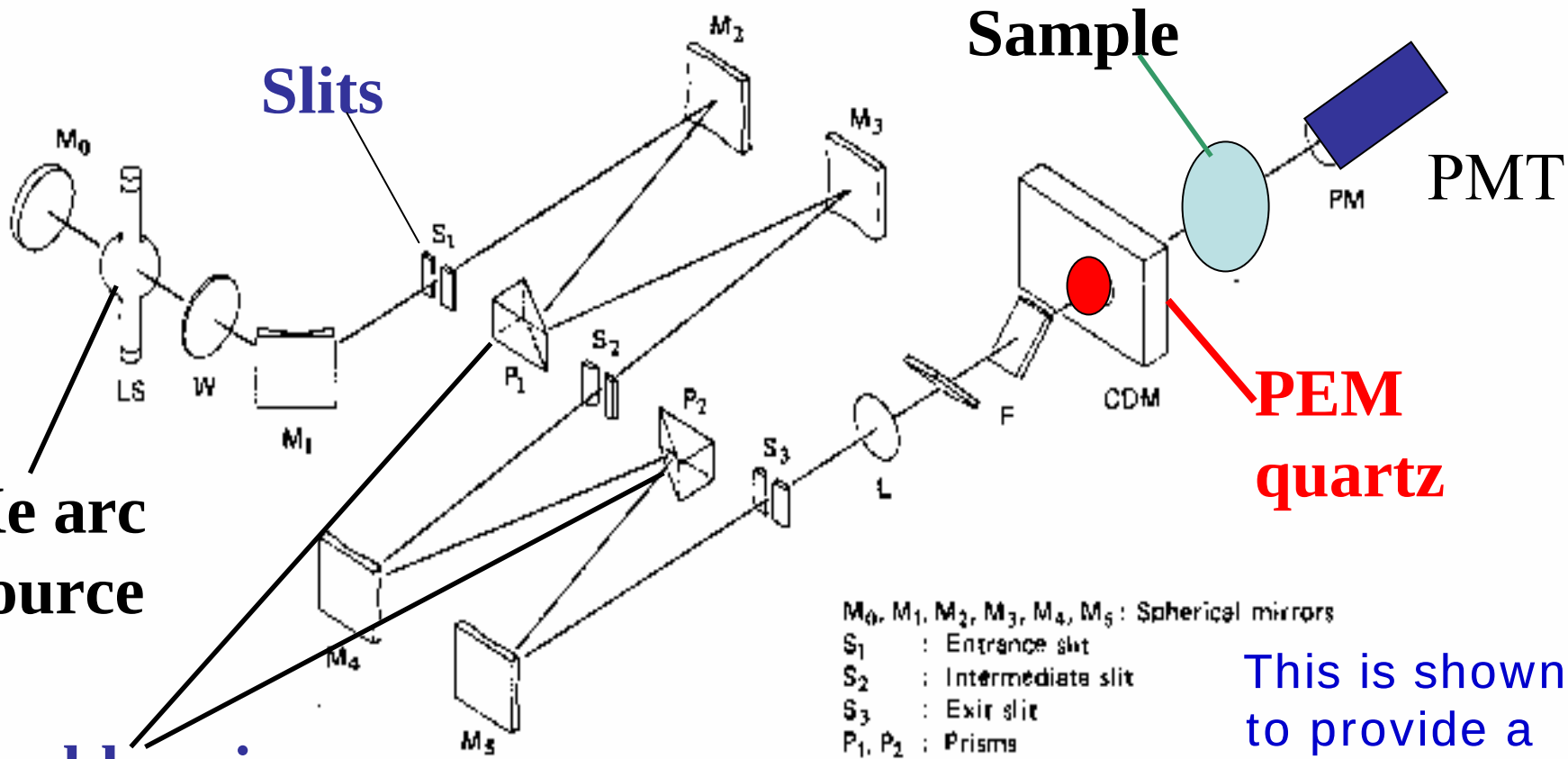
Circular Dichroism

- 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

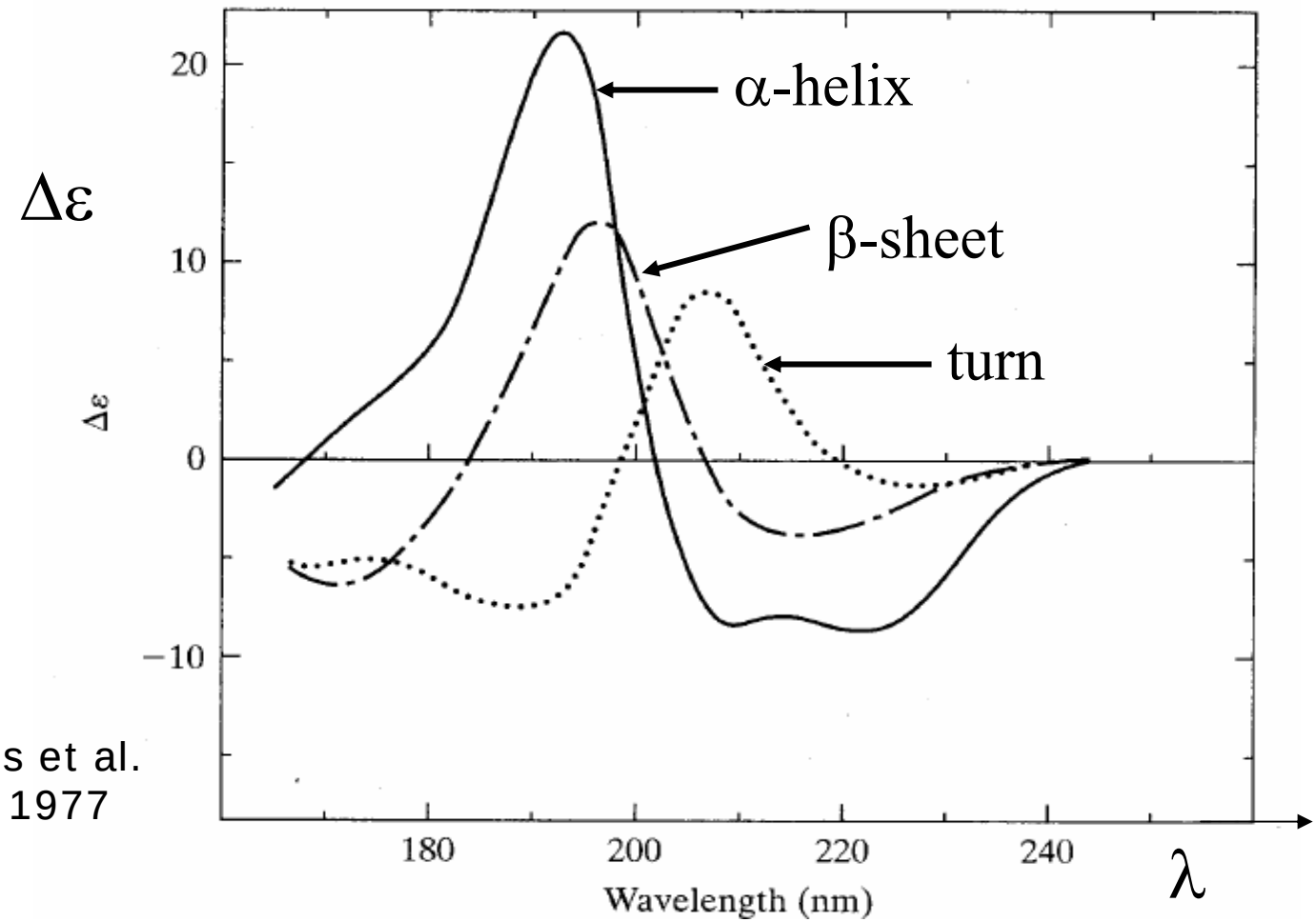
UV-vis Circular Dichroism Spectrometer



JASCO–quartz prisms disperse and *linearly polarize* light

Polypeptide Circular Dichroism

ordered secondary structure types

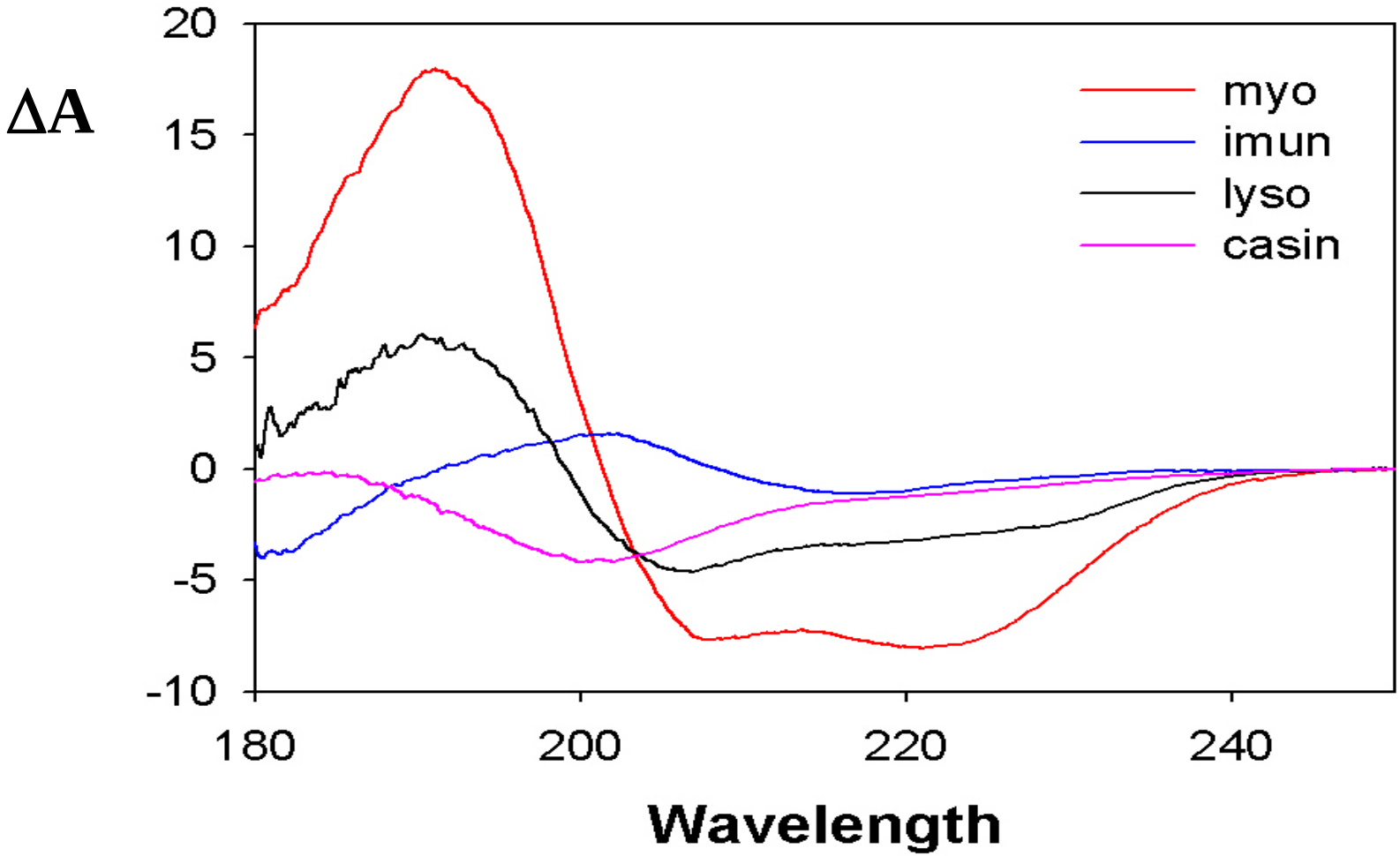


Brahms et al.
PNAS, 1977

poly-L-glu(α,——), poly-L-(lys-leu)(β,- – - –), L-ala$_2$-gly$_2$(turn, · · · · ·)

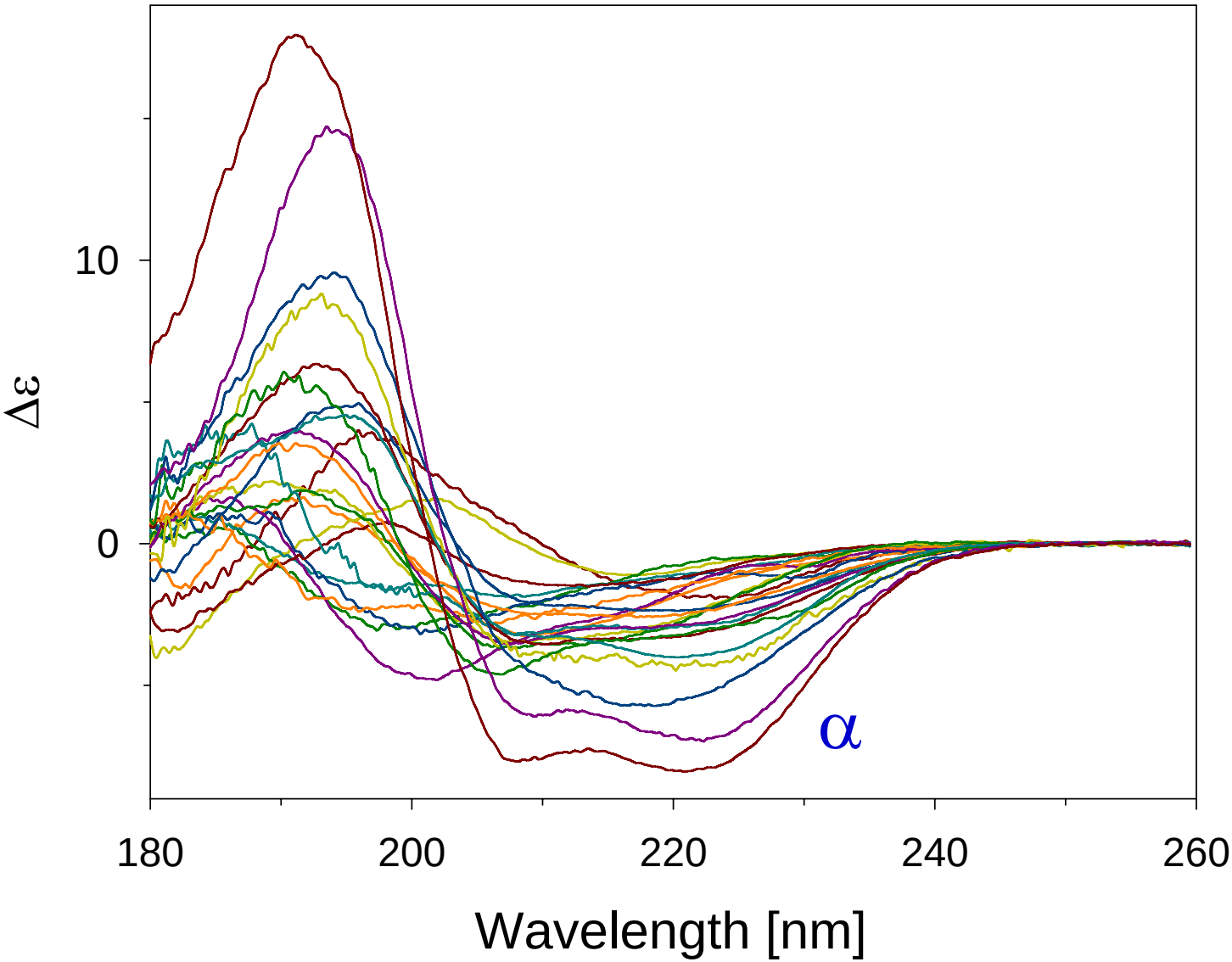
Critical issue in CD structure studies is SHAPE of the Δε pattern

Protein Circular Dichroism

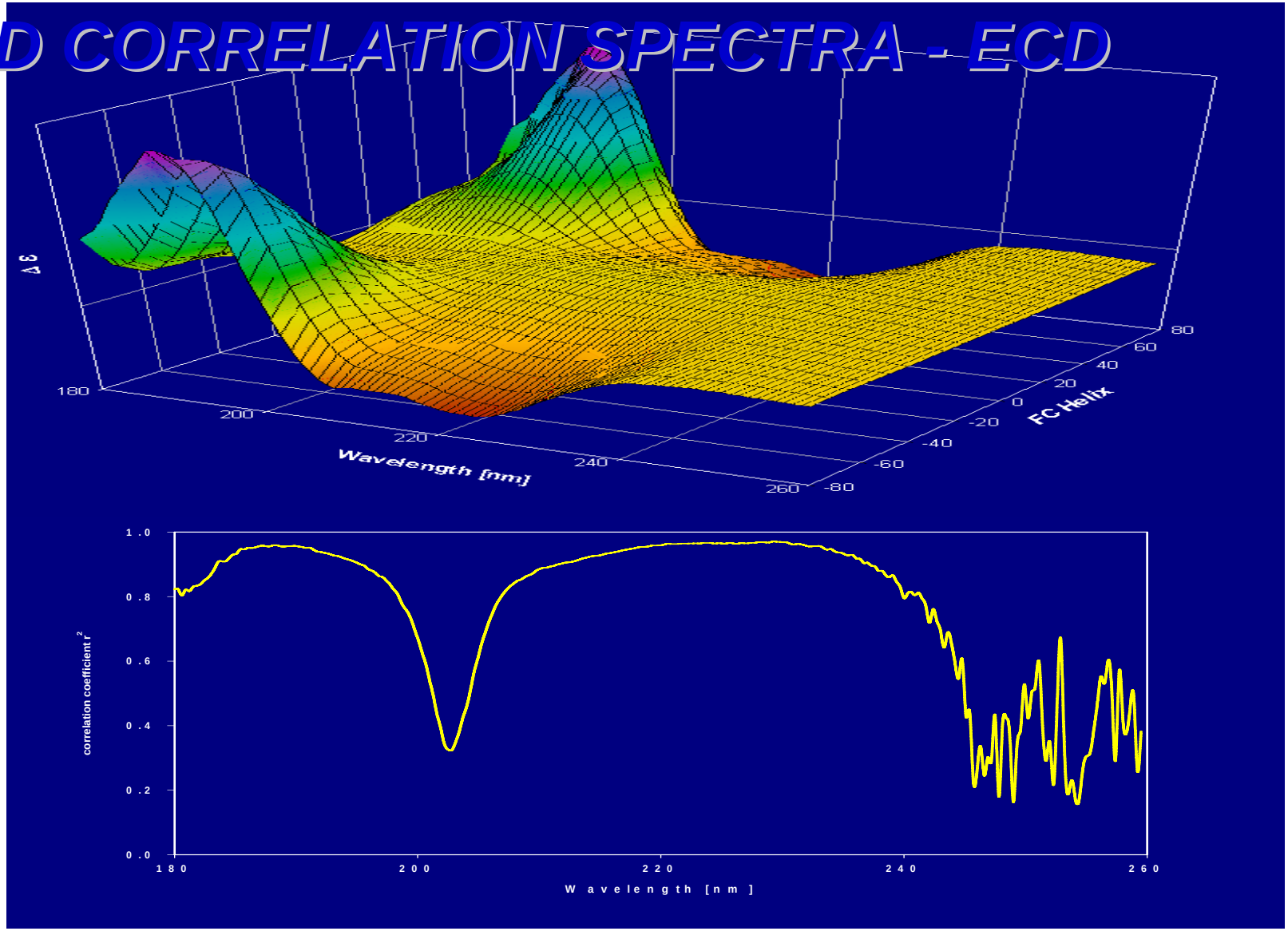


Myoglobin-high helix (——), Immunoglobin high sheet (——)
Lysozyme, a+b (——), Casein, "unordered" (——),

UIC Basis set - 22 proteins ECD

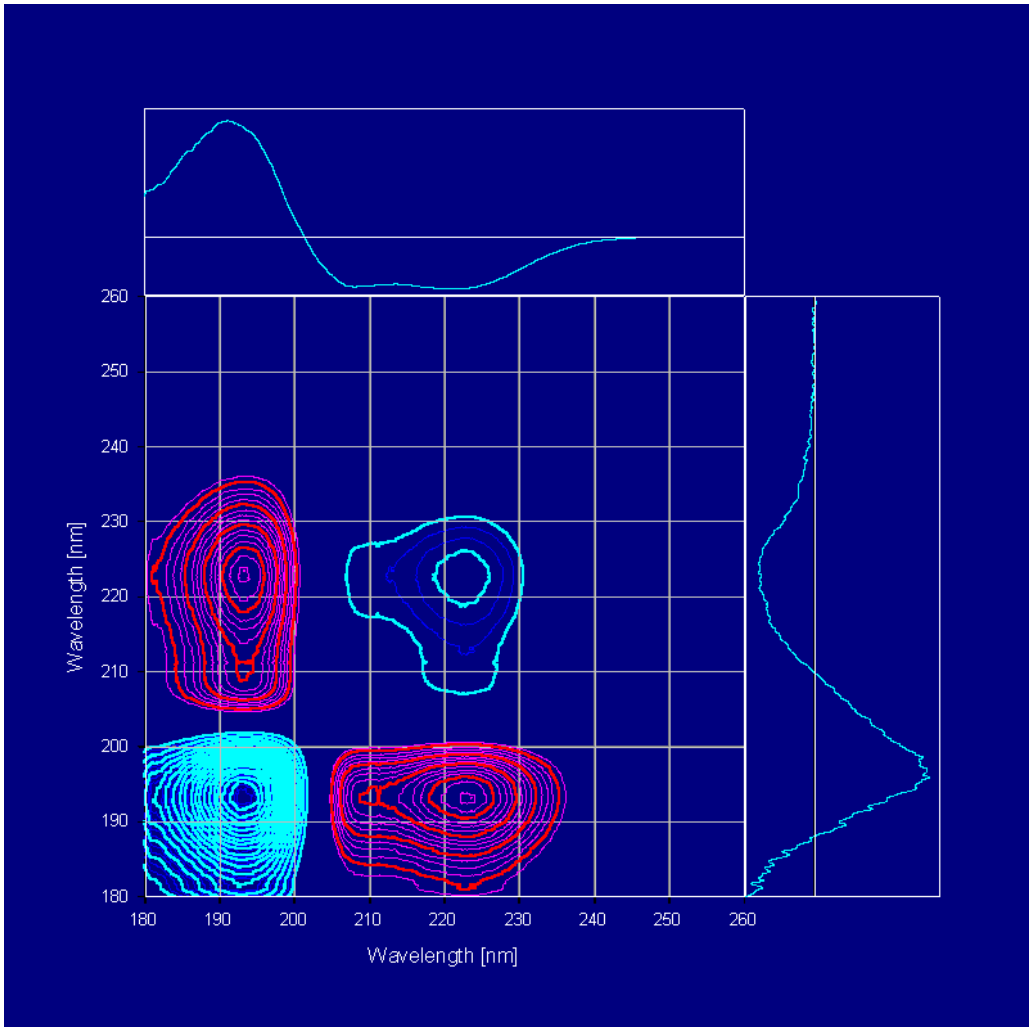


2D CORRELATION SPECTRA - ECD



3D surface obtained by fitting the set of ECD spectra with polynomial Correlation coefficients of the polynomial fit of the ECD spectral intensity as the function of α-helical FC .

2D CORRELATION SPECTRA - ECD



Synchronous correlation map of the protein ECD spectra with respect to α-helix FC perturbation. Positive contours : blue/cyan, negative contours: red/pink.

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

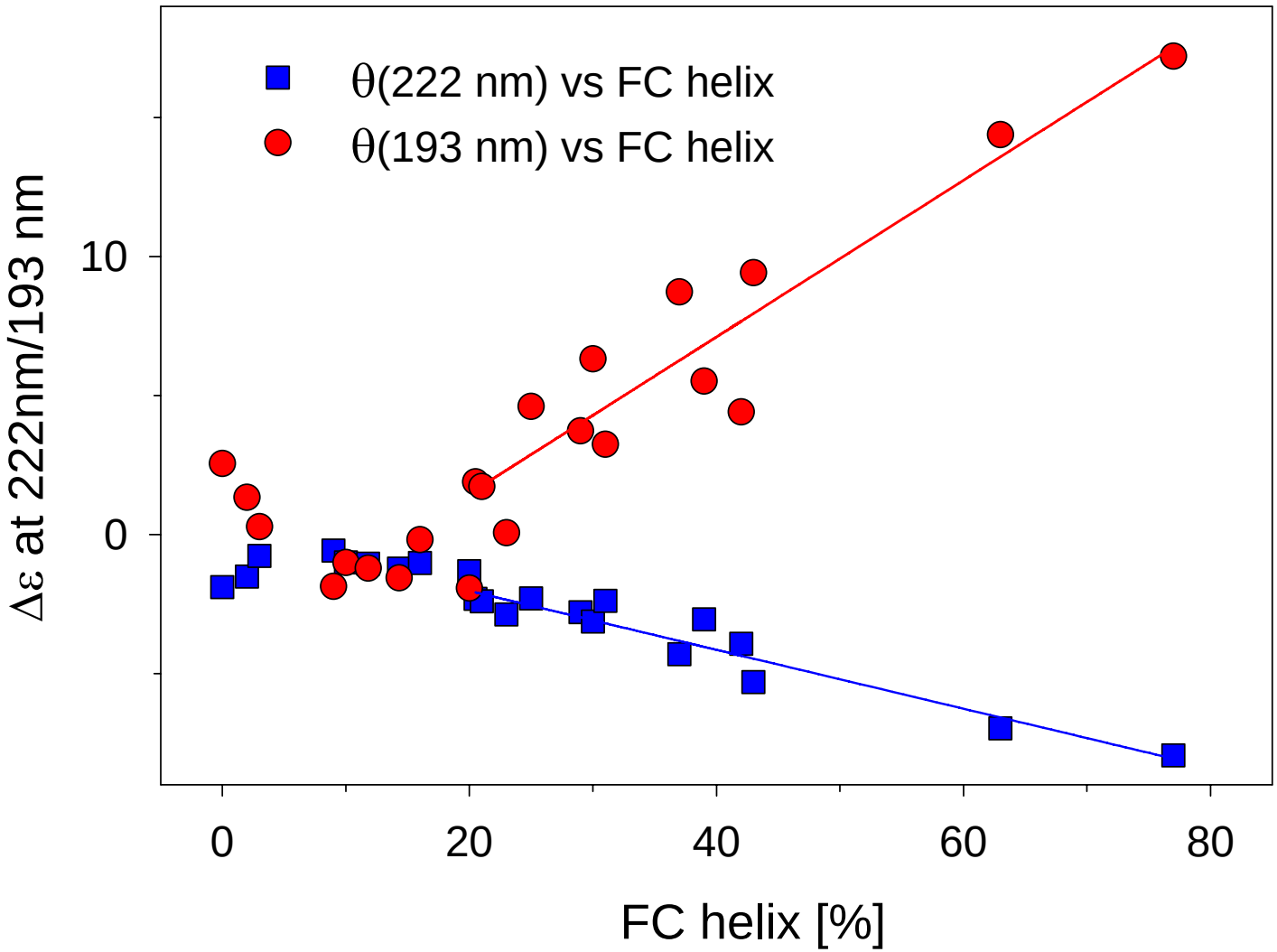
-secondary structure components disperse freq.

Alternate: uv CD - helix correlate to negative intensity at 222 nm, CD spectra in far-UV dominated by helical contribution

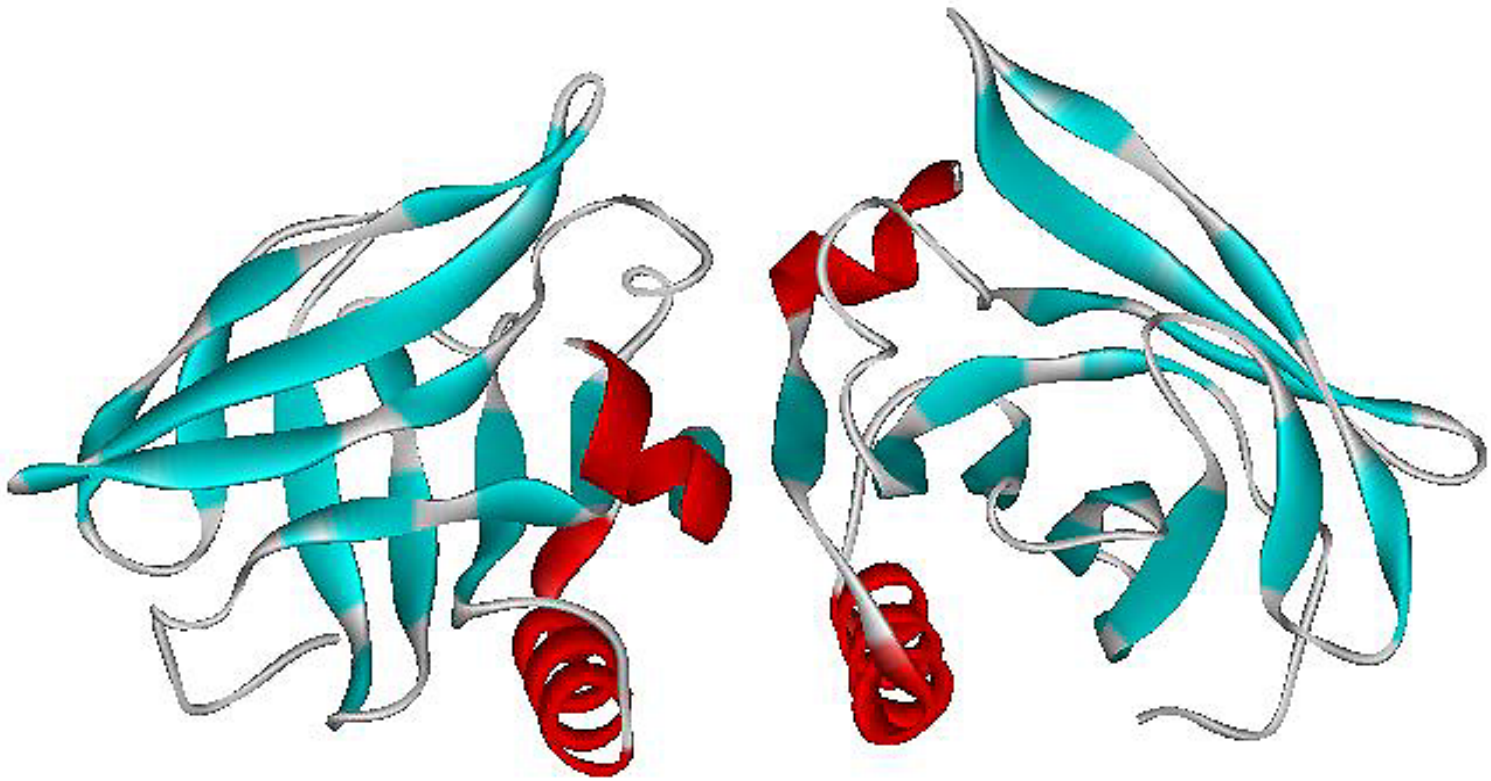
Problem - limited to one factor,

-interference by chromophores]

Single frequency correlation of Δε with FC helix

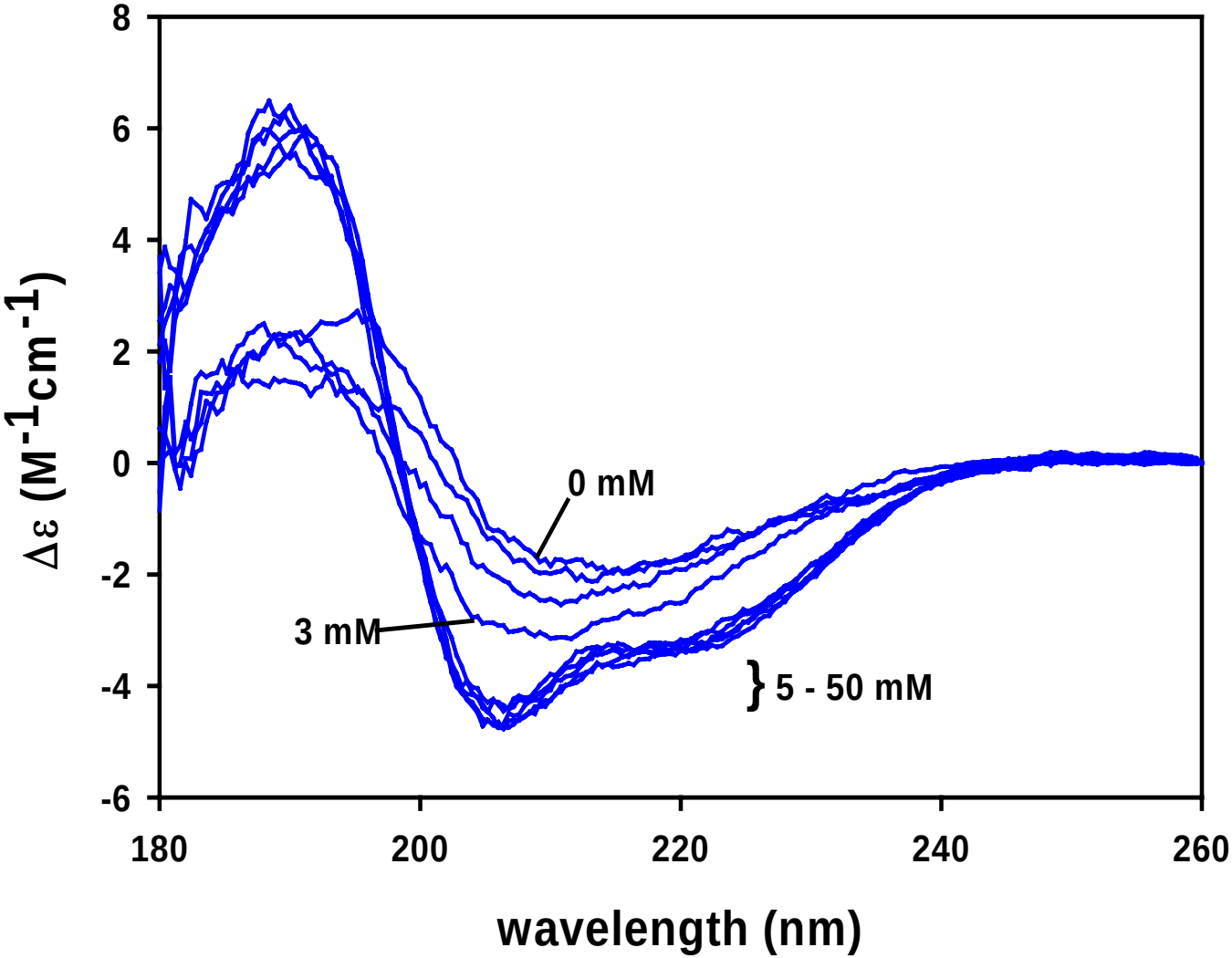


BETA-LACTOGLOBULIN

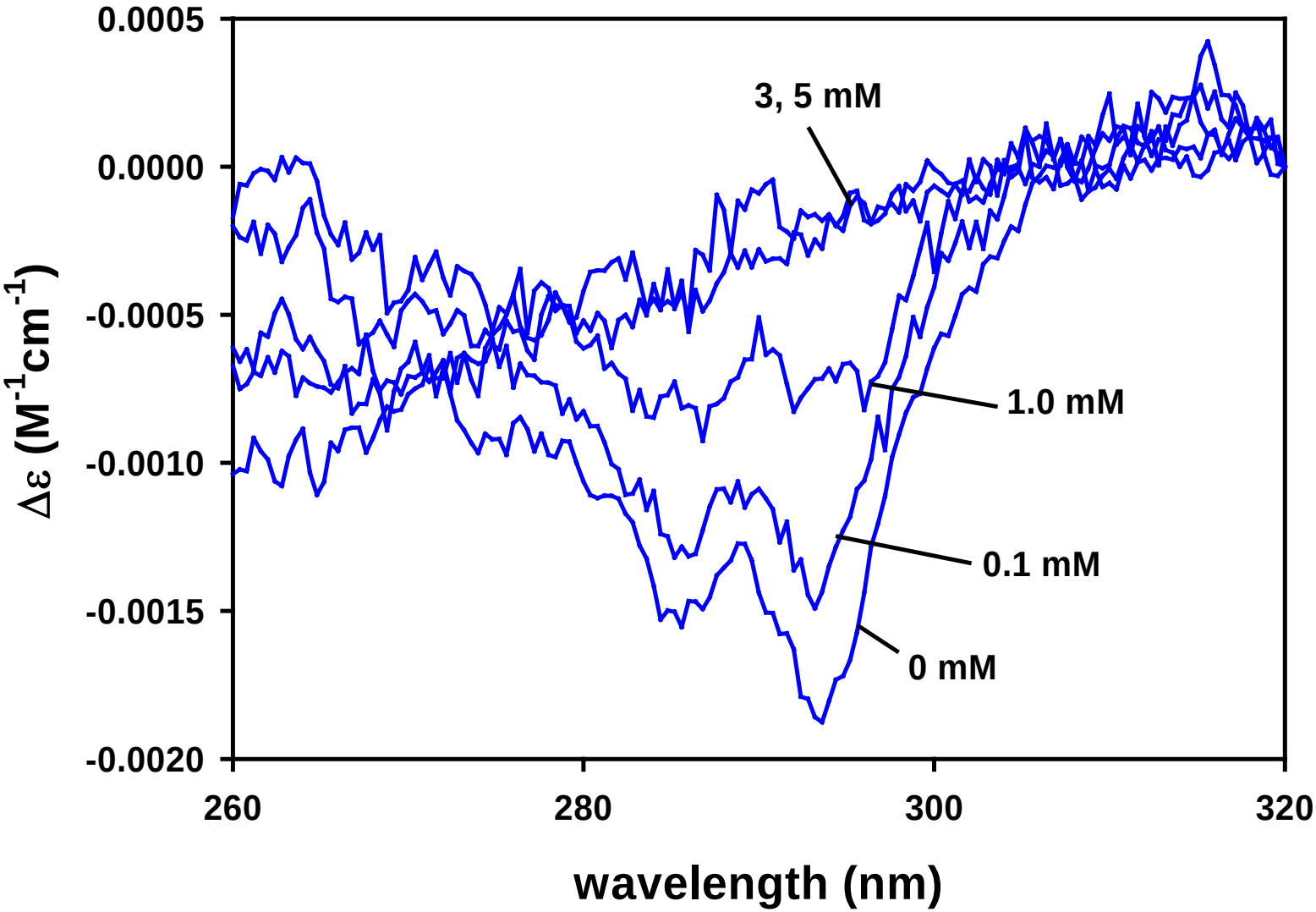


- M_w 18,400 Da, 162 residues
- Primarily β-sheet (42% sheet, 16% helix)
- High propensity for helical conformation
- Structural homolgy to retinol binding protein

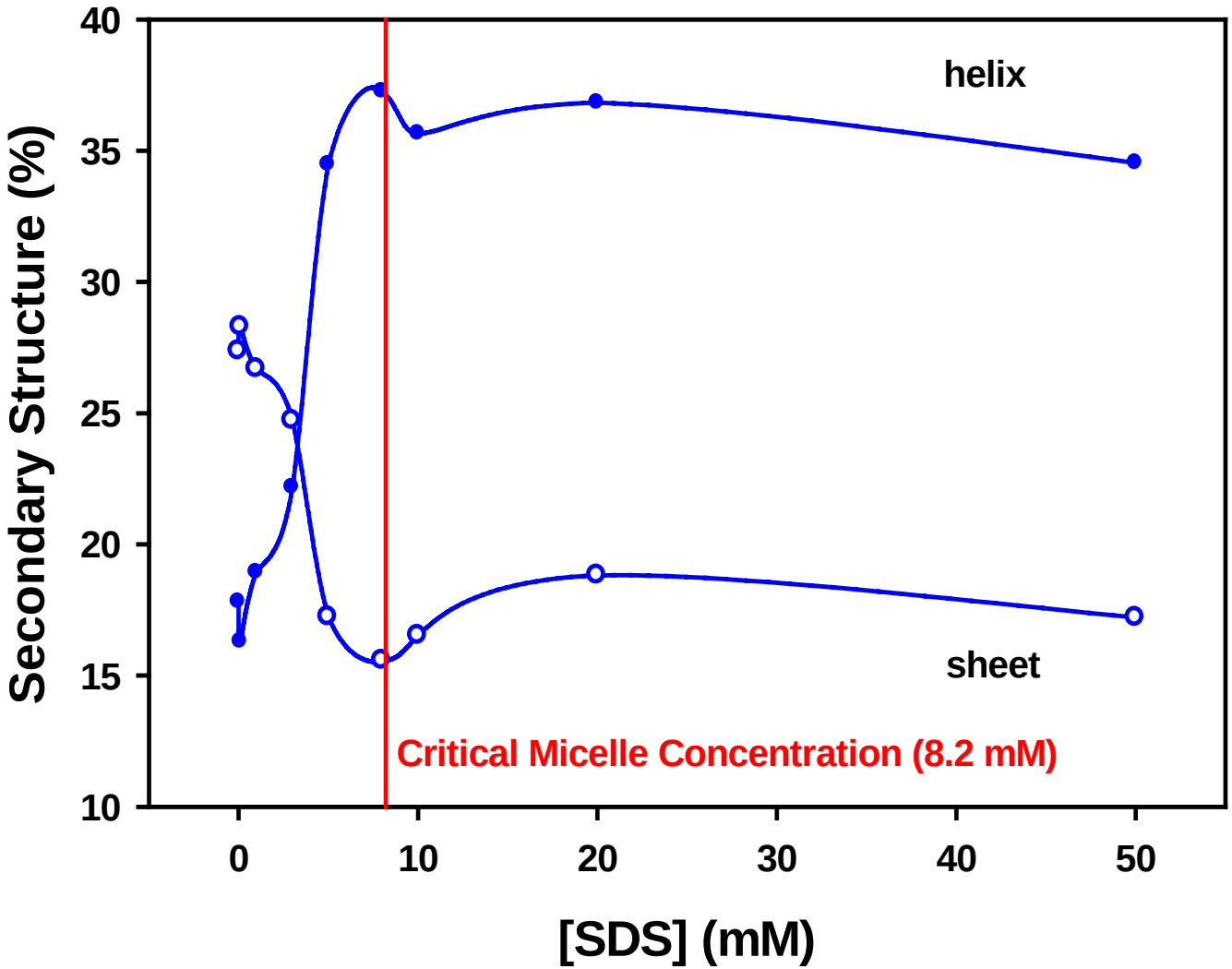
Far-UVCD spectra of BLG titrated with SDS (0-50 mM)



Near-UVCD spectra of BLG titrated with SDS



PC/FA determined secondary structure change

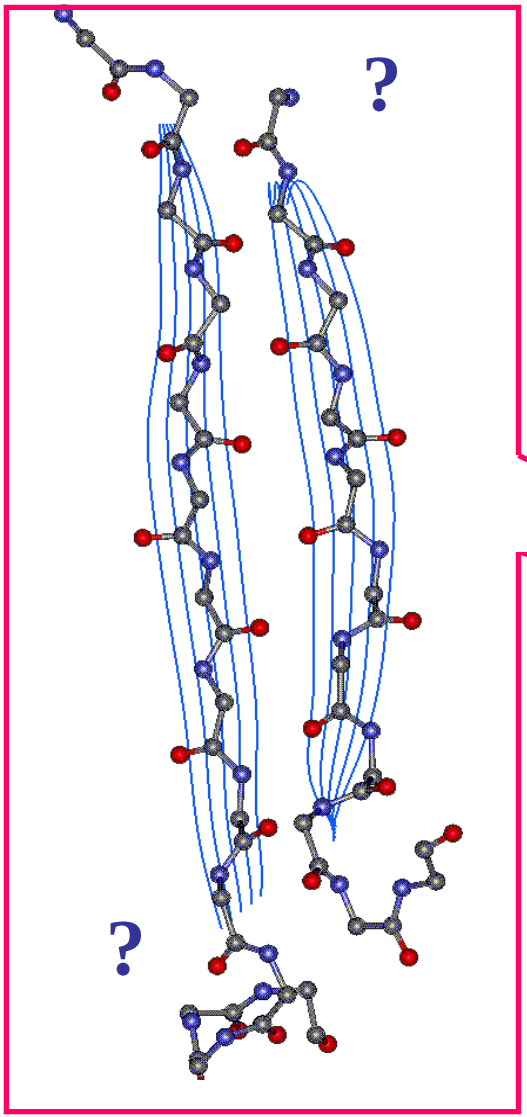


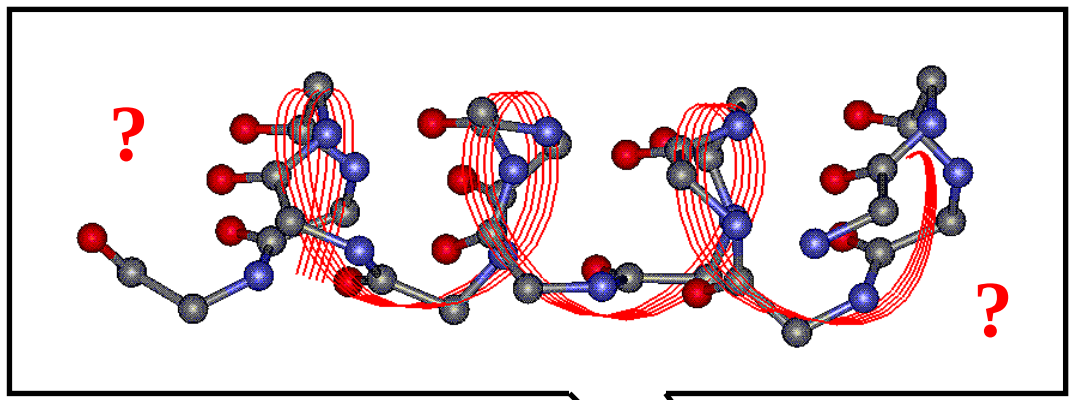
Problem of Secondary Structure Definition

- where do segments begin and end
- what are turns, bends, etc.
- what is basis for helix or sheet -
  ϕ,ψ ***or H-bond pattern***?
- sources:
  X-ray report - non-uniform (visual)
  Levitt-Greer - C_α relationships dominate
  Kabsch-Sander - H-bond patterns dominate (DSSP)
  Frishman-Argos - “knowledge-based” (STRIDE)
  King-Johnson - CD oriented

Problem of secondary structure definition

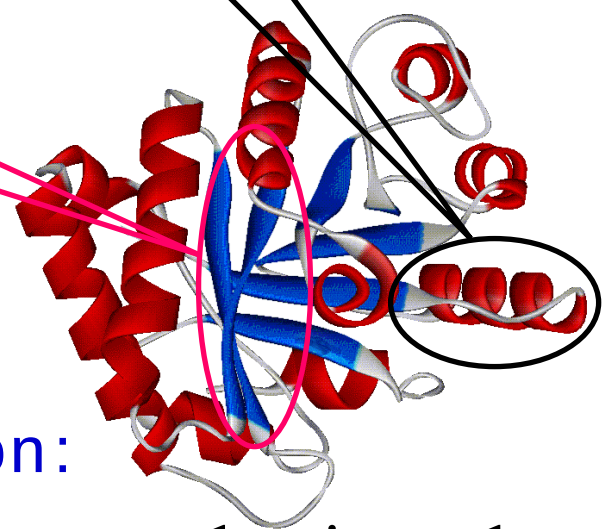
No pure states for calibration purposes





helix

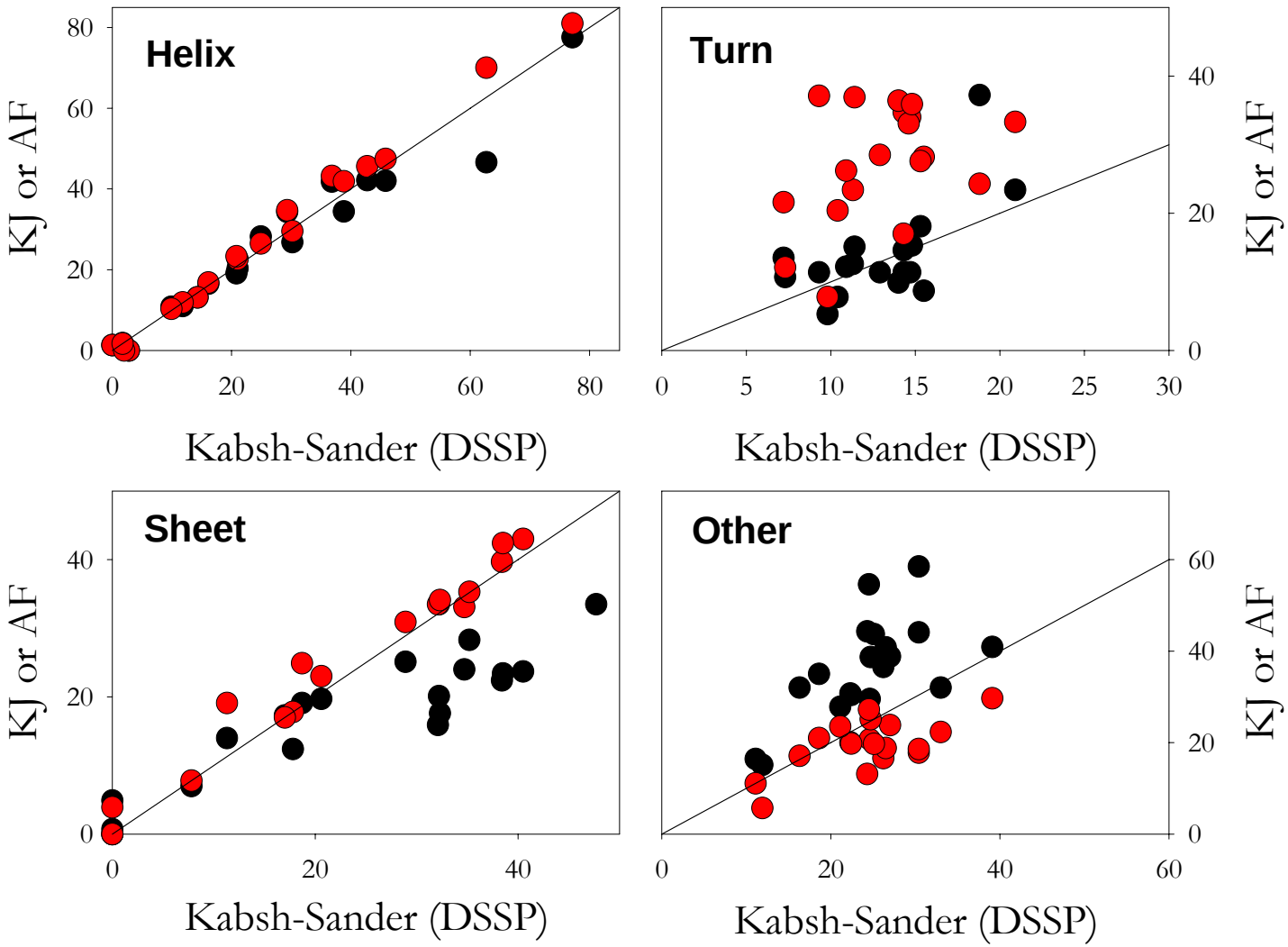
sheet



Need definition:

Where do segments begin and end?

Comparison of secondary structure definitions:



Comparison with DSSP (Kabsh-Sander):

- King-Johnson
- Frishman-Argos (STRIDE)

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
- spectra reflect proteins used as models

Basis set (protein spectra) size and form - major issue

Freedom from model spectra

Series of methods developed assuming:

- spectral response was (fully) related to the secondary structure
- sampling structures with sufficient proteins creates a spectral basis

Milestones:

- Provencher - Glockner --(CONTIN) - ridge regression, no intermediate
- Hennessey - Johnson -- Single value decomposition (SVD)

  initial step is same as principle component or **Factor analysis**
  simplifies spectral variation - monitor component loadings
  5 factors (independent component spectra)

  Fractional structure from (total)inversion of SVD result

$$\mathbf{A = USV^T}$$

$$\mathbf{F = XA}$$

$$\mathbf{X = F(VS'U^T)}$$

Modifications: **Project out model spectra** (Compton -Johnson)
Variable selection - optimize basis (Manavalan-Johnson)
permits analysis of why proteins are outliers.

Variations on a Theme

- **Self-consistent** methods - Sreerama - Woody - (SELCON) –
  probably the most widely used now, Web site connect

- **Restricted multiple regression (RMR)** of Factor Analysis loadings
  Pancoska - Keiderling (et al.) applied to many spectral types

- **Factor analysis is general** - same as SVD
  build correlation matrix of all experimental spectra,
  diagonalize to get eigenvalues, eigenvectors
  yielding weights (singular values), loadings and components
  Useful for analysis of spectral variation with structural variation

- **Quantitative Secondary Structure** application:
  Spectral shape and intensity is influenced by many factors
  eg. solvent, pH, sequence, secondary structure, chromophore
  RMR idea is to find spectral components sensitive to structure

Factor Analysis Method

Decomposition of an experimental spectrum $\theta(\lambda)$ into linear combination of independent component spectra $\phi_j(\lambda)$:

$$\theta_i(\lambda) = \sum_{j=1}^{p} C_{ij}\phi_j(\lambda) = A_i \sum_{j=1}^{p} c_{ij}\phi_j(\lambda)$$

where

$A_i = \sqrt{\int_{\lambda_1}^{\lambda_2} \theta_i^2(\lambda)d\lambda}$ "norm"

C_{ij} / c_{ij} "loadings (expansion coefficients)"

$\phi_j(\lambda)$ "component spectra"

Factor Analysis Method

1. Construct *Correlation Matrix* [R]:

$$[R] = [w_i(\lambda)]^T [w_i(\lambda)] \text{ , where } \quad w_i(\lambda) = \frac{1}{A_i}\theta_i(\lambda) = \sum_{j=1}^{p} c_{ij}\phi_j(\lambda)$$

(normalized spectral data)

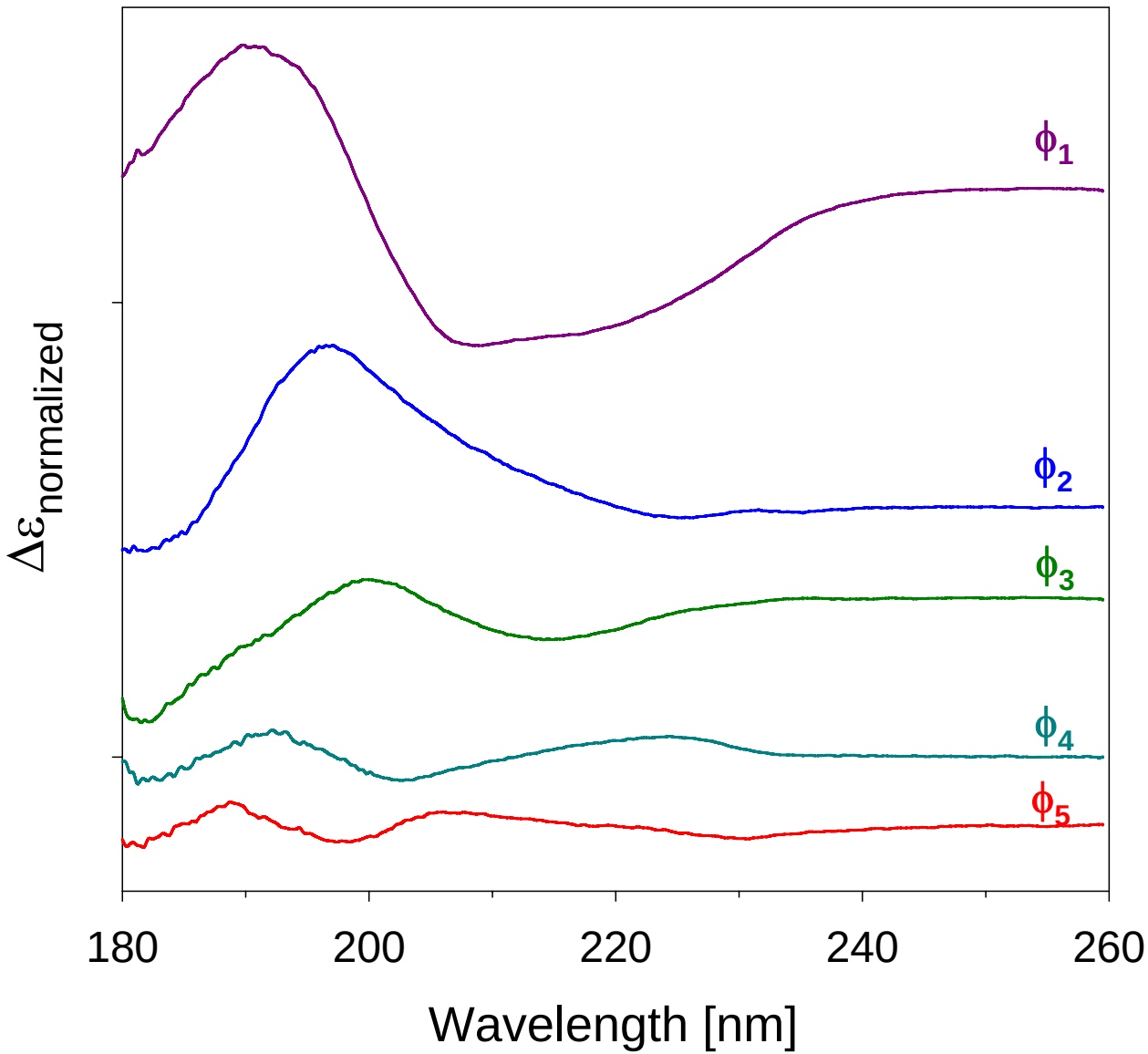
2. Diagonalize [R] to obtain *Principal Components*:

$$[q]^T [R][q] = [\Lambda_{ij}\delta_{ij}]$$

3. Calculate *component spectra* and corresponding *loadings* (coefficients):

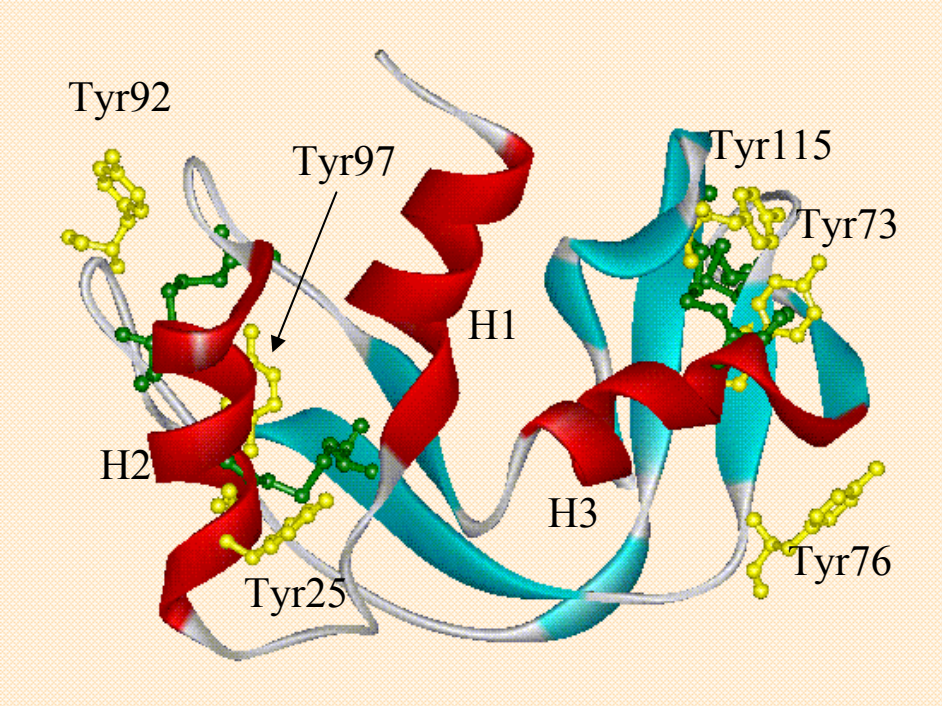
$$[\phi_j(\lambda)] = [w_j(\lambda)][q] \qquad \text{and} \qquad [c_{ij}] = [q]^T$$

FA component spectra - 22 proteins ECD



Factor (Principle Component) Analysis

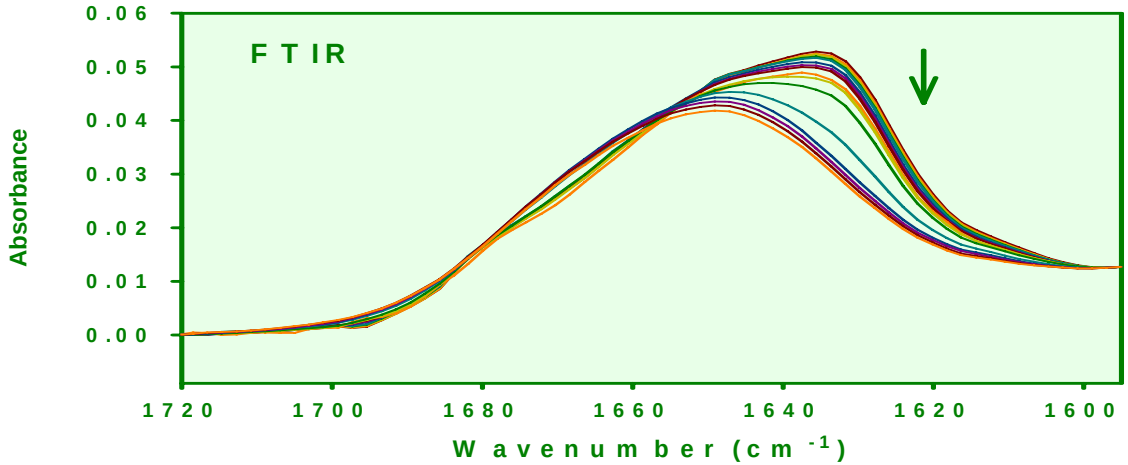
- Approach is functionally equivalent to Principle Component Analysis - Singular Value Decomposition
  - No curve fitting is necessary
  - Band assignments are not necessary
  - Method is general - any technique
- Method:
  - *treat set of protein spectra as basis set of functions,* $[\phi]$
  - Diagonalize the co-variance matrix to
    - find most common elements- ψ_1
    - find most common deviation - ψ_2
    - continue
  - Reconstruct Spectra: $[\phi] = [\psi][\alpha]$, where $[\alpha]$ is a matrix of coefficients, c_{ij} for i^{th} protein and j^{th} subspectrum
  - Use vector of c_{ij} for protein i to characterize protein. Note ψ_i depends on training set, construct to be orthogonal



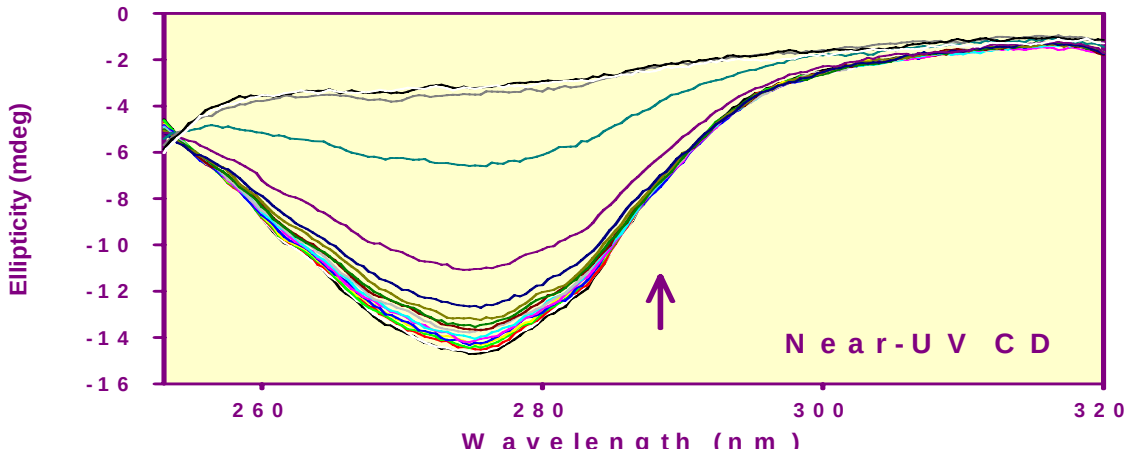
Ribonuclease A combined uv-CD and FTIR study

- 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)

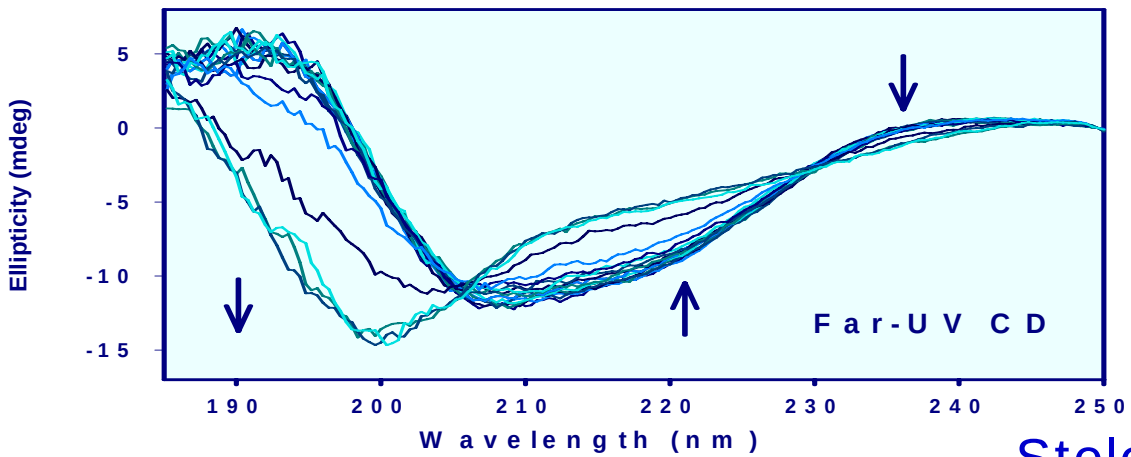
RibonucleaseA



FTIR—amide I
Loss of β-sheet



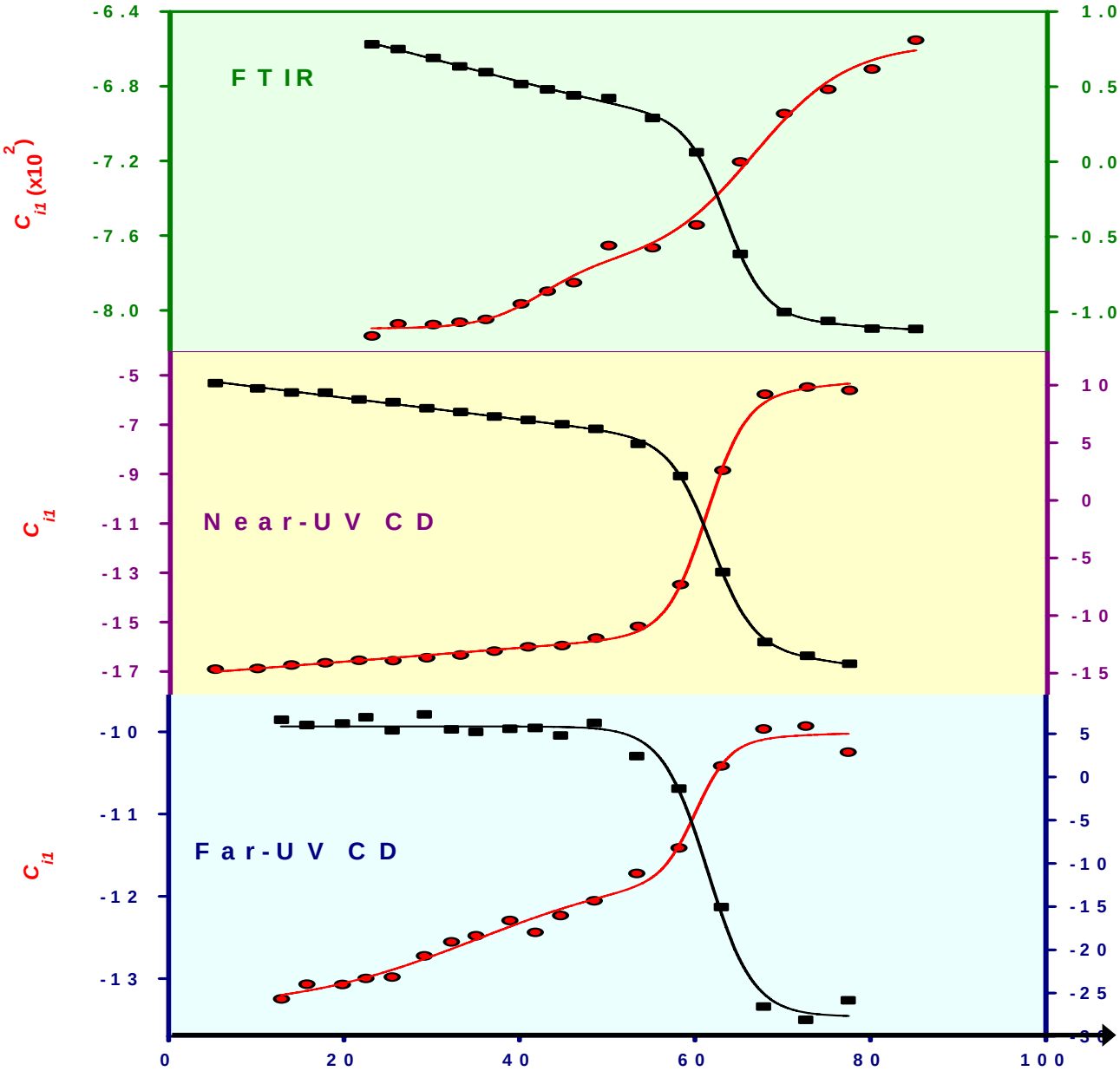
Near –uv CD
Loss of tertiary structure



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)

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

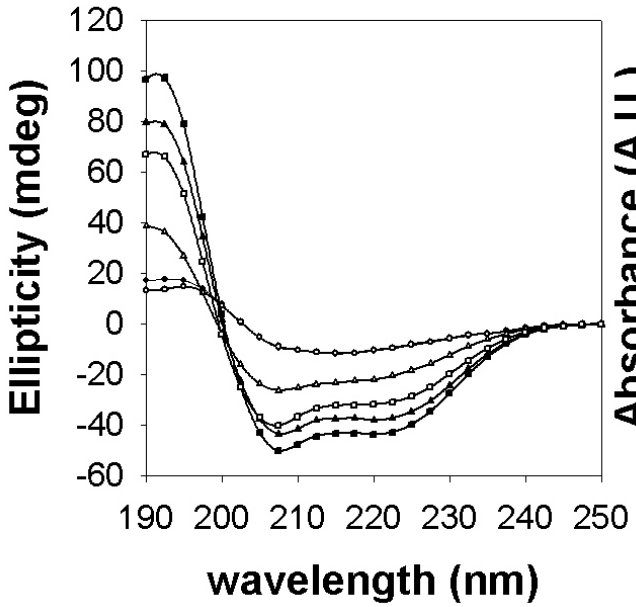
dominantly β-sheet proteins

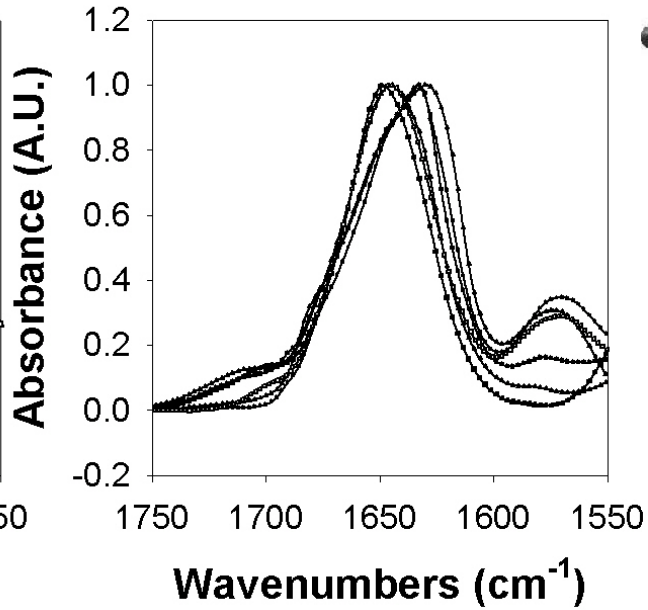
TFE and MeOH behave differently

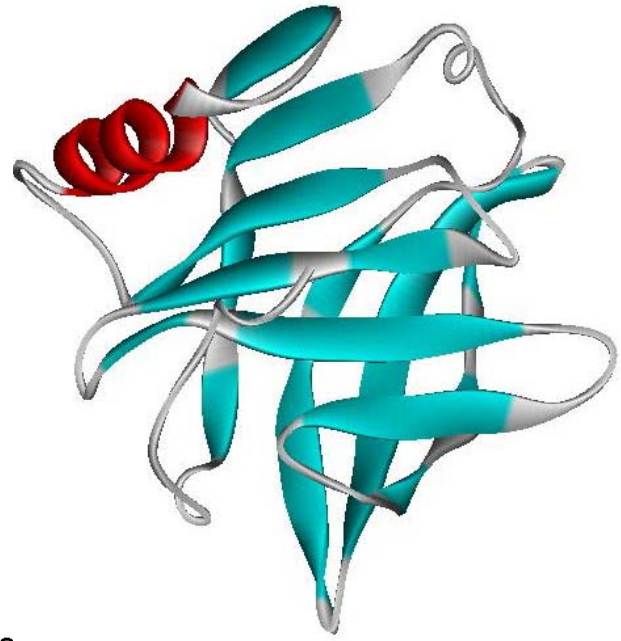
thermal stability key to differentiating states

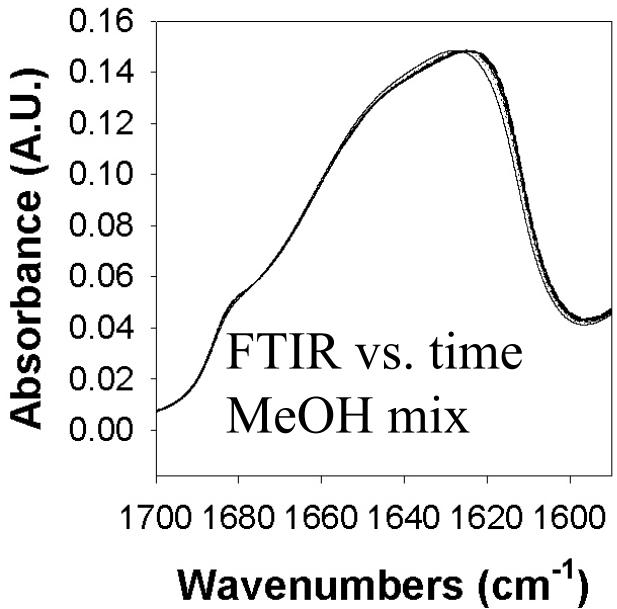
indicates residual partial order

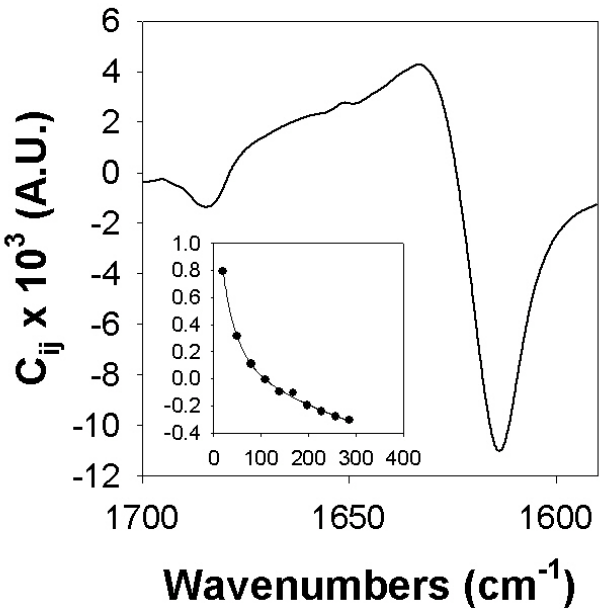
β-lactoglobulin--pH and TFE , MeOH









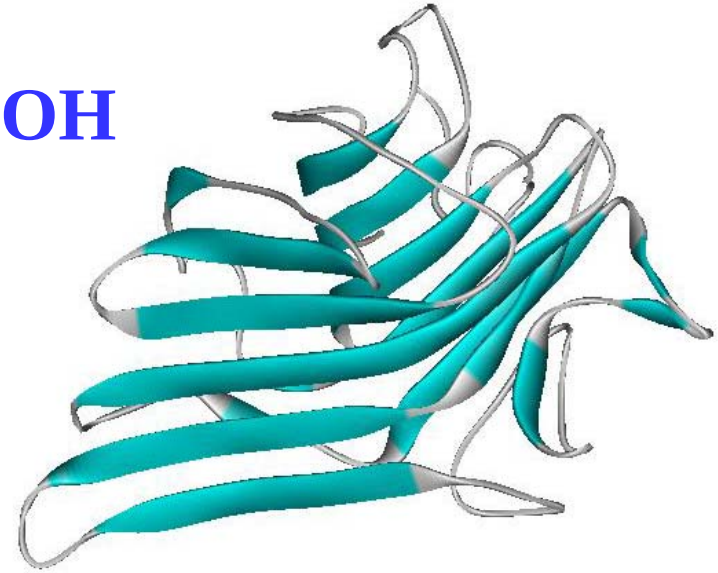


TFE and MeOH both induce helix at both pH 7 and 2

Factor analysis - 2nd component loading shows loss of sheet with time, double exp.

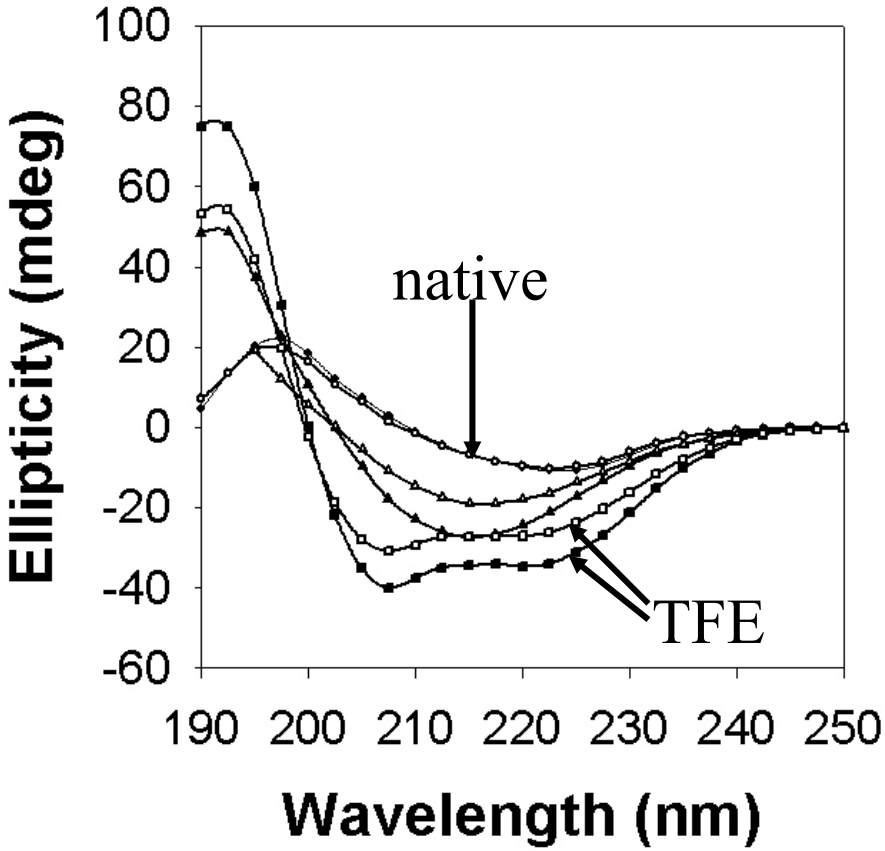
Xu&Keiderling, Adv.Prot.Chem.2002

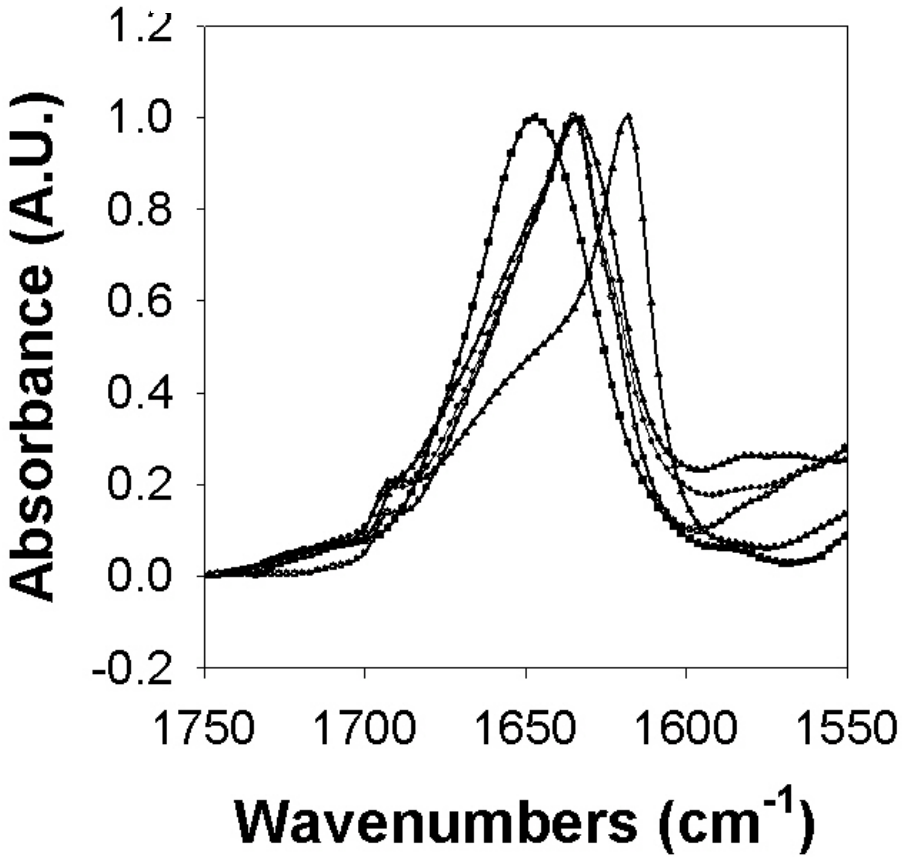
Concanavalin A pH, TFE and MeOH



MeOH normalizes β-sheet ECD,
FTIR indicates aggregated
TFE induces helix

Xu&Keiderling, Biochem, 2005





Lipid-induced Conformational Transition of β-Lactoglobulin: Equilibrium and Kinetic Studies

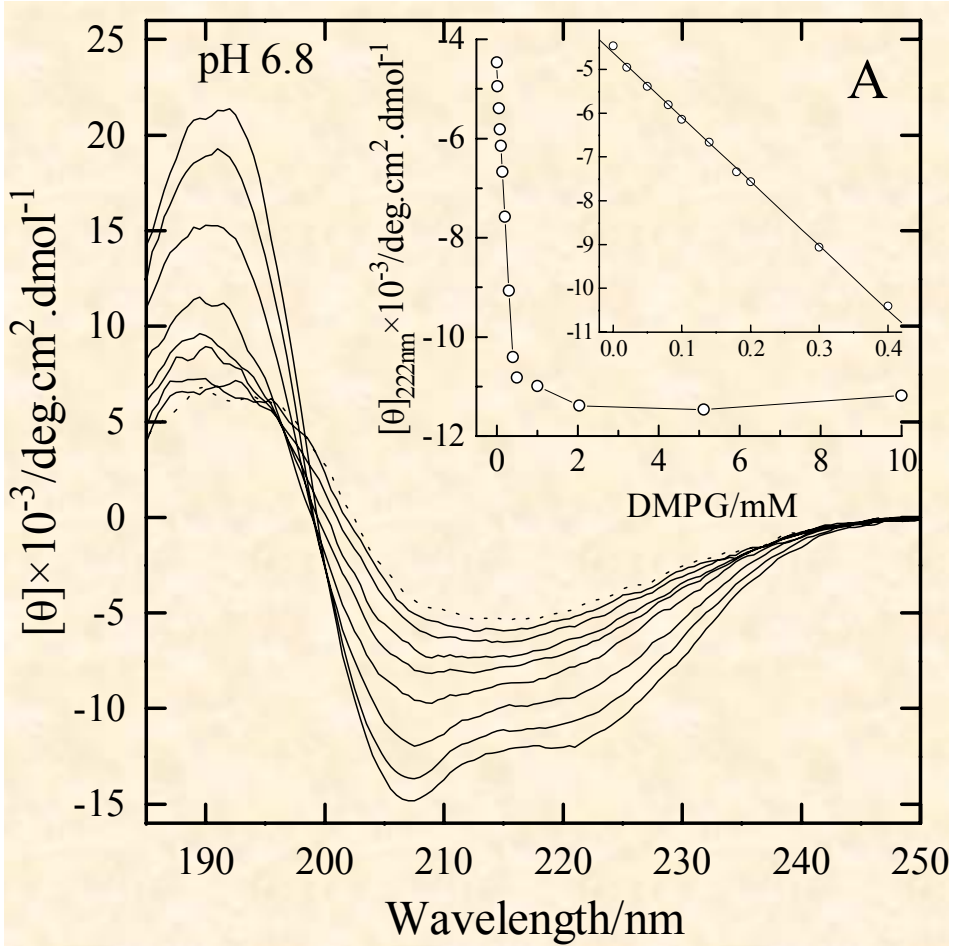
Globular protein with 9-stranded sheet
(flattened β-barrel) and one helical segment
Terminal segments have high helical propensity
Good model for β-to-α conversion

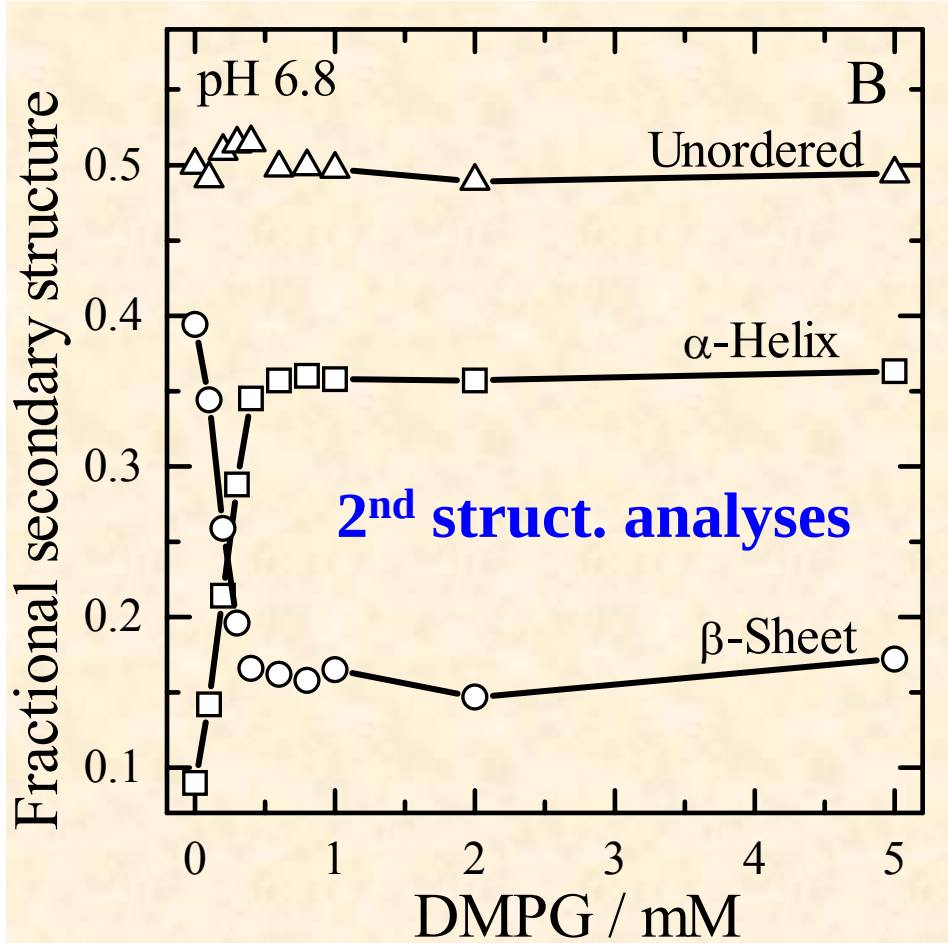
Binding to lipid vesicle acts as perturbation—cell model

Xiuqi Zhang, Ning Ge,TAK Biochemistry 2006/2007

BLG Binding to DMPG at pH 6.8: Circular Dichroism

-β-sheet to α–helix transition, dependence on DMPG

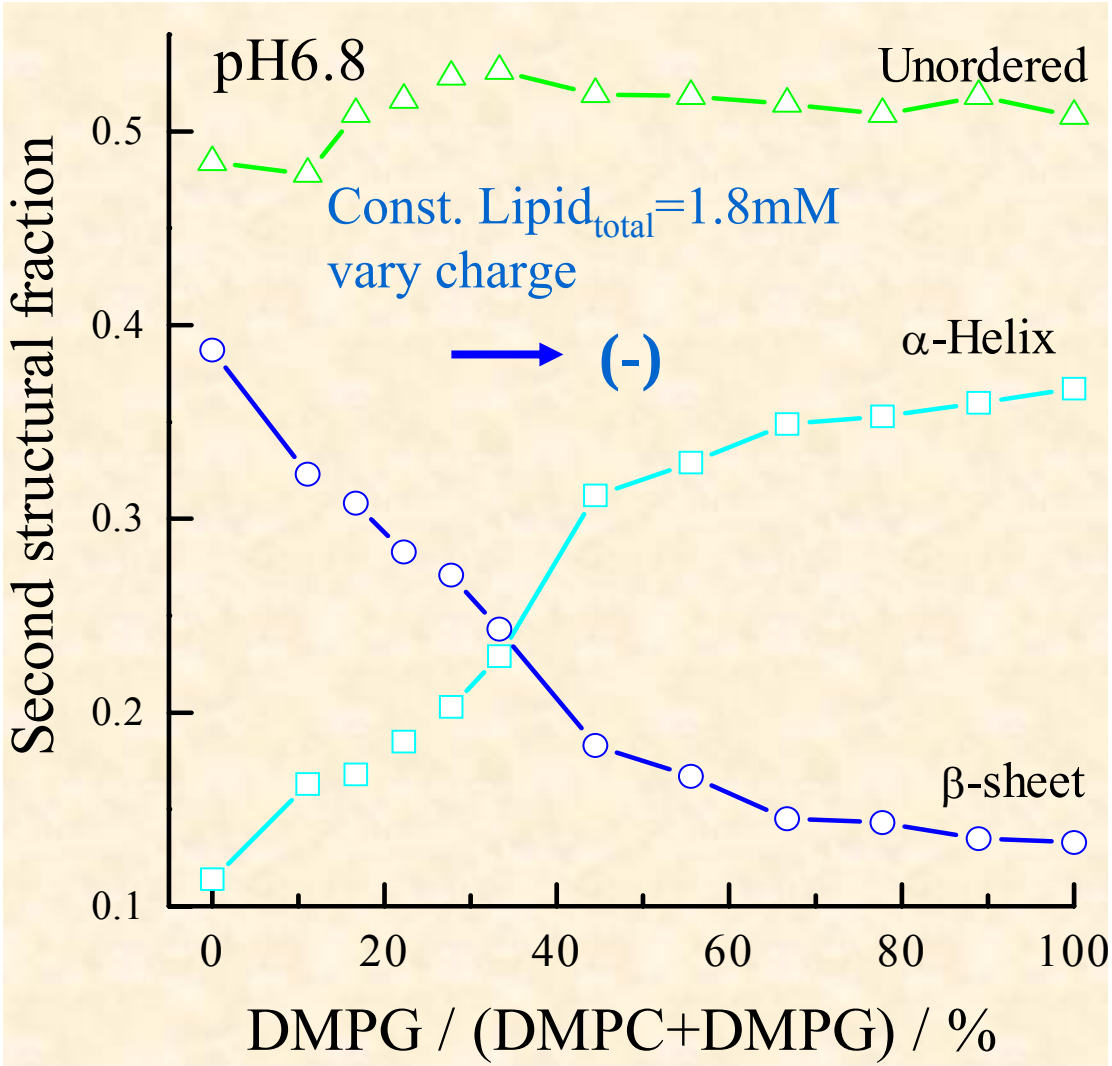




Secondary structure: Binding DMPG at pH6.8, causes BLG conformational change. The α–helix formed with loss of β-sheet.

Effect of lipid charge:

-How does the charge of lipid affect protein binding?



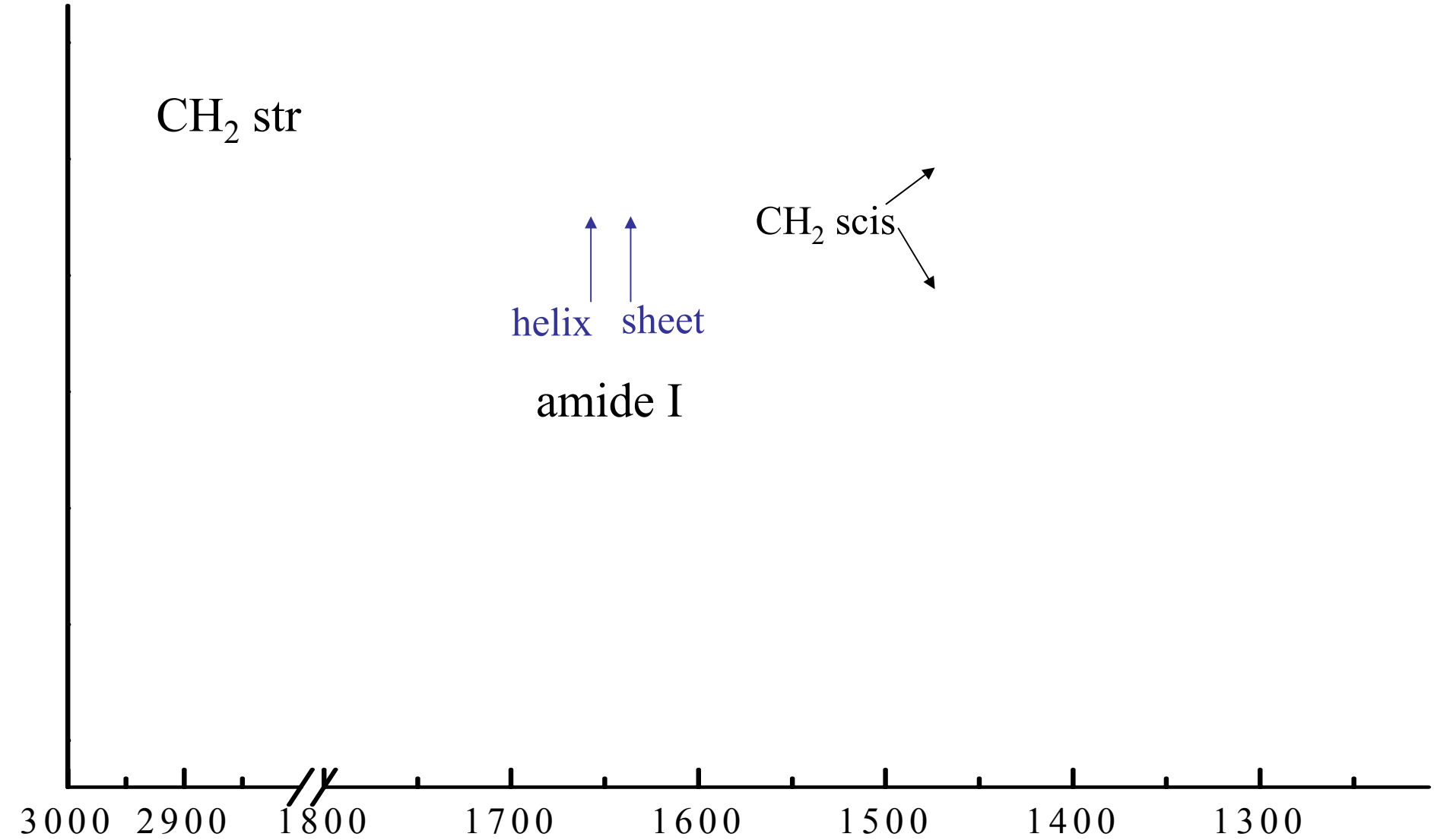
Effect of Charge: Addition of neutral lipid (DMPC) decreases lipid charge and α–helix in BLG:DMPG mixture (left). So negative charge of lipid is necessary for the formation of α–helix (right).

Xiuqi Zhang, TAK
Biochemistry 2006

BLG in varying DMPG / DMPC mixture

Orientation of BLG into lipid membrane:

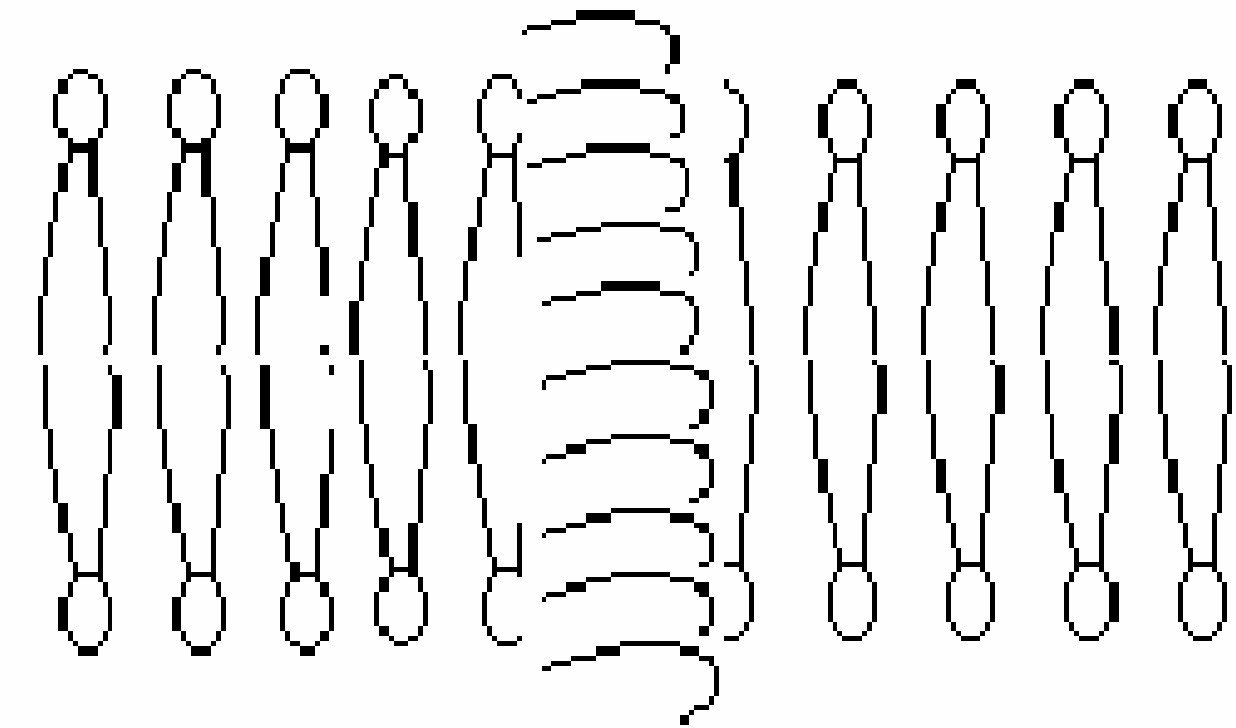
-Polarized ATR-FTIR spectra of DMPG-bound BLG



Summary βLG: **Orientation of protein segments**

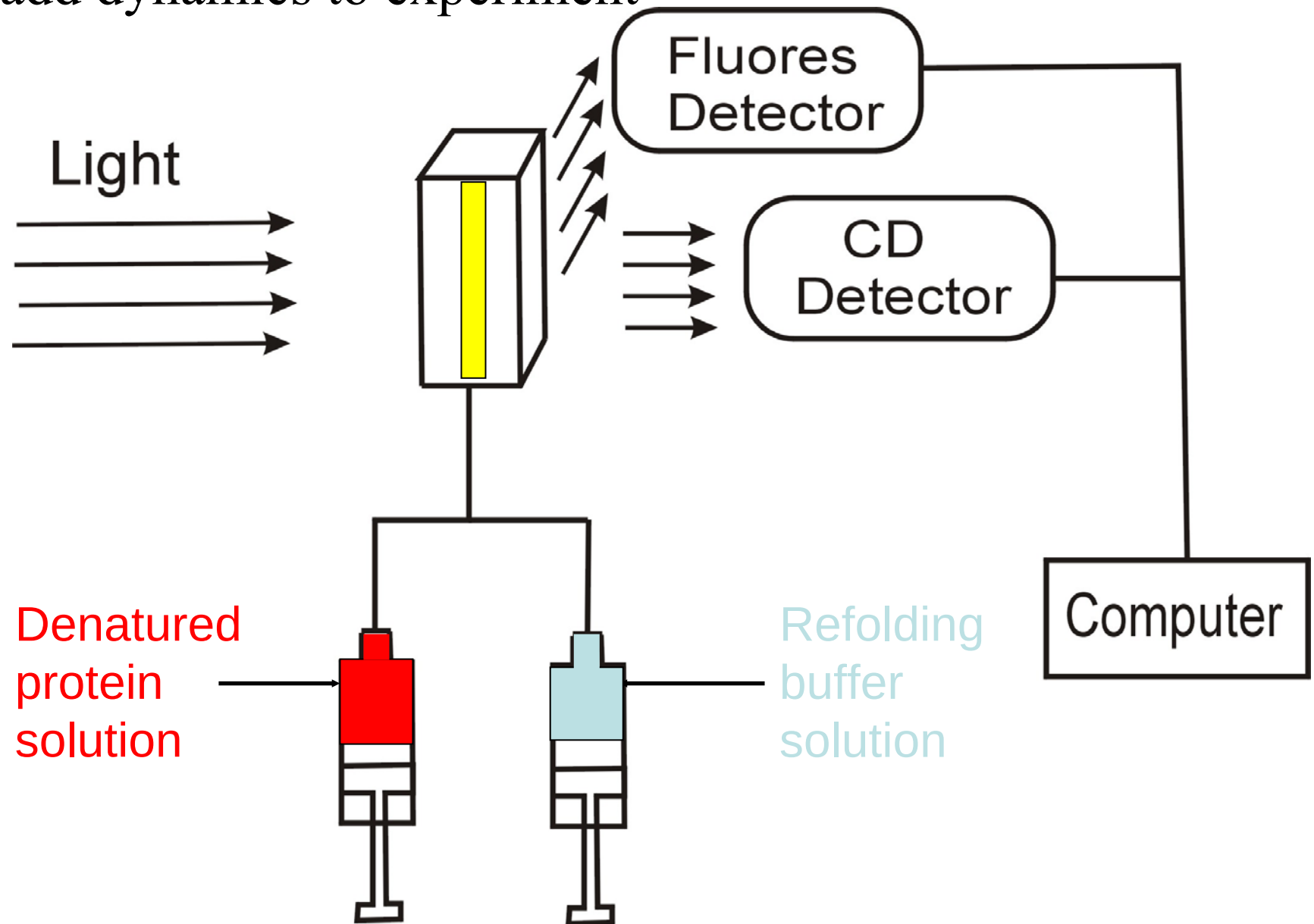
Some portions of BLG inserted into bilayer. The positive amide I peaks at 1654 and 1637 cm^{-1} suggest that α-helices have a preferred orientation perpendicular to the membrane surface, and β-sheets are probably not inserted, at both pHs.

Current studies – Ning Ge various membrane systems

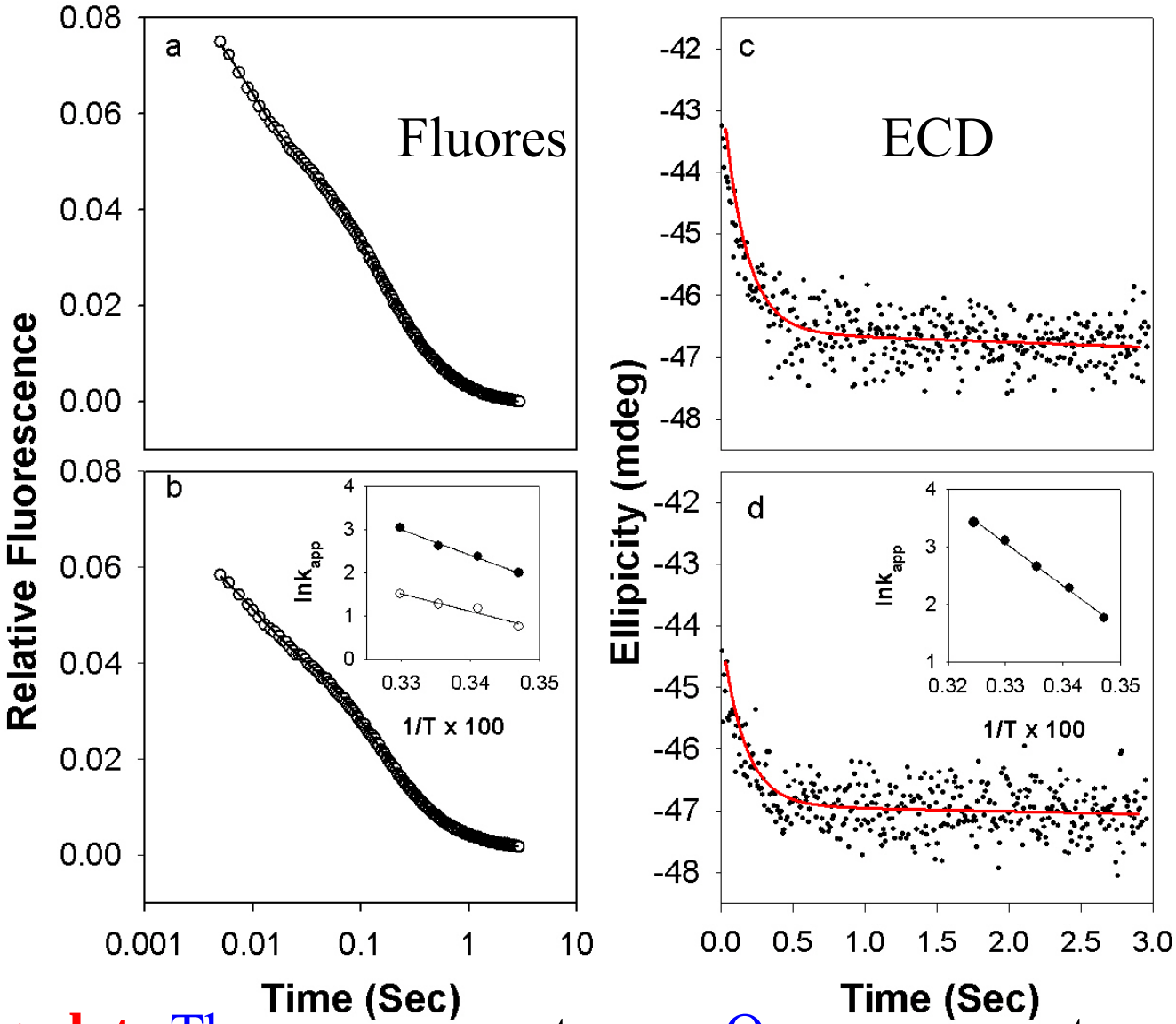


Dynamics--Scheme of Stopped-flow System

- add dynamics to experiment



Stopped-flow ECD and Fluorescence of acid denatured Cyt c refolding by neutralization with phosphate buffer



Without salt

T = 15°C

With 0.5M salt

Log plot--Three components

One component

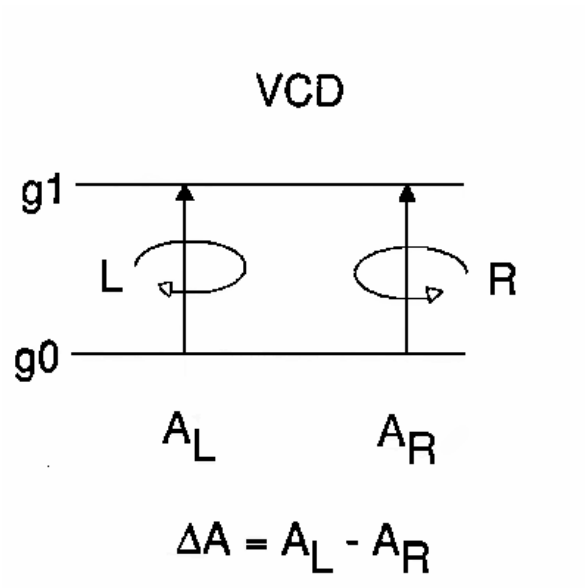
Xu & Keiderling, Proteins 2006

VIBRATIONAL OPTICAL ACTIVITY

Differential Interaction of a Chiral Molecule with Left and Right Circularly Polarized Radiation During Vibrational Excitation

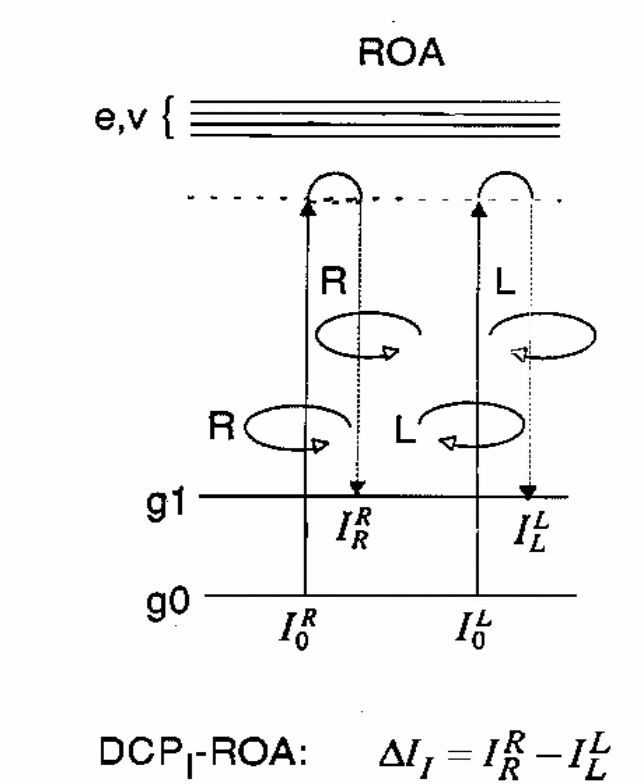
VIBRATIONAL CIRCULAR DICHROISM

Differential Absorption of Left and Right Circularly Polarized Infrared Radiation



RAMAN OPTICAL ACTIVITY

Differential Raman Scattering of Left and Right Incident and/or Scattered Radiation



Combining Techniques: Vibrational CD

"CD" in the infrared region

Probe chirality of vibrations → goal stereochemistry

Many transitions / Spectrally resolved / Local probes

Technology in place -- separate talk

Weak phenomenon - limits S/N / Difficult < 700 cm^{-1}

Same transitions as IR

same frequencies, same resolution

Band Shape from spatial relationships

neighboring amides in peptides/proteins

Relatively short length dependence

AA_n oligomers VCD have $\Delta A/A \sim$ const with n

vibrational (Force Field) coupling plus dipole coupling

Development -- structure-spectra relationships

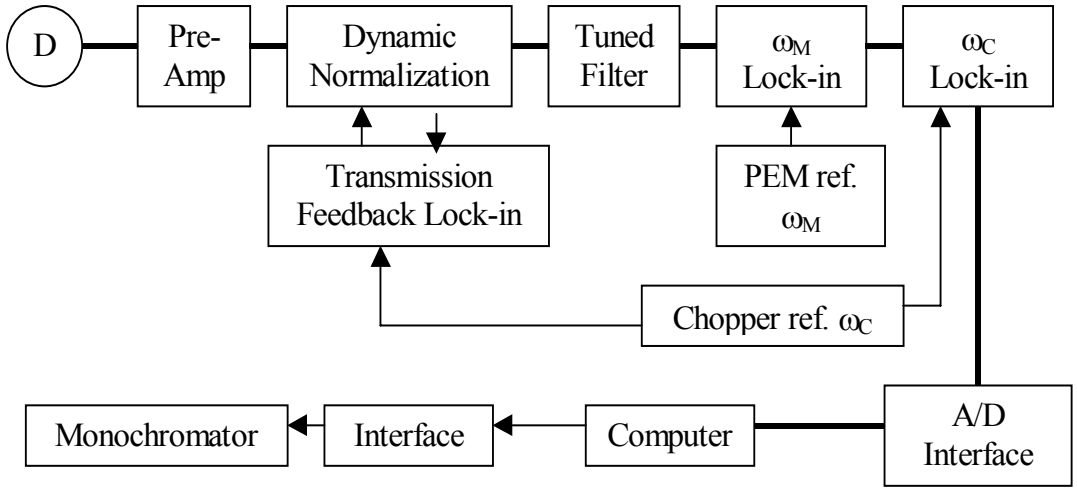
Small molecules – theory / Biomolecules -- empirical,

Recent—peptide VCD can be simulated theoretically

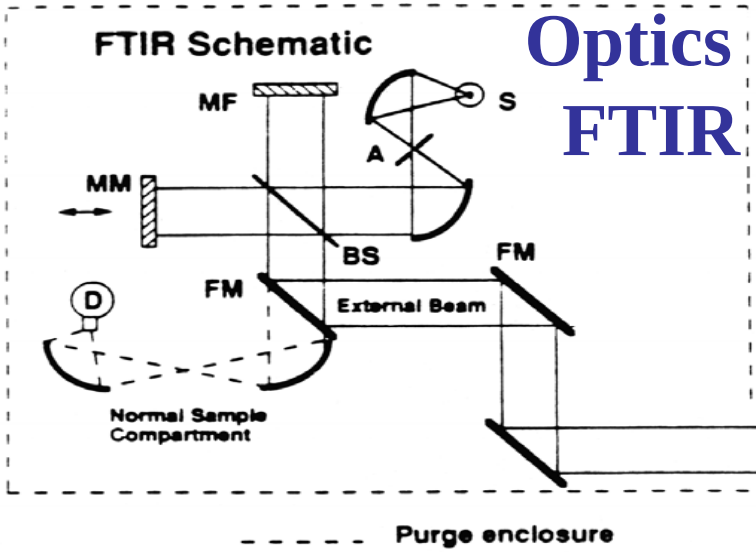
UIC Dispersive VCD Schematic

Yes it still exists and measures VCD!

Electronics

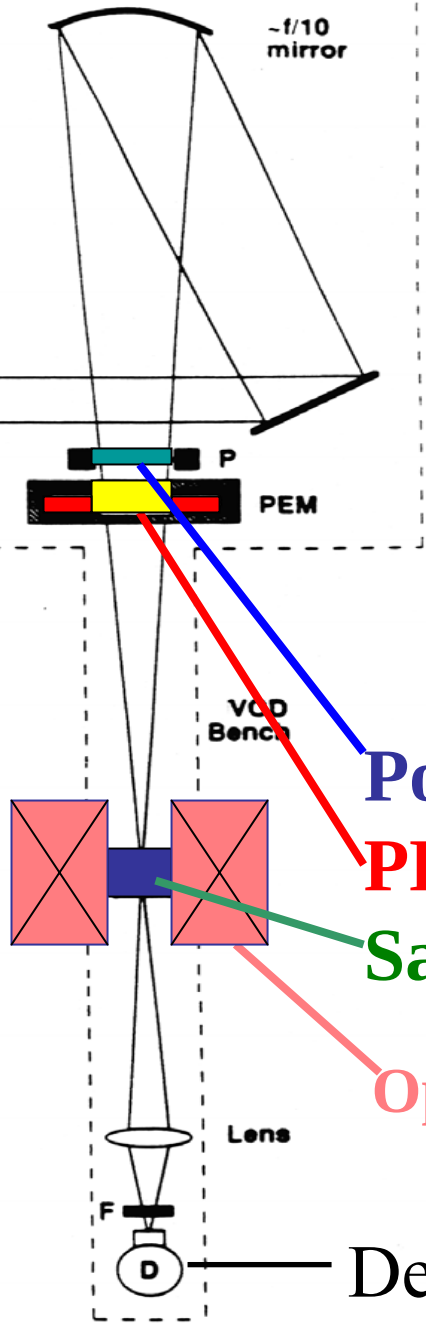


Optics and Sampling



Optics FTIR

Separate VCD Bench



UIC FT-VCD Schematic (designed for magnetic VCD ***commercial ones simpler***)

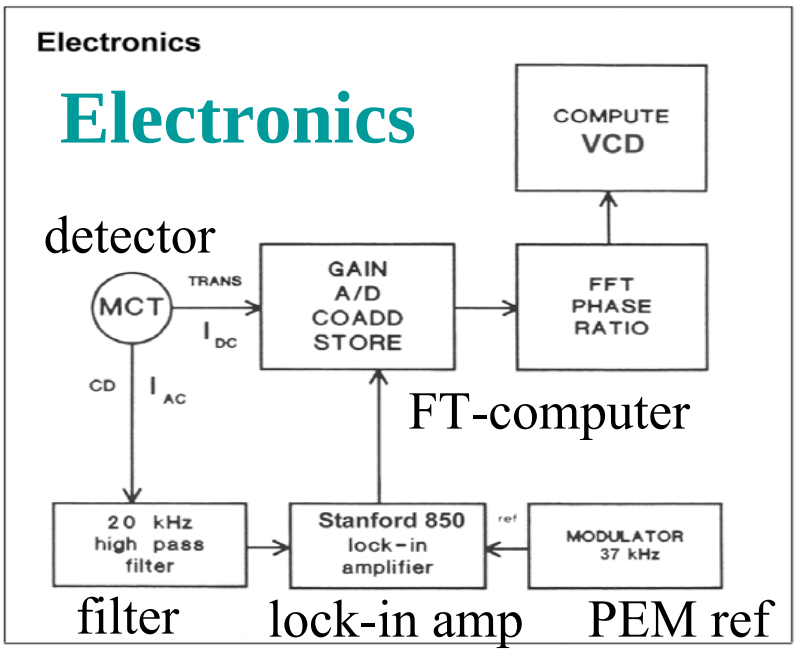
Polarizer

PEM (ZnSe)

Sample

Optional magnet

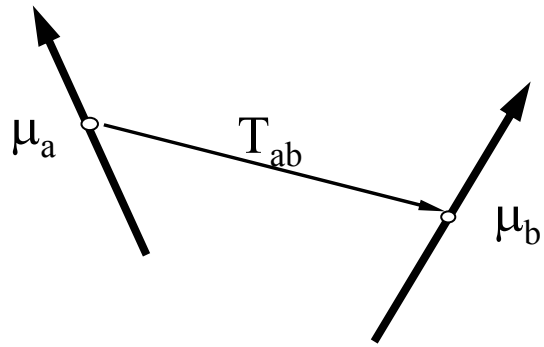
Detector (MCT)



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

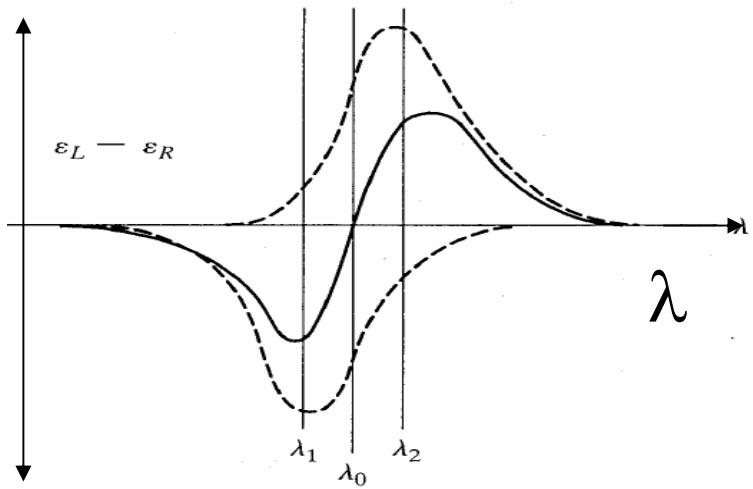
Simplest representation is *coupled oscillator*

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



$\Delta\varepsilon =$

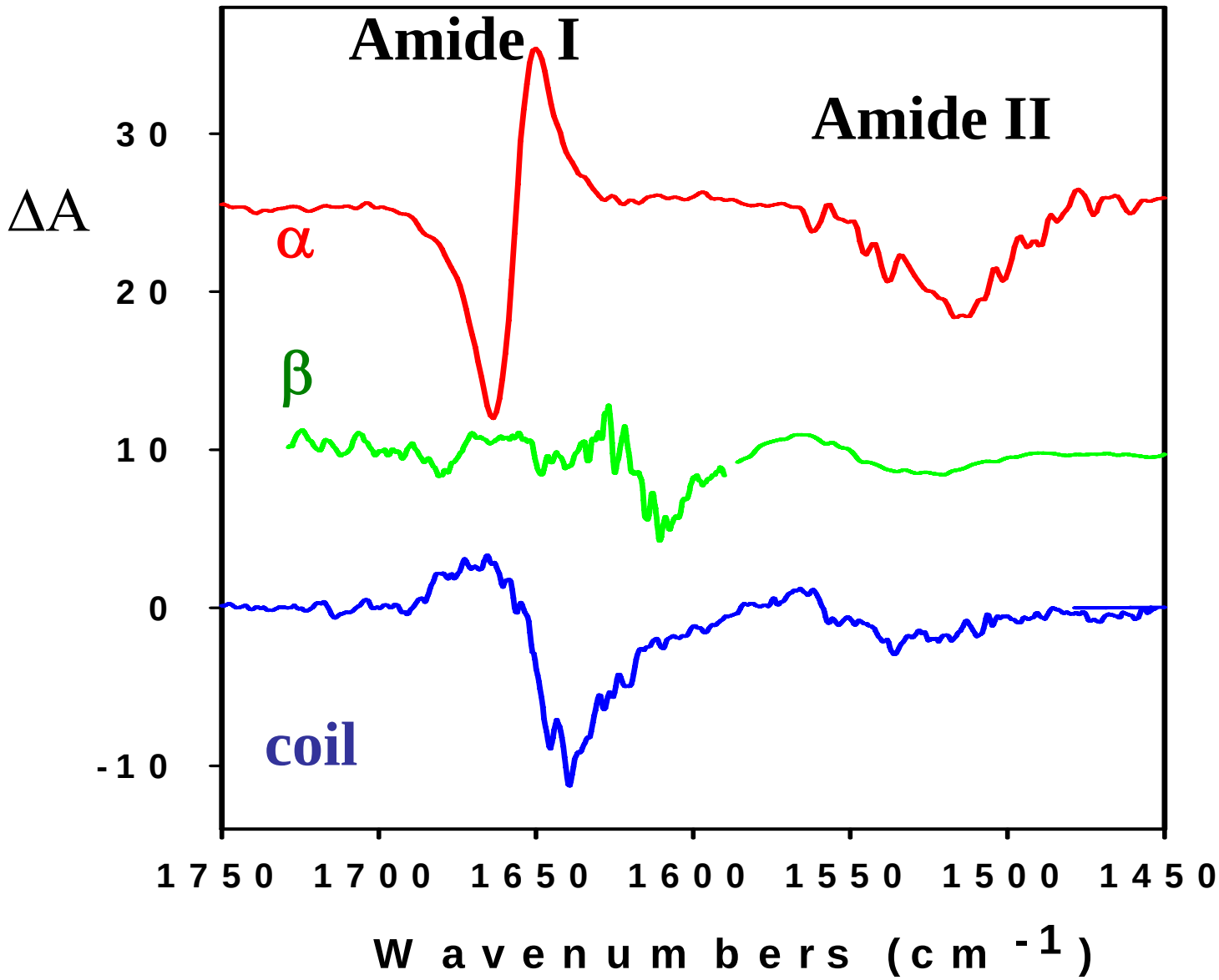
$\varepsilon_L - \varepsilon_R$



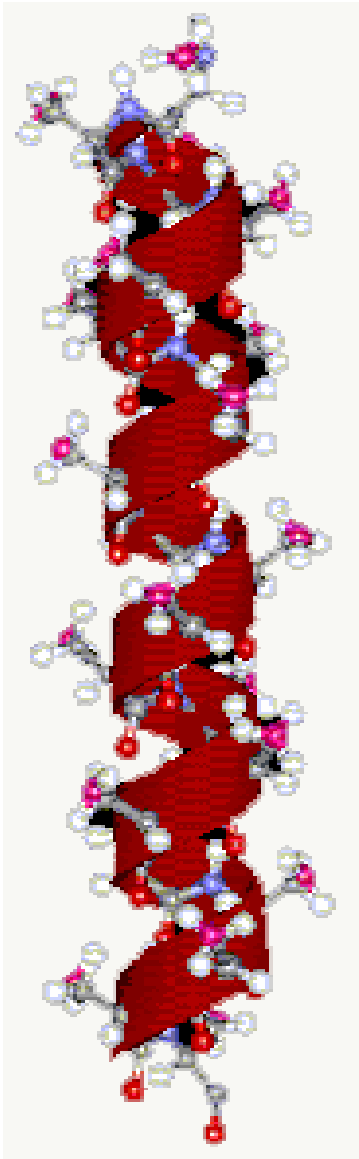
Dipole coupling results in a derivative shaped circular dichroism

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

Selected model Peptide VCD, aqueous solution



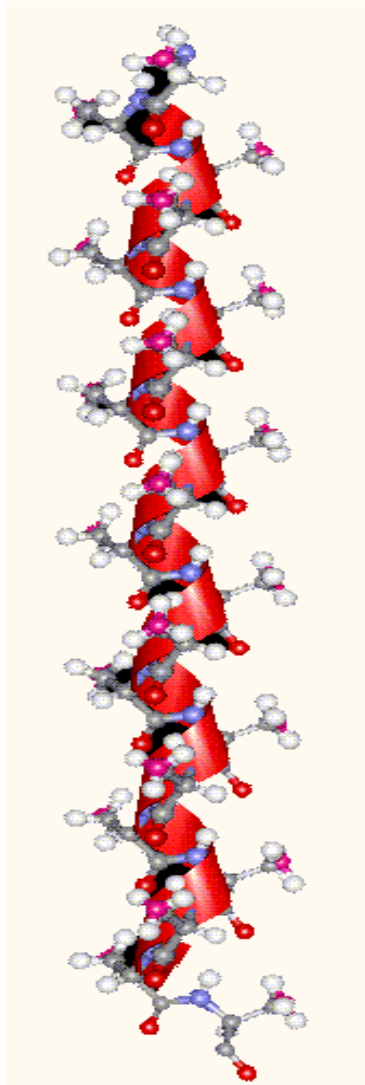
VCD Example: α- vs. the 3_{10}-Helix



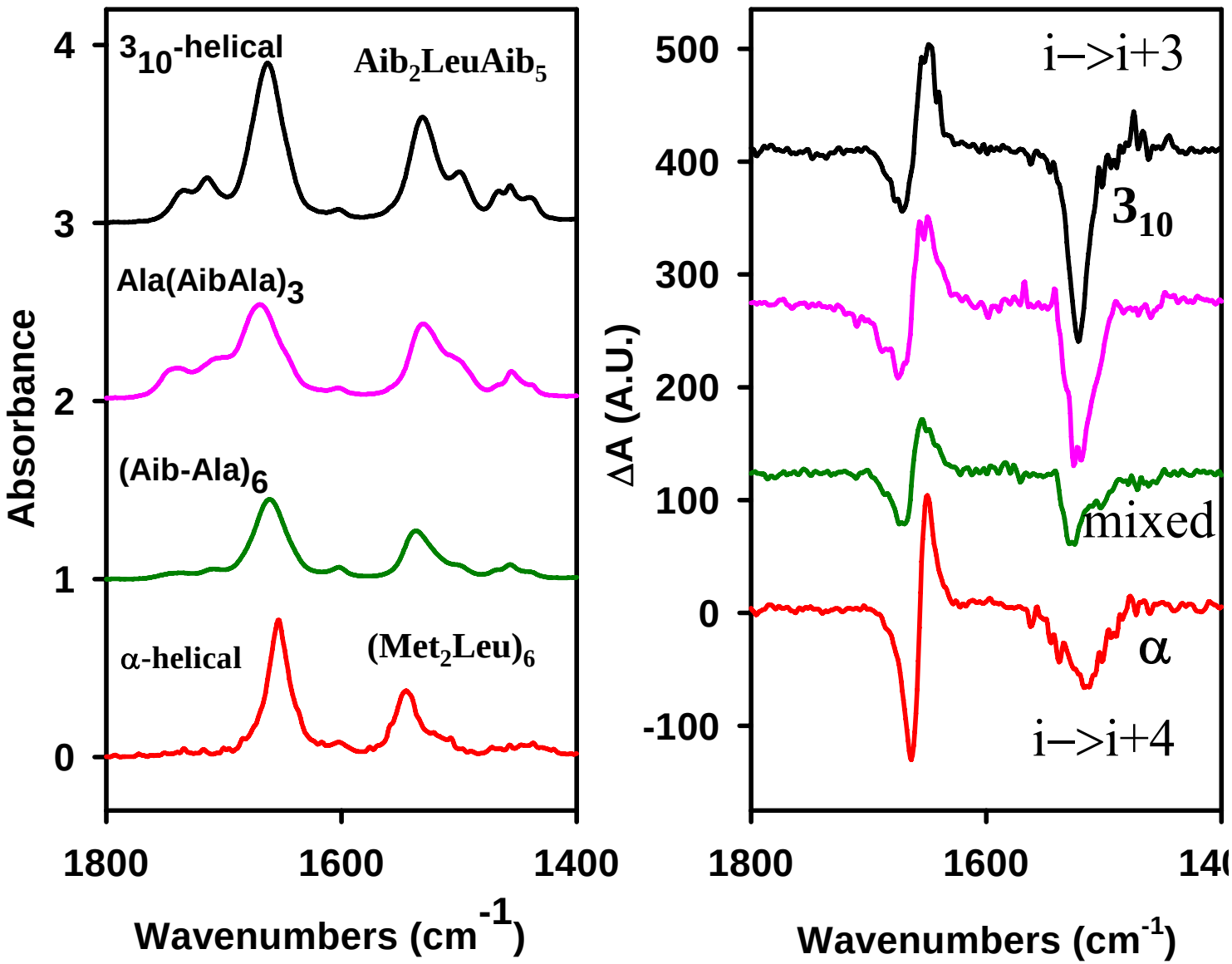
i+4 ← H-bonding → i, i+3

3.6 ← Res./Turn → 3.0

.00 ← Trans./Res (Å) → 1.50

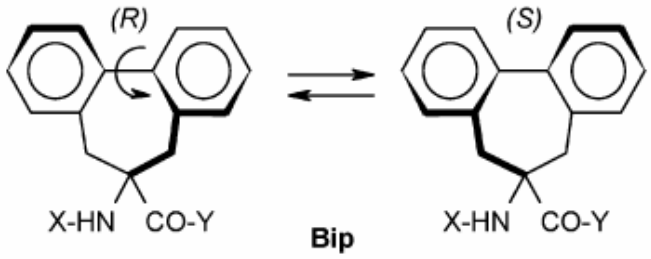


The VCD success example: 3_{10}-helix vs. α-helix



Relative shapes of multiple bands distinguish these similar helices

Silva et al. Biopolymers 2002



Biphenyl bridged residues (Bip)

CD and IR difficult to get structure

CD—all biphenyl

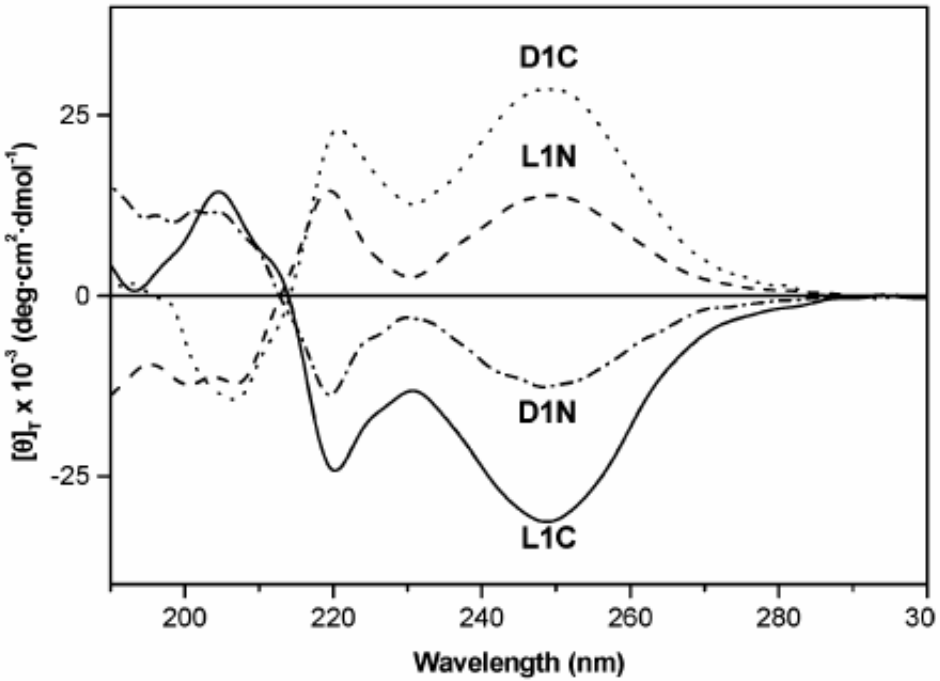


Figure 3. CD spectra of the Boc-L-Val-Bip-OMe (**L1N**), Boc-D-Val-Bip OMe (**D1N**), Boc-Bip-L-Val-OMe (**L1C**), and Boc-Bip-D-Val-OMe (**D1C** dipeptides in MeOH solution.

Amide A shows H-bond form

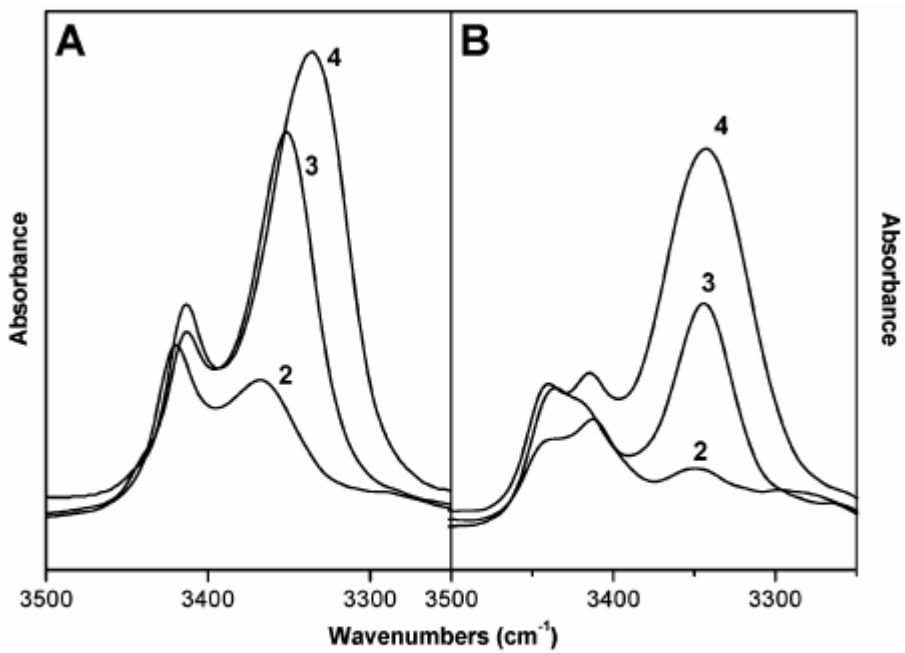
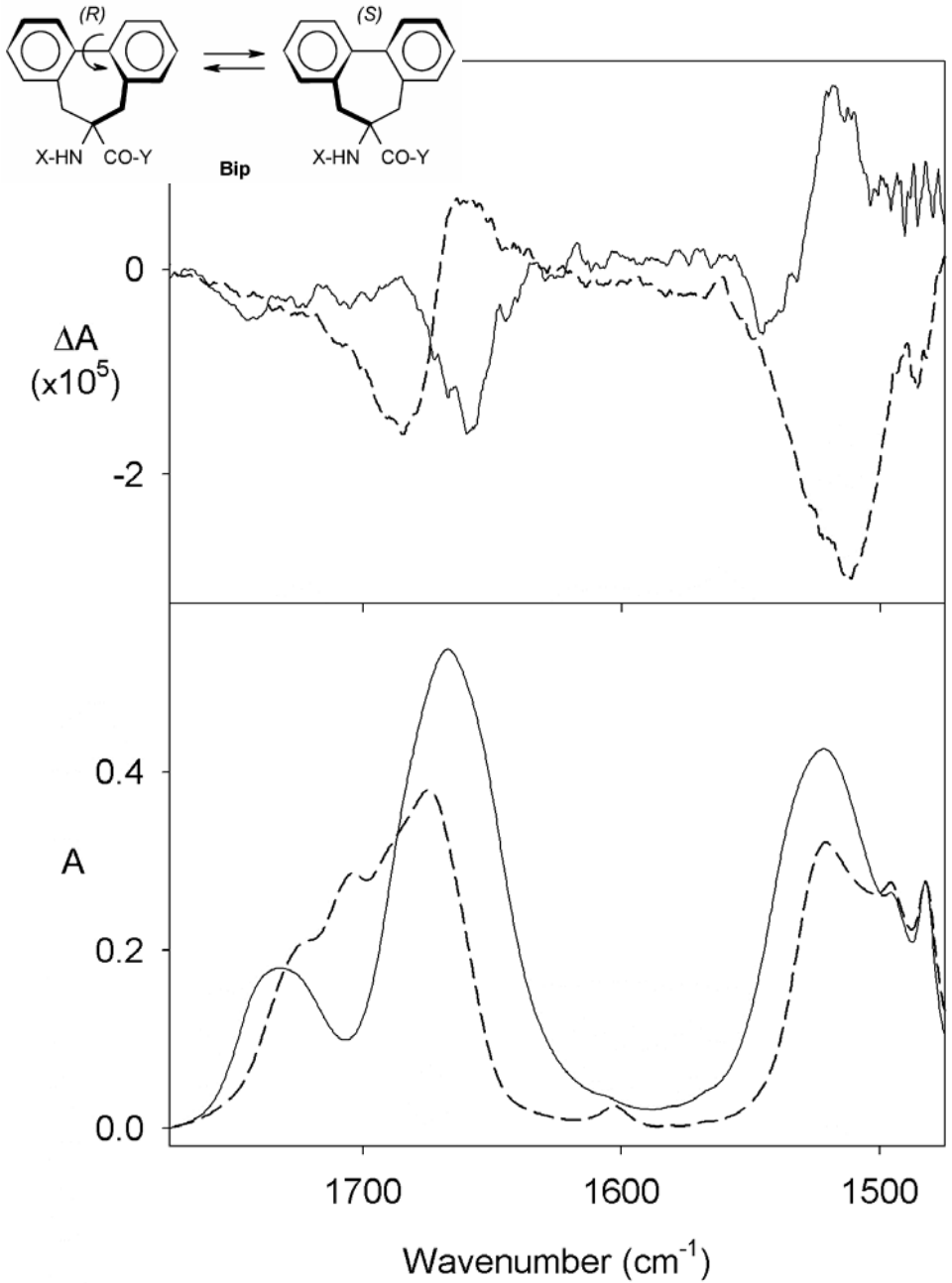


Figure 4. FT-IR absorption spectra (3500−3250 cm^{-1} region) in $CDCl_3$ solution of (A): Z-(Bip)$_n$-L-Val-OMe (n = 2−4) (**L2C**−**L4C**) and (B) Boc-L-Val-(Bip)$_n$-O*t*Bu (n = 2−4) (**L2N**−**L4N**). Peptide concentration: 1 mM.

Toniolo, co-workers JACS 2004

Biphenyl bridged residues (Bip) show inversion



Ac-$(Bip)_3$-L-Val-OMe (———)

left-handed

Boc-L-Val-$(Bip)_4$-O*t*Bu (-------)

right-handed (3_{10}-helix)

Vibrational spectrum separates aromatic and amide transitions

Figure 1 VCD (upper frame) and IR absorption (lower frame) spectra of Ac-$(Bip)_3$-L-Val-OMe (full lines) and Boc-L-Val-$(Bip)_4$-O*t*Bu (dashed lines). Spectra of Ac-$(Bip)_3$-L-Val-OMe were measured in 46/11 (v/v) $CDCl_3$/TFE-OH and Boc-L-Val-$(Bip)_4$-O*t*Bu in $CDCl_3$ solution using the cell pathlength 500 μm and peptide concentration of 9.5 and 8.6 g/L, respectively.

Toniolo, co-workers JACS 2004

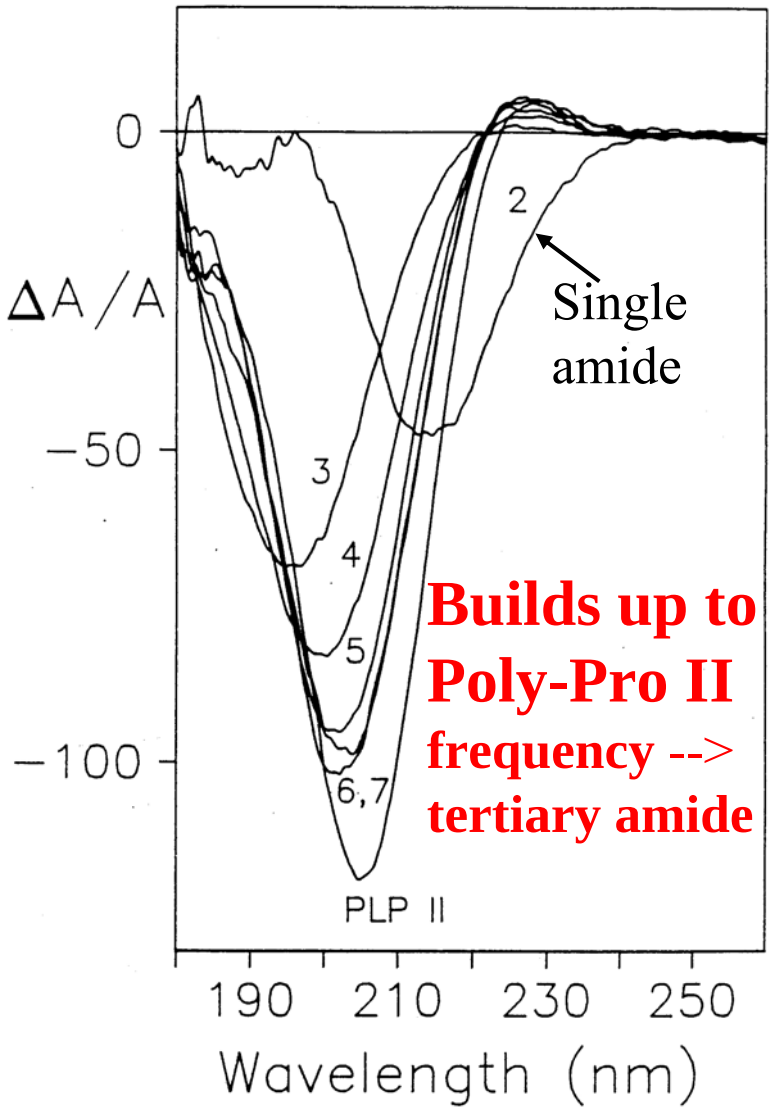
Nature of the peptide random coil form

Tiffany and Krimm in 1968 noted similarity of Proline II and poly-lysine ECD and suggested "extended coil"
Problem -- CD has local sensitivity to chiral site
--IR not very discriminating

Dukor and Keiderling 1991 with ECD, VCD, and IR showed Pro_n oligomers to have characteristic random coil spectra
Suggests -- local order, left-handed turn character
-- no long range order in random coil form

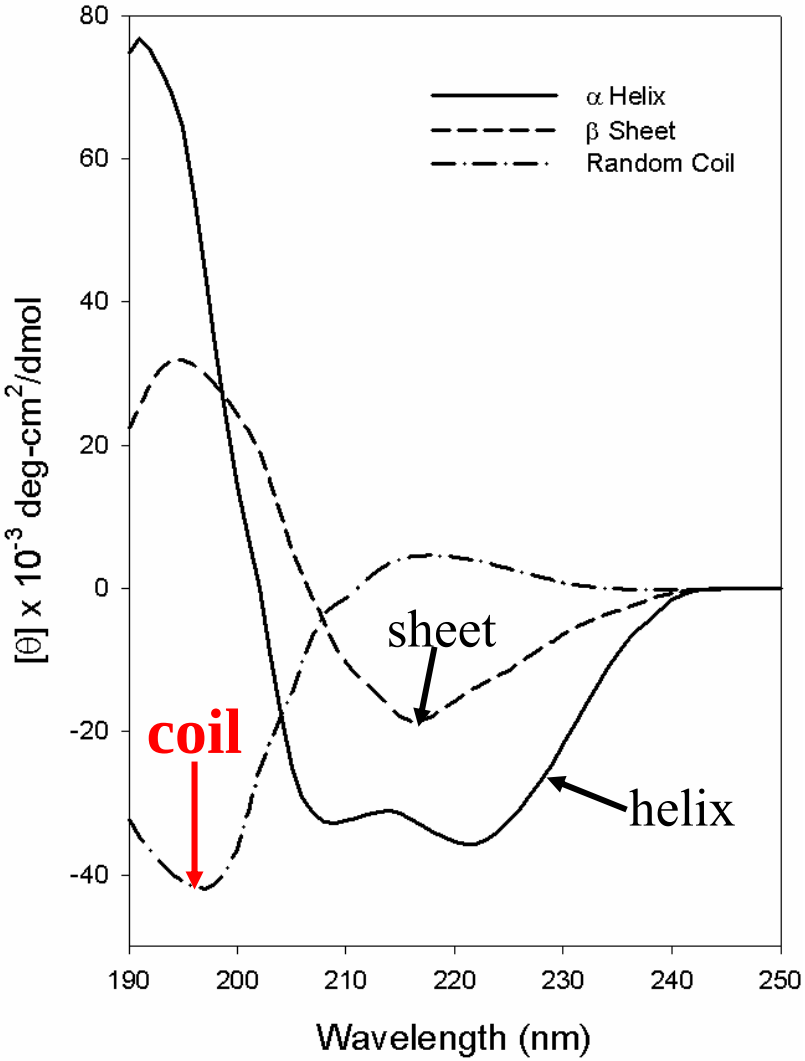
Same spectral shape found in denatured proteins, short oligopeptides, and transient forms

ECD of Pro_n oligomers



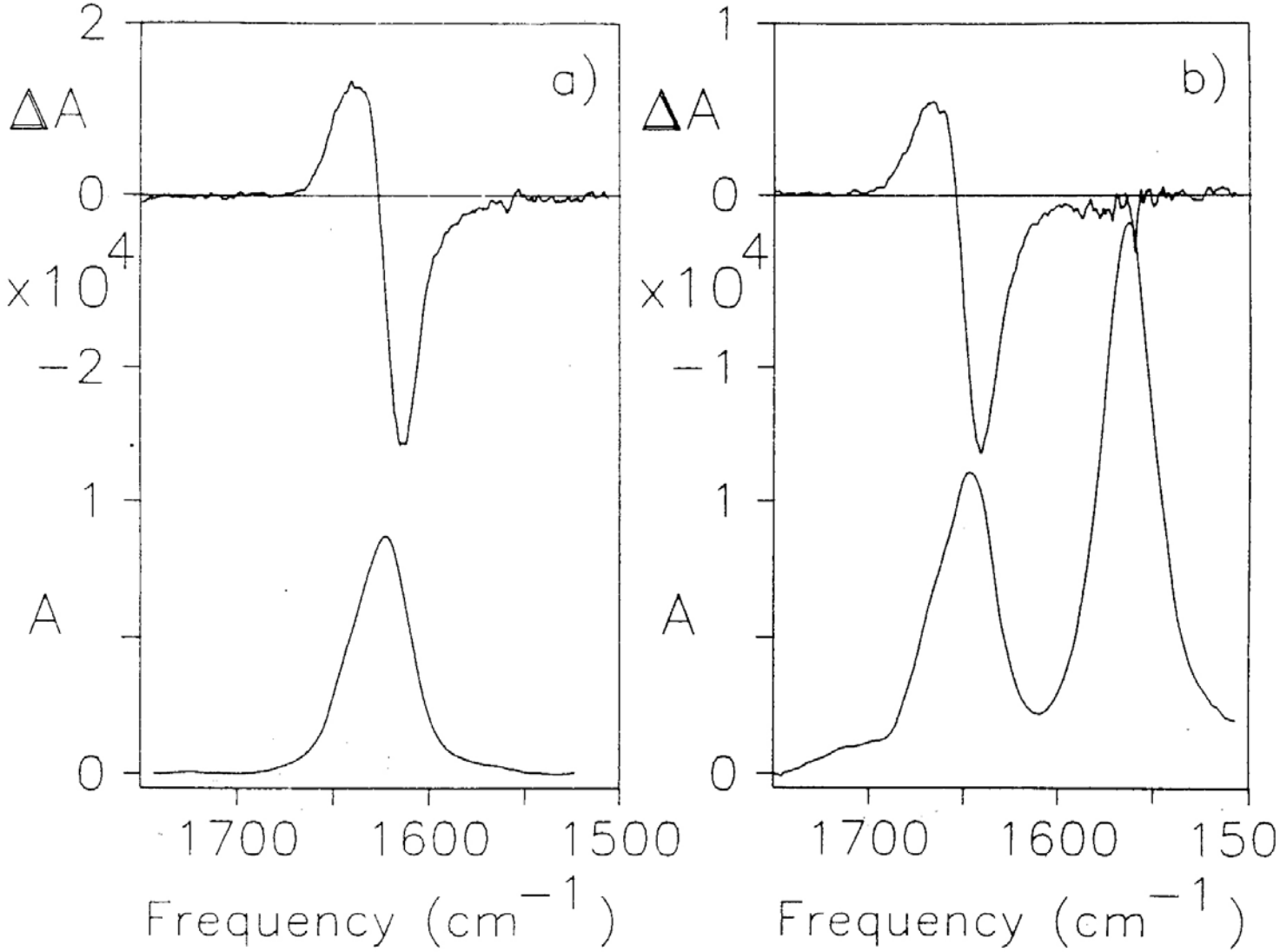
Dukor, Keiderling - Biopoly 1991

Reference: Poly(Lys) - coil, pH 7



Greenfield & Fasman 1969

Relationship to "random coil" - compare Pro_n and Glu_n

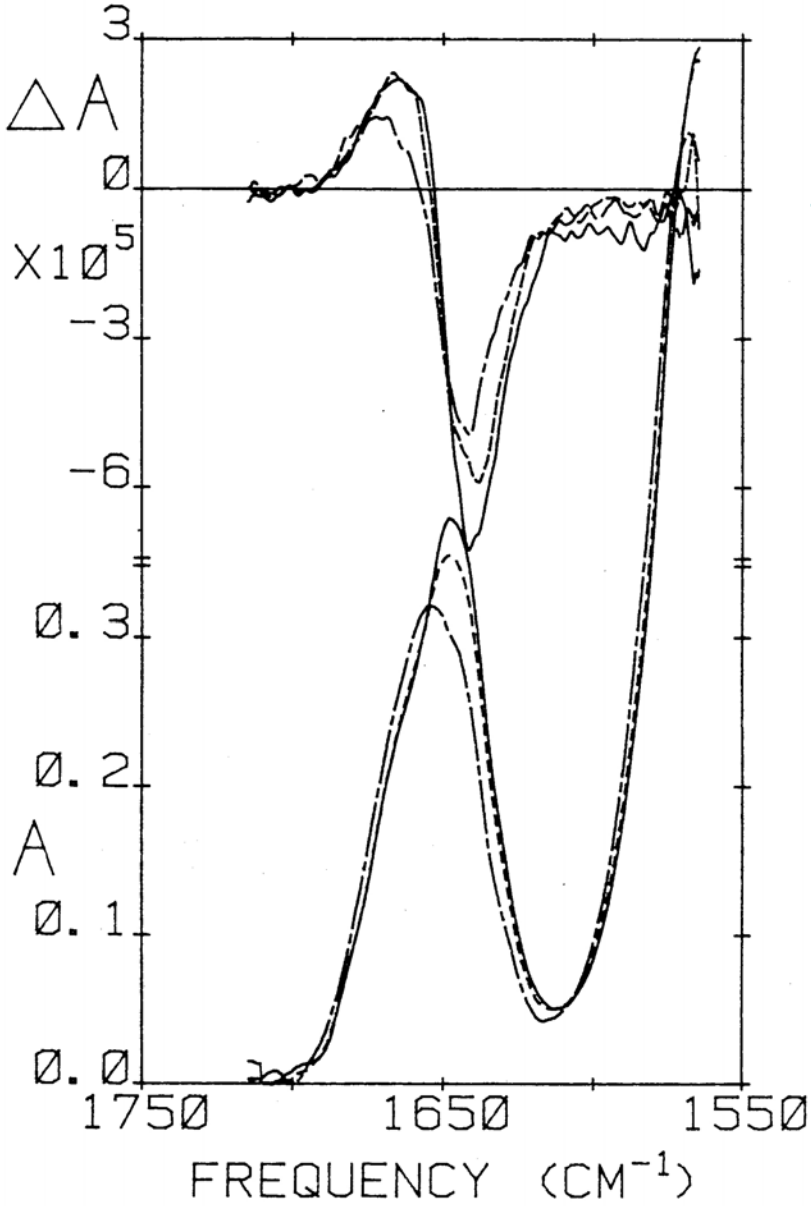


IR ~ same, VCD - same shape, half size -- partially ordered

Dukor, Keiderling - Biopoly 1991

Thermally unfolding “random coil” poly-L-Glu -IR, VCD

T = 5°C (—)
25°C (- - -)
75°C (-.-.-)

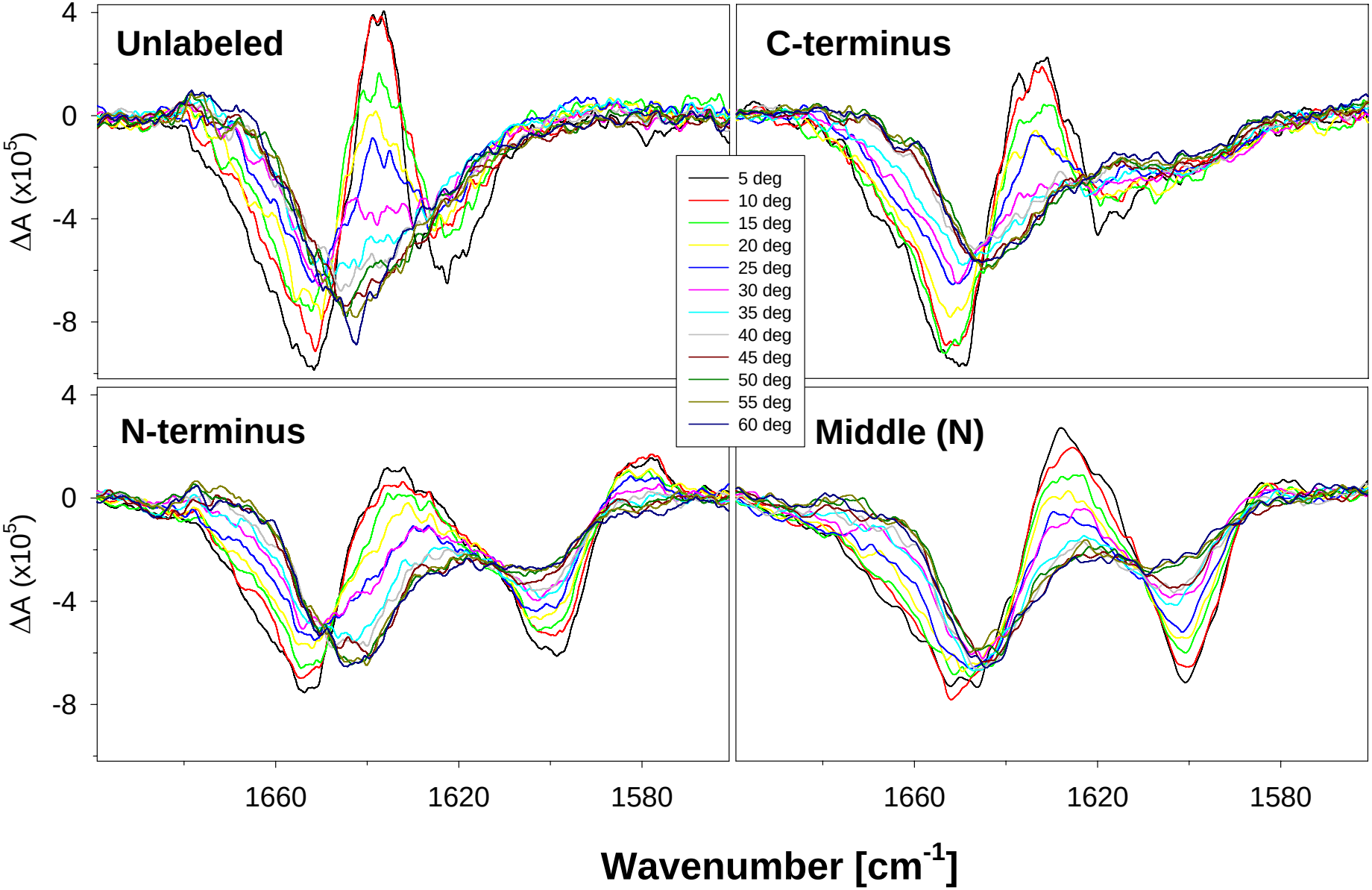


VCD loses magnitude

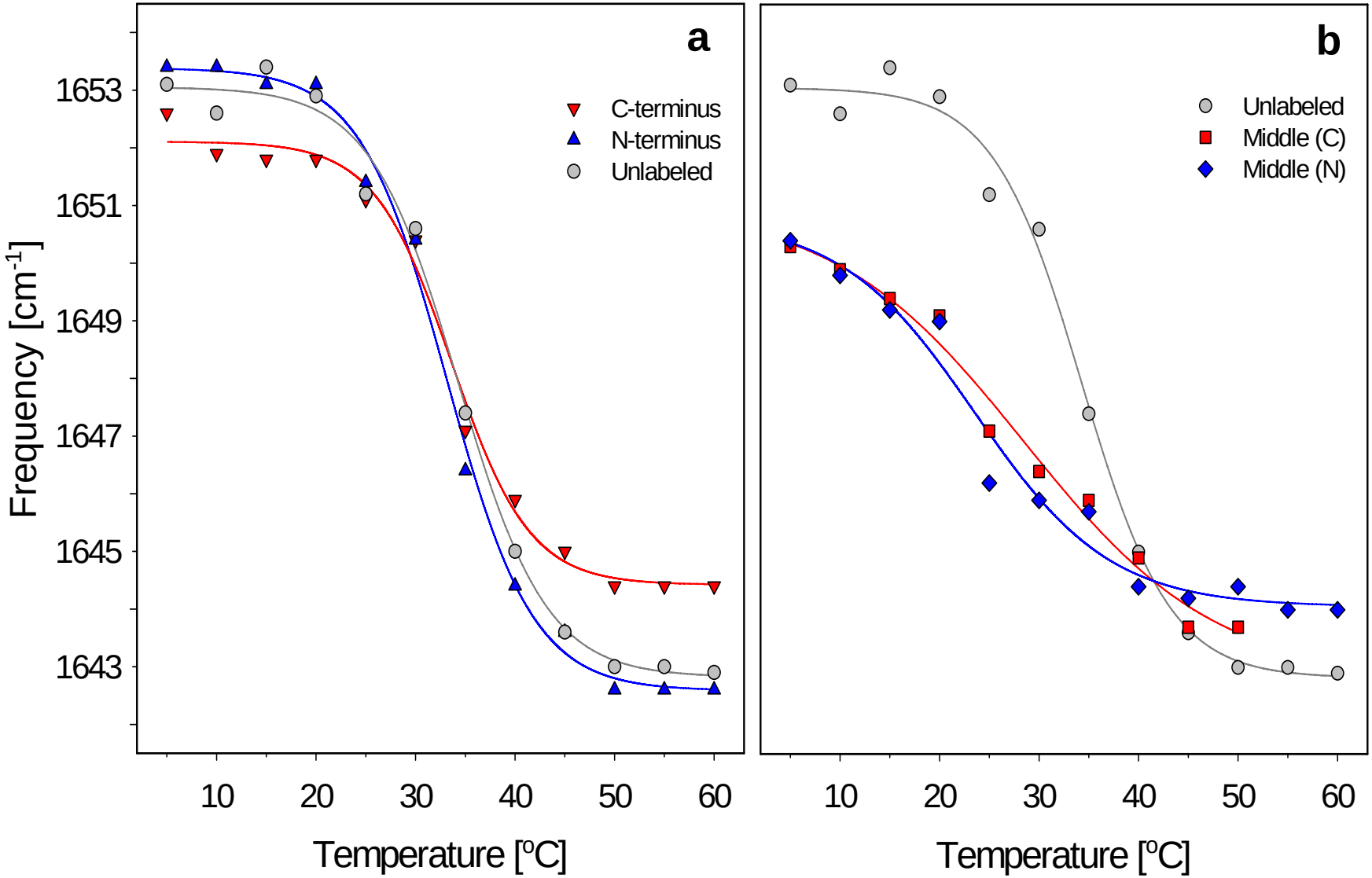
IR shifts frequency

“random coil” must have local order

Keiderling. . . Dukor, Bioorg-MedChem 1999



Temperature dependent amide I' VCD of labeled peptides characteristic of site-dependent helix-coil transition.



Frequency shift of ^{12}C amide I' VCD band minimum with temperature: a) terminal, b) middle labeled. Unlabeled added for comparison.

Relative position of isotope labels

An examination of amide coupling

Ala-rich peptides (25 mer) with a high propensity for helix formation were synthesized and purified at Mount Holyoke. ^{13}C-labels (on the amide C=O) were incorporated into the peptide as follows: (**red** refers to **labeled residues**)

Unlabel: Ac-AAAAKAAAAKAAAAKAAAAKAAAAY-NH_2

2LT: Ac-AAAAKAAAAK**AA**AAKAAAAKAAAAY-NH_2

2L1S: Ac-AAAAKAAAAK**AAA**AKAAAAKAAAAY-NH_2

2L2S: Ac-AAAAKAAAAK**AAAA**KAAAAKAAAAY-NH_2

2L3S: Ac-AAAAKAAA**AKAAA**AKAAAAKAAAAY-NH_2

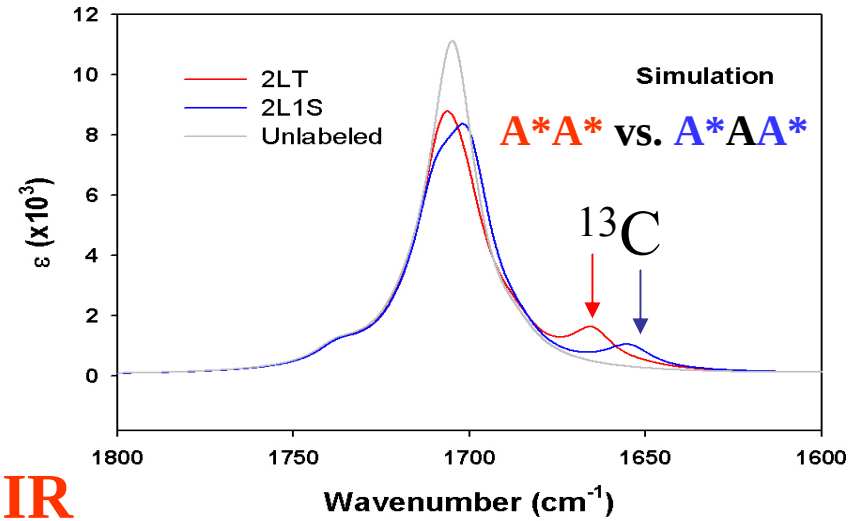
3LT: Ac-AAAAKAAAAK**AAA**AKAAAAKAAAAY-NH_2

3L1S: Ac-AAAAKAAA**AKAAA**AKAAAAKAAAAY-NH_2

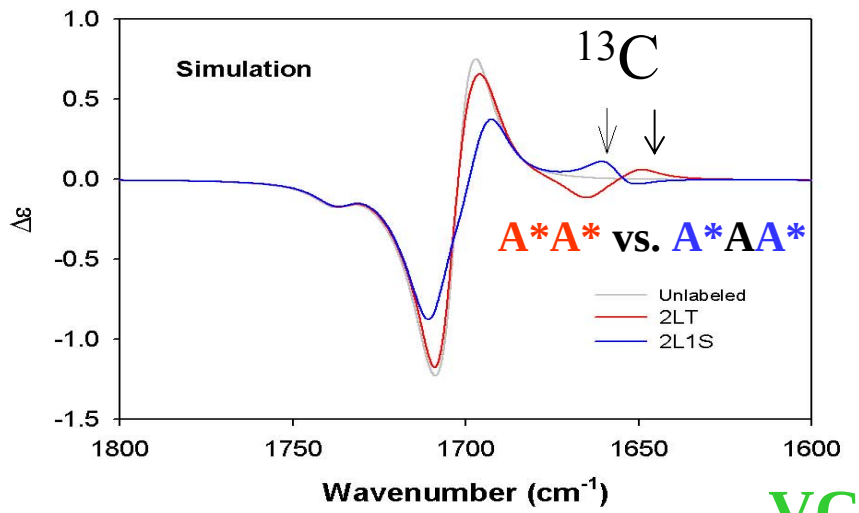
4LT: Ac-AAAAKAAAAK**AAAA**KAAAAKAAAAY-NH_2

4L1S: Ac-AAAAKA**AAAKAAA**AKAAAAKAAAAY-NH_2

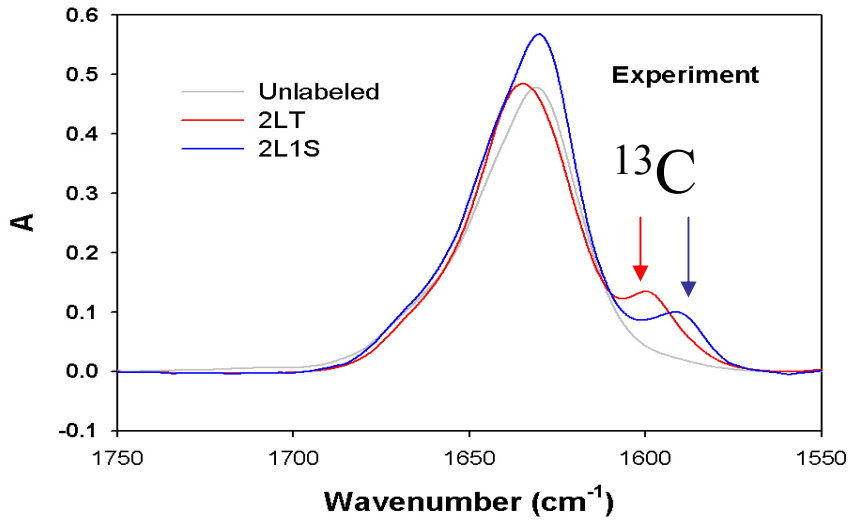
Isotopic labeling-- experiment and theory

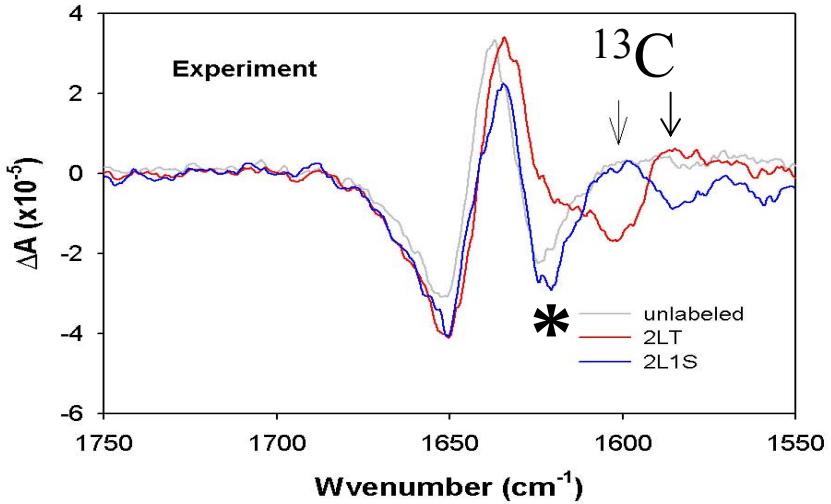


IR



VCD





Two sequential labels have higher IR freq. due to coupling (intensity in high ν mode),
VCD : sequential (2LT) - same sign ^{12}C and ^{13}C, but opposite sign if separated (2L1S)
* since exp. in D_2O a (-)VCD band develops the amide I, not modeled without solvent

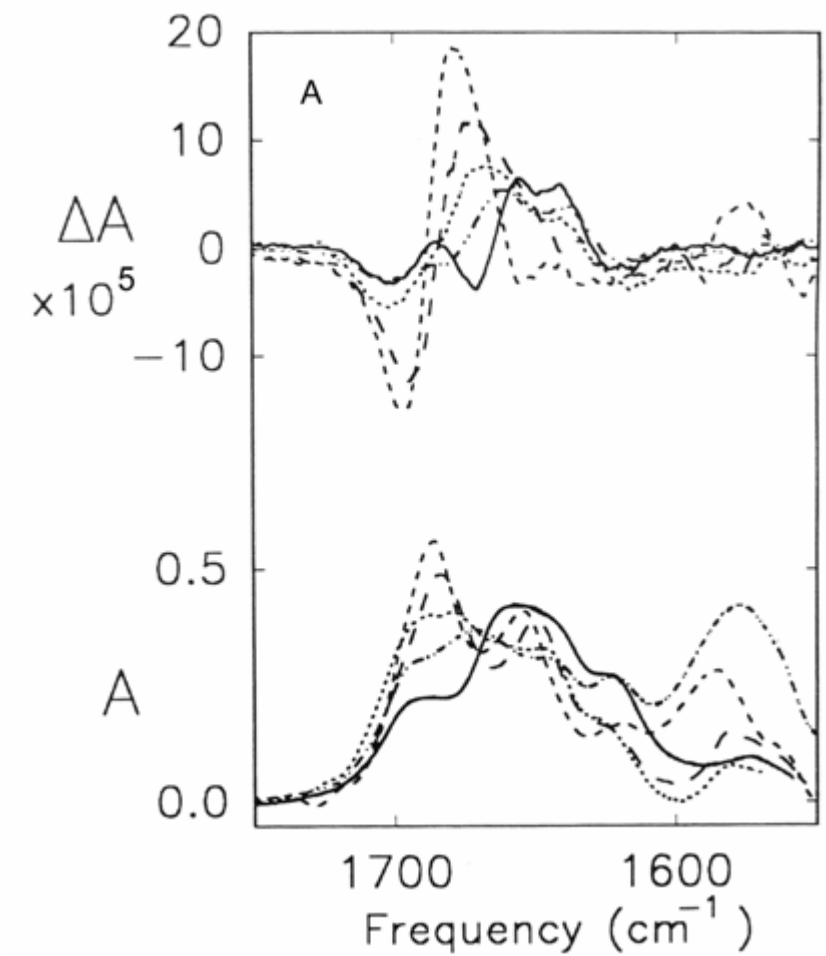
Nucleic Acid VCD

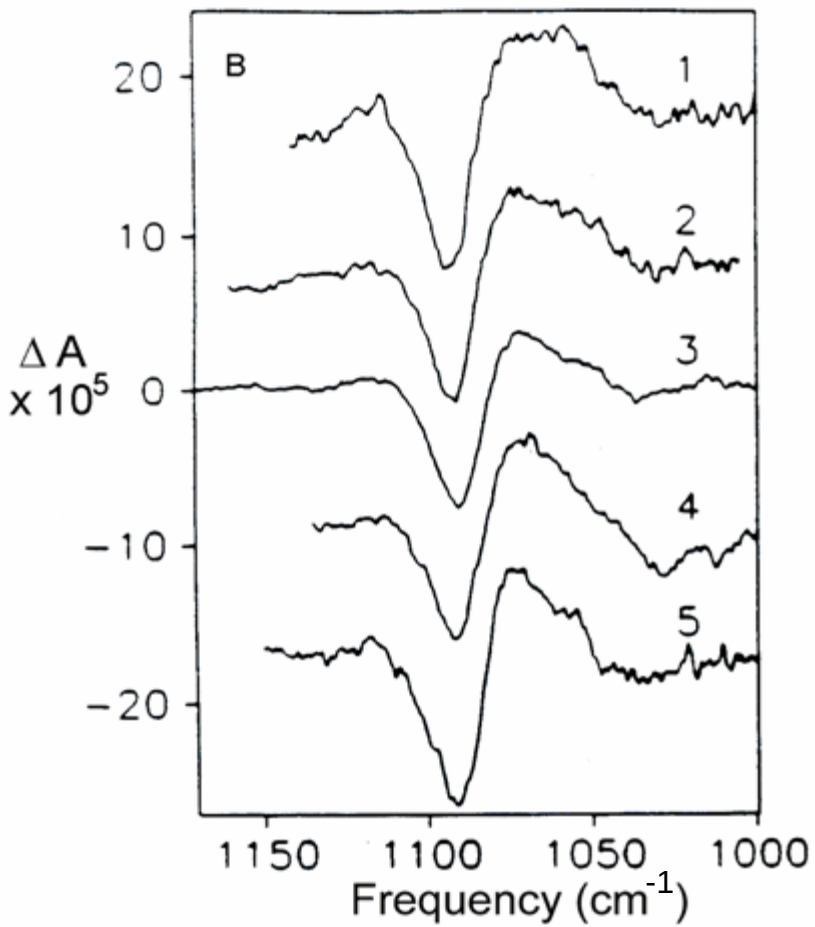
- Wieser and co-workers (Calgary) have made much progress with model systems, including metal interactions and drug binding
- Here give examples of basic spectral response

VCD of DNA, vary A-T to G-C ratio

base deformations

sym PO_2^- stretches

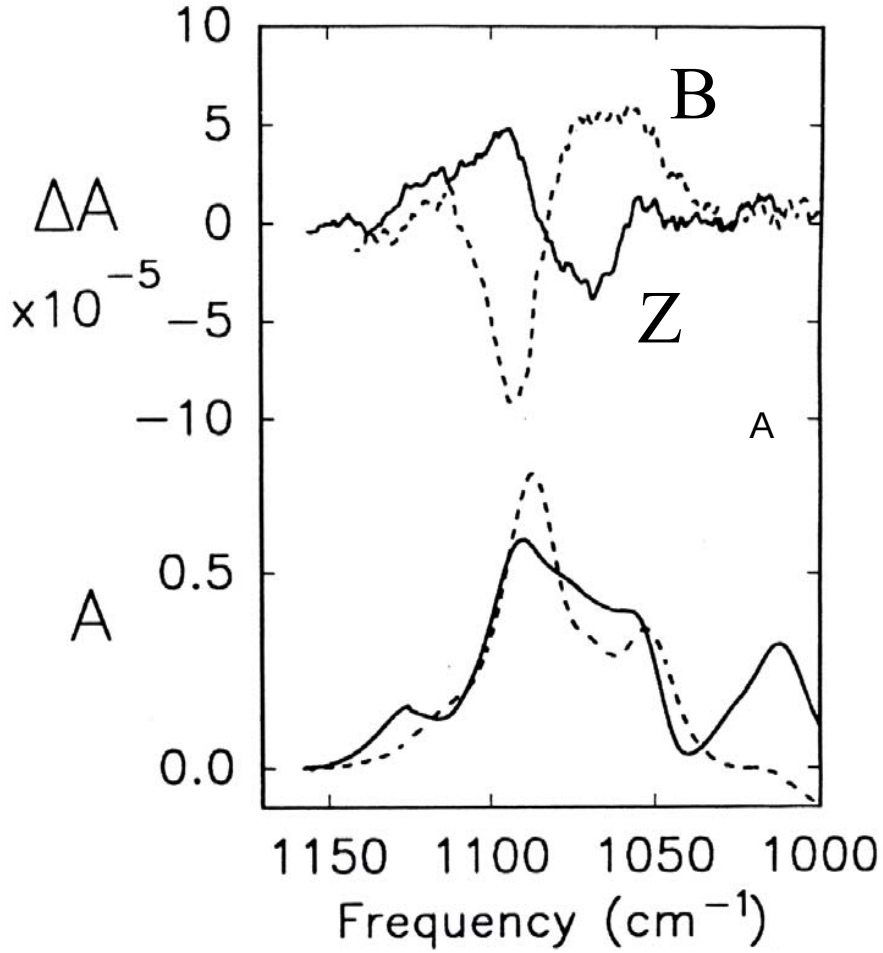




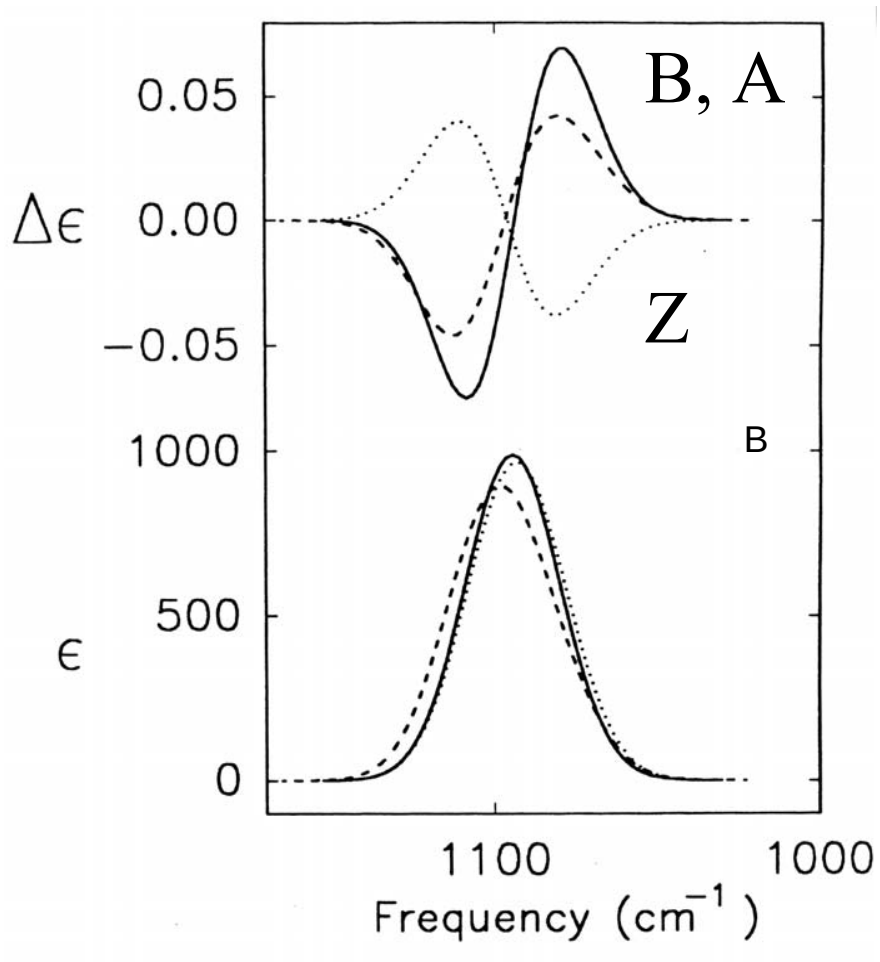
big variation

little effect

DNA VCD of PO_2^- modes in B- to Z-form transition

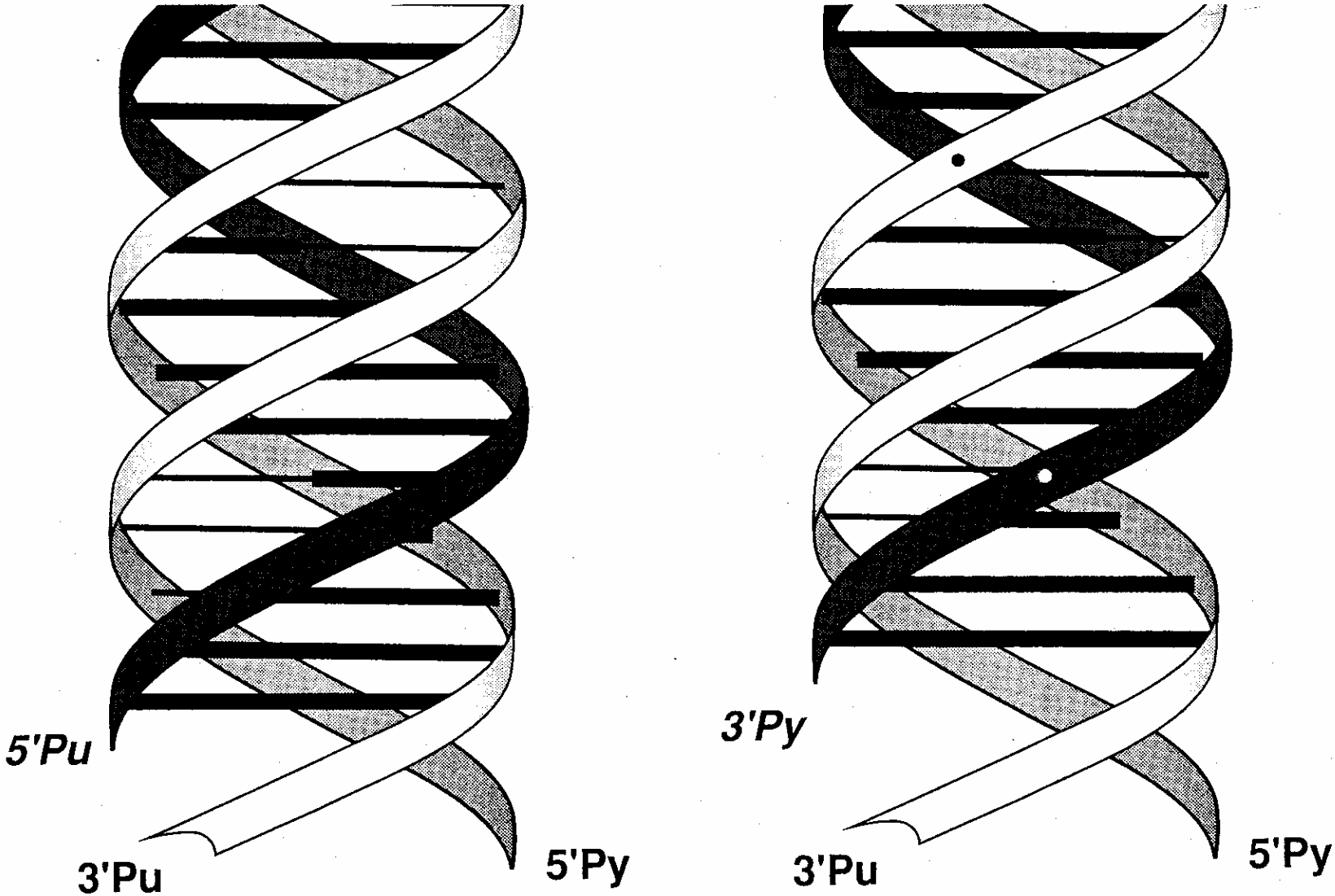


Experimental

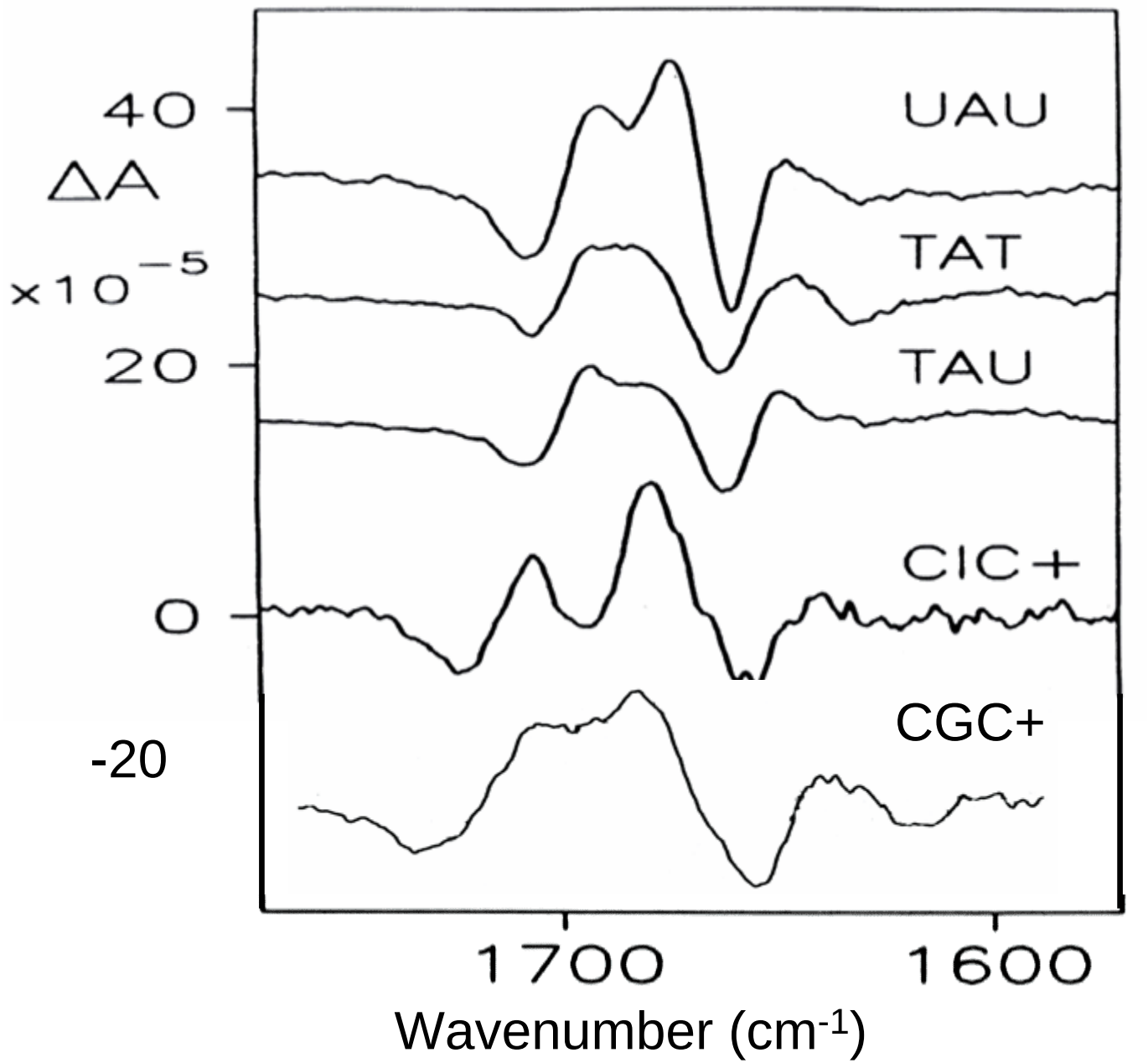


Theoretical

Triplex DNA, RNA form by adding third strand to major groove with Hoogsteen base pairing



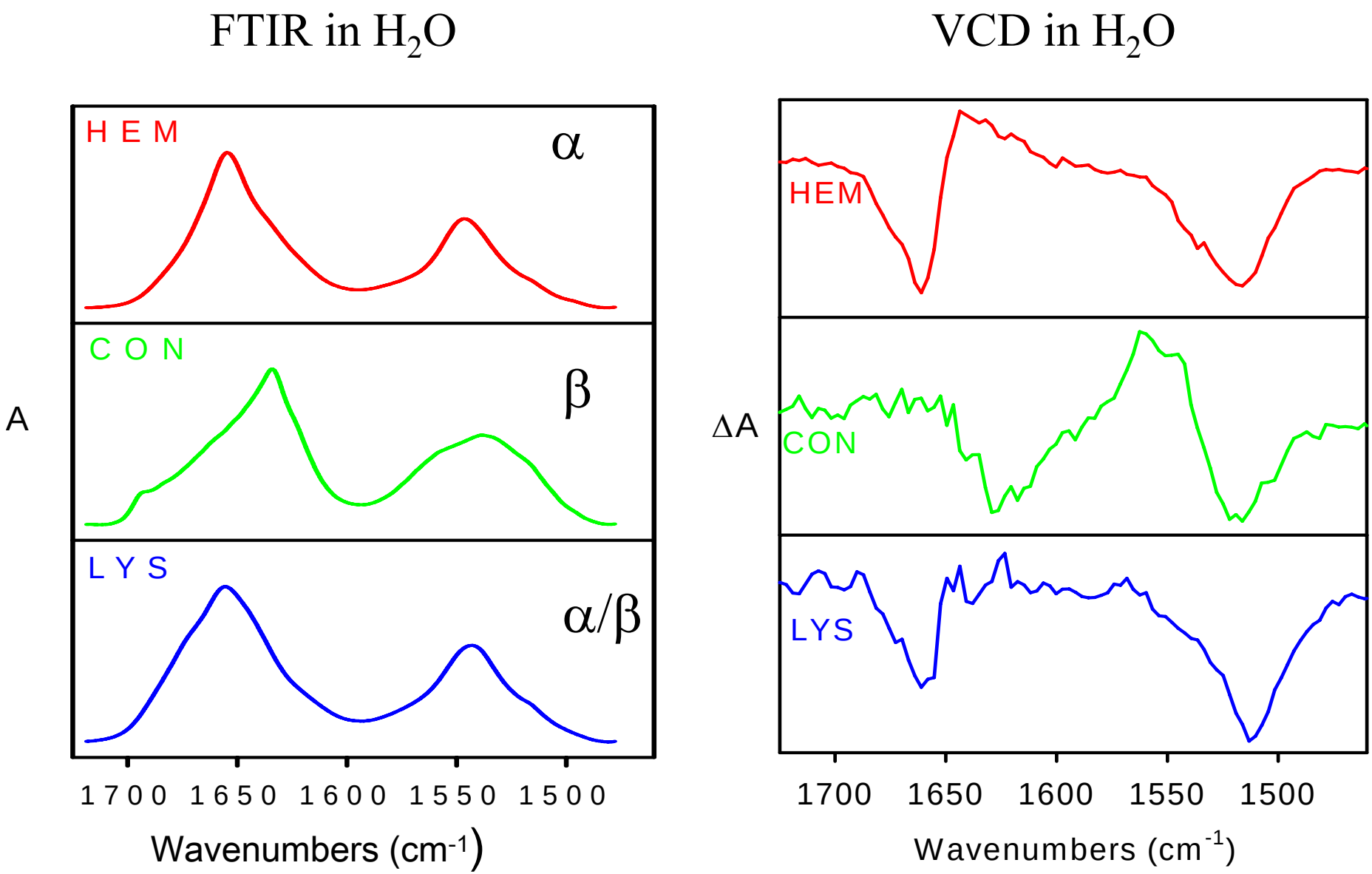
VCD of Triplex formation—base modes



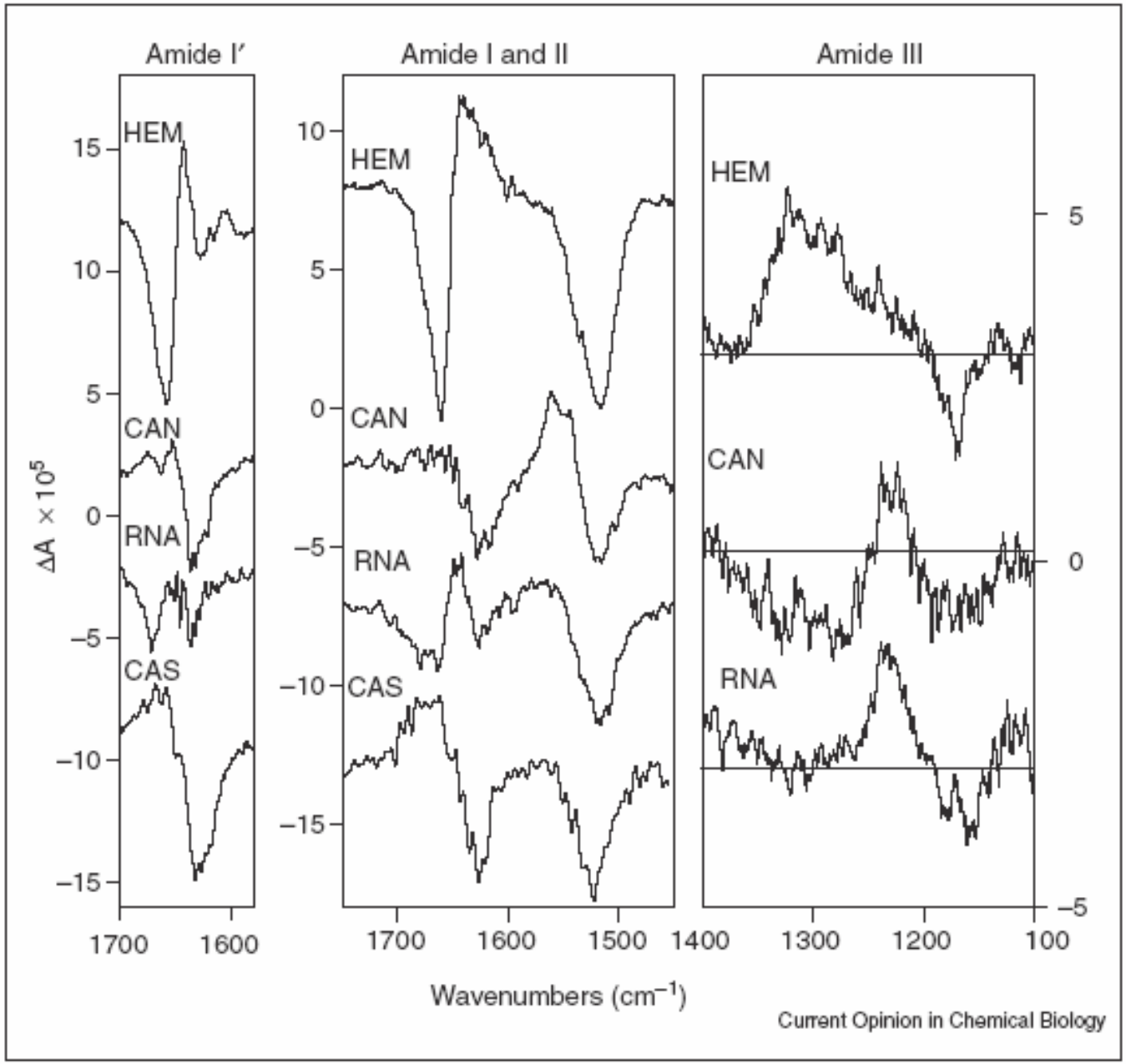
Protein VCD

- Protein CD has been used to develop secondary stucture algorithms (Pancoska et al.) and to follow folding and unfolding processes.
- Due to complexity of the structue and S/N limitations, more quantiative work has been done with peptides

Comparison of Protein VCD and IR



VCD of amide I', I+II an III regions in selected proteins



High helix

High sheet

Mixed

CAS-unstructured

VCD Example: α-Lactalbumin and Lysozyme

- Homologous proteins
- Similar crystal structures
- Lysozyme VCD spectra is *not the same* as that of α-Lac
- α-Lac stabilize by Ca^{+2} needs to bind a co-protein, so flexible