Chem 524 Lecture Notes – CD (Section 18)— 2013

For HTML of 2005 notes, click here

XV. Circular Dichroism

A. Differential absorption of left and right circular polarized light by molecular transition

a. Normally only consider chiral molecules (non-superimposable on mirror image)

b. Can do magnetic CD, any molecule in **B** field collinear with **k**, direction of propagation

1. Measure polarization modulated transmission/need ratio to get transmission absorption

Single beam, but two signals, sum and difference separate due to modulation

$I_{mod} = I_0/2\ (10^{-A_L} - 10^{-A_R})$ and $I_{trans} = I_0/2\ (10^{-A_L} + 10^{-A_R})$

to get ΔA, convert to base e, ratio I_{mod}/I_{trans}, divide by $\exp(-\Delta A/2)$

$I_{mod}/I_{trans} = (\text{const}) \tanh(1.15\ \Delta A)$ - small ΔA $\tanh \Delta A \sim \Delta A$, constant is a gain → calibrated

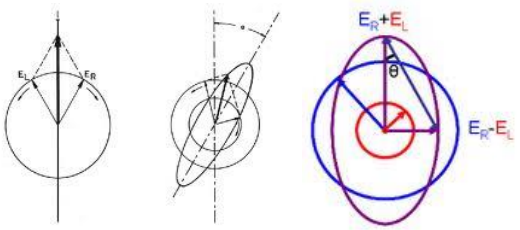
$\Delta A = A_L - A_R$ → CD, or $\Delta A = \Delta\varepsilon\ bc$ but often *quoted* as ellipticity ***(see below)***

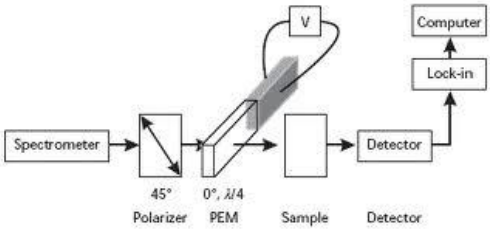
$[\theta] = 3300\ \Delta\varepsilon$ and $[\theta] = 100\ \theta/cb$ is the molar ellipticity in [deg M^{-1} cm^{-1}]

to get free of concentration and path length, express as

$\Delta A/A$ → "g-value" or anisotropy

ΔA ~ ellipticity, absorb R more than L, tilt polarization Typical CD (and LD) modulation design





2. Signals are small compared to absorption

electronic: $\Delta A/A \sim 10^{3}$; vibrational: $\Delta A/A \sim 10^{-4} \sim 10^{-5}$

need careful demodulation (lock-in, higher time constant – DC filter - typical)

3. Polarization created by linear polarizer ***(see below)***

uv — crystal prism — IR — grid

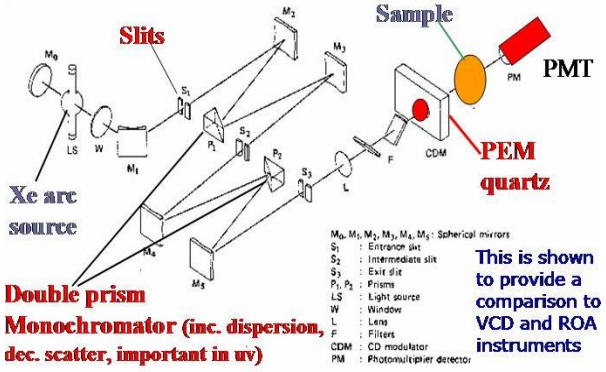
and modulator — old electro-optic (KDP) / modern — photoelastic — stress induce retard

optics, modulator materials: uv — qtz or CaF_2; IR — CaF_2 or ZnSe

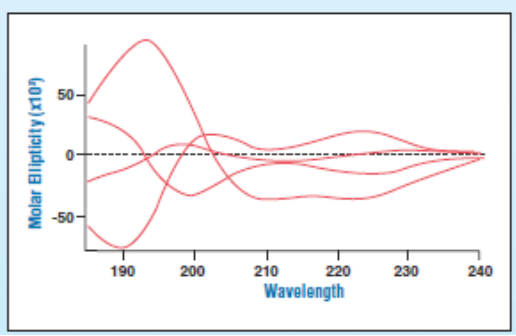
4. Detector: PMT — UV, MCT (or like) — IR

B. UV Instruments

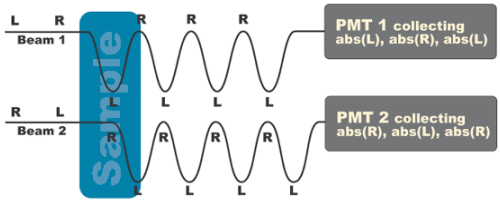
1. JASCO —dominates market — prism monochromator, good for uv also polarize (linear)

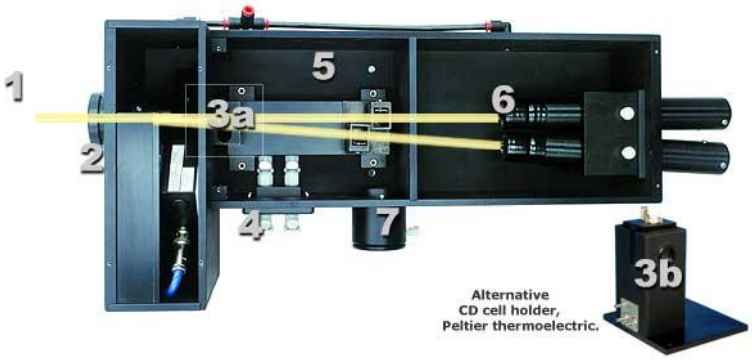


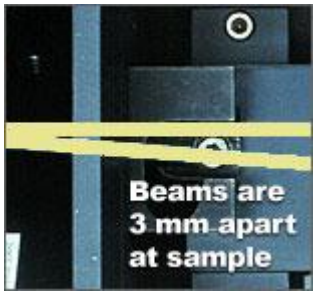
2. AVIV — similar (US), but grating based (originally remake Cary 61, now independent constr.)
Many in Biochem labs, tend to collect spectra in steps, use grating, focus at sample, OK quality



3. OLIS — conceptually different — two beam and two detector operation – (also US)
(beams from Rochan crystal polarizer — out of phase modulation – difference is CD)



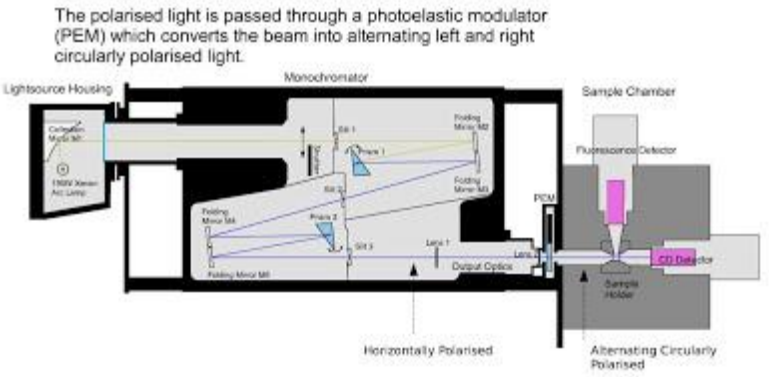


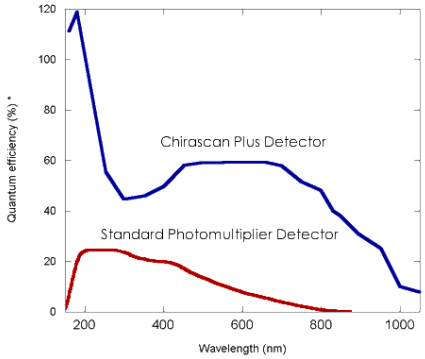


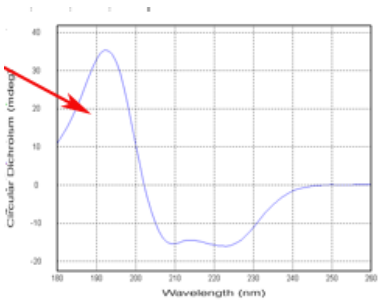
1. light input for spectrometer, several choices available from OLIS now
2. MgF_2 polarizer, followed by PEM modulator (Hinds), beams split ~3^o, 3.5 mm at sample (3)
4. water inlet for Temp. control, 5. alternate samples for absorbance, 7. port for fluorescence det.
6. two detectors (PMT) for difference detection – method fundamentally differs from normal CD

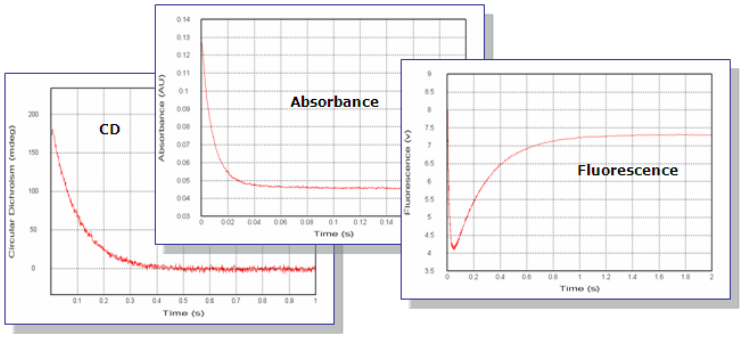
4. Others — Applied PhotoPhysics—especially good for stop-flow (dynamics), use prism optics, solid state detector, superior S/N and far-UV penetration, tends to cost more (British)

Optical arrangement, air cool Xe, prism monochromators, but solid state detector (high quantum eff.)

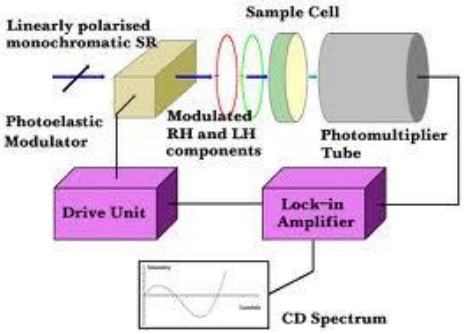


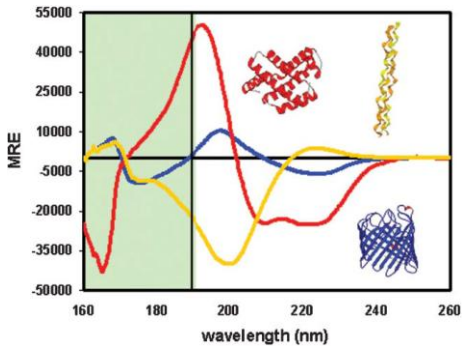


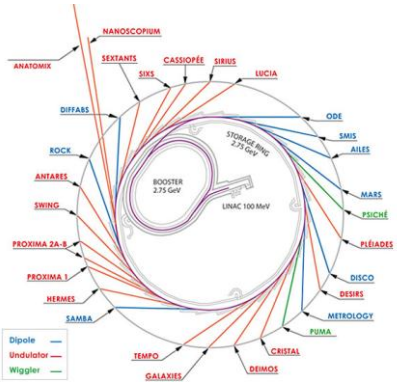




Synchrotron sources can be coupled to CD optics, big advantage, access to vacuum UV



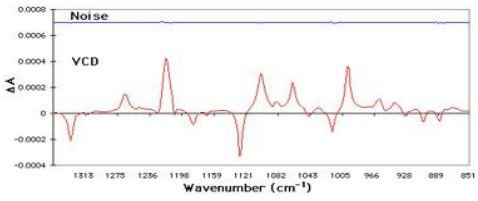


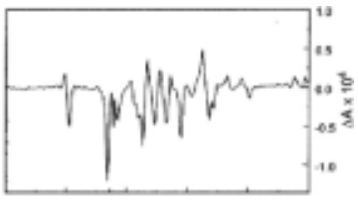


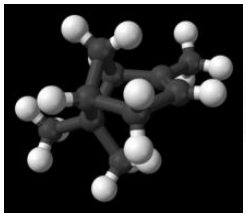
C. IR circular dichroism instruments

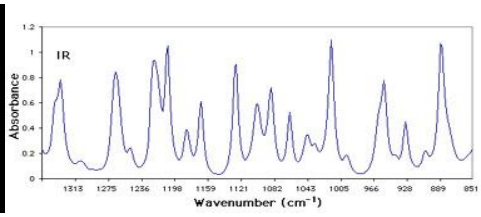
Initially, most were home made until recently, now broad commercial availability

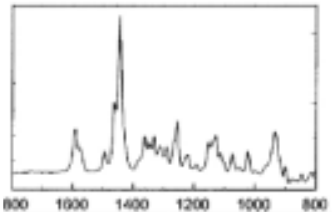
BioTools has dedicated design; Bruker, Varian, Thermo as accessories to FTIR, Jasco less clear

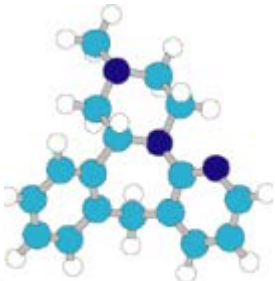






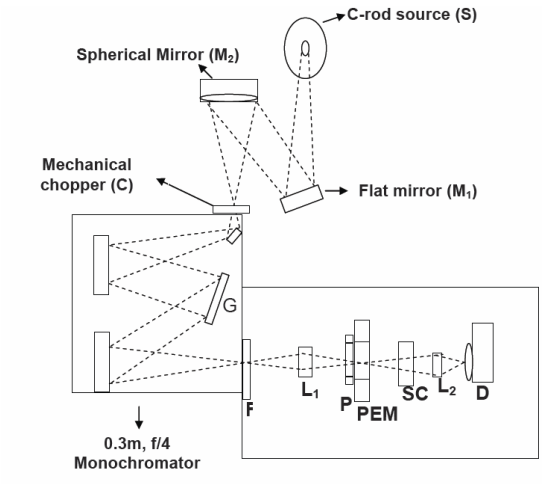


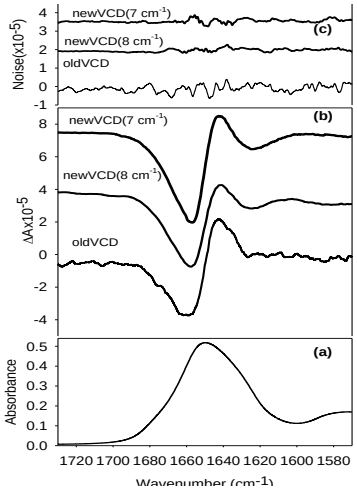


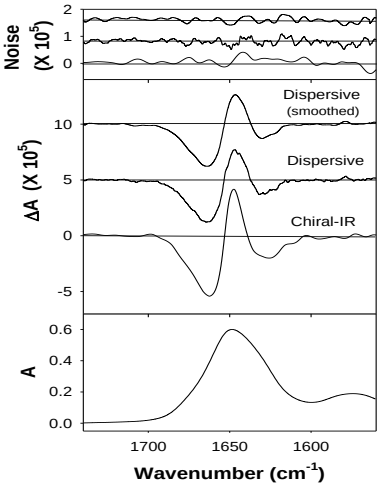


α-pinene used as a standard, mid-IR conformation constraint, means bigger VCD signals

a. Dispersive — TAK lab -- advantage low resolution, single bands, bio systems in water or D_2O





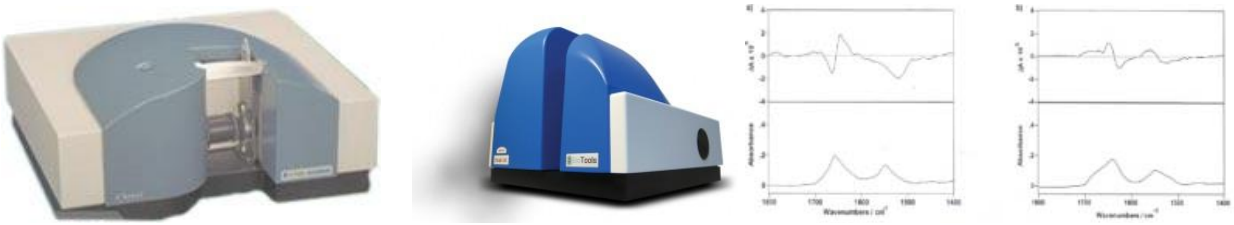


b. FT based — many labs - advantage multiplex (multiple trans), high res. – all commercial are FT

c. Dedicated VCD —Biotools --FT — for mid IR — very good S/N *(see pinene above)*

options available for near IR, also options with dual PEM for better baseline and dual source

No adjustment, drop in sample, electronics originally separate, now integrated (DSP), can do proteins



- DSP-based — eliminate lockin -- full digitize time dependent signal
  - *Hilario et al, Appl Spectrosc, 55, 1435 (2001)*
  - Independent commercial design now available form BioTools

Link to slides from TAK group meeting summary of bio-applications of CD and VCD

Other companies operate VCD as an accessory to FTIR line

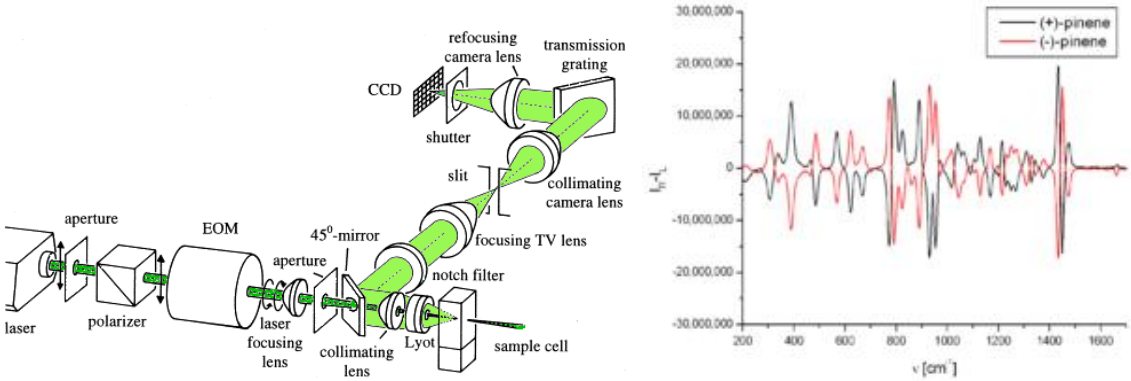
Bruker PMA 50

Thermo-Nicolet PEM mod

Jasco accessory + FTIR, also have stand alone model

d. Raman Optical Activity is a variant of Raman spectroscopy, but measuring differential scattering in left and right circularly polarized light → $\Delta I = I_R - I_L$ (see Raman section Notes 17 for Instruments)

Bio-Tools - commercial version based on Hug design with split beam measured on CCD, can inc. μ-scope



Barron design, CP laser, 180° backscatter, fast spectrograph with CCD, spectra mirror image entire region

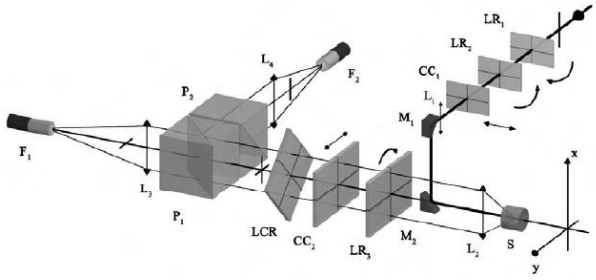


Figure 6.2: Optical arrangement of the backscattering part of our actual ROA spectrometer in Fribourg. M_1, M_2: mirror for 90° polarisation neutral beam deflection. LR_1, LR_2: high-speed counter-rotating linear rotator. LR_3: slow-rotating linear rotator. P_2: second polarising cube used to compensate the difference in extinction of P_1 for the two orientation of linear radiation. L_3, L_4: lens for focusing linear radiation splitted by P_1 and P_2 into the two fiber optics, F_1 and F_2, of our dual-arm design. Other optical elements as in Fig.6.1.

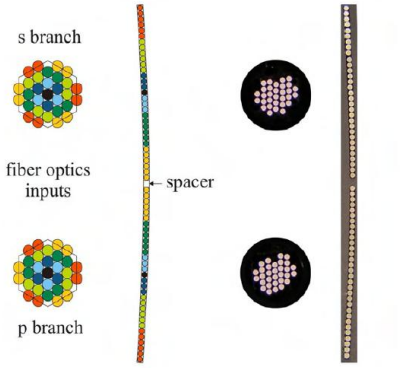
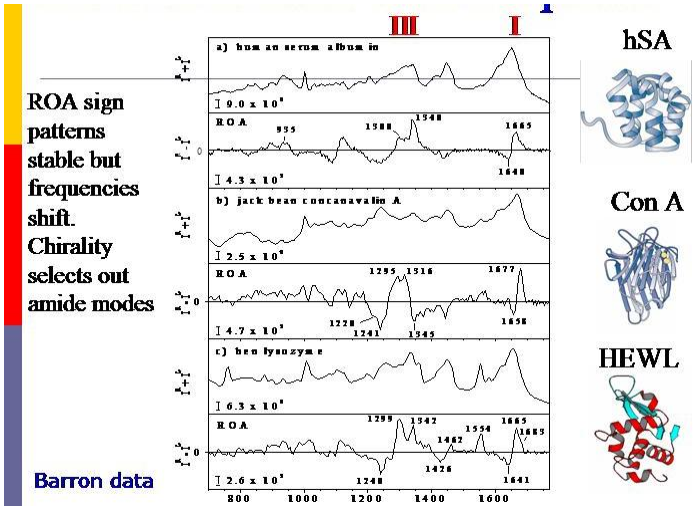


Figure 7.5: Detailed view of the fiber optics cross-section transformer. Left: schematic design of both fiber inputs and of the curved slit (R=108.5 mm). Right: pictures of both fiber inputs and of the curved slit. It looks evident that the input's hexagonal shape has not been manufactured correctly. The vertical alignment of the fibers is correct but not the horizontal one.

Hug design, two branches cancel artifacts

Fiber optic in each, reshape to slit match, commercial instrument (BioTools)↓ based on Hug design



D. CD Applications _Incomplete!! ↓

1. Molecular stereochemistry/absolute configuration

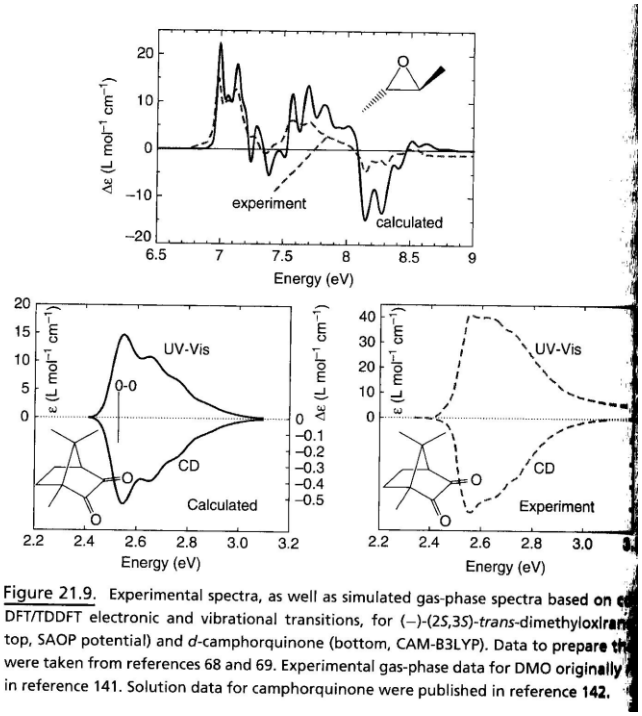
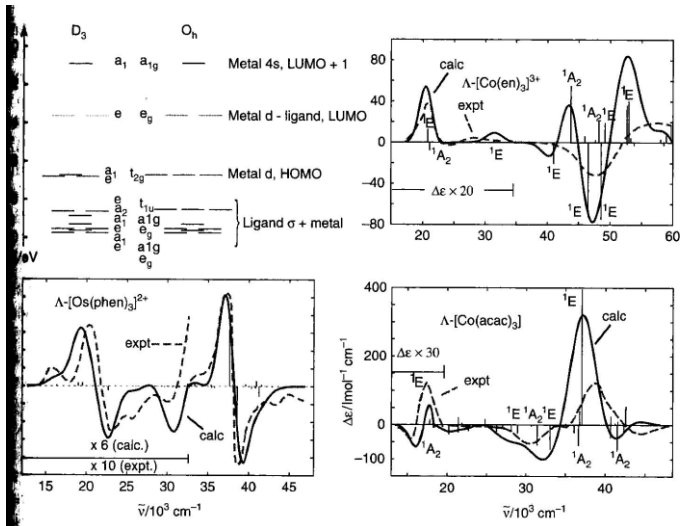
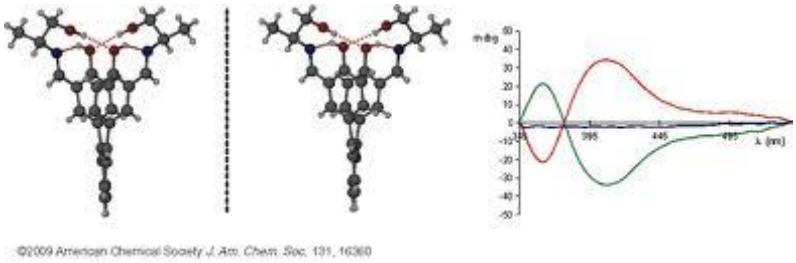


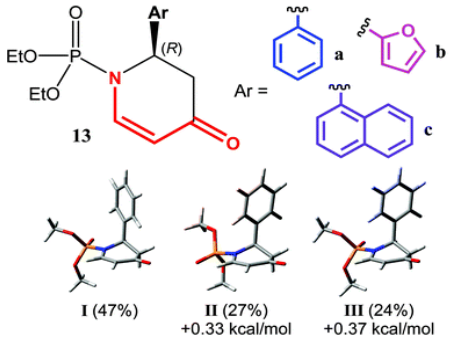
Figure 21.9. Experimental spectra, as well as simulated gas-phase spectra based on c DFT/TDDFT electronic and vibrational transitions, for (−)-(2*S*,3*S*)-*trans*-dimethyloxiran top, SAOP potential) and *d*-camphorquinone (bottom, CAM-B3LYP). Data to prepare th were taken from references 68 and 69. Experimental gas-phase data for DMO originally in reference 141. Solution data for camphorquinone were published in reference 142.



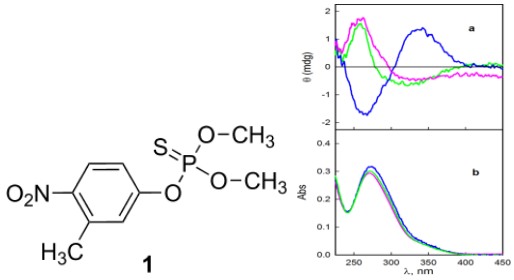
re 21.12. Representative examples of calculated CD spectra of Λ tris–bidentate metal plexes, in comparison with experiment. Data to prepare the figures were taken from ences 25 and 156–159. Also shown is a calculated orbital diagram for $[Co(en)_3]^{3+}$ in D_3 metry, along with the orbital levels of a hypothetical octahedral complex. The first pair lculated *d*-to-*d* transitions for $[Co(en)_3]^{3+}$ was shifted by -6×10^3 cm^{-1}. All calculated itions for $[Co(acac)_3]$ was shifted by -4×10^3 cm^{-1}, and for $[Os(phen)_3]^{2+}$ by $+2 \times 10^3$ cm^{-1}. of the spectra are magnified.

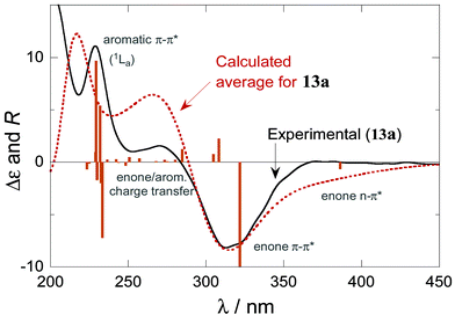


Enatiomers have mirror image CD

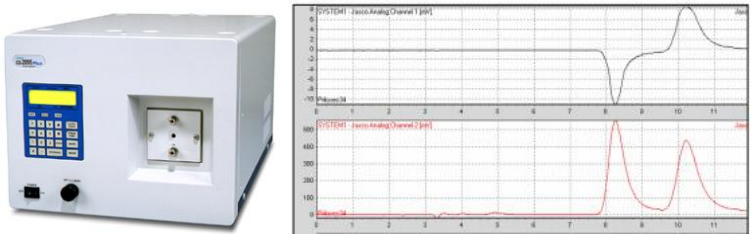


Induced chirality also possible, dissolve in chiral hosts e.g. cyclodextrins





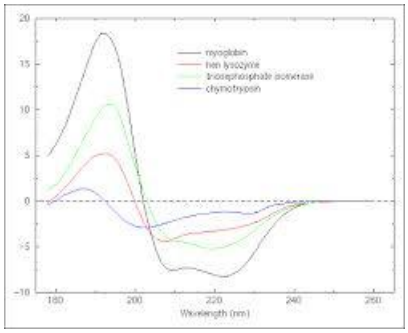
2. Chiral detector in separations, LC, Priosec is mixed enantiomer drug

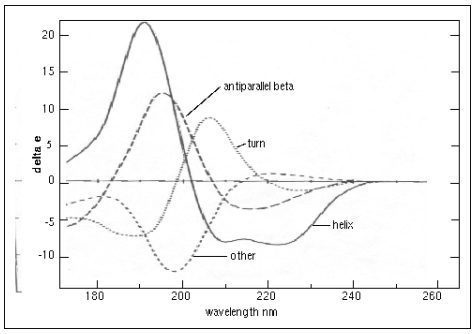


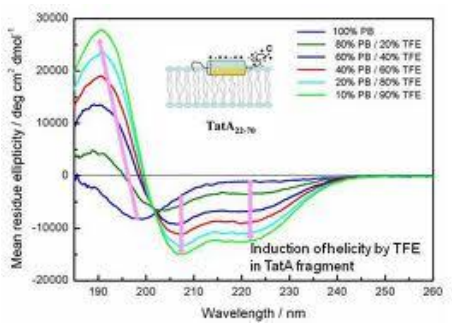
JASCO CD 2095 for HP compare CD and UV detection HPLC

3. Biological polymer — conformational analysis

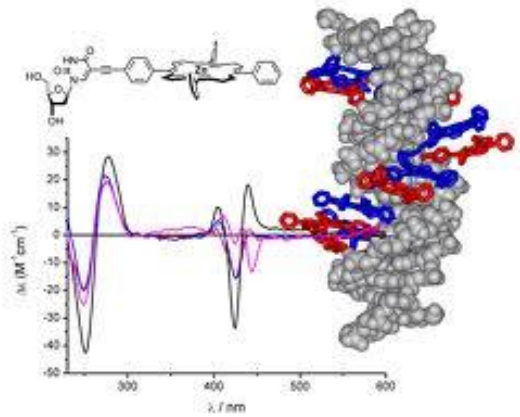
(proteins, peptides, nucleic acid — secondary structure)

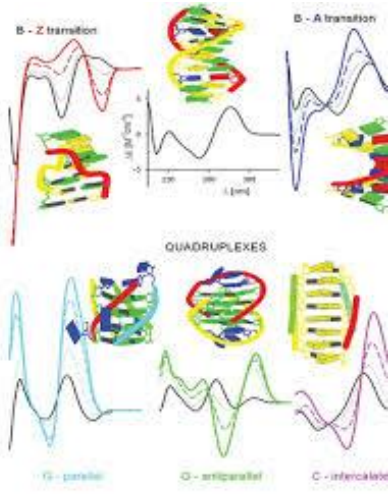


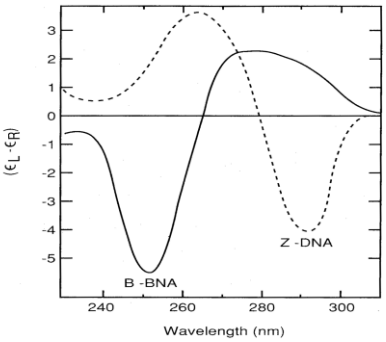




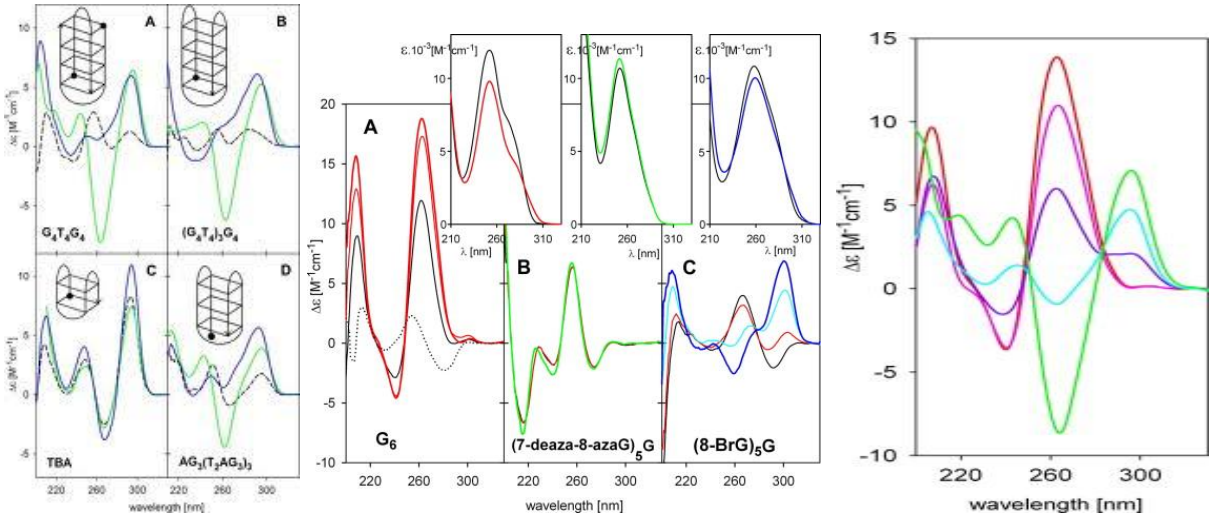
Nucleic acids and interactions of porphyrins (e.g.) with DNAs, B-Z transitions,



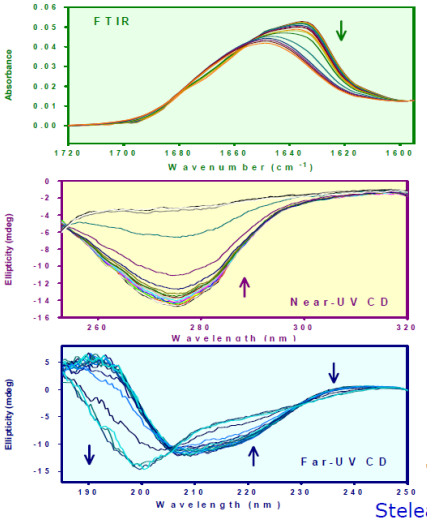




quadruplexes and base sensitive CD results



CD spectra of d(G_6) and of its modified analogs d[(7-deaza-8-aza-G)$_5$G] and d[(8-BrG)$_5$G]. For all oligonucleotides, spectra shown in black were measured in 1 mM sodium phosphate (pH 7), 0.3 mM EDTA. Those in thin red lines were measured in 1 mM sodium phosphate, 0.3 mM EDTA, 3 mM K_2HPO_4 immediately after K^+ addition. (A) d(G_6): spectrum in thick red was taken 24 h after K^+ addition. The black dots show the calculated CD spectrum based on mono- and dinucleotides (i.e., without the influence of secondary structure). CD spectra of (red) d(G_8), (pink) d(G_4TG_4), (violet) d($G_4T_2G_4$), (cyan) d($G_4T_3G_4$) and (green) d($G_4T_4G_4$) measured in 10 mM sodium phosphate (pH 7), 150 mM NaCl seven days after NaCl addition.



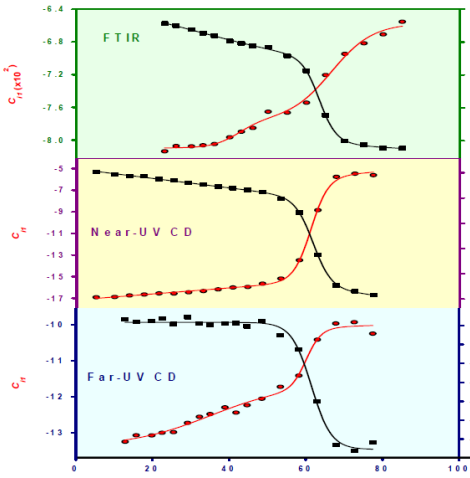
RibonucleaseA

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



Ribonuclease A
PC/FA loadings
Temp. variation

FTIR (α,β)

Near-uv CD (tertiary)

Far-uv CD (α-helix)

Temperature

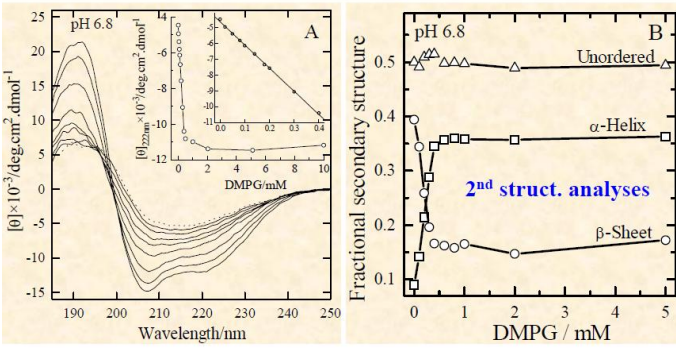
Stelea, et al. Prot. Sci. 2001

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

Stelea, et al. Prot. Sci. 2001

BLG Binding to DMPG at pH 6.8: Circular Dichroism

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

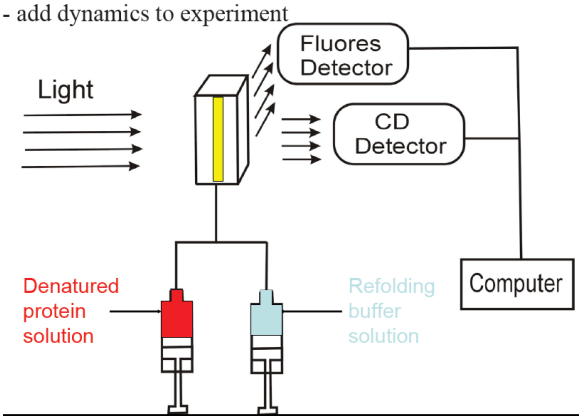


Secondary structure: Binding DMPG at pH6.8, causes BLG conformational change. The α–helix formed with loss of β-sheet.

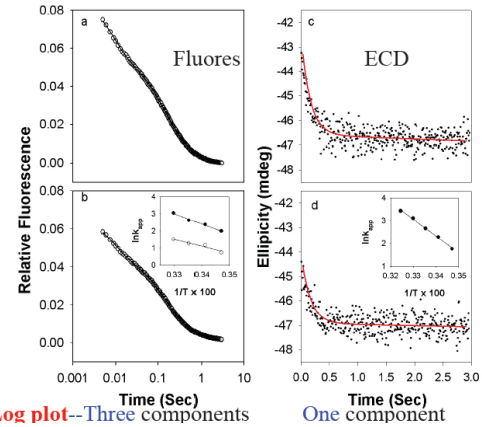
4. Protein folding — stop flow

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

Xu & Keiderling Proteins 2006

Log plot--Three components

One component

5. Time dependent possible — detect ellipticity with ns laser -- photolysis (chromophore)