Optical Spectroscopic Applications in Peptide and Protein structure and folding. Systems of increasing complexity

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1. **Introduction**--Peptide secondary structure & spectra – observations and methods
2. Theoretical modelling of spectra (Petr Bour, J. Kubleka, J. Kapitan)
3. **Isotope** substitution –**helix** background (RongHuang/Gangani Silva/Jan Kubelka/Heng Chi /Ahmed Lakhani/Anjan Roy/Sean Decatur/Claudio Toniolo/Jim Cheeseman)
4. **β-hairpin** data & simulations (Ling Wu/R.Huang /A.Roy /J.Kubelka/ P.Bour /V. Setnicka)
5. **Dynamics, T-jump results (**Karin Hauser, Carsten Kretjschi/Alex Popp**)**
6. **Interacting systems** – aggregation, membrane binding – not enough time (H.Chi/J.Kubleka/W. Welch/G.Zhang/A.Lakhani/ Wojciech Dzwolak)

Olomouc
19-Sept-2016

Motivation: Structural Biology

Principle:

Understanding molecular structure of complex biological systems can lead to control of function

GOALS (Protein/peptide):

- Define Protein structure--conformational
- Determine folding mechanisms.
- Use tools to monitor structural change during biological process
- Relate peptide folding to protein mechanisms

Peptide/Protein Primary and Secondary Structure

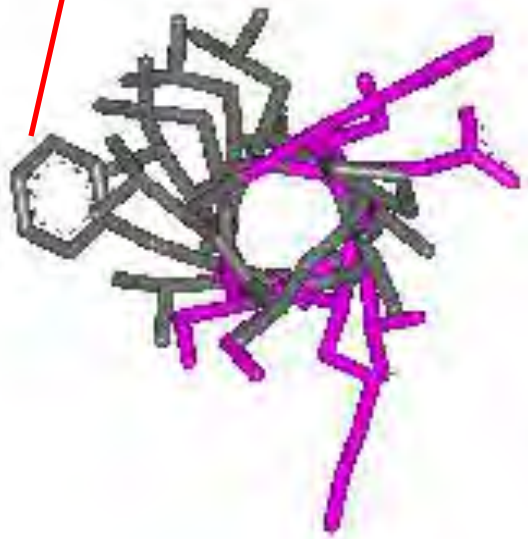
Proteins are polymers of amino acids

Pro-Ala-Val-His-Ala-Ser-Leu-Asp-Lys-Phe-Leu-Ala-Ser-
Val-Ser-Thr-Val-Leu

Primary structure—sequence of residues (amino acids) in chain

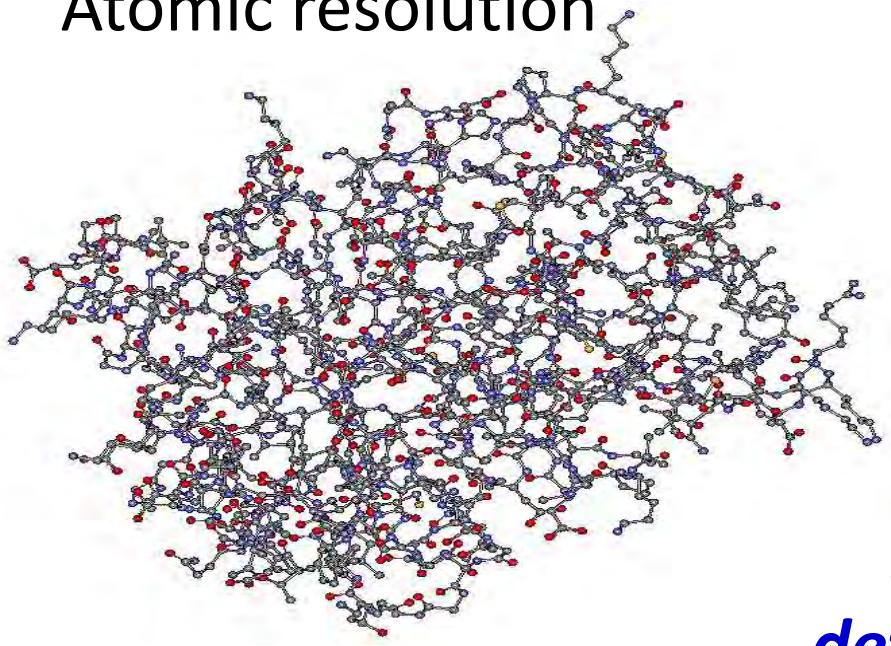
Secondary Structure — stereochemical relation of residues in chain

Helix – side-on

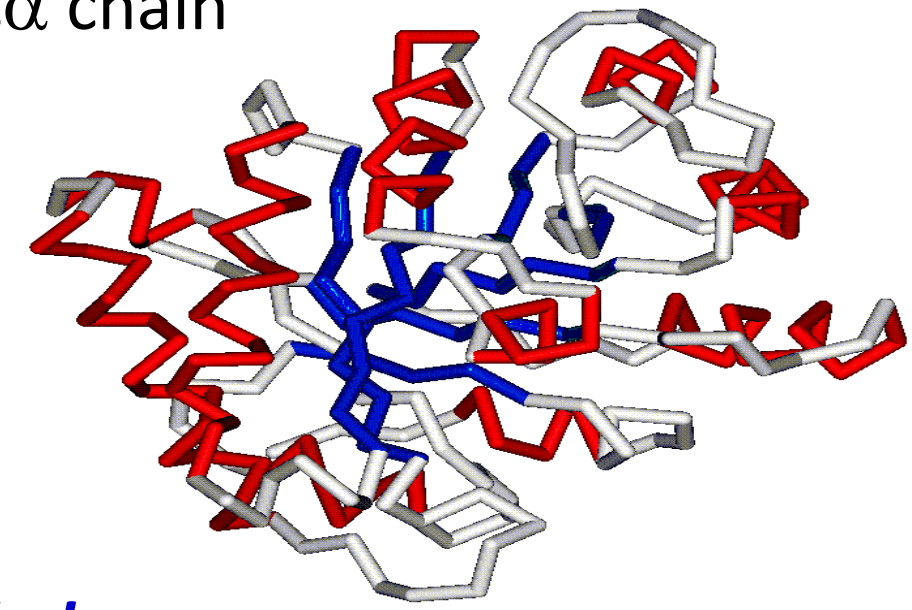


Helix – end-on

Atomic resolution

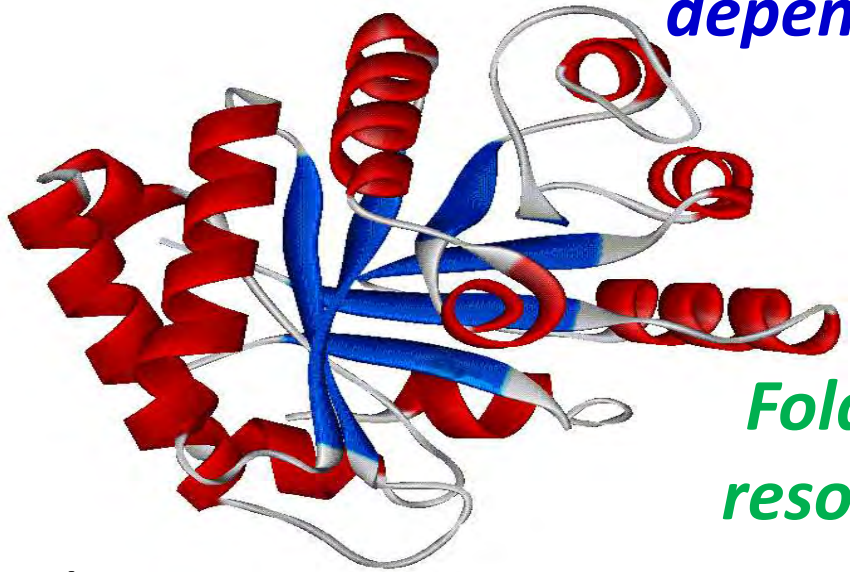


Cα chain



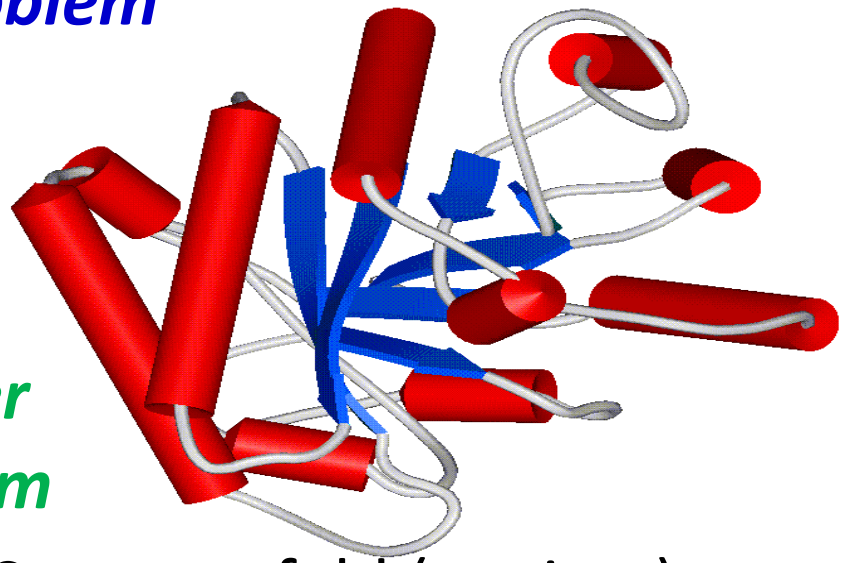
Structural Biology

Level of structure determination needed depends on the problem



Secondary structure

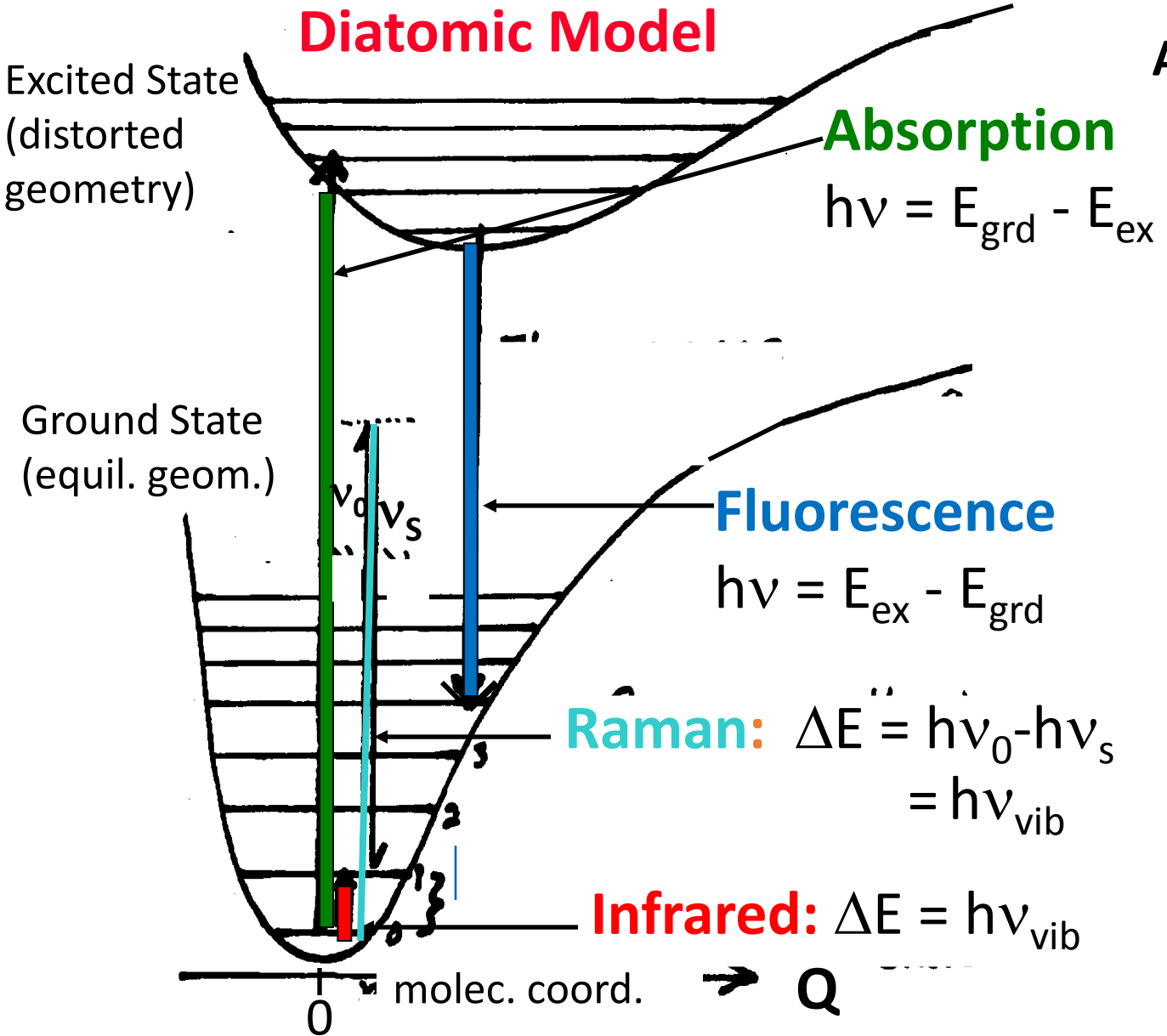
Folding is a lower resolution problem



Segment fold (tertiary)

Lower resolution Optical Spectroscopy - Processes / Methods

UV/ Fluorescence/ IR/ Raman/ Circular Dichroism



Analytical Methods

UV-vis absorp. & Fluorescence.
move e^- (change electronic state)
high freq., intense

CD – circ. polarized absorption, UV or IR

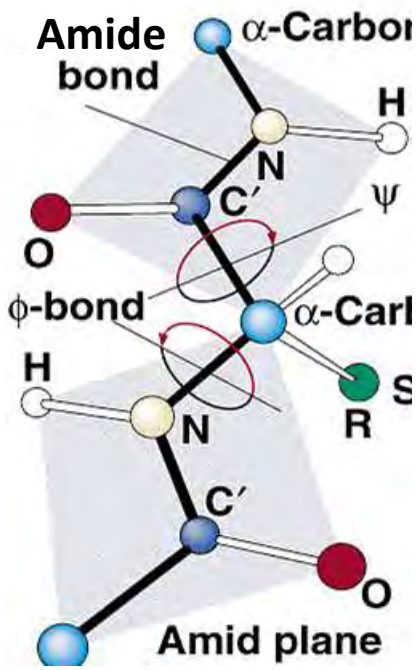
Raman –nuclei, inelastic scatter
very low intensity

IR – move nuclei
low freq. & inten.

Physical method of conformation detection must sense secondary structure — e.g. *couple amides*

IR/Raman— ***coupling comparable to band width***,
intensity maximum is characteristic of structure – **frequency basis**

Circular dichroism --dipole and through-bond ***chiral coupling*** of local modes (excitations) → *circularly polarized transitions*,
$\Delta A = A_L - A_R$ - Develops characteristic **band shapes** (***intensity basis***)



Theoretically try to understand spectra/structure relation

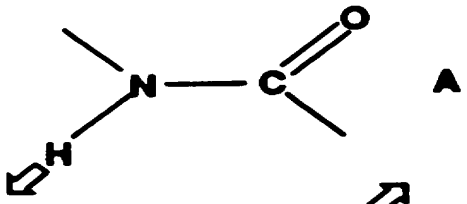
IR ~ D = $\mu \bullet \mu \sim |\delta\mu/\delta Q|^2$ (Raman ~ $|d\alpha/dQ|^2$) – ***square (+)***

ECD, VCD ~ $R = Im(\mu \bullet m)$ -- ***cross term → +/- signs***

} *calculations, for analysis!*

Computable with *ab initio* QM techniques, ECD needs excited states
IR & VCD relatively easy, Raman more basis set sensitive

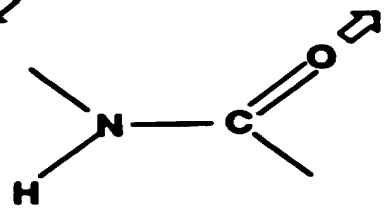
Characteristic Amide Vibrations- for structure sensing



~3300 cm^{-1}

A – often obscured by solvent

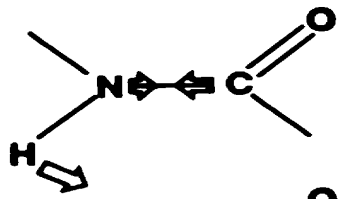
IR focus – polymer/amide



~1650 cm^{-1}

I - Most useful;
IR intense, less interference (by solvent, other modes,etc)
Less mix (with other modes)

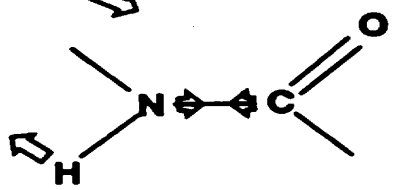
To first order, no magnetic moment for ground state vibrations, *m* = 0. i.e. Coupling must dominate VCD, effect largest for like oscillators



1500-50 cm^{-1}

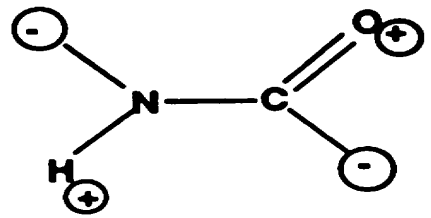
mix

II - IR intense,*broad*



1300-1250 cm^{-1}

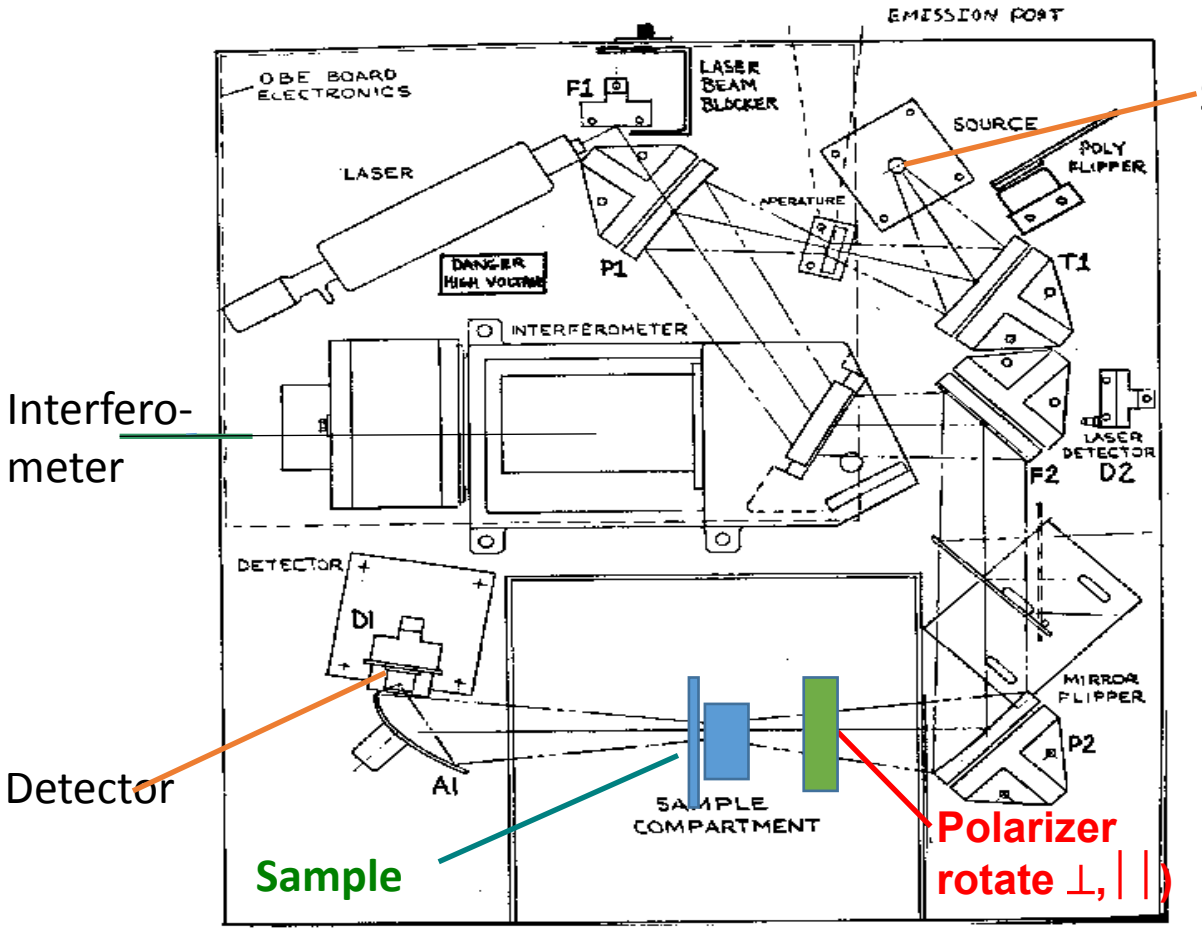
III - Raman Intense,*dispersed*

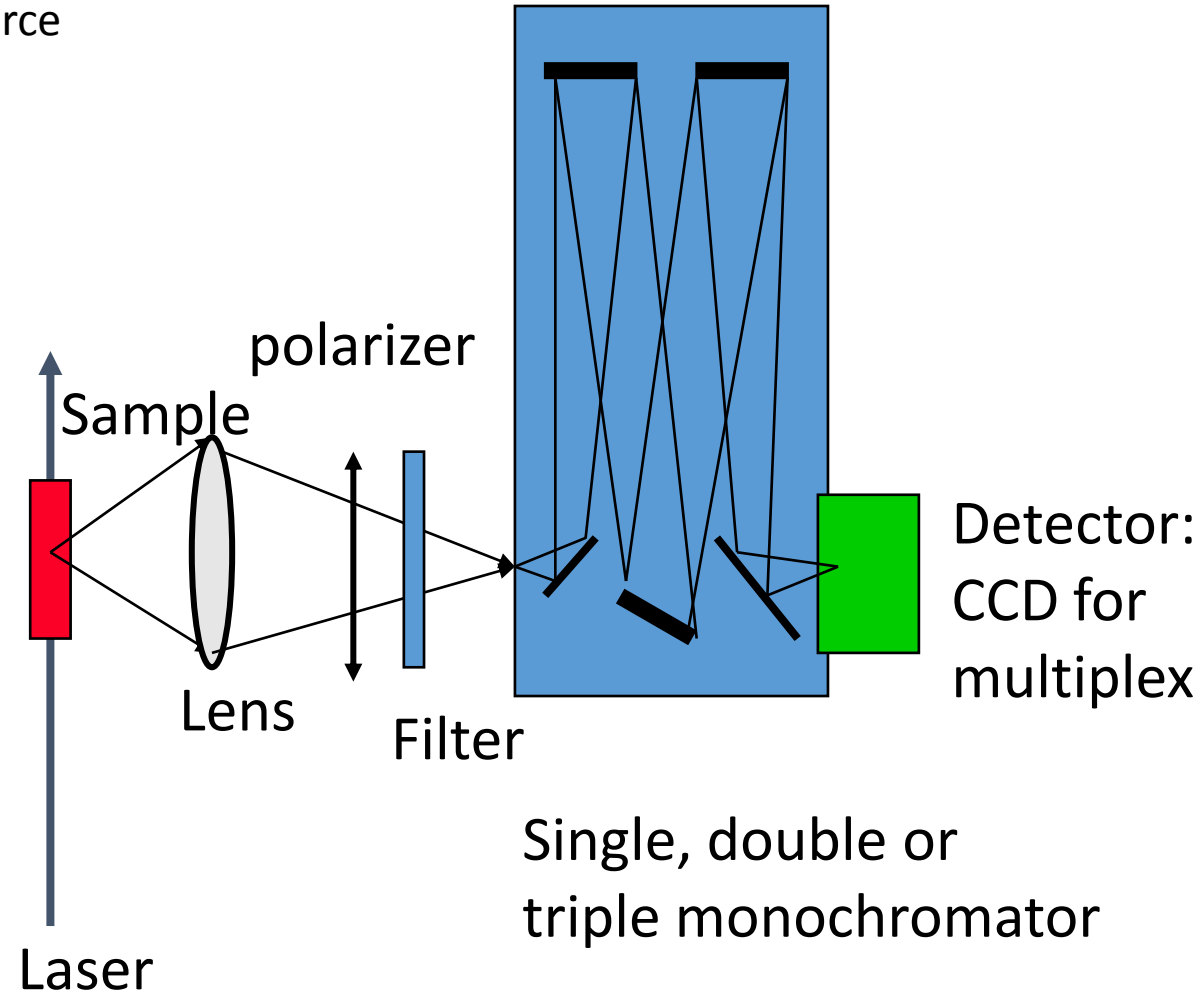


700 cm^{-1}

IV – VII – difficult to detect, discriminate

Infrared (FTIR) and Raman schematic instrumentation

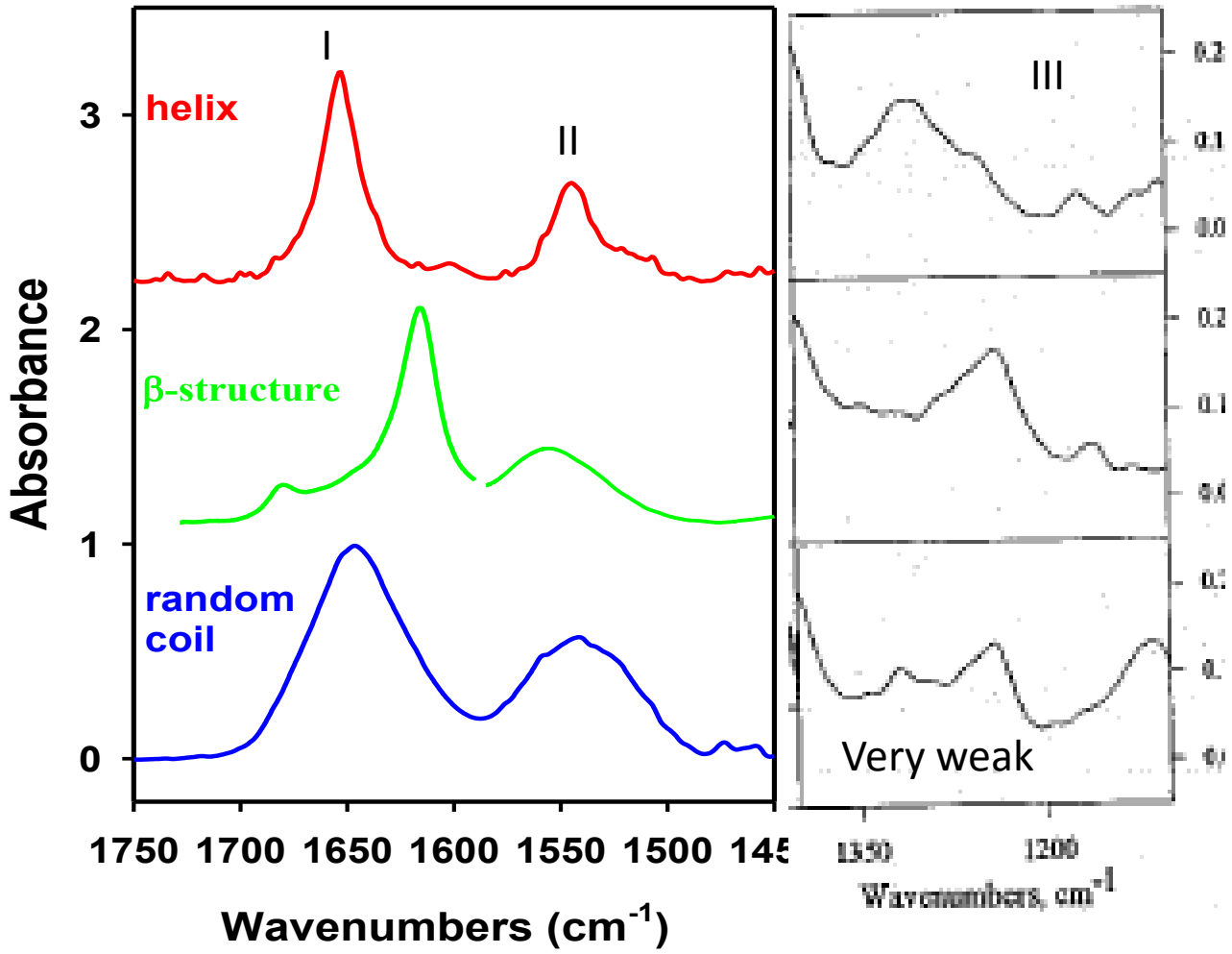




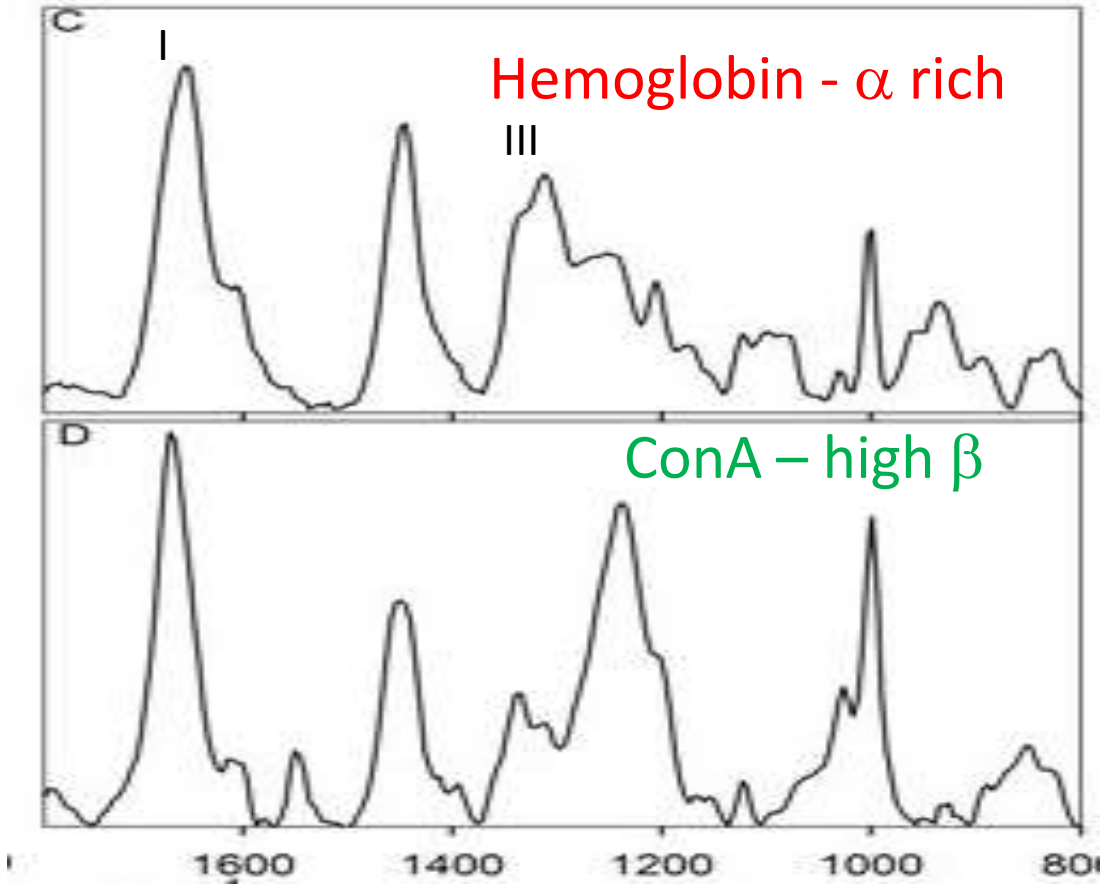
Sensing Secondary Structure with IR and Raman

R absorbance spectra of selected model peptides

IR – peptide amide I and II dominate



Raman – large region, side-chain impact

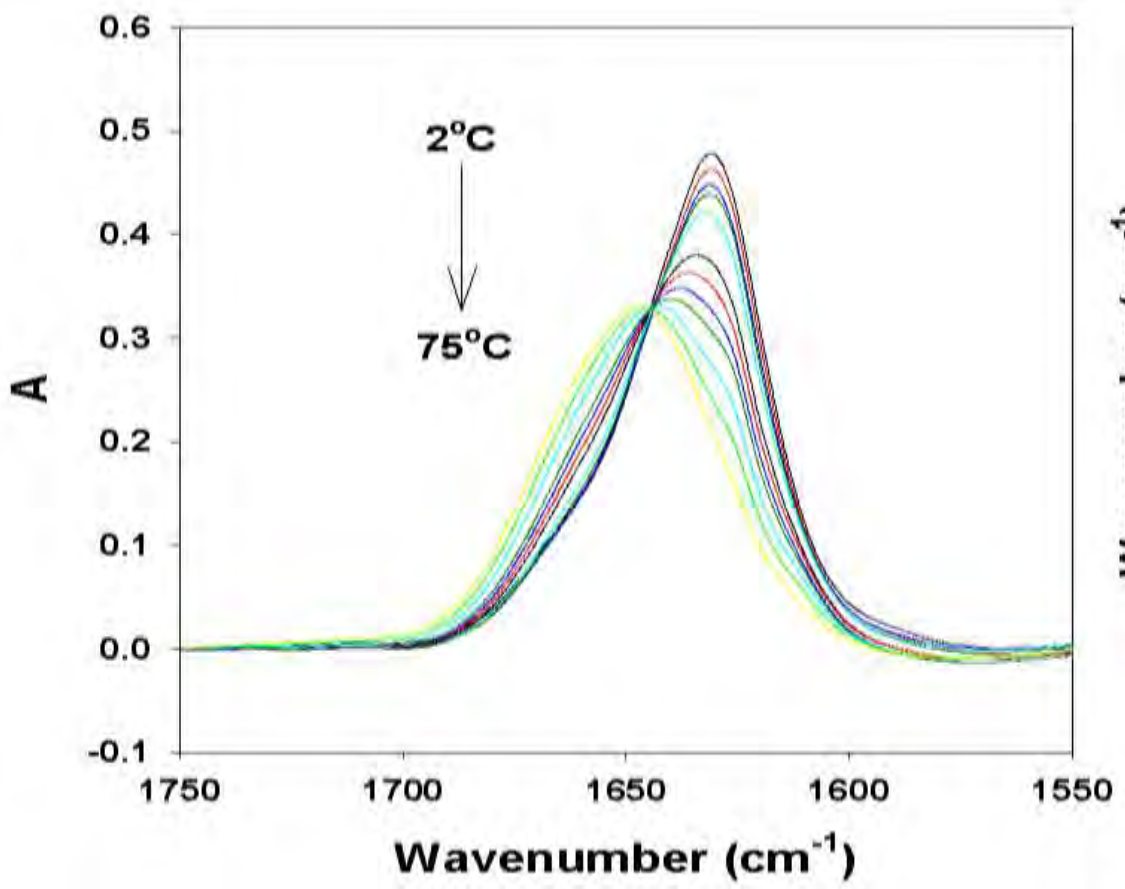


Amide I contrast - IR, Raman same modes differ intensity/shape *due to coupling*

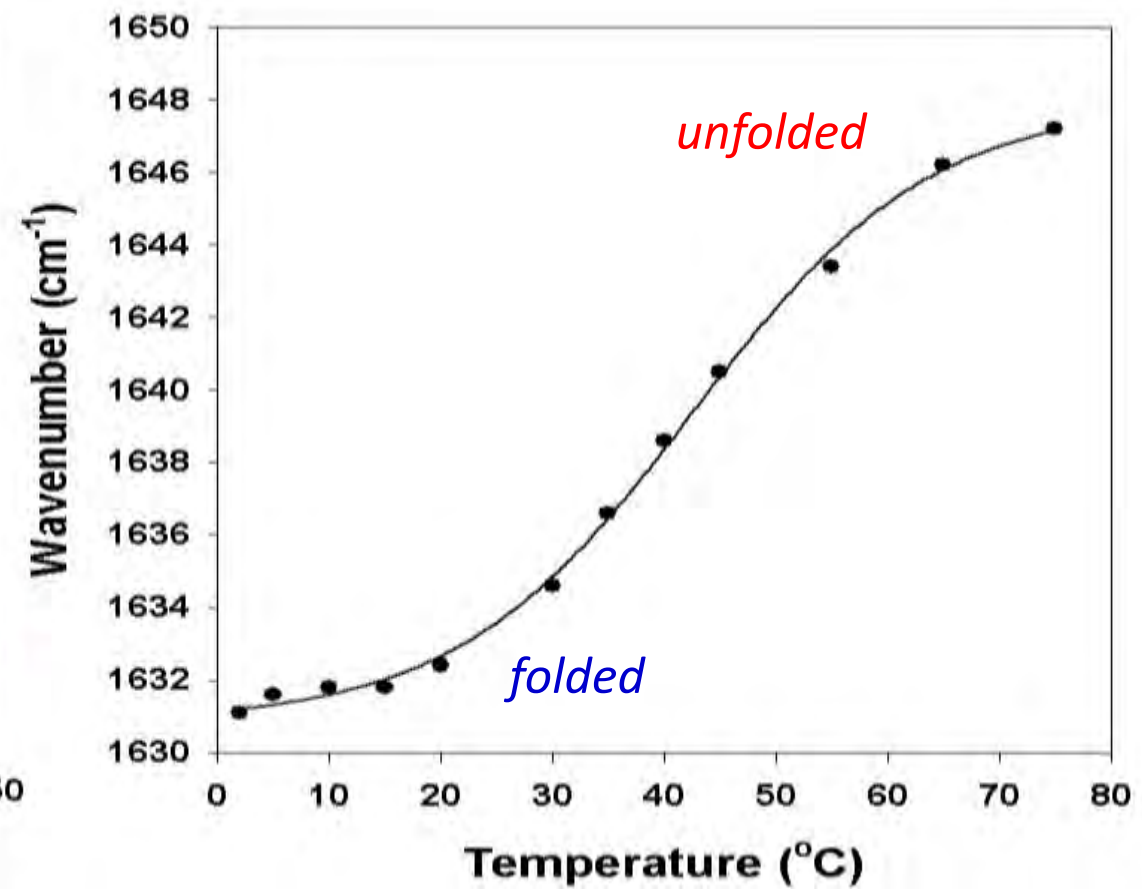
Raman: amide II weak, but amide III has large shift, due to mix with Cα-H

Monitoring structural change - temperature

Temperature dependent IR spectra of the helical peptide



Temperature dependence of amide I' frequency

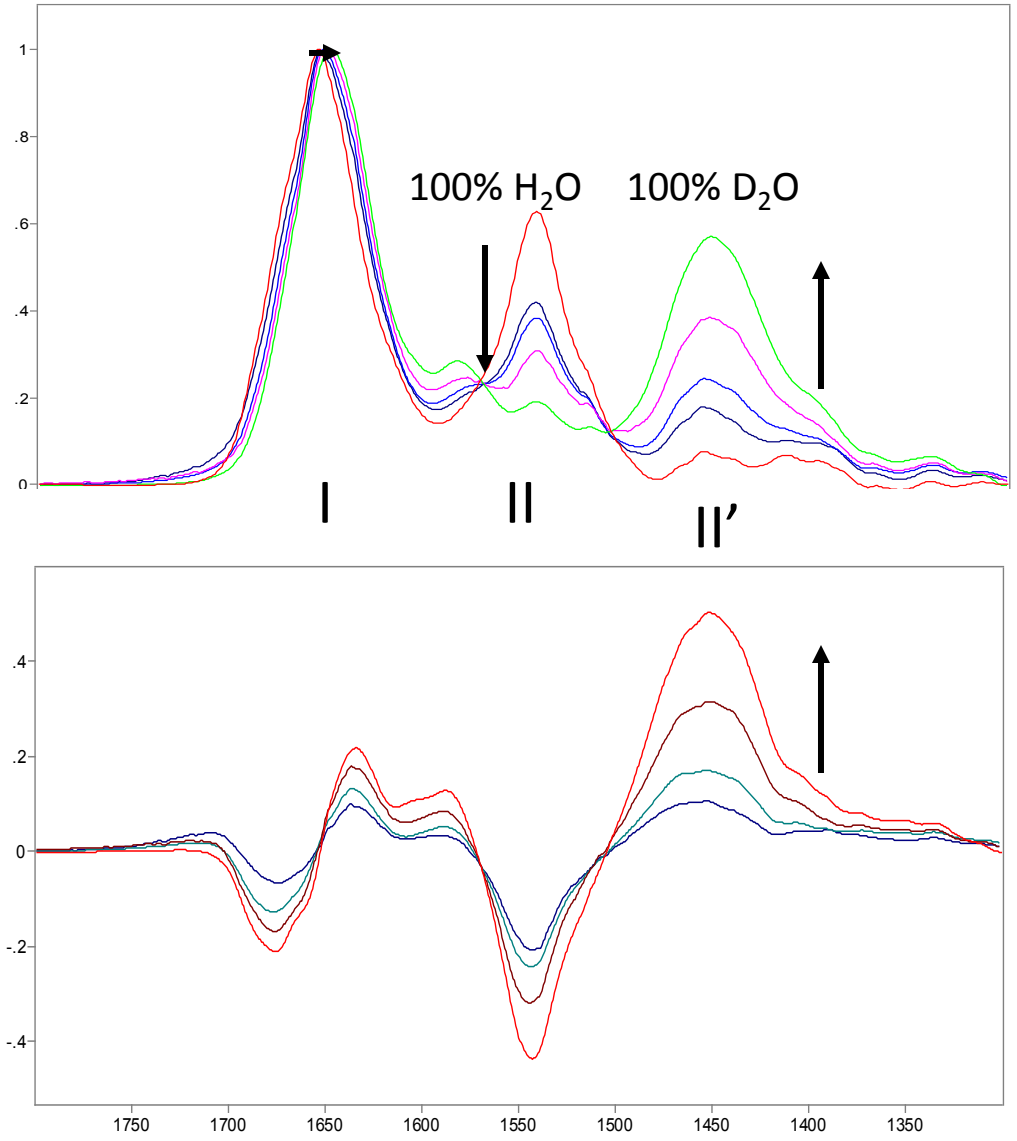


IR frequency shift shows a sigmoidal curve and spectra have an isobestic point for thermal unfolding

However, frequency shift is ~1635 → ~1645 cm^{-1} – solvated helix

FTIR and DFTIR – Lysozyme : H_2O-D_2O Mixtures

"Problem" of H/D exchange is made useful by analyses exposed residues exchange faster



FTIR
Inc. D_2O ↑

Difference IR
"DFTIR"
IR_x-IR_{H2O}

Amide I relatively small change, amide II 100 cm^{-1} shift, amide III more

Circular Dichroism

$$\Delta A = A_L - A_R \sim R = \mathrm{Im}(\underline{\mu} \bullet \underline{m})$$

Small molecules, intrinsically chiral oscillators, $\underline{m} \neq 0$ and $\underline{m}$ not $\perp \underline{\mu}$

Peptide/ Protein - many oscillators locally achiral -*Coupling is central*

in uv - for amide: n-π* or π–π* in planar, *locally achiral* -HN-C=O-

in IR - amide centered vibrations most important, $\underline{m} \sim 0$

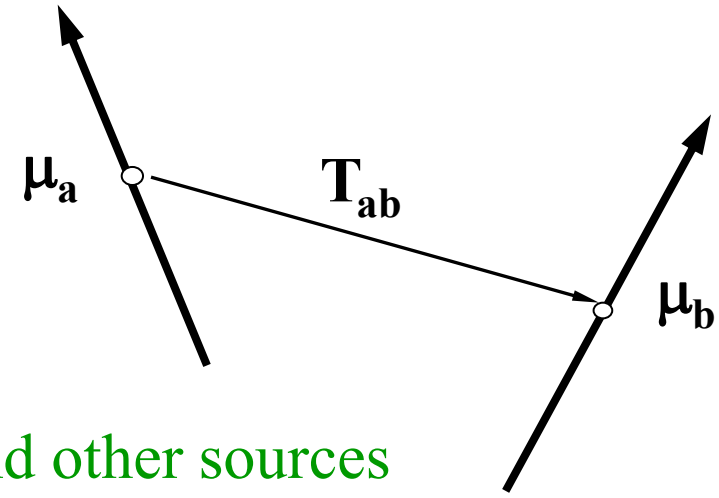
Coupled amide transitions along chain create delocalized $\underline{m}$,

leads to distinctive bandshapes--depend on secondary structure

e.g. dipole coupled (2) oscillators (classical):

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

"$\underline{m}$"



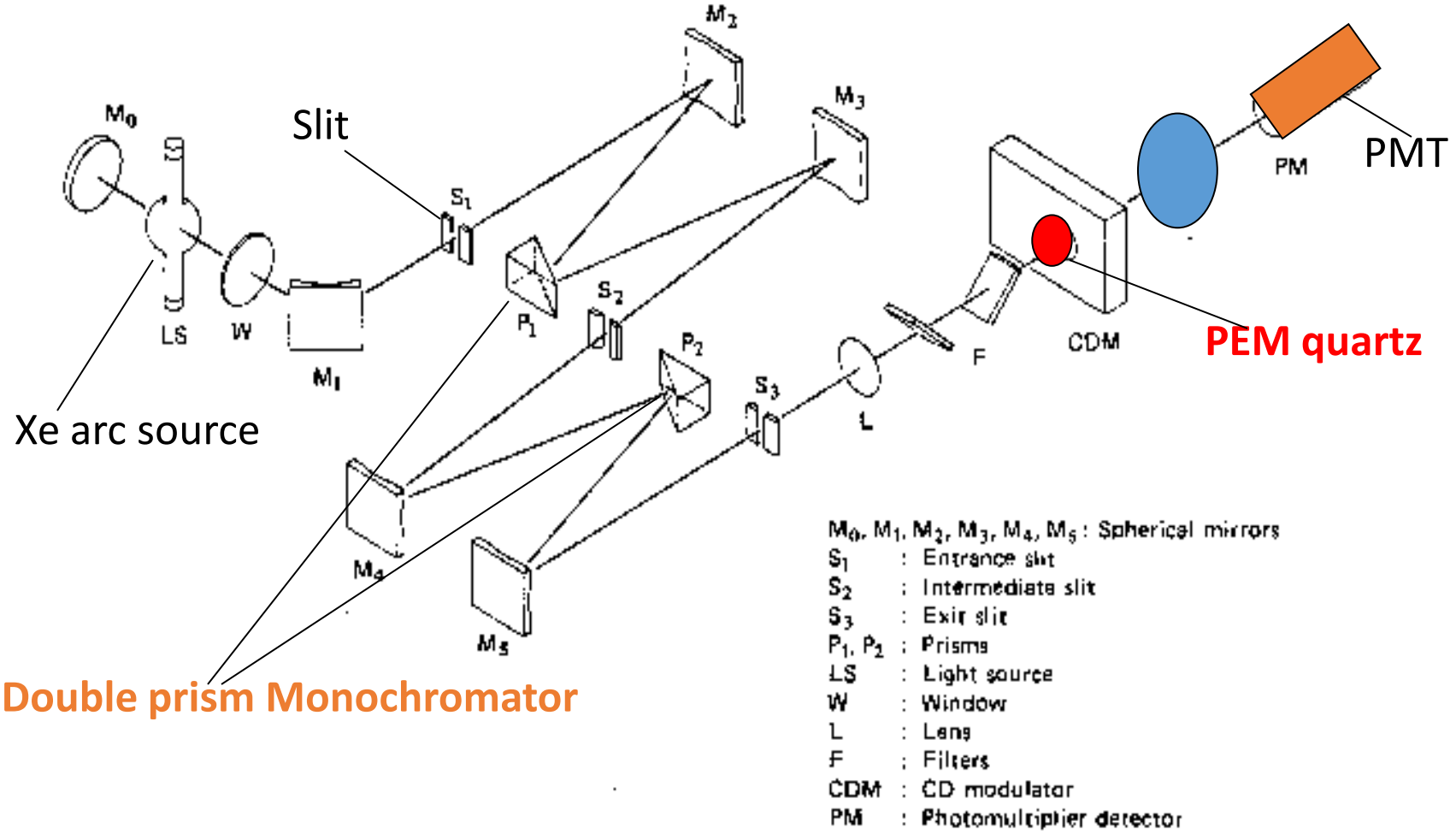
Derivative shape (couplet) CD from overlap of

opposite sign transitions, split by coupling, dipole and other sources

UV-vis Circular Dichroism Spectrometer schematic

$\Delta A = A_L - A_R$ -- **non-zero for chiral molecules**

Commercial design

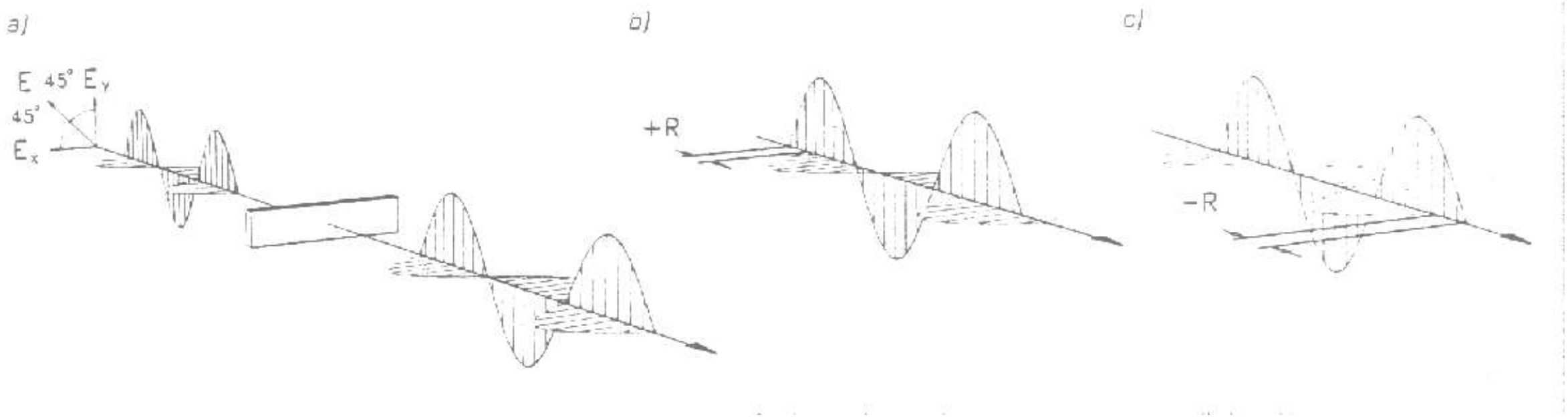


JASCO CD–quartz prisms disperse and *linearly polarize* beam, quartz PEM modulate left-right circular polarizations

PEM – photo elastic modulator

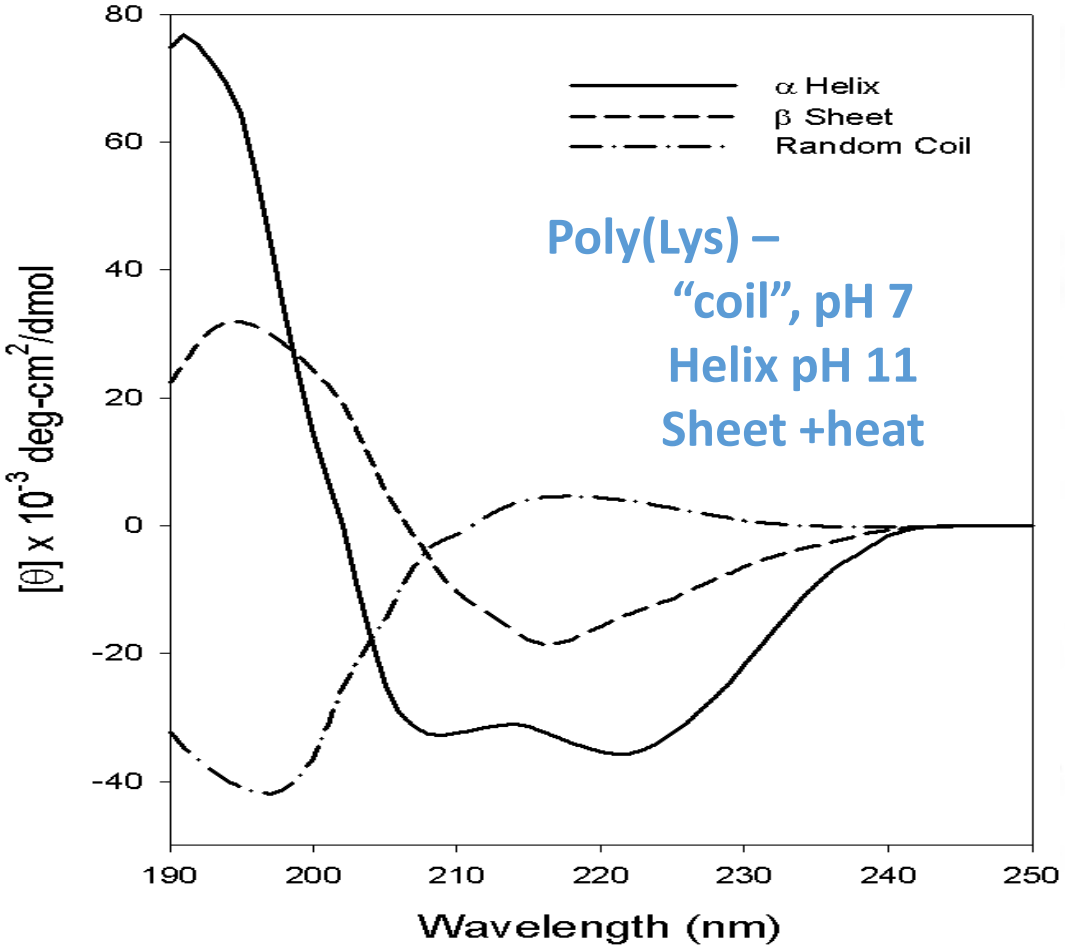
Phase retard linear polarization to form circular polarization

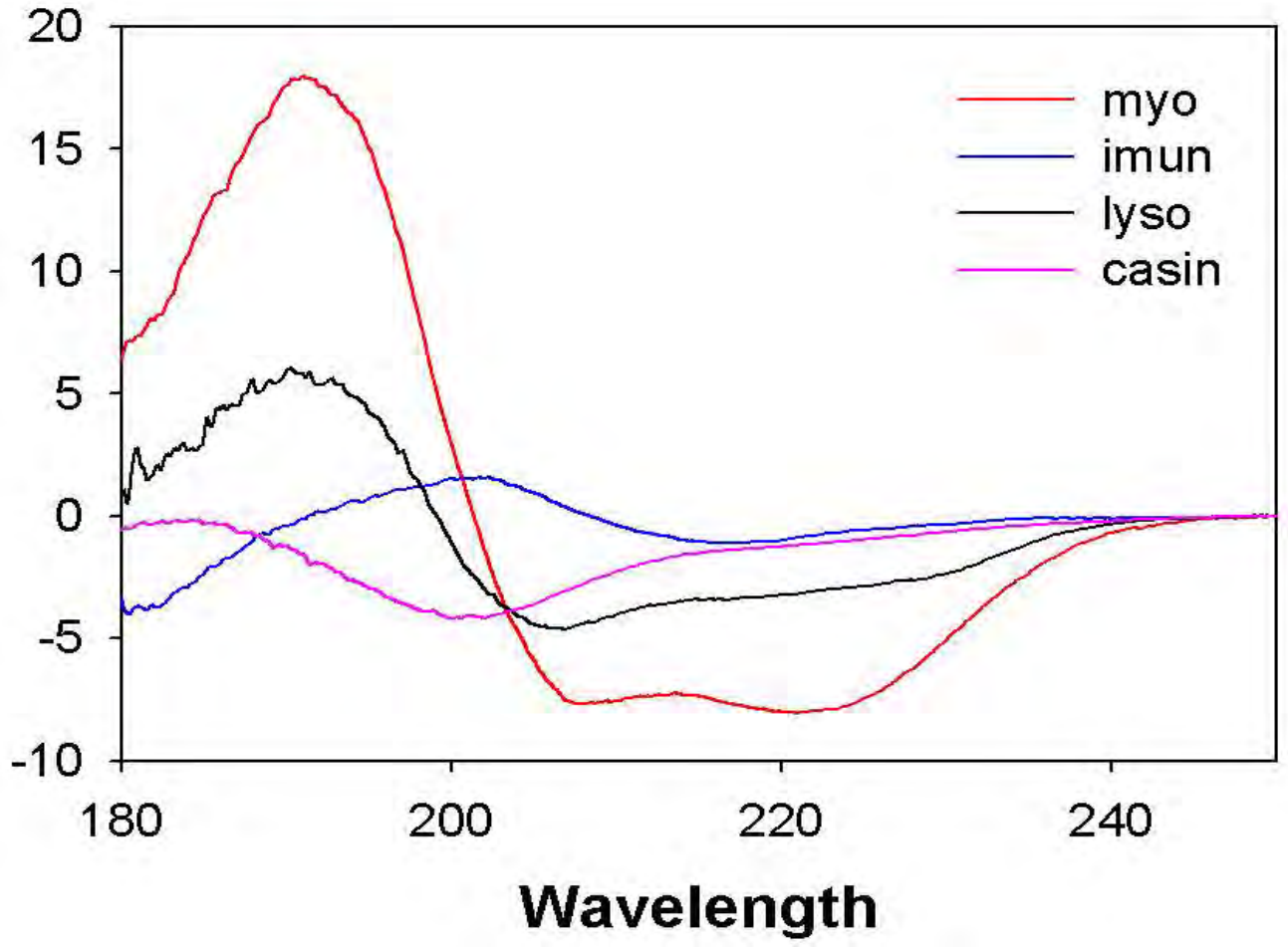
Place alternate stress on isotropic crystal



Polypeptide and Protein Circular Dichroism

yields global/average secondary structure content

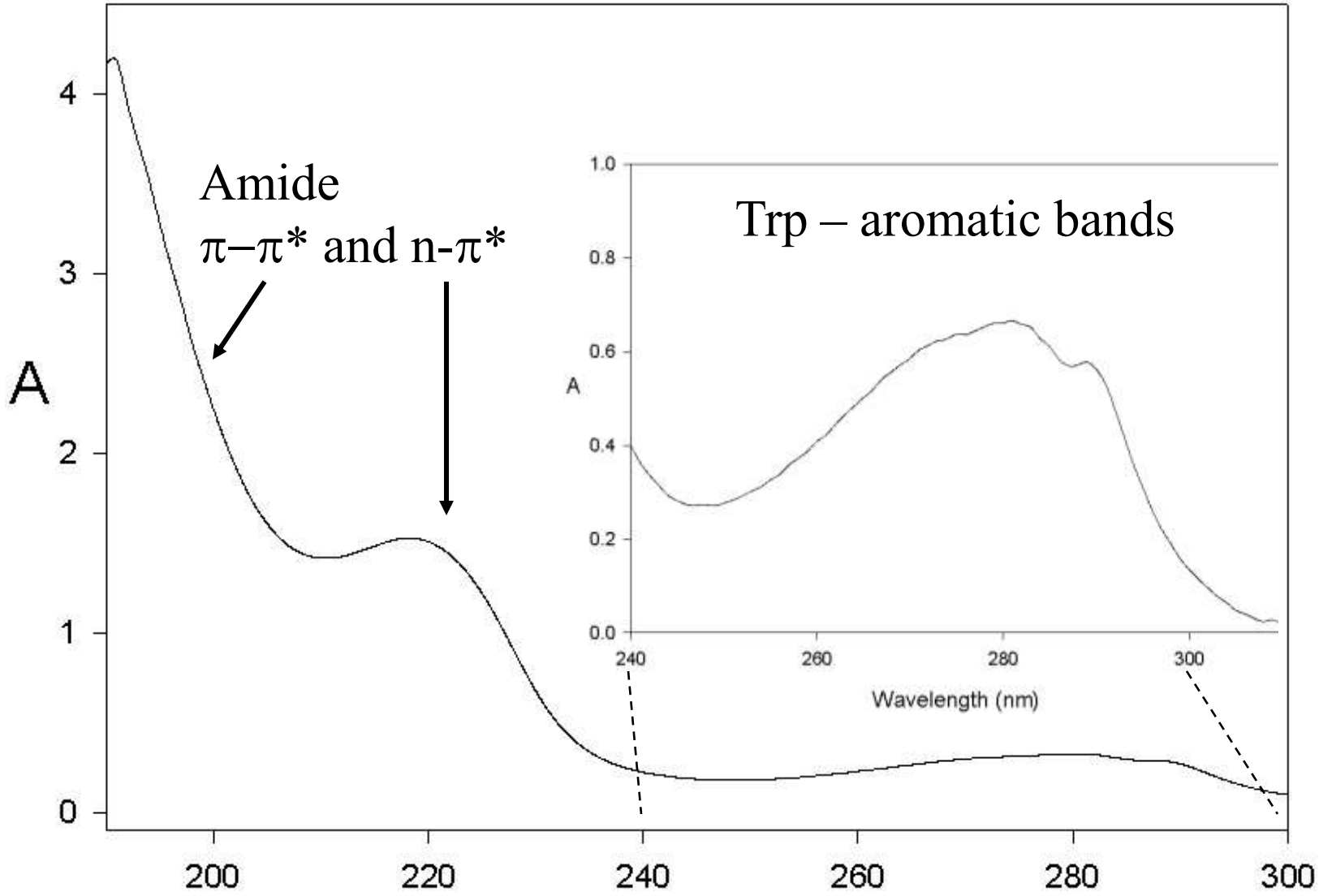




Greenfield-Fasman 1969
poly-L-Lys comparison

Protein CD – helix contribution to mix dominates – θ_{222} correlate to helix

UV absorption of peptides is featureless --except aromatics



TrpZip peptide in water
Rong Huang, unpublished

Electronic Absorption and Fluorescence

Probe method - sense change in fluorophore environment

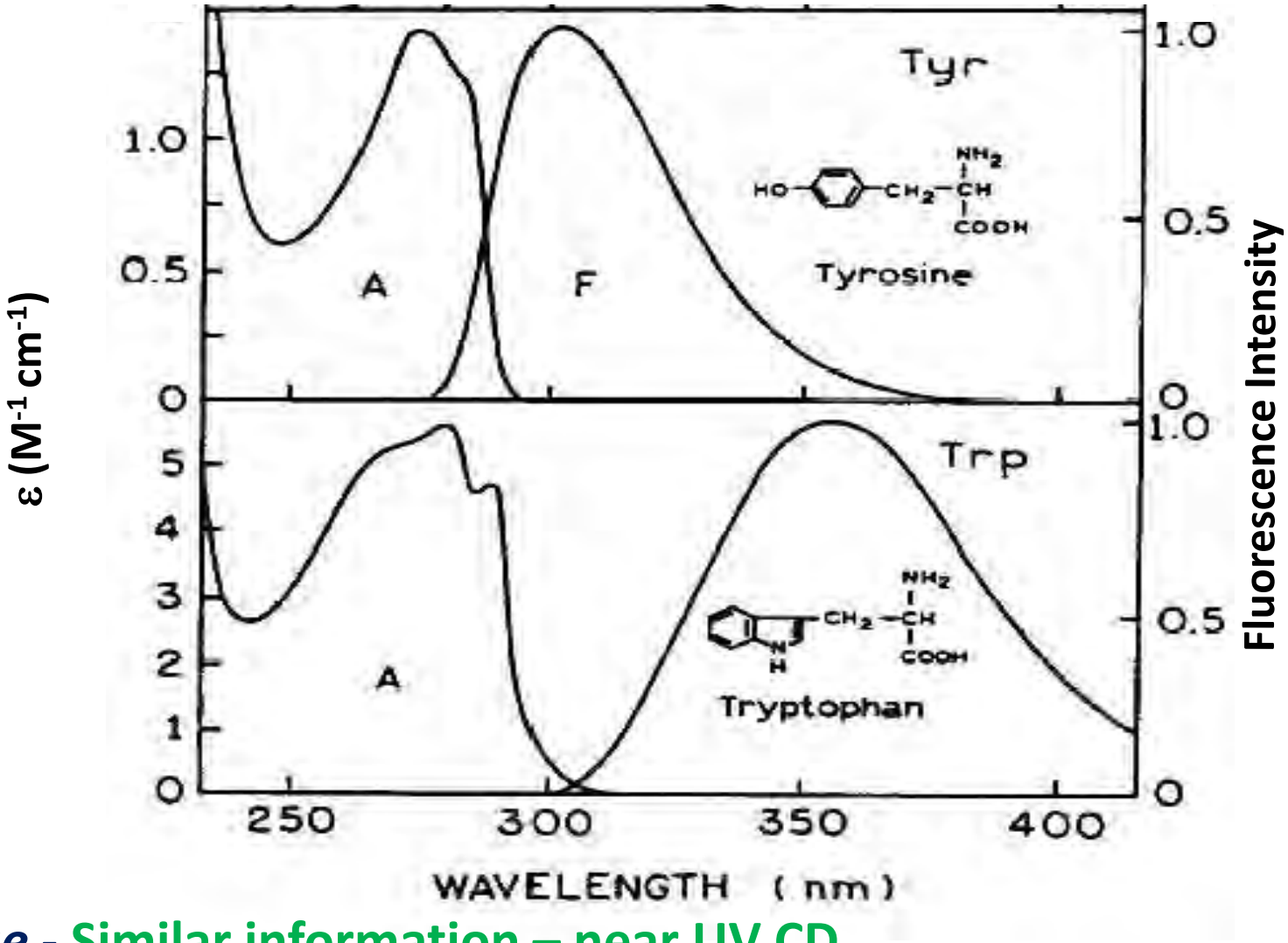
What do you see?
(typical protein)

Intrinsic fluorophores

eg. Trp, Tyr

Change with tertiary structure, compactness

Amide absorption broad, Intense, featureless, far UV ~200 nm and below

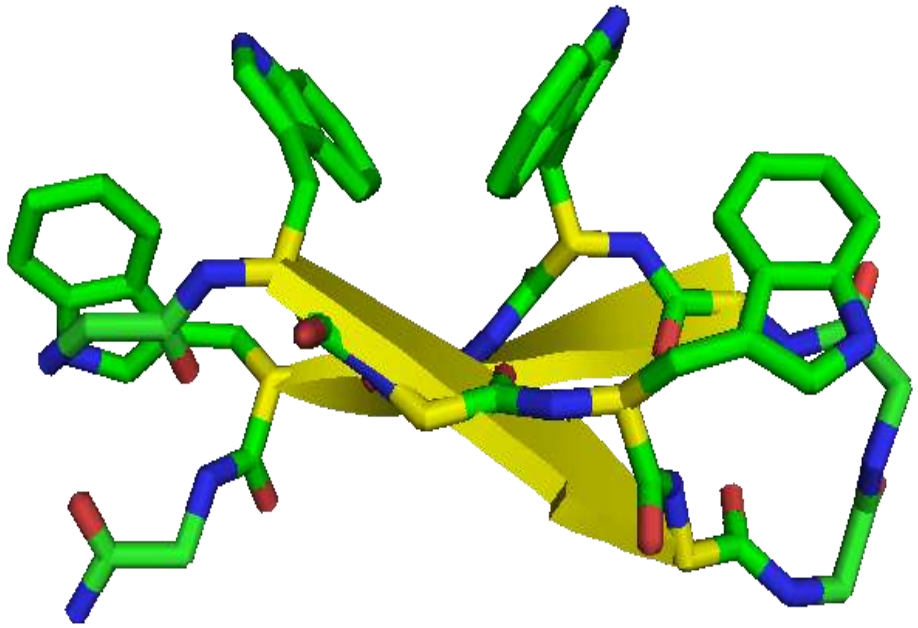


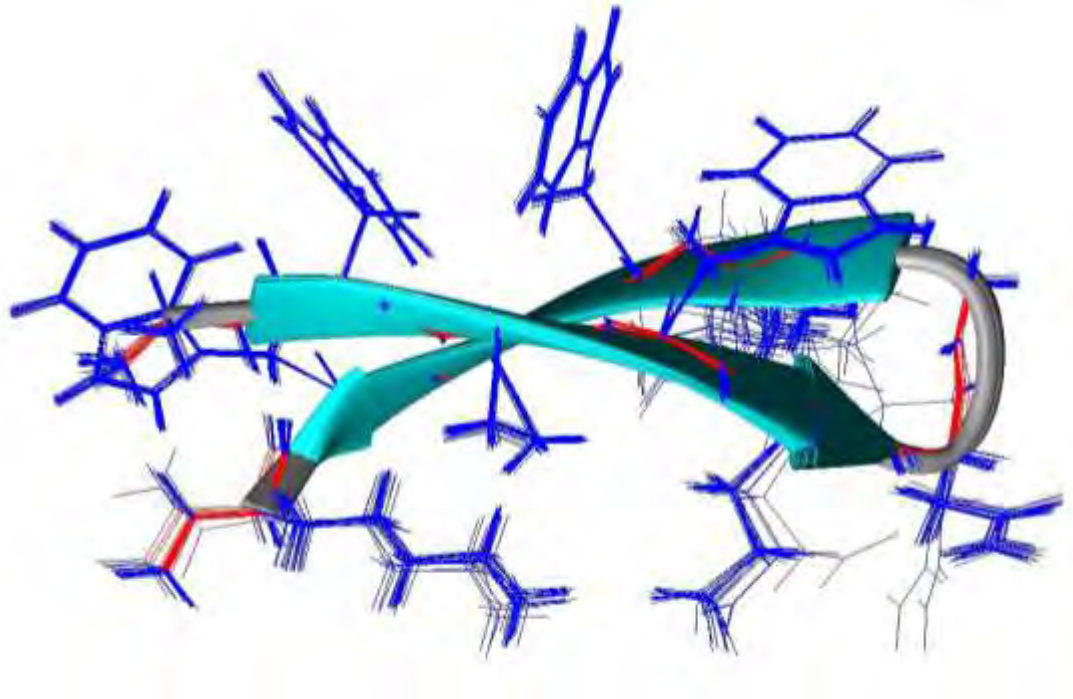
Tertiary structure change **- Similar information – near UV CD**

Side-chain e.g. TrpZip2, NMR equilibrium structure

- High degree of order, especially in Trp interactions
- Twisted beta strands, bit frayed at termini
- CD of Trps – edge-to-face, simulate with TD-DFT – *dominate UV*

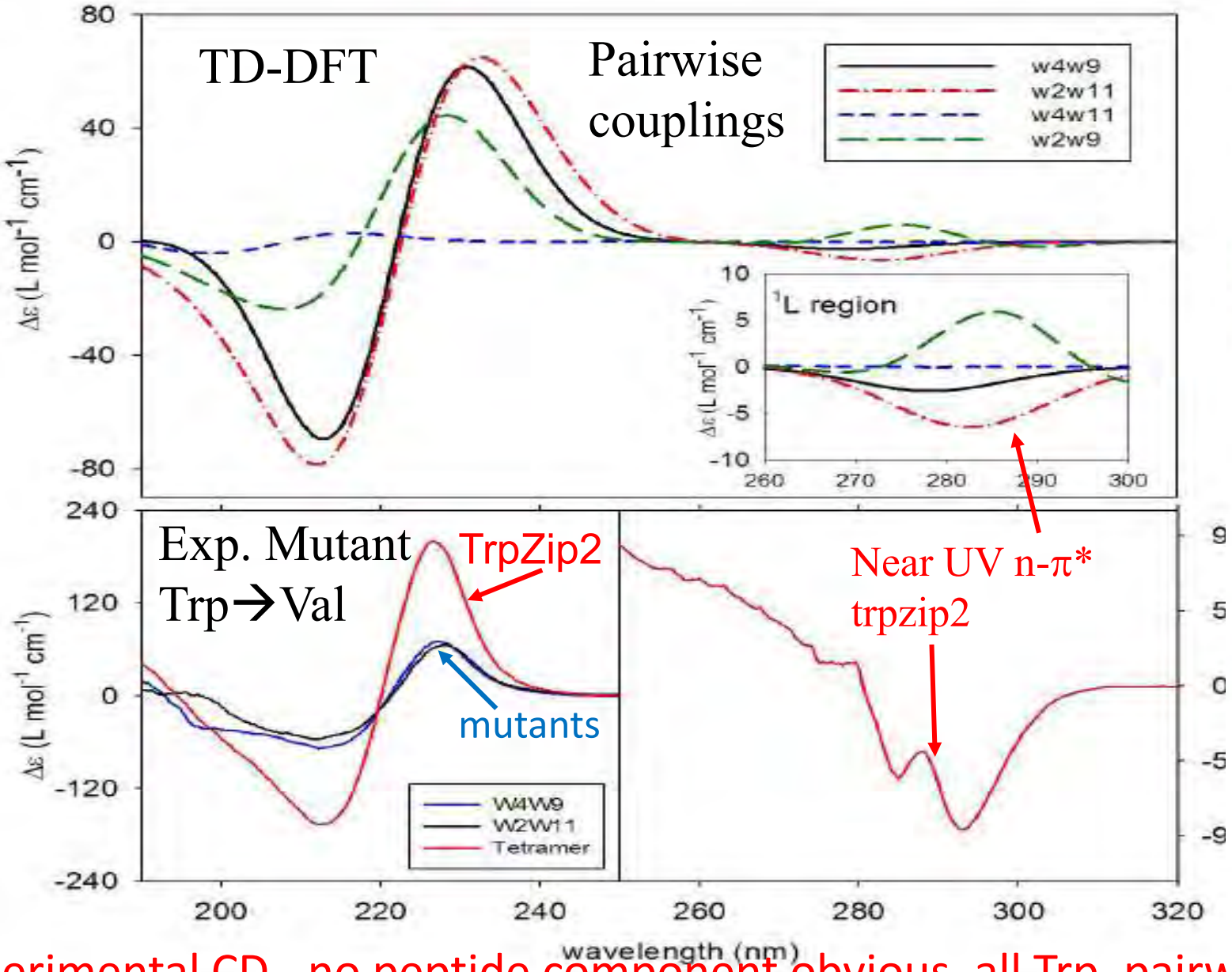
Huang, Wu et al.
JPhysChem B 2009



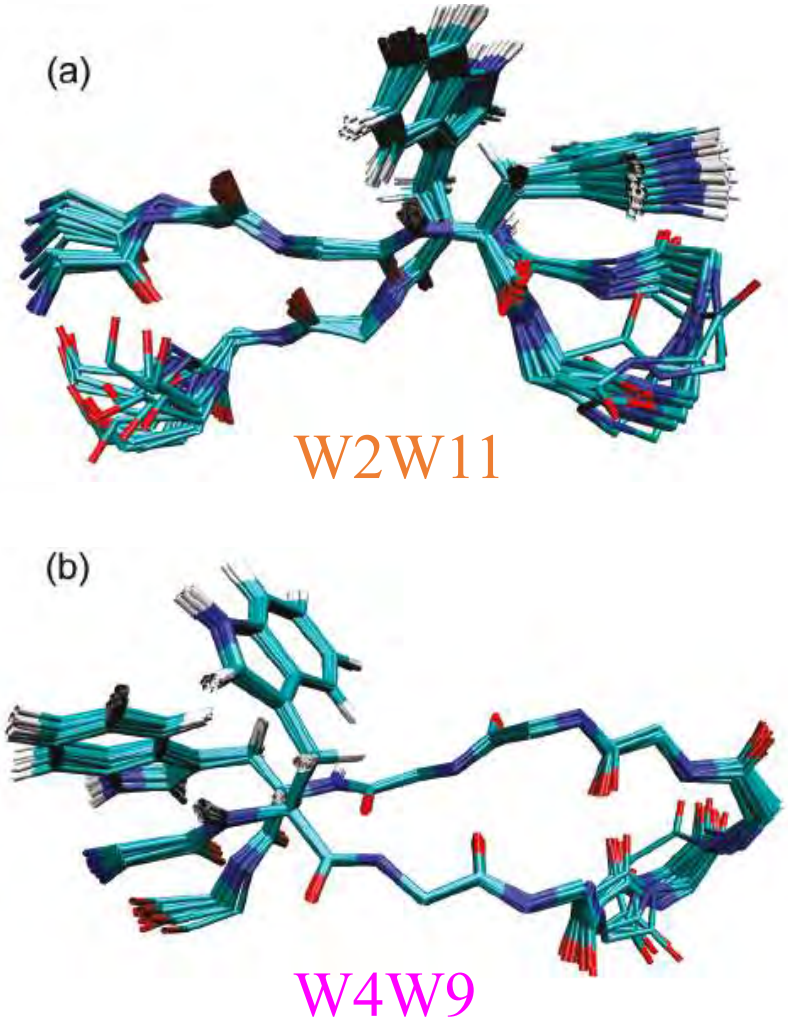


Later: Simulate IR, VCD and Raman - full DFT calculation of hairpin (backbone vs. full)

TD-DFT Calculated CD spectra for coupled Trp (just indols)

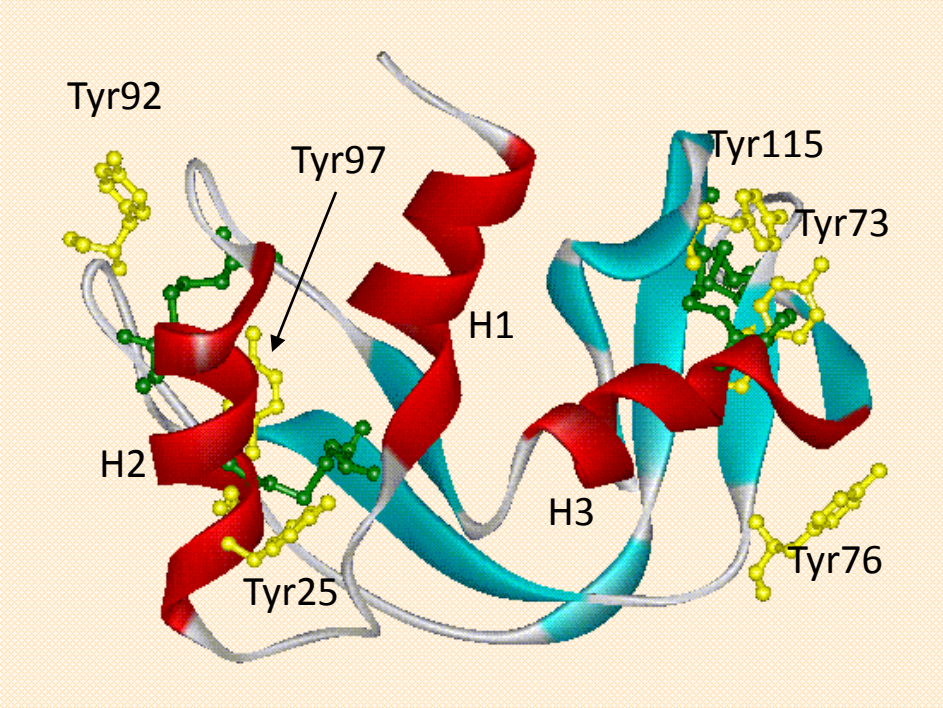


TrpZip2 mutants, Trp→Val



A.Roy et al. Chirality 2009

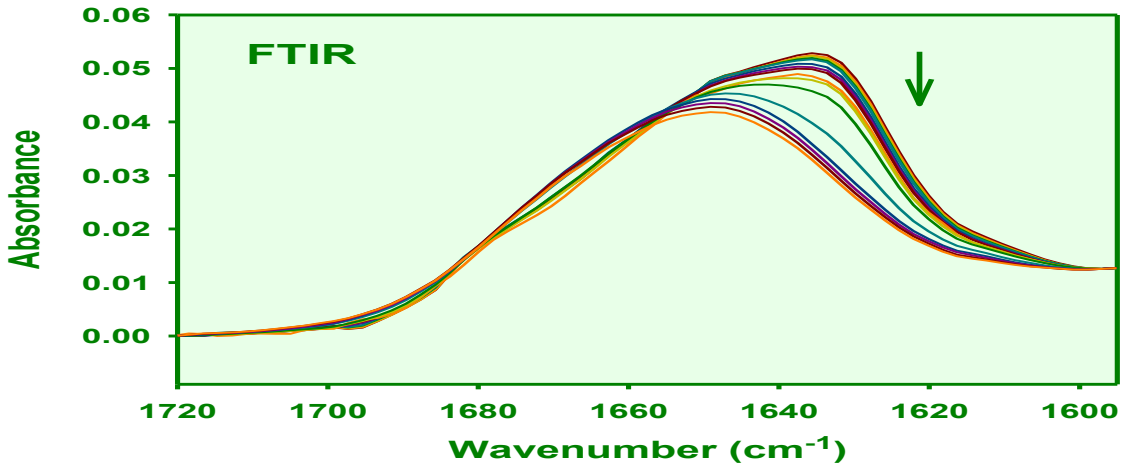
Experimental CD - no peptide component obvious, all Trp, pairwise



Ribonuclease A Thermal unfolding a combined uv-CD and FTIR study

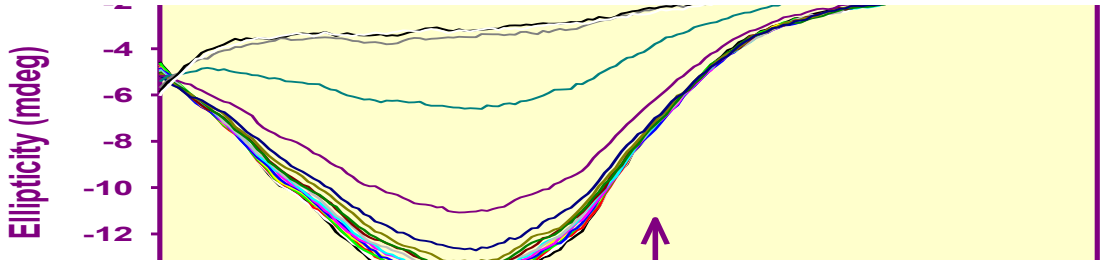
S.Stelea Prot. Sci. 2001

- 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)



Ribonuclease A, multiple probes of thermal unfolding,

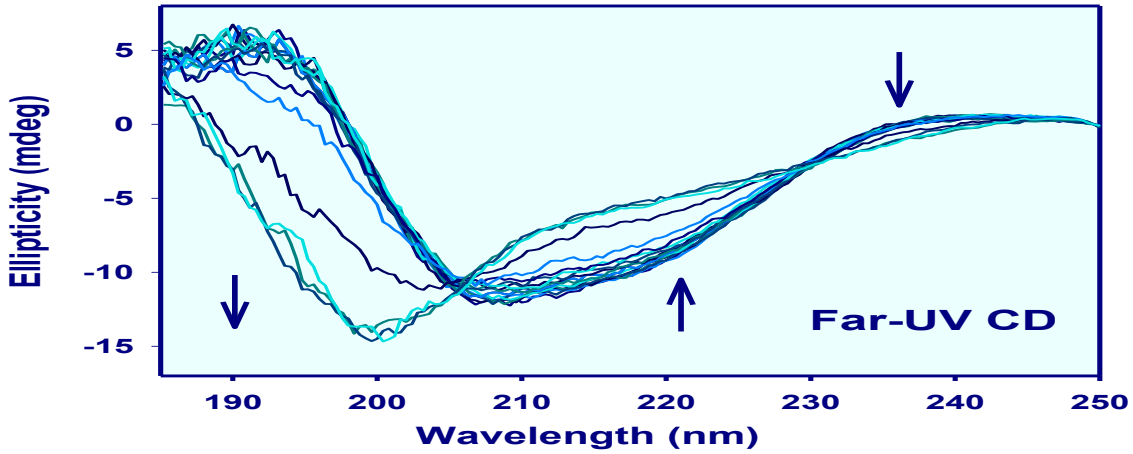
FTIR—amide I
Loss of β-sheet



Spectral Change

Temperature 10-70°C

Near –uv CD
Loss of tertiary struct.

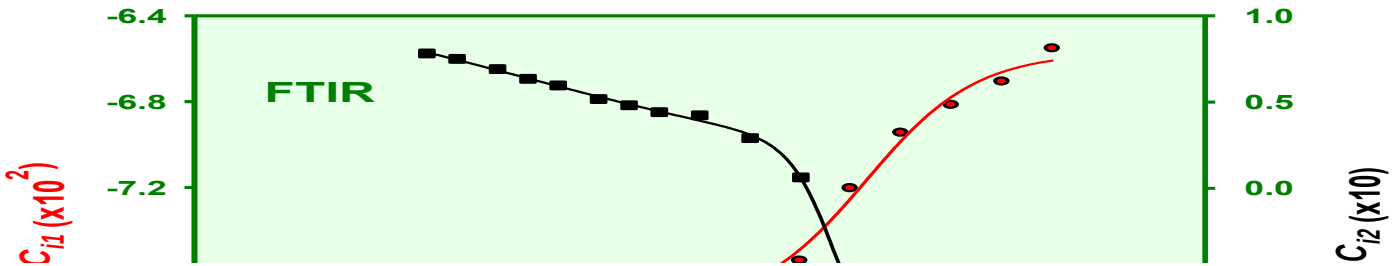


Far-uv CD
Loss of α-helix

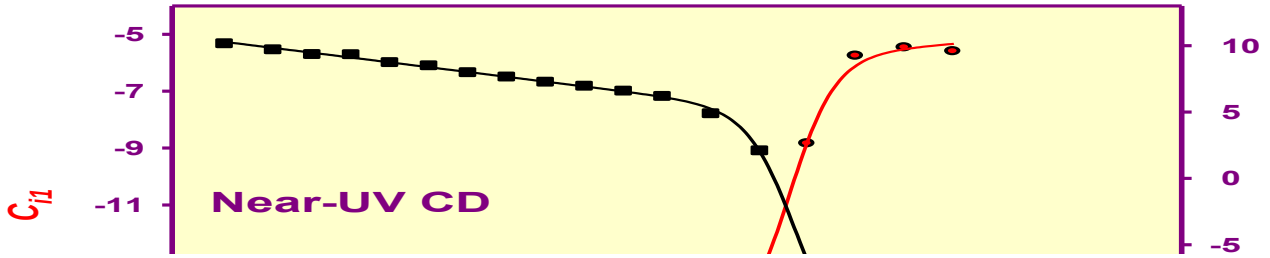
Stelea et al., Prot.Sci. 2001

Ribonuclease A

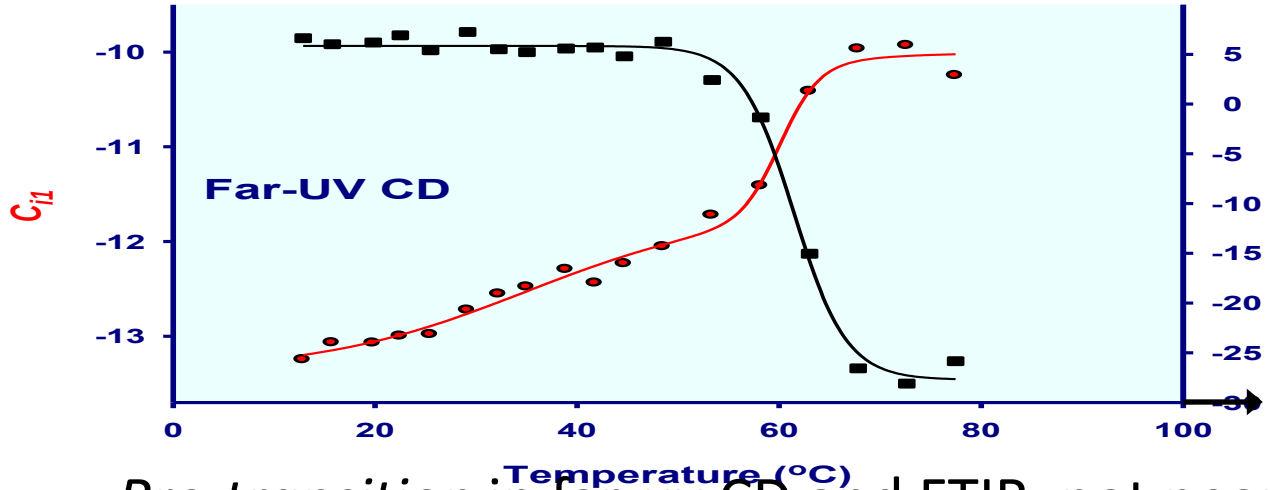
PC/FA loadings
Temp. variation



FTIR (α,β)



Near-uv CD
(tertiary)



Far-uv CD
(α-helix)

Temp.

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

Stelea et al.,
Prot.Sci. 2001

Combining Techniques: Vibrational CD

"CD" in the infrared region: $\Delta A = A_L - A_R \sim R = Im(\underline{\mu}.\underline{m})$

Vibrational chirality →Many transitions / Spectrally resolved / Local

Technology in place → $\Delta A \sim 10^{-5}$ - limits S/N / Difficult < 700 cm^{-1}

Same transitions as IR

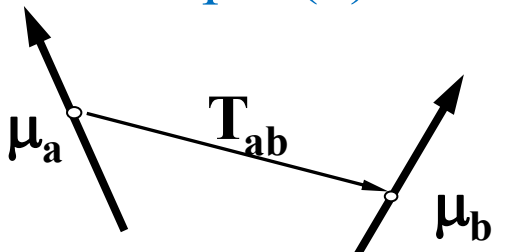
same frequencies, same resolution

Band Shape from spatial relationships

coupling amides in peptides/proteins

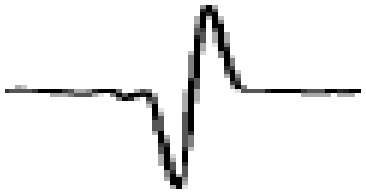
Relatively short length dependence

Classical: couple (2) oscillators:



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

Derivative shape result:



Development -- structure-spectra relationships

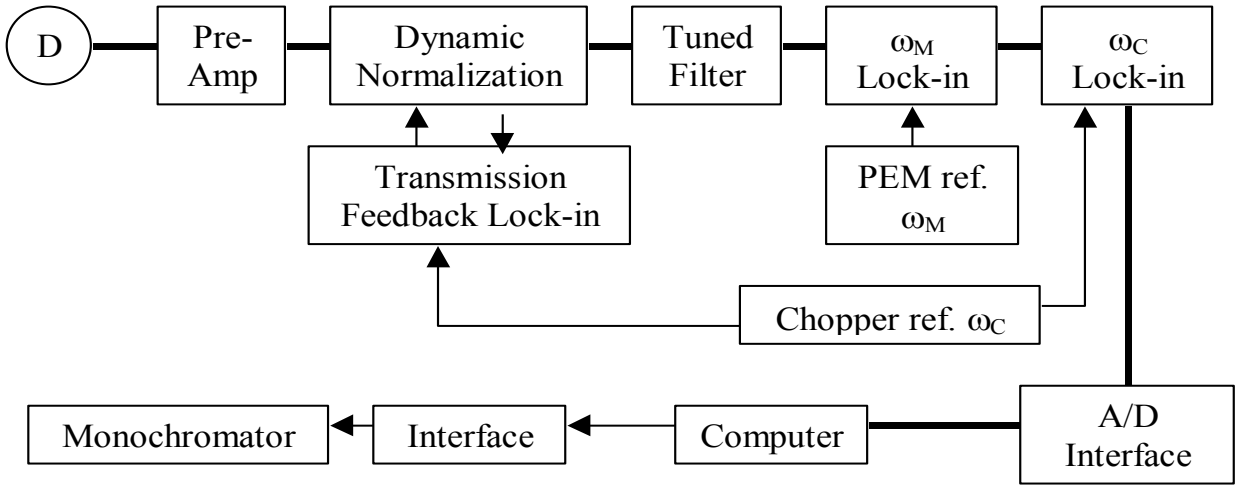
History--Small molecules – theory /

Biomolecules -- empirical,

Status now—oligopeptide VCD - simulated theoretically

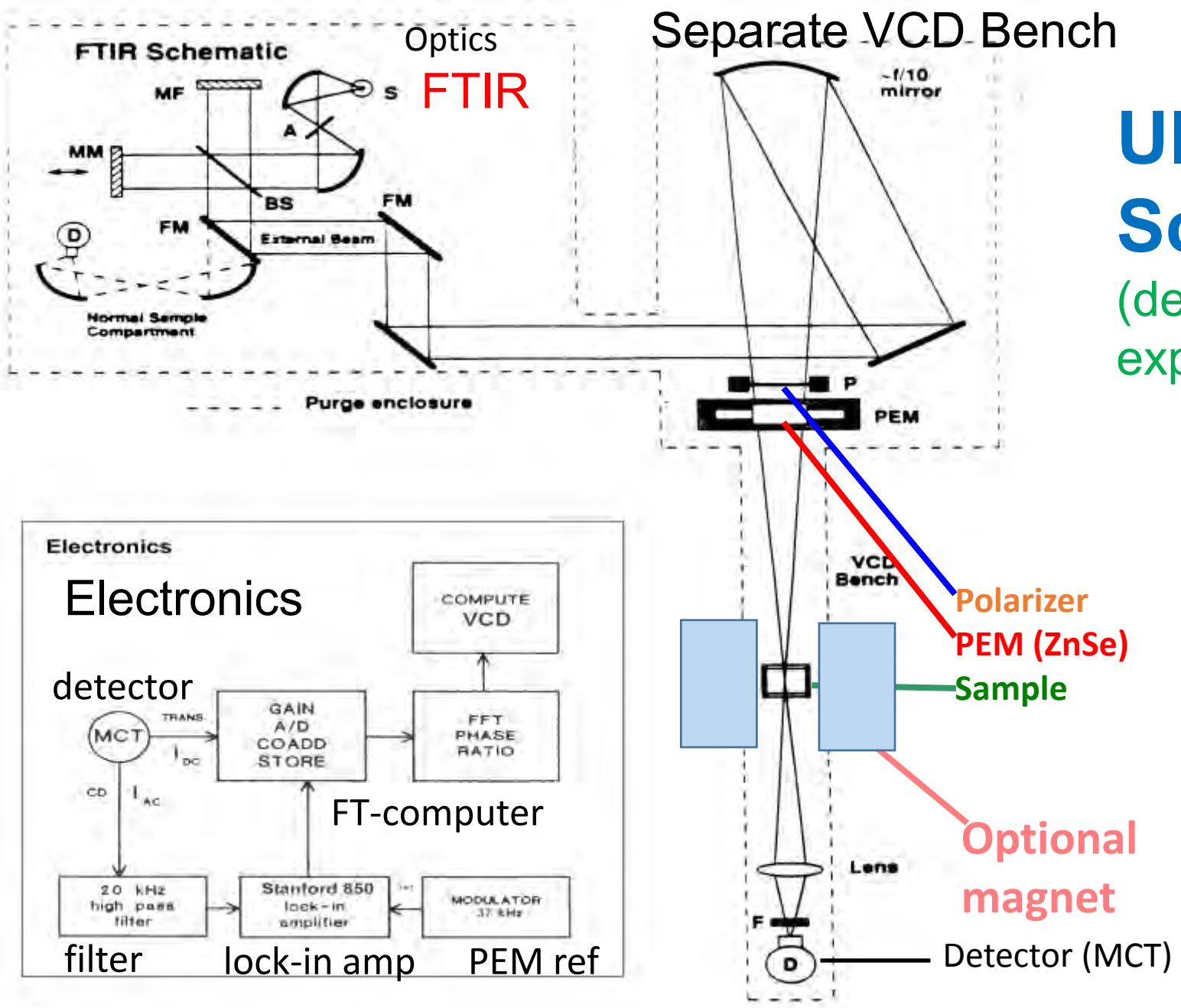
Original UIC Dispersive VCD

Electronics



Optics and Sampling

Infrared makes material challenge for polarization, lenses, cells
Polarizer – grid
Modulator - ZnSe



UIC FT-VCD Schematic

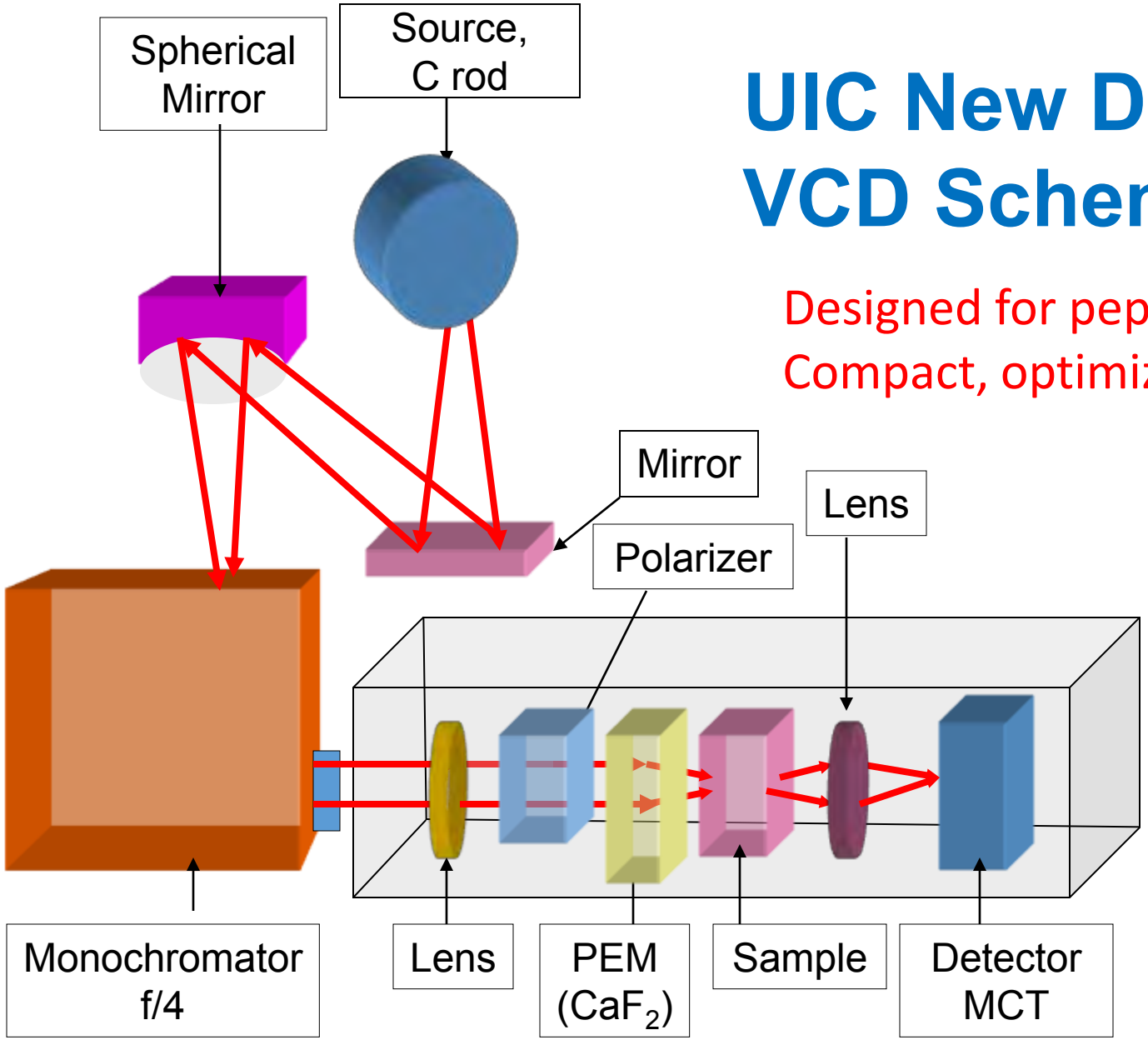
(designed for magnetic experiments as well)

Allows parallel detection of whole range. Can be dedicated or add-on accessory.
Software needs to accommodate differential signals

UIC New Dispersive VCD Schematic

Designed for peptides and proteins

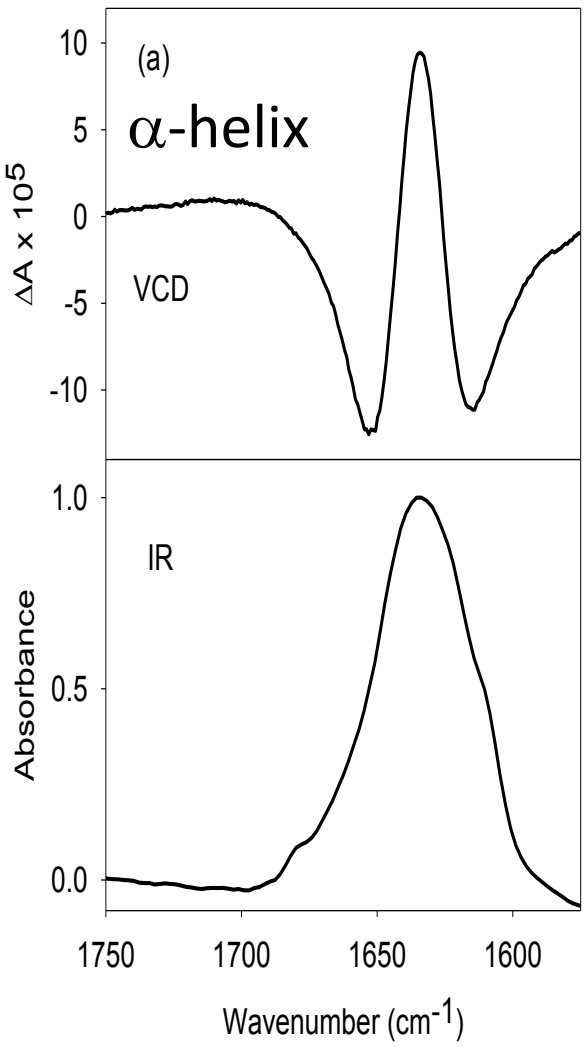
Compact, optimize S/N 1800-1400 cm^{-1}

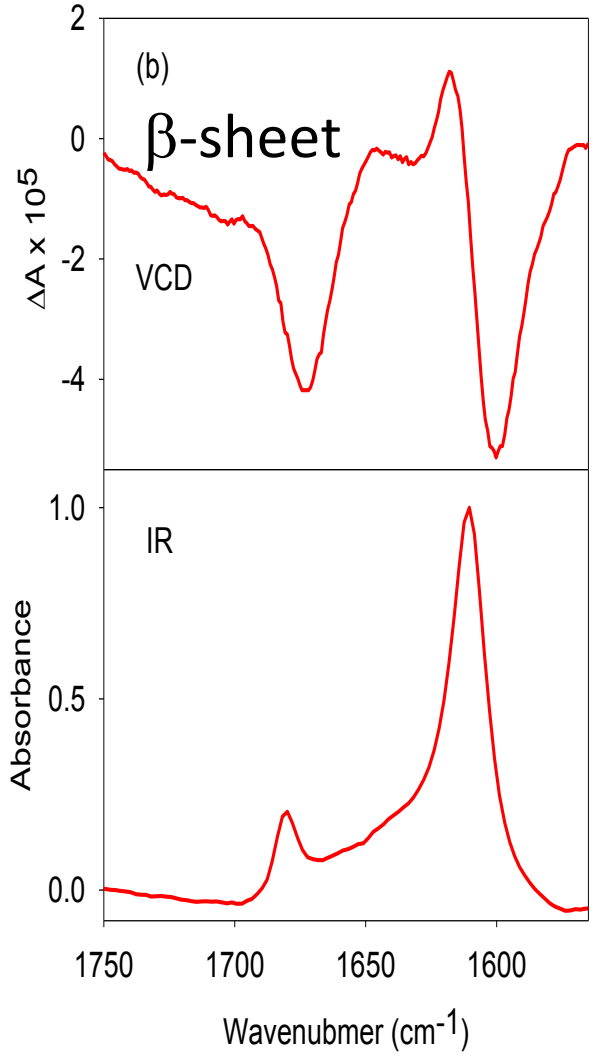


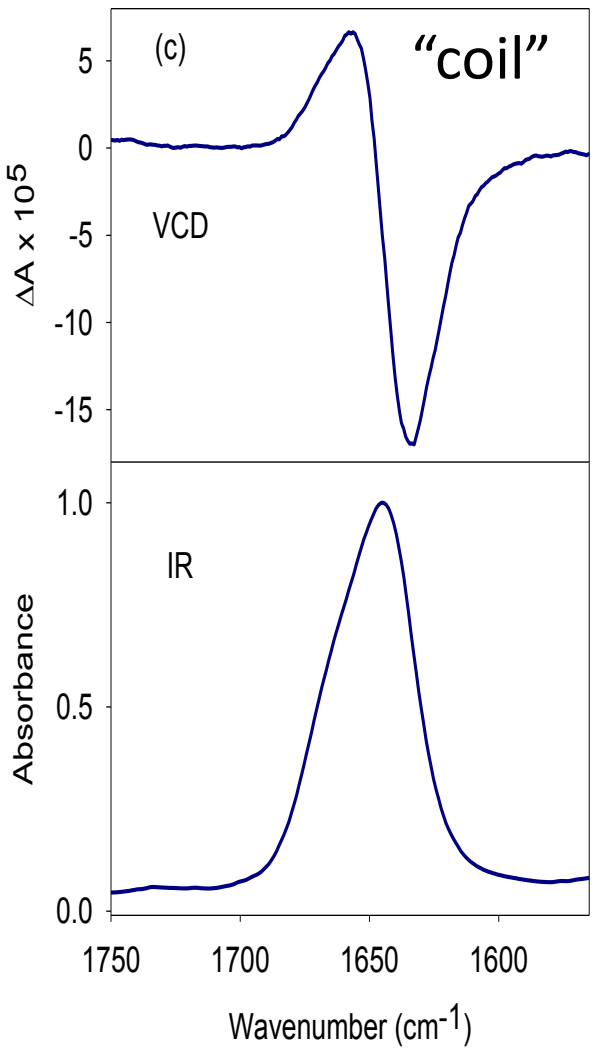
Optics and sampling

Ahmed Lakhani, P.Malon, Appl. Spec 2009

Poly Lysine in D_2O – Amide I'– 2nd structure VCD







If coupling dominate, large VCD for "disordered" or "coil" amide I band implies *local order (PP II)*

Note: demo of S/N, baselines still issue

Raman and ROA similar idea, less variance, different intensity in this mode

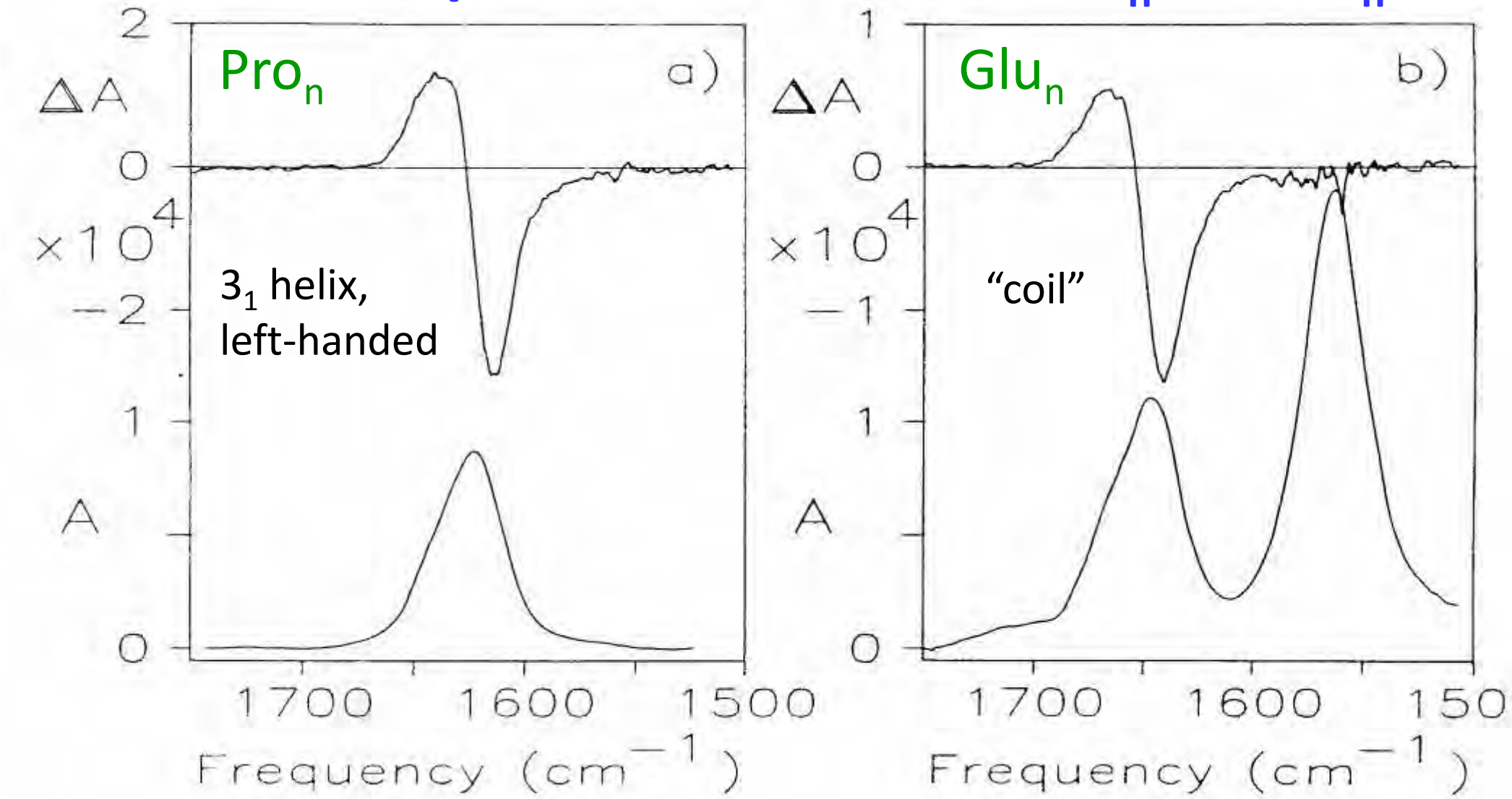
High pH – helix (here maybe mix)

High pH, heating – sheet (maybe aggregate)

Neutral pH - disordered

Lakhani, Malon et al, Appl Spect 2009

Relationship to "random coil" - Pro_n vs. Glu_n

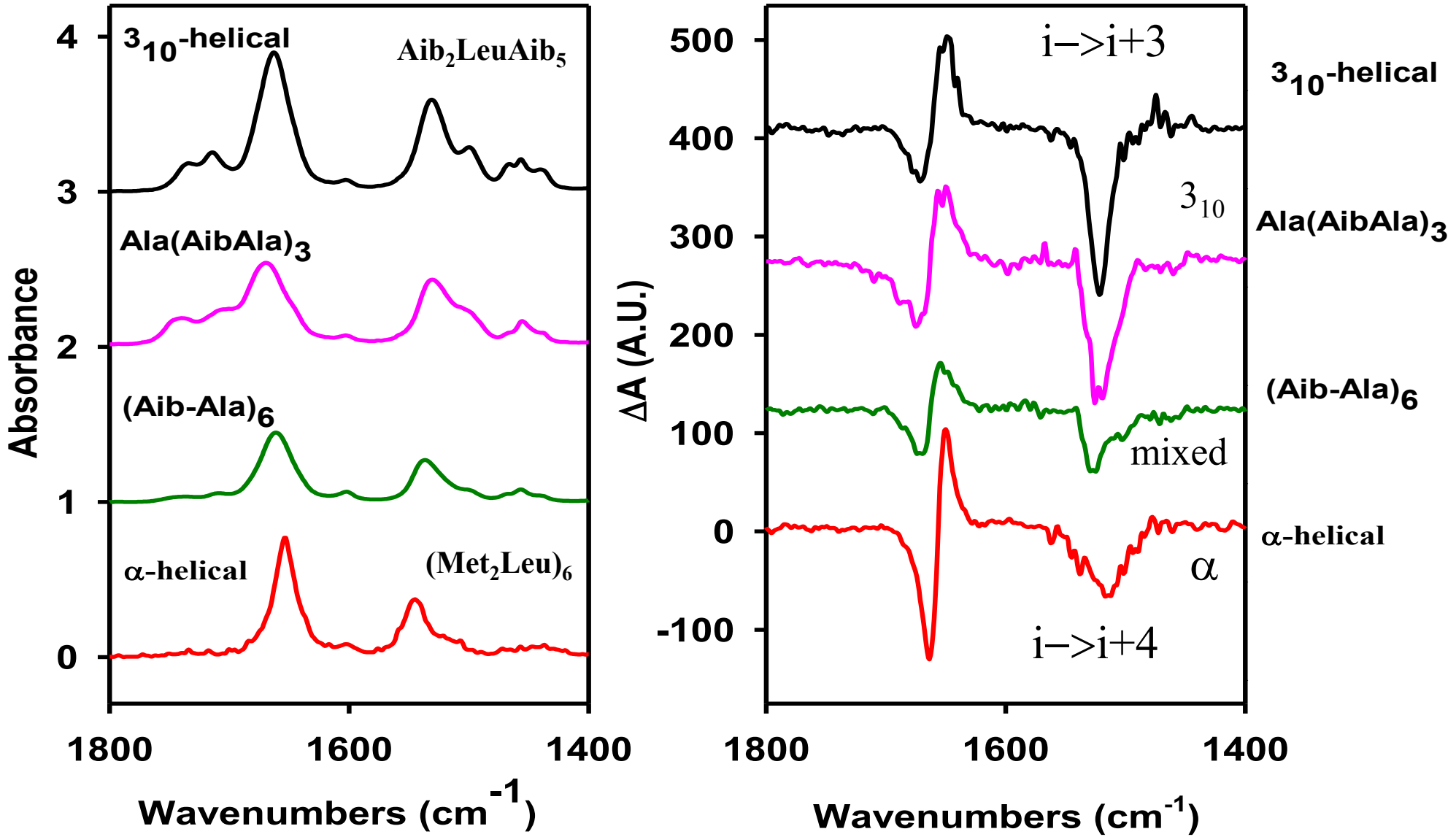


IR ~ differ in ν, VCD - same shape, half size -- partially ordered

Provides a basis for theoretically modeling coil, disorder

Dukor, TAK - Biopoly 1991

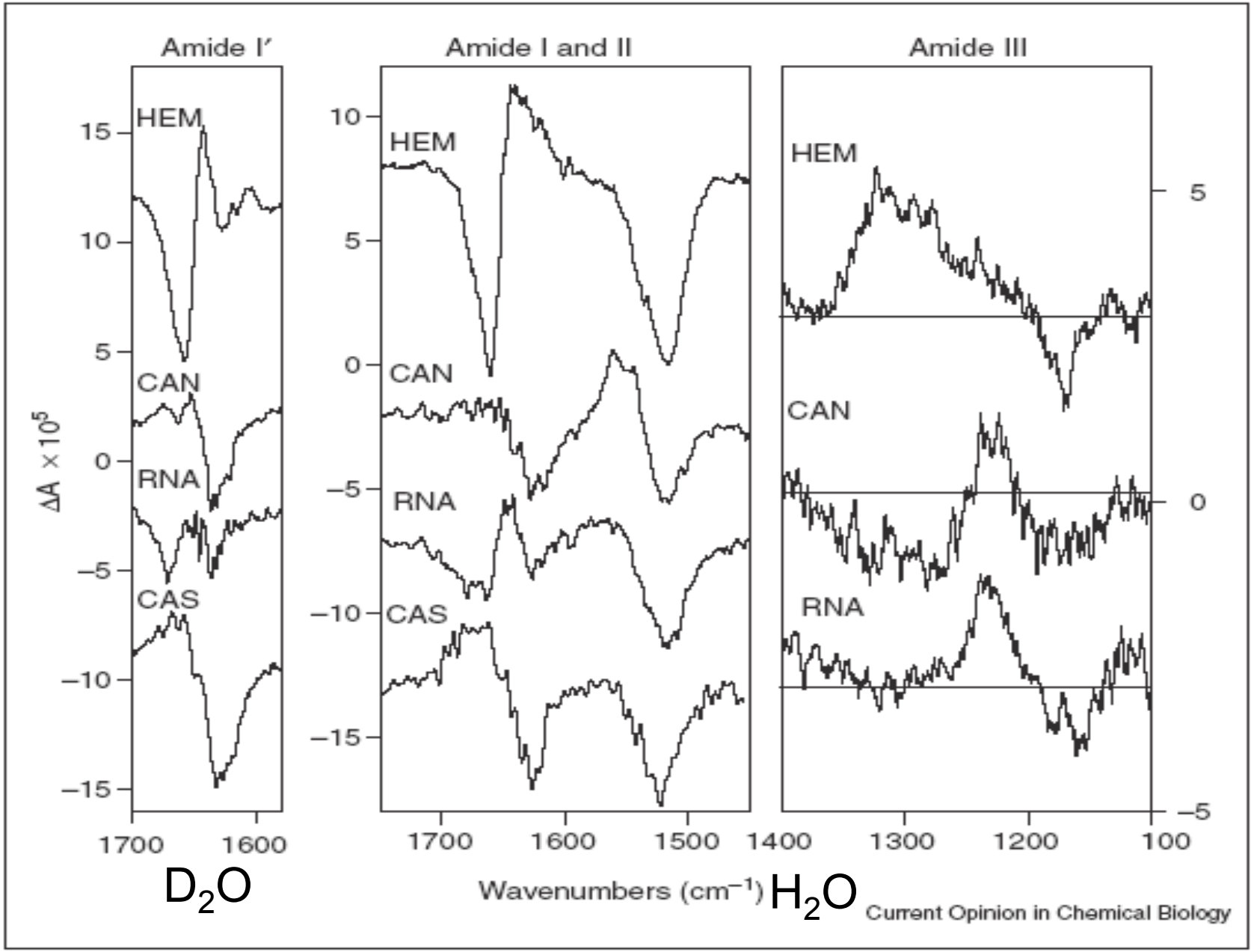
VCD success example: 3_{10}-helix vs. α-helix



Relative bandshapes distinguish similar helices

Yasui, Toniolo, et al. JACS 1986

Proteins - VCD of amide I', I+II and III regions



collaboration with Petr Pancoska

HEM-hemoglobin
High helix

CAN-Concanavalin A
High sheet

RNA –Ribonuclease A
-Mixed

CAS-Casein
unstructured

Summary

IR, Raman, ECD and VCD

-detect structure

-monitor change of structure

with folding processes

Challenge

-Can we understand the spectra-structure link?

Theoretical methods

-Can we use them to model folding mechanism?

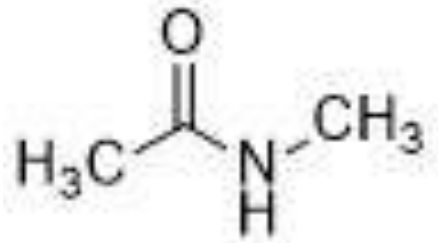
Isotopic substitution

Modeling peptide vibrations, focus: amide I, C=O stretch

Simplest approach, assume all residues identical, *interact with dipole coupling model.* If just consider two amide I modes, can model as C=O stretch oscillator, ν_0, interacting via energy term V, *often assume dipole coupling for V, some small molecule or empirical frequency for* ν_0 -- *example:*

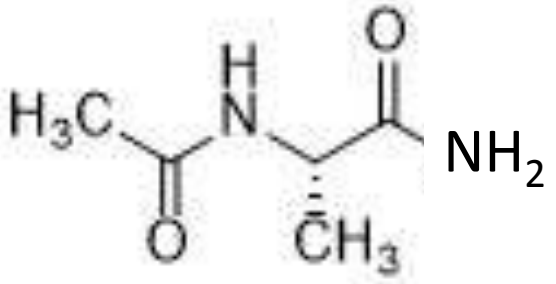
$$\begin{vmatrix} \nu_0 - \nu & V \\ V & \nu_0 - \nu \end{vmatrix} = 0 \quad \text{yields} \quad \nu = \nu_0 \pm V$$

splitting of the two transitions is - 2V



But if not identical, i.e. $\nu_1 \neq \nu_2$, then *splitting no longer gives* V_{12}

$$\begin{vmatrix} \nu_1 - \nu & V_{12} \\ V_{12} & \nu_2 - \nu \end{vmatrix} = 0 \quad \text{yields} \quad \nu = \tfrac{1}{2}(\nu_1 + \nu_2) \pm \tfrac{1}{2}[4V_{12}^2 + (\nu_1 - \nu_2)^2]^{\frac{1}{2}}$$



splitting no longer gives V_{12} *if* $(\nu_1 - \nu_2) >> V_{12}$ intensities differ less

transition dipole coupling : $V_{12} = \{\mu_1 \bullet \mu_2 / R_{12}^3 - 3(\mu_1 \bullet R_{12})(\mu_2 \bullet R_{12})/R_{12}^5\}$

Real peptides - bonds between oscillators, multiple coupling interactions,

dipole coupling is insufficient for near neighbor interactions

Computed IR, Raman and VCD and ROA spectra

The *best practical computations* for the largest possible molecules

Ab Initio (DFT) quantum mechanical calculations – *all modes*

spectra comparable to experiment for ***medium sized*** molecules

Frequencies from force field – *normal modes* - *harmonic*

-diagonalize second derivatives of the energy

Intensities - IR,VCD - change *dipole* moment } *with motion*

- Raman, ROA - change *polarizability* } *along mode*

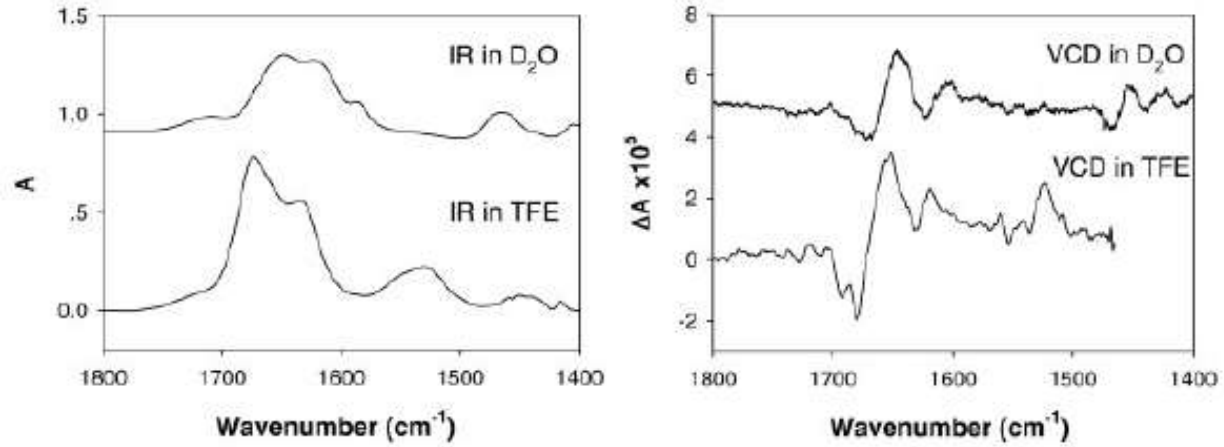
Express as ***atomic properties***, to utilize properties in other calculations

Limitation – medium size (~ hundred 1st row atoms – ***our facility***) and

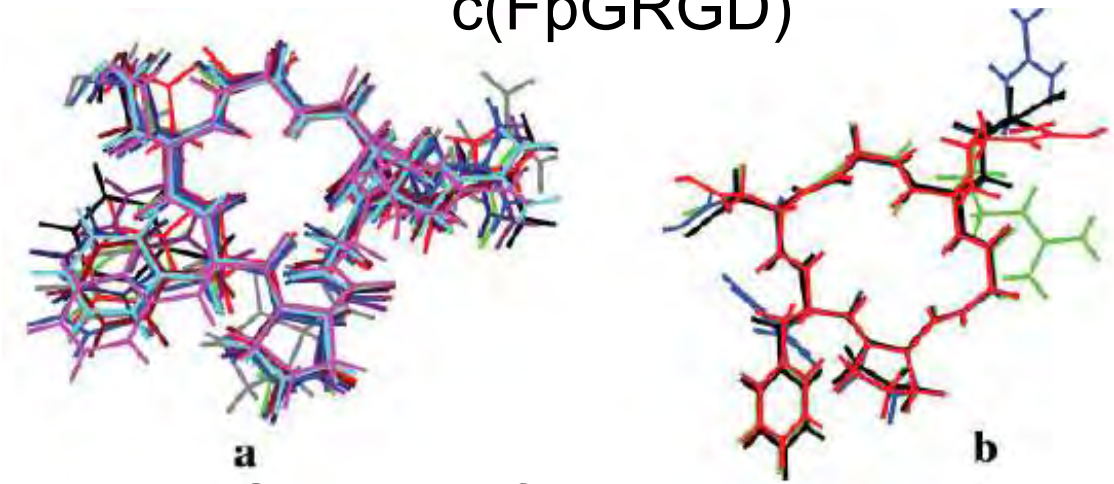
-- static approximation (adding dynamics, greatly expands calc.)

Cyclic pentapeptide: Structure, IR and VCD simulation

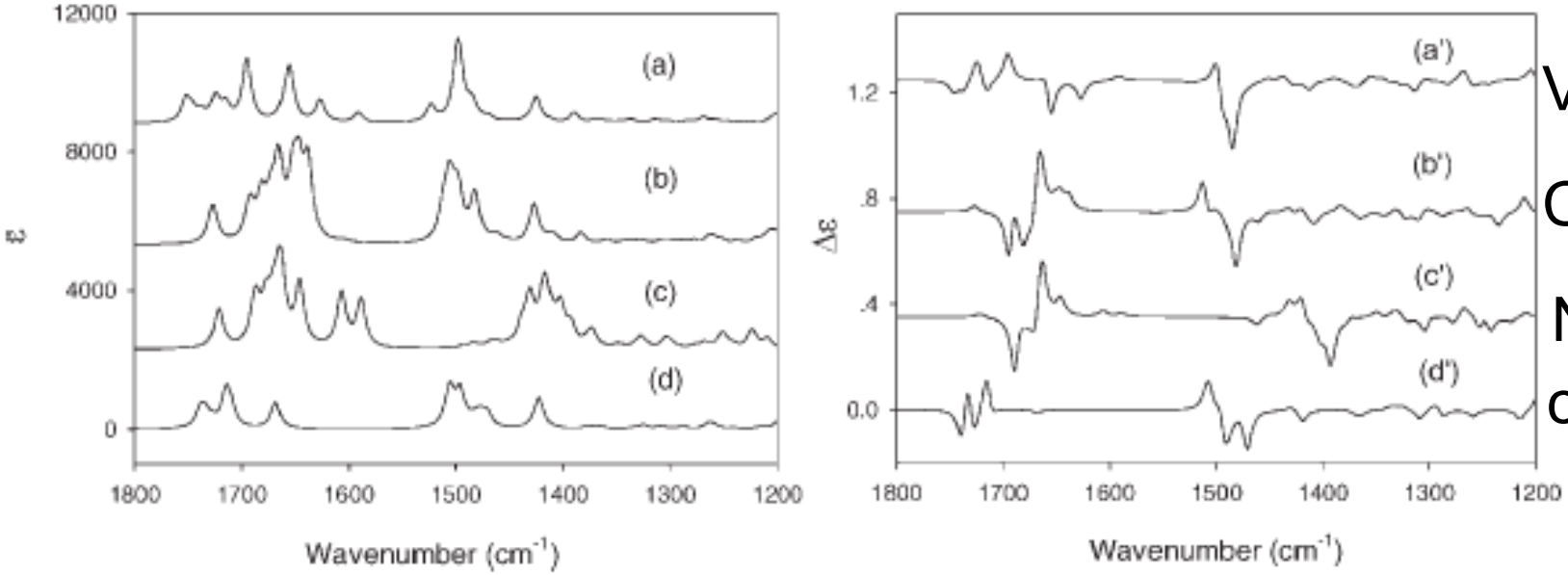
Experimental IR and VCD, D_2O and TFE



c(FpGRGD)



Overlap of MD structures



Vacuum c(FpGRGD)

COSMO c(FpGRGD)

N-deuterated c(ApGAGA)

Bour, Kapitan et al. Chirality 2008

Utilize model DFT parameters for large molecules

Cartesian tensor transfer method of Petr Bour

Large bio-macromolecules -- assume stay ab initio level

-- we use a **trick (**Bour et al. *J.Comp.Chem.* 1997**)**

Transfer atomic properties from "small" model to larger one

i.e. FF, APT-dipole moments, AAT – magnetic dipoles

Method: need polarized basis (e.g. 6-31G*), diffuse help Raman (6-31++G*)

Functional an issue: BPW91 good for amide IR,VCD; B3LYP for Raman

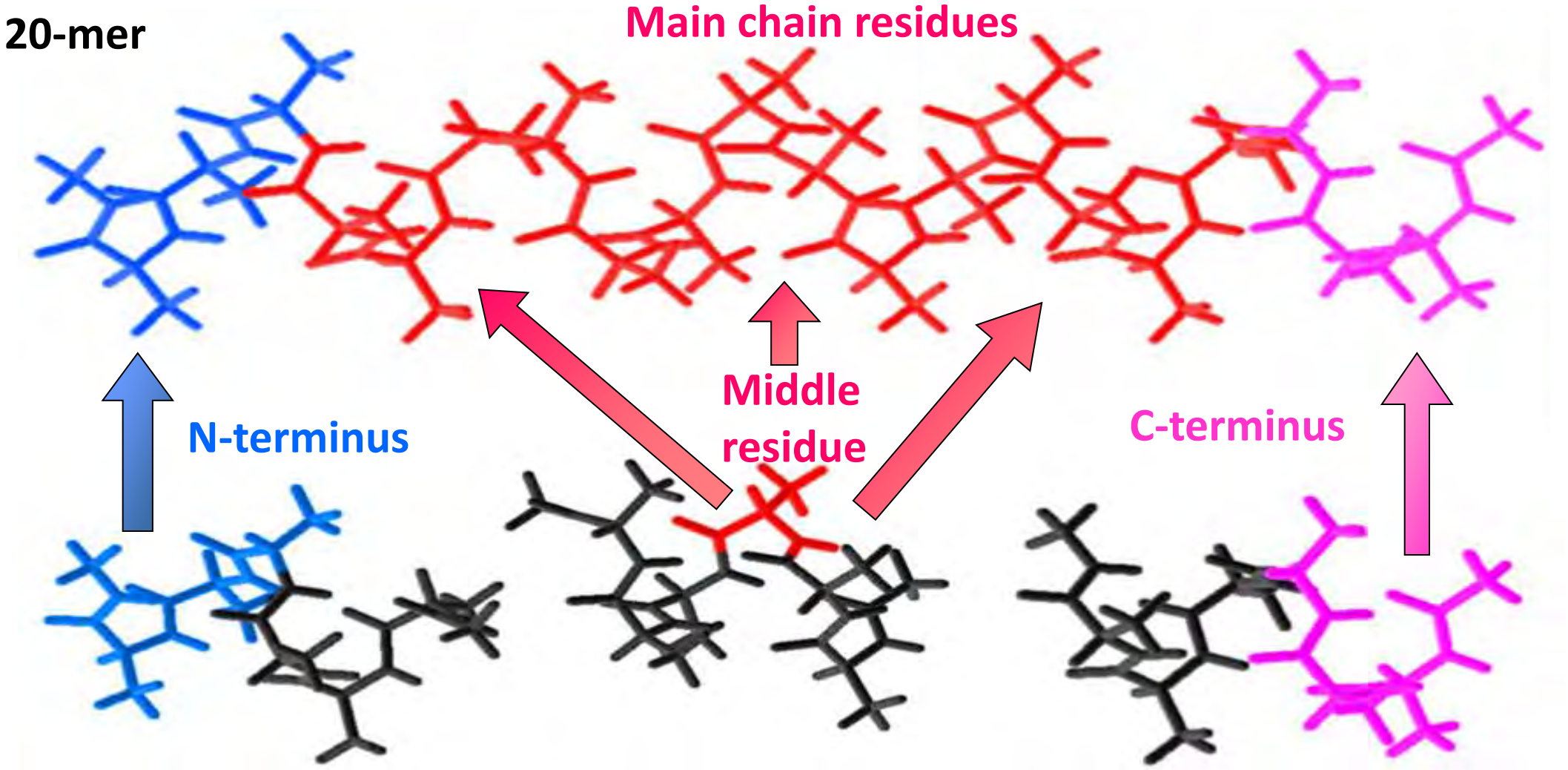
In our case these small model calculations are sometimes "large"—12+ AA

initially tried just tripeptide results, expanded length later

Method best for regular structure (e.g. helices) to propagate model

Transfer of FF, APT and AAT (e.g. Ala_7 to Ala_{20})

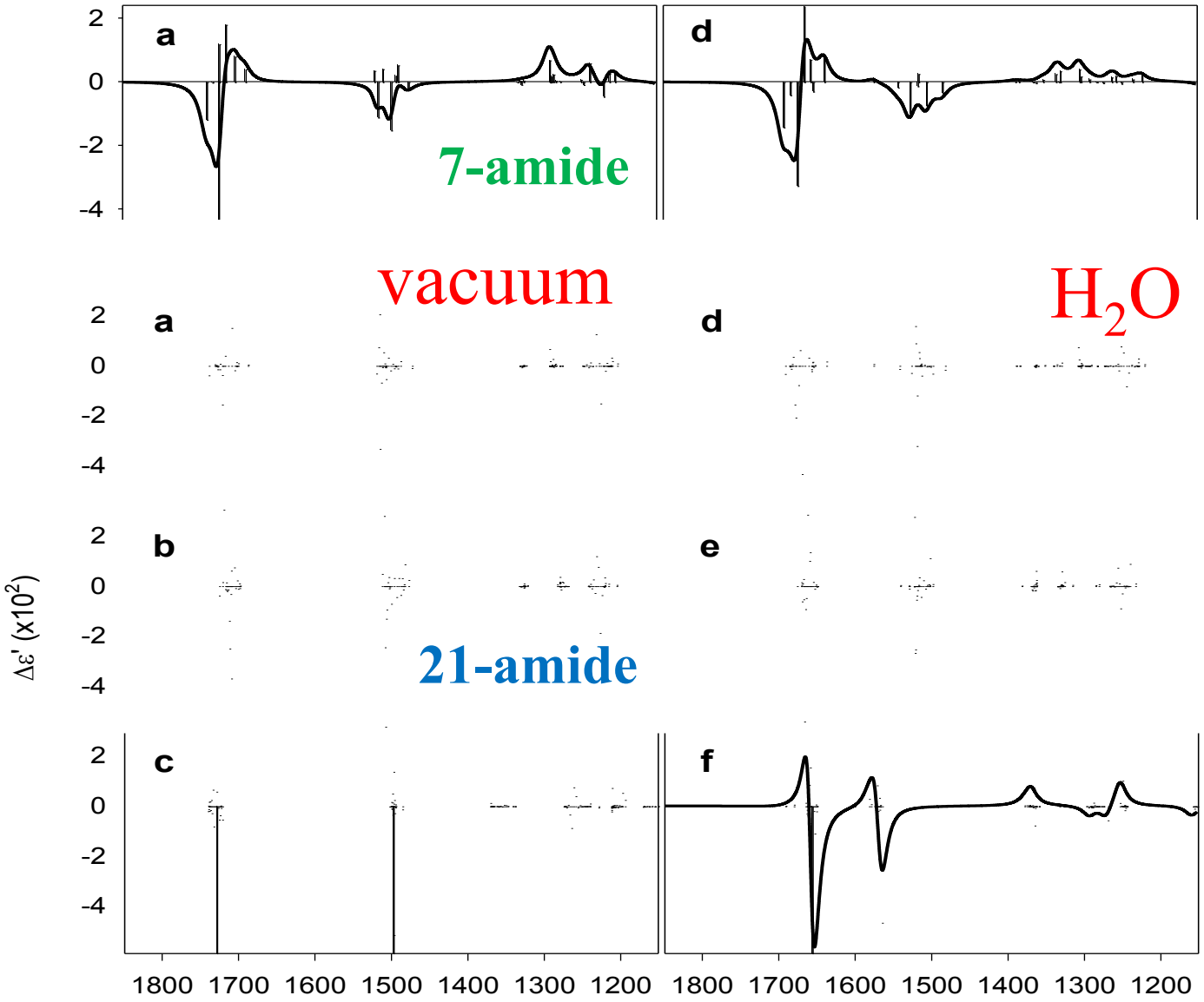
Method from *Bour, et al. J. Comp Chem. 1997*



7-mer: FF, APT, AAT calculated at BPW91/6-31G* level

Kubelka, Bour, et al., ACS Symp. Ser.810, 2002

Uniform long α-helix → characteristic, narrow bands



Frequency error mostly solvent origin

reflect experiment

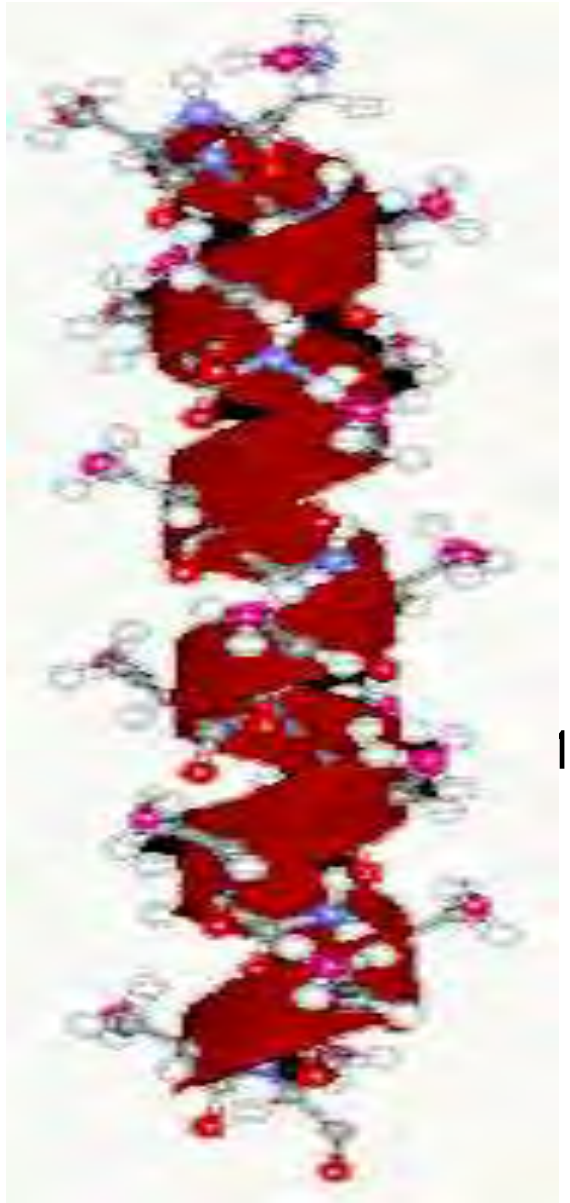
7-amide: dispersed amide I, II bands, *end effects* distort spectra

21-amide: still dispersed, narrow band by *change intensity distribution*, preserve VCD shape,

Solvent -- close amide I-II gap, *improve frequencies*
Preserve band shapes

Kubelka & Keiderling, J.Phys.Chem.B 2005

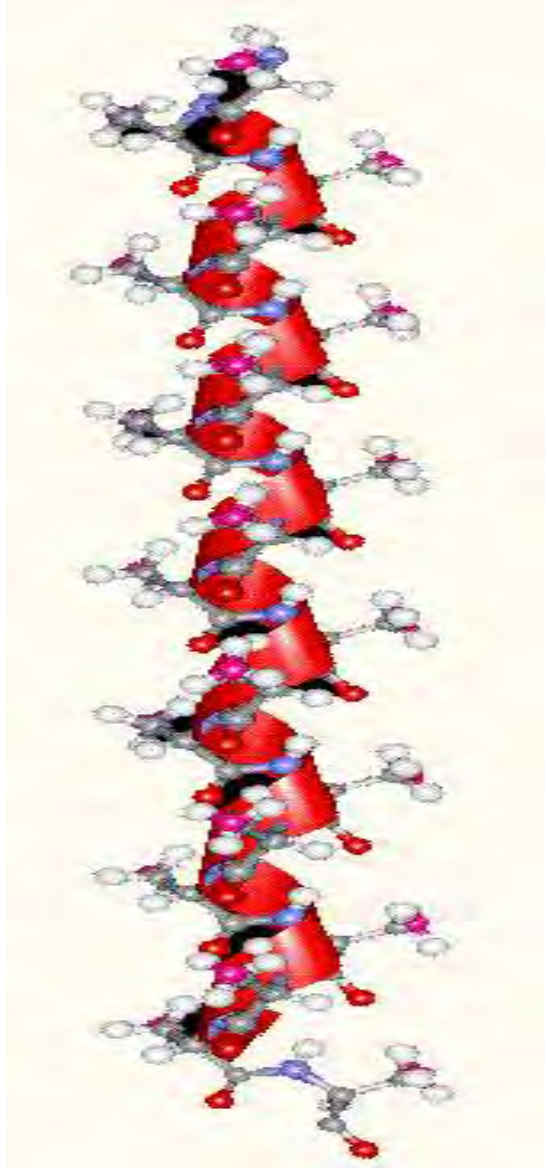
α-Helix vs. 3_{10}-Helix



i+4 ← H-bonding → i, i+3

3.6 ← Res./Turn → 3.0

1.50 ← Trans./Res (Å) → 2.0

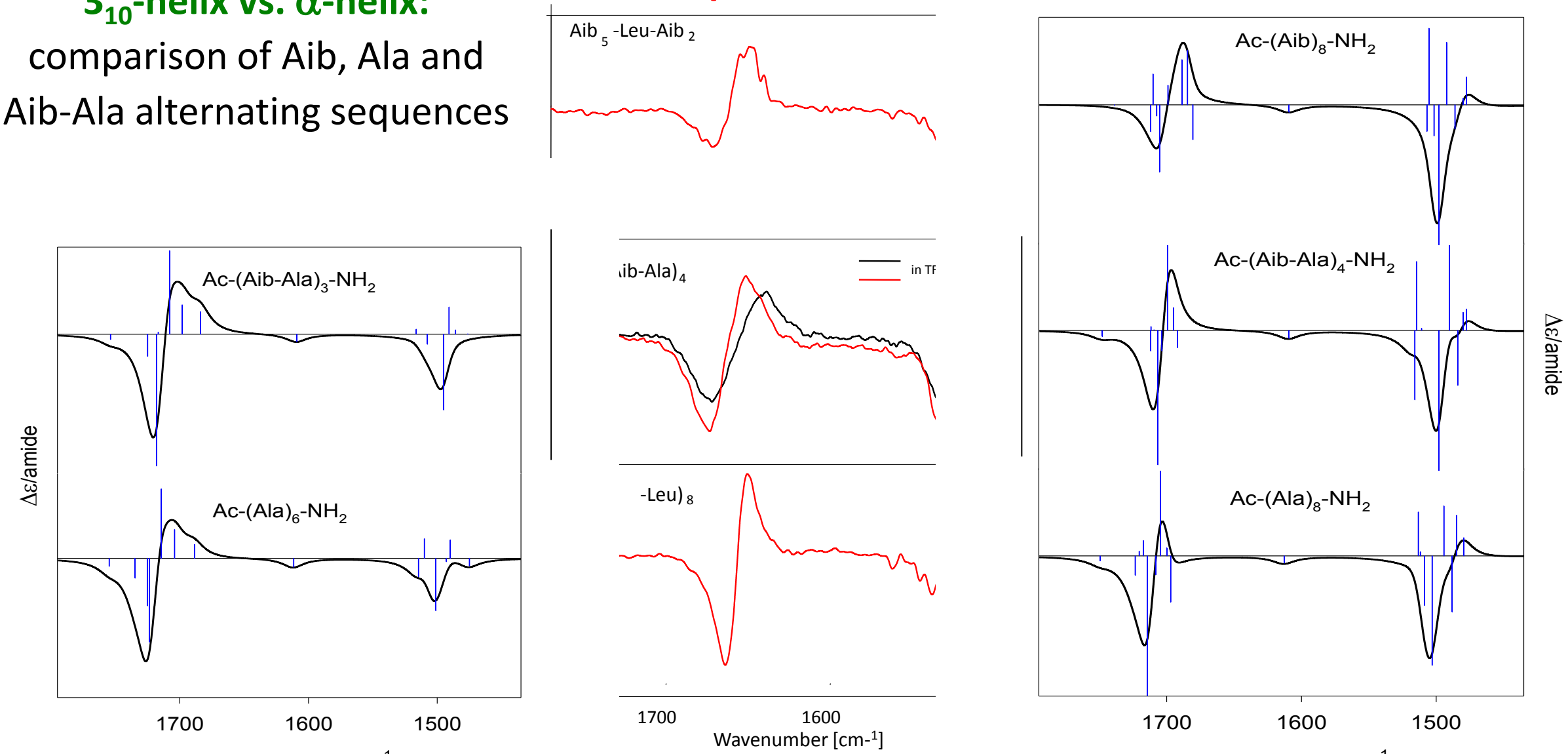


Simulation of Helix IR and VCD Really Works!

3_{10}-helix vs. α-helix:

comparison of Aib, Ala and Aib-Ala alternating sequences

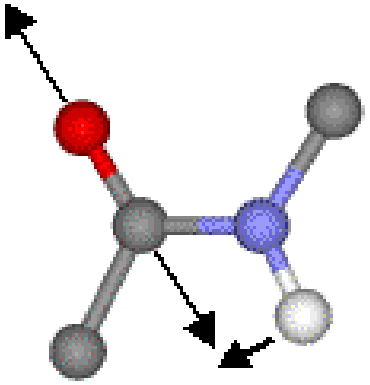
Experiment:



(Kubelka,Silva, Keiderling JACS 2002)

^{13}C Isotopic Labeling

Basic principle of spectra-structure relationships:

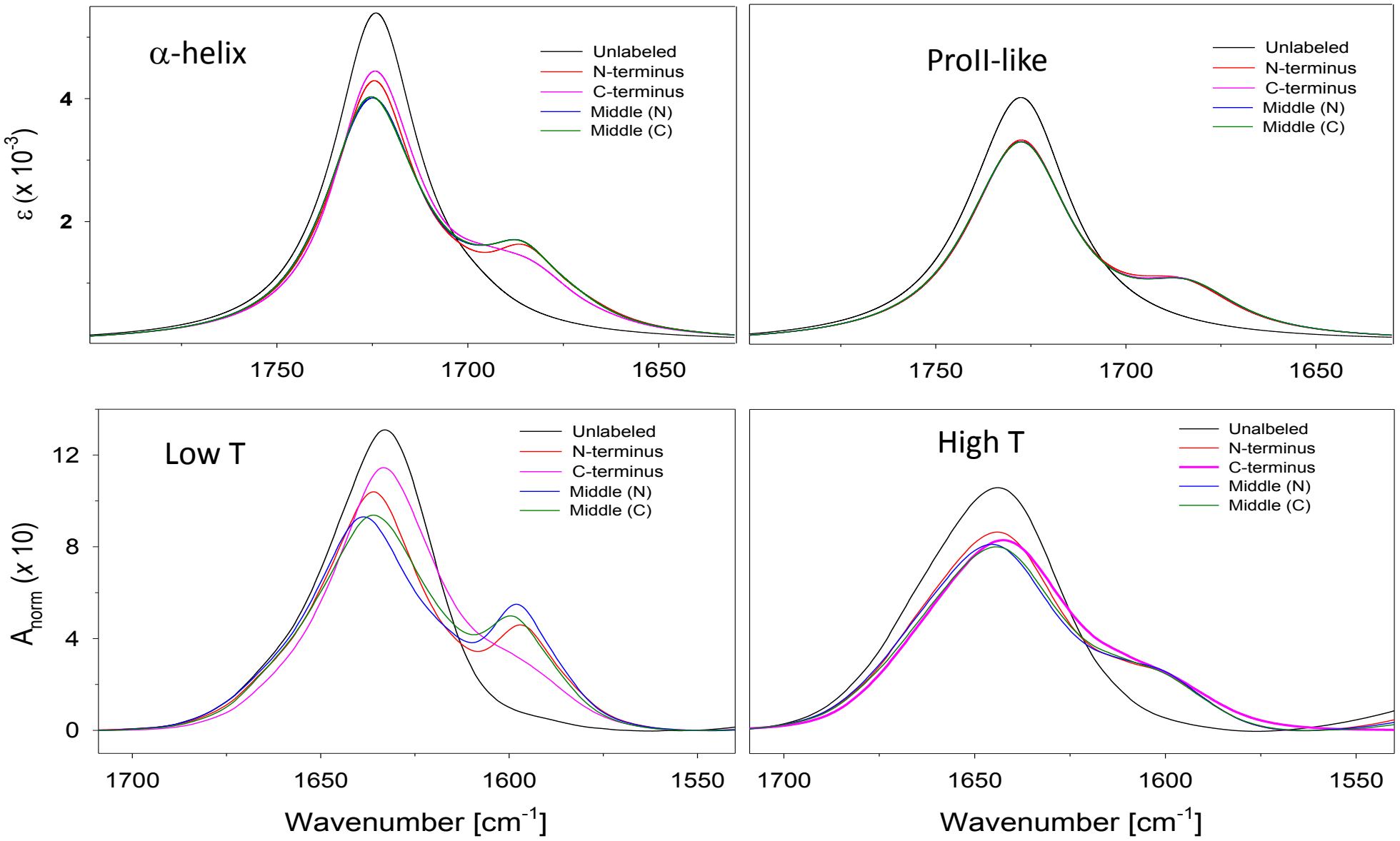


Amide I
(1700-1600 cm^{-1})

coupling → structure

IR limitation: average secondary structure
(delocalization of Amide I vibrational coupling)
Isotopes can break that - give specific sites

Change ^{12}C to ^{13}C on amide C=O
shift amide I down by ~40 cm^{-1} (isotopic shift)
(^{13}C=^{18}O even more ~*70 cm^{-1}*)
Isotopic advantage: **site-specific**
(specific, local Amide I vibrational coupling)



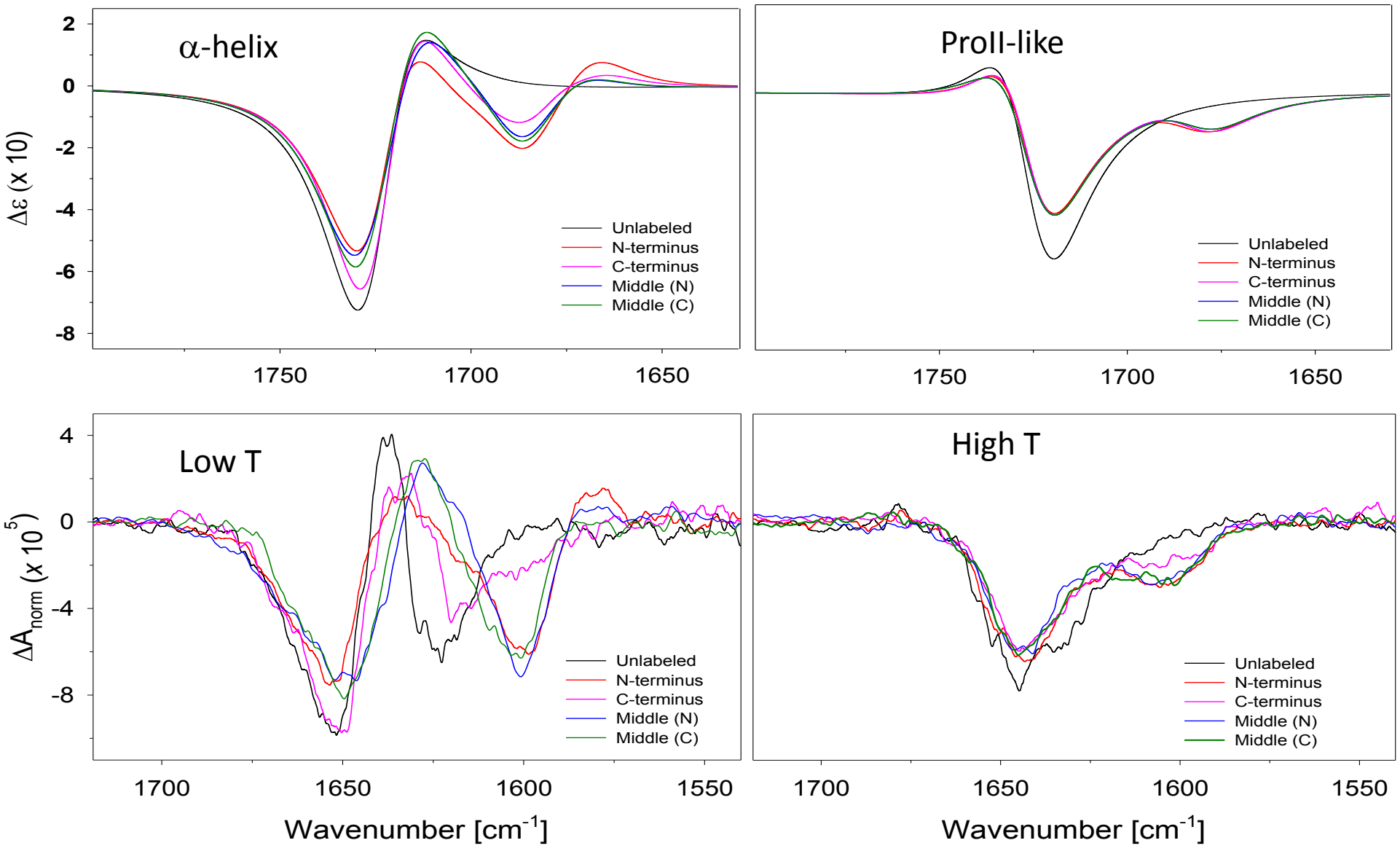
Simulated

Experiment

Simulated & experimental amide I IR : Ala_{20} - ^{13}C labels

IR subtle variation with site

Silva, Kubleka, et al. PNAS 2000



Simulated

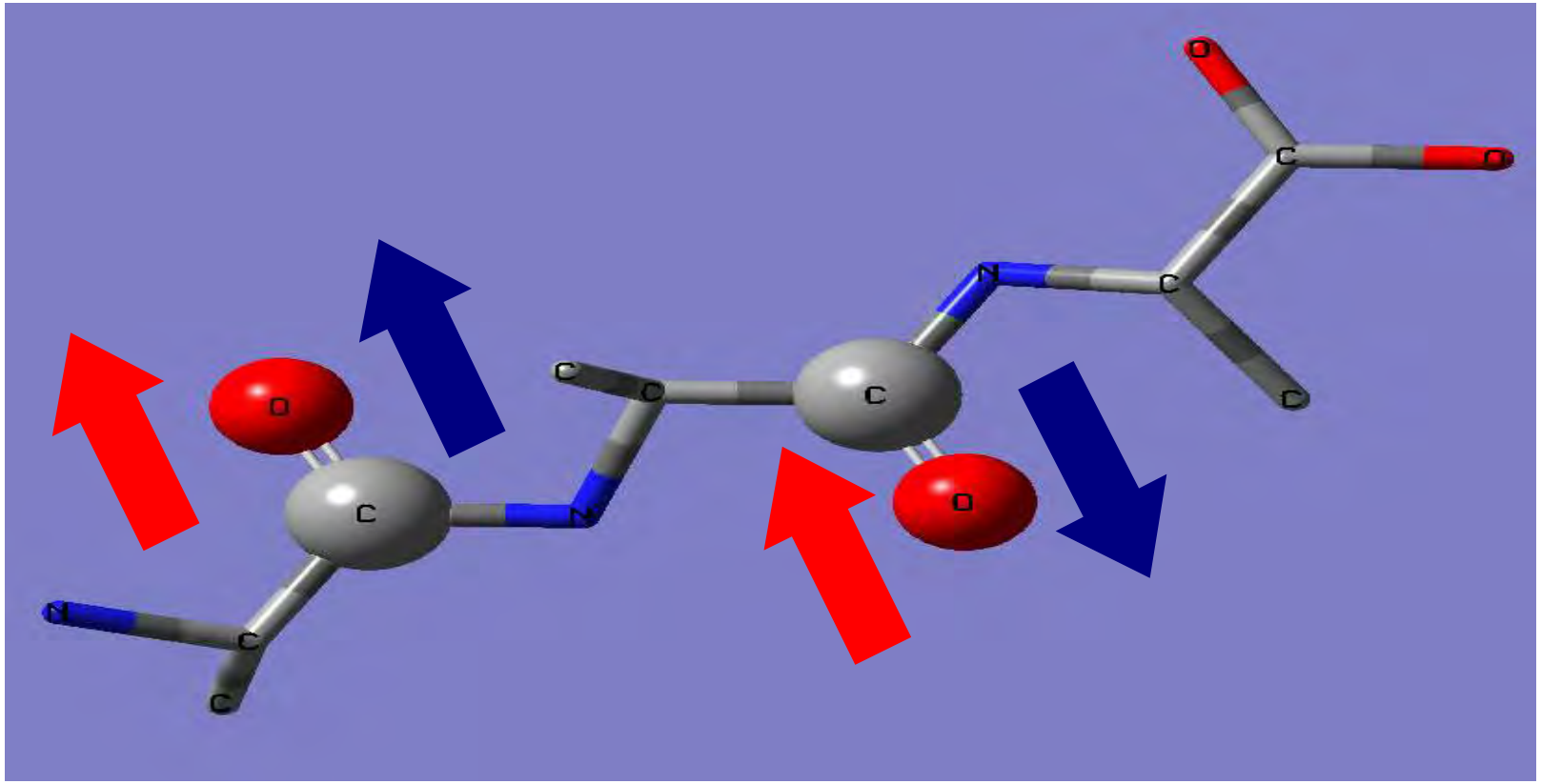
Experiment

Simulated & experimental VCD : Ala_{20} - ^{13}C labels

VCD identify folded/unfolded site

Silva, Kubleka, et al. PNAS 2000

Coupling pairs of modes



Two ^{13}C labeled carbonyls in the amide I mode:

Blue arrows indicate symmetric stretching modes

Red arrows indicate the anti-symmetric stretching.

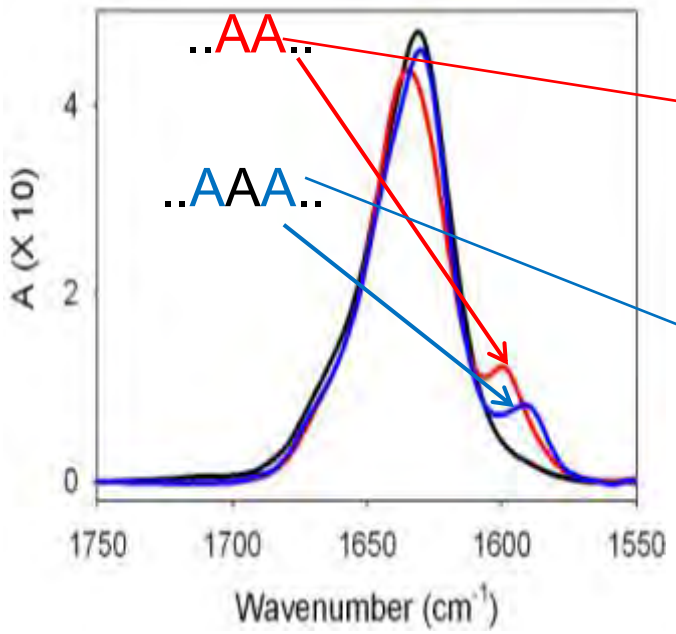
Helix - sym. pair has large dipole, sheet – anti-sym. large

Computation of ^{13}C Amide I IR absorption - α-helical Ac-(A_{25})-NH_2

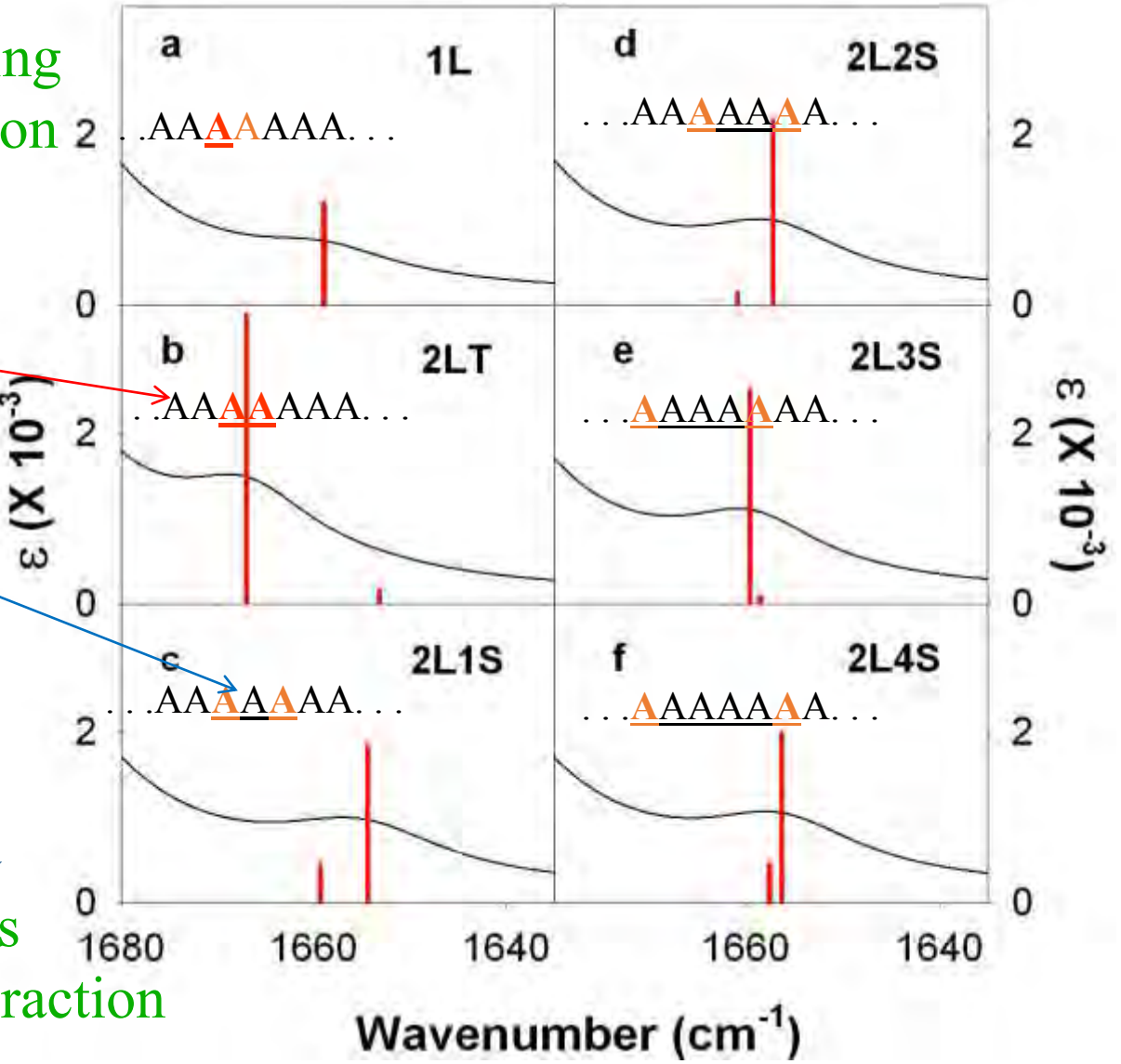
Two labels coupled

Increasing separation

Experiment



Ac-$(AAAK)_4AAAAY$-NH_2



Less interaction

Calc. All Ala residues ideal helix dipolar coupling OK beyond 2-3 residue decrease with distance.

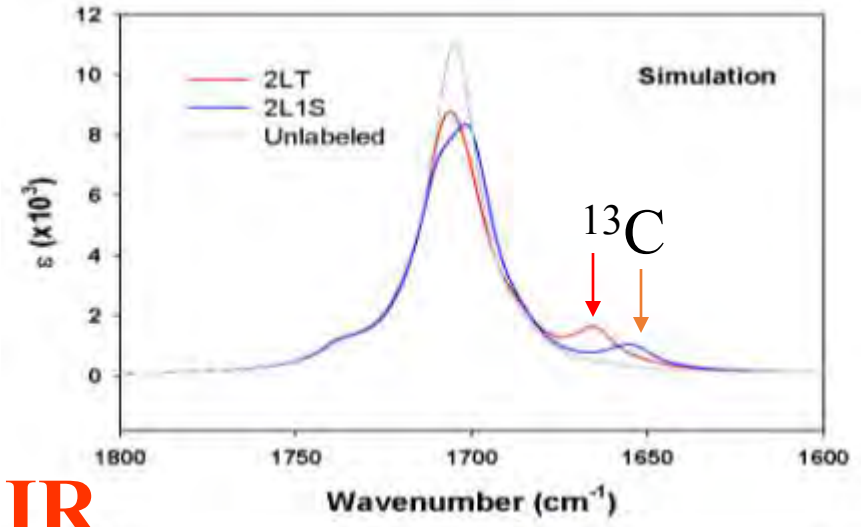
Sign flip depends on geometry

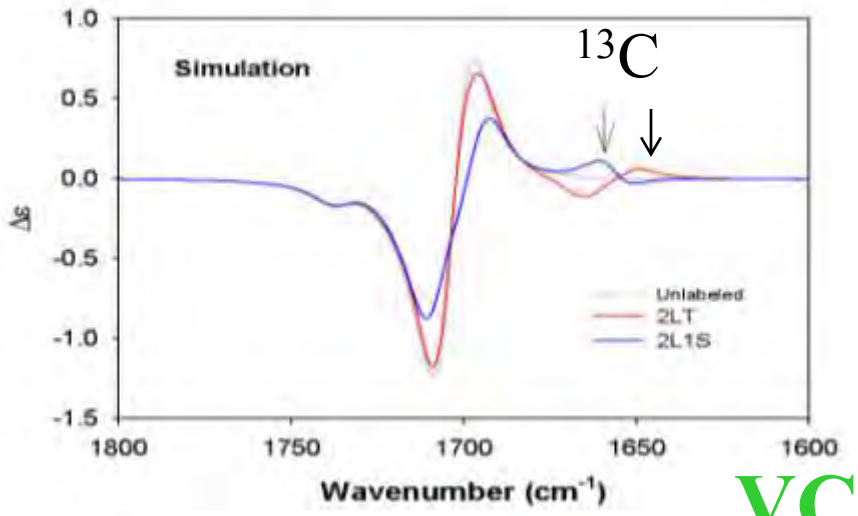
IR experiment sees one component, intense one of pair

Position of band is positon of one mode, not measure of coupling

Simulated IR bands for <u>labels in different relative positions</u> with different isotopic shifts for sequential and alternate labels due to sign change in coupling constant.

Relative position – 2 labels - experiment and theory (transfer)

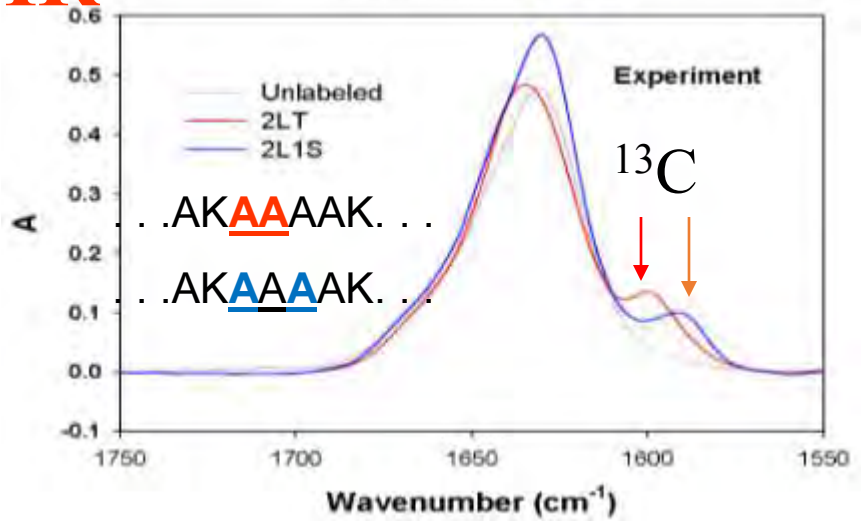


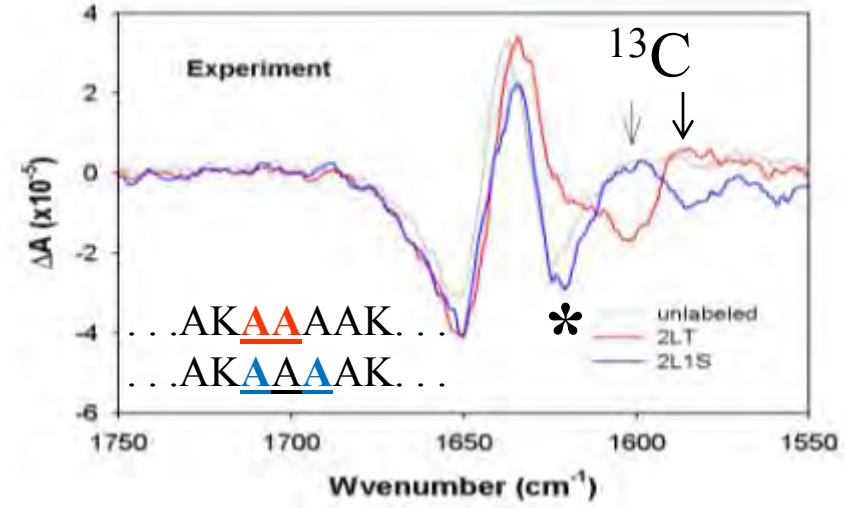


IR

VCD

Ideal, all Ala helix Ac-A_{25}-NH_2





Ac-$(AAAK)_4$-AAAAY-NH_2

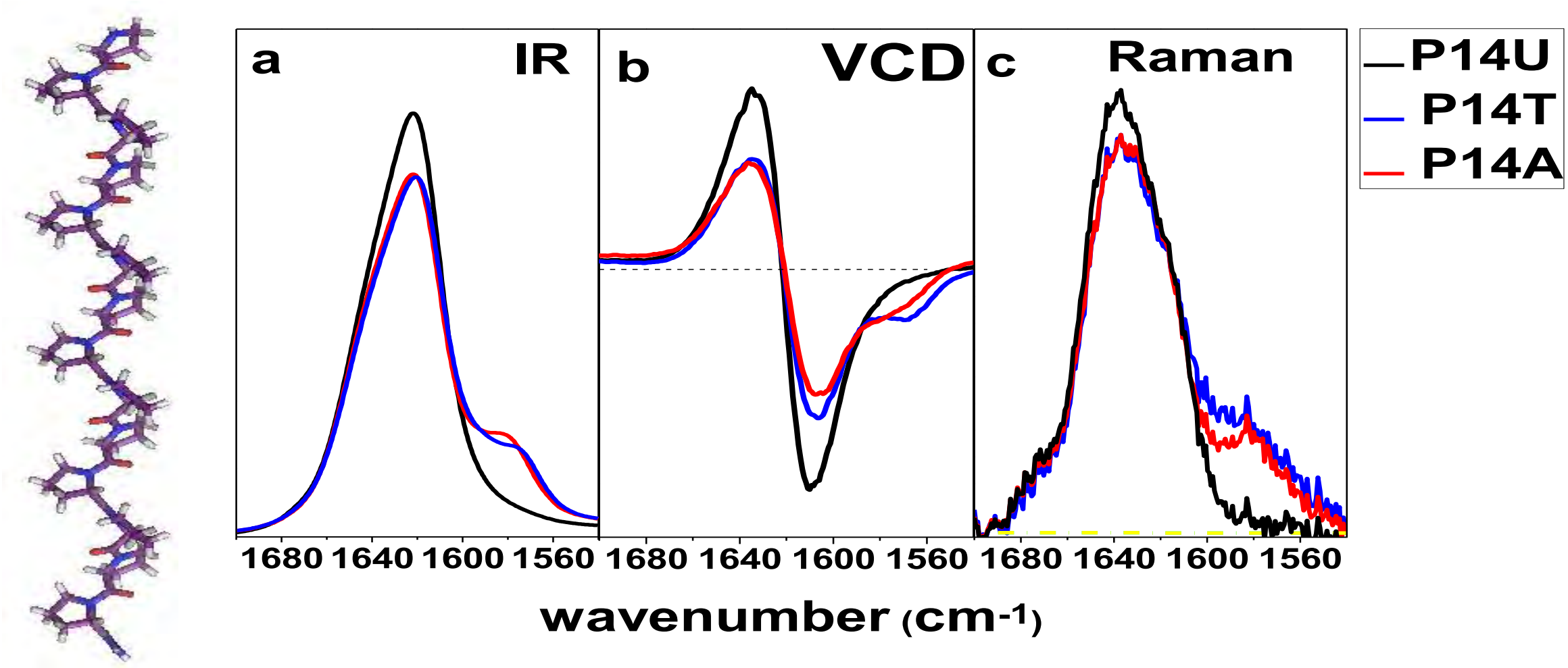
Two sequential labels have higher IR freq. due to coupling (intensity in high ν mode),

VCD : sequential (2LT) - same sign ^{12}C and ^{13}C, but opposite sign if separated (2L1S)

* since exp. in D_2O a (-)band develops the amide I, but not modeled without solvent

Coupling sign is source of variation – VCD directly measure *Huang, et al. JACS 2004*

Labeling PPII Helices – Less Coupling – Pro_{14} example

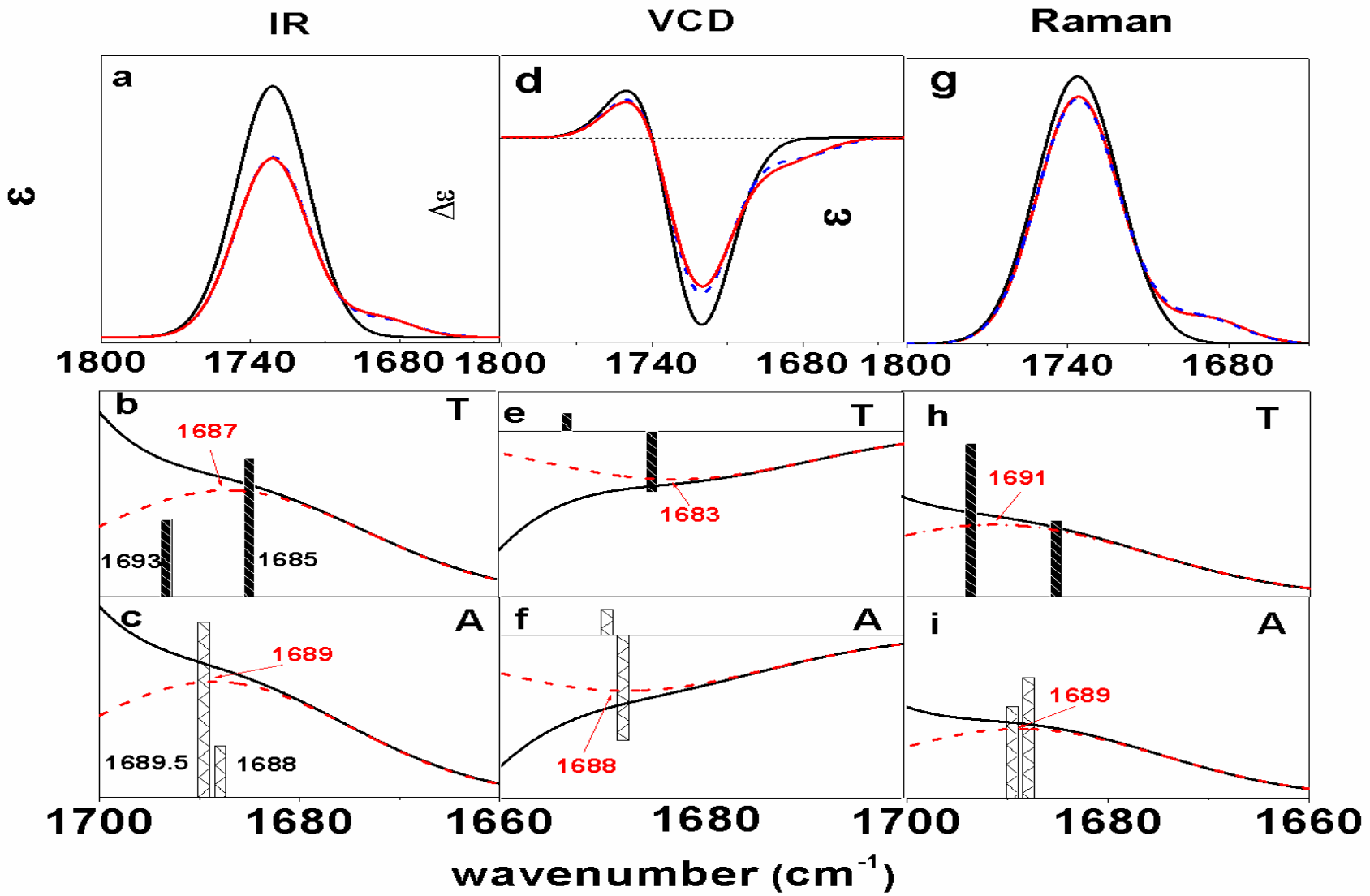


See much less difference in adjacent and separated labels

But shift can be interpreted and fits theoretical prediction

H.Chi, et al. JPCB 2010

Theoretical model of PPII coupling



Full amide I like experiment

Computed Labeled bands

Adjacent

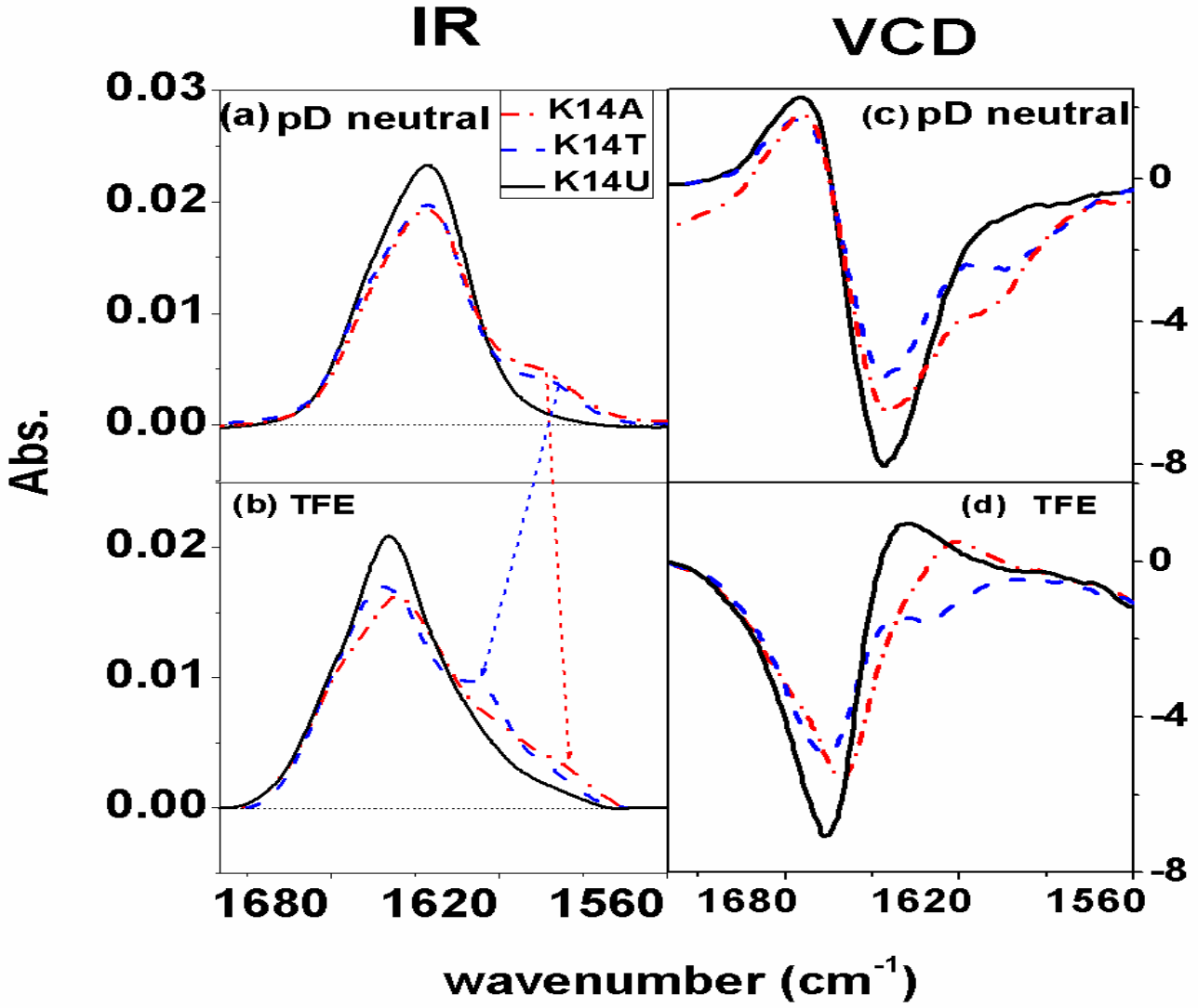
Separated

H.Chi, A. Roy, et al. JPCB 2010

Separated labels weaker coupling, opposite sign from adjacent

Same approach works with 3_{10}-helices, except signs, length – *Lakhani, et al JPCB 2011*

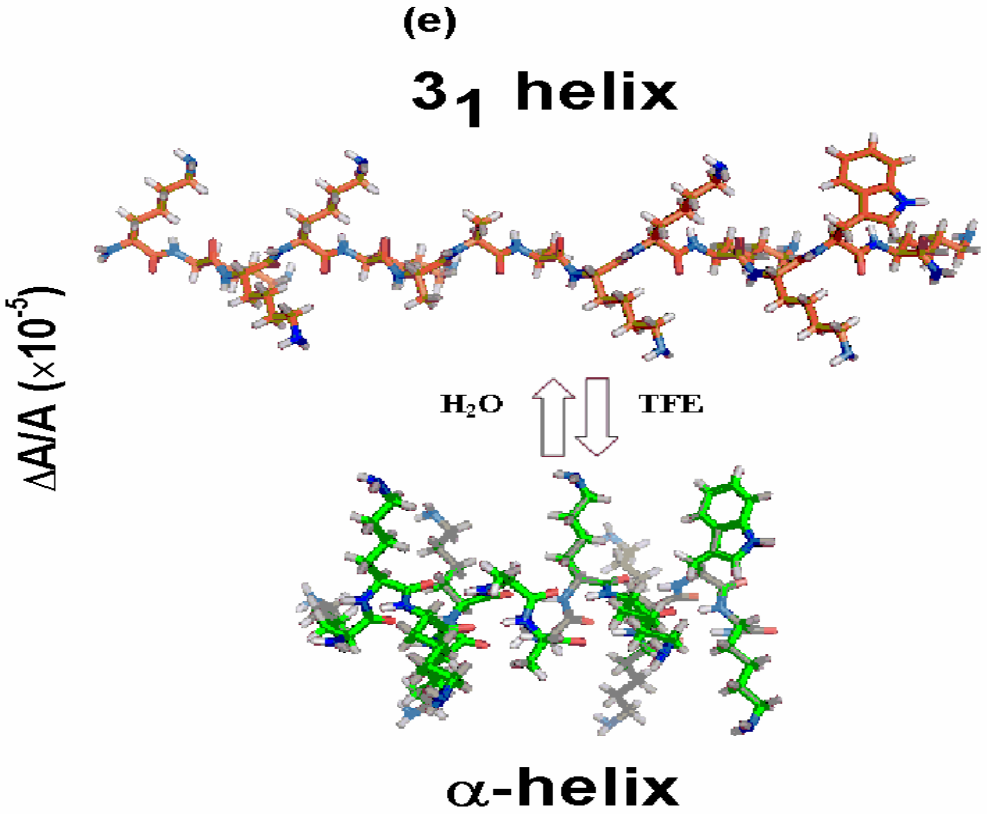
Same for "Coil" – Poly Lys coupled like PPII



Titration with TFE induce coil → helix transition

Big change in coupling seen

But can convert with TFE



Proof - coil is locally PPII

Lys_n same coupling as Pro_n

H.Chi, et al. J Phys Chem 2010

β-sheets 2nd common secondary structural type

Problem : Multiple types:

- Parallel and anti-parallel
- In- and out-of register
- Flat and twisted
- Stacked, aggregates and fibrils

All can affect spectra, IR, Raman, VCD etc.

Theory & isotopes can help sort out effects

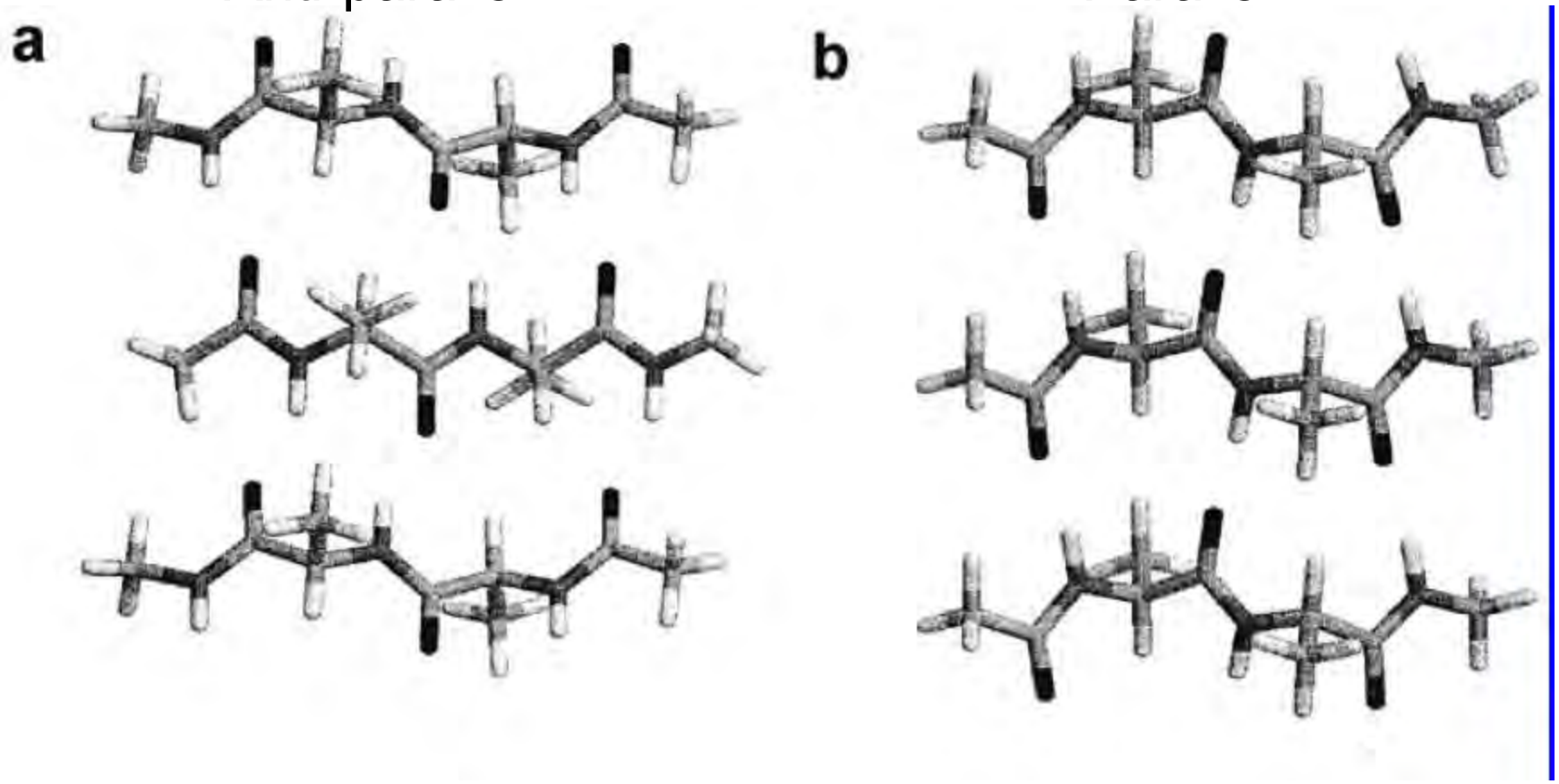
Hairpins offer small, monomers to start study

Hydrophobic interactions or turn design stabilize

Strand alignment in β-sheets

Anti-parallel

Parallel



H-bonds straighter, two kinds of rings

Identical rings, bent H-bonds

Transfer of property tensors – β sheet a bit different

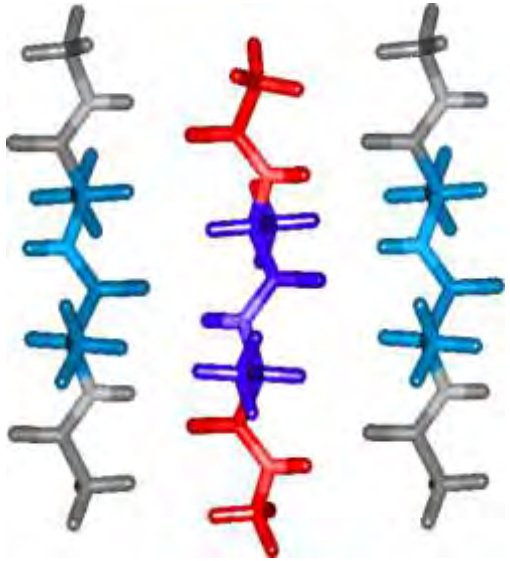
(Bour et al, *J. Comp. Chem.* **18**, 646, 1997)

source "small" molecule: → **target "LARGE" molecule**

FF, APT, AAT from DFT: BPW91/6-31G** → empirical couple FF, APT, AAT

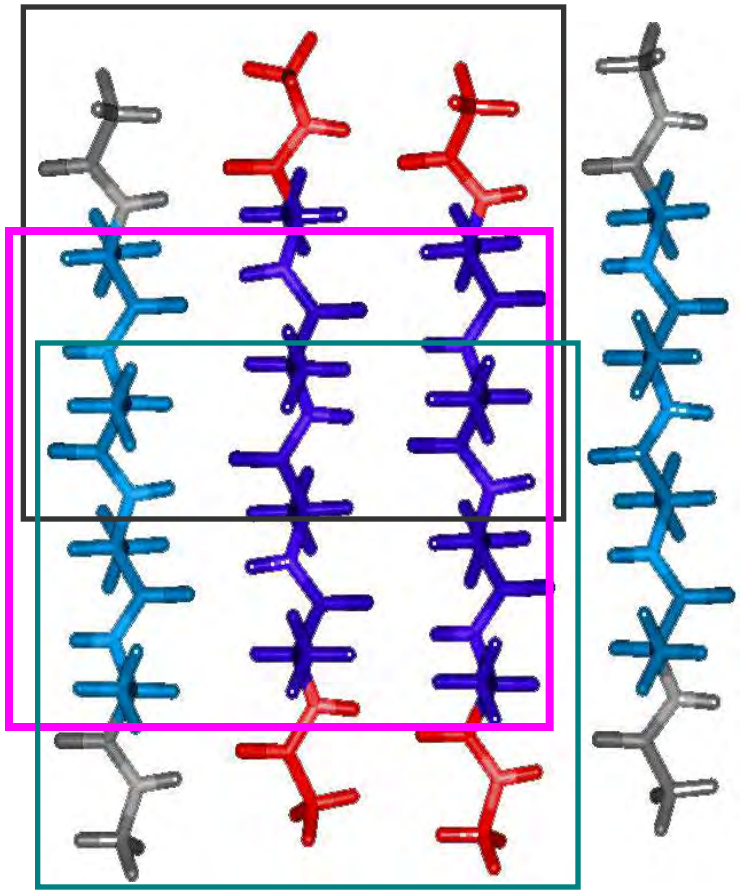
The small molecule "overlaps" residue type with all corresponding parts of the target structure - local interactions between fragments and in strand are included .

Parameters from the ***edge ends*** are transferred onto the ***edge corners***.



Parameters from ***inner center*** residue transfer onto the ***inner strand residues (bulk of the sheet amides)***.

Parameters from the ***inner strand ends*** are transferred onto the ***inner ends***.

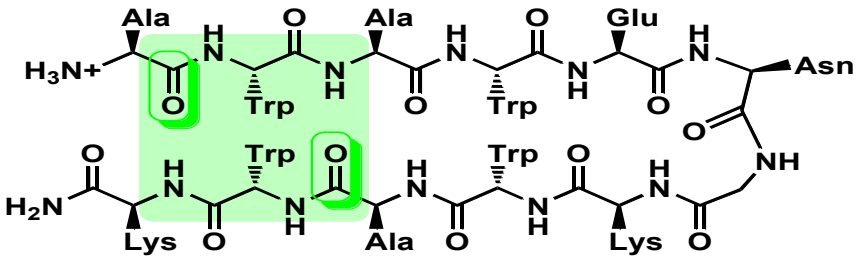


β-sheet applications: Kubelka & Keiderling *JACS* 2001.

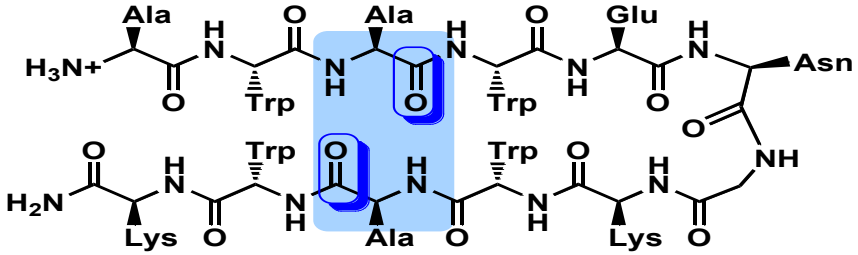
$^{13}C=O$ isotopic labeling of TZ2C

Simulations predict $^{13}C=O$ coupling

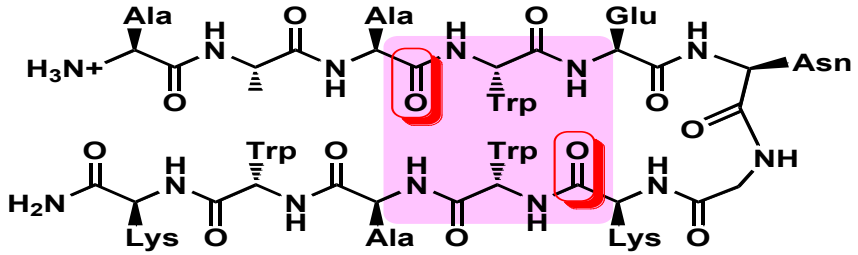
A1A10

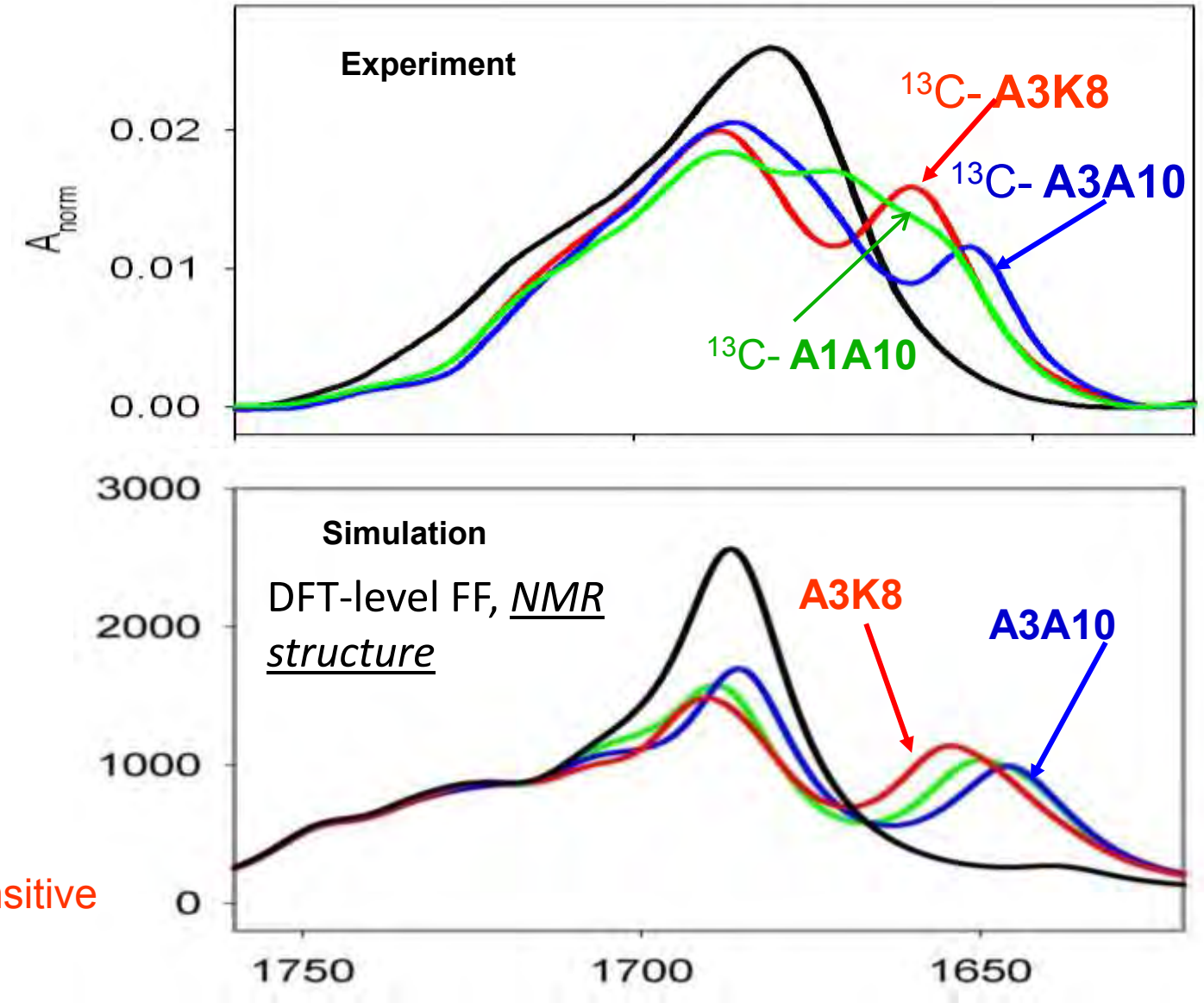


A3A10



A3K8





Cross-strand vibrational coupling is position sensitive

Huang, Wu et al. JPhysChem B 2009

Summary – equilibrium variation

Tm values differ for ^{12}C and ^{13}C modes
Also differ for frequency shift and intensity change

Inconsistent with 2-state model for unfolding

Fits earlier conclusions, folding multistate ensemble

Dynamics might give mechanistic insight

Acknowledgements

UIC work:

Hairpin/helix studies

Dr. Rong Huang

Dr. Ling Wu

Dr. Heng Chi

Fibril studies:

Dr. Heng Chi (Aurora) – Huai'an

Dr. Ge Zhang

Dr. Ahmed Lakhani

Fernando (Ralph) Tobias

Modelling

Dr. Anjan Roy

Yue Wei

Dr. Ahmed Lakhani, Allen Walker *(Coll.St.Joseph)*

NMR:

Dr. Dan McElheny *(UIC)*

Fibrils – Prof. Wojciech Dzwolak – *Warsaw*

Theory collaborators:

Prof. Jan Kubelka, Will Welch – *Wyoming*

Prof. Petr Bour, Jiri Kessler – *Czech Academy*

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