Laser induced Temperature jump

IR pulse heats the solvent

(Raman shifted YAG to 1.9 μ for D_2O)

Probe heated spot with tunable IR laser

(Pb-salt diode, FTIR experiments proposed)

Fast MCT needed for ns response

Repetition rate limited by cooling back initial state

Analysis is relaxation kinetics, $k_{rel} = k_f + k_r$

Signal average thousands of shots,

single frequency (diode laser) normal method

Callender/Dyer general T-jump setup

Fluorescence use cavity doubled, lots cw power

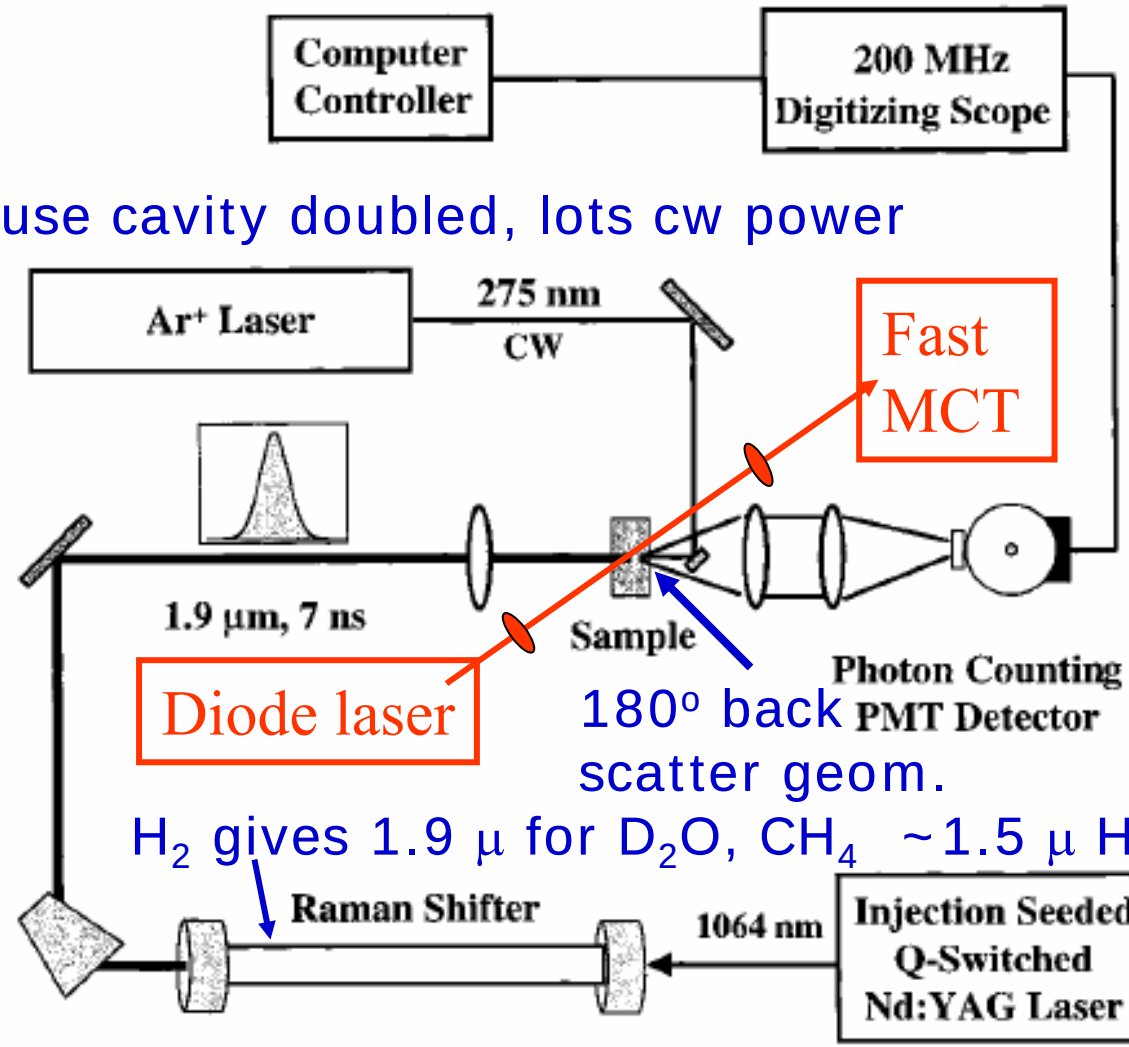


FIGURE 2: Schematic of the fluorescence *T*-jump spectrometer. See the text for details.

Generic design for T-jump, IR diode laser detection transmit to MCT

Brian Nolan*, Edward Gooding*, Sapna Sharma♦, Martin Volk♦

Chemistry and Biochemistry, Swarthmore College ♦Surface Science Research Centre, University of Liverpool

Variant of Dyer design uses difference frequency generation, shorter pulse smaller ΔT

Temperature-jump experiments used direct D_2O excitation via a pulsed 1.9 μm pump and a CW probe with fast detection and a digitizing scope

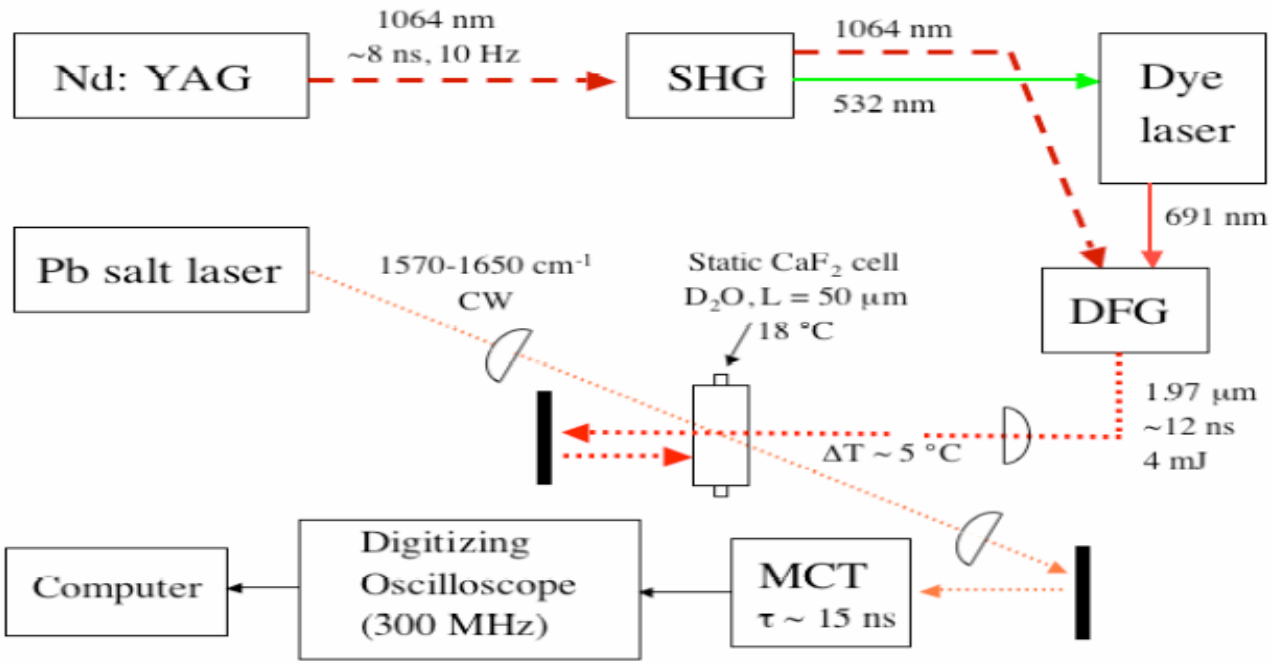


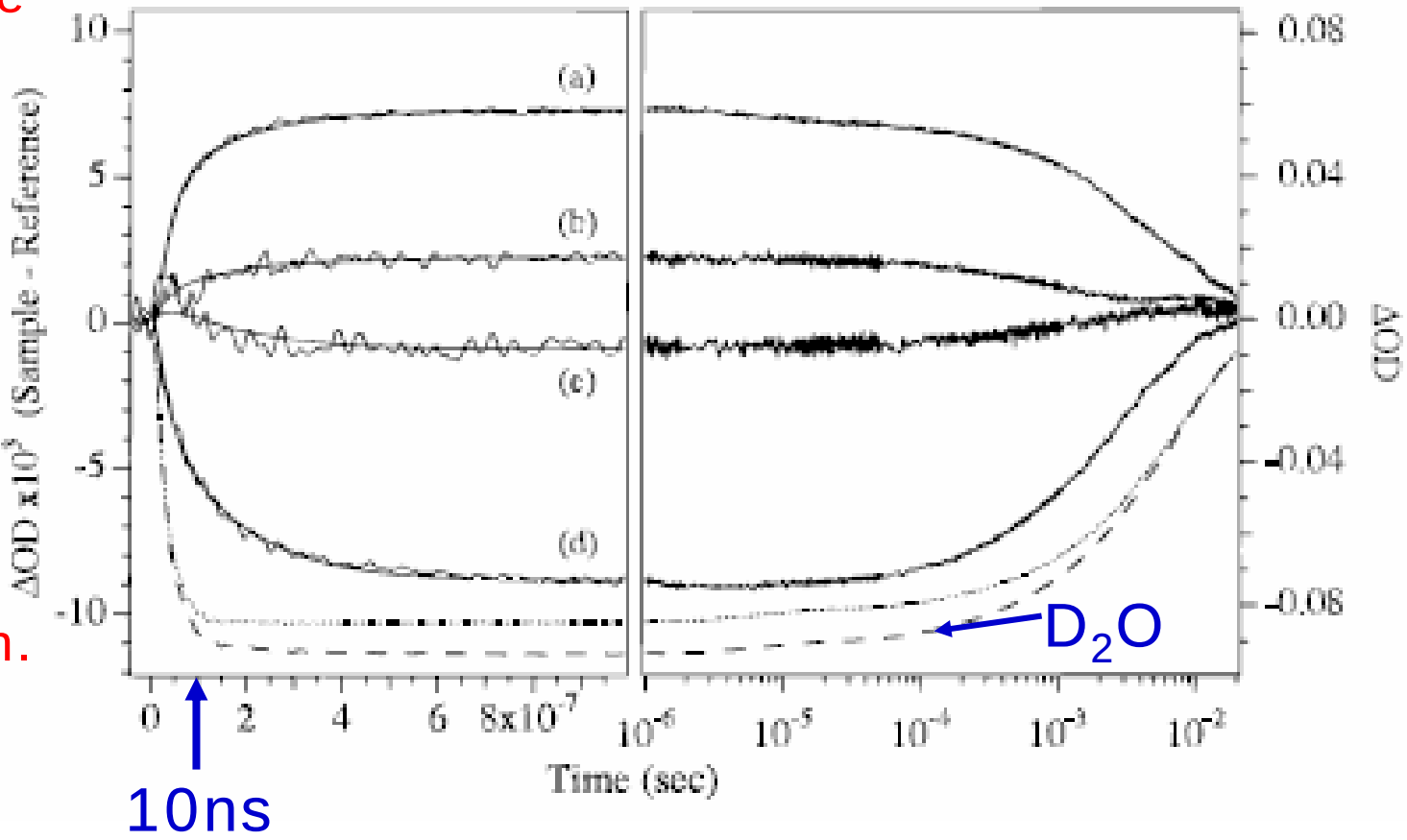
Fig. 2. The pump beam (heavy lines) at 1.9 microns directly pumps a D_2O absorption band. The excitation is converted to a temperature rise of about 5 °C on a subnanosecond timescale. We monitor the change in absorbance of the (CW) probe beam in the amide I' region with a fast digitizing oscilloscope.

Character of Temperature jump--timing

Helix example

D_2O - - -
Sample .....
Difference:
a-1655 cm^{-1}
b-1644 cm^{-1}
c-1637 cm^{-1}
d-1632 cm^{-1}

Fit to biexpon.
<10 ns
160+/-60 ns



Pump ΔT at 2μm focus to 300μm, 110 μm path use split cell
fast (50MHz) MCT detector, avg. 9000 shots, 10 Hz

T-jump calibrated by change of D_2O absorption with temperature
$3.0x10^{-5}$ to $4.0x10^{-5}$ (OD)/°C.μm for 1700 and 1632 cm^{-1}

Apo-Mb kinetics, T-jump Fluorescence & IR

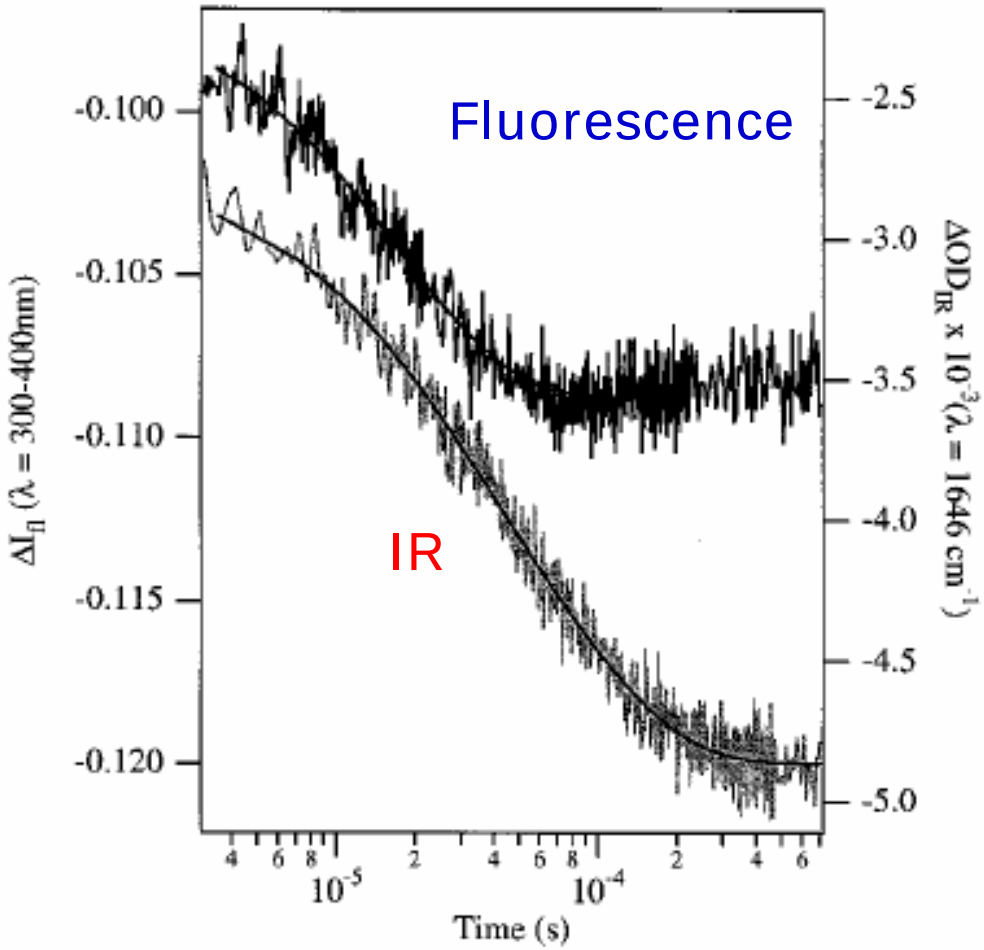


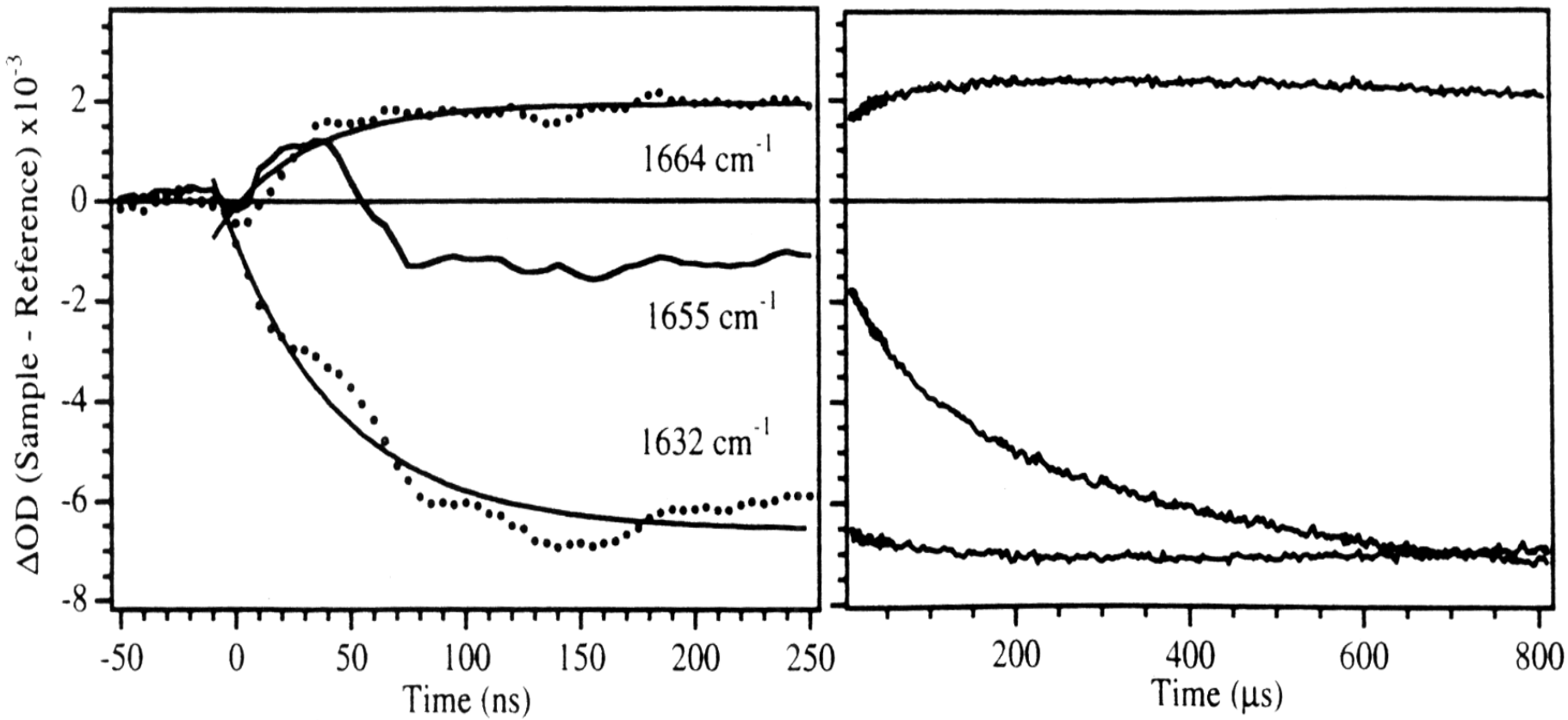
FIGURE 4: Fluorescence (top trace) and IR (bottom trace) kinetics traces for the E state of apoMb following a *T*-jump from 42 to 54 °C. Both samples were in 20 mM NaCl at pH* 3.0 (D_2O). The concentration of apomyoglobin used in the fluorescence studies was 2 mg/mL. The concentration used for the IR studies was 4−8 mg/mL.

Follow different processes, μs response

Fluorescence – tertiary structure unfold

IR – secondary structure - helices

Kinetic IR response to T-jump (45-60 C) - apo Mb



Solvated helix (1632 cm^{-1}) lost very fast, ~100 ns, as is 1664 (turns?)
protected helices (1655 cm^{-1}) slower. Laser pulse heat water in 10's ns

Gilmanshin, et al. PNAS 1997

T-dependence of rates (Arrhenius)

IR-two phases
Fluorescence (+)
match fast IR (x)

Activation energy can be determined

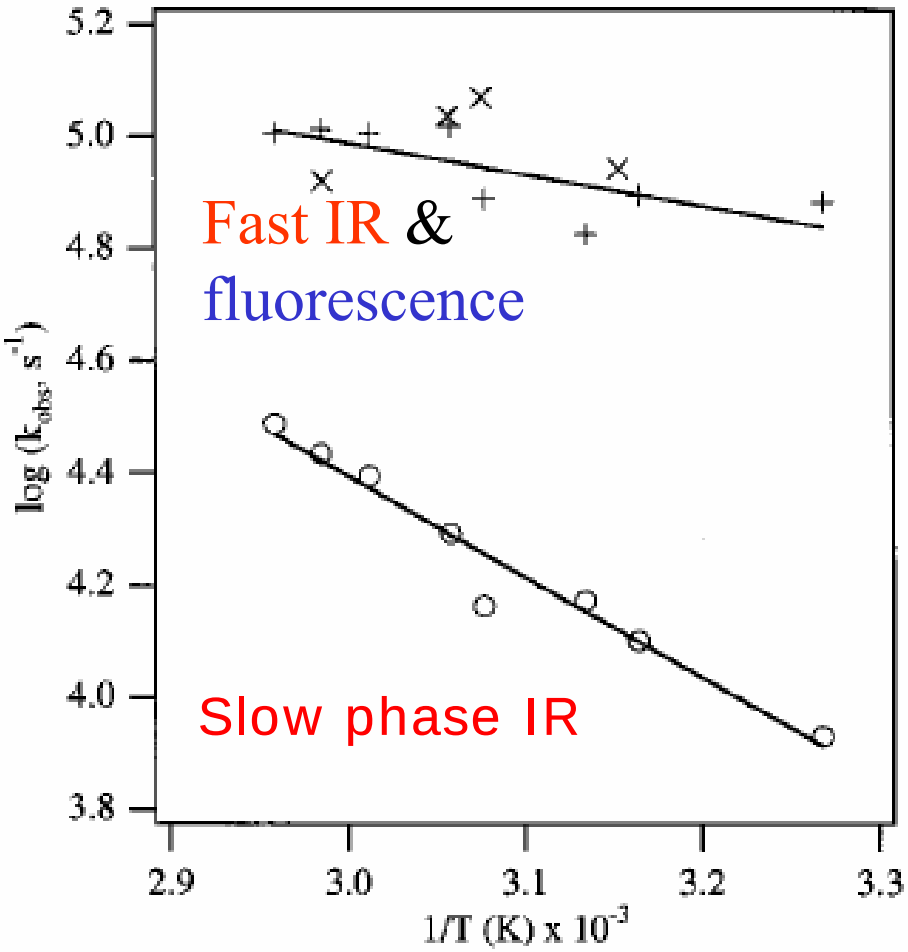


FIGURE 5: Temperature dependence of the two phases. The $1/T$ axis represents the temperatures reached during the T-jump. The data points show the rate dependence for the fluorescence data (+), the fast phase in the IR data (×), and the slow phase in the IR data (○). A linear least-squares regression algorithm using an Arrhenius function was used to determine the best straight line fit to the data (both the emission and IR data being used in the fit). The apparent activation energies obtained from the fit are 2 and 8 kcal/mol to

Vilin head-piece – very fast folder

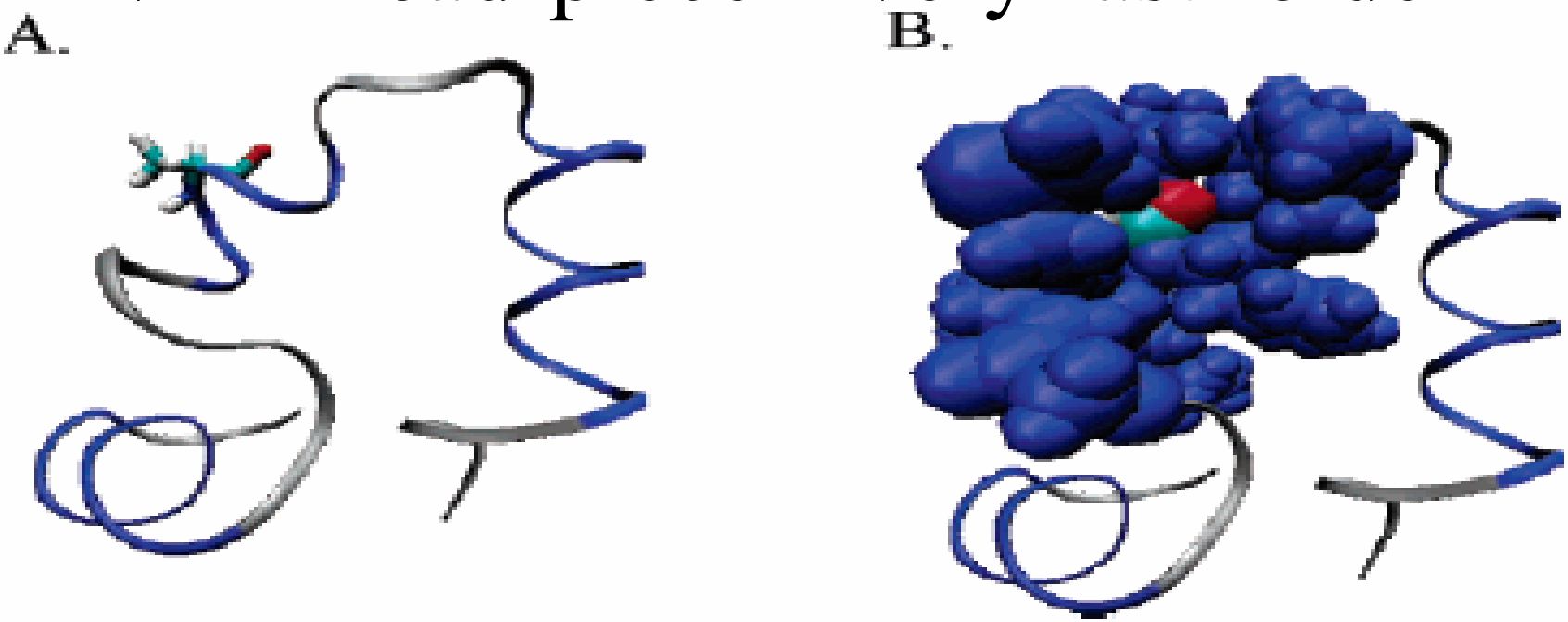


FIGURE 1: Structure of the villin headpiece subdomain (HP36) (Protein Data Bank structure 1VII) highlighting the alanine residue (A57) containing the amide $^{13}C{=}^{18}O$ isotopic label (A). The four residues on either side of A57 are shown in a space-filling representation (blue) to illustrate the solvent accessibility of the carbonyl group of A57 shown in cyan (carbon atom) and red (oxygen atom) (B). The sequence of the protein is MLSDED-FKAVFGMTRS*A*FANLPLWKQQNLKKEKGLF with A57 in italics. The number of the first residue is 41 using the numbering system from previous publications. The figure was generated using the program VMD (*38*).

Biochemistry **2007**, *46*, 3279−3285

A57 ^{13}C labeled Vilin Head-piece Results (IR/T-Jump)

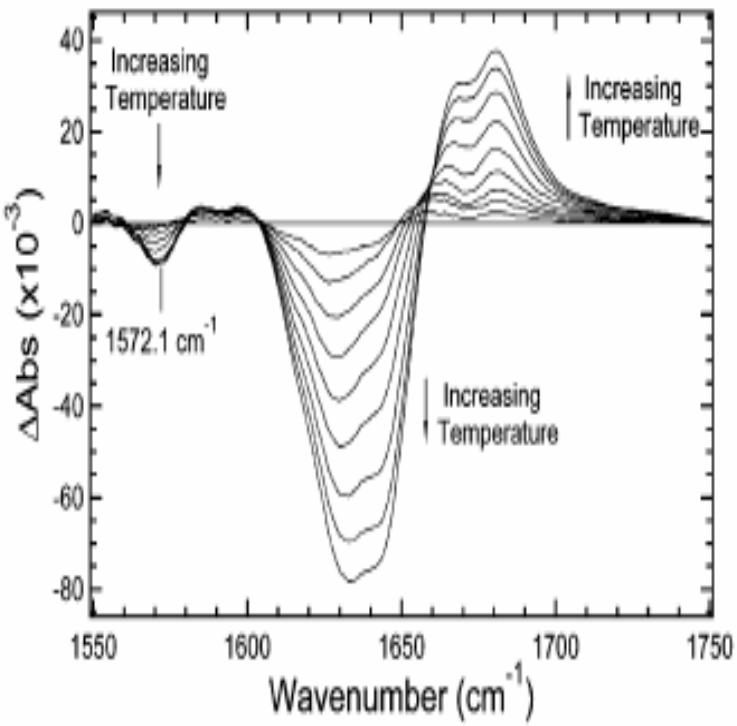


FIGURE 2: Temperature-dependent difference FTIR spectra of A57-(^{13}C=^{18}O) HP36 from 7 °C to 86 °C in ~10 °C increments. The difference spectra are produced by subtracting the lowest temperature absorbance spectrum from the higher temperature spectra. The peak corresponding to the carbonyl stretch of the ^{13}C=^{18}O label for residue A57 appears at 1572.1 cm^{-1} in the folded state.

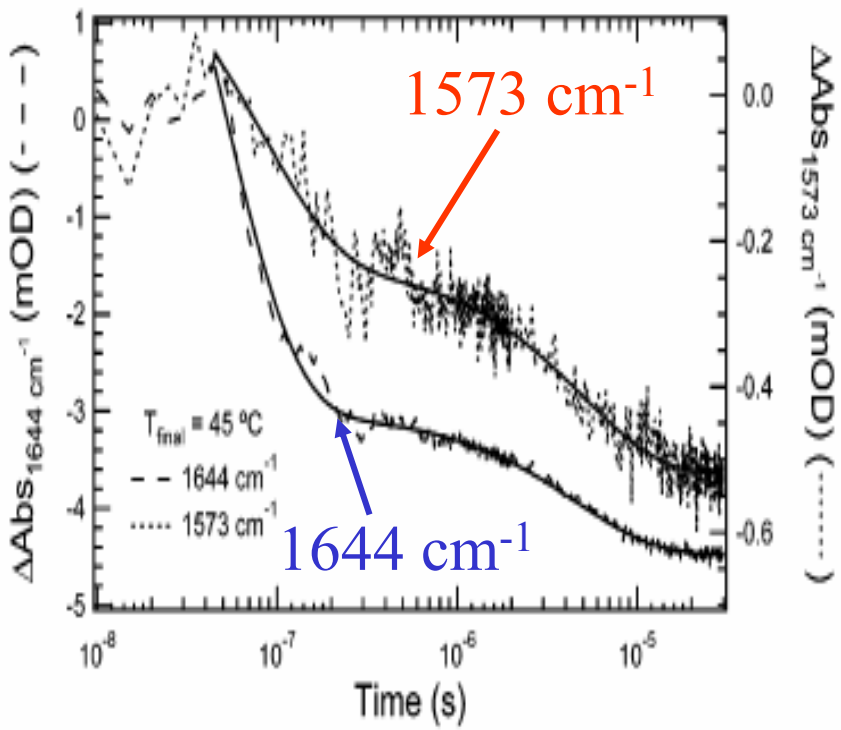


FIGURE 5: Temperature-jump relaxation kinetics monitored at 1573 cm^{-1} (short dashed) and 1644 cm^{-1} (long dashed) for A57(^{13}C=^{18}O) HP36 in response to a T-jump from 41 °C to 45 °C. The kinetic traces at 1573 and 1644 cm^{-1} are the result of 16 000 or 10 000 laser shots, respectively. The kinetic traces are fit with a biexponential function (solid curves).

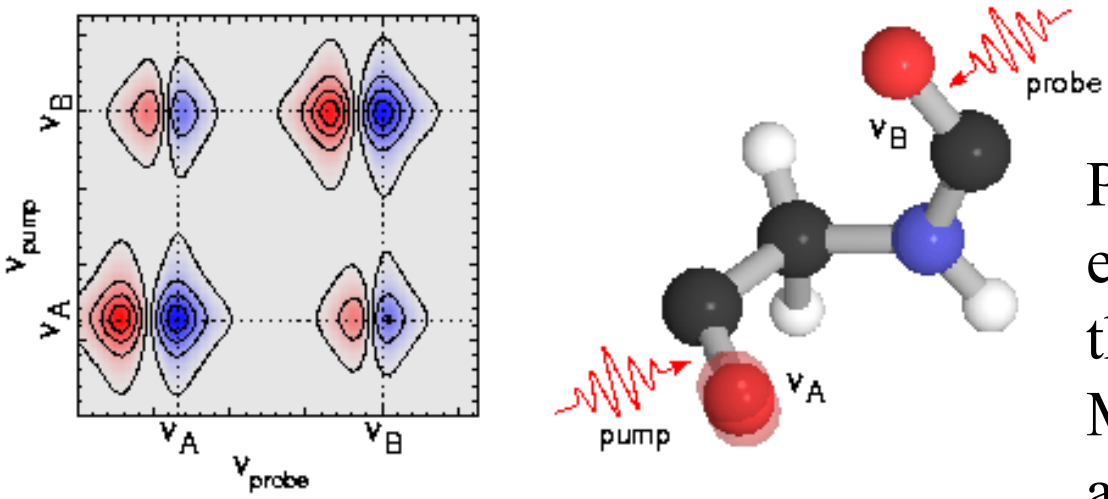
Dyer

Biochemistry, Vol. 46, No. 11, 2007 3281

Advanced techniques

Multidimensional (2D)
TeraHertz (farIR)
Modulation Spectra (VCD)

2D IR Coherence Spectra—like NMR COSY



Pump one mode, see effect on other mode through time evolution
Modes must be anharmonic and best if resolved

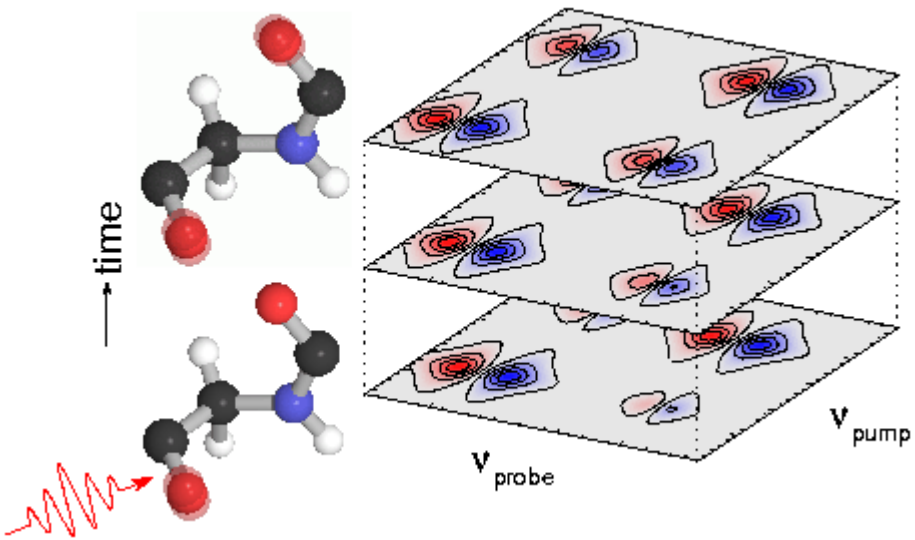
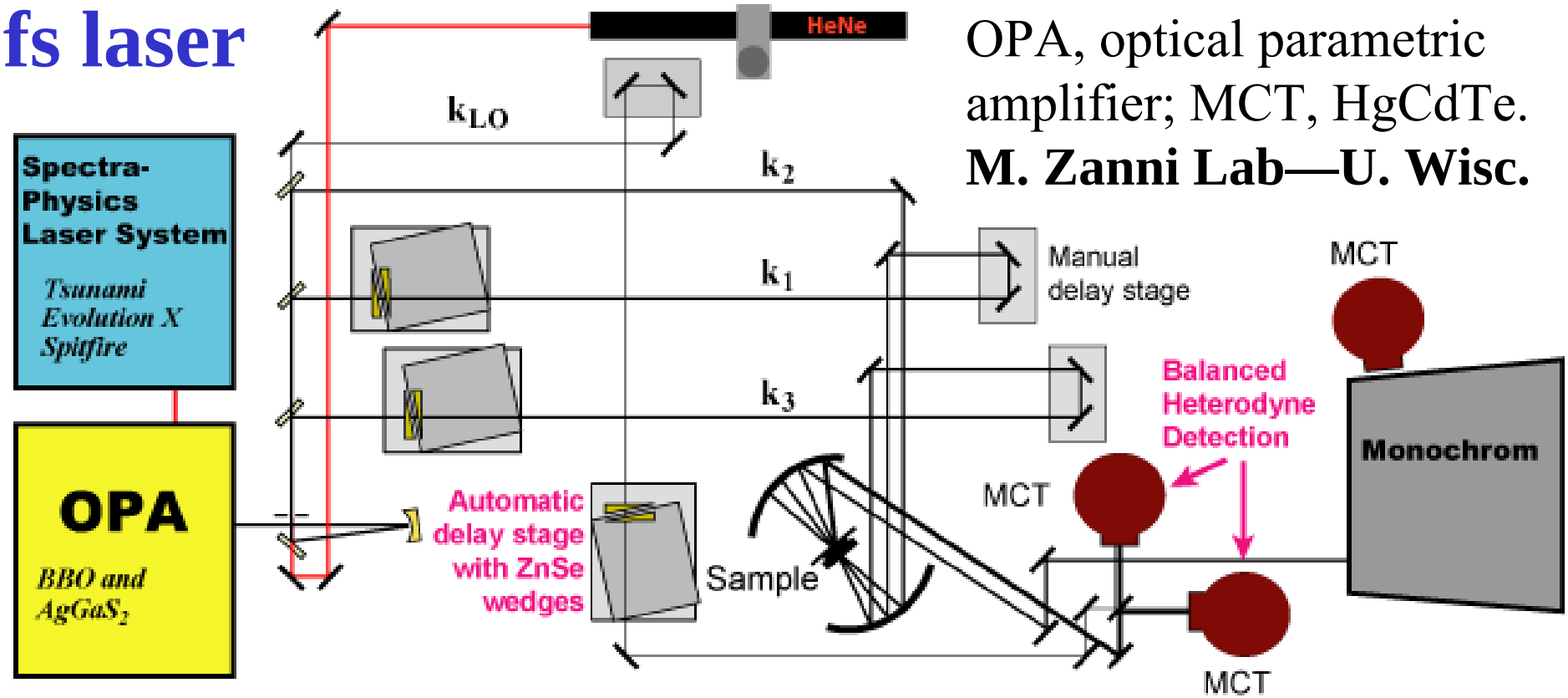


Figure from Woutersen web site

Experimental 2D IR setup

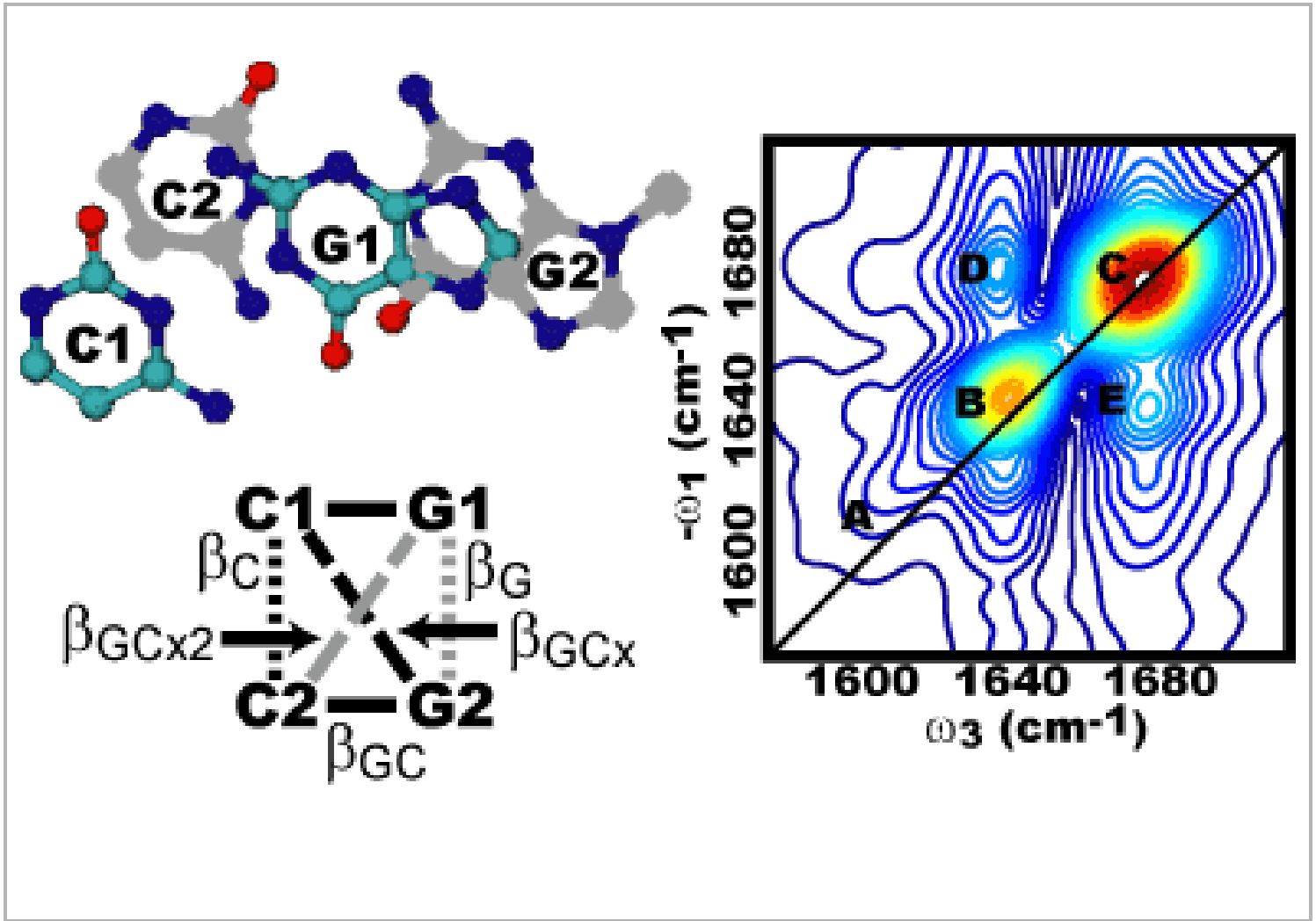
fs laser



OPA, optical parametric amplifier; MCT, HgCdTe.

M. Zanni Lab—U. Wisc.

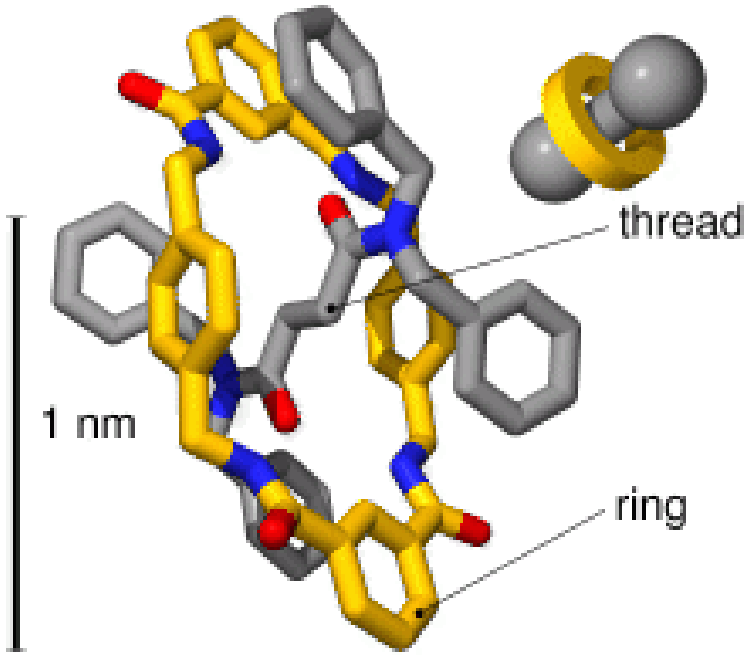
2D IR uses 3 fs pulses, so 2nd excited states are measured. After heterodyning the response signal with a local oscillator pulse, 2D data set is collected and a FT along two time axes gives the 2D IR spectrum. Because overtone and combination bands are measured, 2D IR spectra exhibit cross peaks between coupled vibrational modes.

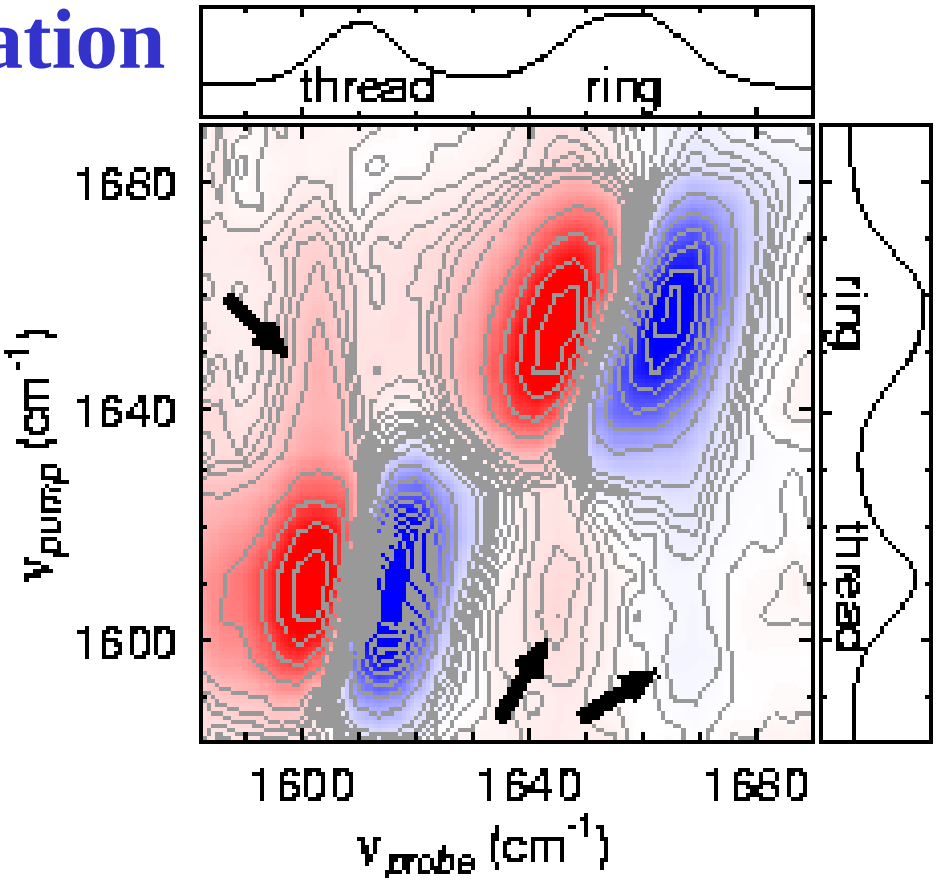


Rephasing <VVVV> 2D IR spectrum of (dG5C5)2 and a model of the coupling interactions for calculation the 2D IR spectrum

M. Zanni, Univ. Wisconsin

2D IR dynamic conformation In a molecular complex





Cross-peaks (indicated by arrows) reflect couplings between C=O groups. From the couplings and anisotropies, solution conformation of the ring-thread system is determined on a sub-ps time scale. Fluctuations in conformation are observed with time-delayed 2D-IR.

Probing the structure of a rotaxane with 2D infrared spectroscopy - PNAS 2005
O.F. A. Larsen, P.Bodis, W. J. Buma, J. S. Hannam, D. A.Leigh, S. Woutersen

Single shot 2D IR spectroscopy

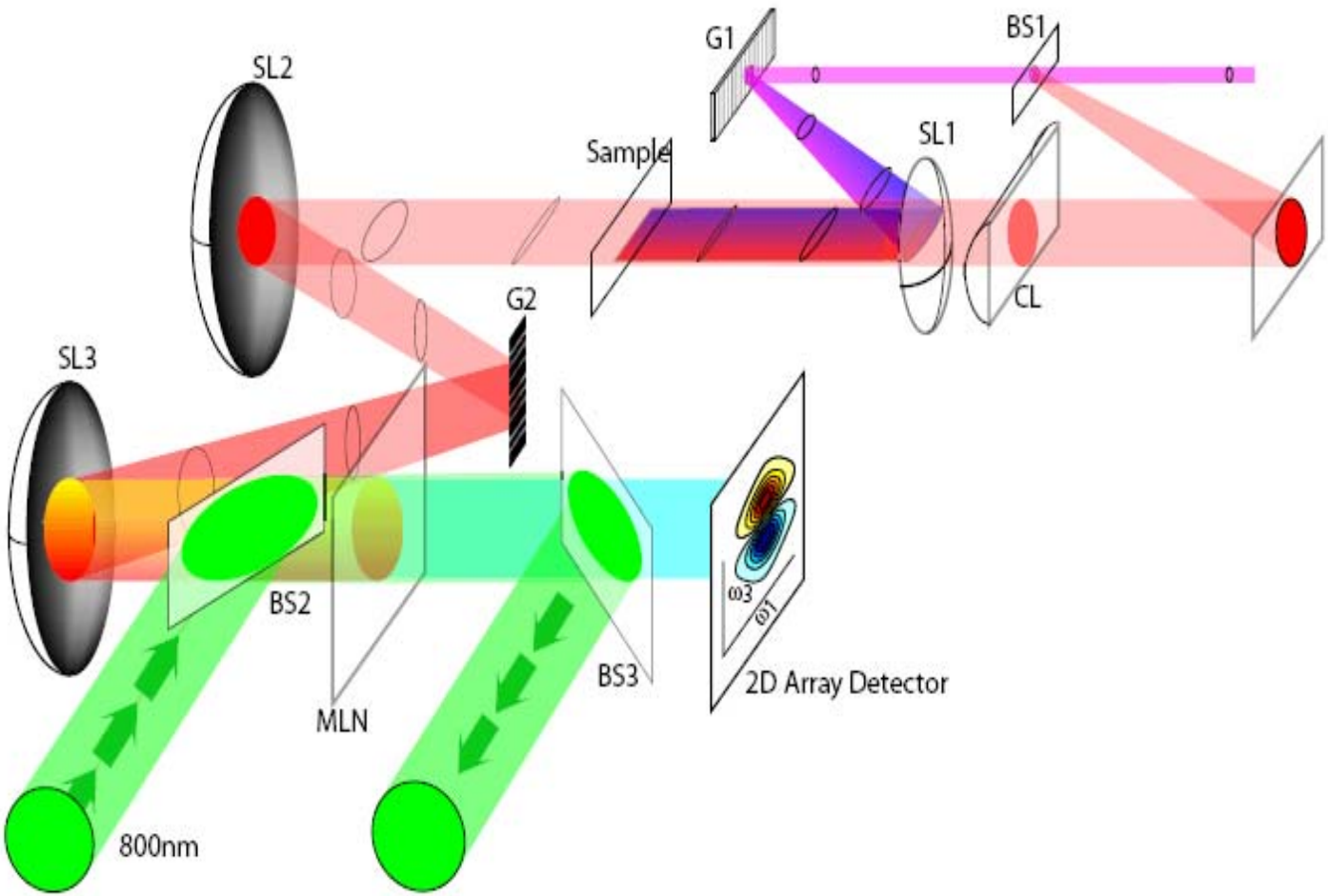


Fig. 1. 2DIR spectrometer. G is diffraction grating, SL is a spherical focusing mirror, CL is a cylindrical lens, BS is a beamsplitter, and MLN is the upconversion crystal.

Tokmakoff and co-workers, Optics Express, 15, 233, 2007

Single shot 2D IR spectroscopy

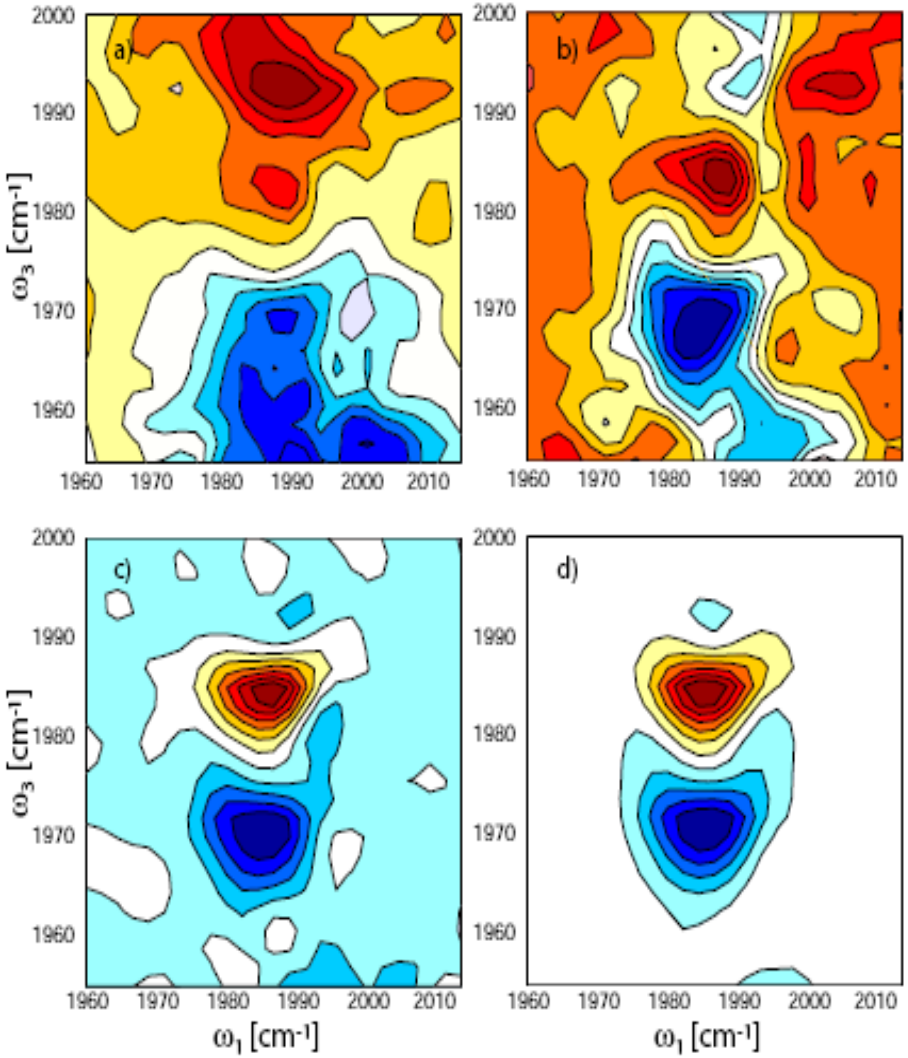


Fig. 4. Time series of 2DIR difference spectra of WHC in hexane using (a) 1 (b) 16 (c) 256 (d) and 4096 pump-probe pulses.

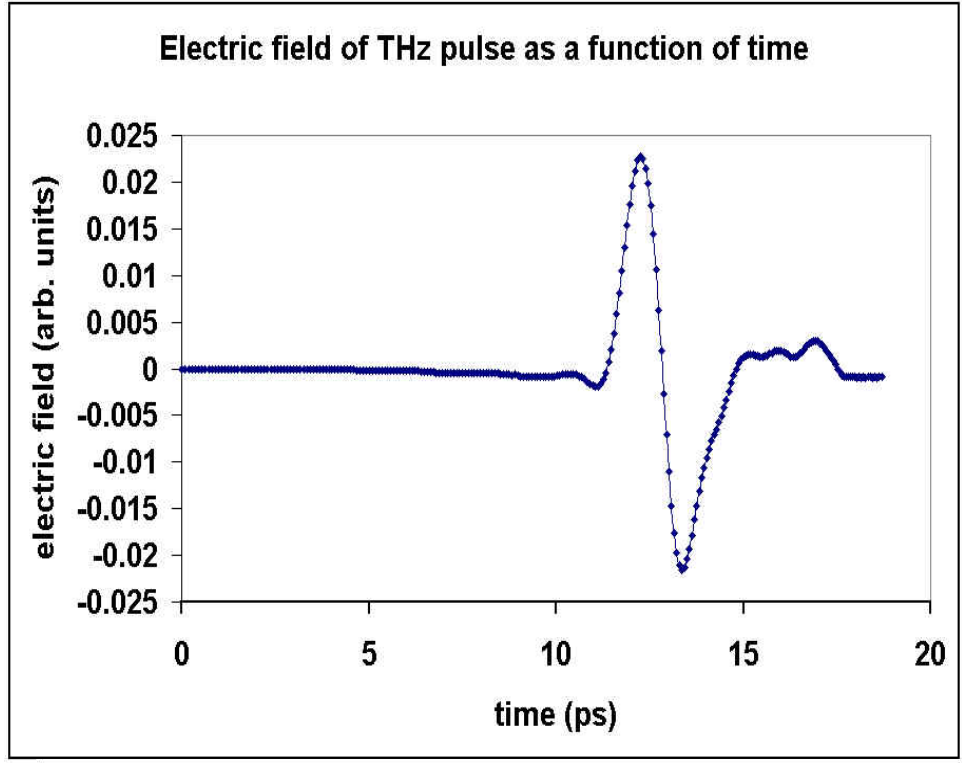
Tokmakoff and co-workers, Optics Express, 15, 233, 2007

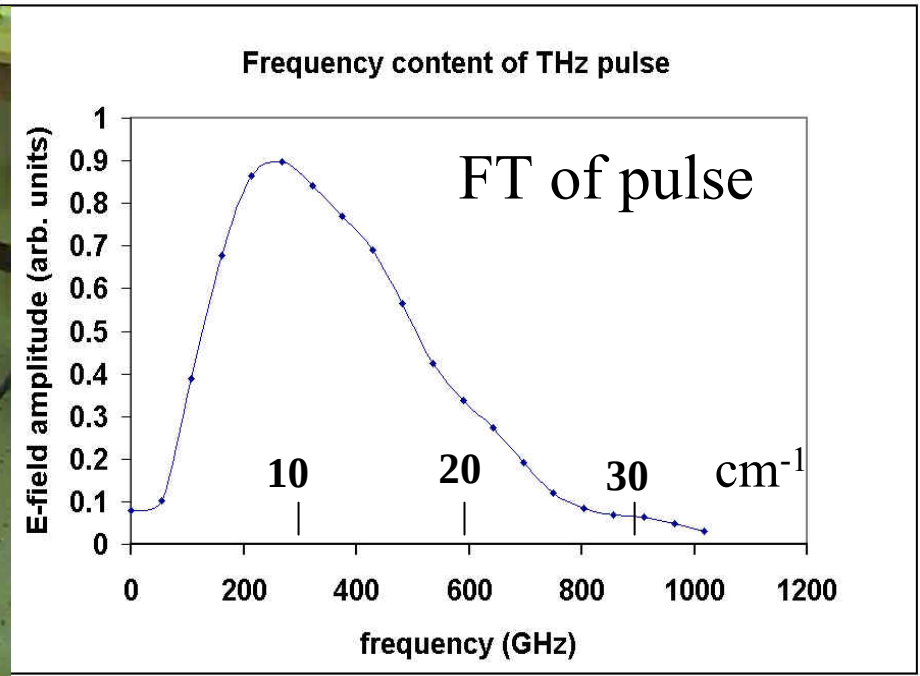
- **TeraHertz Spectroscopy**
- **The new Far-IR spectra with fs time response**

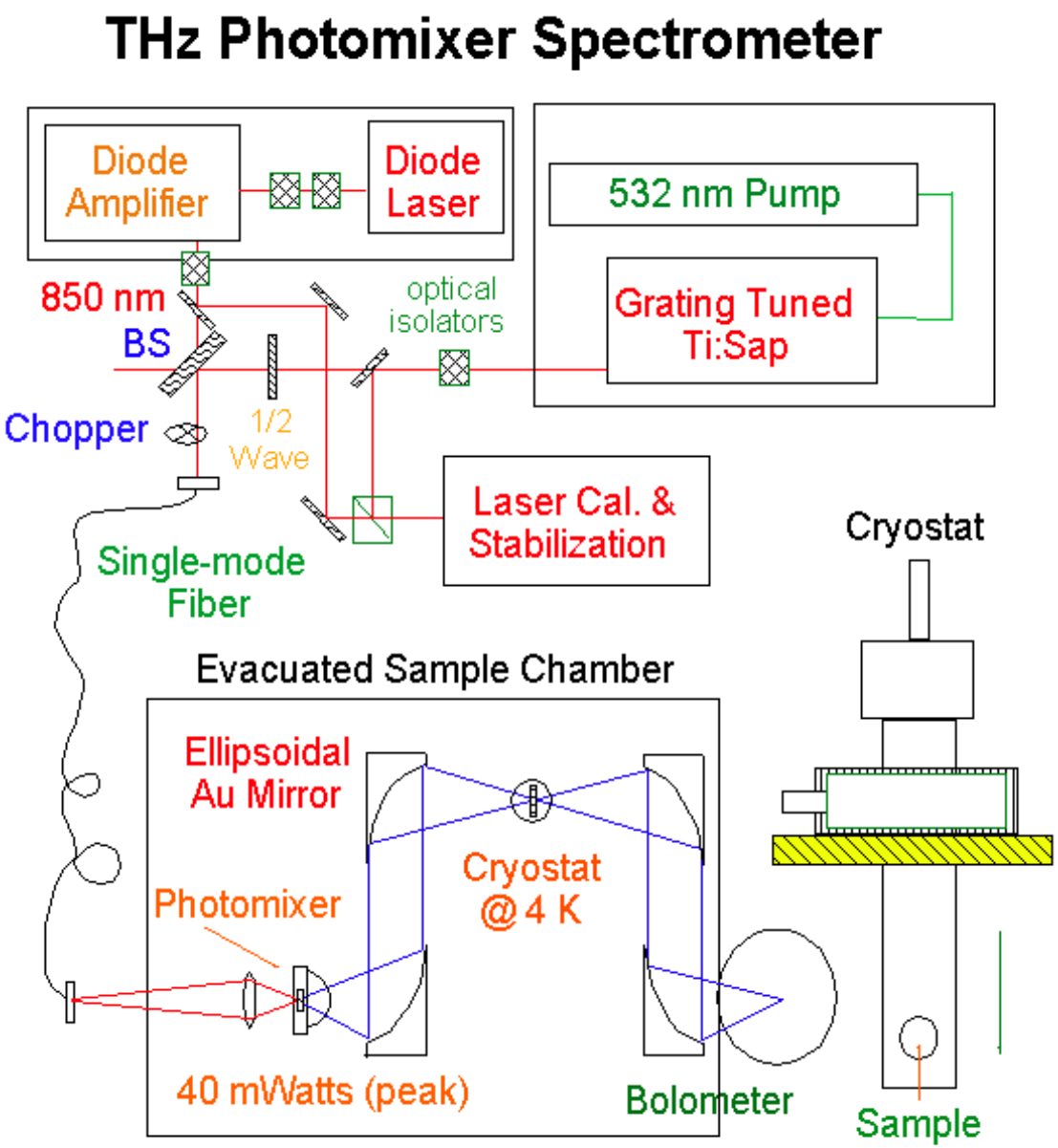
Colgate THz Spectrometer

fs laser pulses hit a “THz antenna," GaAs xtal. generating THz pulse

The antenna is in the brass holder located in the lower left. Parabolic mirrors focus the THz to sample and back to a receiver antenna.

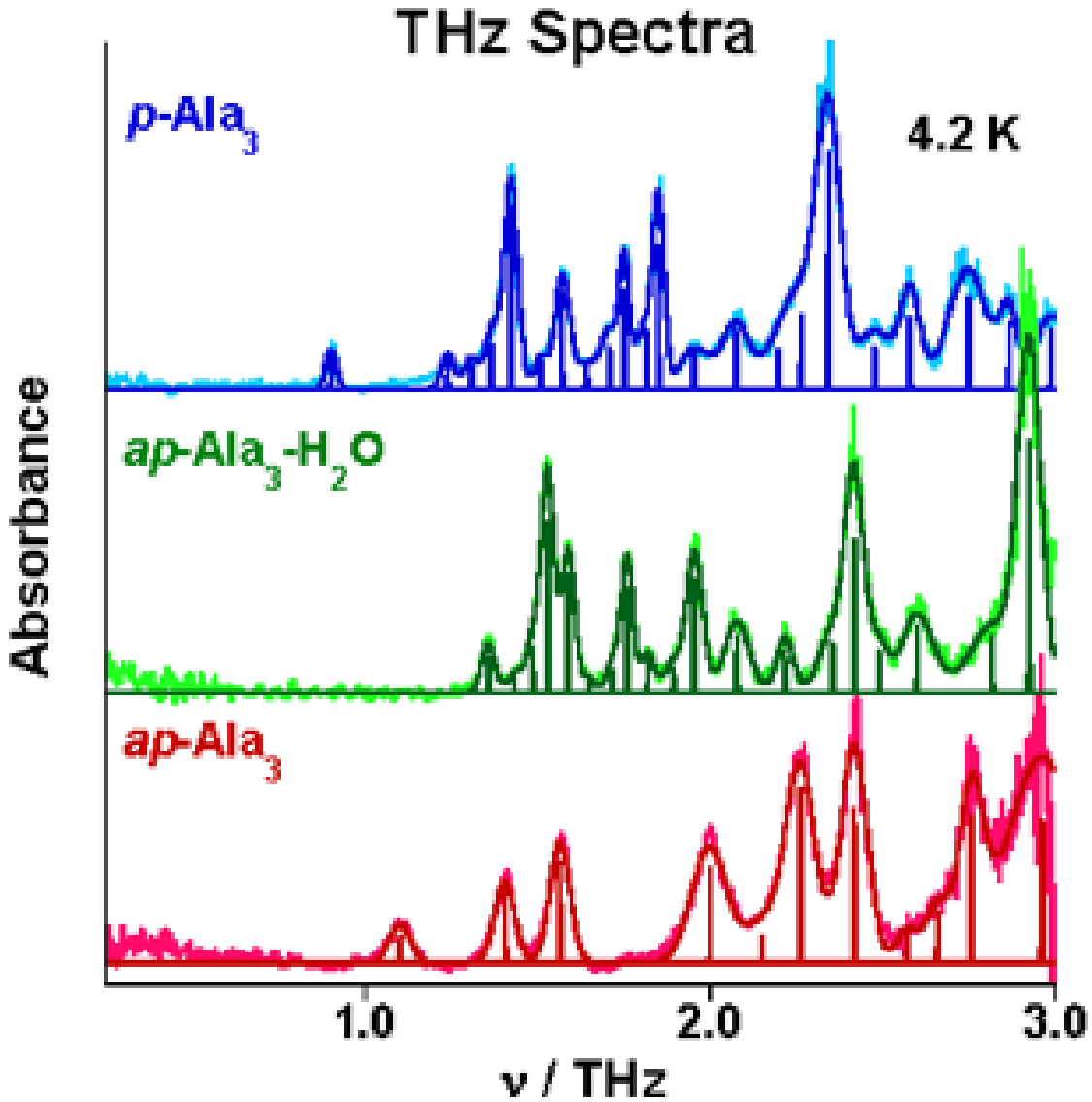




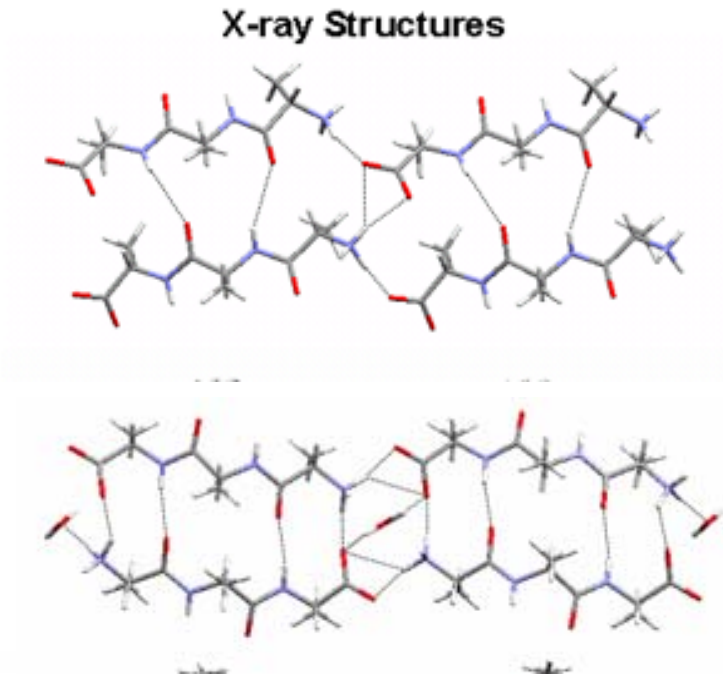


T.M. Korter and D.F. Plusquellic, Chem. Phy. Lett. **385** 45-51 (2004

NIST cw THz spectrometer. The system consists of a low-temperature-grown GaAs photomixer driven at the difference frequency of two near-infrared lasers. The two lasers include a fixed frequency diode laser operating near 850 nm and ($\Delta\nu_{FWHM} \sim 0.0001$ cm^{-1}) and a standing-wave Ti:Sapphire (Ti:Sapp) laser having a resolution of ($\Delta\nu_{FWHM}$ 0.04 cm^{-1}). The laser is seeded by feedback from an external grating-tuned cavity for absolute frequency stability.

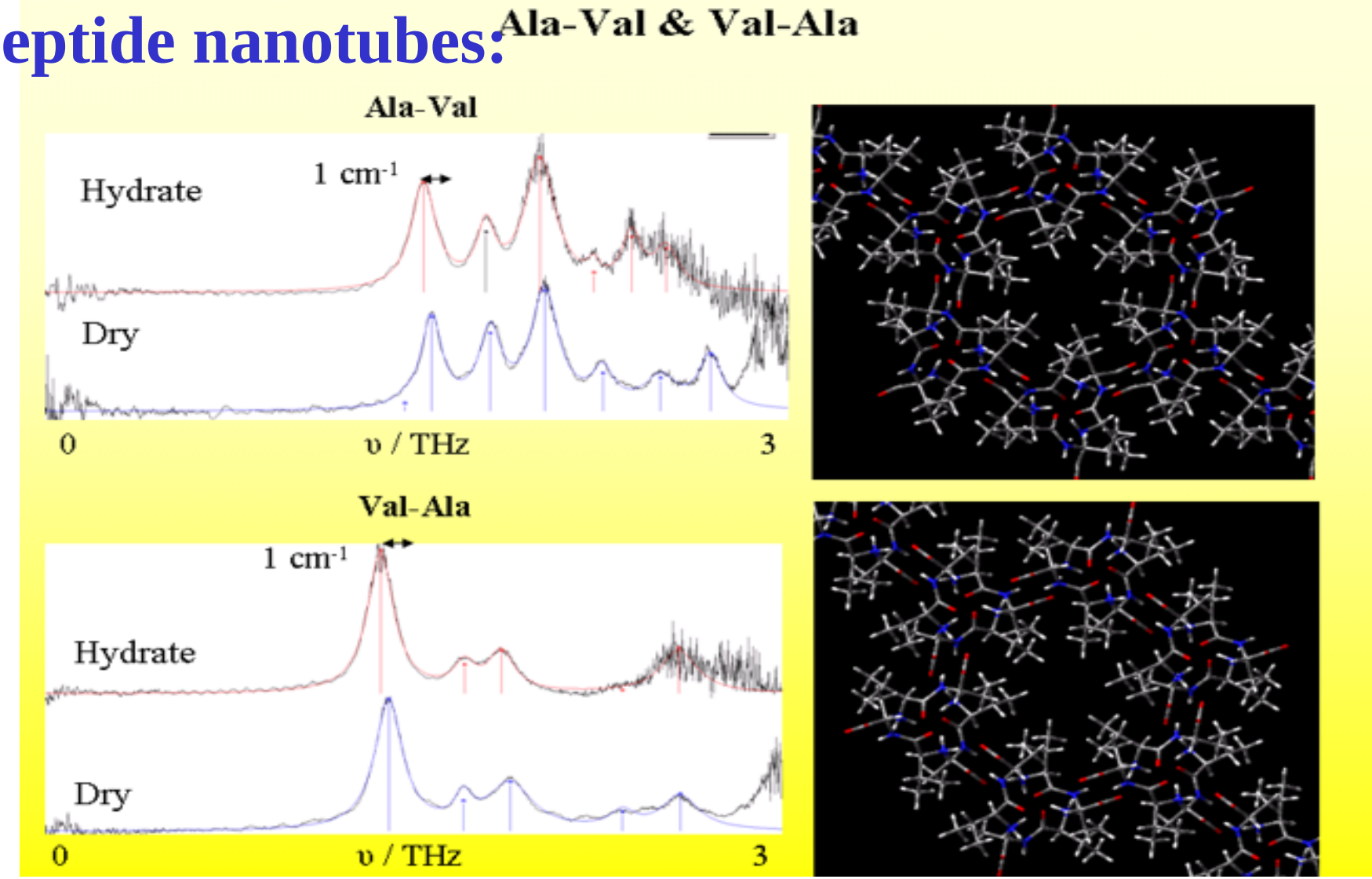


X-ray structures of the parallel (top) and anti-parallel (bottom) β-sheet forms of trialanine.



THz spectra of three crystalline forms of Ala3. All three are unique and illustrate the sensitivity in this region to changes in the β-sheet form and the co-crystallized water weakly bound in the lattice.

Dipeptide nanotubes:



THz spectra of AV and its retro-analog, VA. The two spectra in each panel were obtained for dehydrated samples (blue) and for samples where water is present in the hydrophobic core region (red)
K. Siegrist and D.F. Plusquellic, NIST Web Site (in prep)

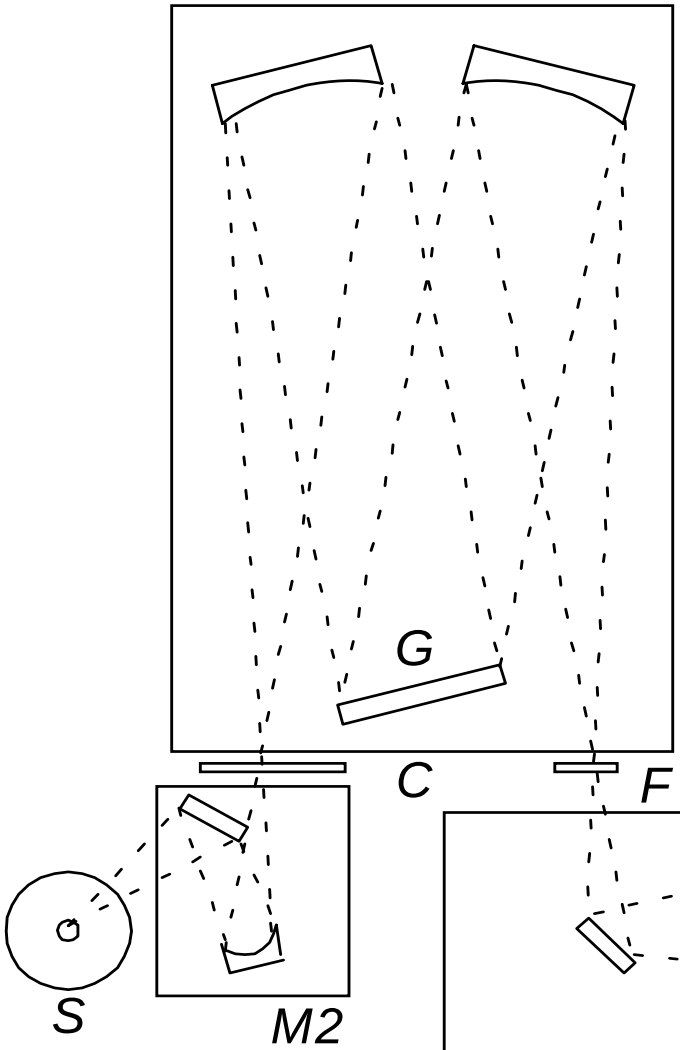
Vibrational Circular Dichroism: VCD

Builds on long tradition of Circular Dichroism (CD) studies in the UV, which still have a large impact on Biomolecular research—comparisons are useful

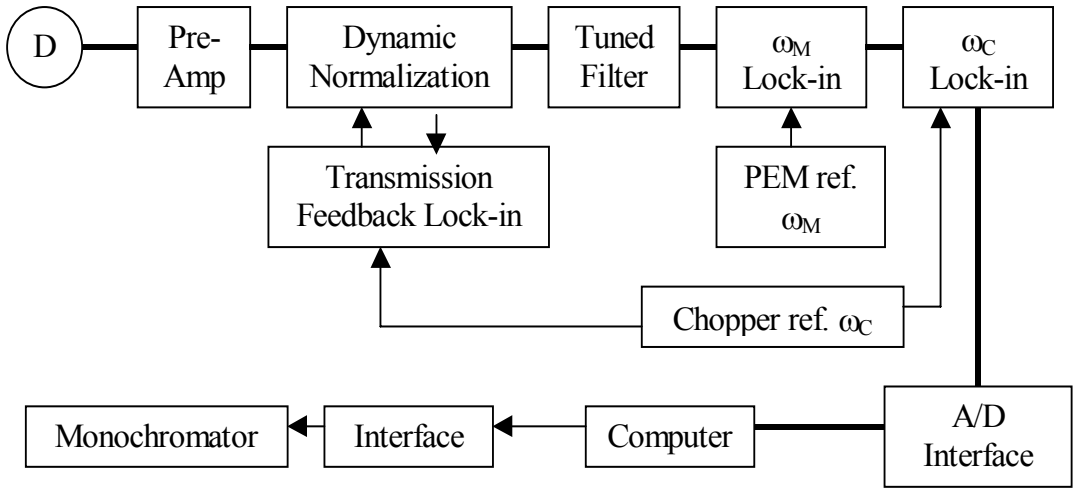
Combine inherent resolution of vibrational spectra with conformational sensitivity of CD

UIC Dispersive VCD Schematic

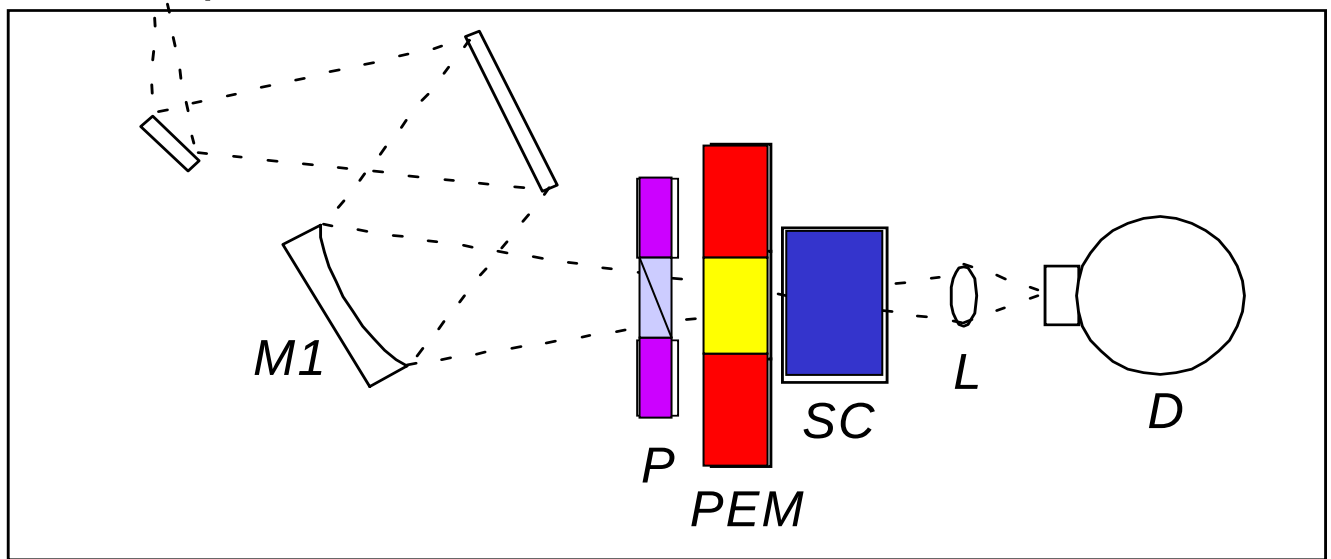
Yes it still exists and measures VCD!

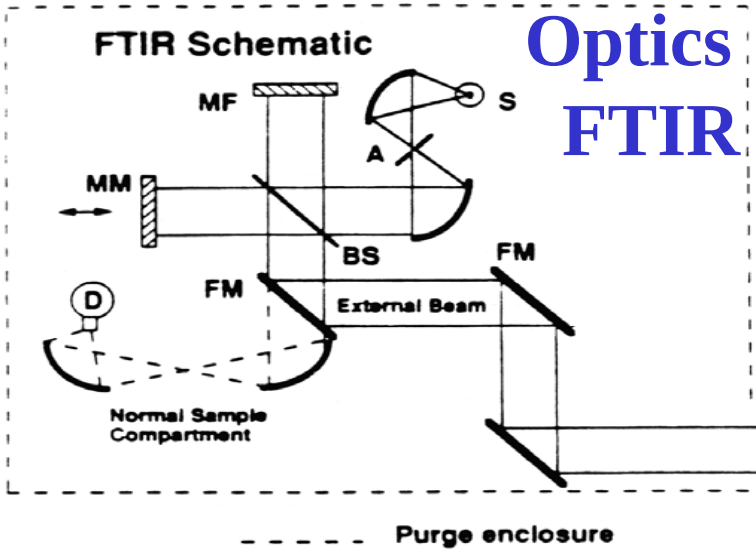


Electronics



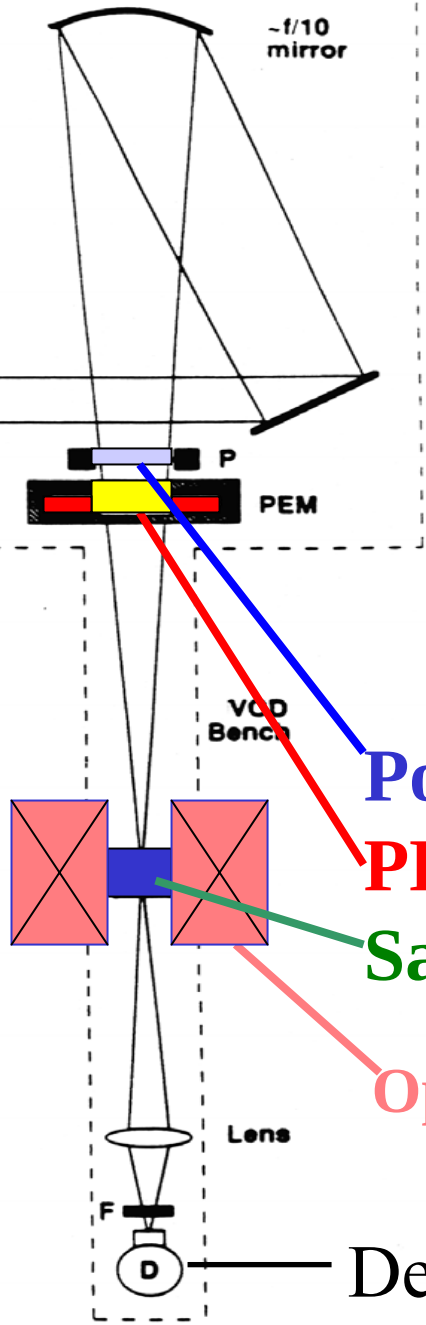
Optics and Sampling





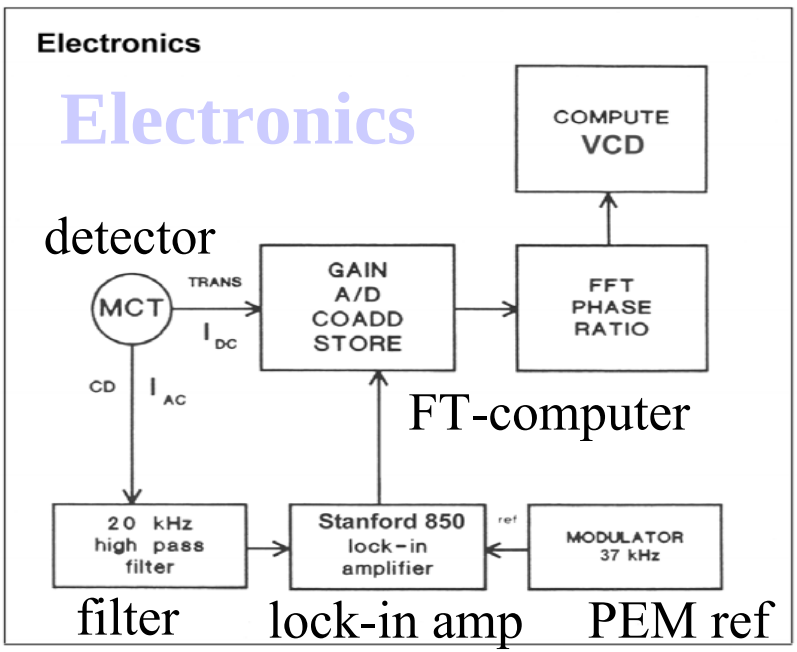
Optics FTIR

Separate VCD Bench



UIC FT-VCD Schematic

(designed for magnetic VCD ***commercial ones simpler***)



Polarizer

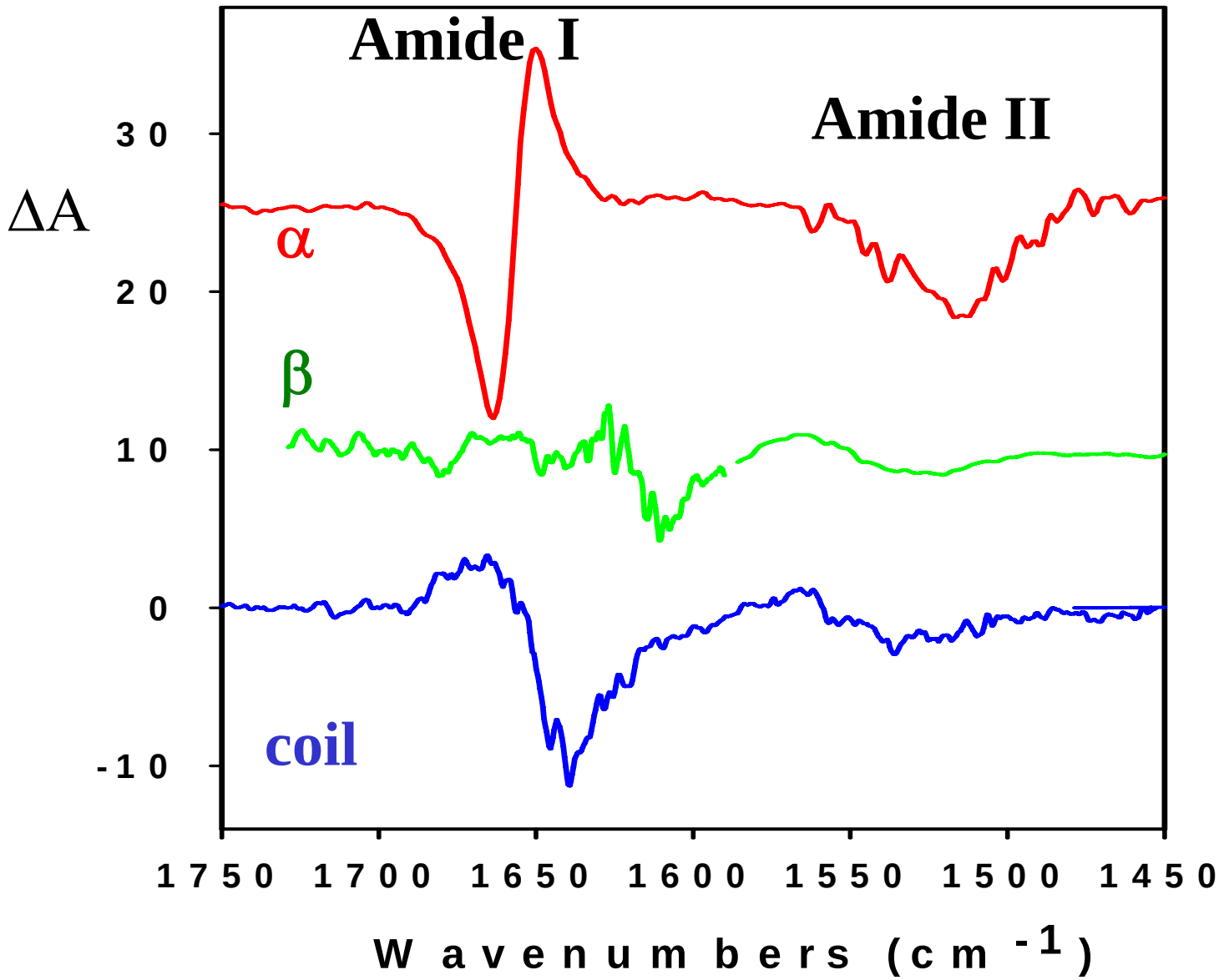
PEM (ZnSe)

Sample

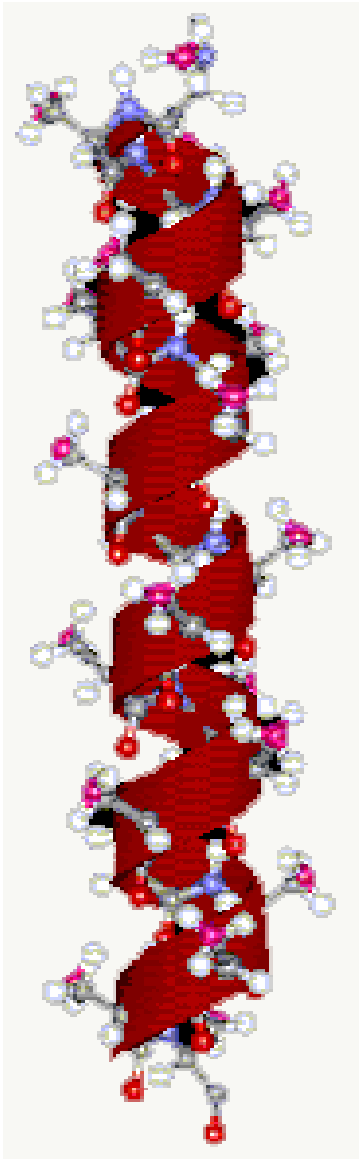
Optional magnet

Detector (MCT)

Selected model Peptide VCD, aqueous solution



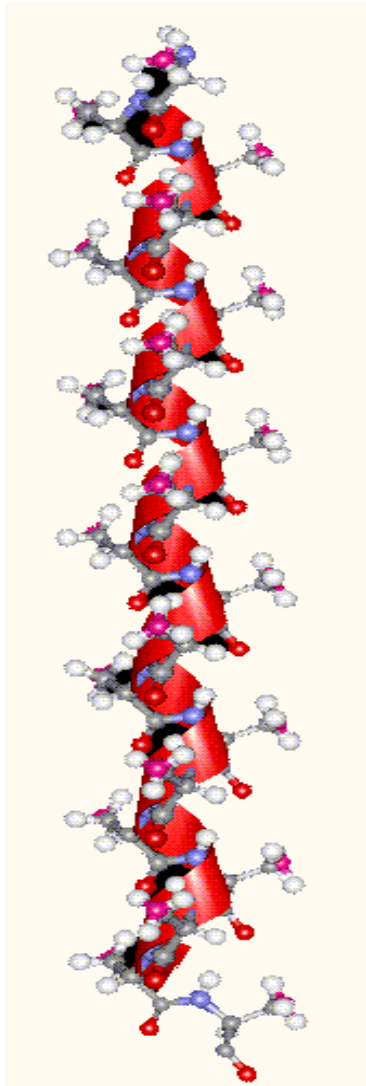
VCD Example: α- vs. the 3_{10}-Helix



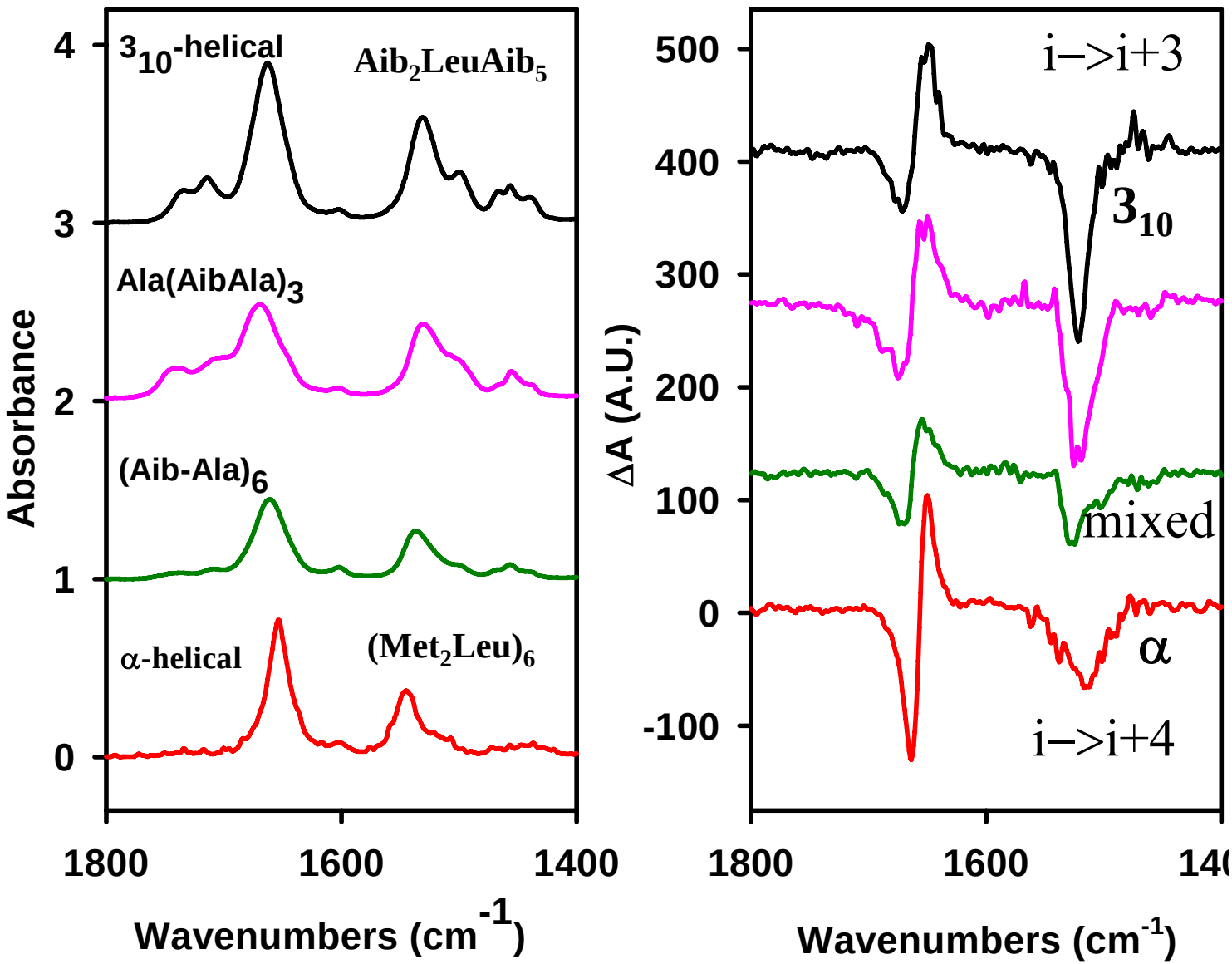
i+4 ← H-bonding → i, i+3

3.6 ← Res./Turn → 3.0

.00 ← Trans./Res (Å) → 1.50



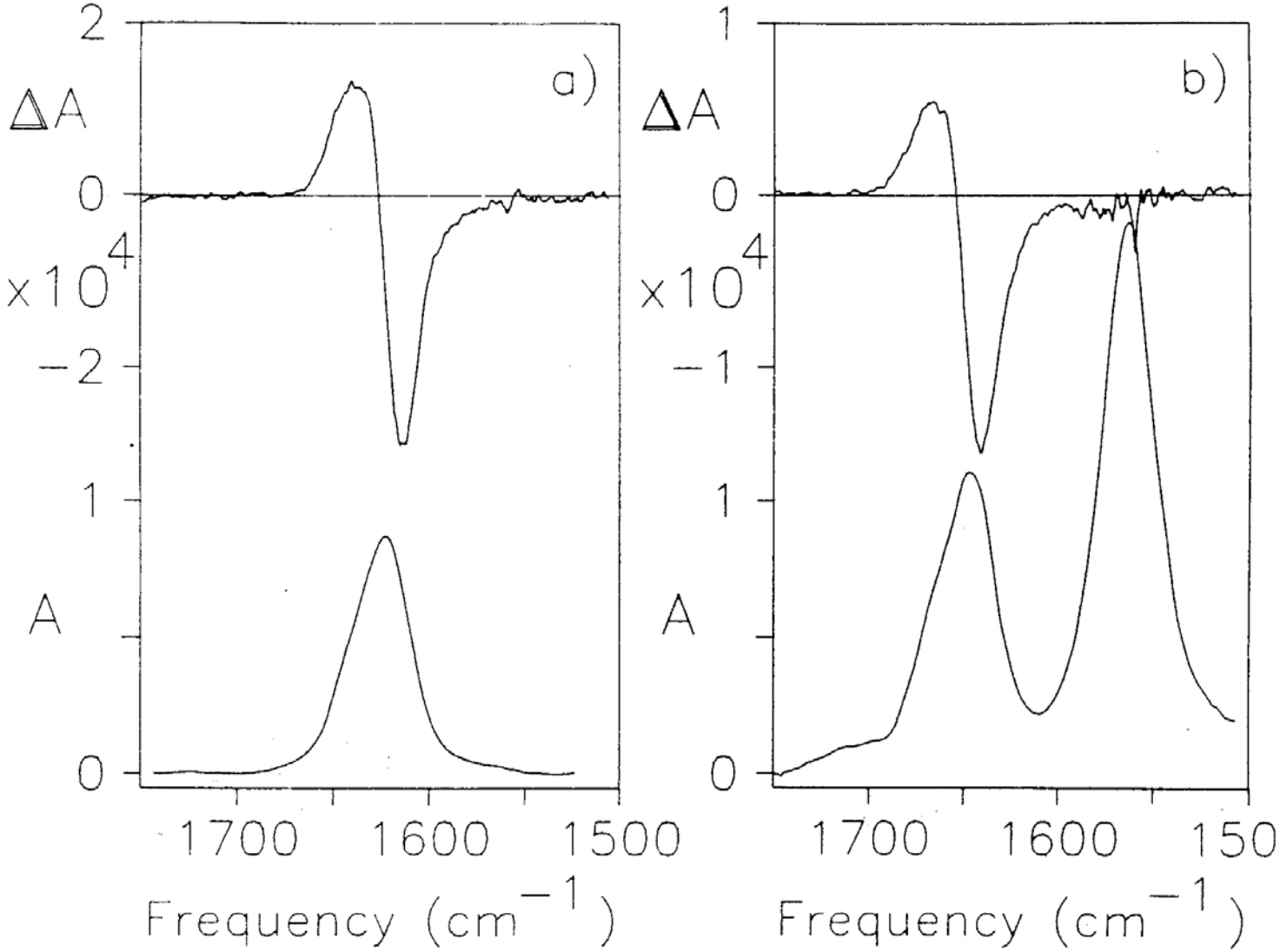
The VCD success example: 3_{10}-helix vs. α-helix



Relative shapes of multiple bands distinguish these similar helices

Silva et al. Biopolymers 2002

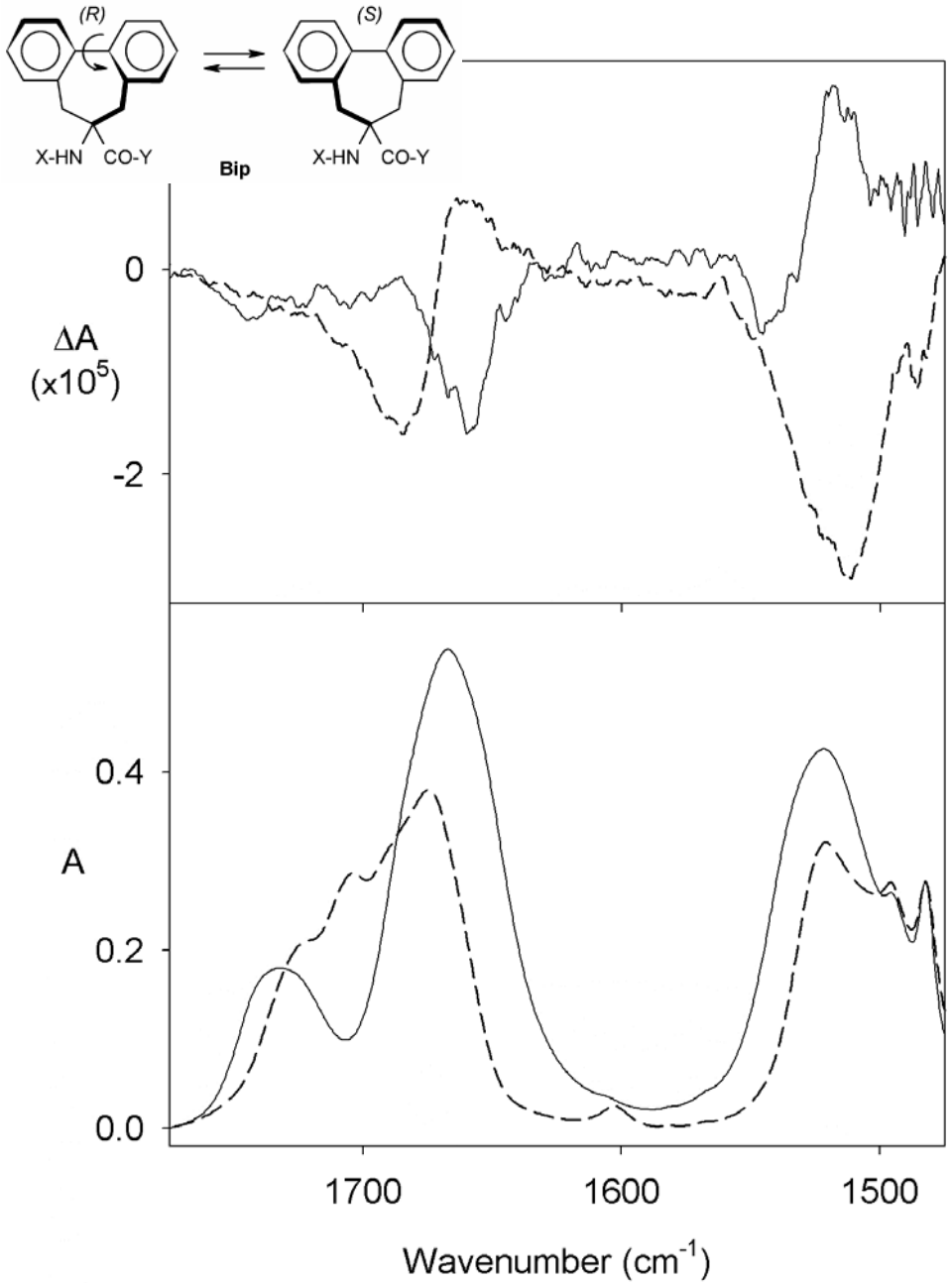
Relationship to "random coil" - compare Pro_n and Glu_n



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

Dukor, Keiderling - Biopoly 1991

Biphenyl bridged residues (Bip) show inversion



Ac-$(Bip)_3$-L-Val-OMe (———)

left-handed

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

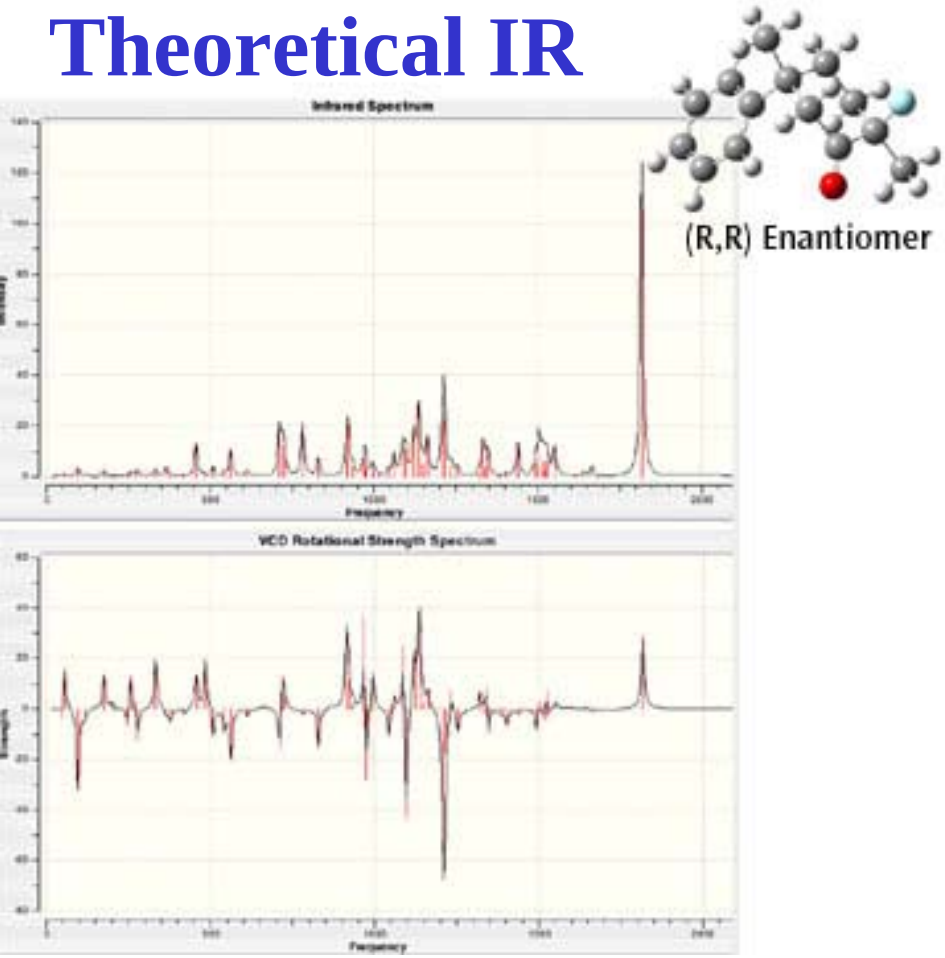
right-handed (3_{10}-helix)

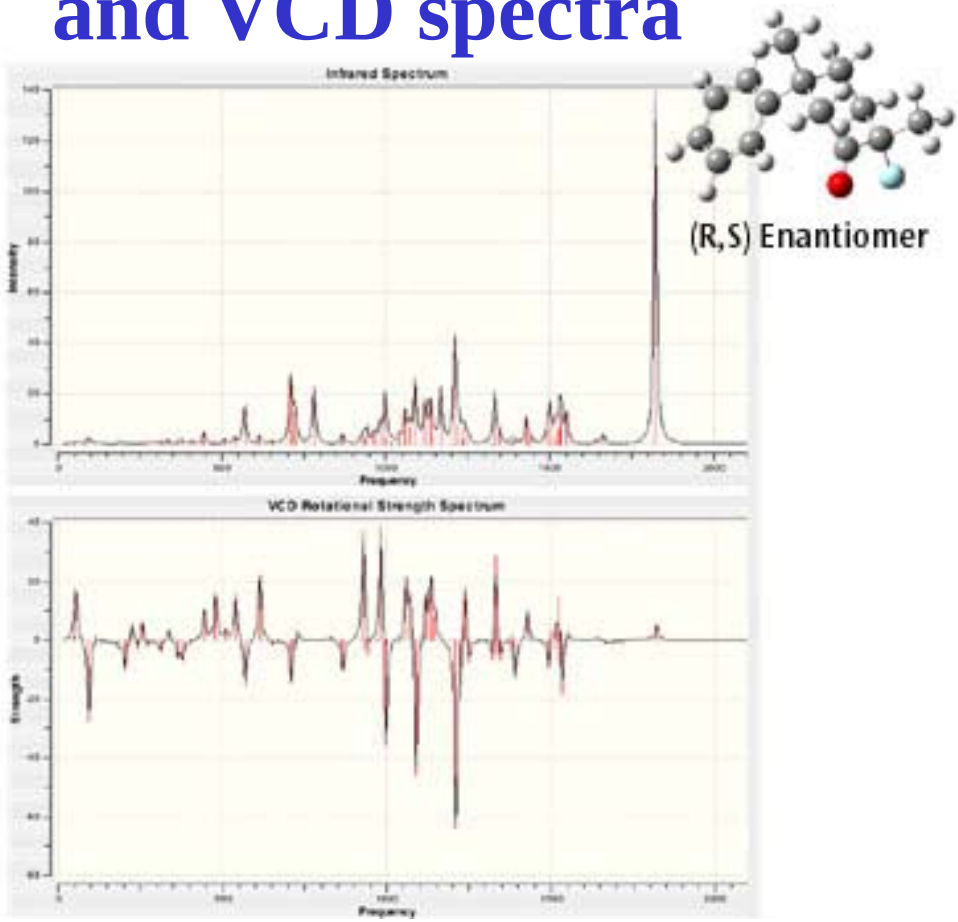
Vibrational spectrum separates aromatic and amide transitions

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

Theoretical IR and VCD spectra

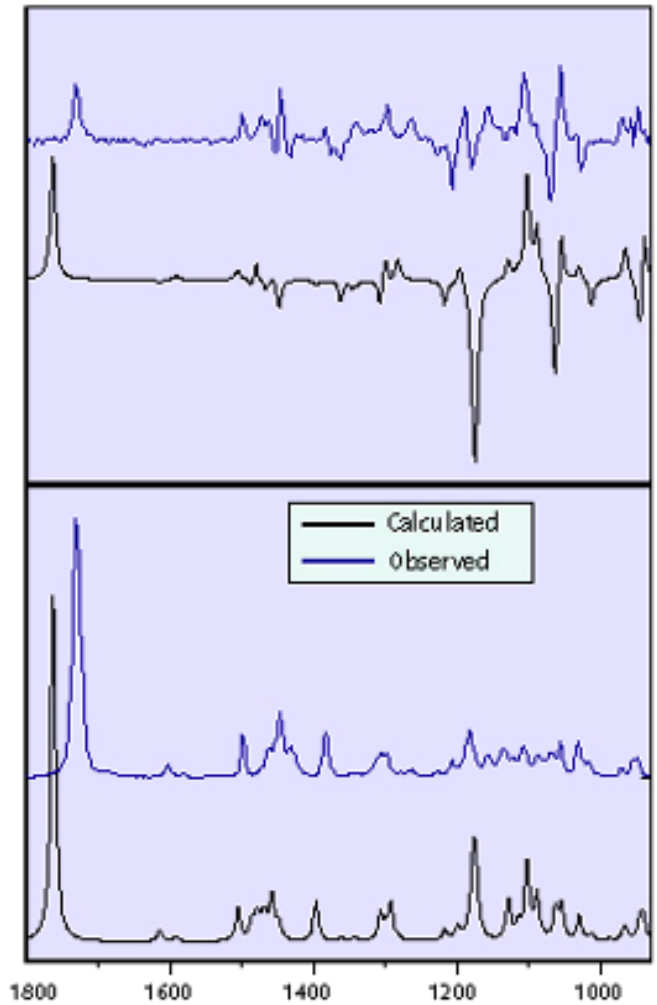


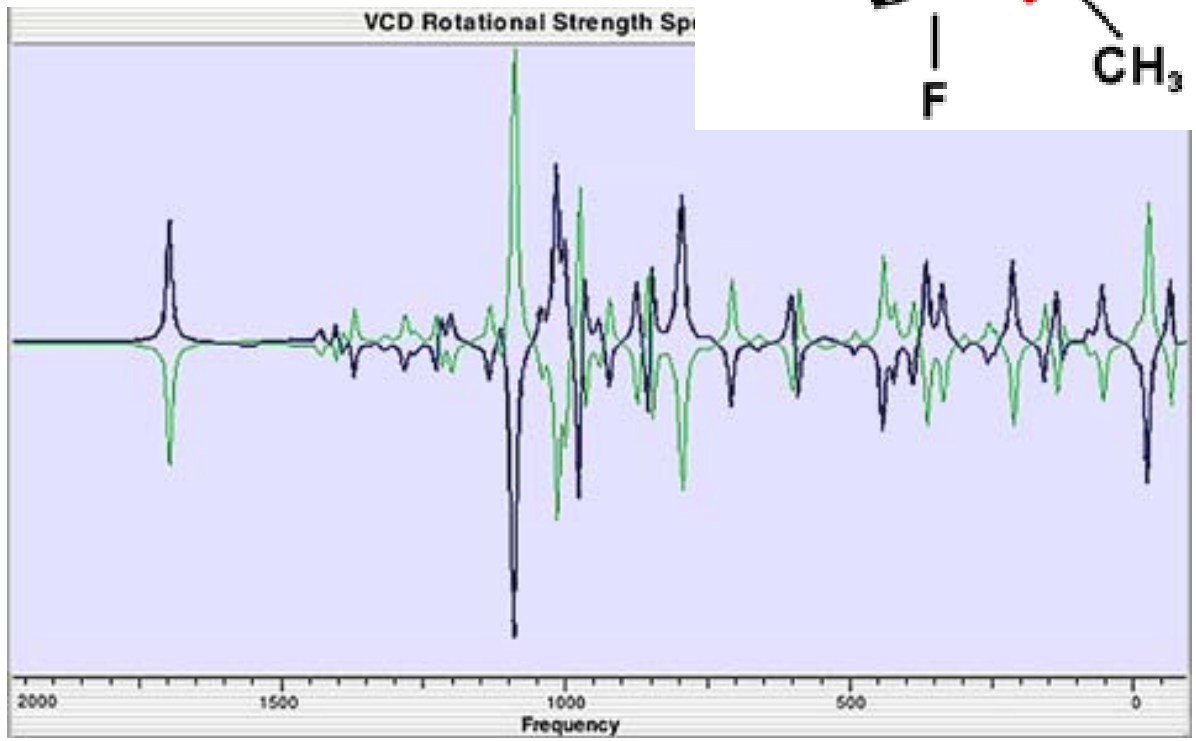


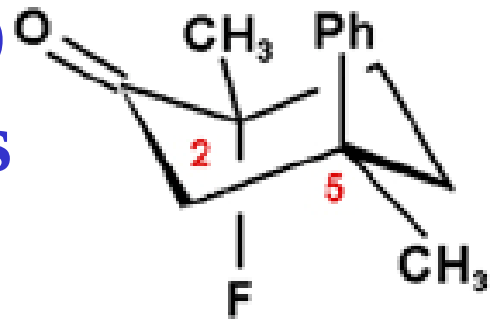
The predicted IR and VCD spectra for the (R,R) and (R,S) enantiomers. IR spectra are similar, with small differences. The VCD spectra are clearly different. All spectra are truncated at 2100 cm^{-1} (there are additional peaks in the 3000-3300 range)

P. J. Stephens, Gaussian Web Site

Fluoro-ketone VCD Theoretical analysis





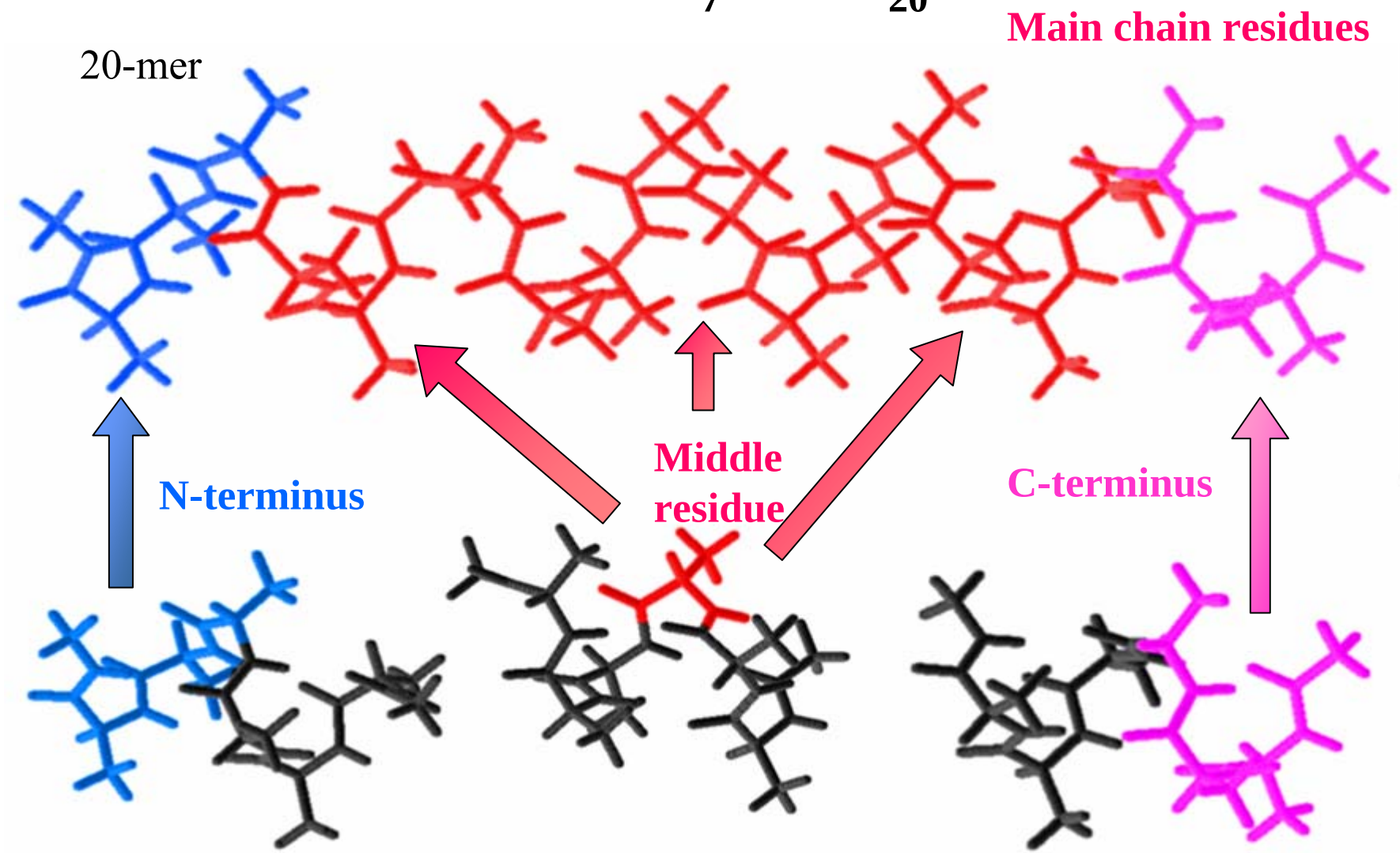


Comparison of the observed and calculated IR (bottom) and VCD (top) spectra for the (R,R) enantiomer.

Predicted VCD spectra for the (R,R) and (S,S) enantiomers (blue and green, respectively).

P. J. Stephens, Gaussian Web Site

Scheme of the transfer of FF, APT and AAT from Ala_7 to Ala_{20}



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

Simulation of Helix IR and VCD Really Works!

3_{10}-helix vs. α-helix:

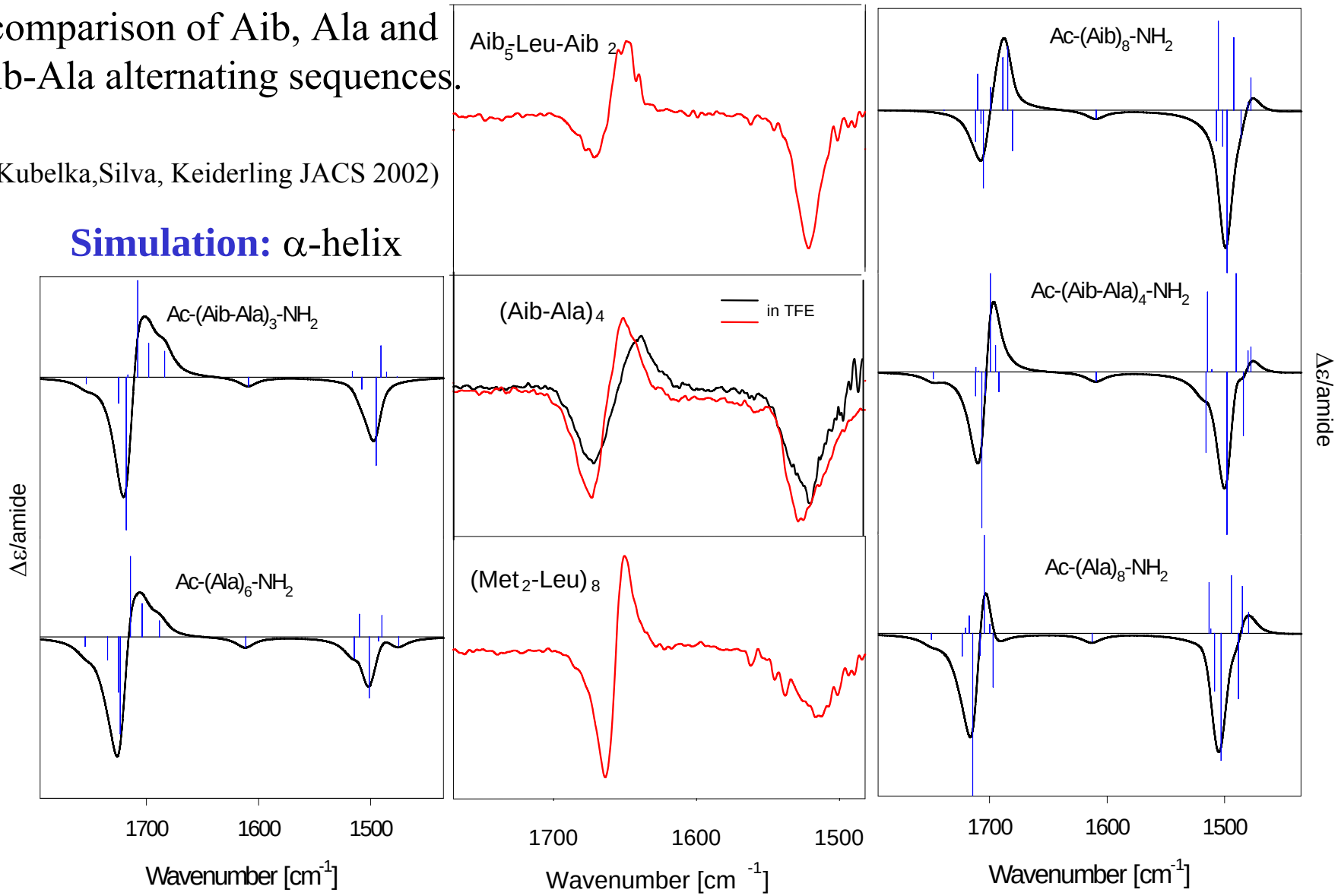
comparison of Aib, Ala and Aib-Ala alternating sequences.

(Kubelka,Silva, Keiderling JACS 2002)

Experiment:

Simulation: 3_{10}-helix

Simulation: α-helix



Isotope labeling → site specific structure

Method - Change ^{12}C to ^{13}C on amide C=O

Shift frequency down by ~40 cm^{-1}

Decouple from rest of the chain--test here

IR can detect **differences**, but frequencies alone not reliable

VCD can determine the **type of secondary structure**

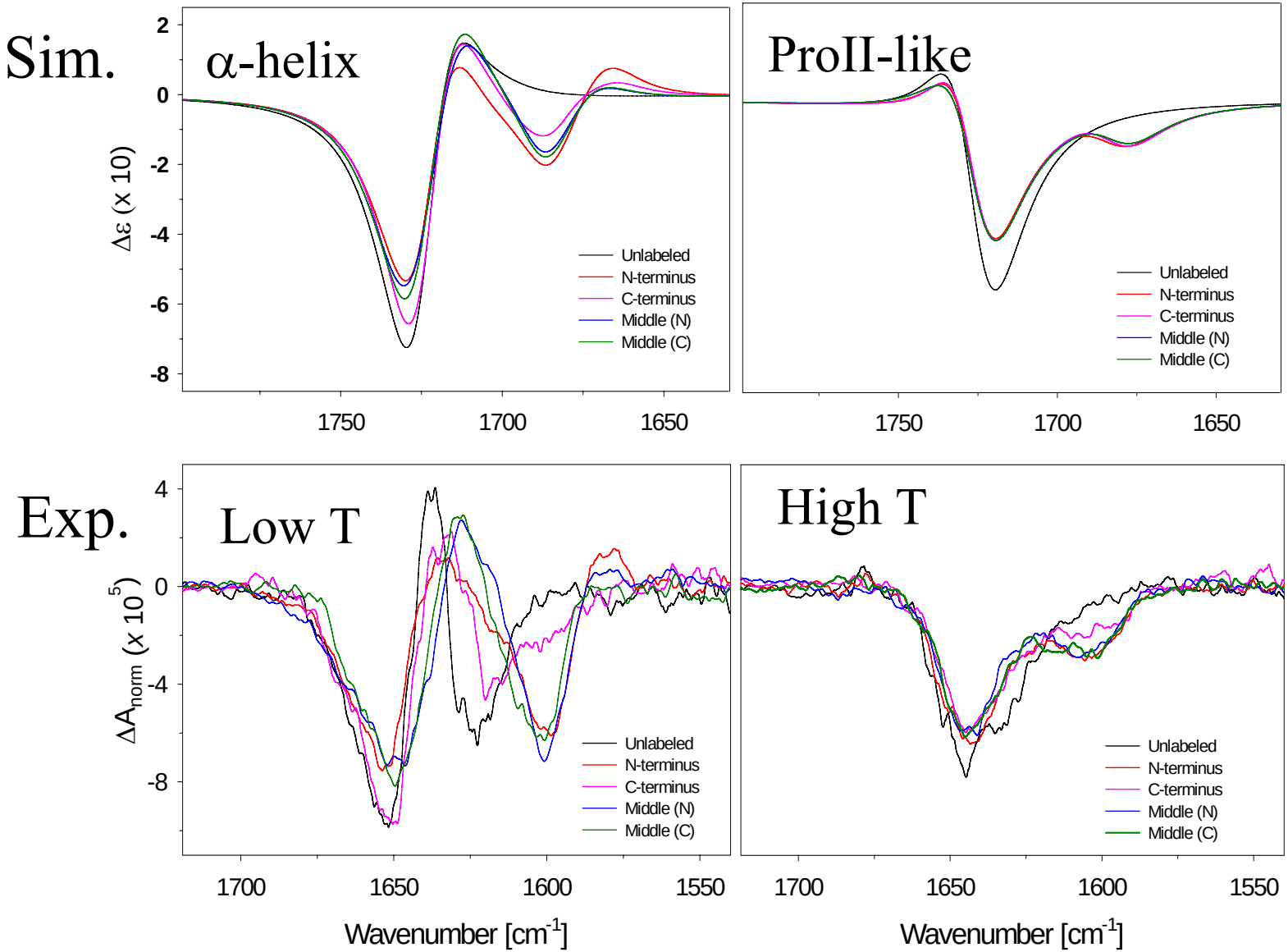
With peptide synthesis, straightforward especially for Ala

Our studies coupled Experiment and Theory - observational

1. α-helix thermal denaturation (Silva et al, *PNAS* 2000)
2. β-sheet formation - aggregation (Kubelka & TAK, *JACS* 2001)

Alanine 20-mer ^{13}C labeling scheme

Notation	Label position	Peptide sequence
unlabeled	none	Ac-AAAAKAAAAKAAAAKAAAAY-NH_2
L1	N-terminus	Ac-**AAAA**KAAAAKAAAAKAAAAY-NH_2
L2	Middle (closer to N-terminus)	Ac-AAAAK**AAAA**KAAAAKAAAAY-NH_2
L3	Middle (closer to C-terminus)	Ac-AAAAKAAAAK**AAAA**KAAAAY-NH_2
L4	C-terminus	Ac-AAAAKAAAAKAAAAK**AAAA**Y-NH_2



Simulated and experimental VCD spectra for labeled Ala_{20}

Vary from N- to C terminal, 4 ^{13}C sequential

Silva, Kubleka, et al. PNAS 2000

Thanks to you and to SAS!

- Most information is from the literature – thanks to all to those many groups noted
- My students helped get data:
- Rong Huang, Ling Wu, Ning Ge, Ahmed Lakhani, Anjan Roy, Weiying Zhu, and
- Research Associates: Zhenjia Wang and George Papadonakis

- There is a lot more I cut out - Have a look!.