CHIRAFUN
Chiralité et Multifonctionnalités

Stereochemical analysis by circular dichroism spectroscopies

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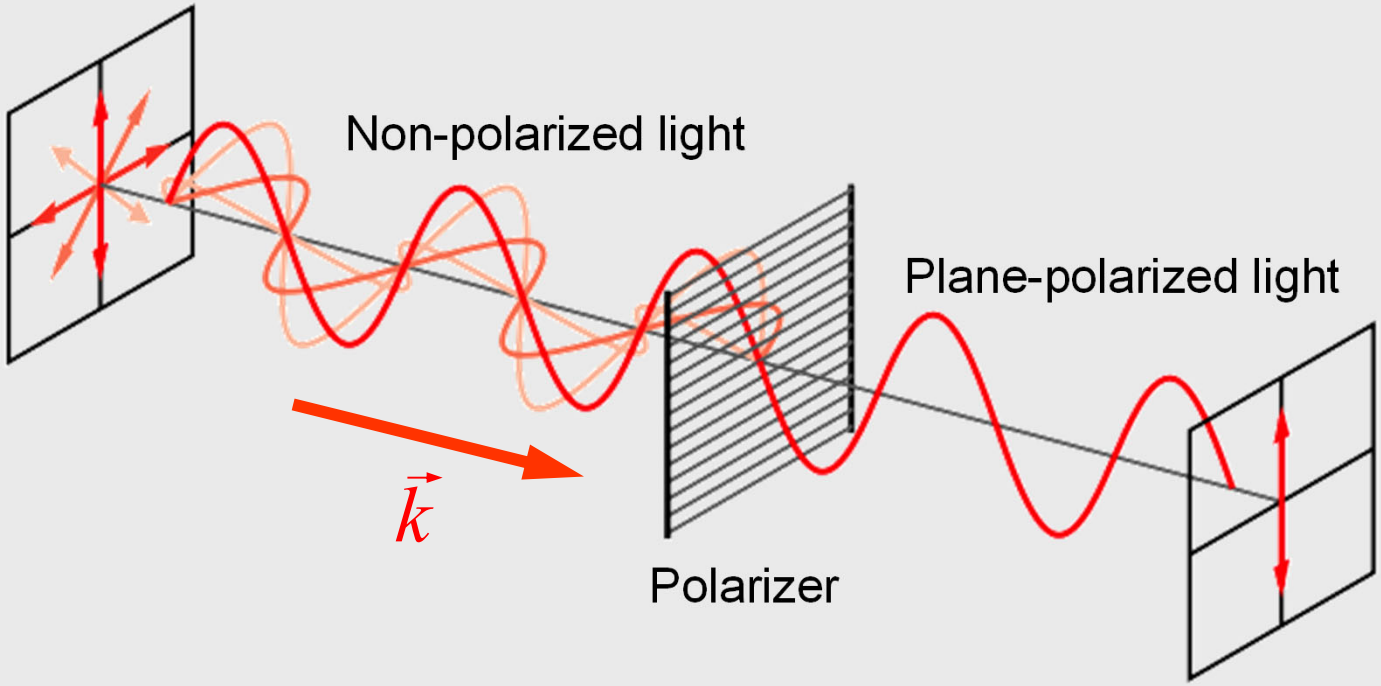
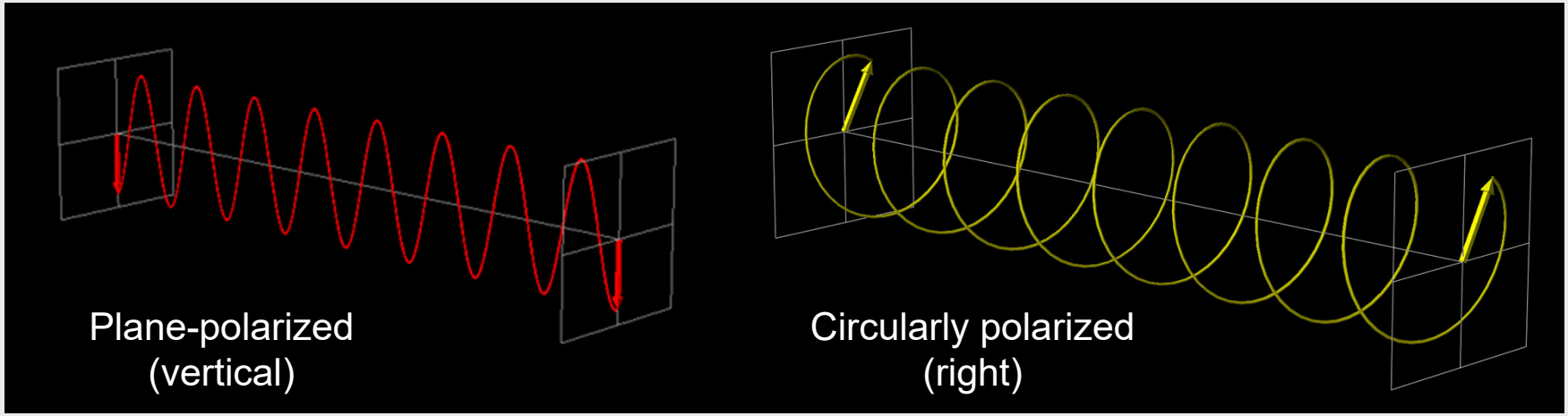
Synopsis

1. Electronic Circular Dichroism (ECD) and related spectroscopies
   a. Instrumentation & basics
   b. Major factors determining ECD spectra
   c. ECD applications on small molecules and biopolymers
      i. Absolute configuration assignment
      ii. Conformational analysis
2. ECD and supramolecular chirality
   a. Chiral supramolecular systems
   b. Assessment of supramolecular helicity
3. ECD of aggregated states of conjugated polymers
   a. Aggregate formation and evolution
   b. Intrachain vs interchain helicity
   c. Multiple aggregation pathways
   d. CD imaging
4. Vibrational Circular Dichroism (VCD)
   a. Parallel with ECD
   b. Main applications

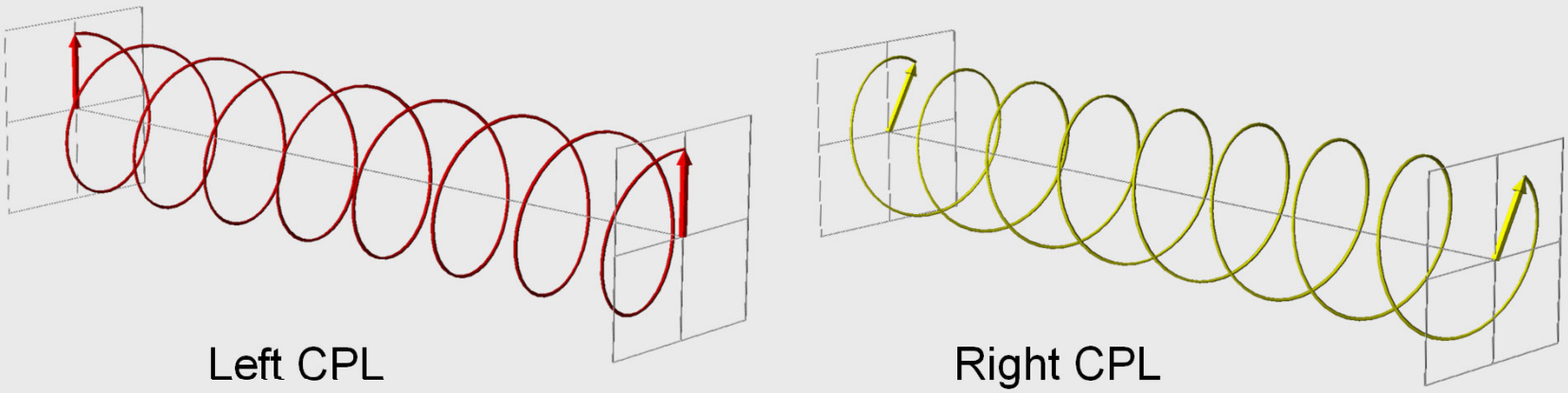
Light polarization



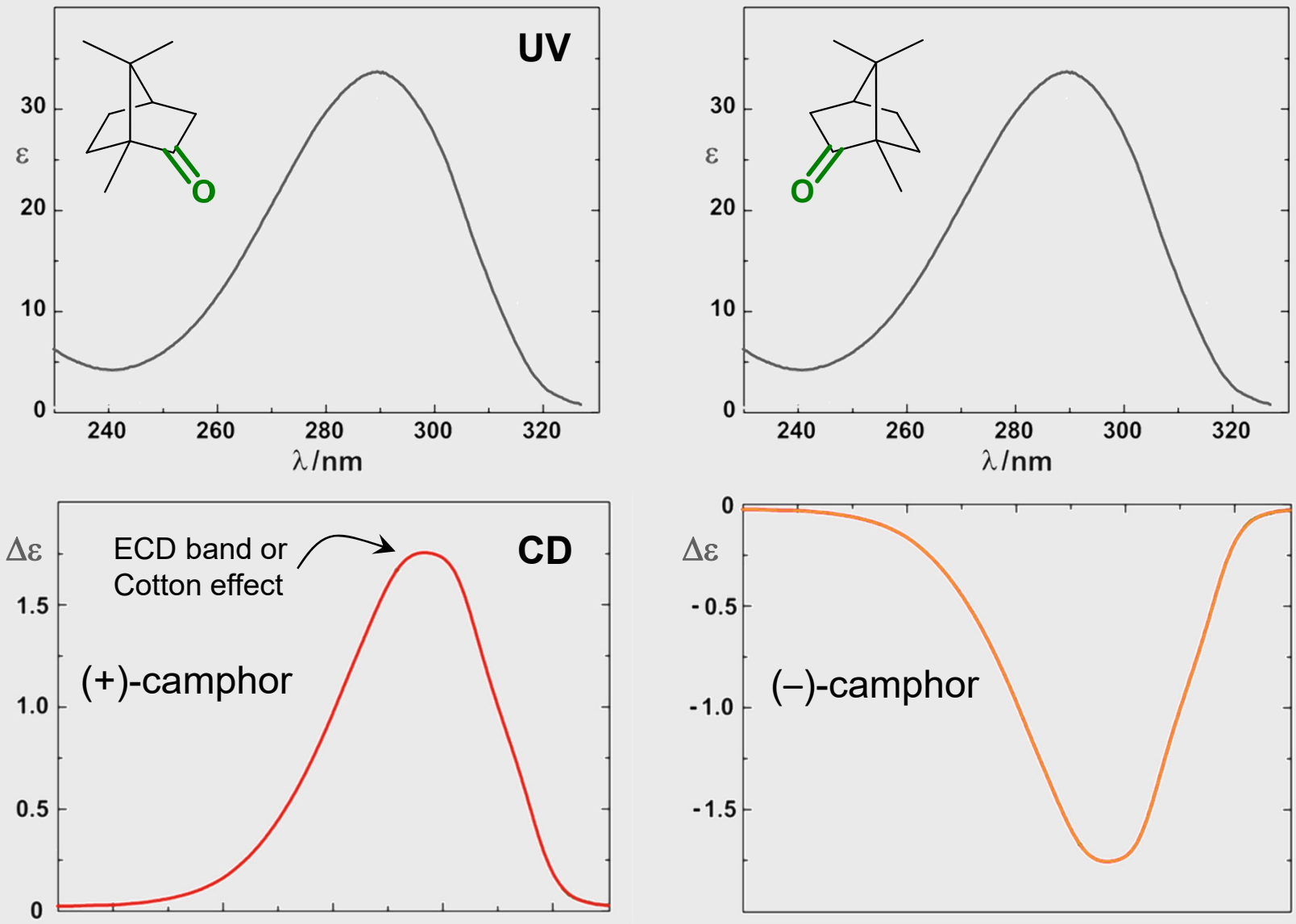


Chiroptical vs. non-chiral spectroscopies

- UV-vis absorption spectroscopy:
  absorption of isotropic (non-polarized) light
- Electronic circular dichroism (ECD):
  differential absorption of left vs. right
  circularly polarized light in the UV-Vis-NIR range
- Both allied with (low-lying) electronic transitions



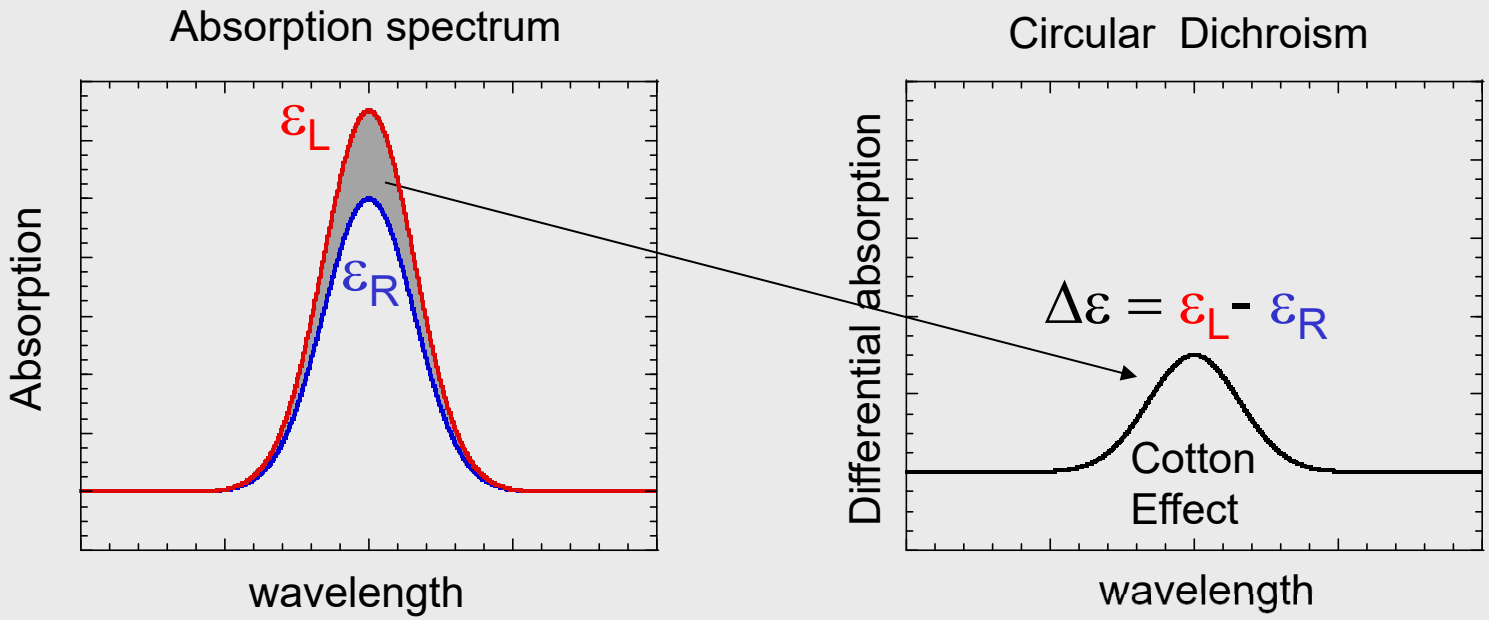
Chiroptical vs. non-chiral spectroscopies



Chiroptical vs. non-chiral spectroscopies

- Electronic circular dichroism (ECD or CD)
  - UV-vis absorption spectroscopy
- Optical rotatory dispersion (ORD)
  - Refractive index dispersion
- Vibrational circular dichroism (VCD)
  - IR spectroscopy
- Raman optical activity (ROA)
  - Raman spectroscopy
- Circularly polarized luminescence (CPL)
  - Fluorescence (emission spectrum)

Circular dichroism: phenomenology



$$\mathrm{Abs} = \frac{1}{2}(A_L + A_R) = \varepsilon \cdot \ell \cdot c$$

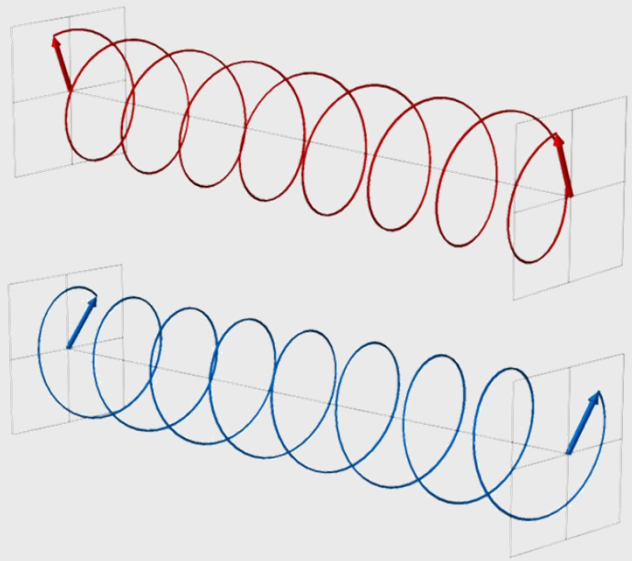
$$\mathrm{CD} = A_L - A_R = (\varepsilon_L - \varepsilon_R) \cdot \ell \cdot c$$

$$= \Delta\varepsilon \cdot \ell \cdot c$$

$$\theta = 33000\ \Delta\varepsilon \cdot c \cdot \ell$$

$\Delta\varepsilon$ and ε in $M^{-1}\,cm^{-1}$, c in M, ℓ in cm

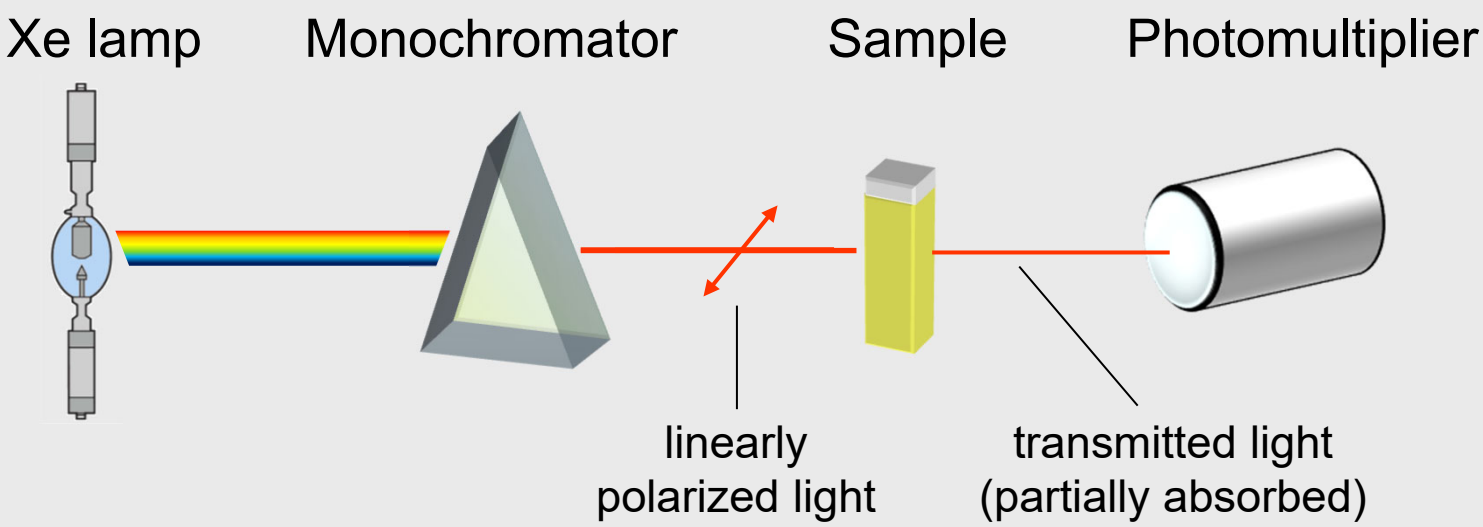
θ ellipticity in mdeg

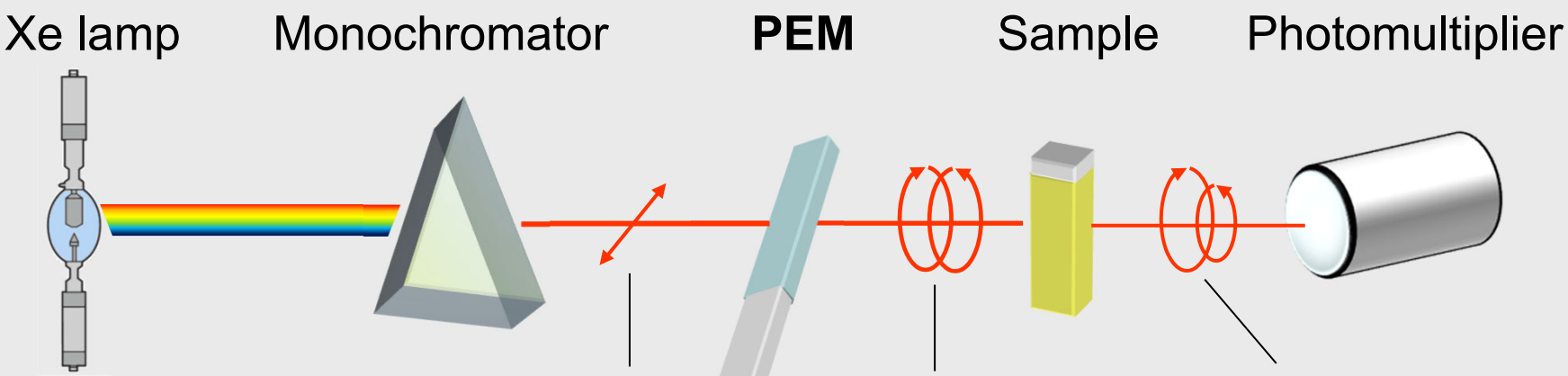


Left CPL

Right CPL

Circular dichroism: instrumentation



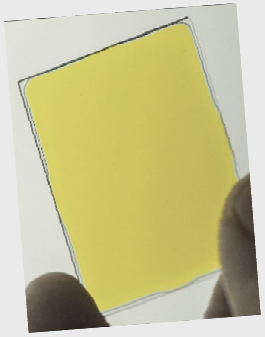


PEM = Photoelastic modulator

Circular dichroism: measurement

Measurement conditions:

- Near UV-transparent solvent
  (water, CH_3OH, CH_3CN, dioxane, hexane)
- Sample absorbance < 1 (ideally ≈0.8)
- Commonly observed range 185-700 nm, up to 1300 nm
- State of the matter: mainly solutions, but also solid state
  (thin films, powders, microcrystals)
- Optional: variable-temperature module, stopped-flow etc.



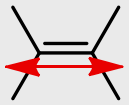
Non-negligible ECD spectrum requires:

- Resonance: presence of one or more light-absorbing units (chromophores)
- Chirality: structure devoid of symmetry elements (planes, centers)

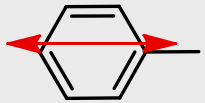
Chromophores and symmetry

Chromophore

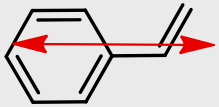
- A light-absorbing unit composed by one or more atoms
- Conjugated π-system, or atom with high-lying *n*, *d* or *f* orbitals undergoing π-π^*, *n*-π^*, *n*-σ^*, *d*-*d* or *f*-*f* transitions



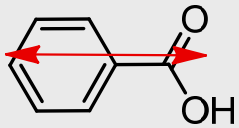
190 nm
alkene



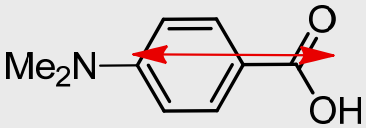
210 nm
benzene



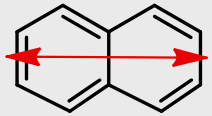
250 nm
styrene



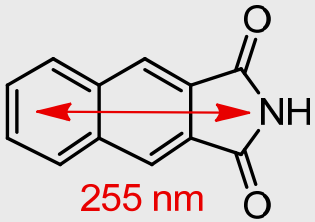
230 nm
benzoate



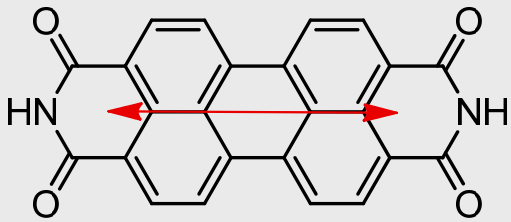
310 nm
p-DMA benzoate



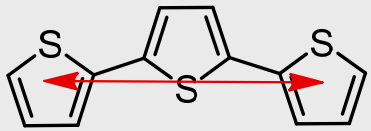
220 nm
naphthalene



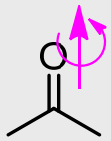
255 nm
naphthalimide



500 nm
perylenediimide



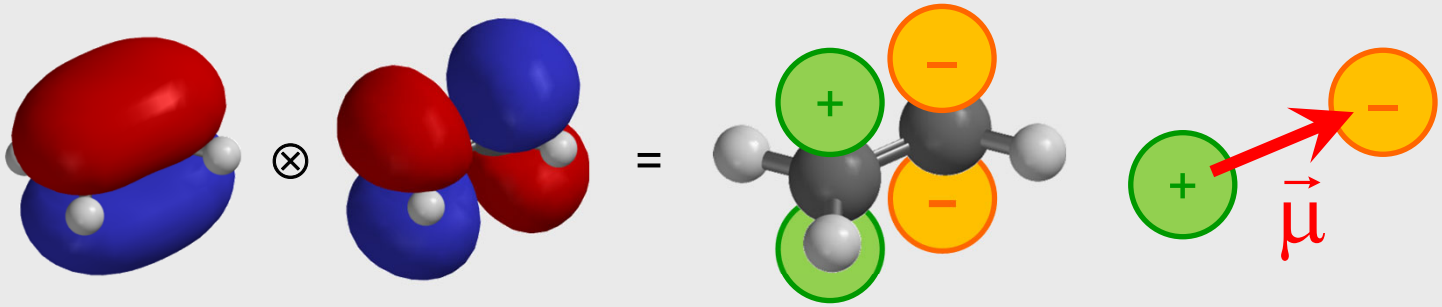
350 nm
tertthiophene



280 nm
carbonyl

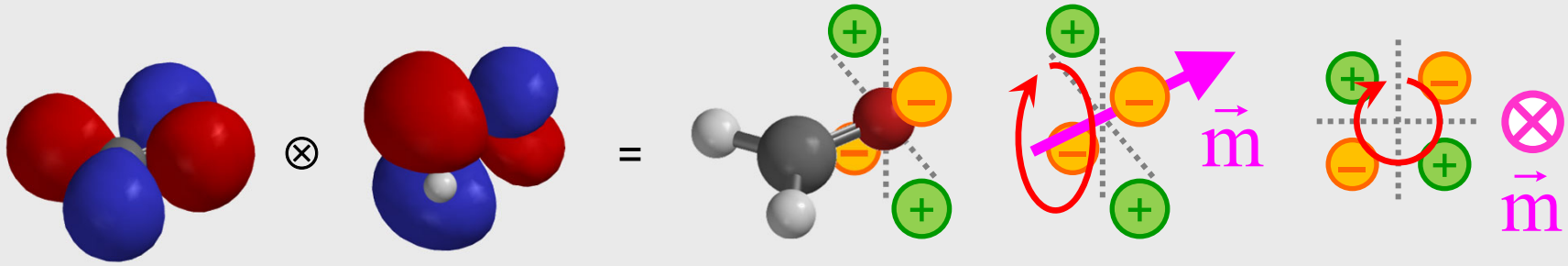
Chromophores and symmetry

- An **electric-dipole allowed** transition: alkene π → π* (190 nm)



HOMO (π) LUMO (π*)

- A **magnetic-dipole allowed** transition: carbonyl n → π* (290 nm)



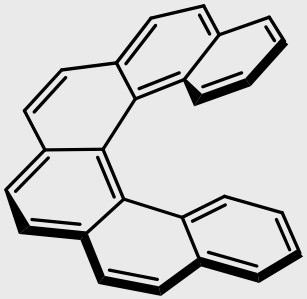
HOMO (n) LUMO (π*)

Both are oscillating dipoles!

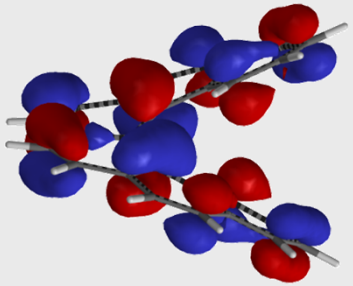
Rotational strength and transition moments

$$R_{0j} = \vec{\boldsymbol{\mu}}_{0j} \cdot \vec{\mathbf{m}}_{j0}$$ Rosenfeld equation

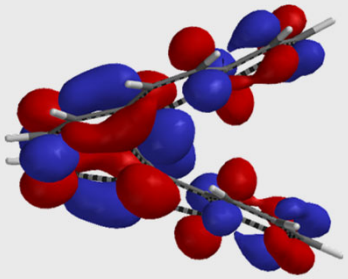
rotational strength — *electric transition dipole* — *magnetic transition dipole*



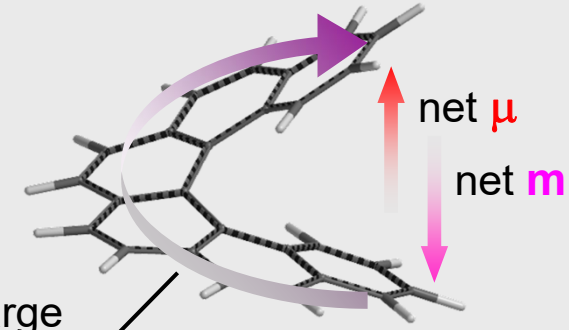
(*M*)-[6]helicene



HOMO

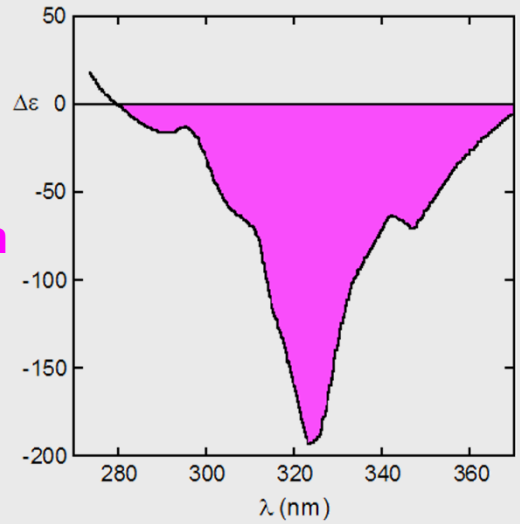


LUMO



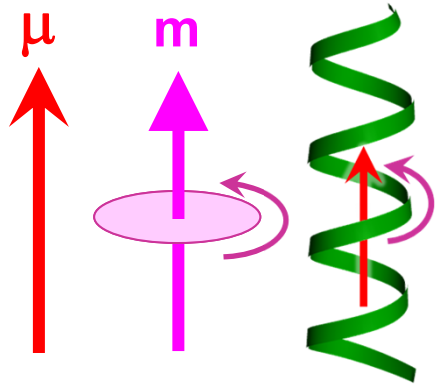
net charge displacement (left-handed **helix**)

$$R = \boldsymbol{\mu} \cdot \mathbf{m} < 0$$



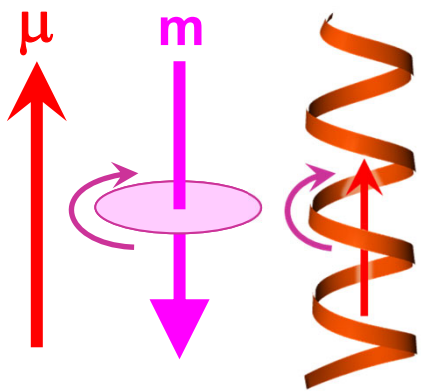
right-handed helix

$$R = \boldsymbol{\mu} \cdot \mathbf{m} > 0$$



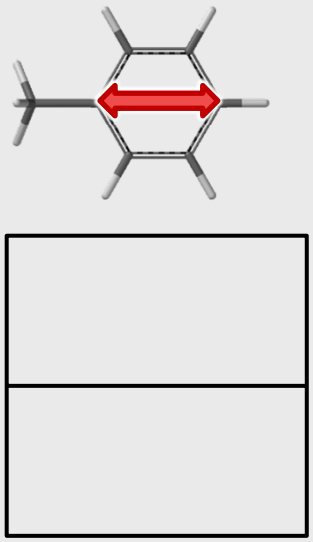
left-handed helix

$$R = \boldsymbol{\mu} \cdot \mathbf{m} < 0$$

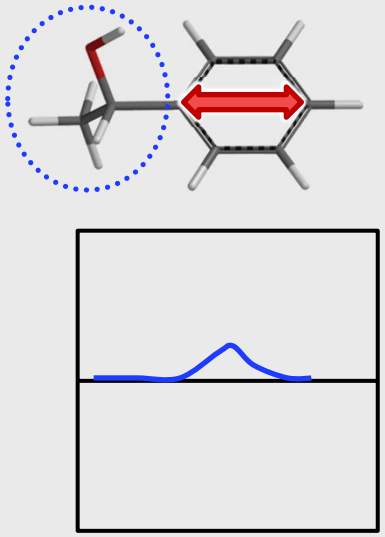


Optical activity mechanisms

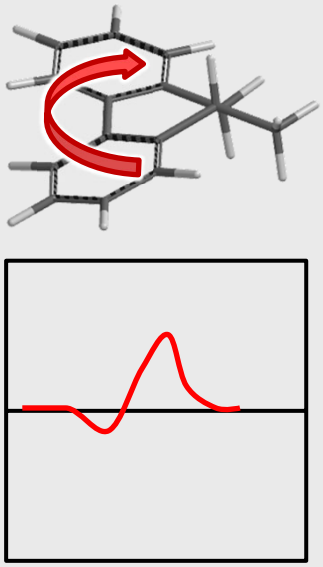
Chromophore in achiral molecule



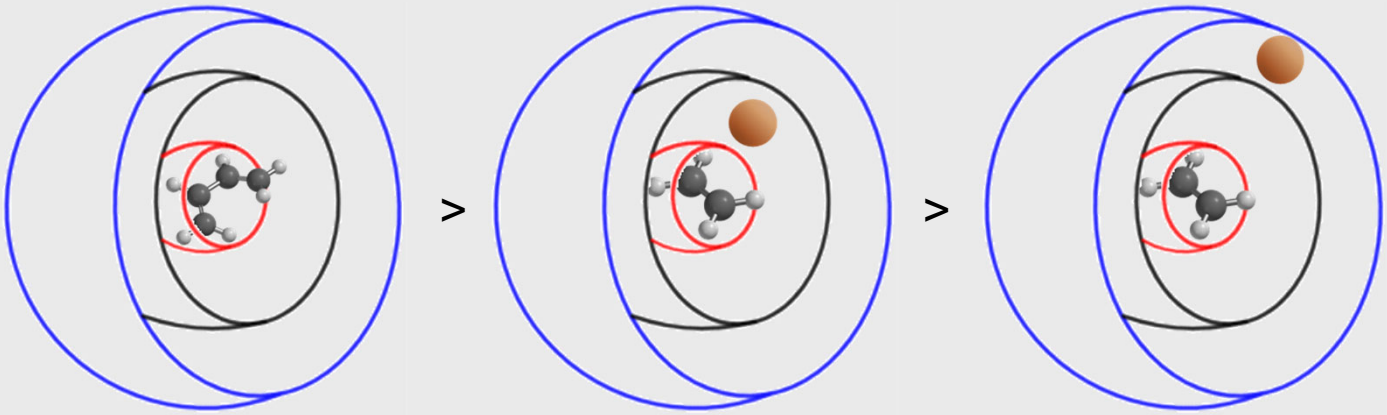
Chromophore with perturber



Twisted chromophore

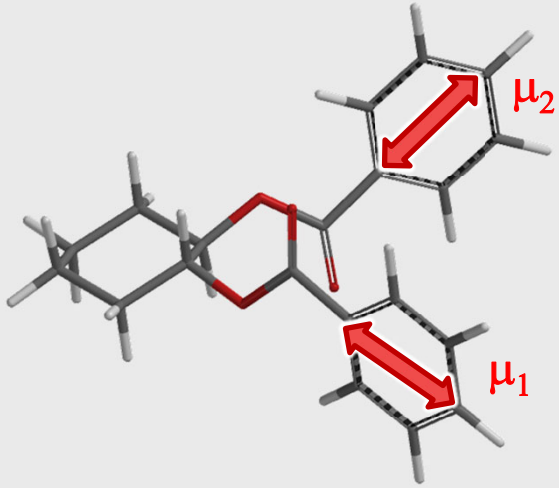


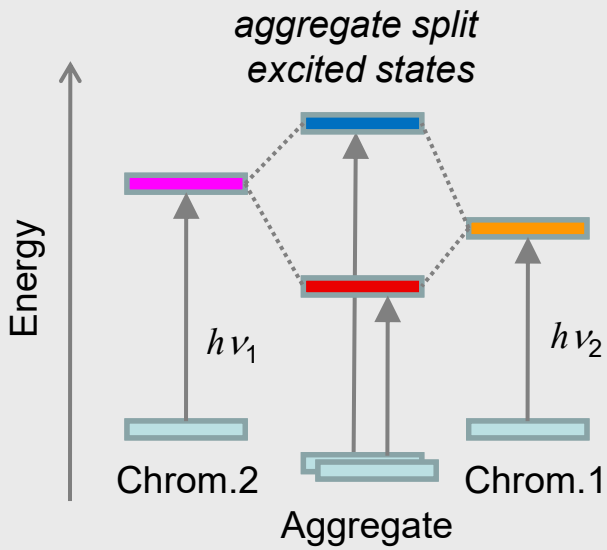
Chirality spheres

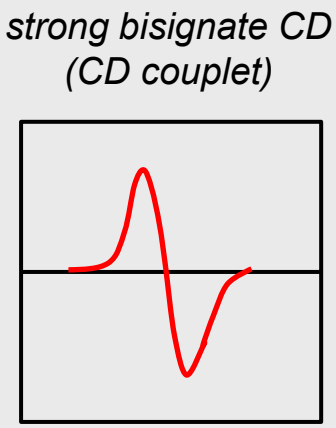


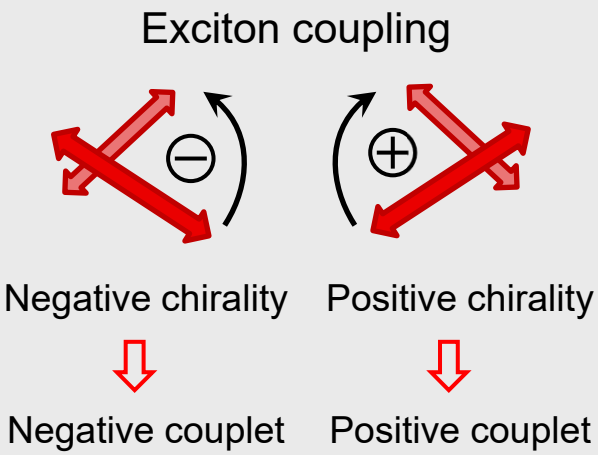
Exciton coupling and exciton chirality

- Two or more chromophores with electric-dipole allowed transitions
- Electronically isolated chromophores (no conjugation, resonance, charge-transfer)
- Skewed moments (not coplanar or collinear)





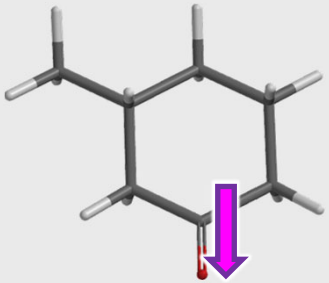




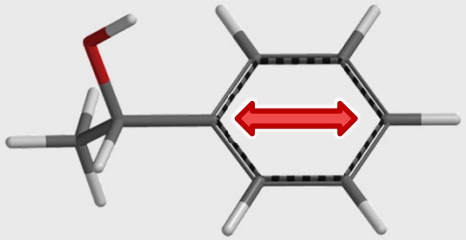
Quantifying CD spectra: the g-factor

Dissimmetry ratio or Kuhn's ratio or g-factor:

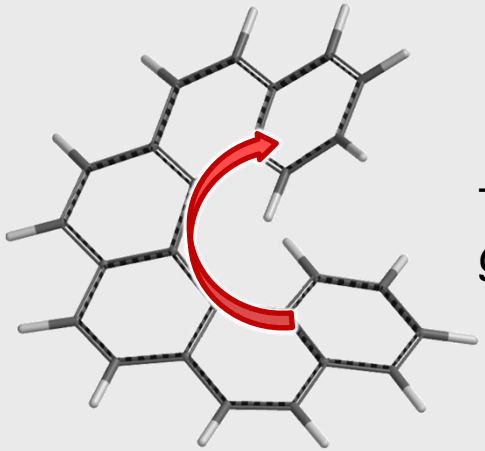
$$g = \frac{\Delta\varepsilon}{\varepsilon} = \frac{\Delta Abs}{Abs} \propto \frac{\mathbf{m}_{i0}}{\boldsymbol{\mu}_{0i}}$$



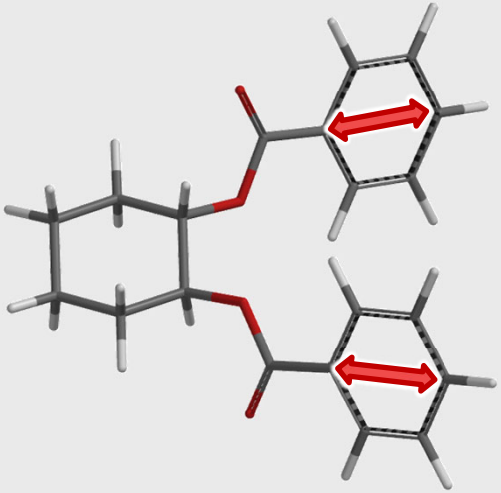
m-allowed
$g \approx 5 \cdot 10^{-2}$



μ-allowed
$g \approx 10^{-4}$



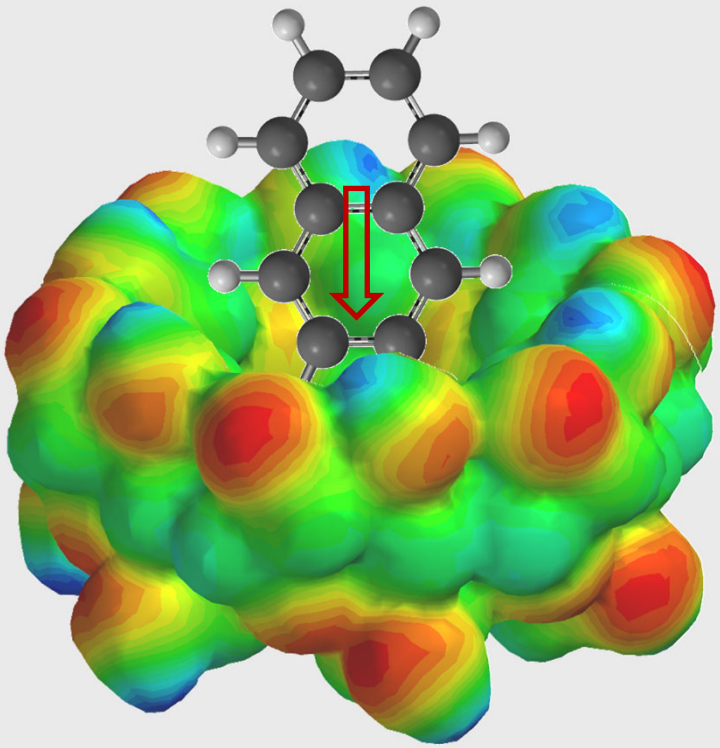
π-twisted
$g \approx 10^{-2}$

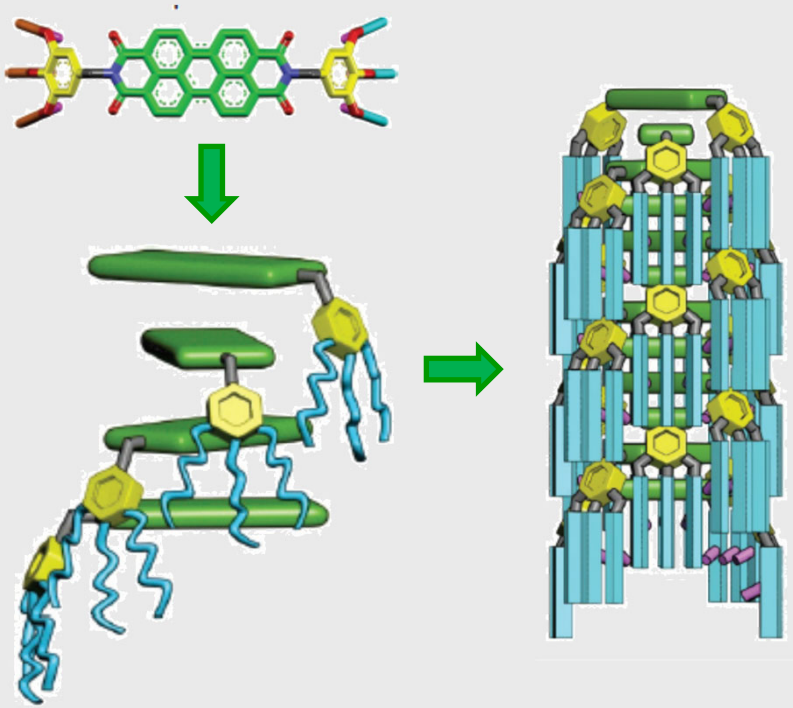


exciton-coupled
$g \approx 10^{-3}$

Chromophores and pertubers

- The perturbation by a chiral surrounding may act non-covalently,
  e.g.: an achiral dye embedded in a chiral host
- Exciton coupling may act intermolecularly,
  e.g.: a chiral supramolecular aggregate of (a)chiral dyes



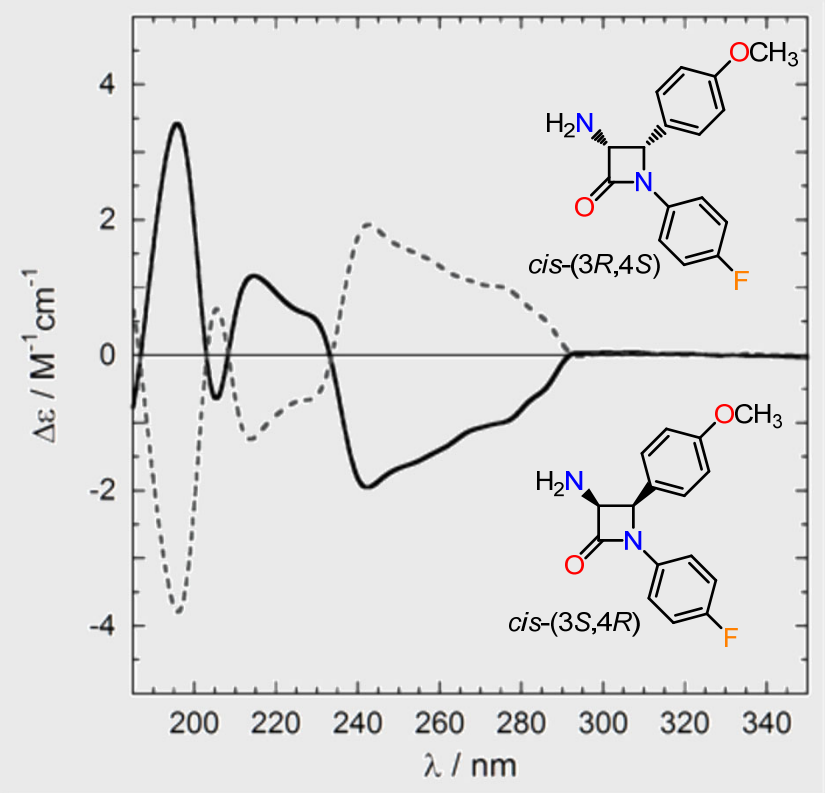


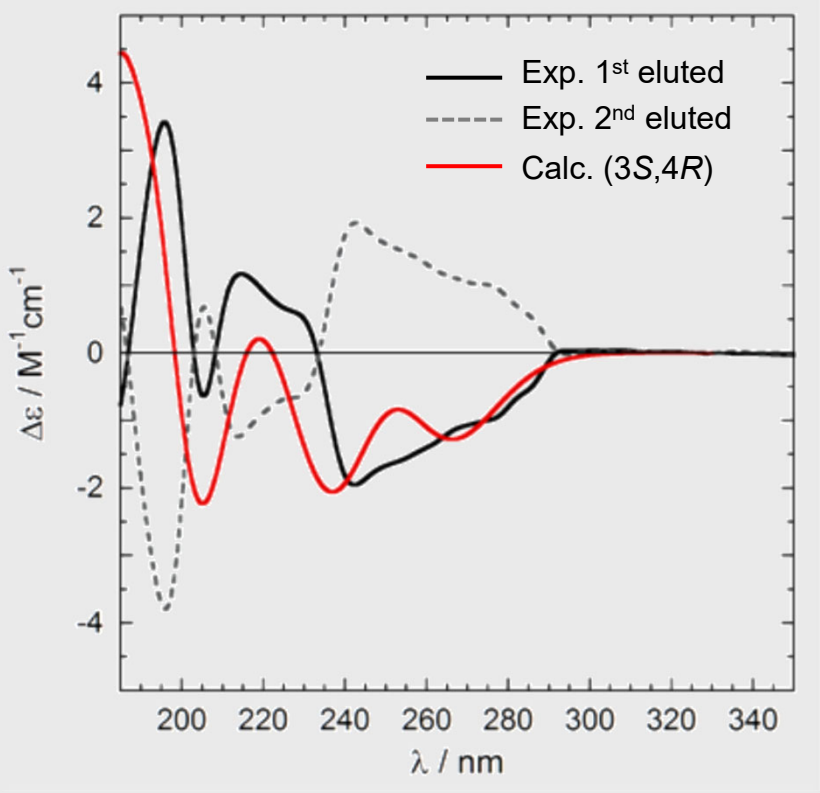
Main applications of ECD spectroscopy

- Assignment of absolute configurations, especially of (small) organic molecules and organometallic compounds
- Conformational investigations (small molecules, polymers, biopolymers)
- Detecting the on/off state of chiroptical switches
- Analytical tool to detect and quantify molecular adducts and supramolecular species
- Helicity and other structural details of chiral supramolecular assemblies
- Chromatographic detector (direct e.e. measurement)

CD, configuration and conformation

- CD spectra are sensitive to the **overall** stereochemistry
- The two enantiomers of a chiral species always have mirror image CD spectra ⇨ absolute configuration assignment

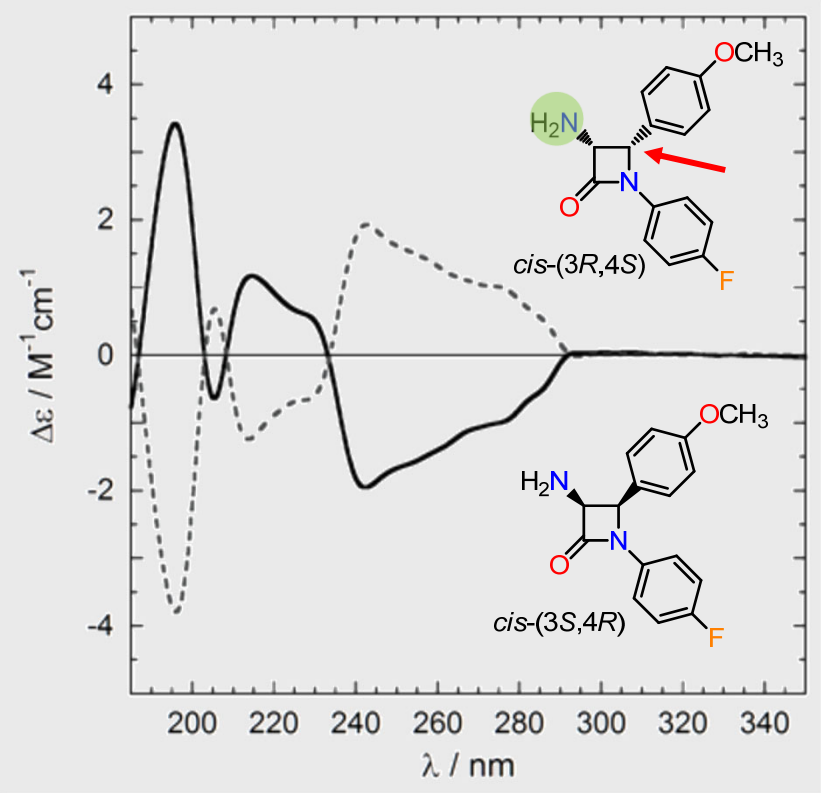


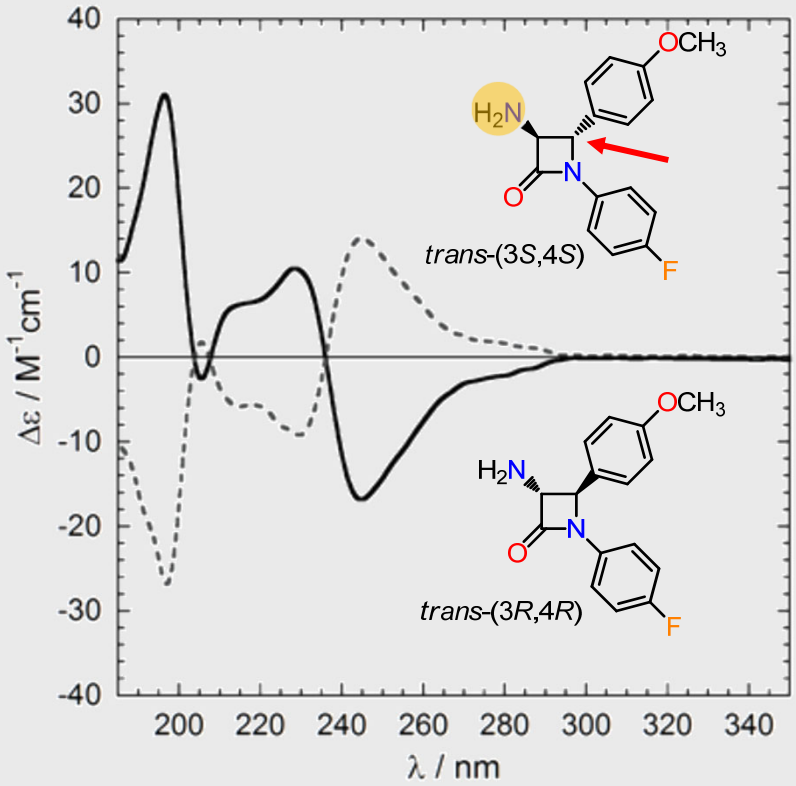


Dražić et al, *Eur J Org Chem* **2016**, 4189

CD, configuration and conformation

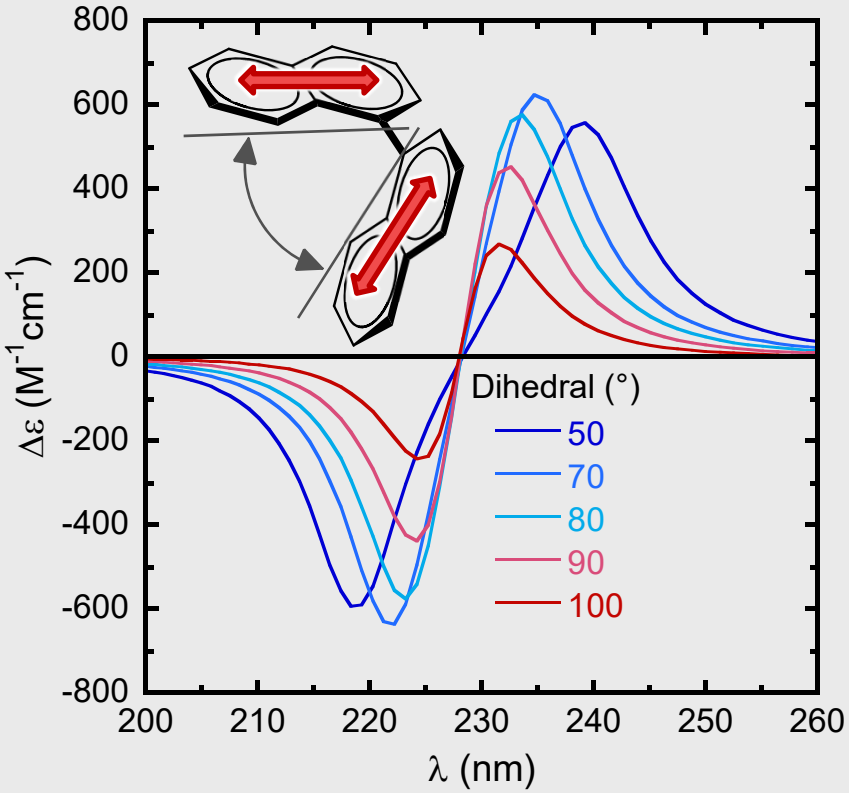
- CD spectra are sensitive to the **overall** stereochemistry
- The diastereomers of a chiral substance with multiple chirality elements have in general different CD spectra

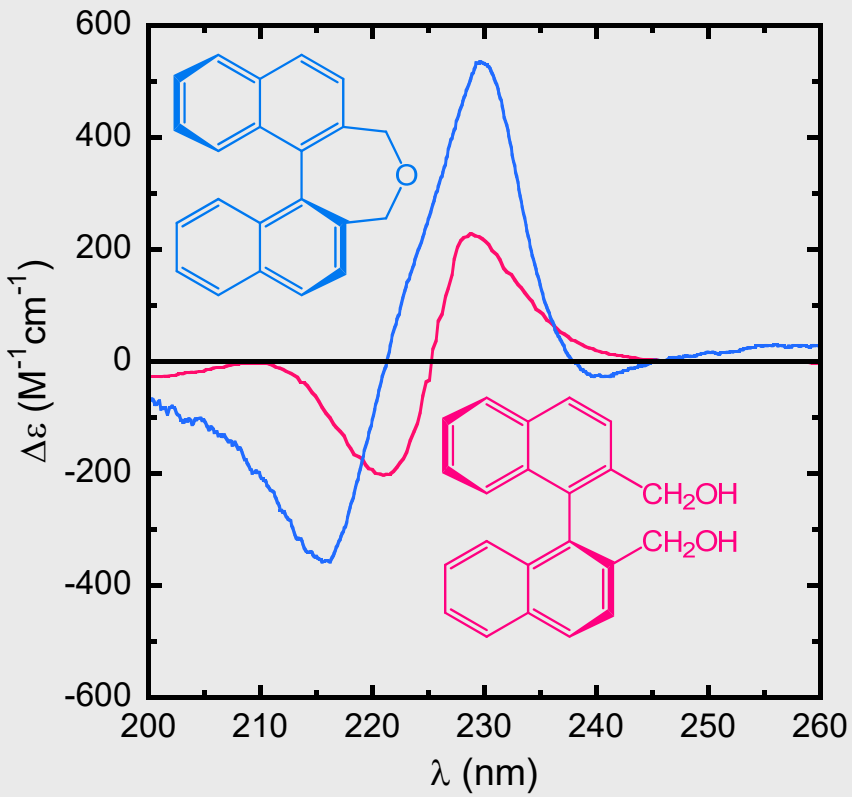




CD, configuration and conformation

- CD spectra are sensitive to the **overall** stereochemistry
- CD is very conformation-dependent, especially for exciton-coupled systems ⇨ conformational studies



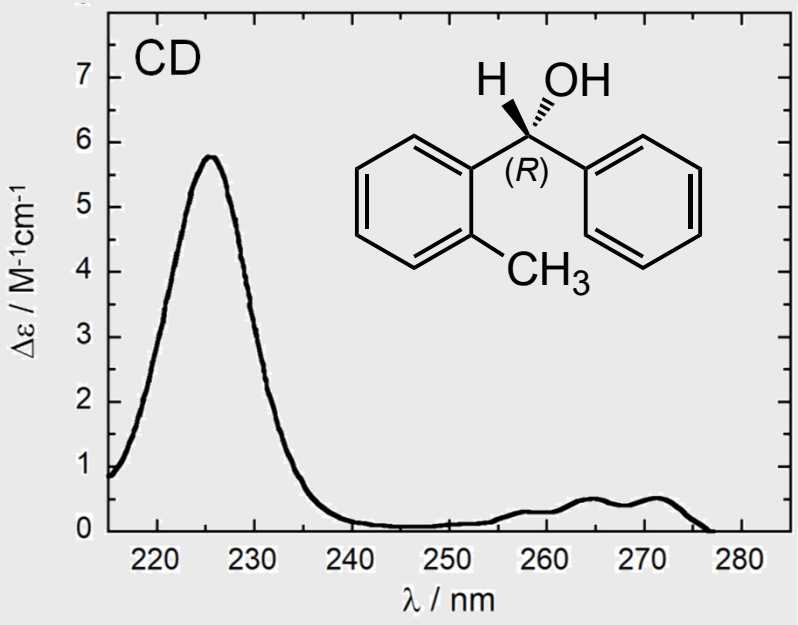


Pescitelli et al, *JACS* **1999**, *121*, 7998

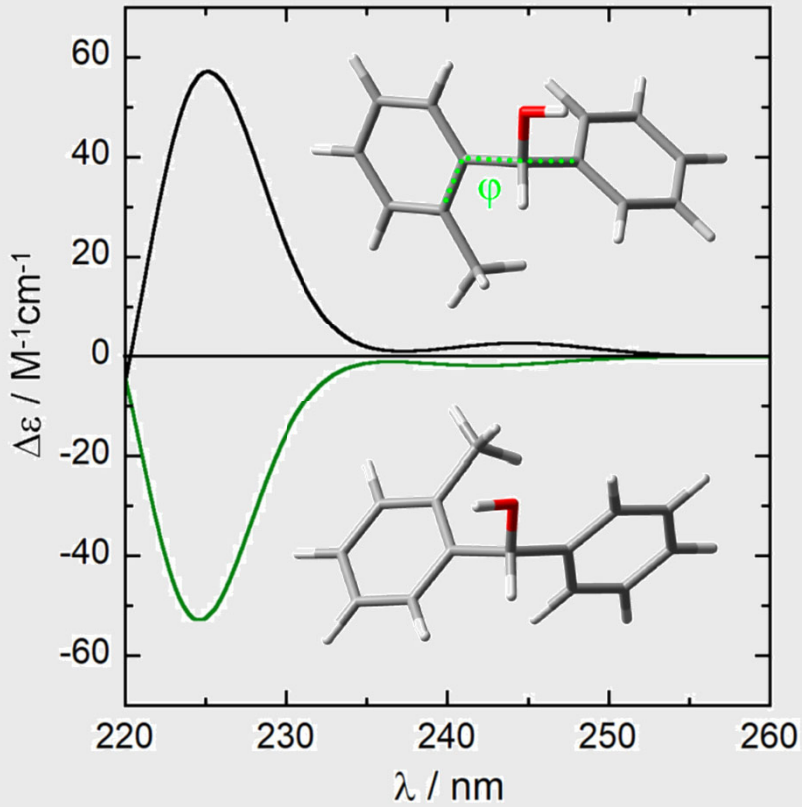
CD, configuration and conformation

- CD spectra are sensitive to the **overall** stereochemistry
- Different conformers of the same compound have in general different CD spectra

Experimental



Calculated CD



Padula et al, *Molecules* **2018**, *23*, 128

Quantum-mechanical ECD calculations

Conformational Analysis

- Efficient sampling of the conformational space with molecular mechanics methods

↓

Geometry Optimization

- Accurate DFT functionals, large basis sets, solvent models
- Check against NMR data (δ, NOE, J-couplings)

↓

Single-Structure ECD Calculation

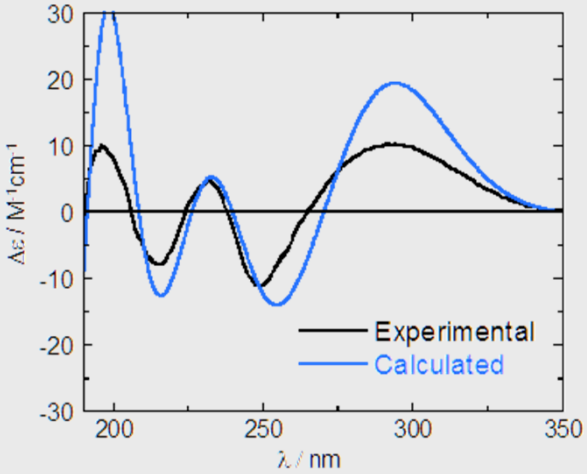
- Use multiple DFT functionals, robust basis sets, solvent models

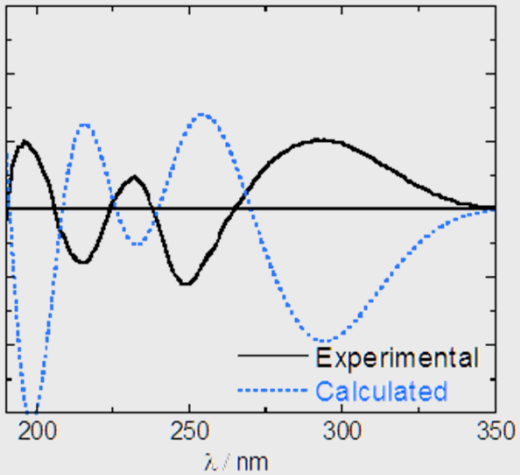
↓

Average Spectra

↓

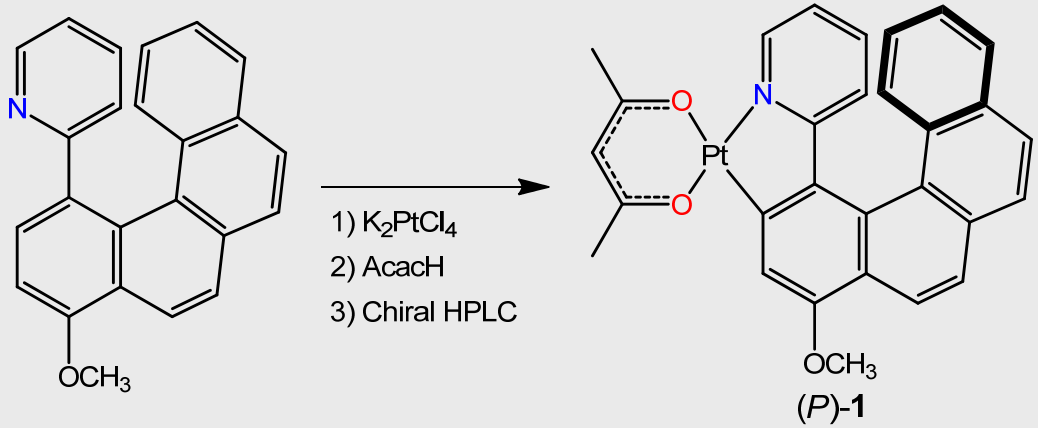
Final Comparison

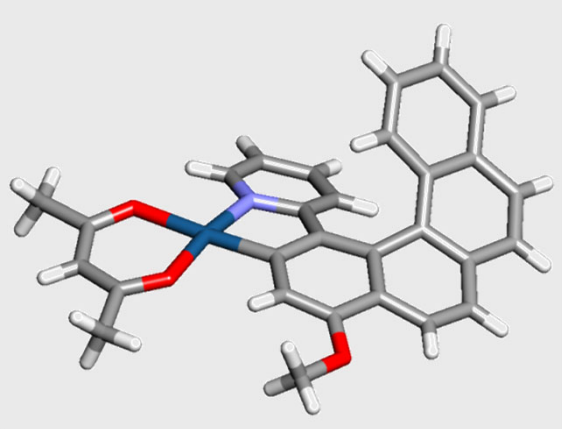


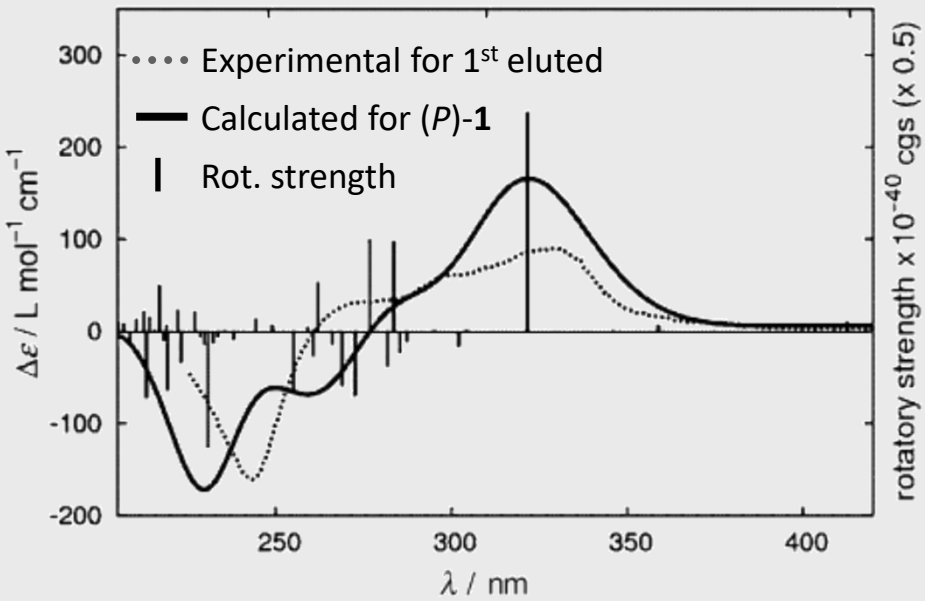


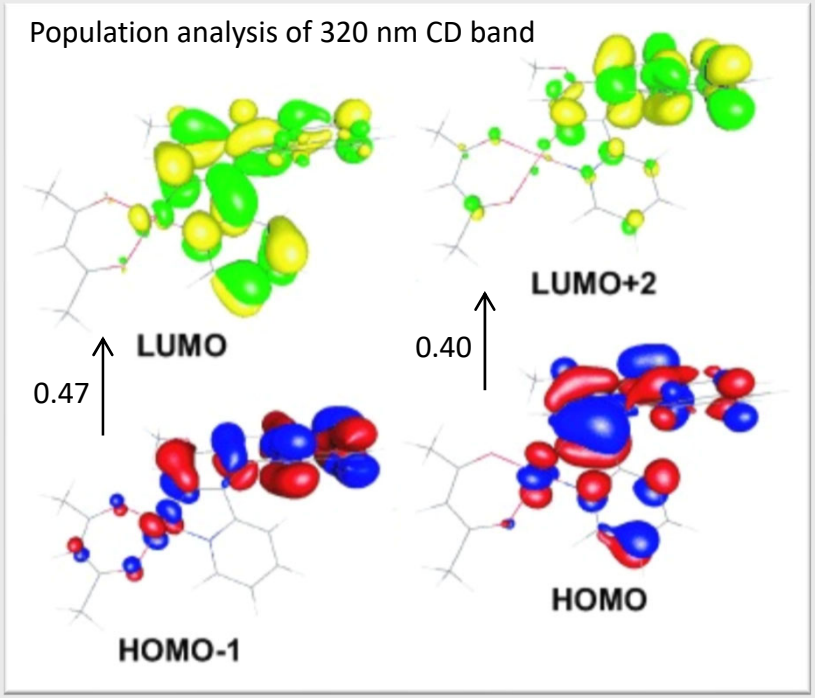
Pescitelli, Bruhn *Chirality* **2016**, *28*, 466

Helicenes and helicenoids





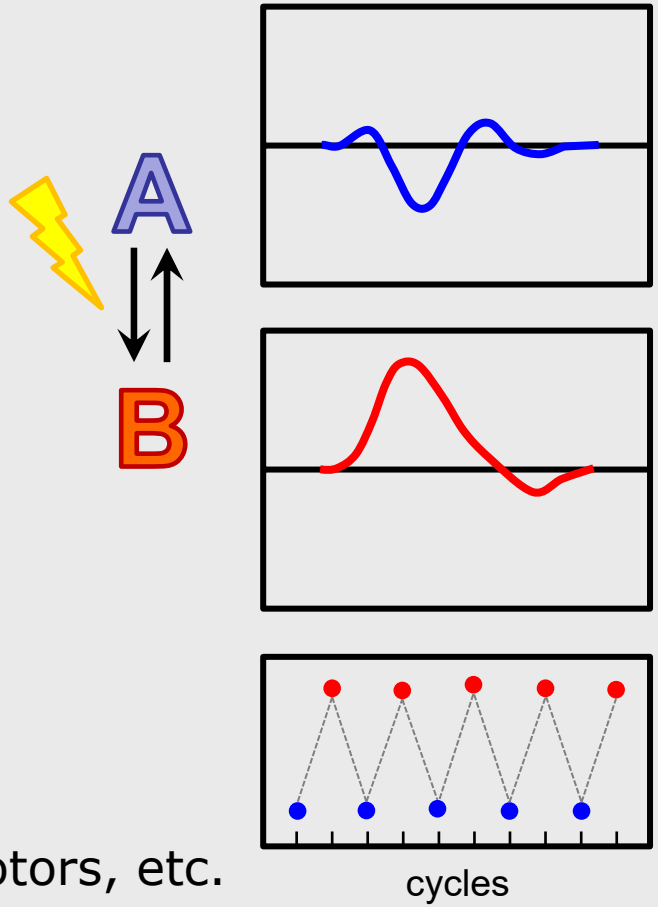




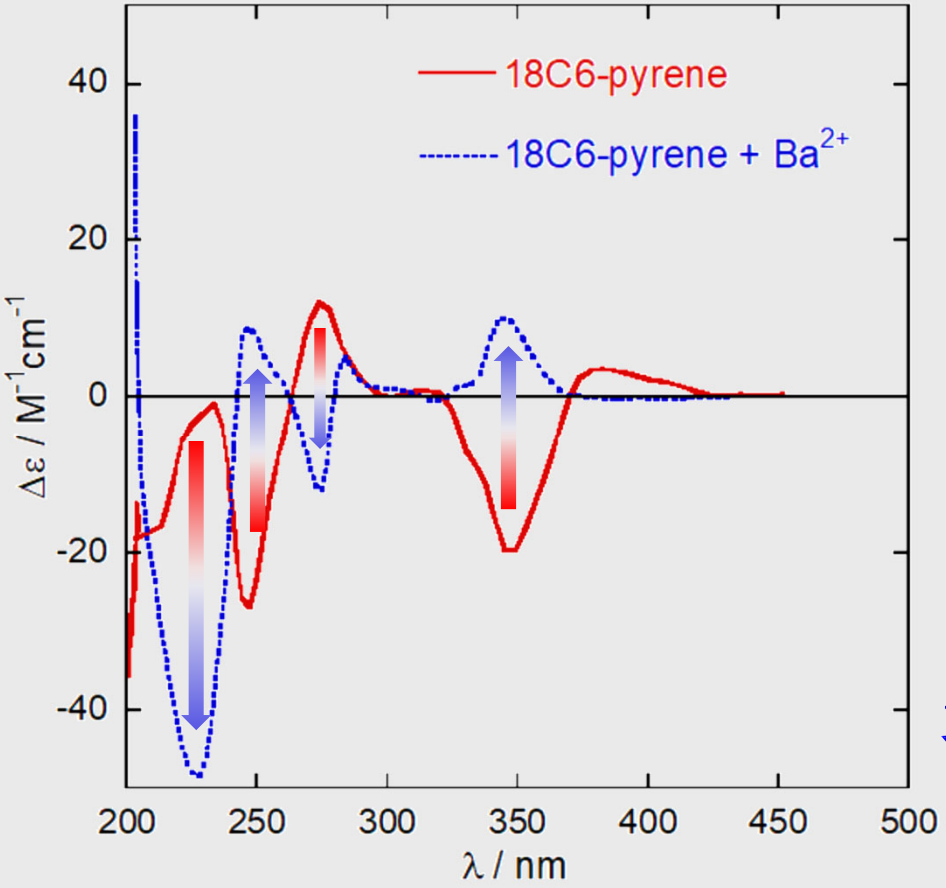
Norel et al. *Angew. Chem. Int. Ed.* **2010**, *49*, 99

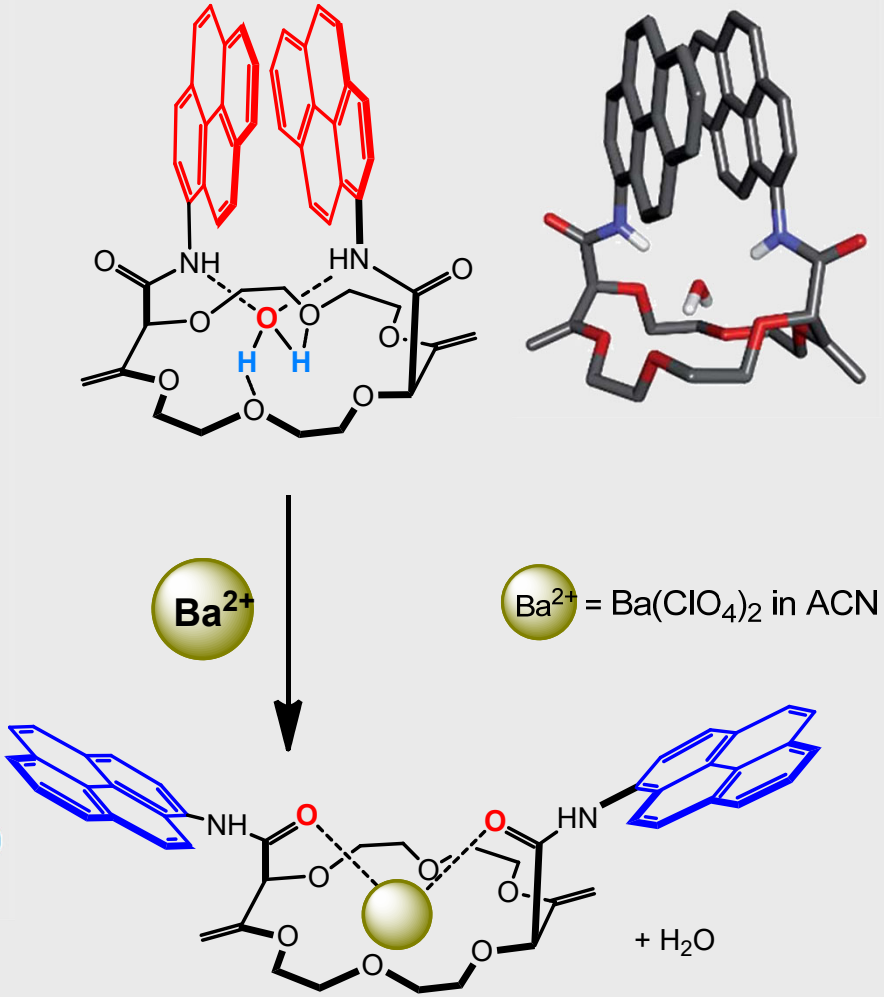
Chiroptical switches

- Chiral molecules adopting two interconvertible states with different chiroptical properties
- Interconversion modulated by various stimuli
  - Host-guest binding
  - Metal cation / anion coordination
  - Solvent exchange
  - Redox triggering
  - Temperature variation
  - pH variation
  - Irradiation (photoswitches)
- Different sizes and complexity
  - Molecular
  - Macromolecular
  - Supramolecular
- Applications: sensing, molecular motors, etc.



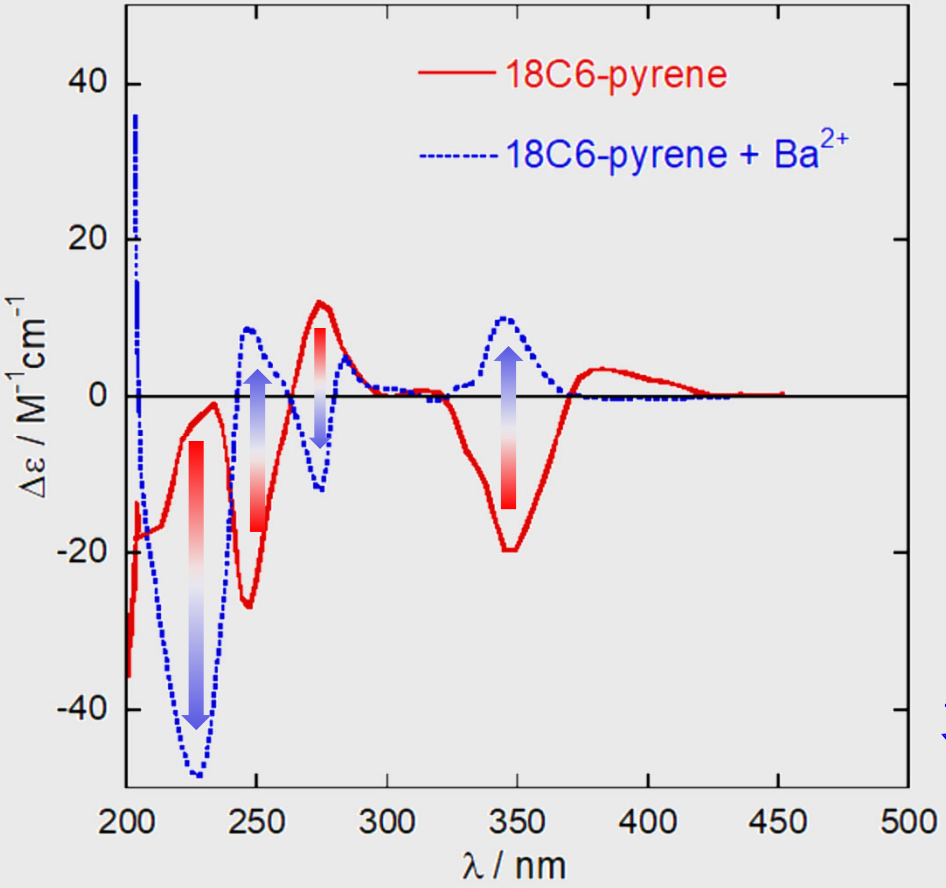
Chiroptical molecular switches

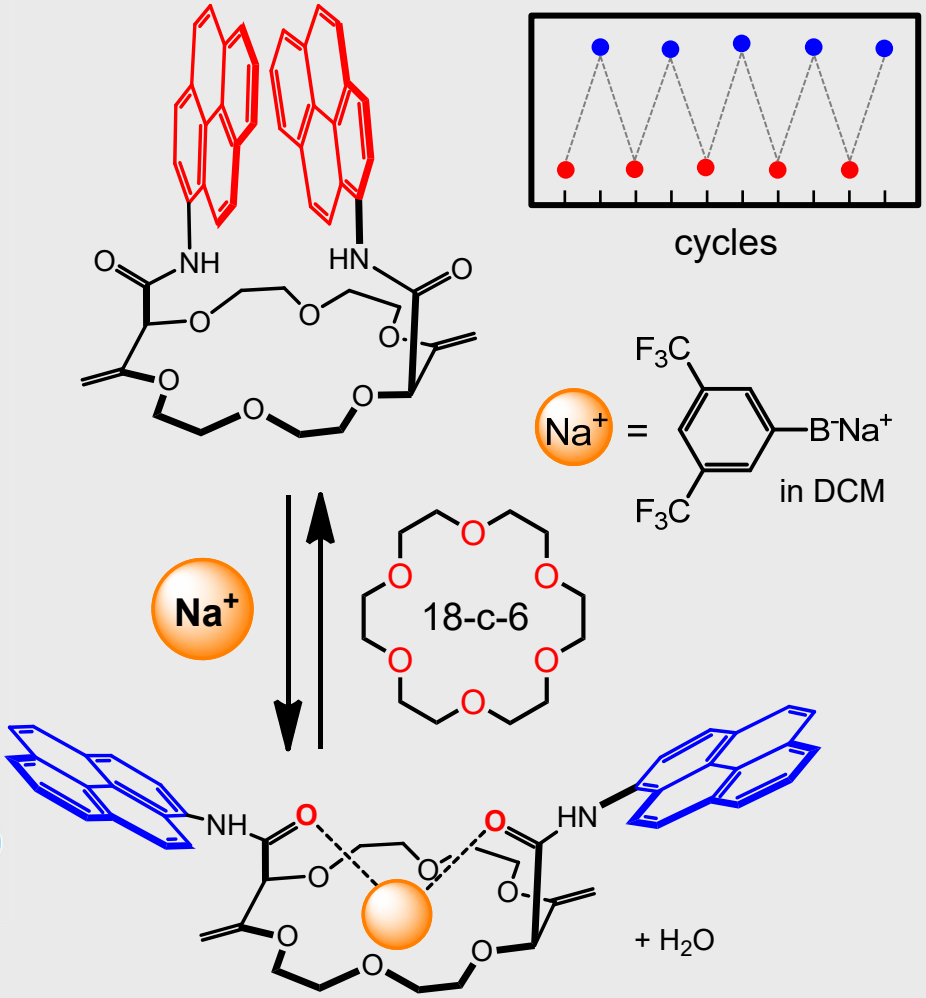




Homberg et al. *Chem. Sci.* **2018**, *9*, 7043

Chiroptical molecular switches

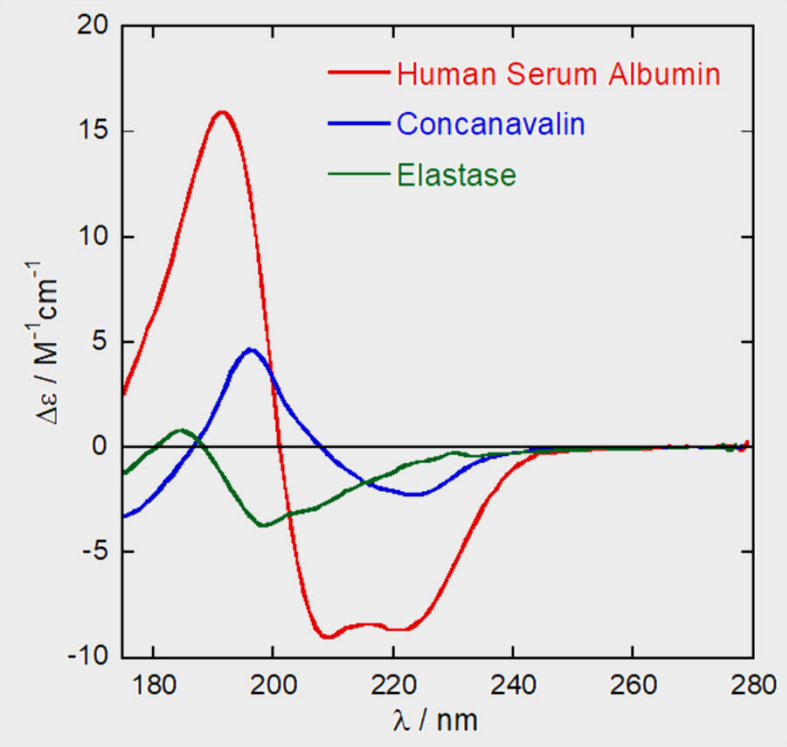


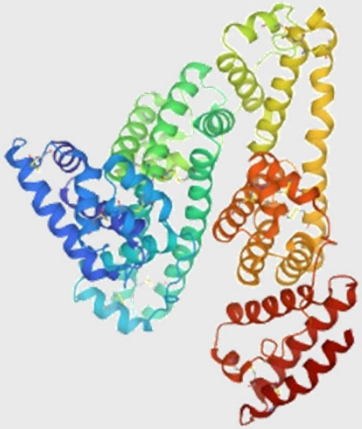


Homberg et al. *Chem. Sci.* **2018**, *9*, 7043

Proteins and CD

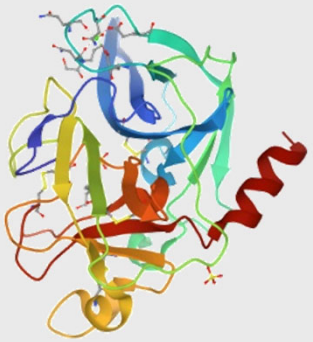
- CD of peptides and proteins is very sensitive to secondary structure
- Used as a tool for several purposes:
  - secondary structure fraction estimation
  - folding/unfolding
  - conformational changes





Human serum albumin

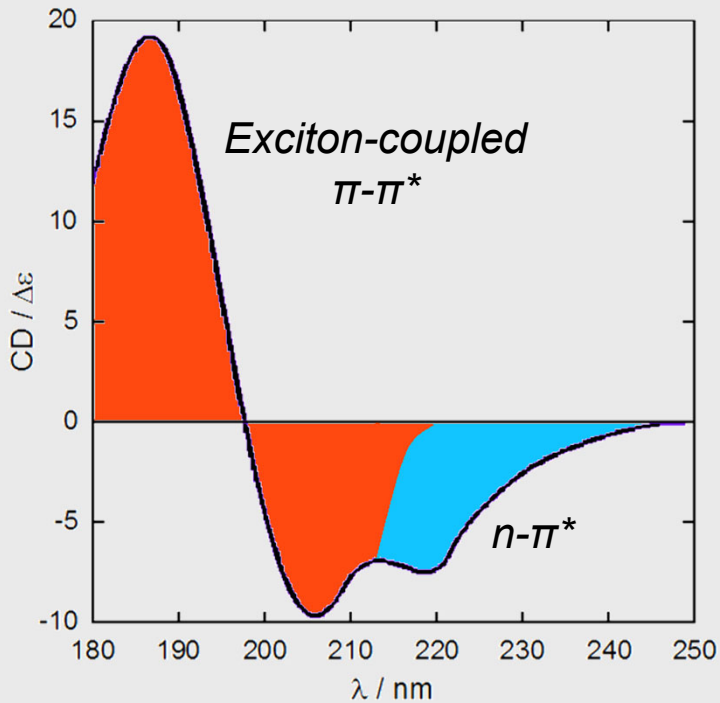
Concanavalin

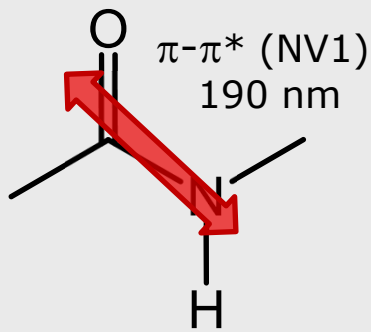


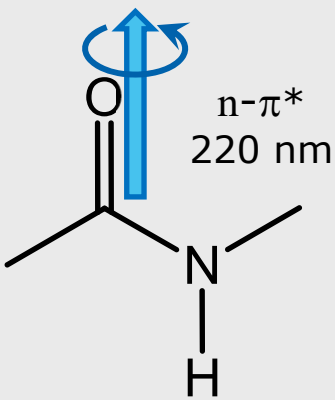
Elastase

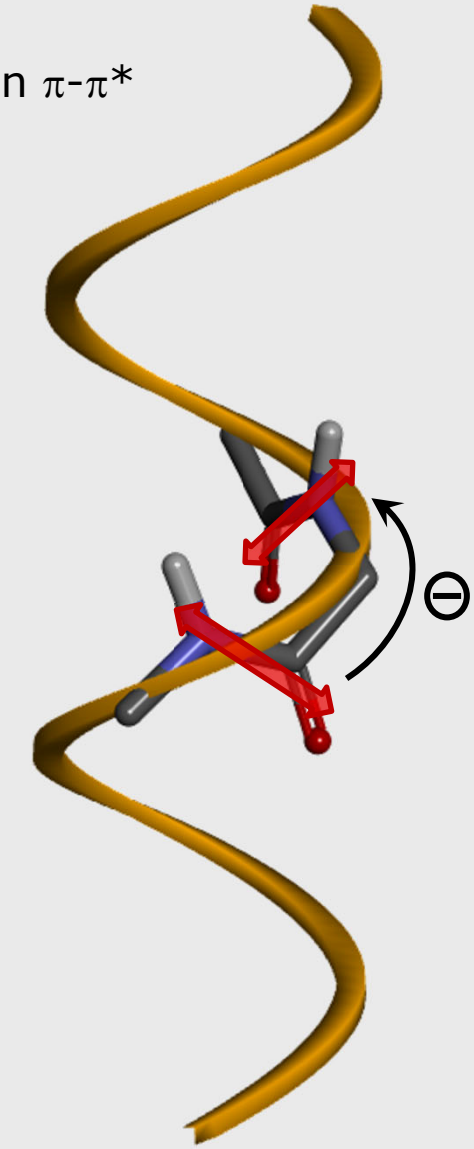
Proteins and CD

- α-Helix CD is dominated by exciton coupling between π-π* transitions, plus a contribution from n-π* transitions
- Side-chain chromophores contribute in the near-UV



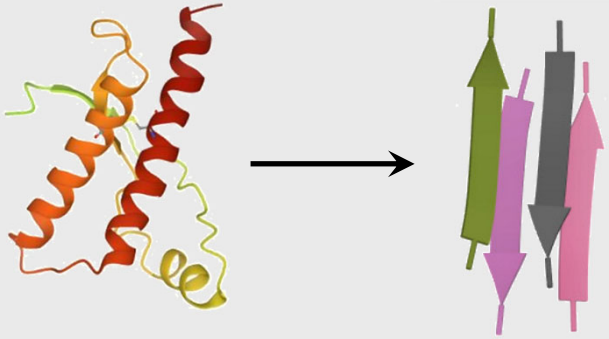


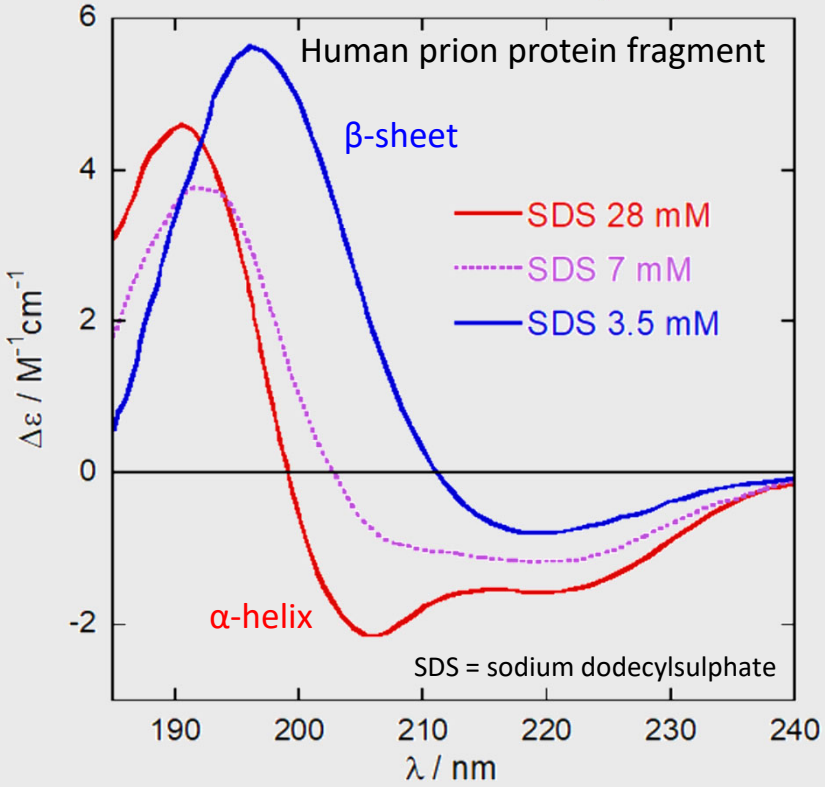




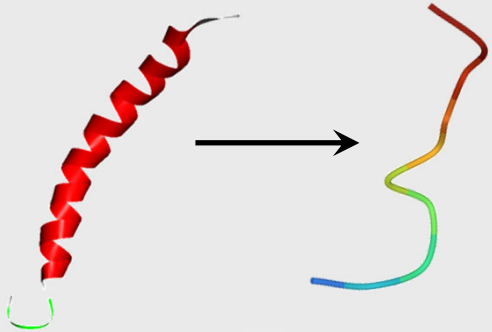
Protein conformational changes by CD

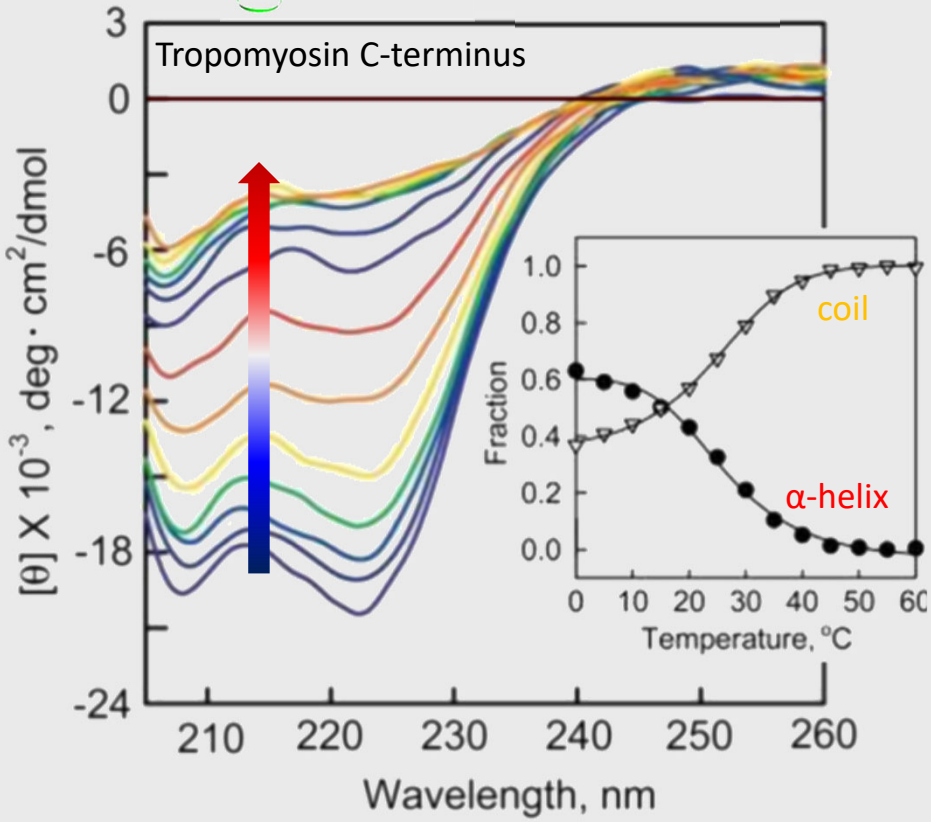
α ⇌ β transition upon stimulus





Melting (thermal denaturation)

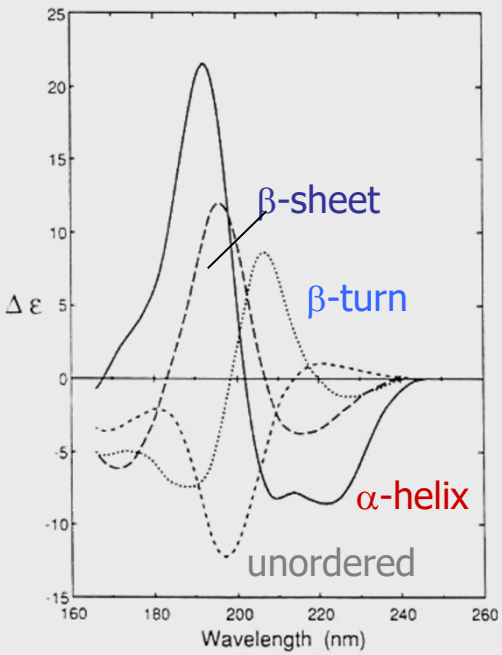


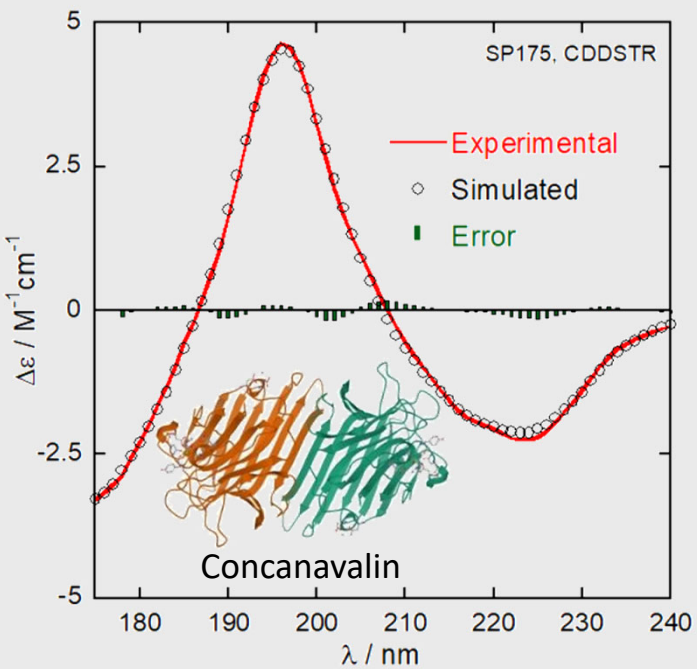


Secondary structure estimation

- Observed CD = weighted sum of CDi of secondary structure elements
- Component CD spectra extracted from model systems (regular peptides) or from a dataset of proteins with known secondary structure
- Yields secondary structure fraction: regular + distorted helix (H), regular + distorted sheet (S), turn (T), unordered (U)

$$\mathrm{CD}_\lambda = \sum_i f_i \mathrm{CD}_\lambda^i$$

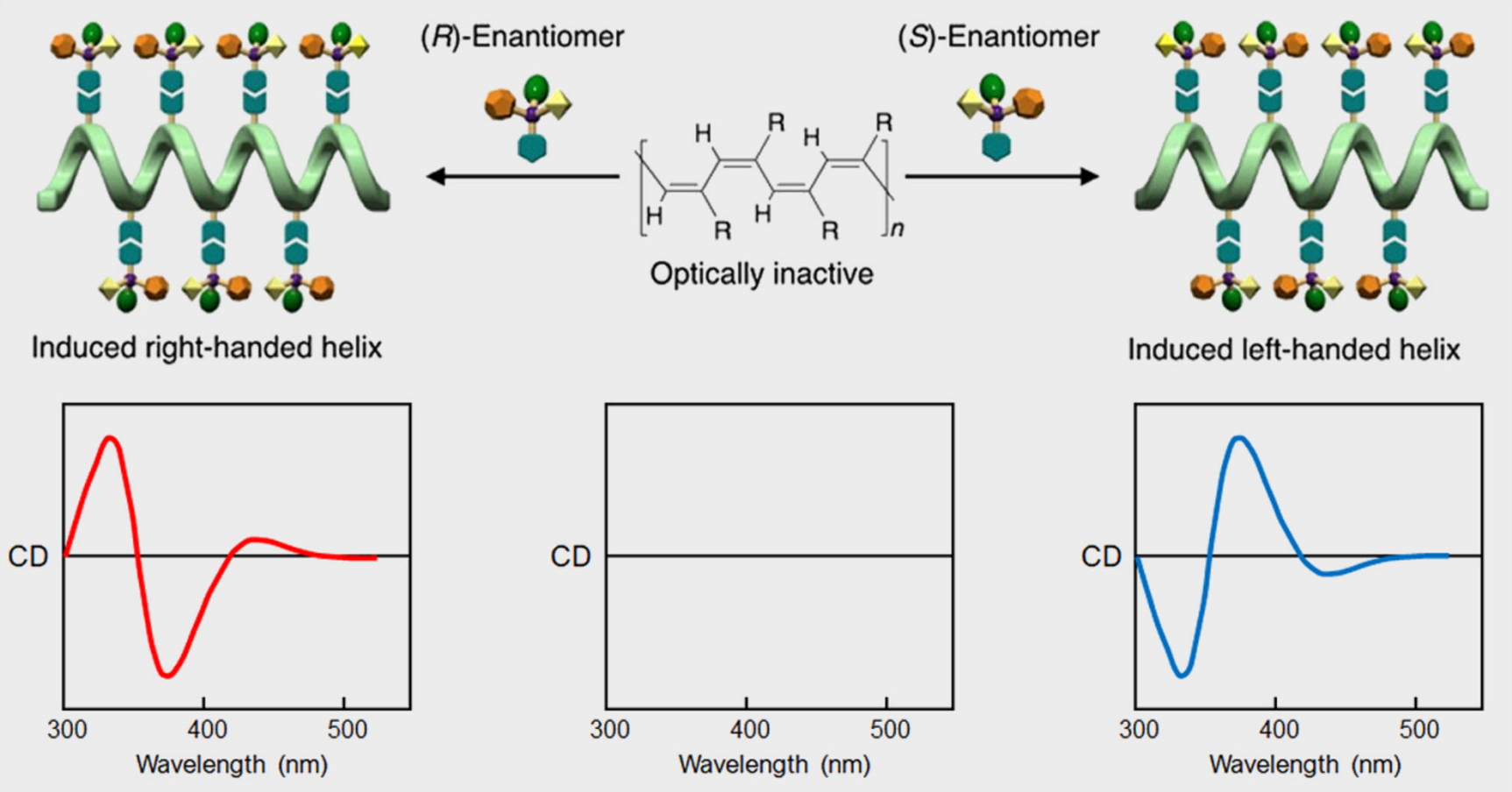




Helix R	Helix D
0.06	*0.11*
Turn	Unordered
0.11	*0.36*
Sheet R	Sheet D
0.24	*0.12*
Total	
1	

Macromolecular chirality: helical polymers

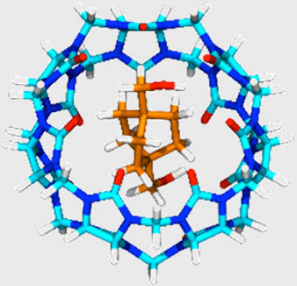
- Chiral polymers folding as helical structures
- Achiral polymers assuming preferential helicity by interaction with chiral dopants
- Main CD applications: detection of helix formation, helicity (handedness)

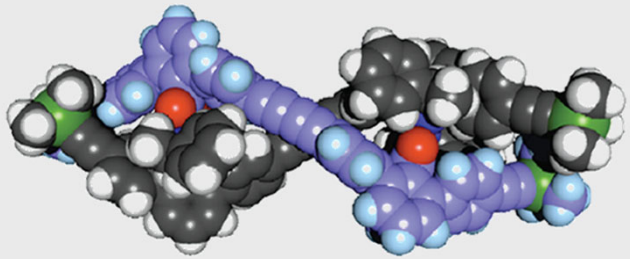


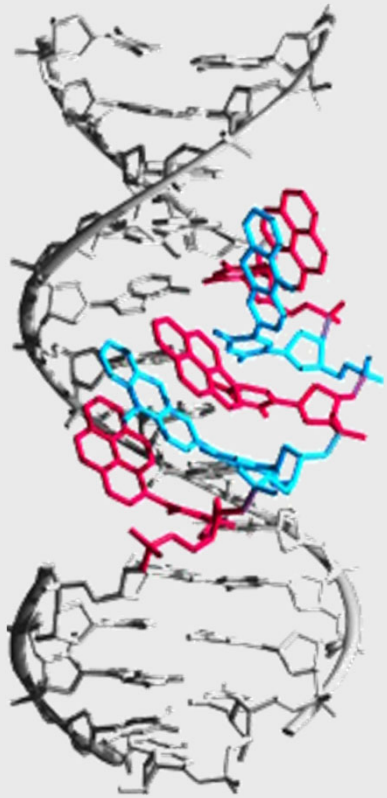
Maeda et al. *Top. Curr. Chem.* **2017**, *375*, 72

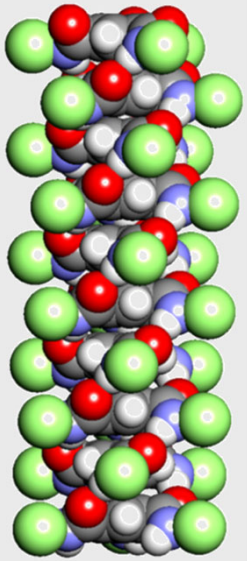
Supramolecular chirality

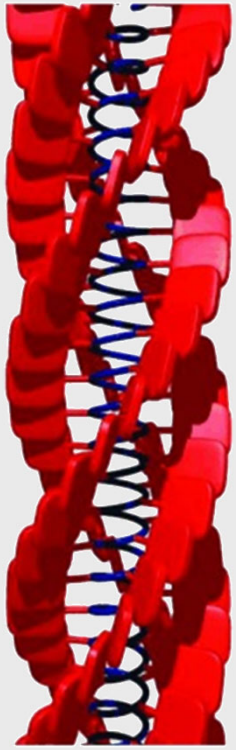
- Many supramolecular architectures inspired by Nature, or designed following a bio-mimetic approach
- Related to chiral symmetry-breaking (Nature homochirality)











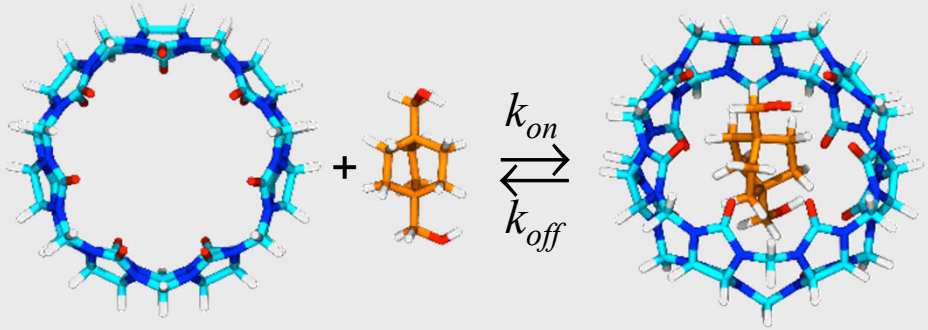
CD and supramolecular chirality

Main applications:

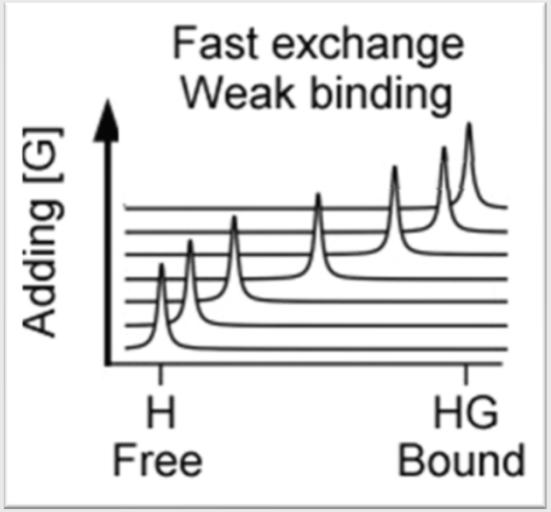
1. As an analytical tool
   - Detection of chiral supramolecular objects
   - Quantification of chiral supramolecular objects
     - Binding isotherms, K_{eq}, ΔH^0 and ΔS^0
     - Stoichiometries and Hill coefficients
     - $T_{sol/gel}$ or T_{melt} and other transition parameters
     - Detection of chirality amplification phenomena
2. Qualititative structure/property relationships
   - Assignment of supramolecular helicity
   - Geometry of host/guest complexes
   - Geometry of (bio)macromolecule/dye adducts
3. Quantitative structural information

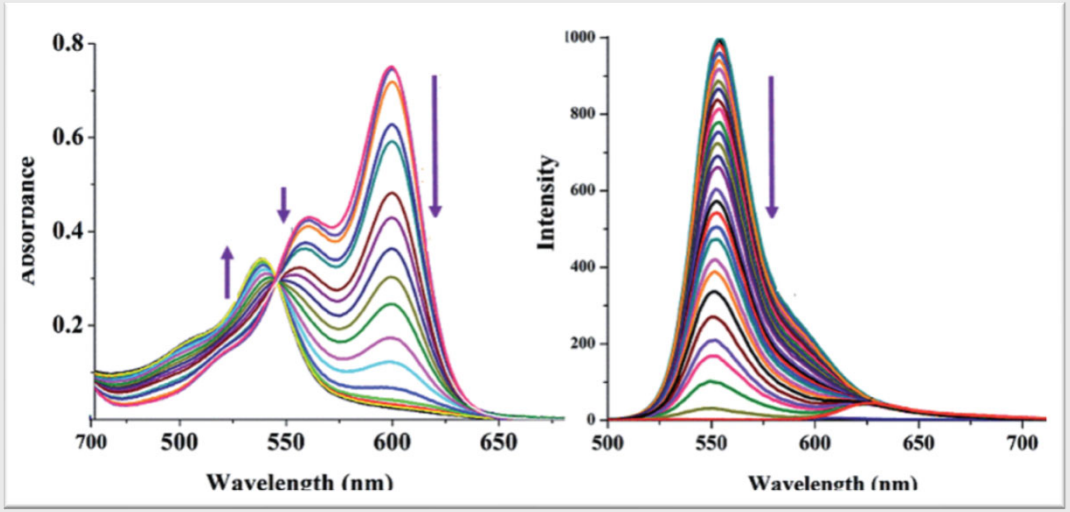
Detection of supramolecular species

$$K_a = \frac{[HG]}{[H][G]} = \frac{k_{on}}{k_{off}}$$



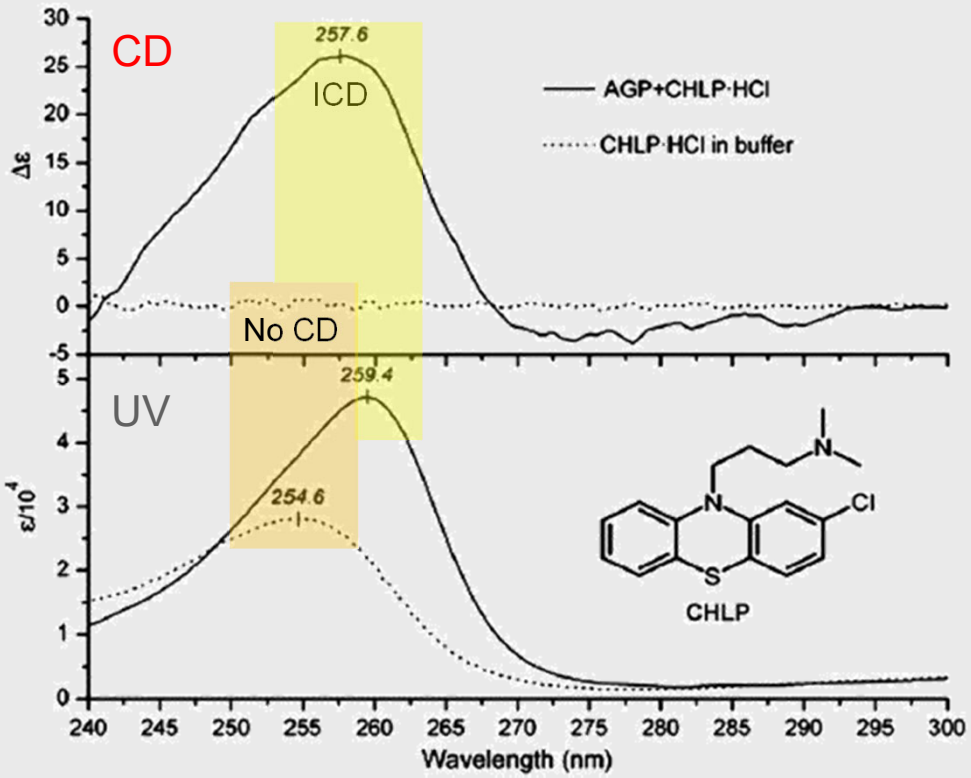
- HG complexes with small association constants (K_a~10^4) have short lifetimes $\tau = k_{off}^{-1} \leq 100$ μs
- "Slow" spectroscopies (NMR) give a single average signal for H (or G) and HG
- Fast spectroscopies (UV, CD, Flu) give independent signals for H and H

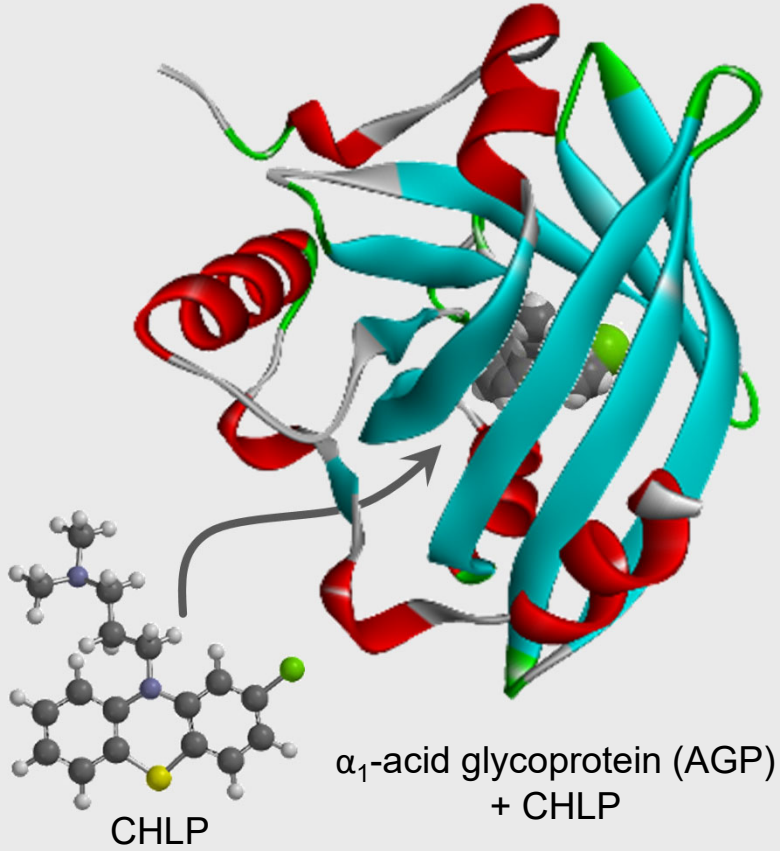




Induced Circular Dichroism (ICD)

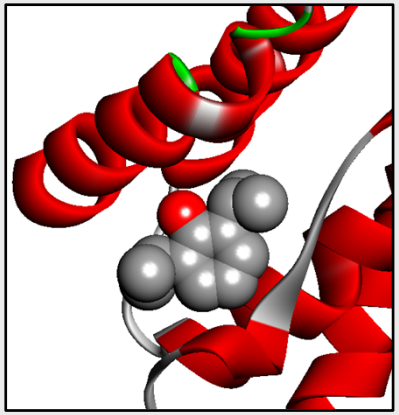
- Achiral chromophoric ligand: Abs bands > 240 nm but no ECD
- Proteins: no Abs bands > 240 nm
- Protein/ligand complex: ECD-active Abs bands > 240 nm ⇨ **induced** CD (ICD)



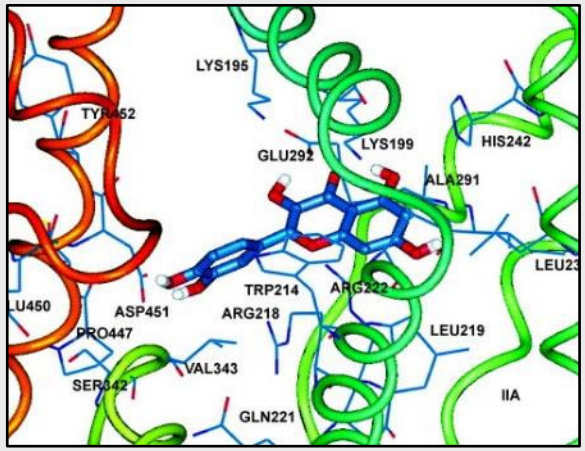


Induced Circular Dichroism (ICD) mechanisms

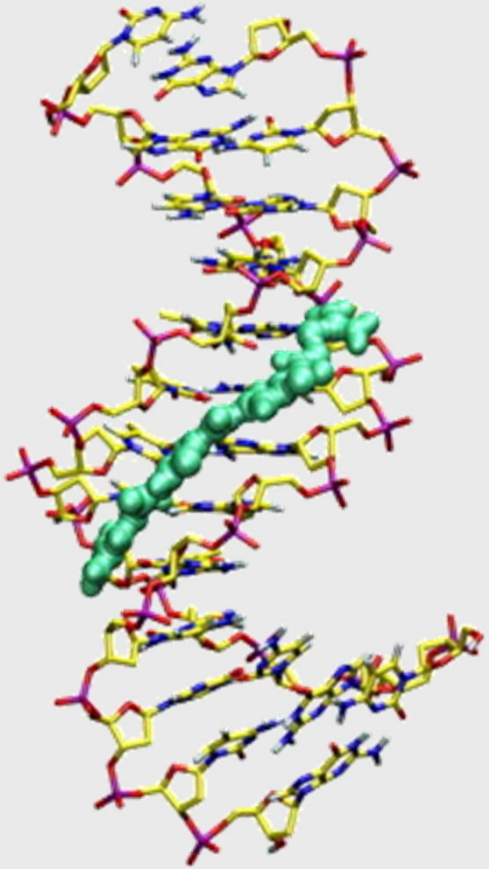
1. Chiral perturbation from the surrounding



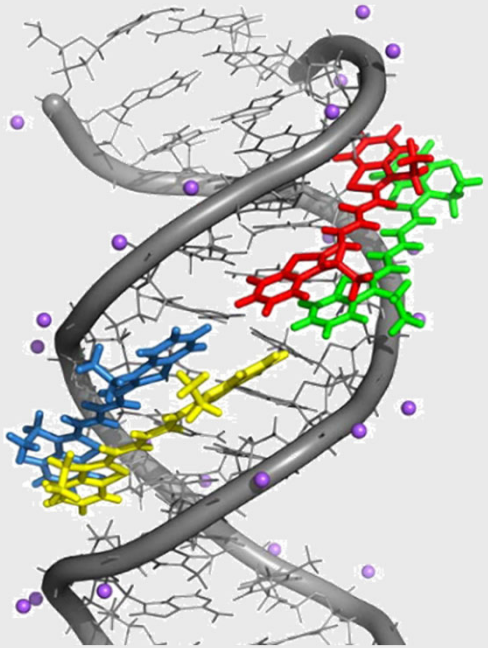
2. Chiral conformation assumed upon binding



3. Exciton coupling between host and ligand chromophores

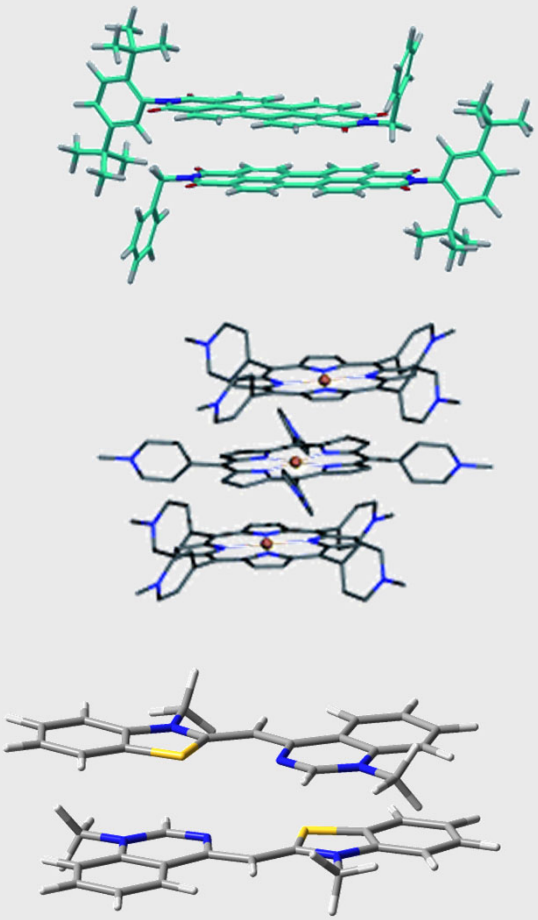


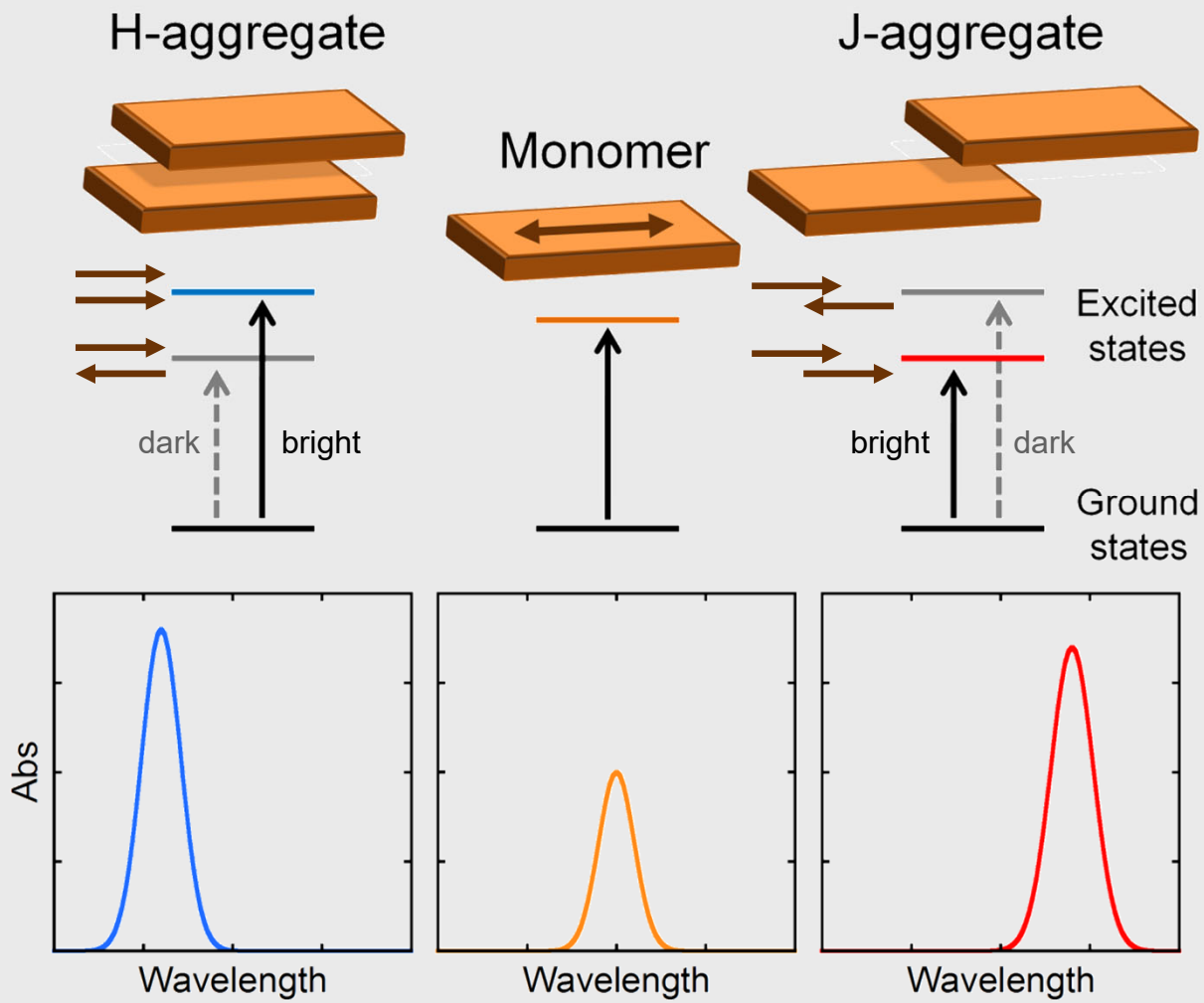
4. Exciton coupling between multiple ligands



H- and J-aggregates: absorption spectra

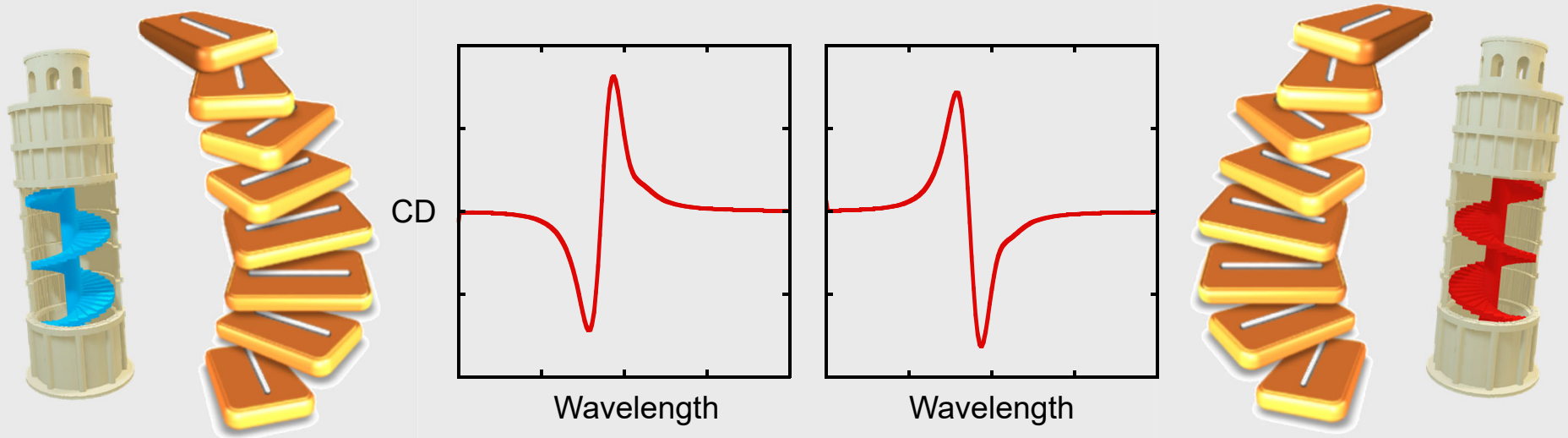
- Typical response to aggregation due to π-stacking
- In- and out-of-phase combinations of transition dipole moments





Exciton CD and supramolecular helicity

- For many supramolecular arrangements of chromophoric units, the relation between helicity (handedness) and CD is straightforward
- Example: spiral staircase stack

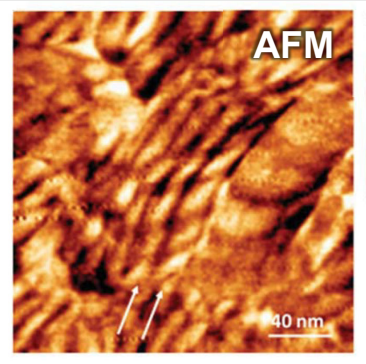


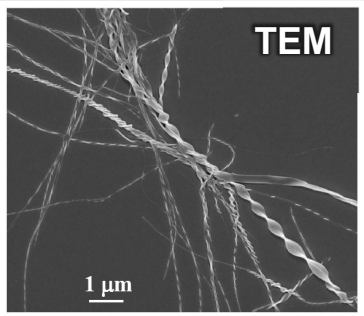
right-handed helix ⟺ ⊕ couplet

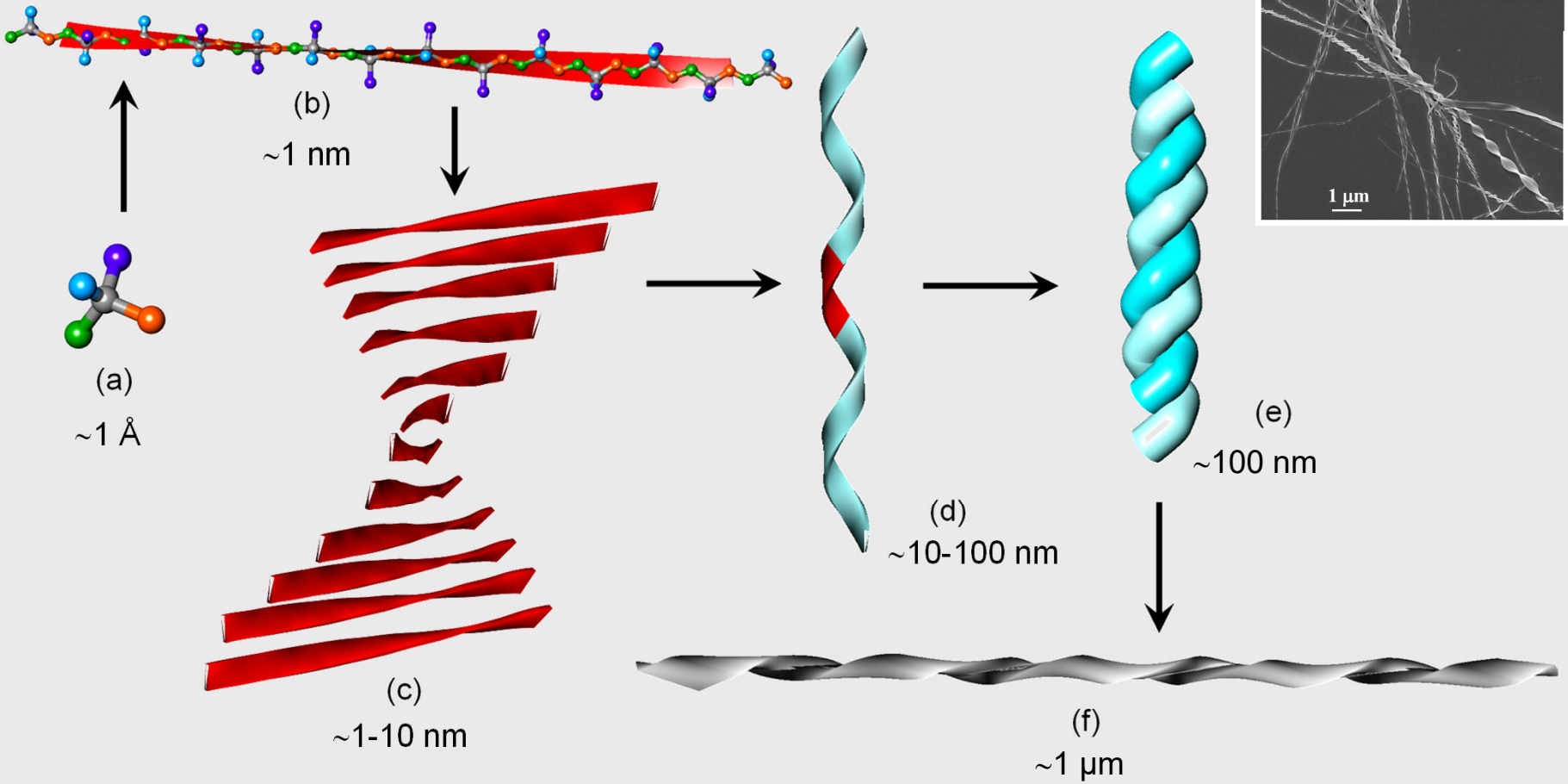
⊖ couplet ⟺ left-handed helix

Helicity at different scales

- Chirality may be encountered at different levels of hierarchy, i.e., at different size scales
- No straightforward of univocal relationship between the chirality or helicity at different levels of hierarchy

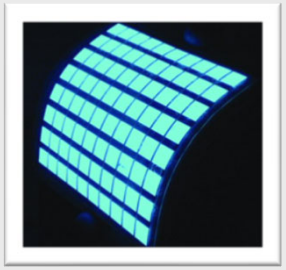


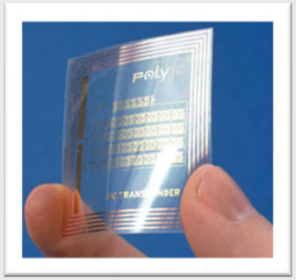


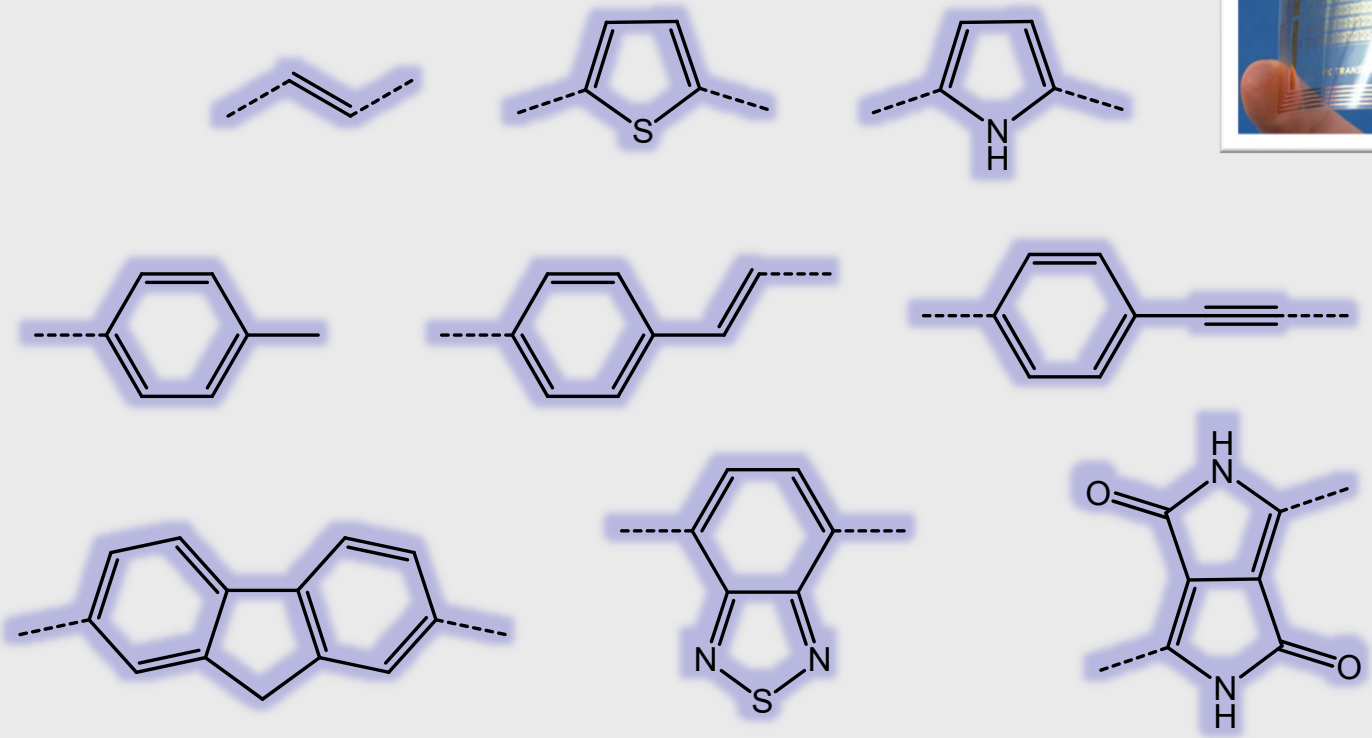


Conjugated polymers

- Key components of organic electronics
- Applications in optoelectronic devices such as
  - Organic light-emitting diodes (OLEDs)
  - Organic photovoltaic (OPV) cells
  - Organic field-effect transistors (OFETs)



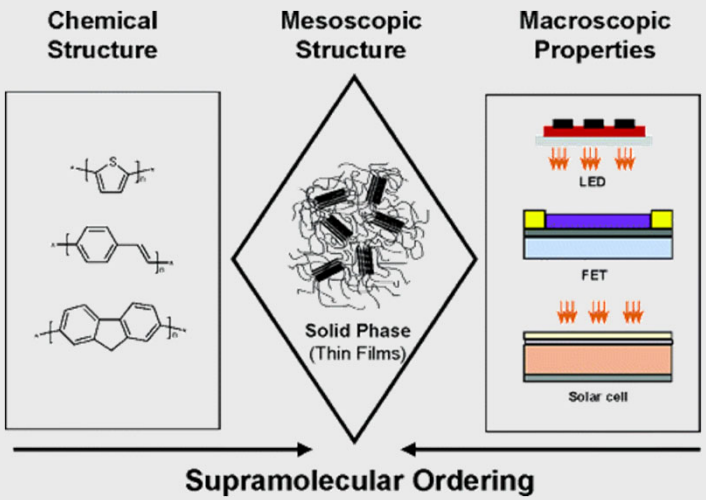




Conjugated polymers

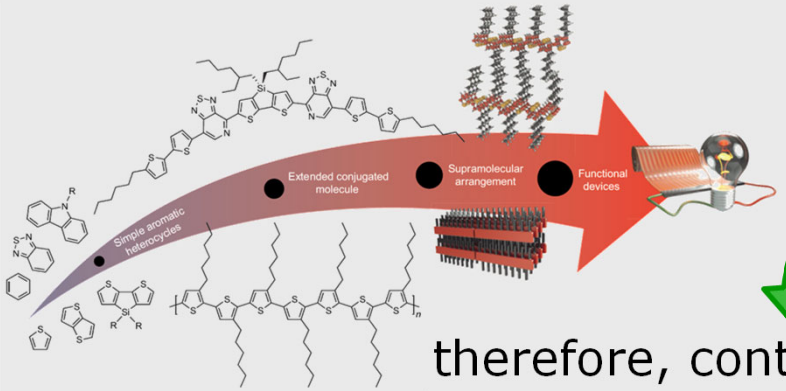
Fundamentals properties such as

- HOMO-LUMO bandgap
- Absorption/transmission profile
- Luminescence quantum yield
- Charge transport
- Magnetism



depend on

- Constituent chemical structure
- Solid-state organization (from first-level to nano/mesoscale)



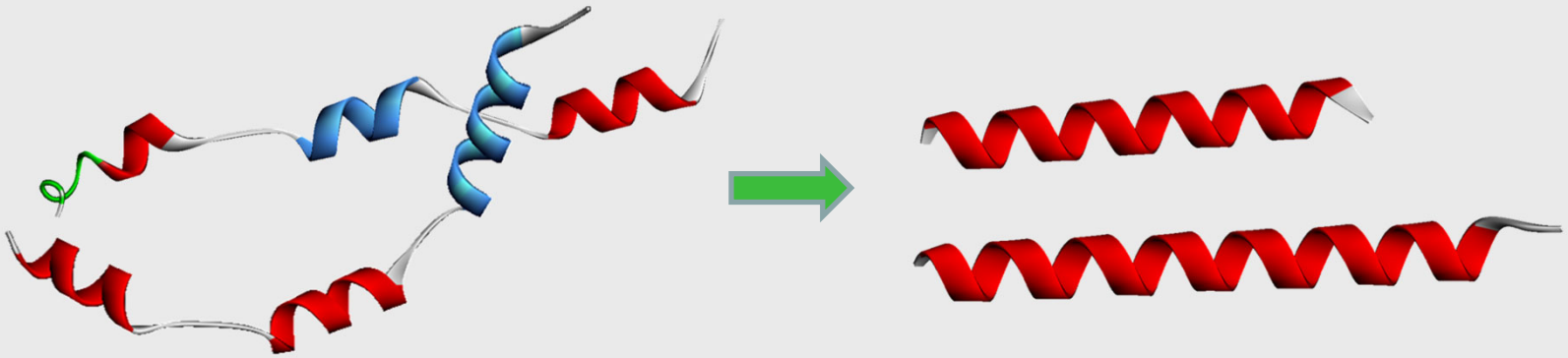
therefore, control is needed on

- Design of the starting material
- Film deposition
- Post-deposition processing

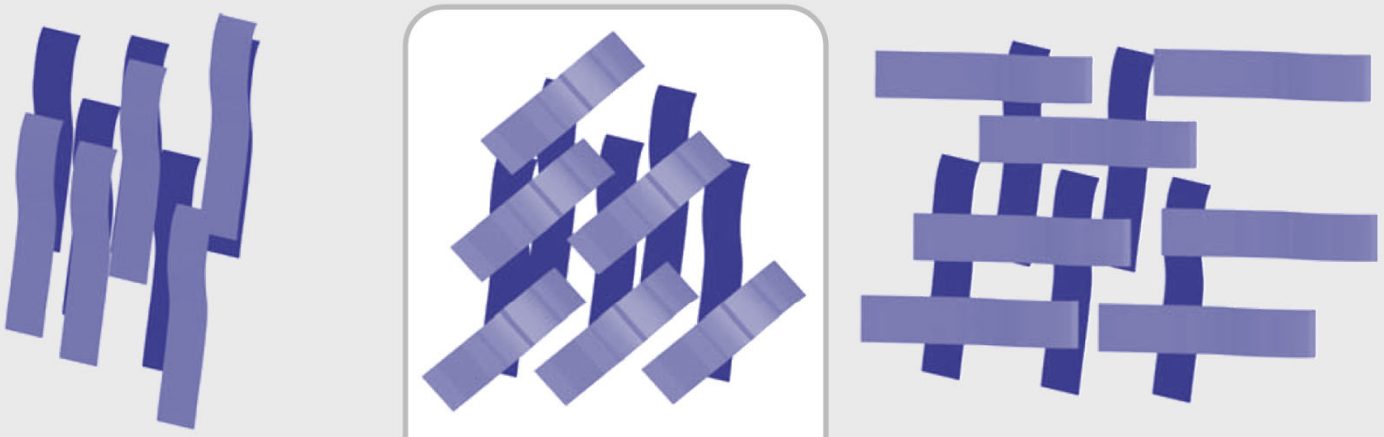
Hoeben et al., *Chem. Rev.* **2005**, *105*, 1491
Henson et al., *Nature Chem.* 2012, *4*, 699

Chiral conjugated polymers

- Control over supramolecular structures, in particular helical ones



- Favorable impact of chirality on material properties

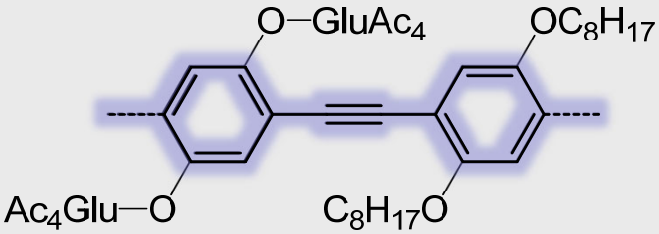


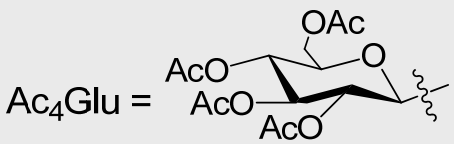
Parallel
Efficient exciton transport but dipole-dipole cancellation leads to fluorescence quenching

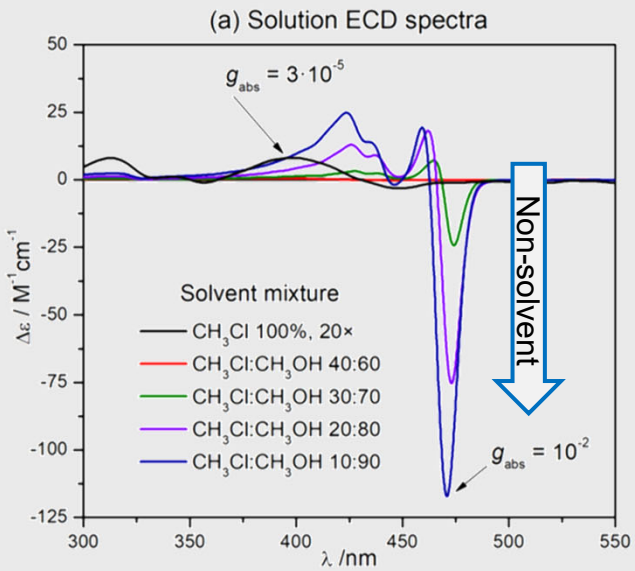
Oblique
Compromise between efficient exciton transport and luminescence

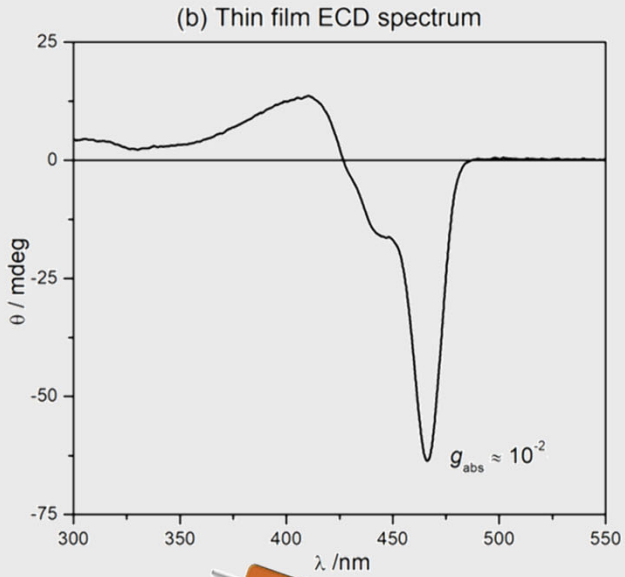
Orthogonal
High luminescence but minimal interchain exciton migration

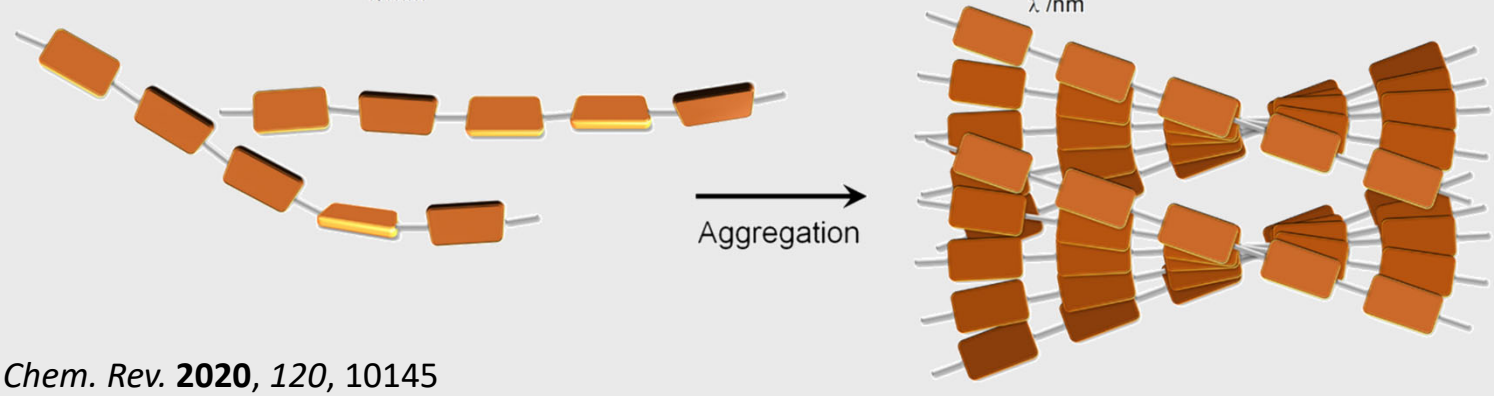
Chiral conjugated polymers





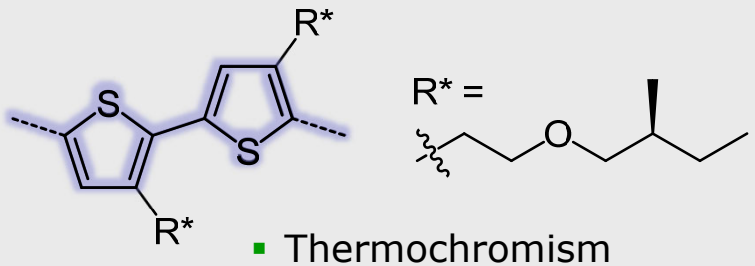




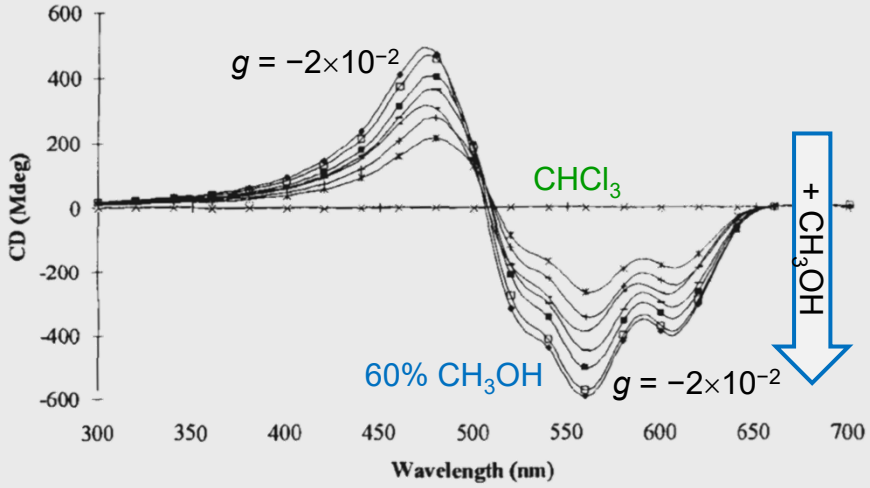


Albano et al., *Chem. Rev.* **2020**, *120*, 10145

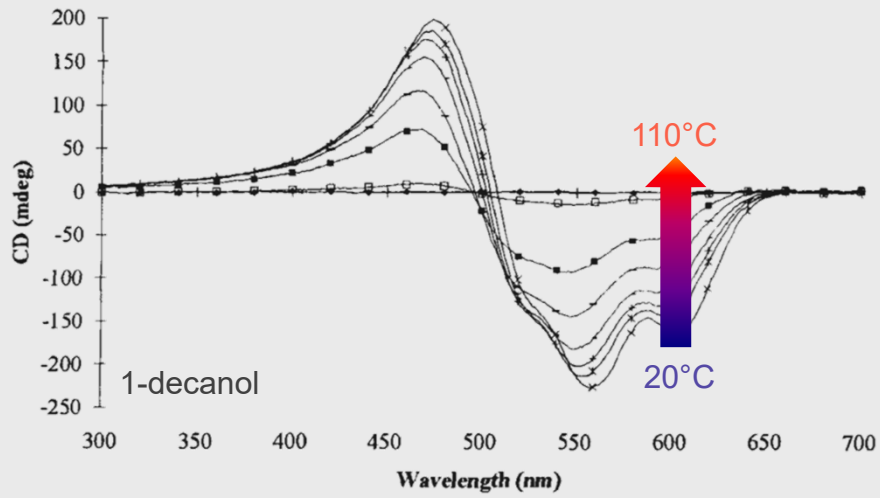
Chiral polythiophenes



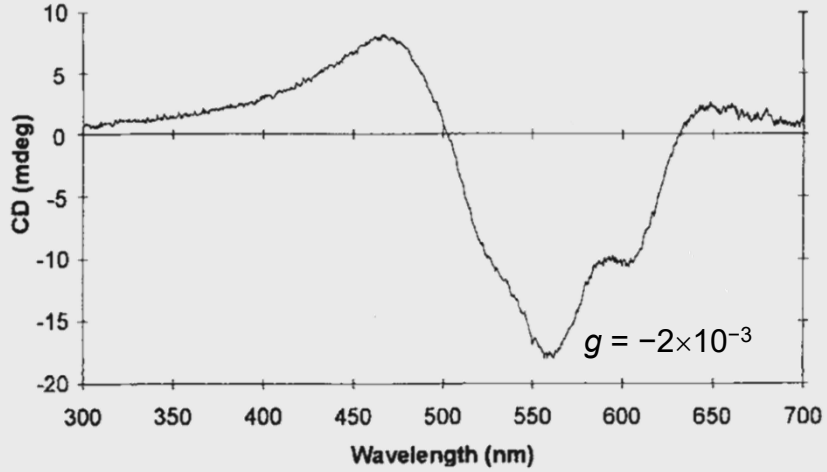
- Solvatochromism



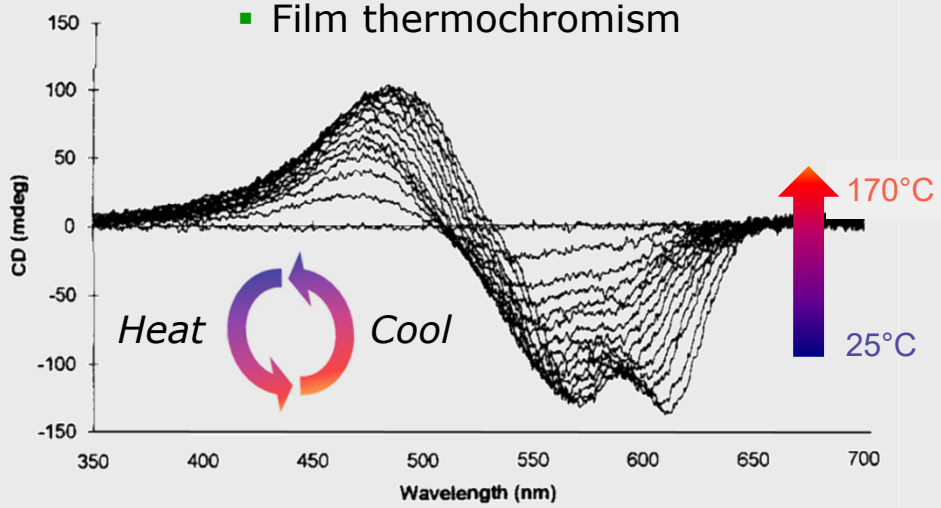
- Thermochromism



- Thin film spectra

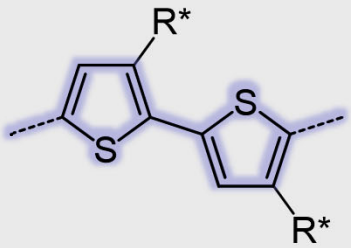


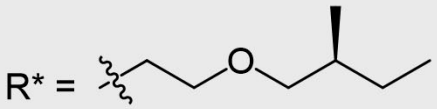
- Film thermochromism

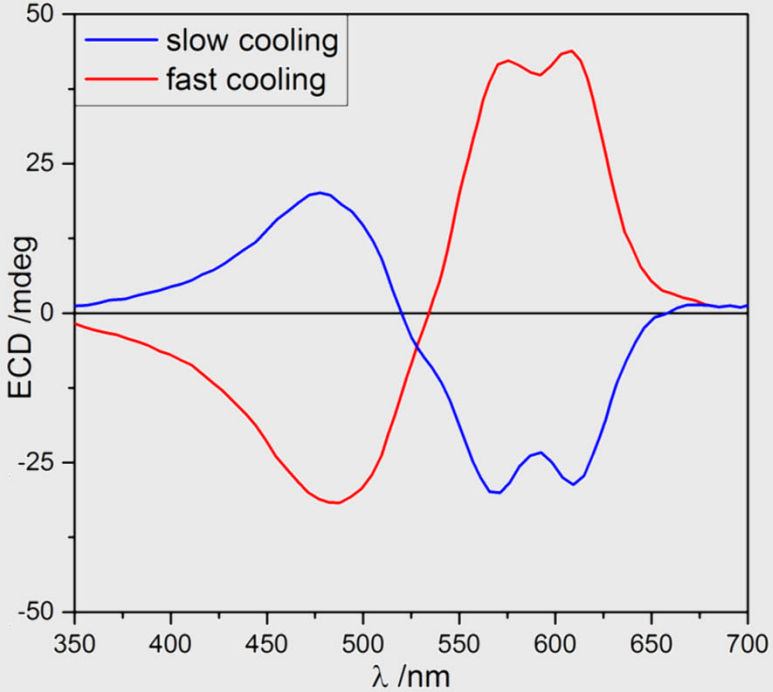


Bouman et al., *Mol. Cryst. Liq. Cryst. A* **1994**, *256*, 439; *Adv. Mater.* **1995**, *7*, 385

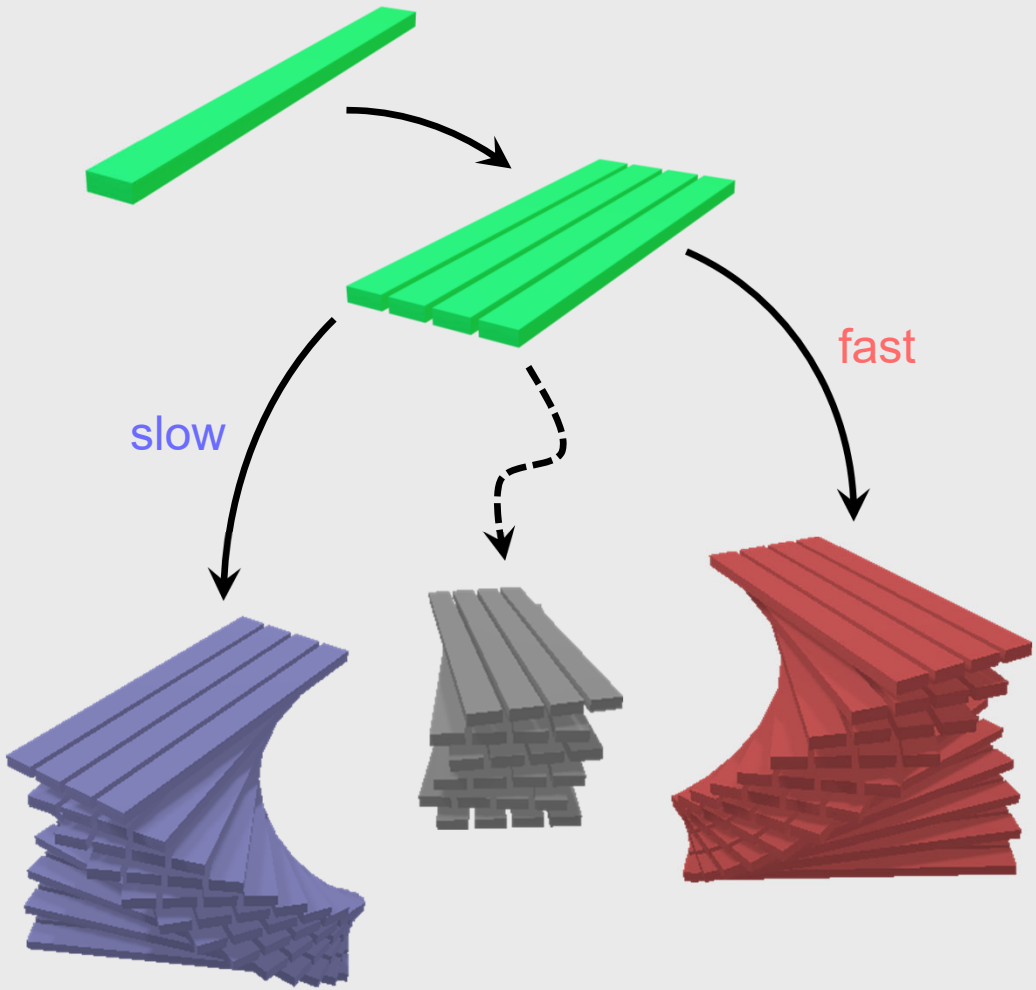
Chiral polythiophenes







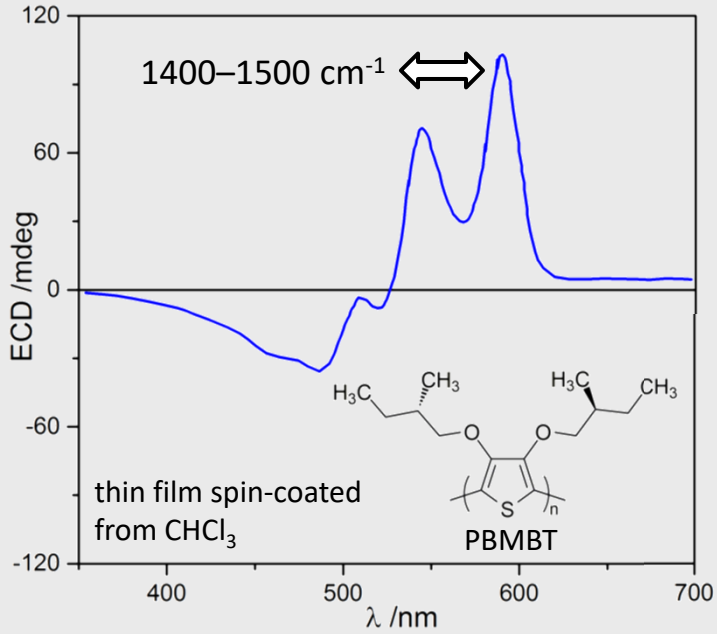
Multiple aggregation pathways*

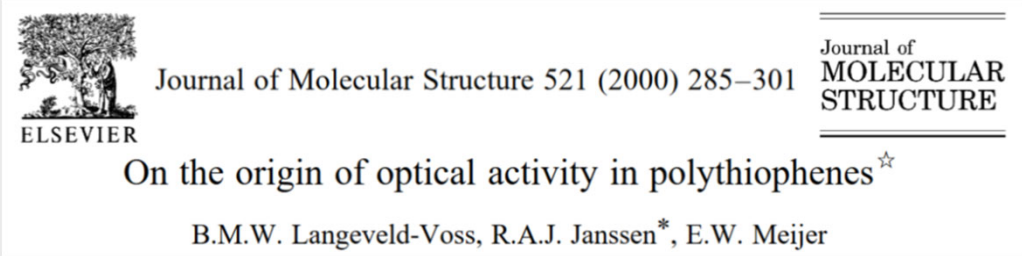


Bouman et al., *Adv. Mater.* **1995**, *7*, 385

* Korevaar et al. *Chem. Mater.* **2014**, *26*, 576

Intrachain vs. interchain chirality

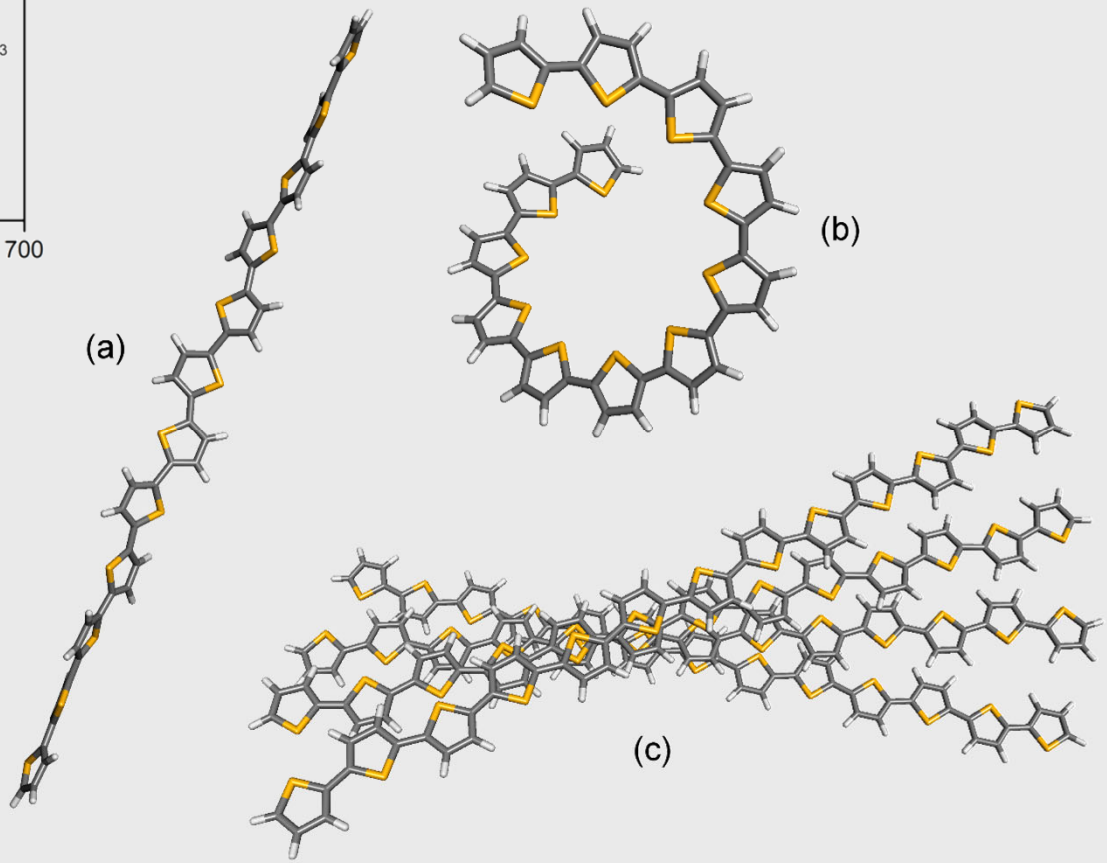




(a) Twisted ribbon (intrachain)

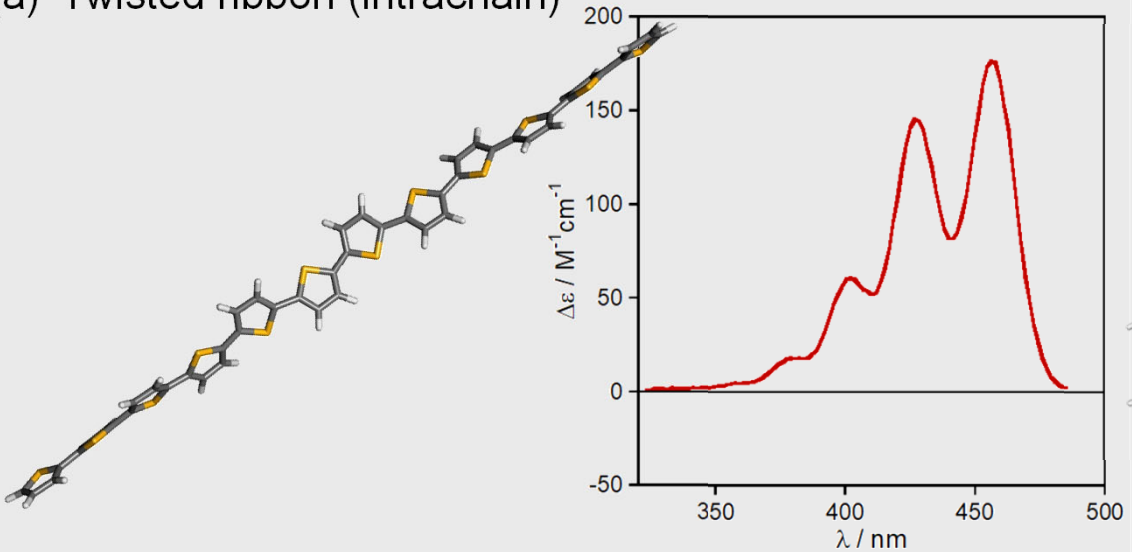
(b) Halical cisoid (intrachain)

(c) Stacked planar (interchain)

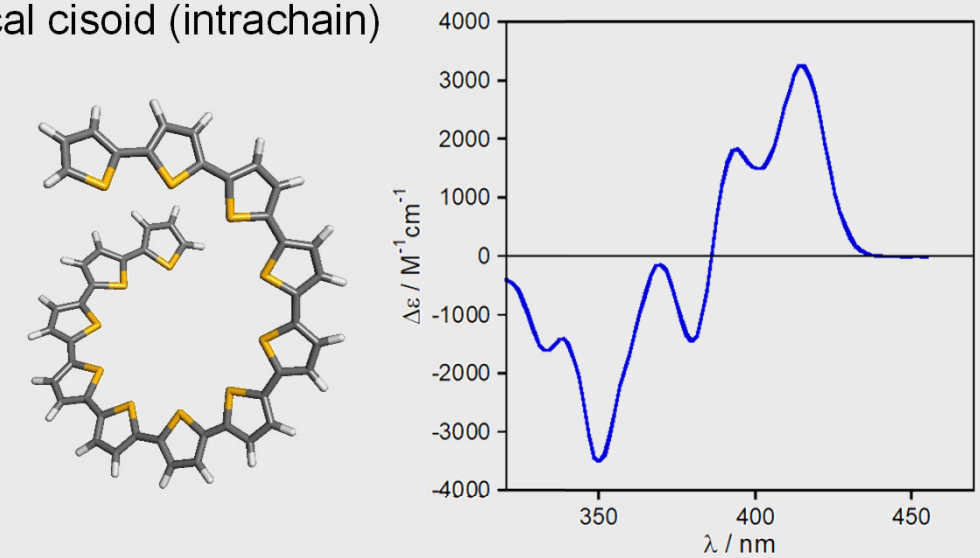


Intrachain vs. interchain chirality

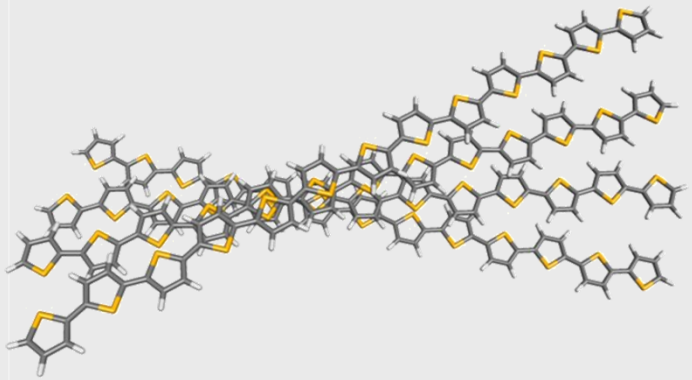
(a) Twisted ribbon (intrachain)

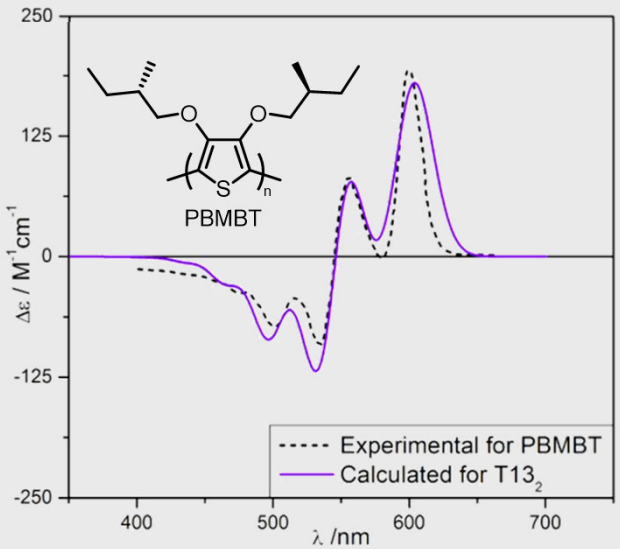


(b) Helical cisoid (intrachain)



(c) Stacked planar (interchain)

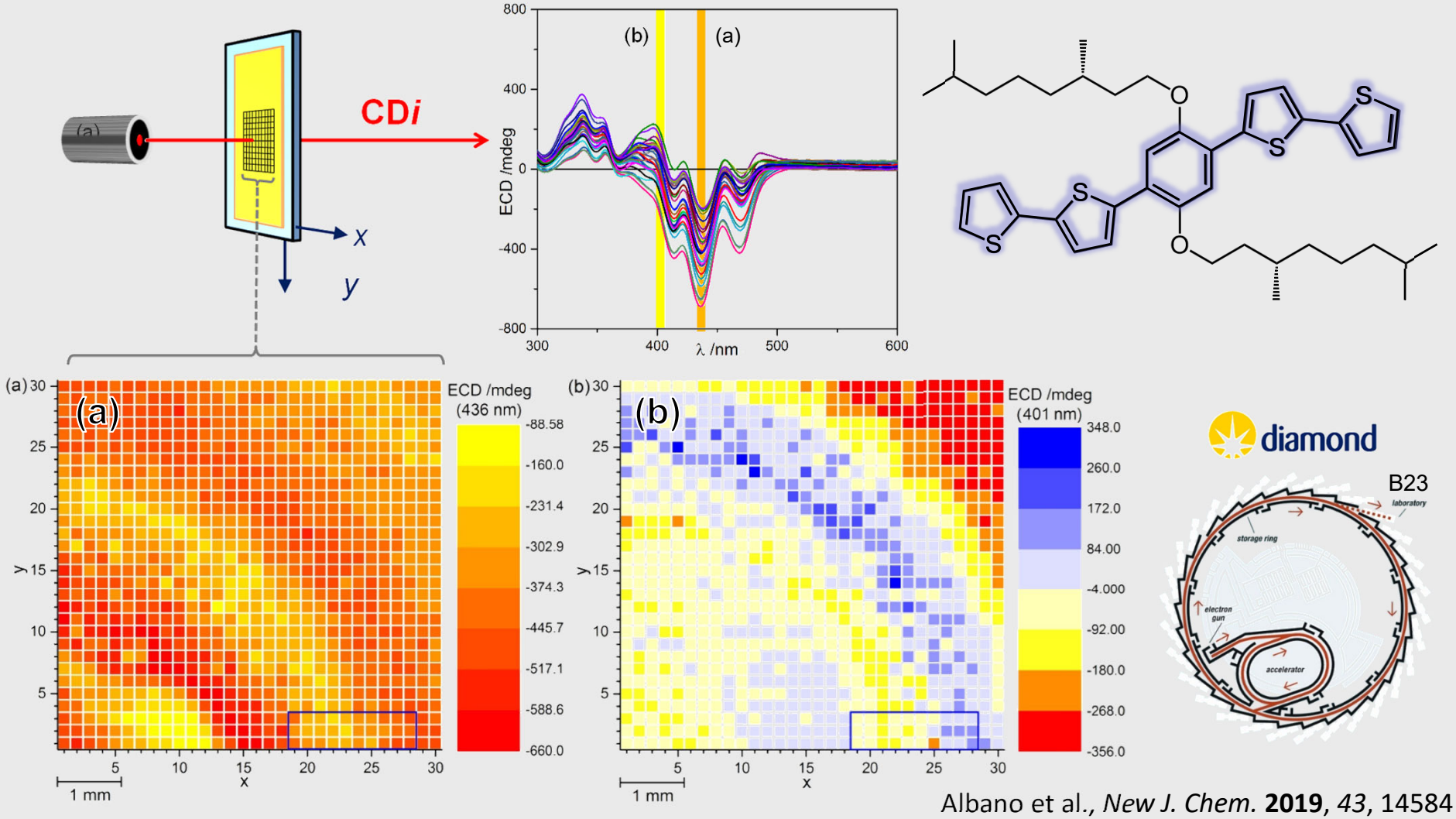




Padula et al., *RSC Adv.* **2016**, *6*, 37938; Aranda et al., *PCCP* **2018**, *20*, 21864

CD imaging (CD_i)

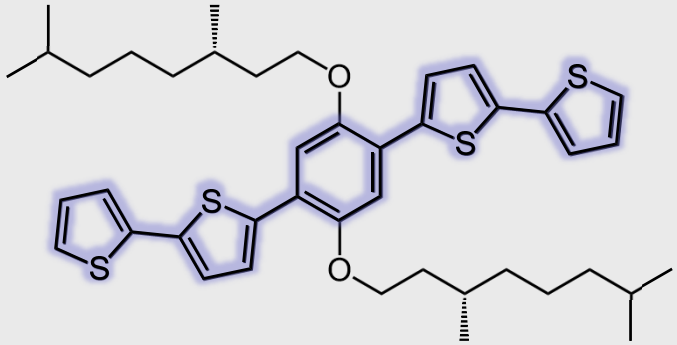
- Spatially-resolved CD measurements with resolution ~0.1 mm
- Combines CD and optical microscopy



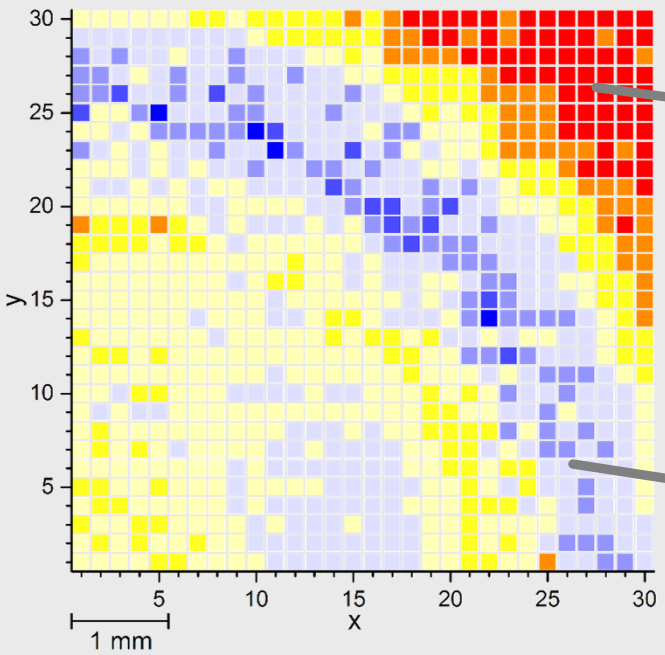
Albano et al., *New J. Chem.* **2019**, *43*, 14584

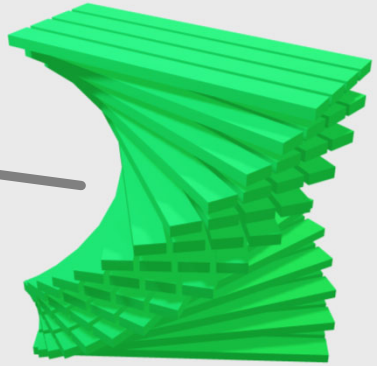
CD imaging (CD_i)

- Provides information on local ordering
- Unveils multiple aggregation phenomena

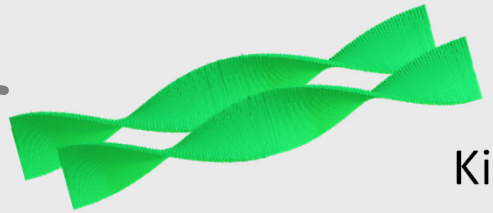


CD imaging and
data analysis



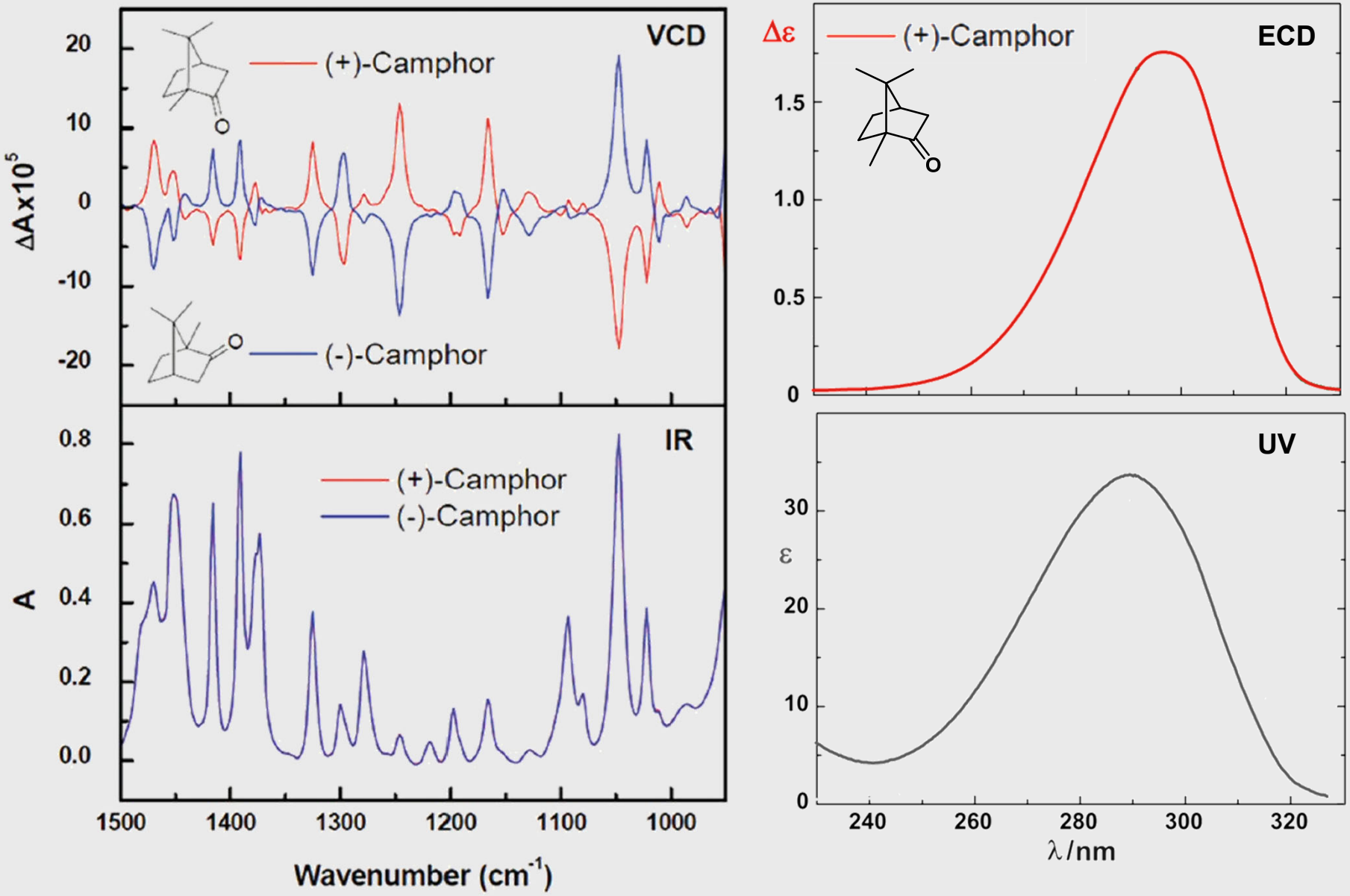


Thermodinamically
stable aggregates



Kinetically entrapped
aggregates

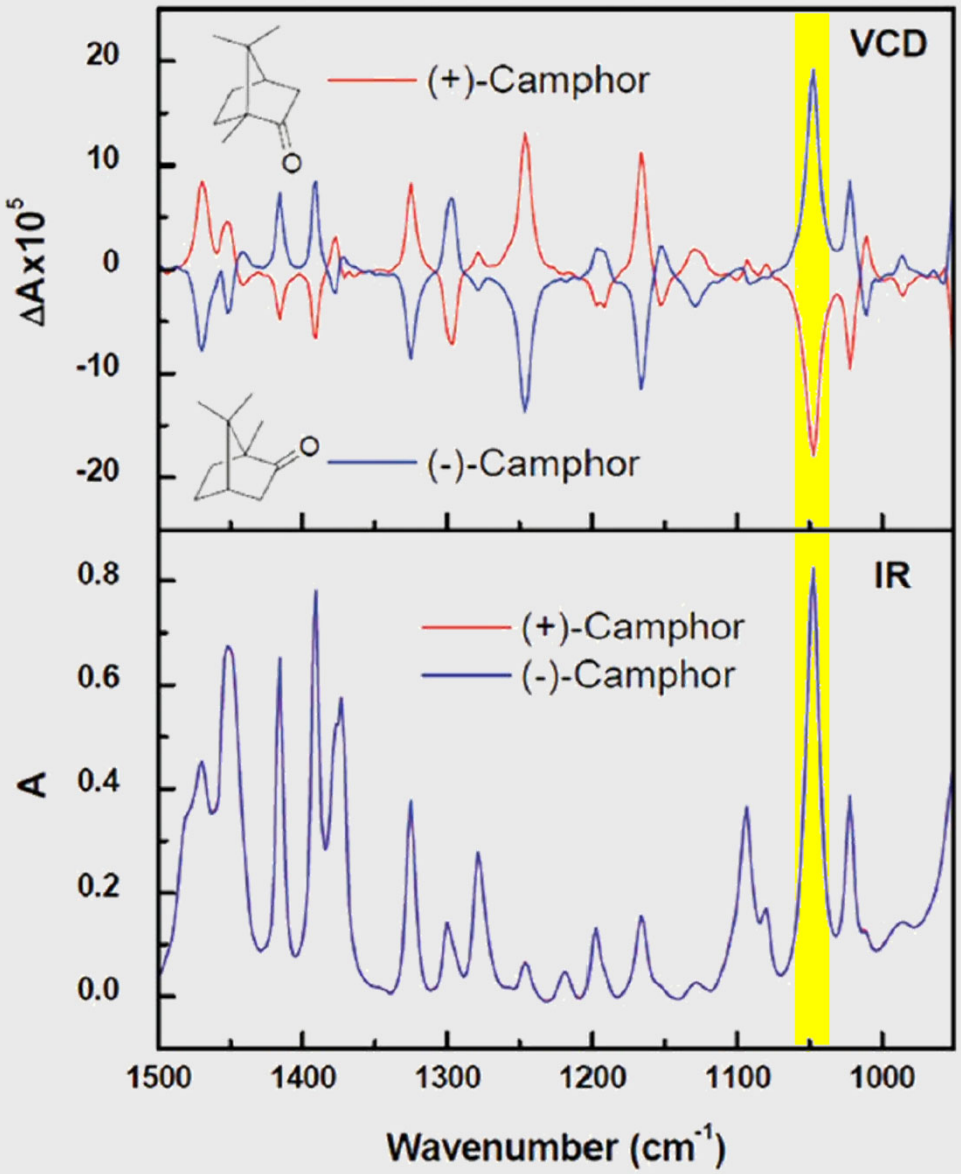
Vibrational vs Electronic Optical Activity

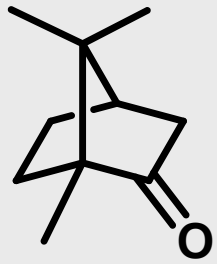


Vibrational vs Electronic Optical Activity

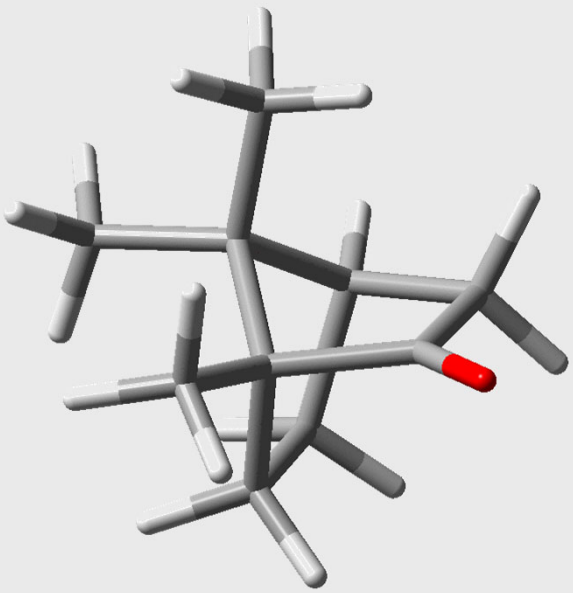
	Vibrational CD	Electronic CD
Range	800-4000 cm^{-1} (esp. 800-1500 cm^{-1})	190-800 nm
Measurement mode	Fourier transform	Continuous
Sample requirements	None	Must have chromophores
Sample concentration	≈0.1 M	< mM
Solvent	CCl_4, $CDCl_3$, CD_3OD, D_2O	H_2O, alcohols, ethers, alkanes, CH_3CN, $CHCl_3$, DMSO, etc (and mixtures)
Cell path length	10-100 µm	0.01-1 cm
Cell material	KBr (hygroscopic) BaF_2, CaF_2	Optical quartz
Typical g-factor	10^{-4}-10^{-5}	10^{-2}-10^{-4}
Typical accumulations	2000-8000	1-8
Typical measurement time	30-60 min	5-10 min

Vibrational vs Electronic Optical Activity





(+)-camphor

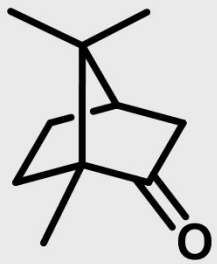


normal mode no. 32

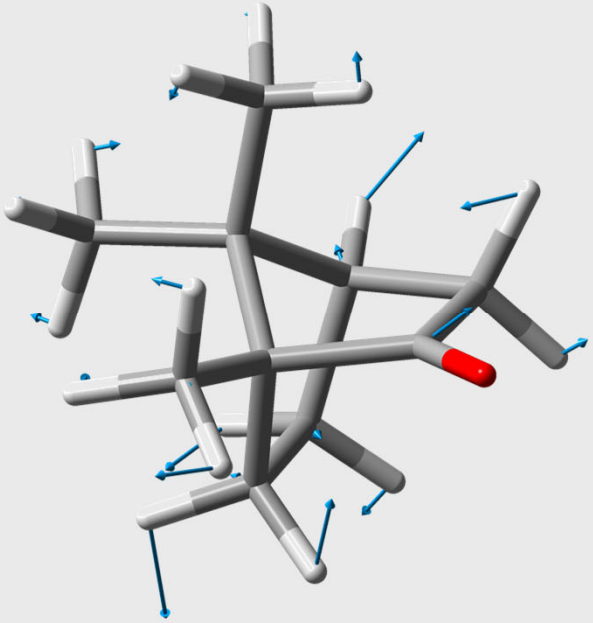
Main applications of VCD spectroscopy

- Assignment of absolute configuration, virtually of any molecule, also in combination with ECD
- Conformational investigations (molecules, biopolymers)
- Enantiomeric excess measurement
- Detection of aggregated species, especially when leading to enhanced VCD signals
  - Gels
  - Fibrils

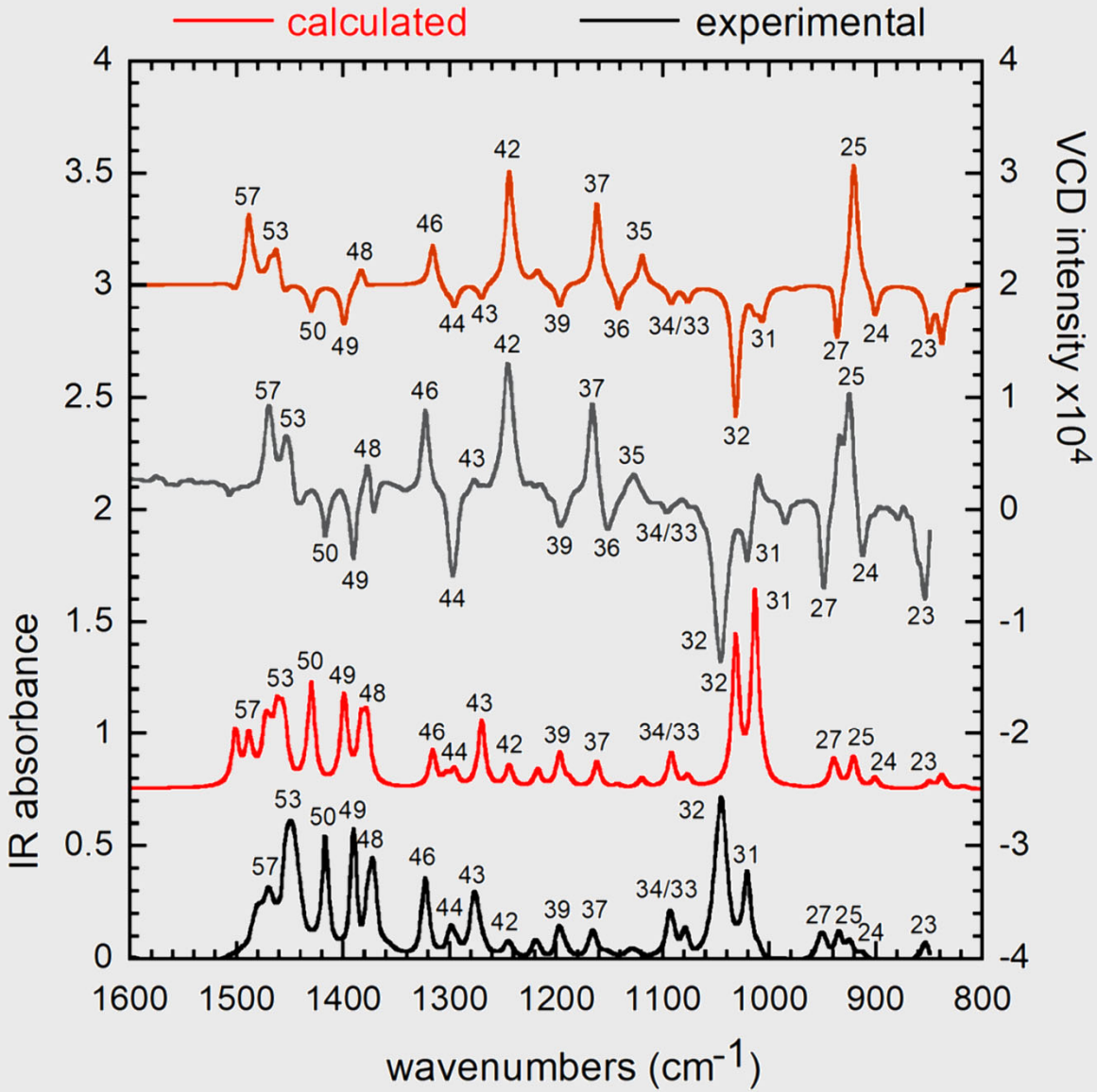
VCD calculations



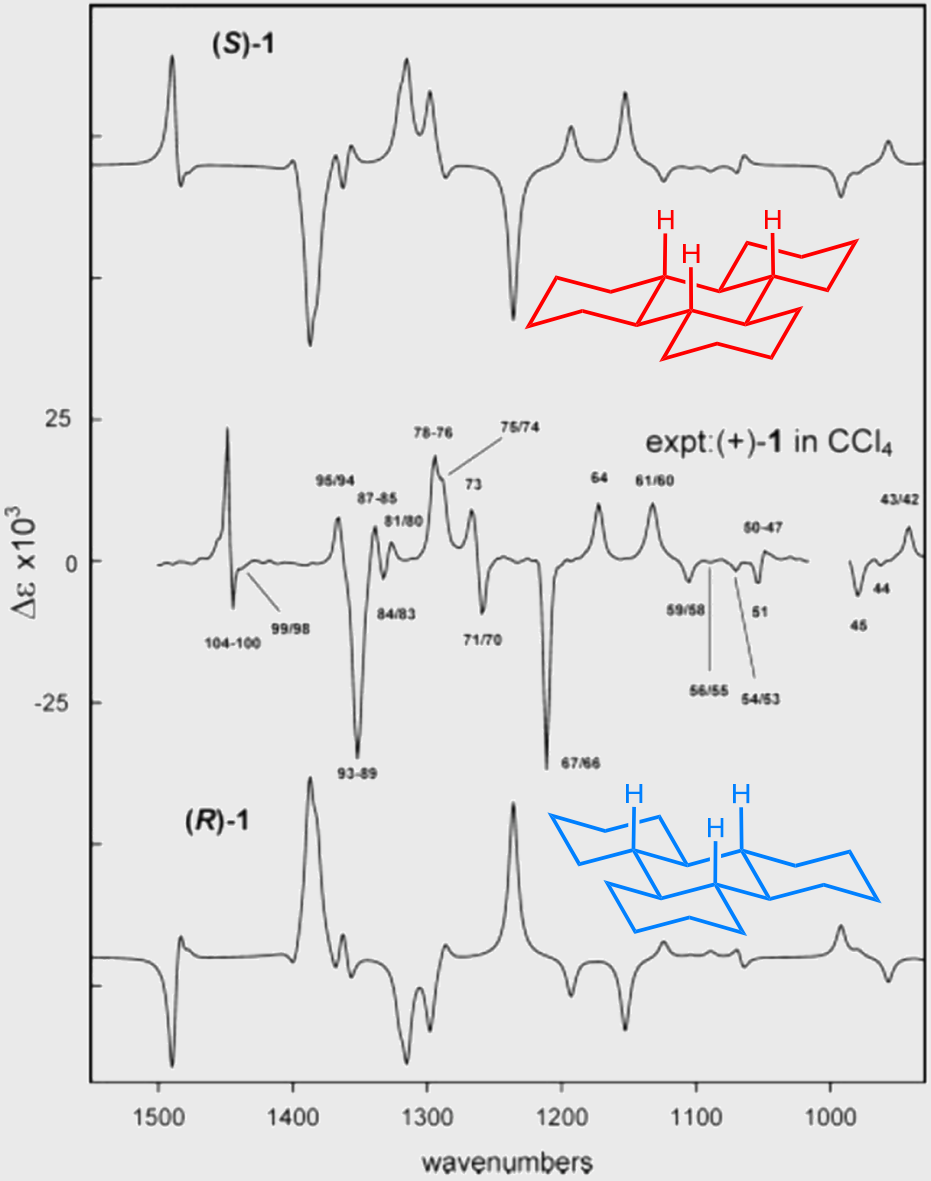
(+)-camphor

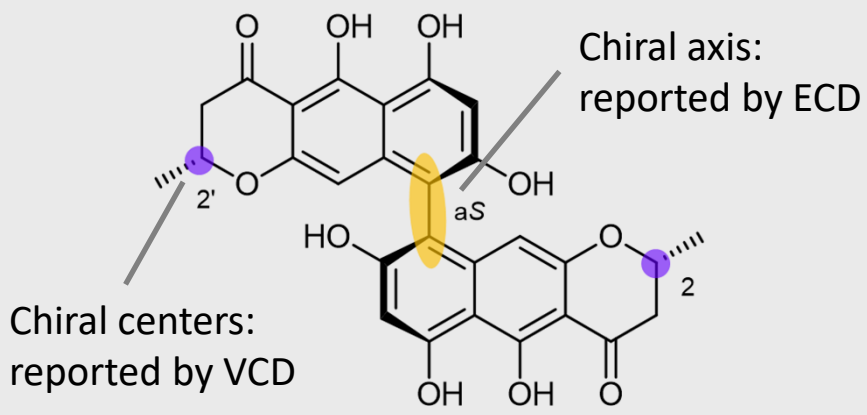


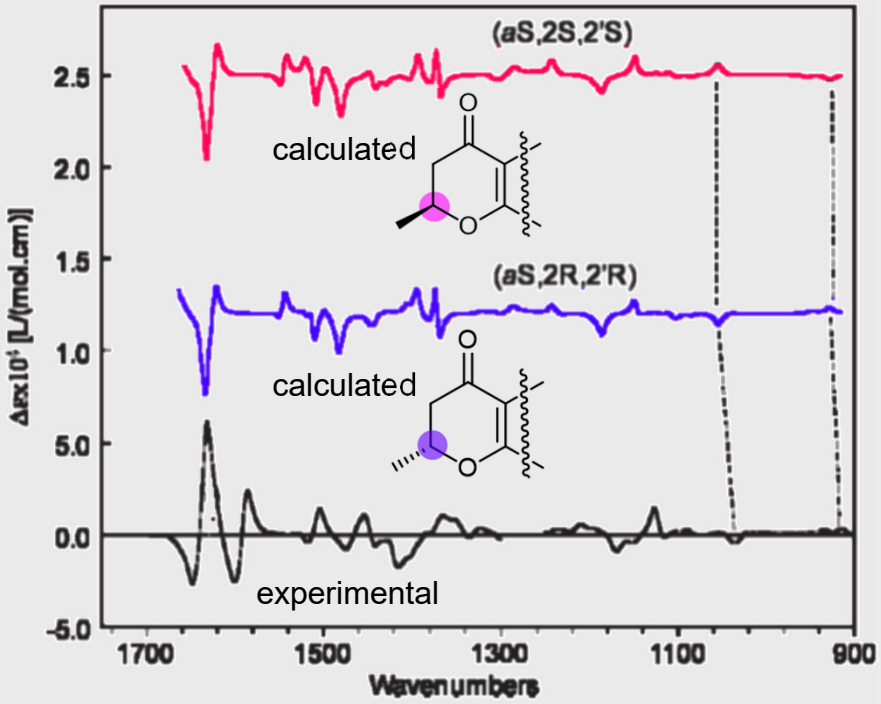
mode 32



VCD calculations







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Torsten Bruhn @ Uni Würzburg

References

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- Chem. Soc. Rev. **2011**, *40*, 4603-4625 [conformational aspects]
- Chem. Soc. Rev. **2014**, *43*, 5211-5233 [supramolecular systems]
- Chirality **2016**, *28*, 466-474 [calculations]