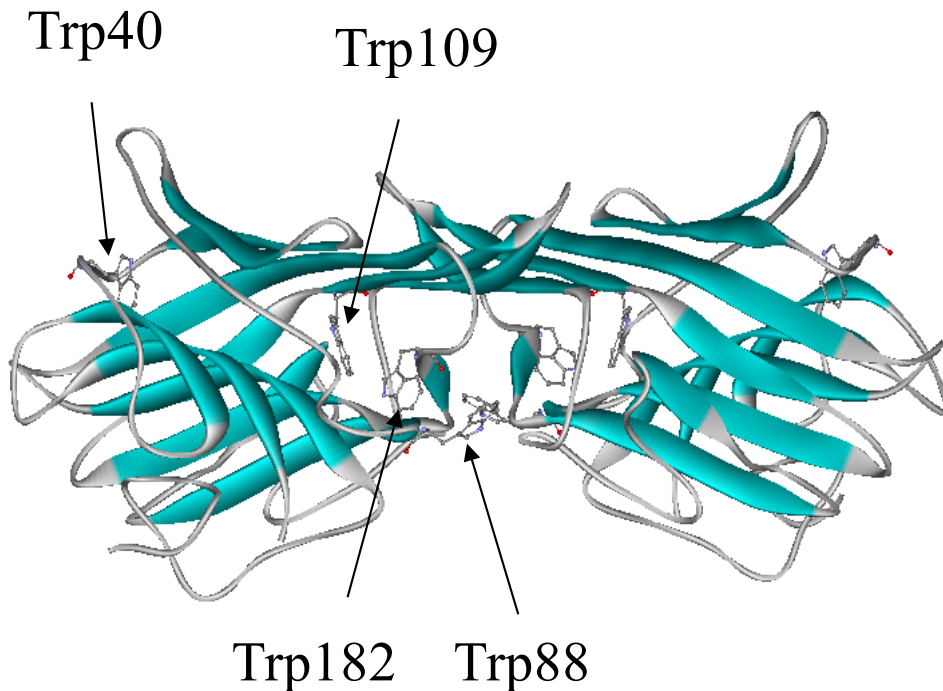
- sometimes thought to mimic membrane
- reported that the consequent unfolding can lead to aggregation and fibril formation in selected cases

Examples presented show solvent perturbation of
dominantly β -sheet proteins

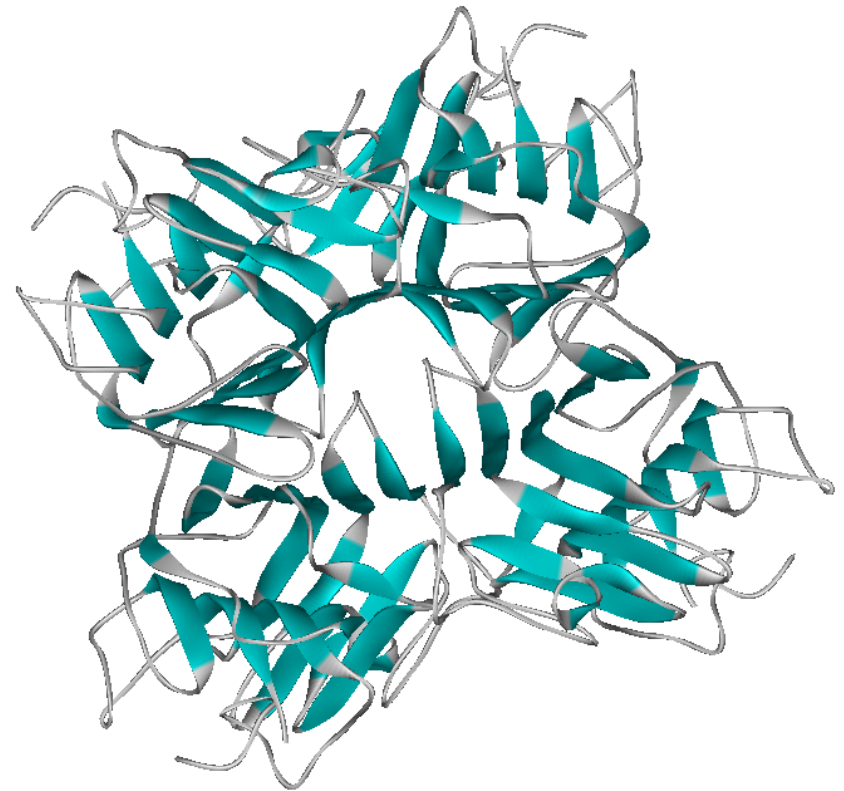
TFE and MeOH behave differently
thermal stability key to differentiating states
indicates residual partial order

3D Structure of Concanavalin A

Dimer (acidic, pH<6)



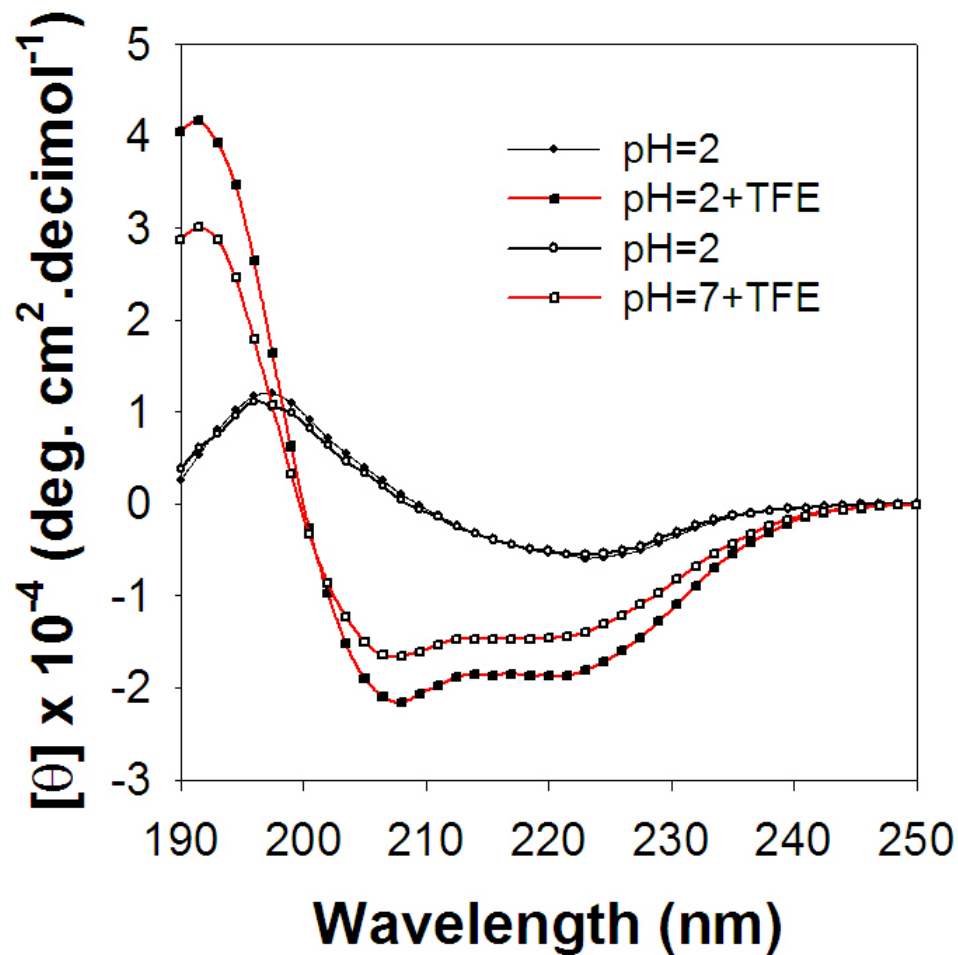
Tetramer (pH=6-7)



High β -sheet structure, flat back extended, curved front
Monomer only at very low pH, 4 Trp give fluorescence

Effect of TFE (50%) on Con A in Far and Near UV- CD

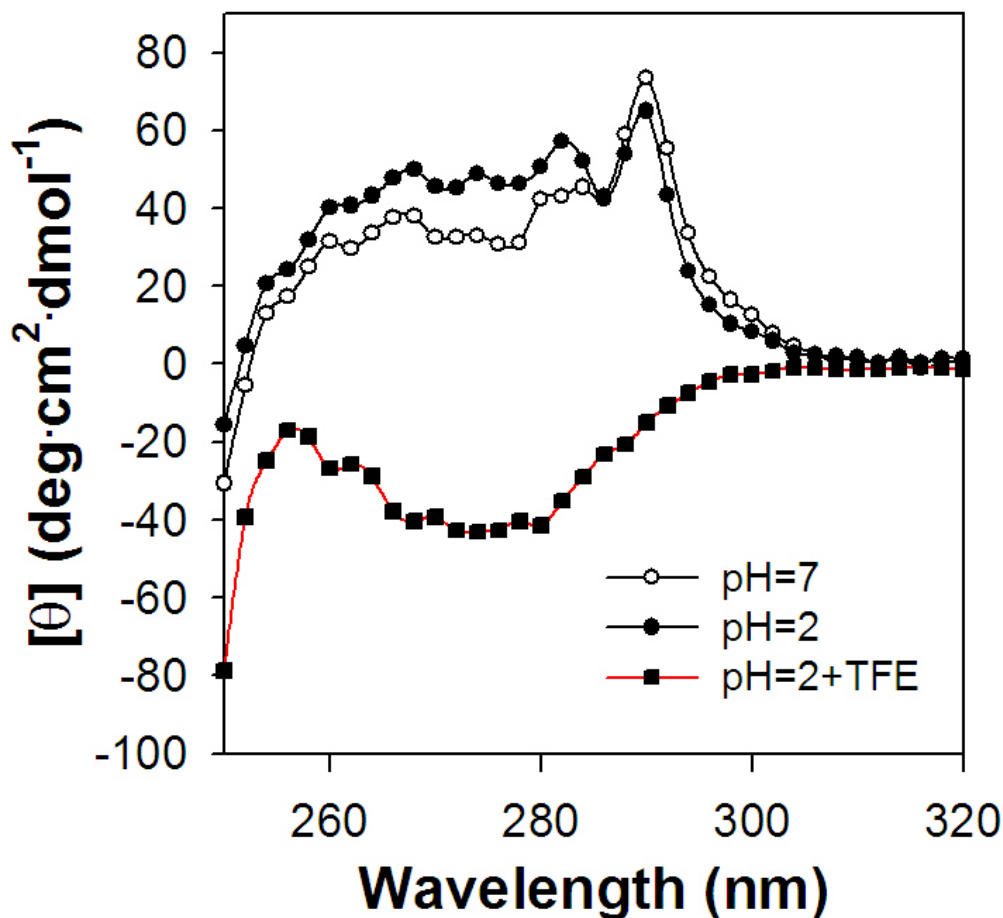
Far UV-CD



Helix induced with
TFE addition

	Helical Content
pH=7	43%
pH=2	57%

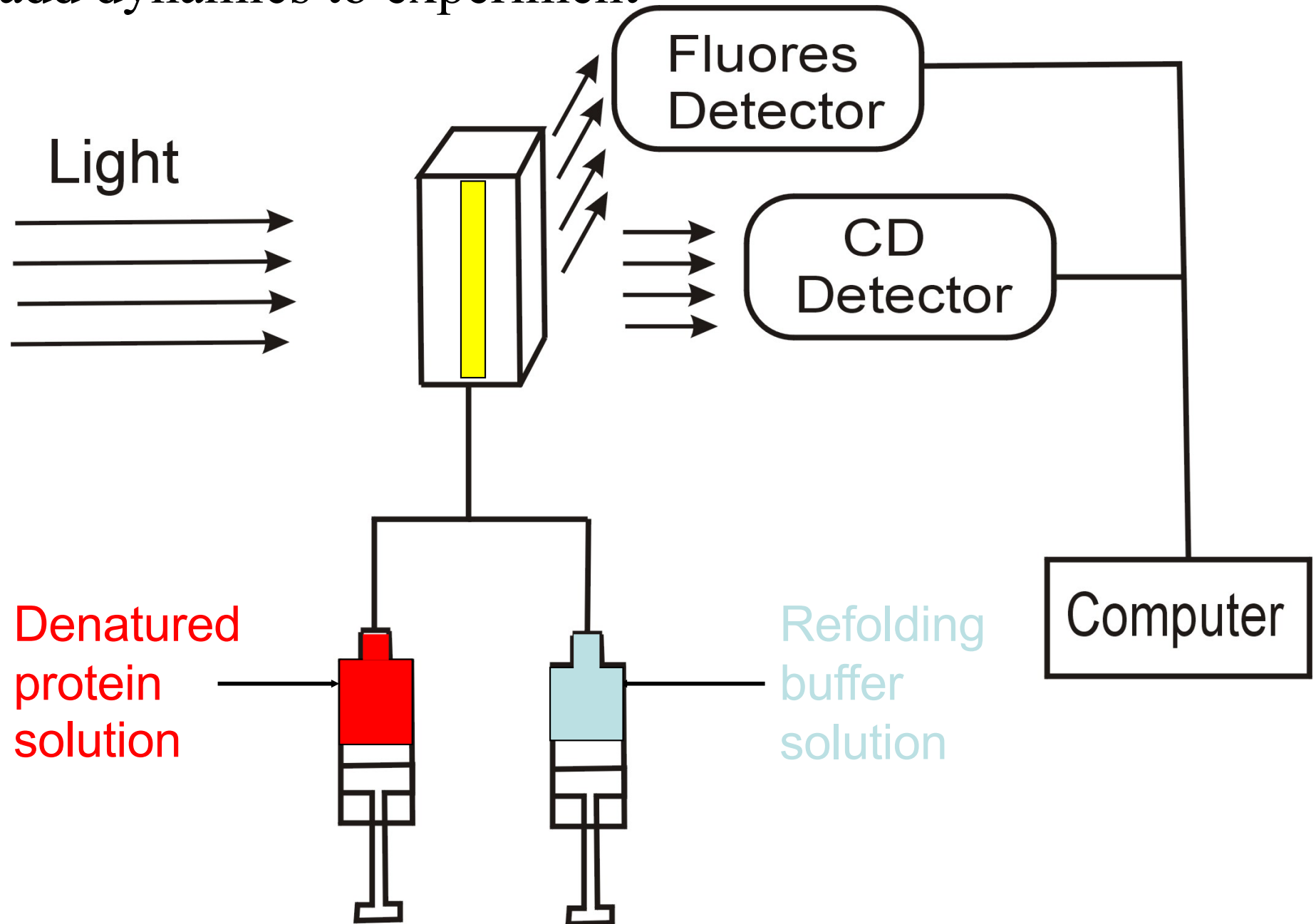
Near UV-CD



**Tertiary change
with TFE - loosen**

Dynamics--Scheme of Stopped-flow System

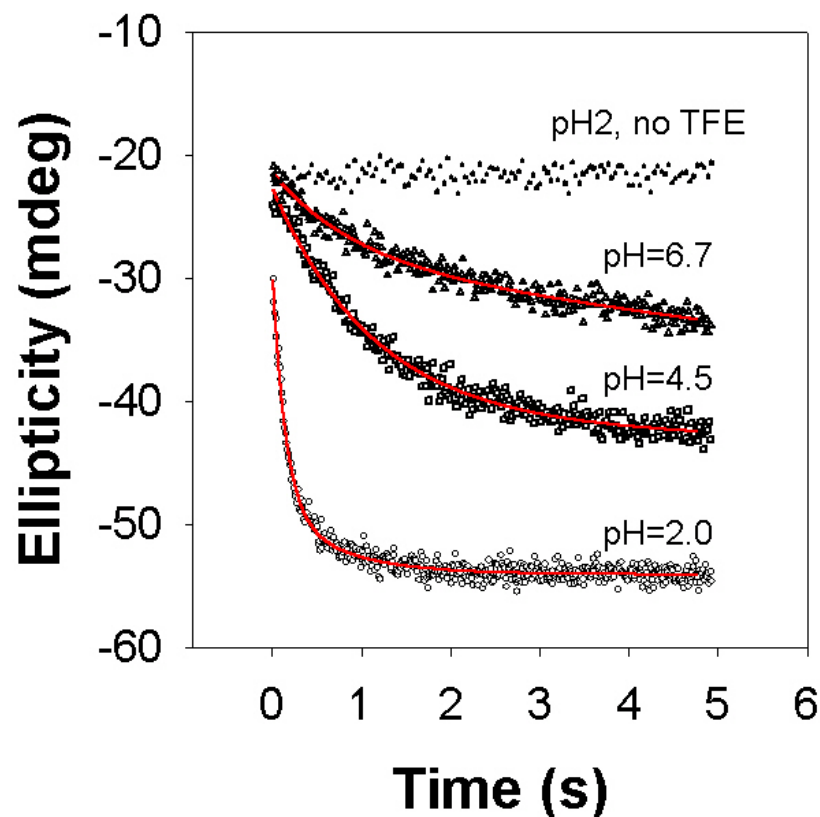
- add dynamics to experiment



Stopped-Flow CD for Con A Unfolding with TFE (1:1) at Different pH Conditions

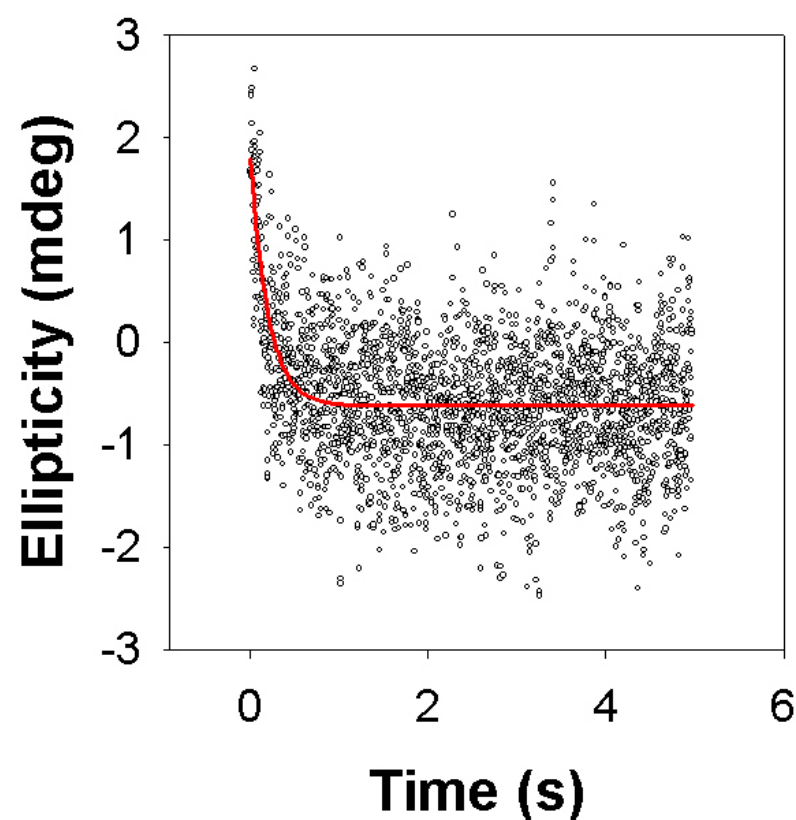
Far UV (222 nm);

$[\text{Con}]_f = 0.2 \text{ mg/ml}$



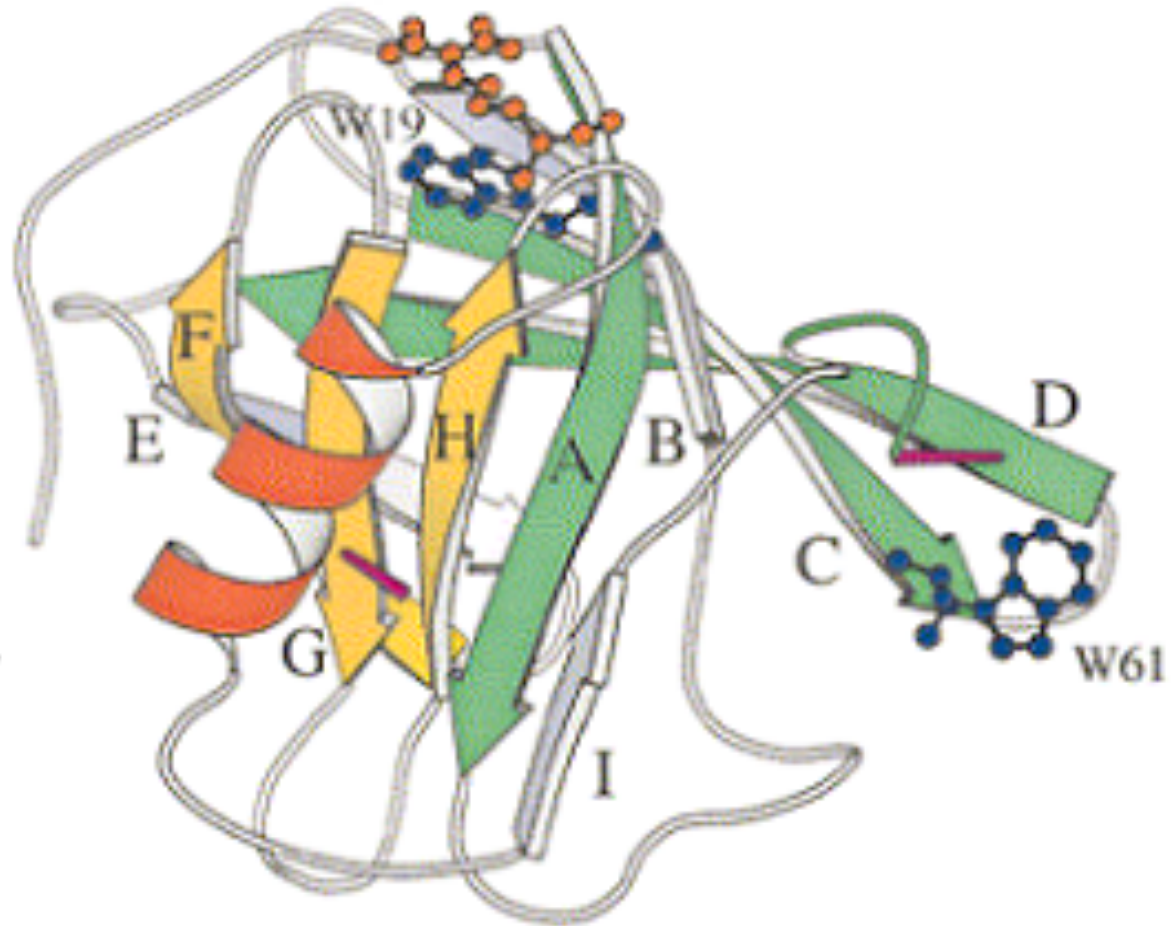
Near UV (290 nm);

$[\text{Con}]_f = 2 \text{ mg/ml}$



β -lactoglobulin: a protein that goes both ways!

Red, α -helix;
green, regions to have
helical propensities;
purple, disulfide bonds
Trp19 & 61 are indicated

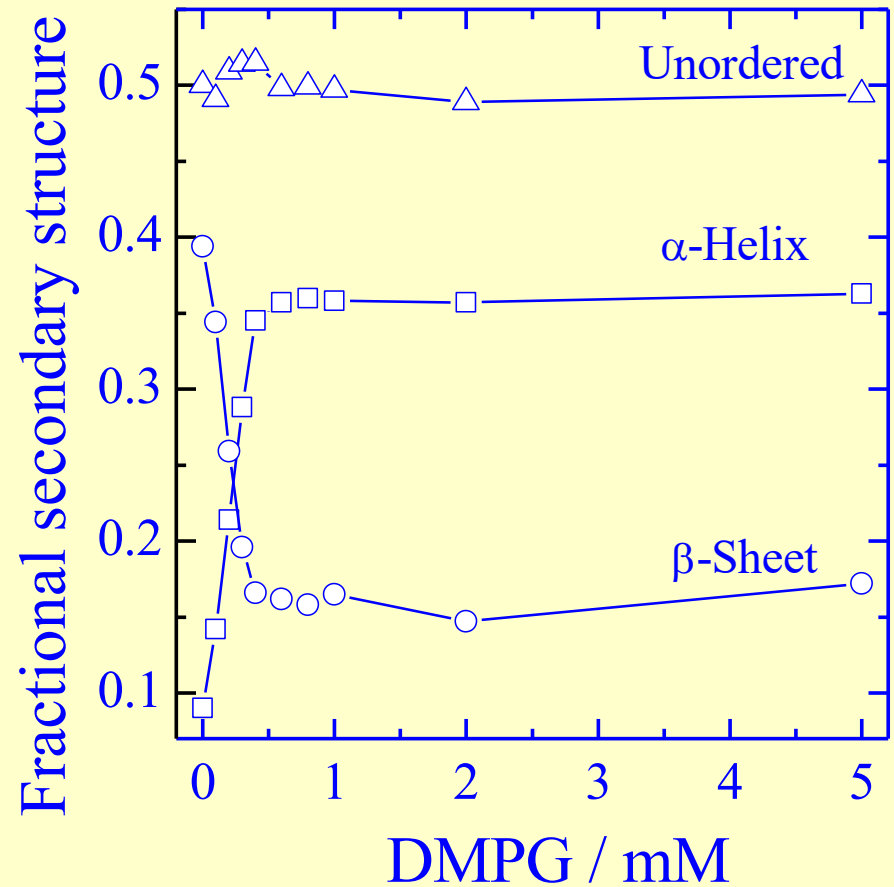
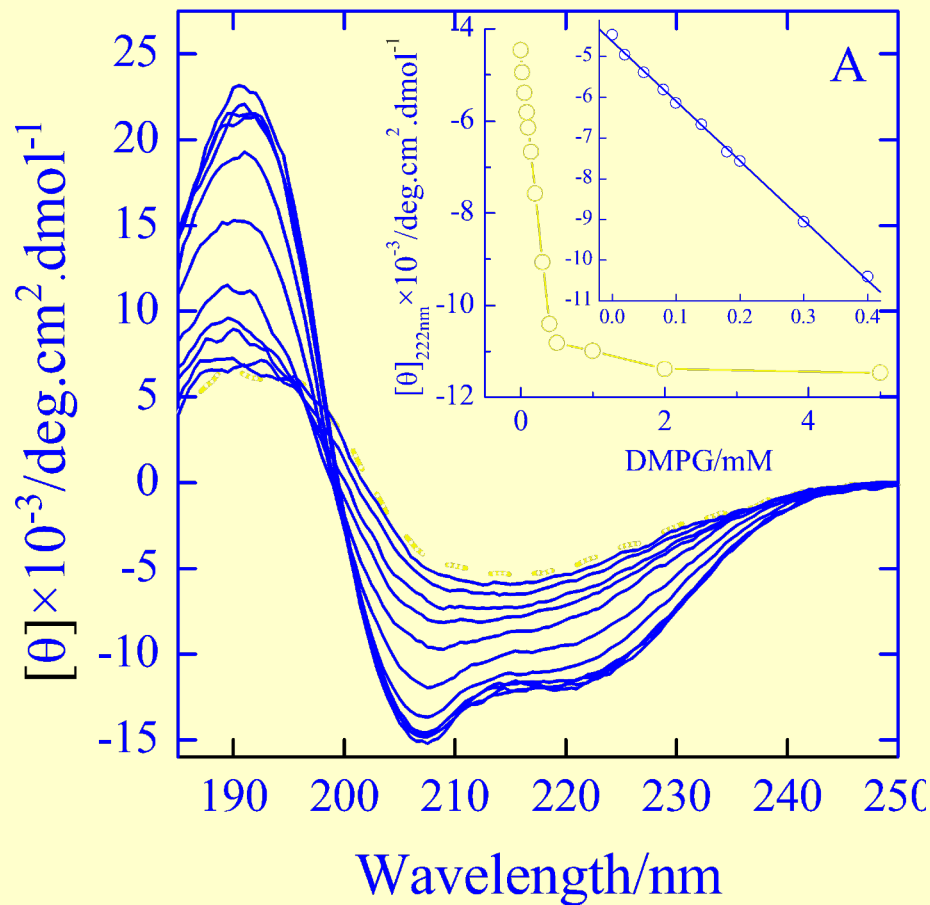


Native state: β -sheet dominant, but high helical propensity.

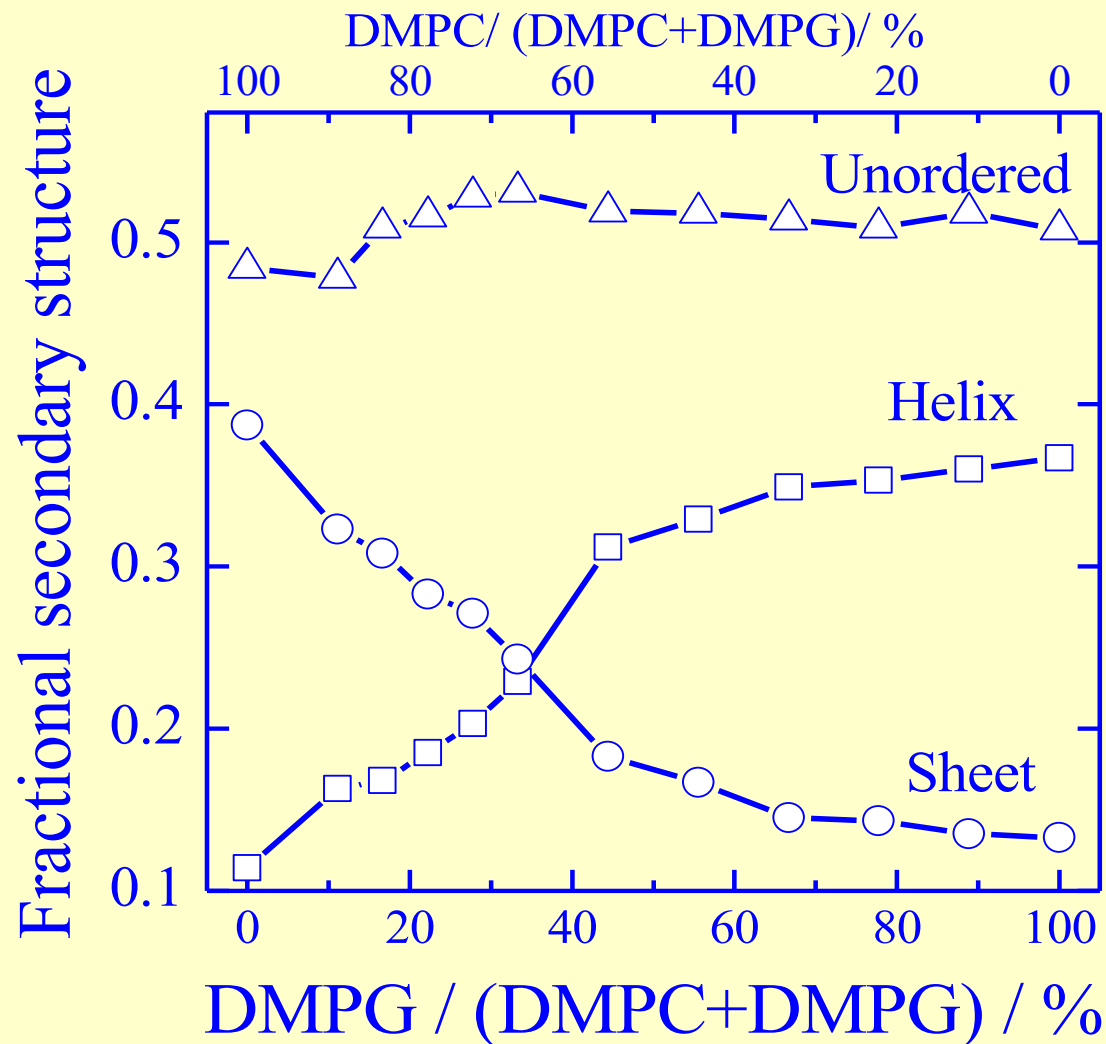
Model: intramolecular $\beta \leftrightarrow \alpha$ transition pathway as opposed to folding pathways from a denatured state.

Lipid-induced Conformational Transition β -Lactoglobulin

1. DMPG-dependent $\beta \rightarrow \alpha$ transition at pH 6.8



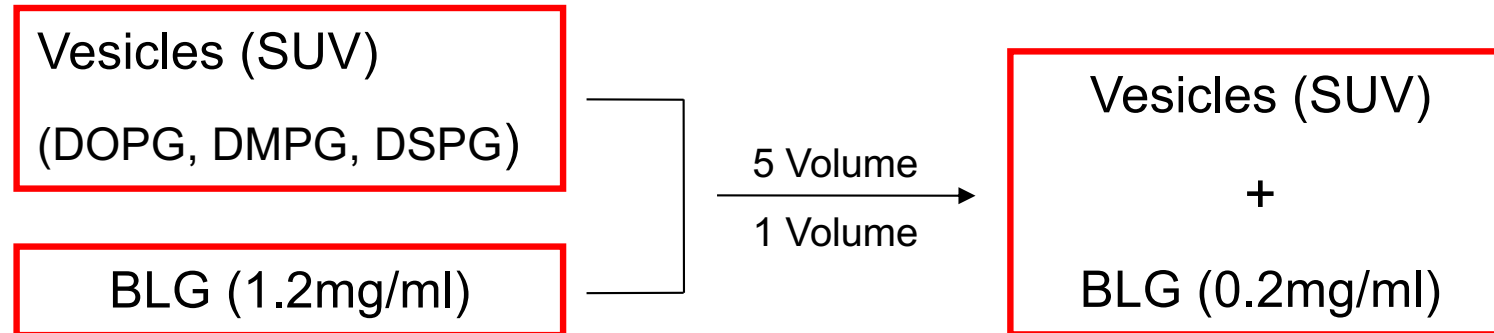
Charge-induced Lipid -- β -Lactoglobulin Interaction



Zhang &
Keiderling,
Biochemistry 2006

Increase DMPG, increases helix at expense of sheet

Stopped Flow Experiments : (pH 4.60)



CD: 222nm to monitor alpha-helix

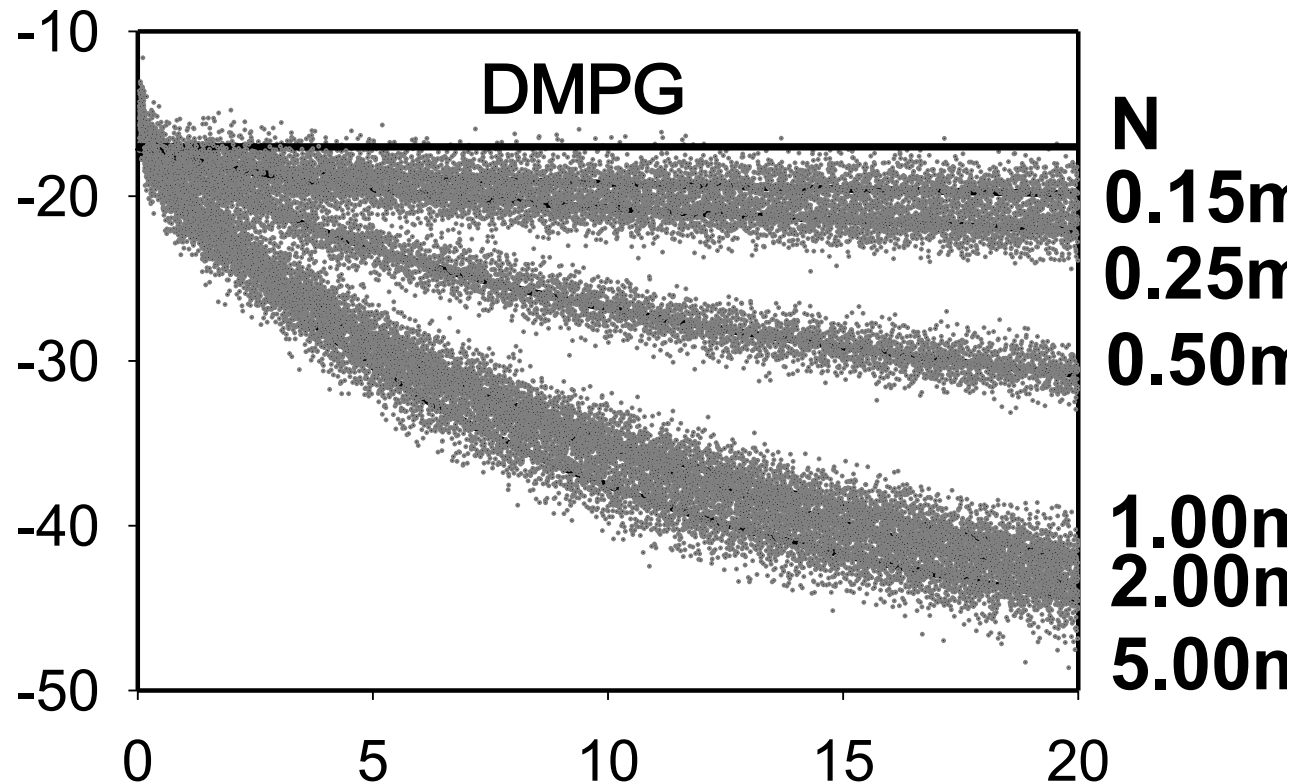
Fluorescence: filter with a 320nm cutoff (Trp Tertiary Structure)

10-15 kinetic traces are collected and averaged

Analysis: Multi-exponential function using Simplex Method:

$$S(t)=a*t+b+\sum_i(c_i \text{ Exp}(-k_i*t))$$

Stopped-Flow CD kinetic traces

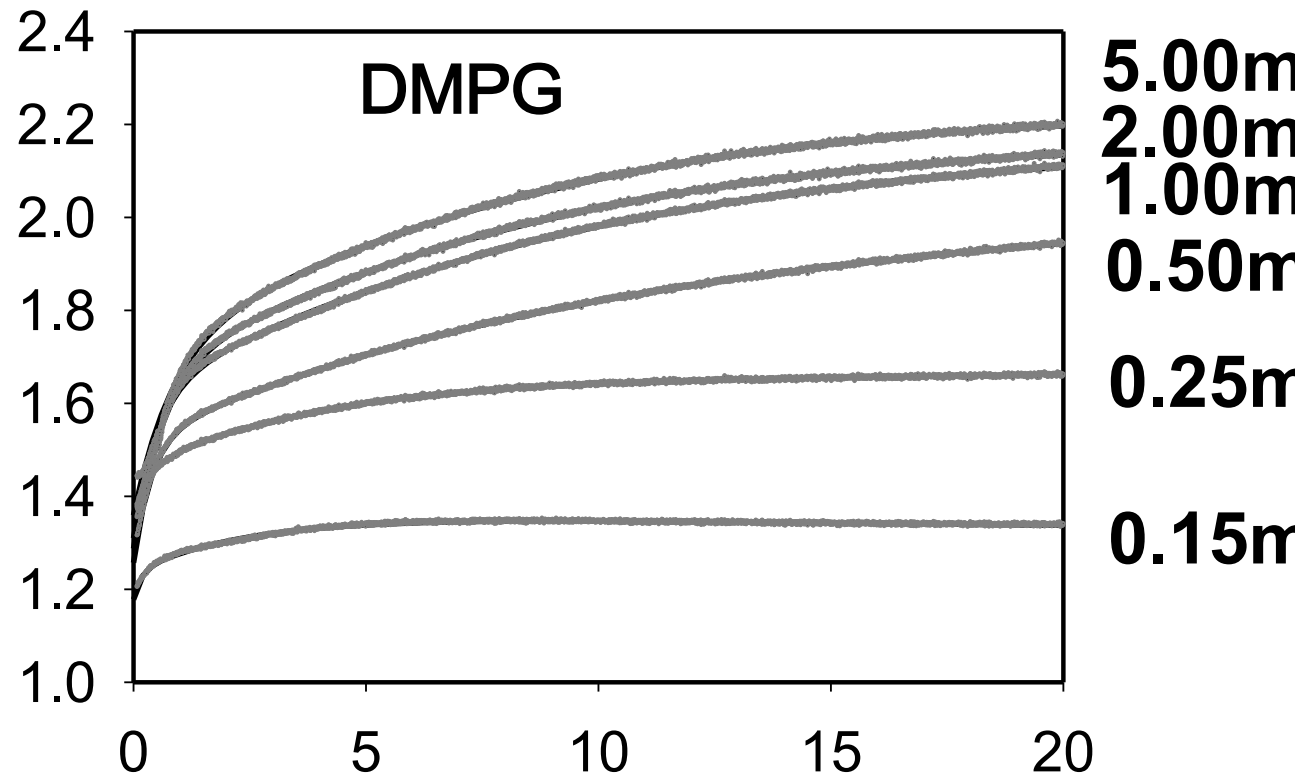


Record at 222nm;

N: trace without lipid vesicles;

Traces are fitted to single-exponential function

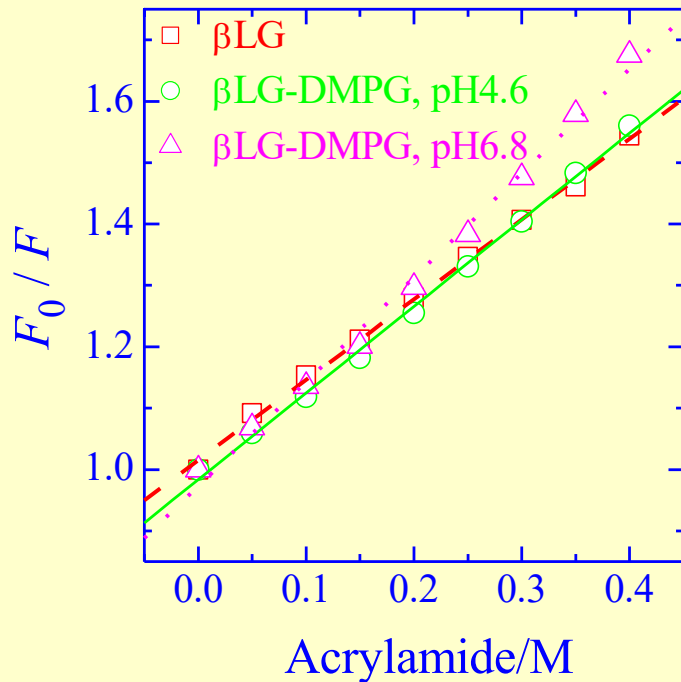
Stopped-Flow fluorescence kinetics



Total fluorescence > 320nm;
Each trace has been divided
by kinetic trace without lipid
vesicles;
Traces are fitted to two-
exponential function

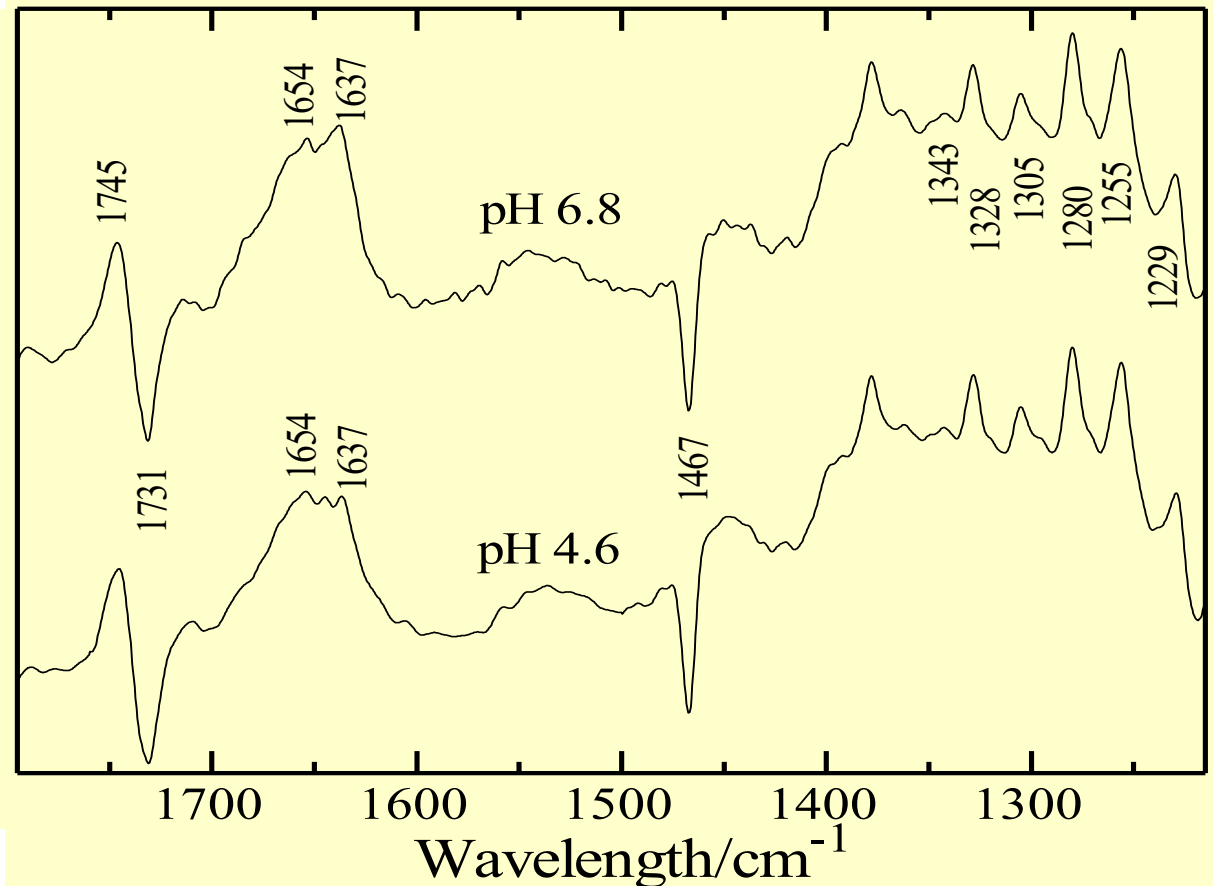
Lipid bilayer insertion of β -Lactoglobulin

Fluorescence quenching



At pH 6.8 & 4.6,
4 & 6 nm blue
shift in $\lambda_{\max}$.

ATR-FTIR orientation



α -helix $\perp$ Membrane surface

Summary: Lipid - β -Lactoglobulin Interaction

