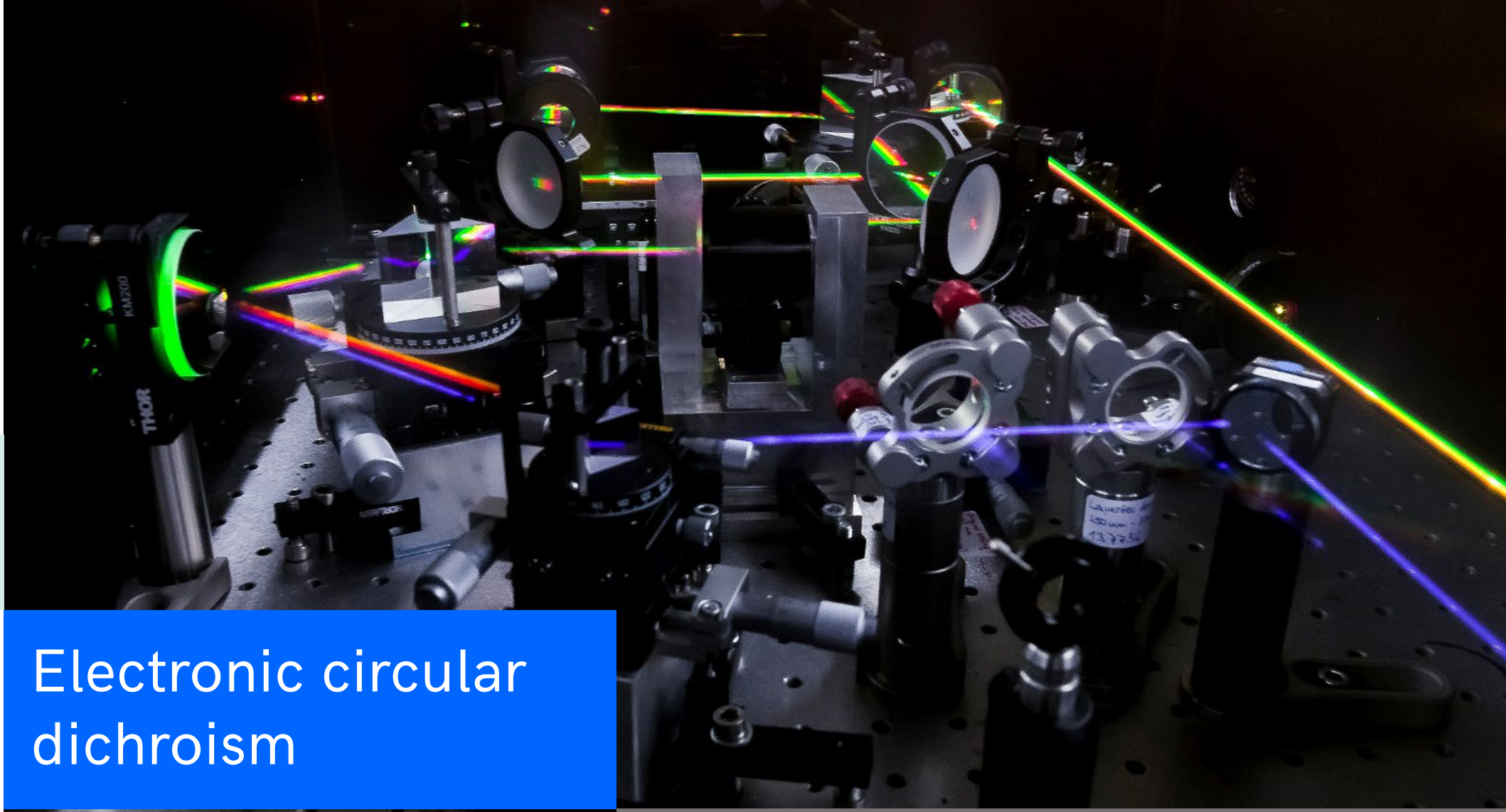
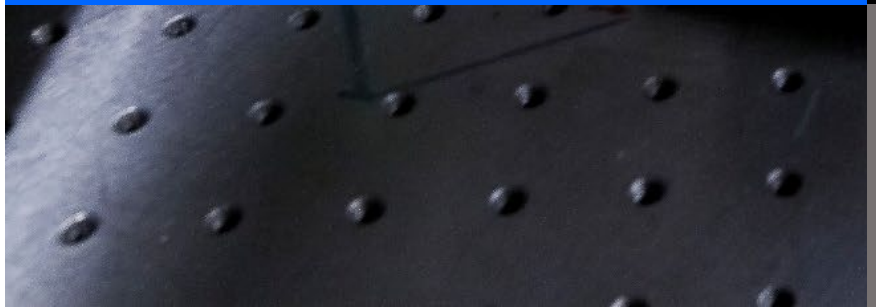
Universität Basel



Electronic circular dichroism



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Web: oppermann.chemie.unibas.ch

TODAY'S OUTLINE

1. Motivation & theory

- Molecular chirality, the polarization of light
- Electronic transitions, Rosenfeld equation, excitonic coupling model

2. Instrumentation & methods

- ECD instrument, sample preparation, spectra

3. Applications

- ECD for chromatography, vibrational CD
- DNA and proteins

4. Ultrafast ECD

- Ultrafast measurements
- Chirality of excited states

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Electronic circular dichroism

Part I:

Motivation & theory

Chiral objects

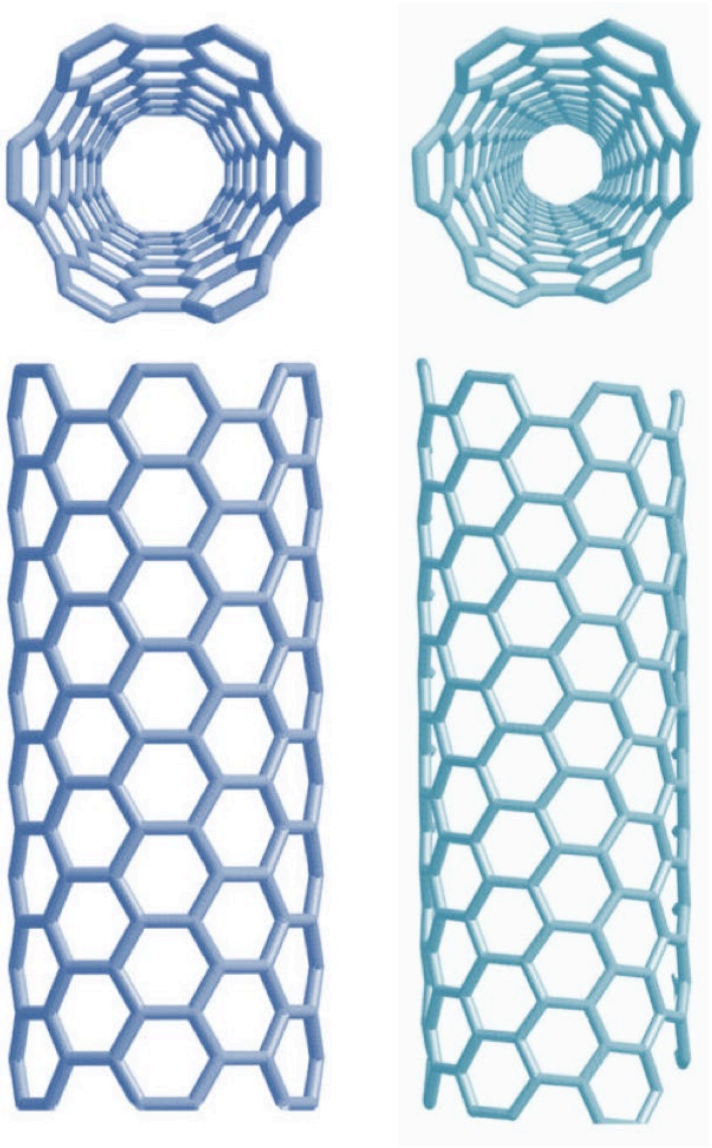
D-(-)-Fusilli

L-(+)-Fusilli

Achiral objects

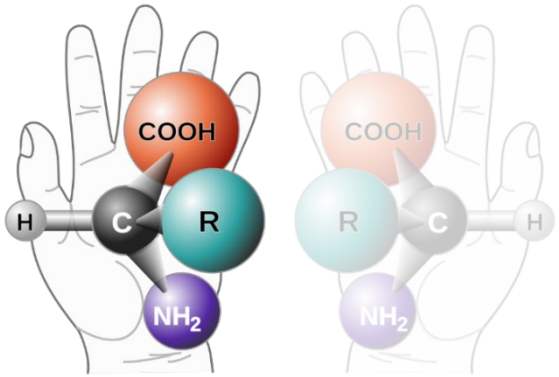
Penne

CHIRALITY

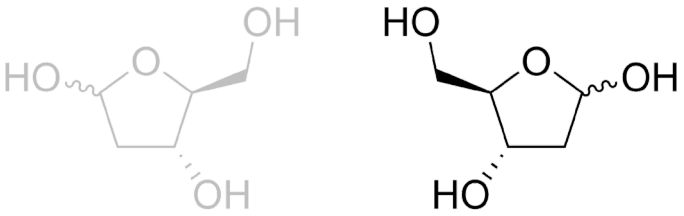



Barilla
RIGATONI
CHIRAL
SERVING SUGGESTION
"AL DENTE" PERFECTION IN 12-13 MINUTES
ITALY'S #1 BRAND OF PASTA
ENRICHED MACARONI PRODUCT
NET WT. 1 LB (454 g)
NON GMO Project VERIFIED
Nutrition Facts

Homochirality

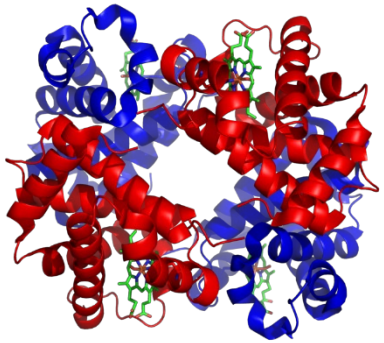


L-amino acid D-amino acid

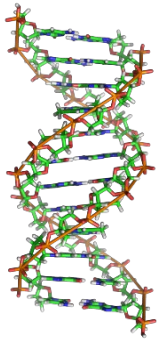


L-deoxyribose D-deoxyribose

Molecular structure



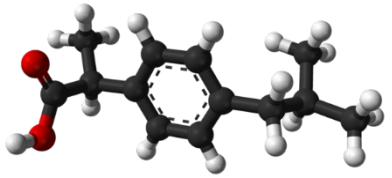
Proteins & Enzymes



DNA & RNA

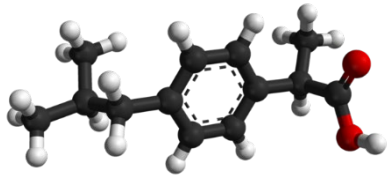
Structural marker

Functional property



active

S-Ibuprofen



mostly inactive

R-Ibuprofen

Design feature

Figures: en.wikipedia.org

Guidelines for drug development

The U.S. Food and Drug Administration (FDA) issued drug development guidelines in 1992 that „require that absolute stereochemistry be known for compounds with chiral centers and that this information should be established early in drug development in order that the analysis can be considered valid. From exploration of structure space to governmental regulations, the question of chirality in drug design is of vital importance."

CHIRAL BLOCKBUSTERS

In nine of top 10 drugs, the active ingredients are chiral

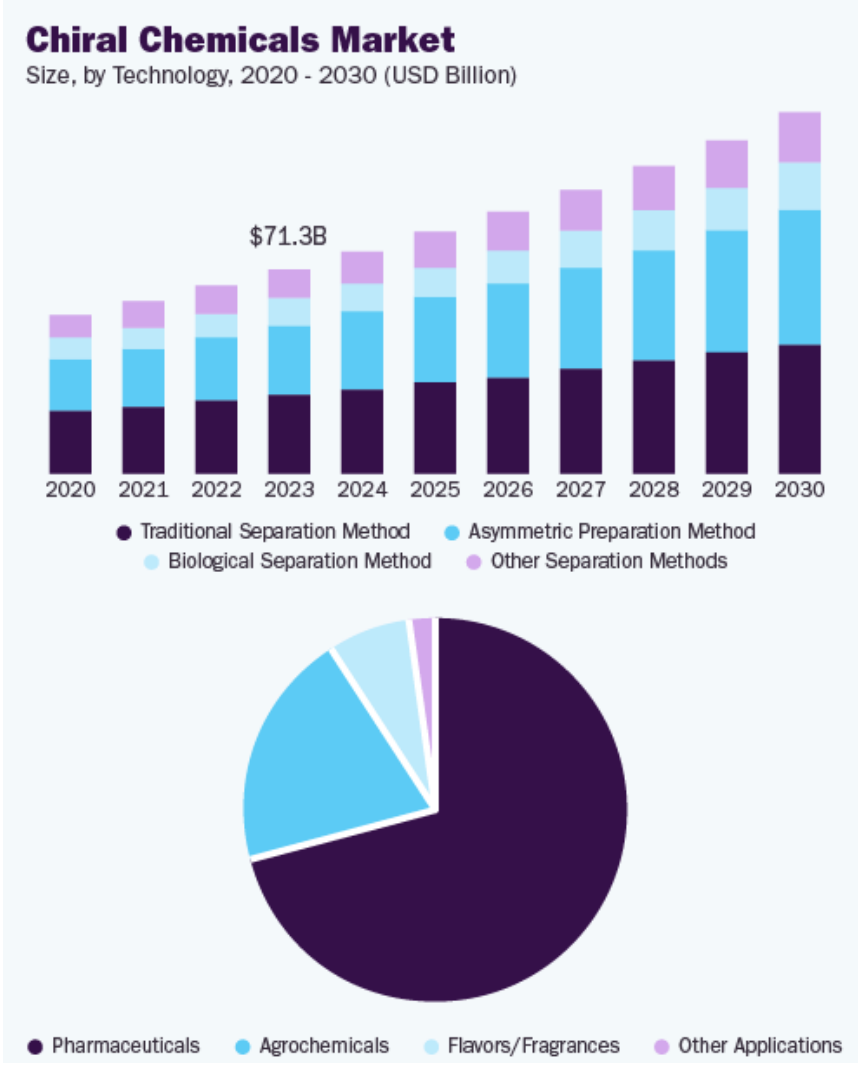
BRAND	GLOBAL 2003 SALES ($ BILLIONS)	ACTIVE INGREDIENT(S)	FORM OF ACTIVE INGREDIENT(S)	THERAPEUTIC EFFECT
Lipitor	$10.3	Atorvastatin	Single enantiomer	Lipid-lowering agent
Zocor	6.1	Simvastatin	Single enantiomer	Lipid-lowering agent
Zyprexa	4.8	Olanzapine	Achiral	Psychotropic agent
Norvasc	4.5	Amlodipine	Racemate	Calcium channel blocker
Procrit	4.0	Epoetin a	Protein	Stimulant of blood cell production
Prevacid	4.0	Lansoprazole	Racemate	Inhibitor of gastric acid secretions
Nexium	3.8	Esomeprazole	Single enantiomer	Inhibitor of gastric acid secretions
Plavix	3.7	Clopidogrel	Single enantiomer	Inhibitor of platelet aggregation
Advair	3.7	Salmeterol	Racemate	b_2-adrenergic bronchodilator
		Fluticasone	Single enantiomer	Anti-inflammatory agent
Zoloft	3.4	Sertraline	Single enantiomer	Selective serotonin reuptake inhibitor
TOTAL	$48.3			

NOTE: Sales figures from IMS Health.

Chiral technology:

1. Enantioselective synthesis
2. Separation of enantiomers
3. Chiral analysis (identification of enantiomers) ⟶ Chiral interaction!

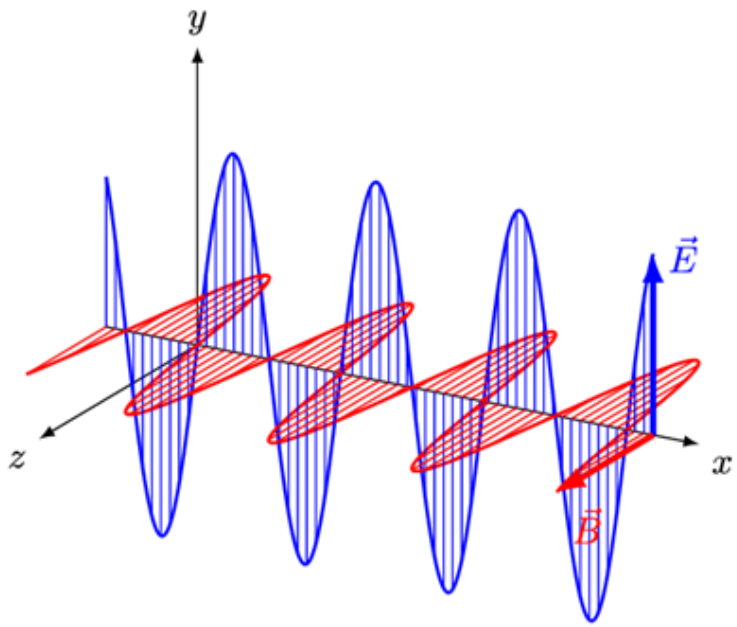
Chiral chemicals market



FDA: Curr. Top. Med. Chem 2011:DOI 10.2174/156802611795165098

https://www.grandviewresearch.com/industry-analysis/chiral-chemicals-market

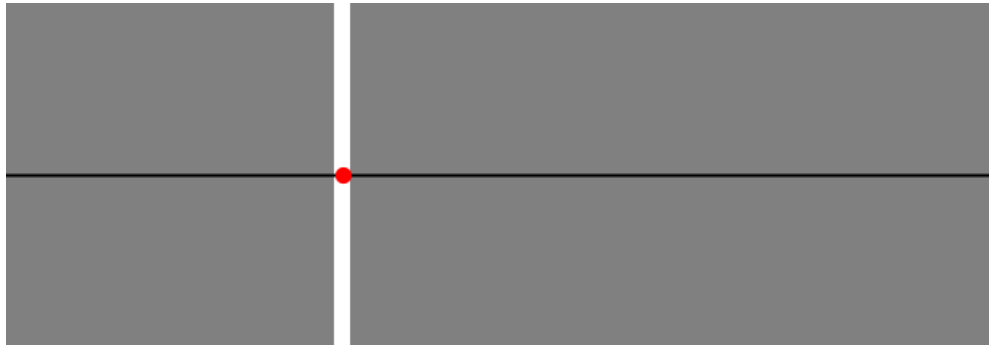
Electromagnetic waves



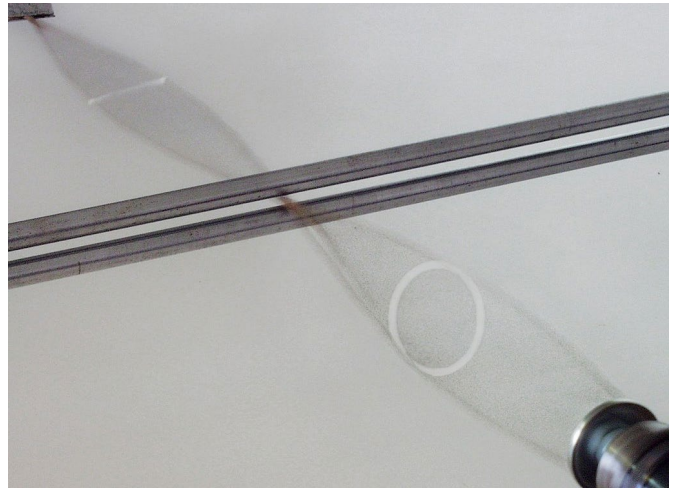
The main features of the electromagnetic wave:

1. The oscillation is sinusoidal
2. Wavelength and frequency of the sine wave
3. Speed and direction of propagation
4. Amplitude of the oscillation
5. Polarization (transversal wave)
6. Phase

Transverse waves



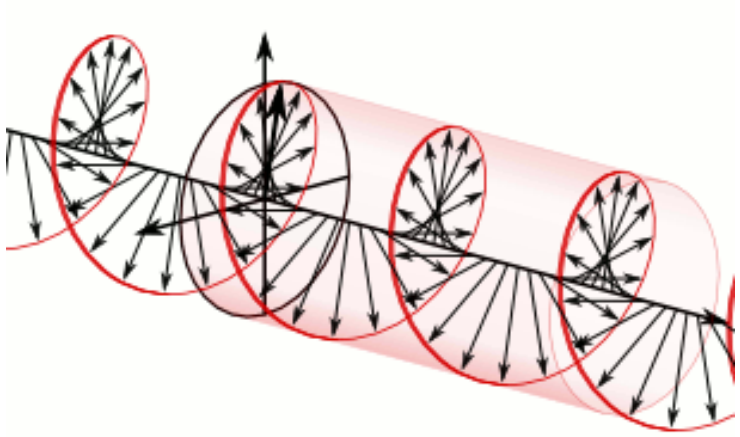
A transverse wave is a wave whose oscillations are perpendicular to the direction of the wave's advance. This contrasts with a longitudinal wave which travels in the direction of its oscillations.



Polarization is a property of transverse waves which specifies the geometrical orientation of the oscillations. The photo shows the circular polarization of an oscillating thread converted linear polarization.

Circular polarization

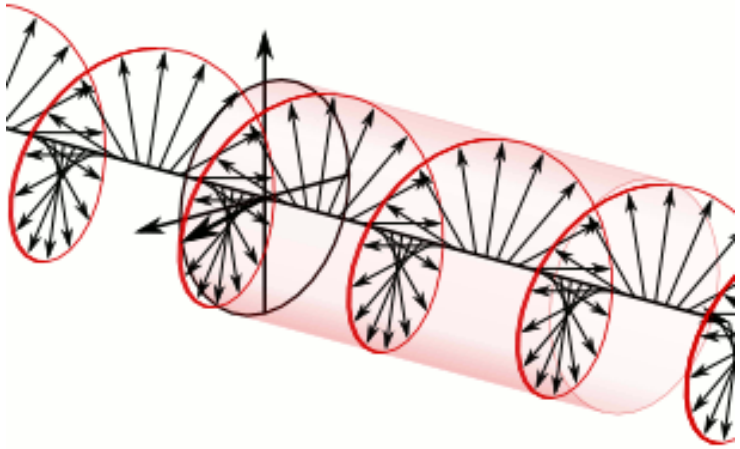
In order to distinguish chiral molecules, the interaction between the light and the molecule needs to be chiral. Linearly polarized light is not chiral, but circularly (or elliptically) polarized light is chiral:



Right-handed circularly polarized (RCP) light:

Right-handed spiral in the direction of light propagation

Electric field vector rotates clock-wise

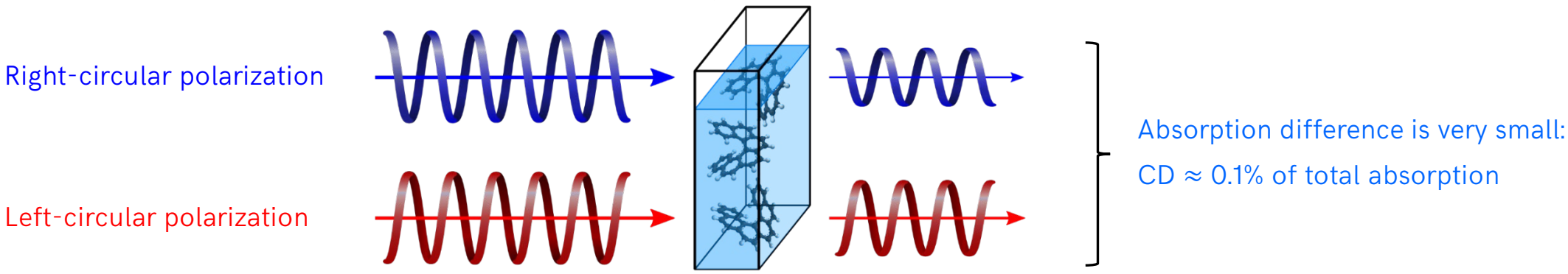


Left-handed circularly polarized (LCP) light:

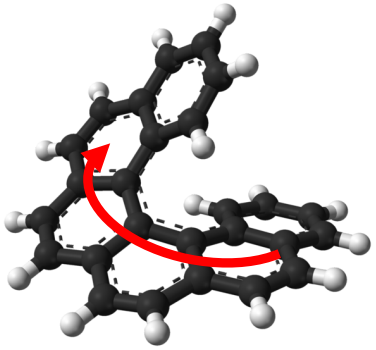
Left-handed spiral in the direction of light propagation

Electric field vector rotates counterclock-wise

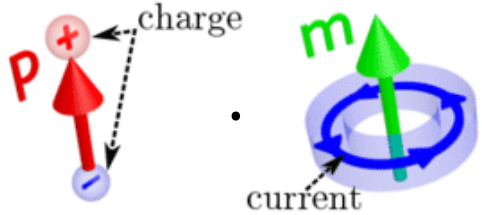
Absorption difference of left- & right-circularly polarized light



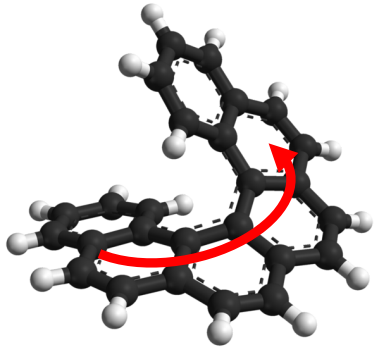
Origin: helical charge displacements in chiral molecules



Chiral M-[6]helicene

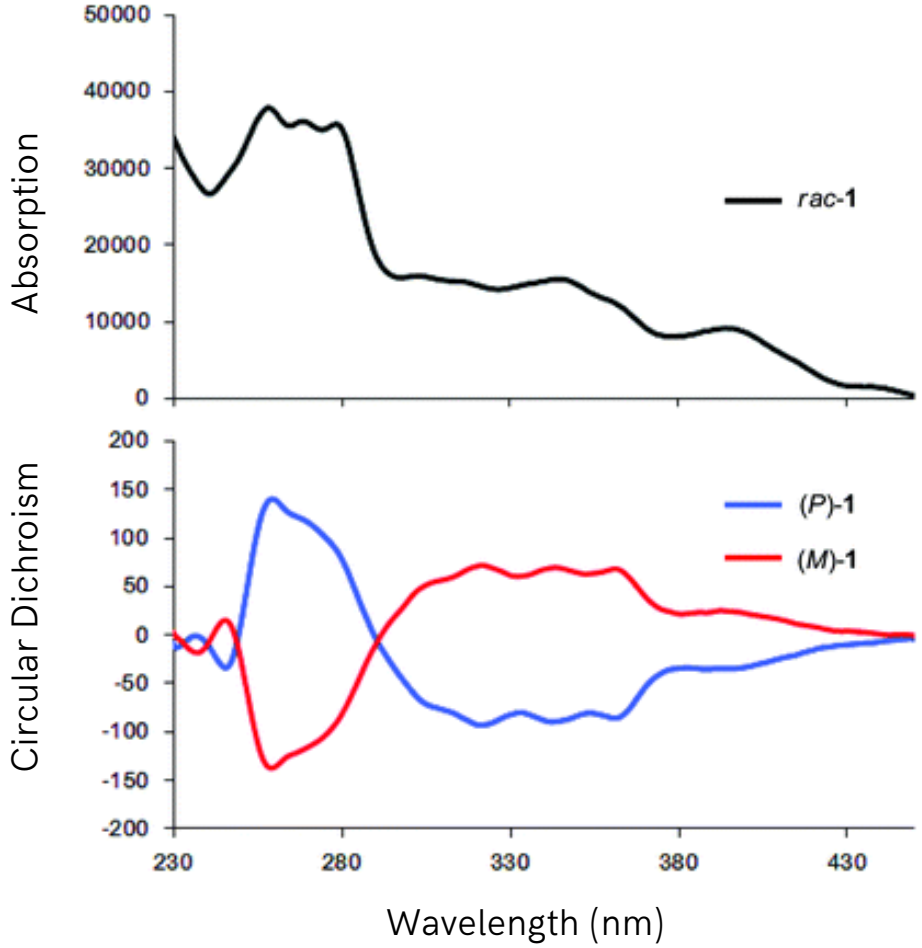


Interference of electric and magnetic transition dipoles



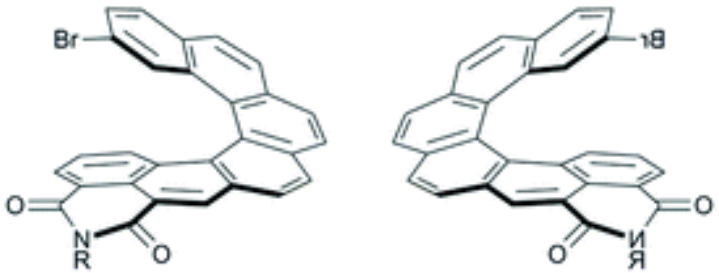
Chiral P-[6]helicene

ECD spectra



Sample

P M



Chiral [6]helicene derivative

Terminology

Enantiomers: Mirror image forms

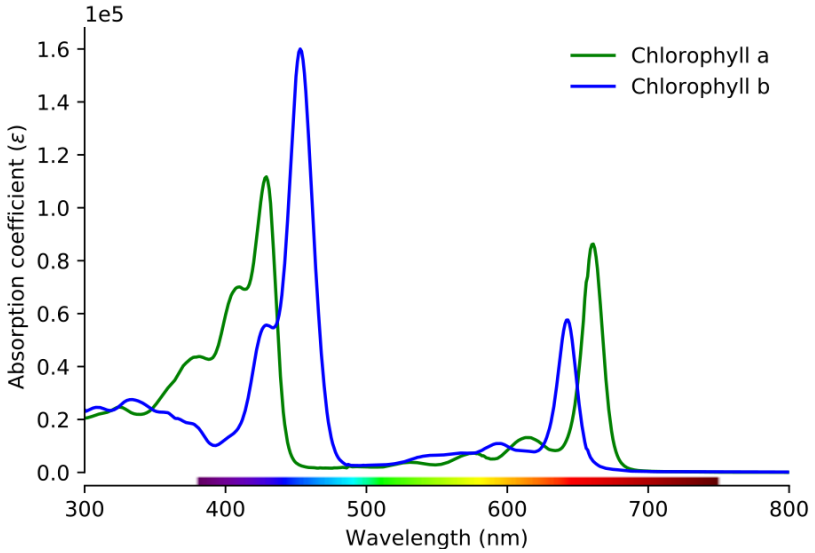
Racemic mix: Equal mix of enantiomers

Observations

- Enantiomers have identical absorption spectra
- Enantiomers have mirror-image ECD spectra
- ECD bands reproduce some absorption features but not all

Figures from DOI 10.1039/C2OB25223F

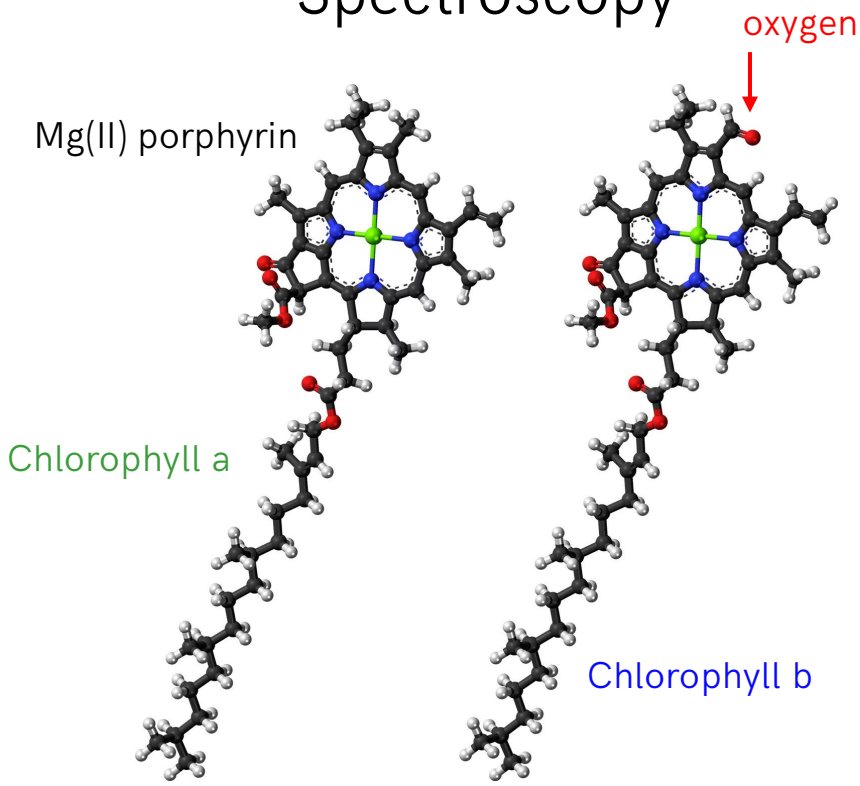
Example: chlorophyll



Chlorophyll is the green pigment in plant leaves.
Why does it look green?

What is its molecular structure?

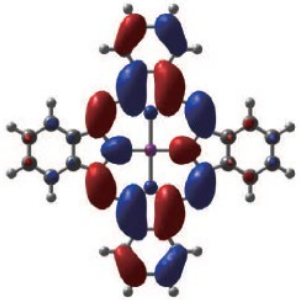
Spectroscopy



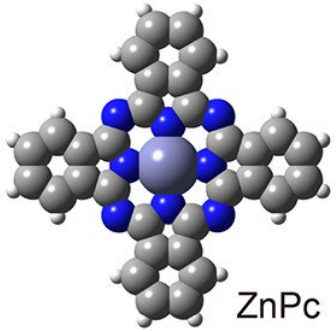
The absorption spectrum of the pigment is determined by:

- Its electronic structure…
- … which is determined by its molecular (= nuclear) structure
- The interactions of the electrons with the environment: the protein pocket, solvent, and adjacent pigments – recent research shows they account for spectral redi-shifts of up to 70 nm!

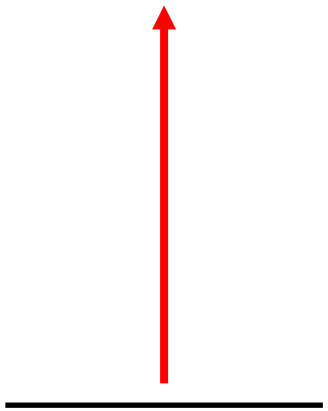
Chlorophyll electronic interactions: https://www.mpsd.mpg.de/42776/2015-04-chlorophyll-rubio



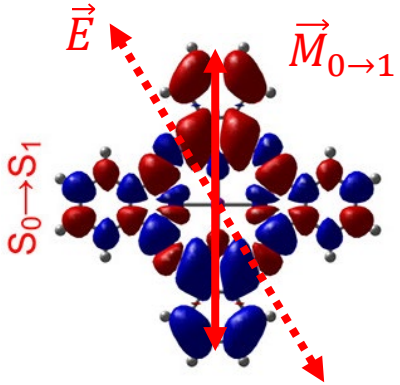
LUMO: S_1



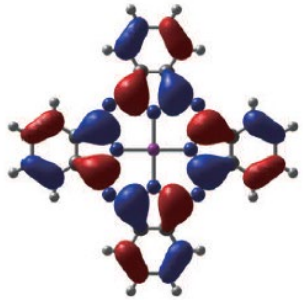
Molecular structure



HOMO: S_0



Transition dipole moment



Electronic energy

Charge distribution

Quantum mechanical approach

The transition dipole moment $\vec{M}_{0\to1}$ for an electronic transition is defined as:

Light-matter interaction: electric dipole operator $\vec{\mu}$

$$\vec{M}_{0\to1} = \int \psi_1^* \vec{\mu} \psi_0 \, dV$$

Wavefunctions of stationary states S_1 und S_0

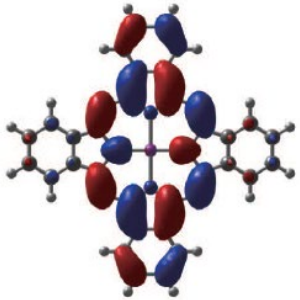
It can be associated with the change in electronic density caused by the interaction of the light with the electrons. It is a vector and has three important properties:

1. It is linked to a transition of a specific photon energy
2. Its magnitude is related to the probability of the transition
3. Its direction is determined by the molecular structure

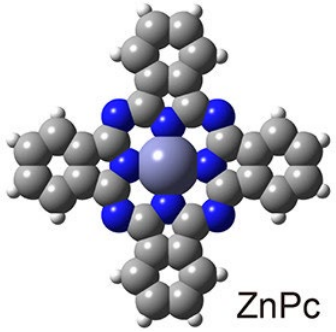
Fermi's Golden Rule tells us how we get the transition probability:

$$A_{0\to1} \propto \left|\vec{E} \cdot \vec{M}_{0\to1}\right|^2 = \left| \int \psi_1^* \vec{E} \cdot \vec{\mu} \psi_0 \, dV \right|^2$$

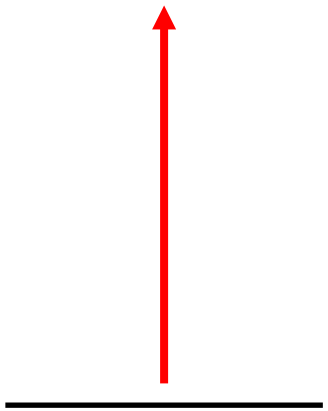
zinc phthalocyanine calculations: DOI 10.1021/acs.jpcc.9b10256



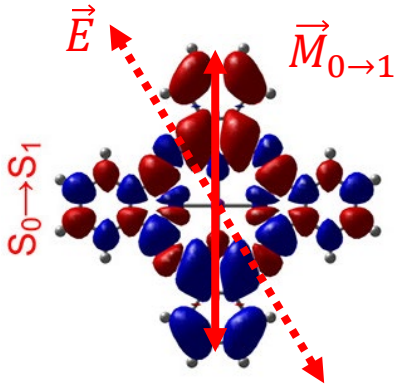
LUMO: S_1



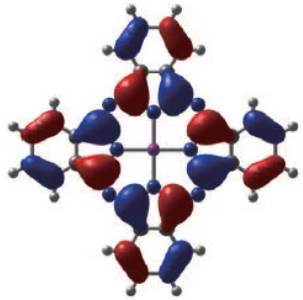
Molecular structure



HOMO: S_0



Transition dipole moment



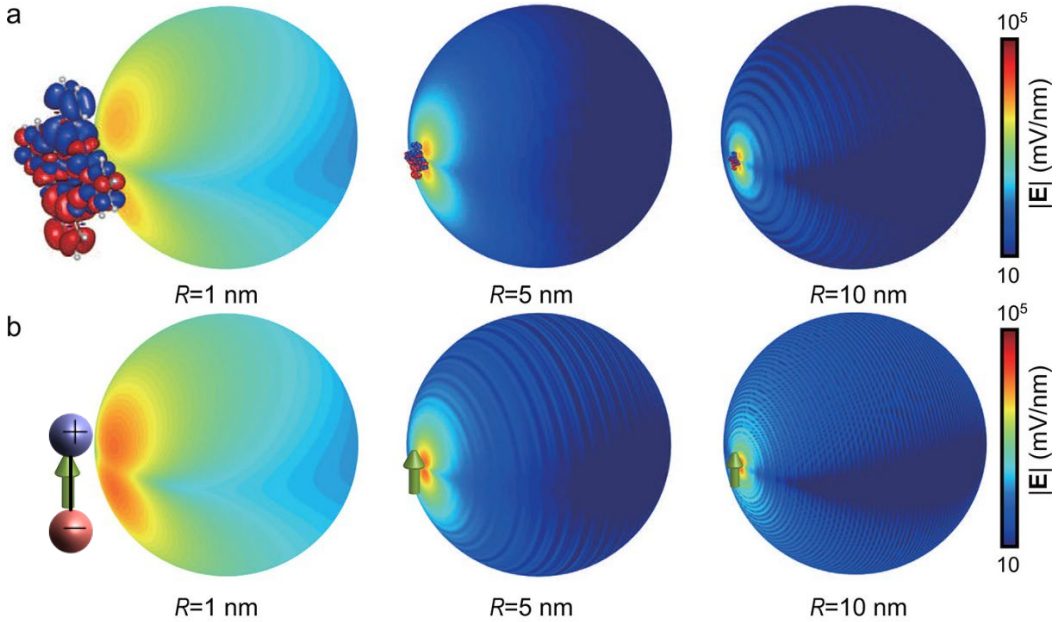
Electronic energy

Charge distribution

Dipole approximation

In molecular spectroscopy we usually do not interact with point charges, but with complicated charge distributions. To simplify this situation, we typically make use of the dipole approximation, which assumes:

1. The interaction with the magnetic field is neglected
2. The charge distribution change is expressed as a (osciallating) dipole



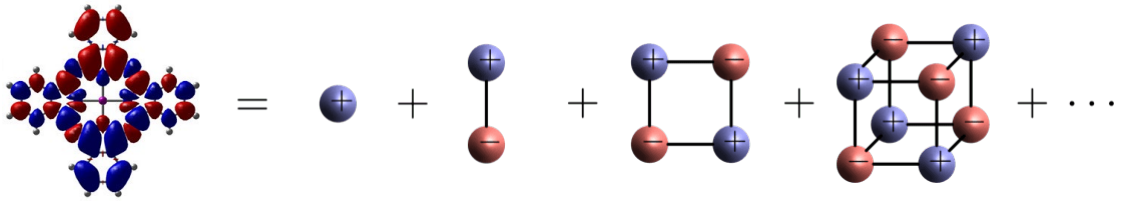
Comparison of local electric field distribution on a spherical nanoparticle induced by the molecular transition dipole and a point dipole.

zinc phthalocyanine calculations: DOI 10.1021/acs.jpcc.9b10256

Figure: DOI 10.1021/acs.jpcc.9b10256

FERMI'S GOLDEN RULE FOR ECD

Origin of the dipole approximation is a so-called multipole expansion, which expresses a three-dimensional charge distribution as a linear combination of electric multi-poles, much like an electrostatic polynomial fit.



Monopole Dipole Quadrupole Octopole

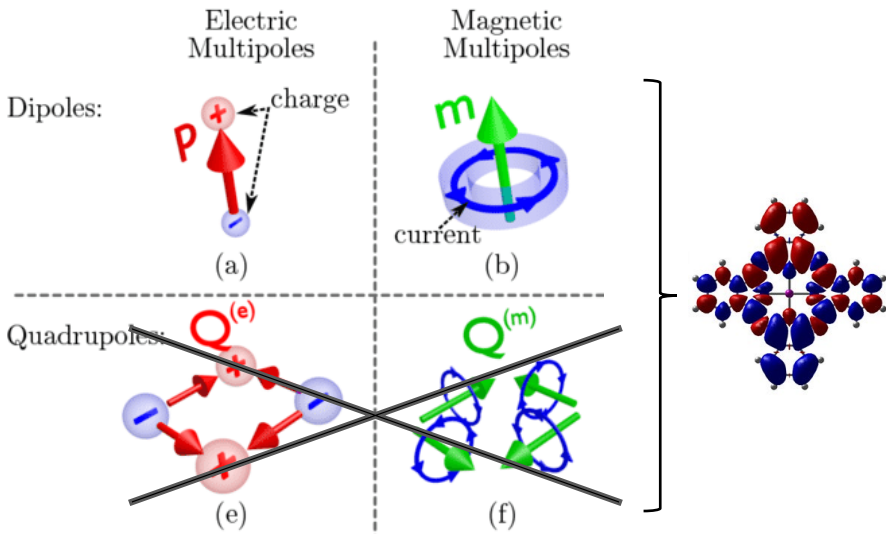
The same expansion can be performed for magnetic multipoles.

Why is it impossible to describe chiral light-matter interactions in the dipole approximation?

To derive the transition probability of circular dichroism, we need to go beyond the dipole approximation:

$$A_{0\to1} \propto \left| \int \psi_1^* (\vec{\mu}_{mol} \cdot \vec{E} + \vec{m}_{mol} \cdot \vec{B}) \psi_0 dV \right|^2$$

Fermi's Golden Rule for complete electro-magnetic interaction with electric and magnetic multipoles



ECD transitions are chiral light-matter interactions:

We must keep at least the electric and magnetic dipole terms. The magnetic interaction breaks the cylindrical symmetry of the electric dipole!

Note: Electric quadrupole transitions also contribute to ECD but usually average out in randomly oriented samples (e.g. molecules in solution).

Multipoles from V. Savinov, PhD thesis, University of Southampton (2014)

FERMI'S GOLDEN RULE FOR ECD

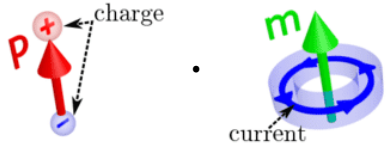
A CD transition is associated with the difference in absorption of left- and right-handed circularly polarized light, which we abbreviate as LCP and RCP, respectively:

$$CD_{0\to1} = A_{0\to1}^{LCP} - A_{0\to1}^{RCP}$$

$$CD_{0\to1} \propto \left| \int \psi_1^* (\vec{\mu} \cdot \vec{E}^{LCP} + \vec{m} \cdot \vec{B}^{LCP}) \psi_0 dV \right|^2 - \left| \int \psi_1^* (\vec{\mu} \cdot \vec{E}^{RCP} + \vec{m} \cdot \vec{B}^{RCP}) \psi_0 dV \right|^2$$

We can now use explicit vector forms for LCP and RCP light and after some fun with algebra, we get:

$$CD_{0\to1} \propto \int \psi_1^* \vec{\mu} \psi_0 dV \cdot \int \psi_0^* \vec{m} \psi_1 dV = \vec{M}_{0\to1}^{el} \cdot \vec{M}_{1\to0}^{mag}$$



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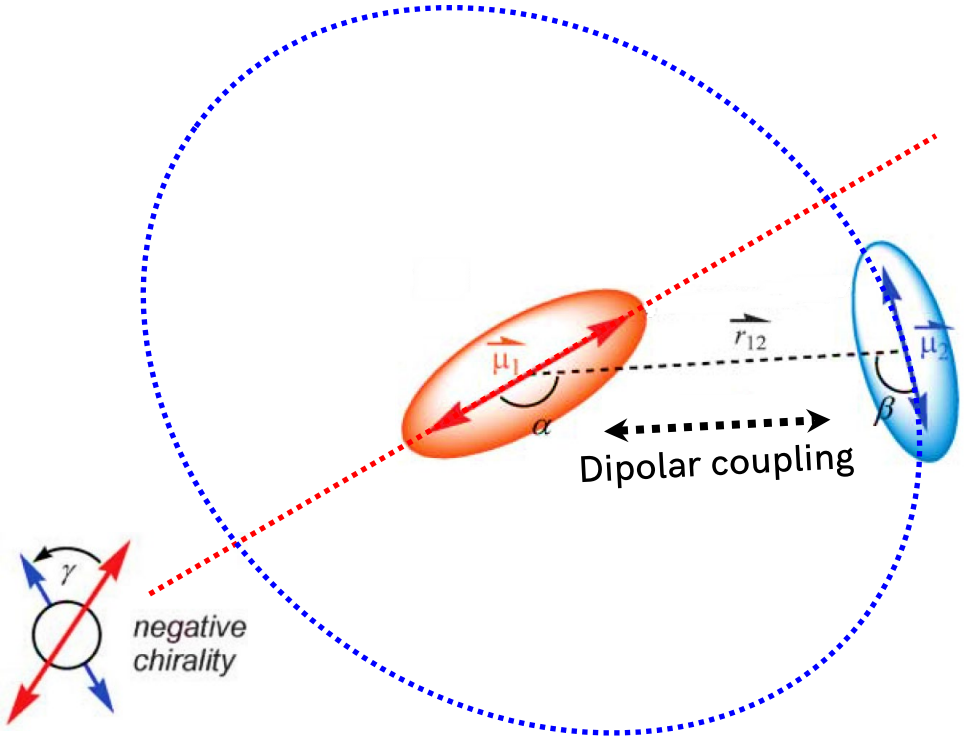
Rosenfeld equation

- CD is proportional to the scalar product of an electric and a magnetic transition moments
- Consequence: CD is accompanied by a helical charge displacement!
- Parallel moments: positive CD band
- Anti-parallel moments: negative CD band
- Tiny signals represent deviations from dipole approximation: typically 0.1 % of total absorption

Full derivation in: Norden, Rodgers, Dafforn - Linear Dichroism and Circular Dichroism (2010)

Chiral two-chromophore system



The electrostatic interaction between dipoles is often referred to as excitonic coupling in molecular photochemistry.

Excitonic ECD

A linear charge displacement from an electric transition dipole can be associated with the tangential component of a circular charge motion, which in turn generates a magnetic transition dipole moment:

$$\boldsymbol{m}_2^{jk} = \left(k\middle|\hat{\boldsymbol{m}}_2\middle|j\right) = \frac{e}{2m_e}\left(k\middle|\hat{\boldsymbol{R}}_2 \times \hat{\boldsymbol{p}}_2\middle|j\right) = \frac{i}{2\hbar}\boldsymbol{R}_2 \times \left(\varepsilon_k - \varepsilon_j\right)\boldsymbol{\mu}_2^{jk}$$

$$\left(k\middle|\hat{\boldsymbol{p}}_2\middle|j\right) = \frac{im_e}{e\hbar}\left(\varepsilon_k - \varepsilon_j\right)\boldsymbol{\mu}_2^{jk}$$

Electron momentum comes from transition dipole:

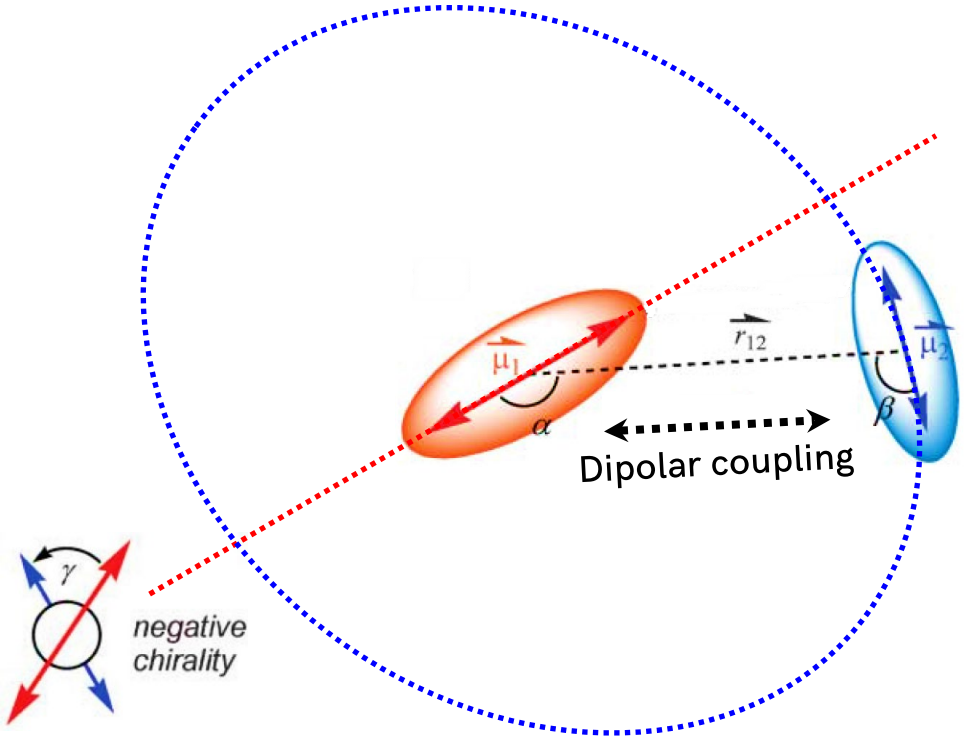
This magnetic transition dipole moment can be plugged into the Rosenfeld equation. This gives a CD that depends on the vector product of the two electric transition dipoles and their geometrical arrangement:

$$CD_{1,2} \propto \pm \vec{r_{12}} \cdot \vec{\mu_1} \times \vec{\mu_2}$$

ECD encodes the geometrical arrangement of excitonically coupled chromophores within a molecular system!

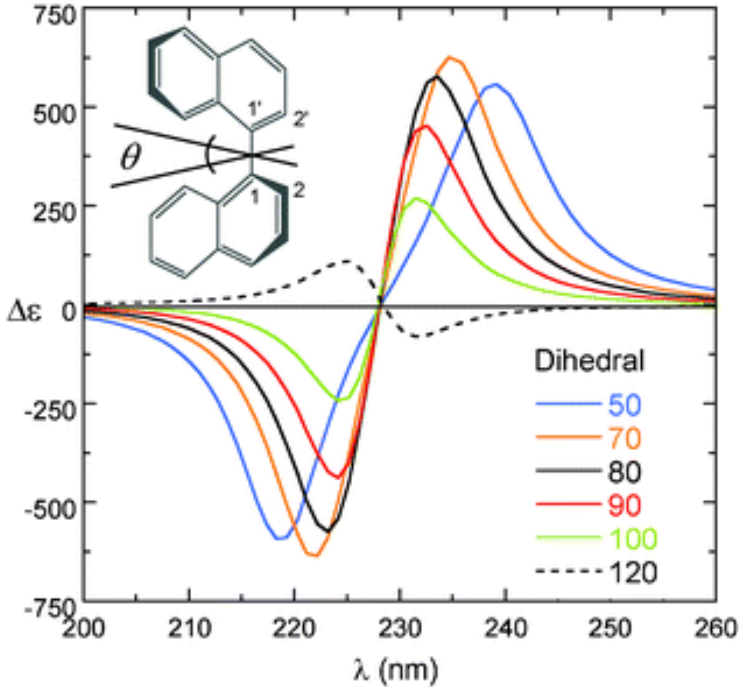
Figures adapted from: Berova et. al, *Chem. Soc. Rev.* 36, 914 (2007)

Chiral two-chromophore system



Excitonic ECD

Example calculation for bi-naphtalene:



The electrostatic interaction between dipoles is often referred to as excitonic coupling in molecular photochemistry.

$$CD_{1,2} \propto \pm \overrightarrow{r_{12}} \cdot \overrightarrow{\mu_1} \times \overrightarrow{\mu_2}$$

Figures adapted from: Berova et. al, *Chem. Soc. Rev.* 36, 914 (2007)

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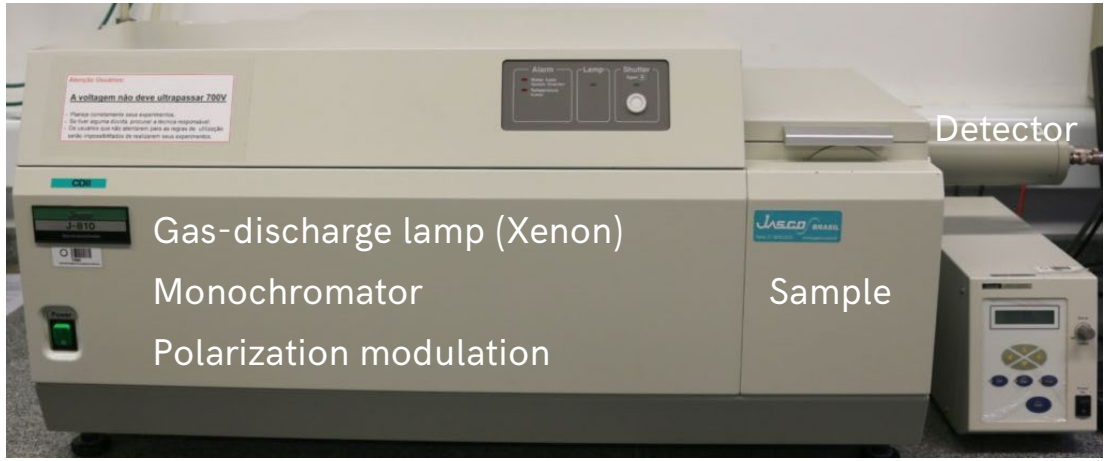
Electronic circular dichroism

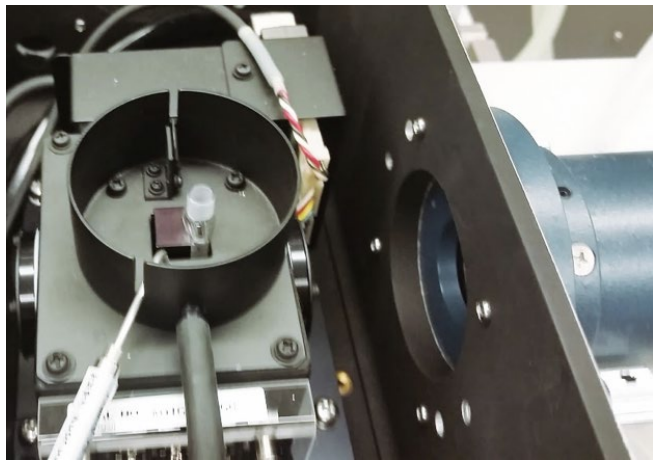
Part II:

Instrumentation & methods

Overview

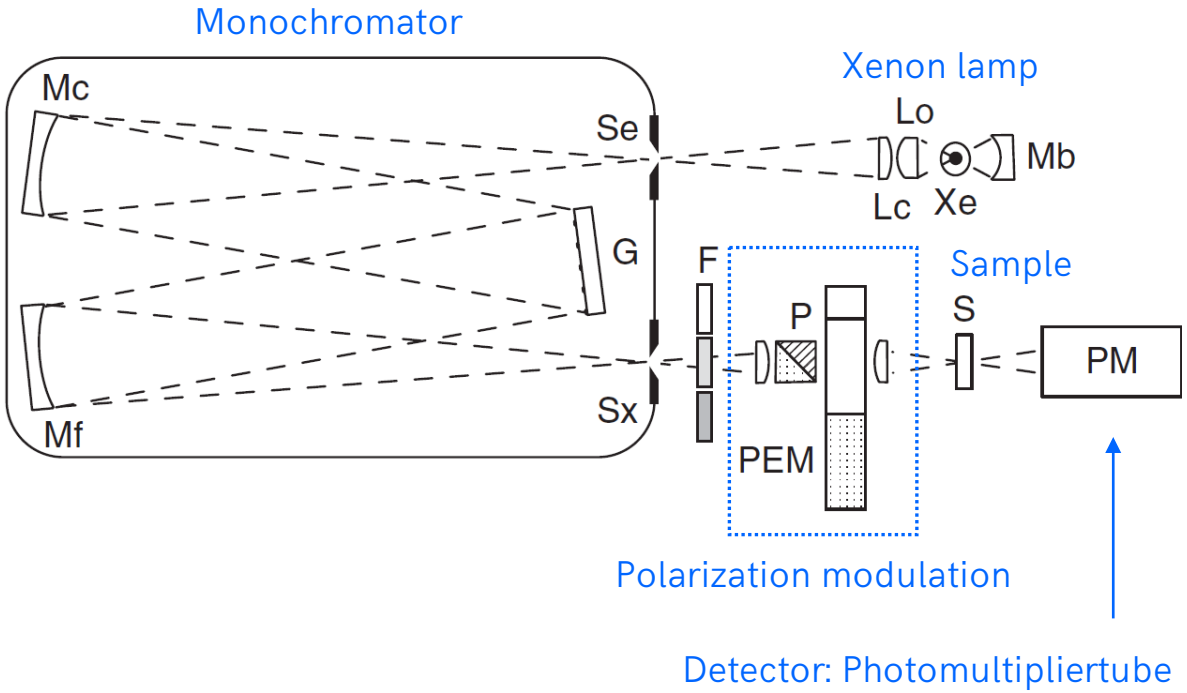


Somebody's J-810 CD spectrometer (cost: 50k to 200k $)



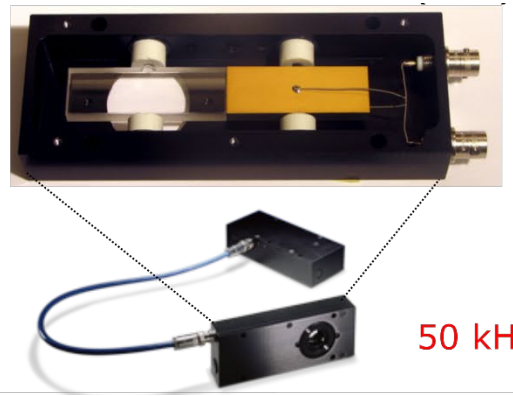
Someone else's sample compartment

Schematic

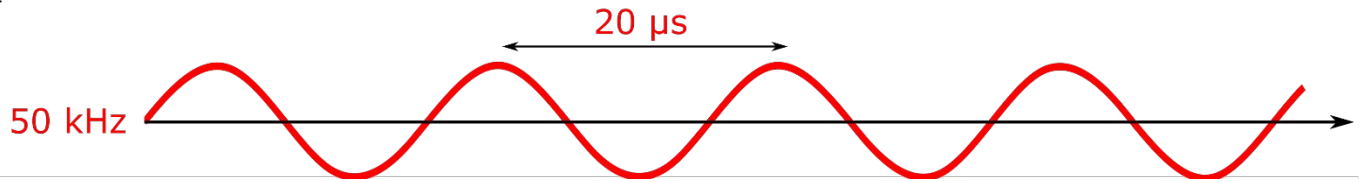


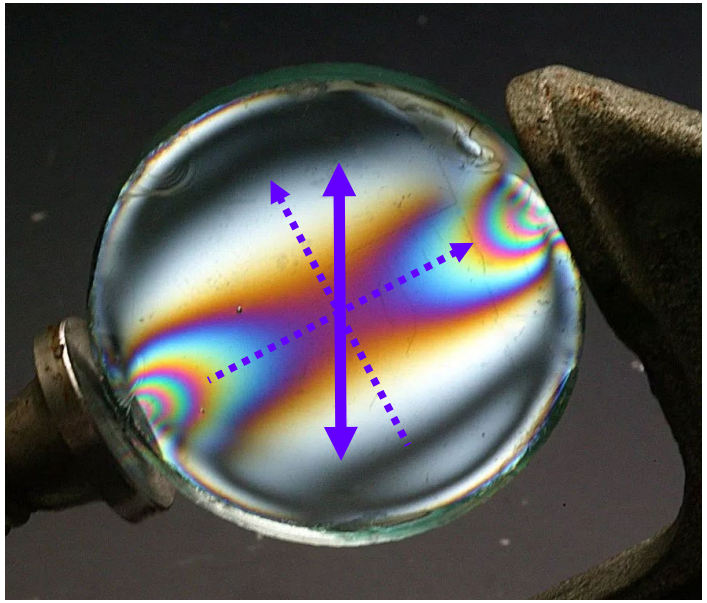
Schematic drawing of a simple CD spectrometer from DOI 10.1002/9781118120392

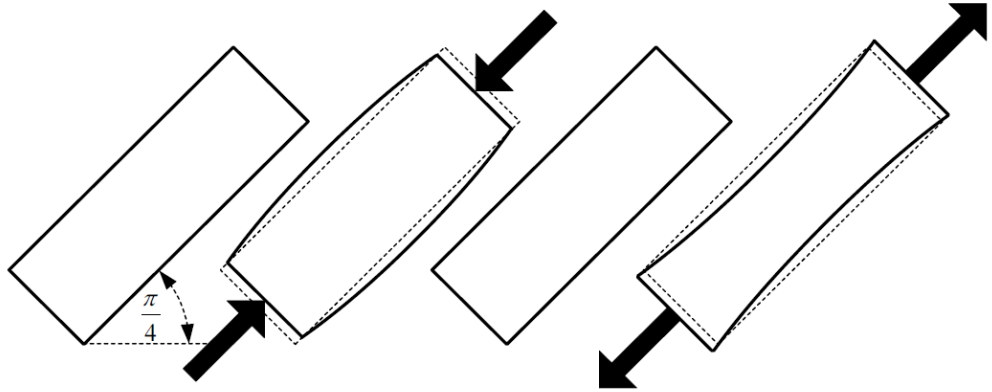
Photoelastic modulator (PEM)



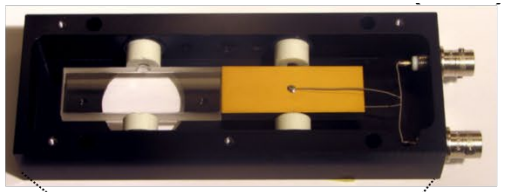
A piezo-electric tranducer modulates the birefringence at the quartz block's resonance frequency



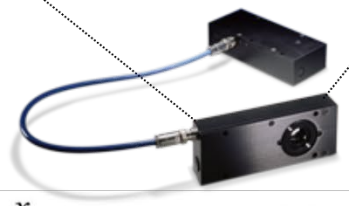




Photoelastic modulator (PEM)

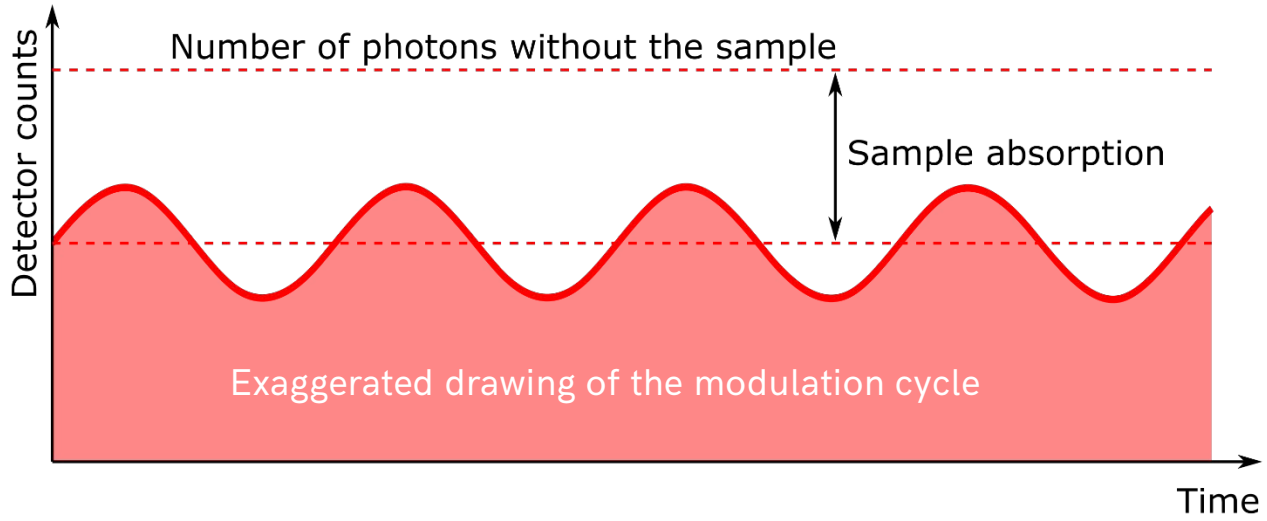


A piezo-electric tranducer modulates the birefringence at the quartz block's resonance frequency



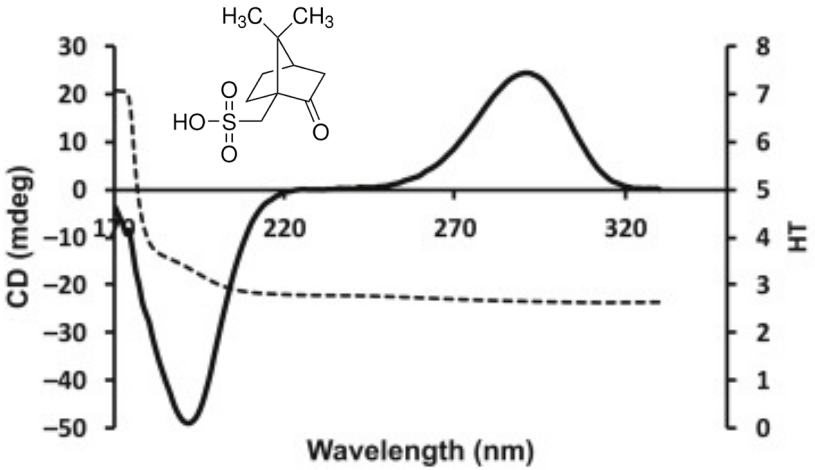
Polarizer

Pay attention to the total absorption of the sample! If it is too high, there are no photons to measure CD!



Best practice

- Use cuvettes made from low birefringence material with high UV transmission
- Use buffers and solvents with low absorption in the UV and/or short pathlength cuvettes
- Total absorption should be around 1 OD if possible, but not more
- Always take a solvent blank measurement and check detector voltage
- Regularly check the wavelength calibration of your instrument



Calibration standard:
d-10-camphorsulfonic acid

Example

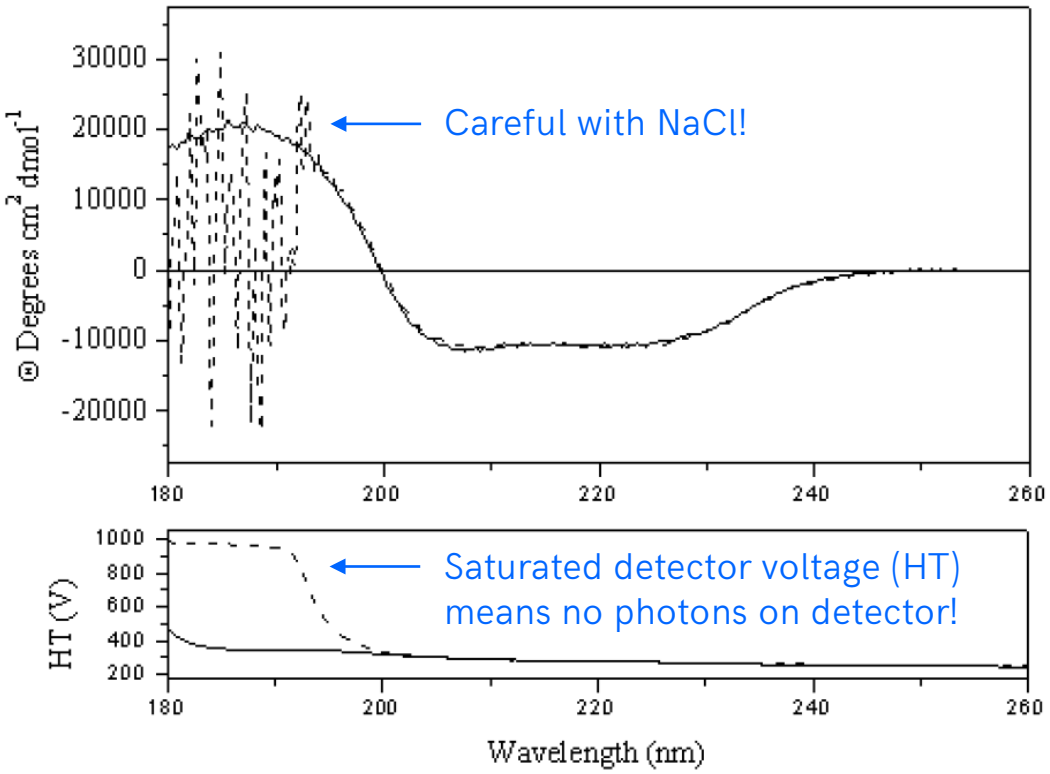


Figure 7. The far UV CD spectrum of α-lactalbumin. The protein was at a concentration of 0.45 mg/ml and spectra were recorded under the conditions described for Figure. 6. The solid and dashed lines refer to spectra recorded in 50 mM sodium phosphate buffer, pH 7.5 or 50 mM sodium phosphate buffer, pH 7.5 to which 150 mM NaCl has been added respectively.

Figure from Sharon Kelly's book chapter in ISBN 978-1-60750-000-1

Absorbance vs ellipticity

From ↓ To →	Absorbance[1]	Milliabsorbance[2]	Molar Extinction[3]	Degrees[4]	Millidegrees[5]	Molar Ellipticity[6]
(A)	A	A*1000	A*M/(C*L)	A*32.98	A*32980	A*M*3298/(L*C)
(mA)	mA/1000	mA	A*M/(C*L*1000)	mA*0.03298	mA*32.98	mA*M*3.298/(L*C)
(ε)	ε*C*L/M	ε*C*L*1000/M	ε	ε*C*L*32.98/M	ε*C*L*32980/M	ε*3298
(°)	°/32.98	°/0.03298	°*M/(C*L*32.98)	°	°*1000	°*M*100/(L*C)
(m°)	m°/32980	m°/32.98	m°*M/(C*L*32980)	m°/1000	m°	m°*M/(10*L*C)
[Θ]	[Θ]*C*L/(3298*M)	[Θ]*C*L/(3.298*M)	[Θ]/3298	[Θ]*C*L/(100*M)	[Θ]*C*L*10/M	[Θ]

[1]Units are Absorbance (Abs)
[2]Units are milliabsorbance (mAbs)
[3]Units are A*L/mol*cm
[4]Units are degrees (°)
[5]Units are millidegrees (m°)
[6]Units are deg*cm2/dmol

C is concentration in g/L
M is average molecular weight (g/mol)
L is path length of cell (cm)

Beer-Lambert law:

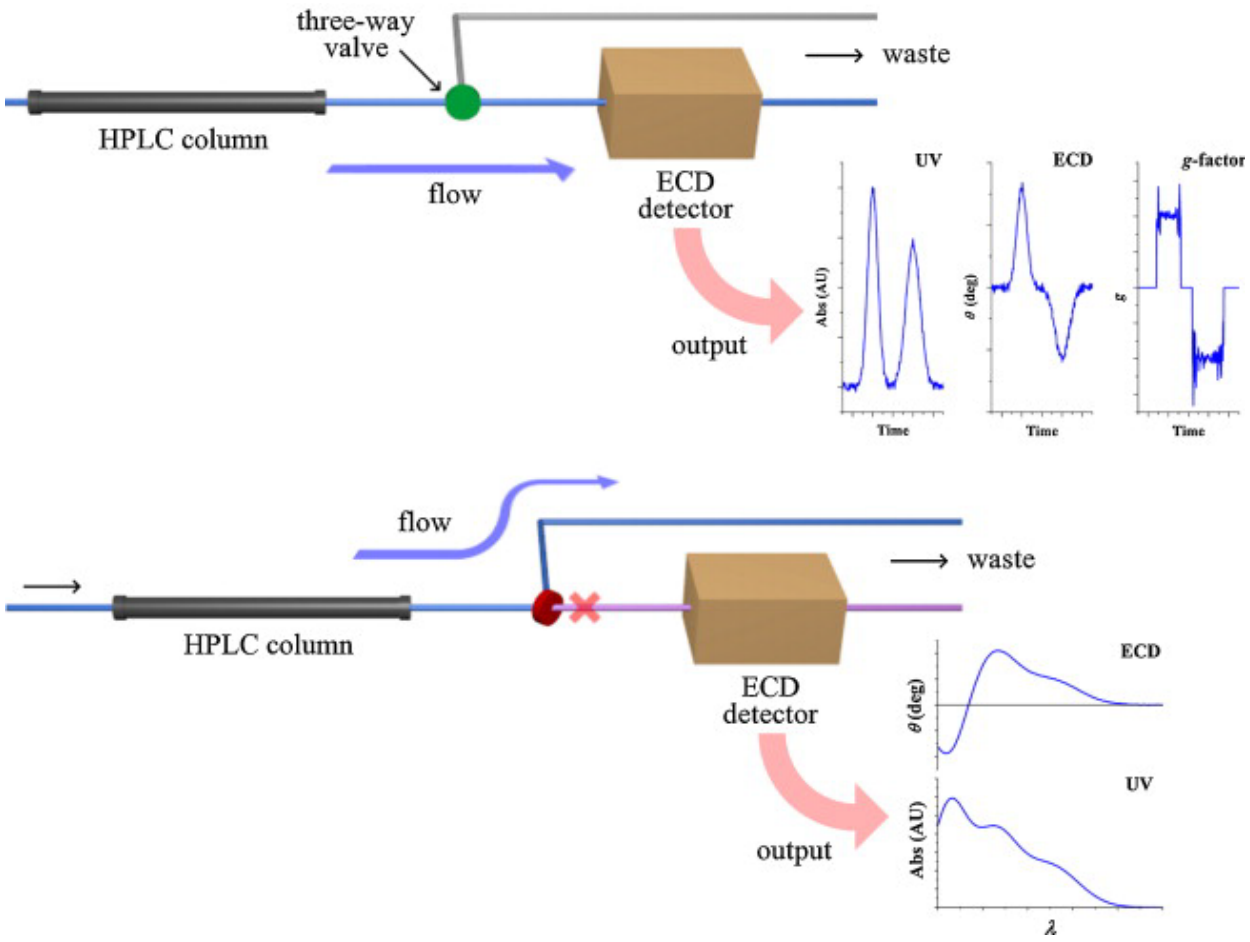
A = (molar exctinction) [L mol^{-1} cm^{-1}] * (path length) [cm] * (molar concentration) [mol L^{-1}]

Universität Basel

Electronic circular dichroism

Part III:
Applications

Setup



Chiral discrimination

"[…] in principle, the absolute configuration (AC) of an unknown sample can be determined from the value and sign of the [ECD] even at a single wavelength. All the information can then be obtained simultaneously from a single analysis, since the enantiomeric excess (ee) value is related to the areas of the stereoisomeric peaks, and the AC of the single stereoisomeric fractions is related to the sign of chiroptical properties at the wavelength of monitoring and in the environmental conditions used for the separation (i.e., the solvent mixture used as mobile phase)."

Asymmetry factor in absorption:

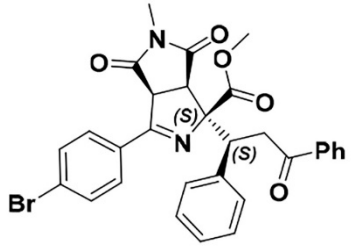
$$g_{abs} = \frac{\Delta\varepsilon}{\varepsilon}$$

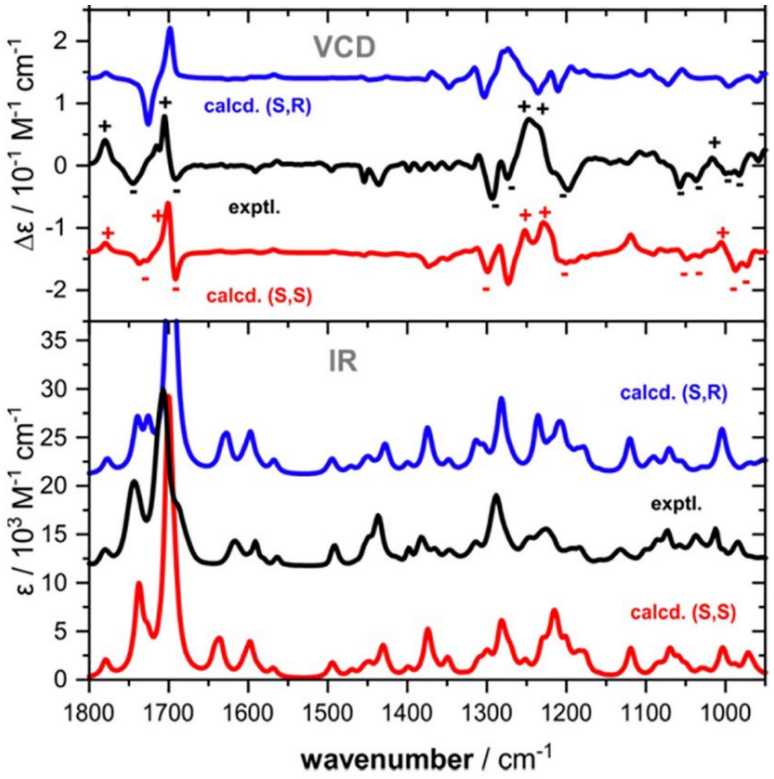
Enantiomeric excess:

$$ee = \frac{R - S}{R + S}$$

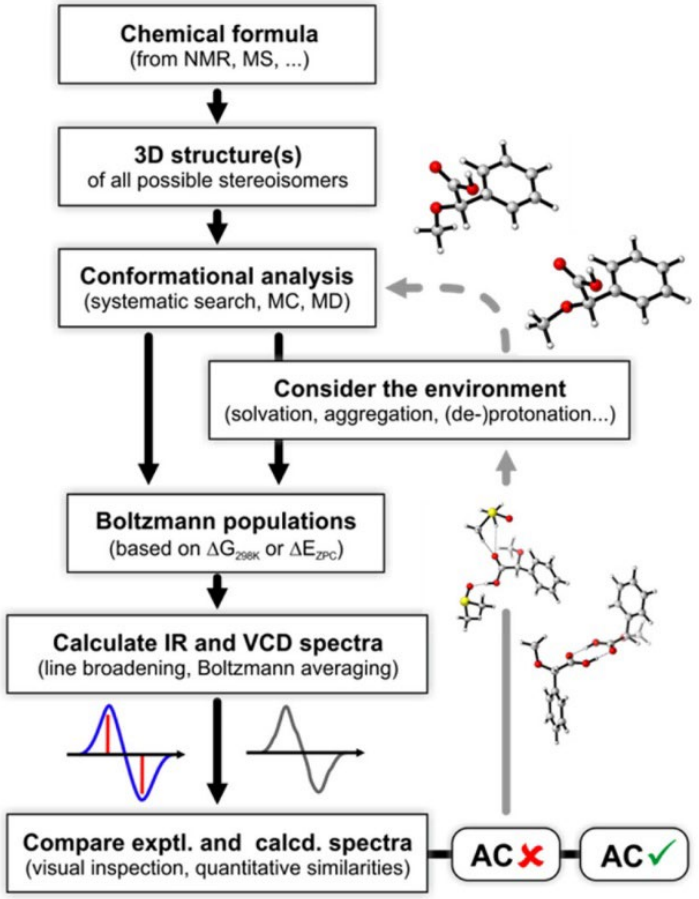
Application of HPLC-CD for chiral analysis: DOI 10.1016/j.chroma.2012.09.046

Example VCD spectrum





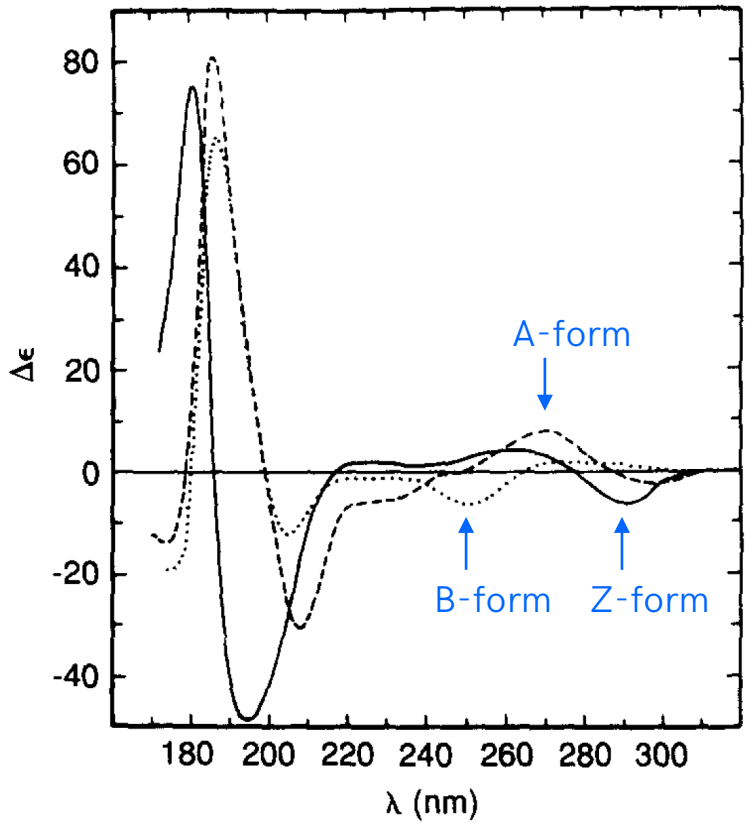
Absolute configuration determination



The coupling of vibrational transitions leads to vibrational CD spectra. Due to the spectral specificity of vibrational modes, VCD is a powerful tool for structure determination when coupled to numerical calculations.

Review on the application of VCD for structure analysis: DOI 10.1021/acs.joc.9b00466

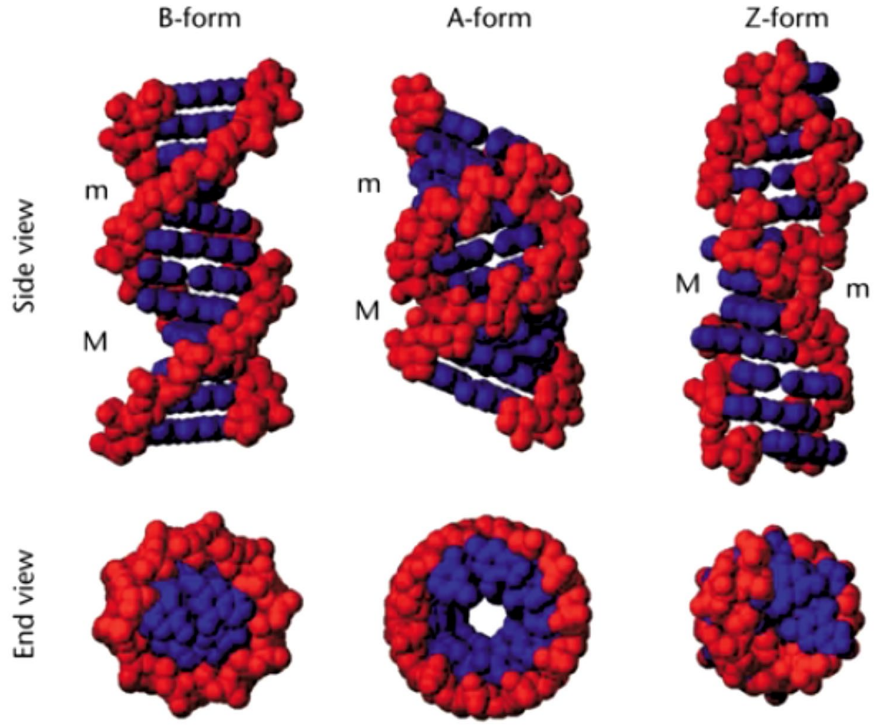
ECD of poly[d(G-C)]

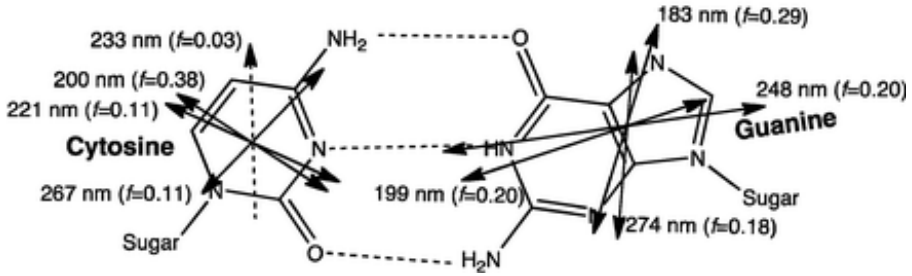


- Distinguish DNA motifs
- DNA stability vs temperature and pH
- Small-molecule binding

Excitonic coupling

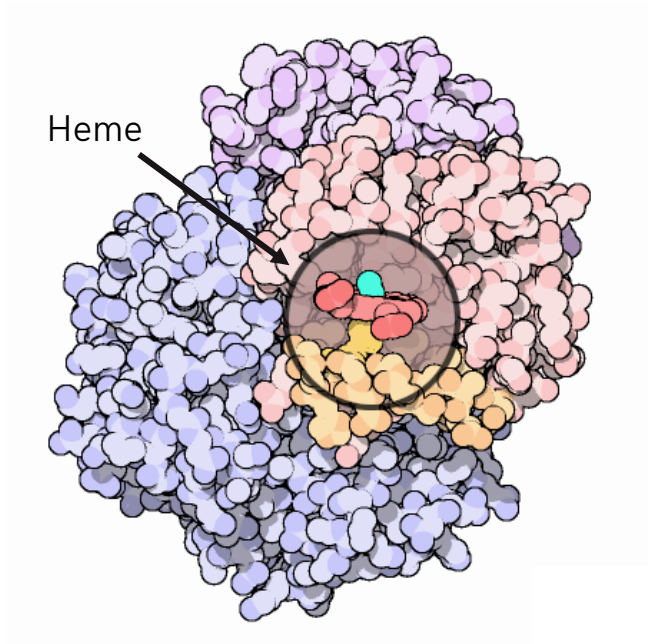
Structural information



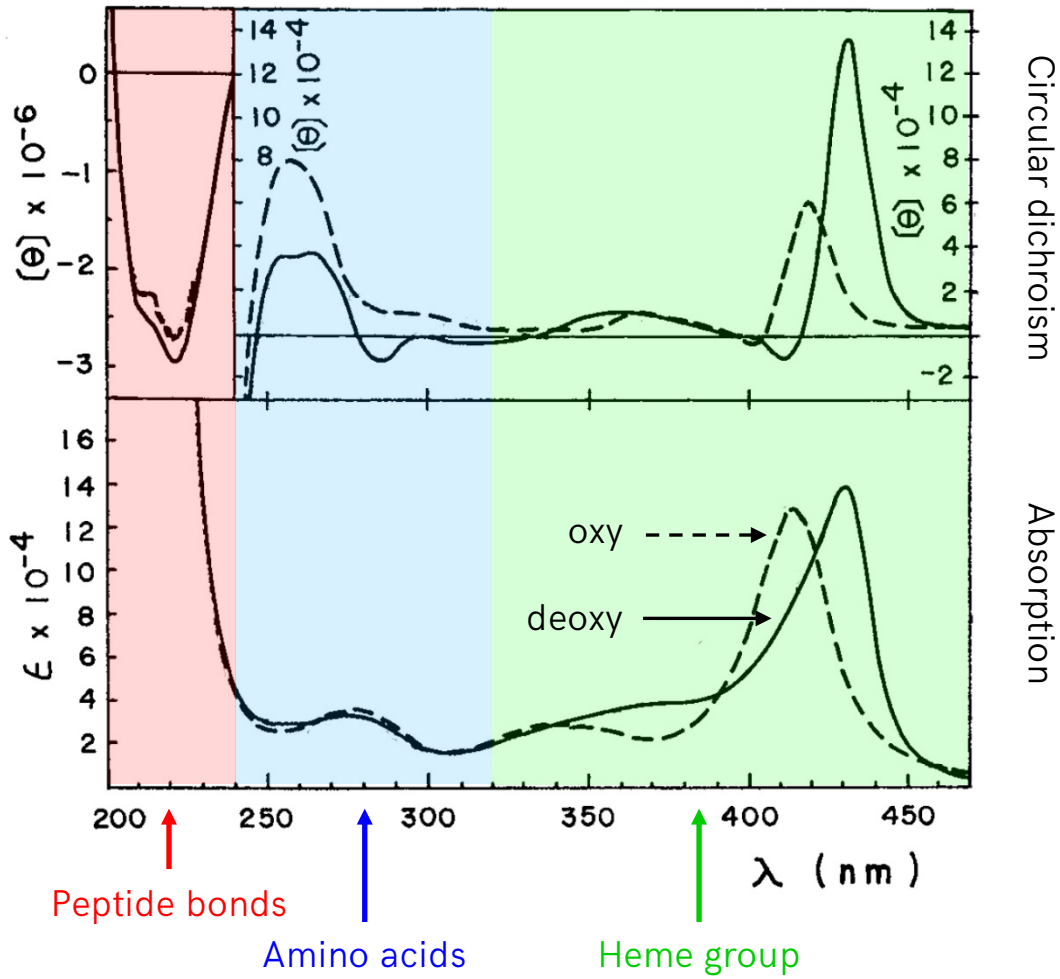


Figures: Ranjbar and Gill, Chem. Biol. Drug Des. 74, 101 (2009), J.H. Riazance et al. Nucleic Acids Res. 13, 4983 (1985)

Hemoglobin heterotetramer



Structural information

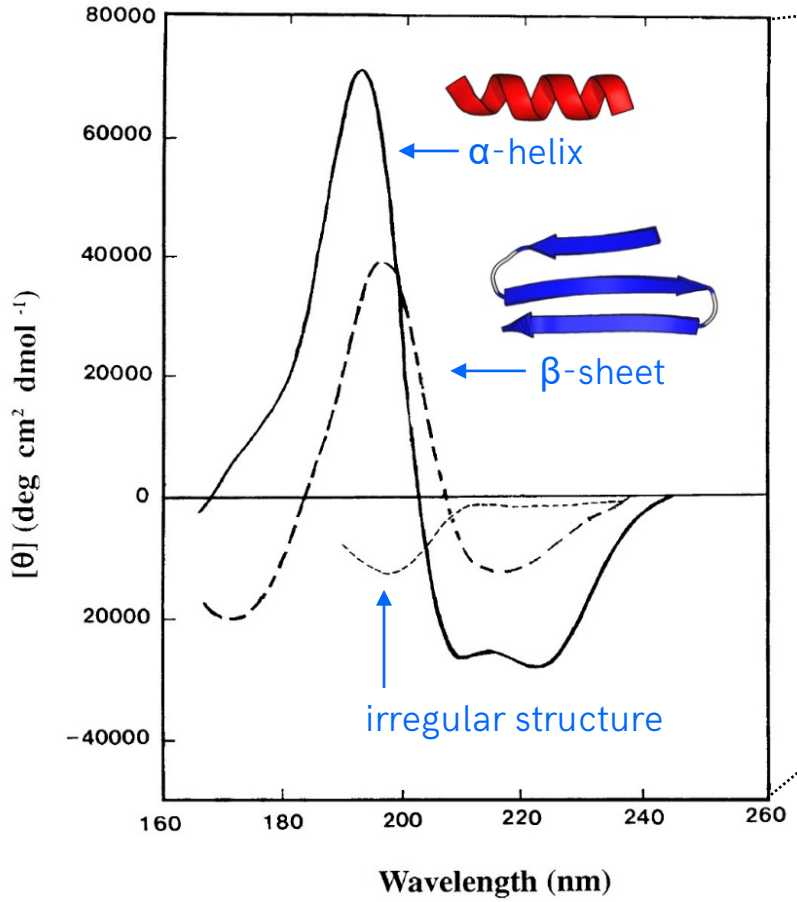


Hemoglobin: http://pdb101.rcsb.org/motm/41

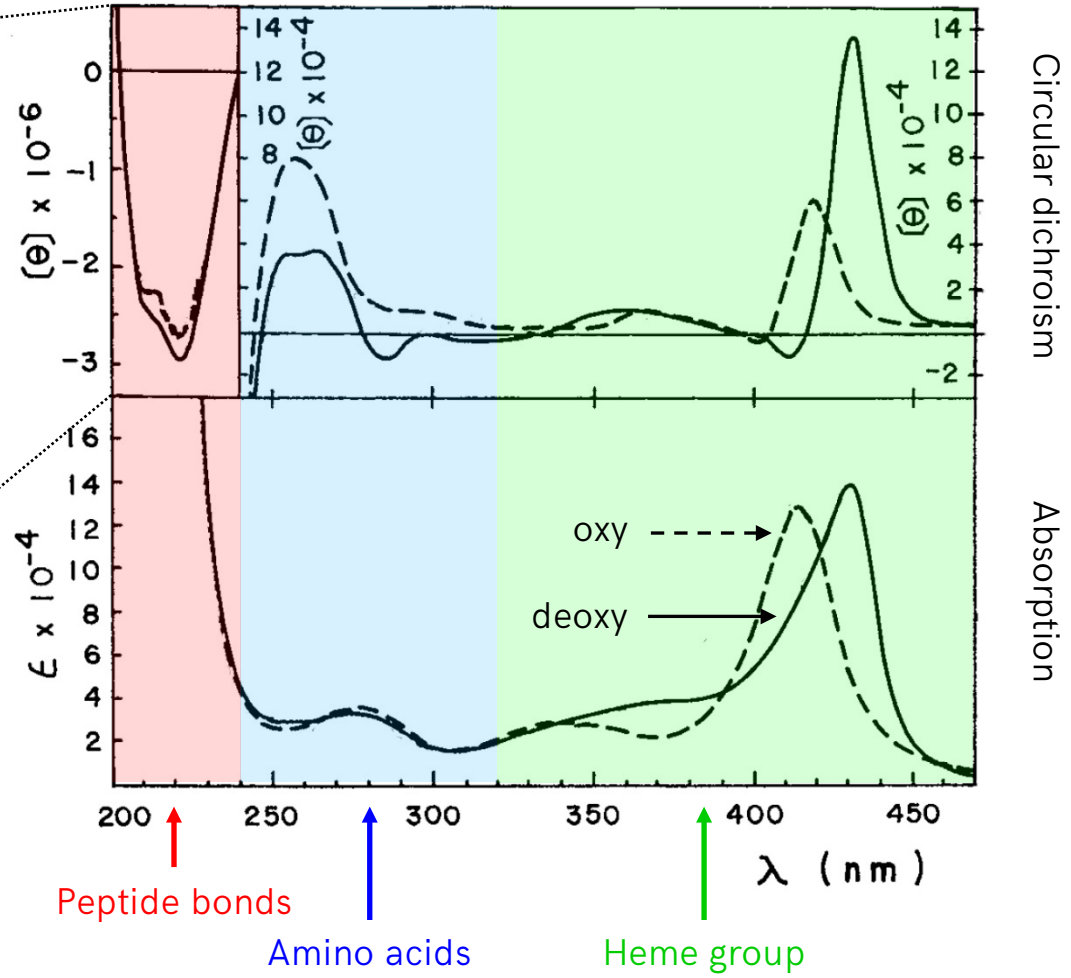
Hb spectra: Y. Sugita et al. *J. Biol. Chem.* 246, 383 (1971)

Protein secondary structures

Far to middle ultraviolet region



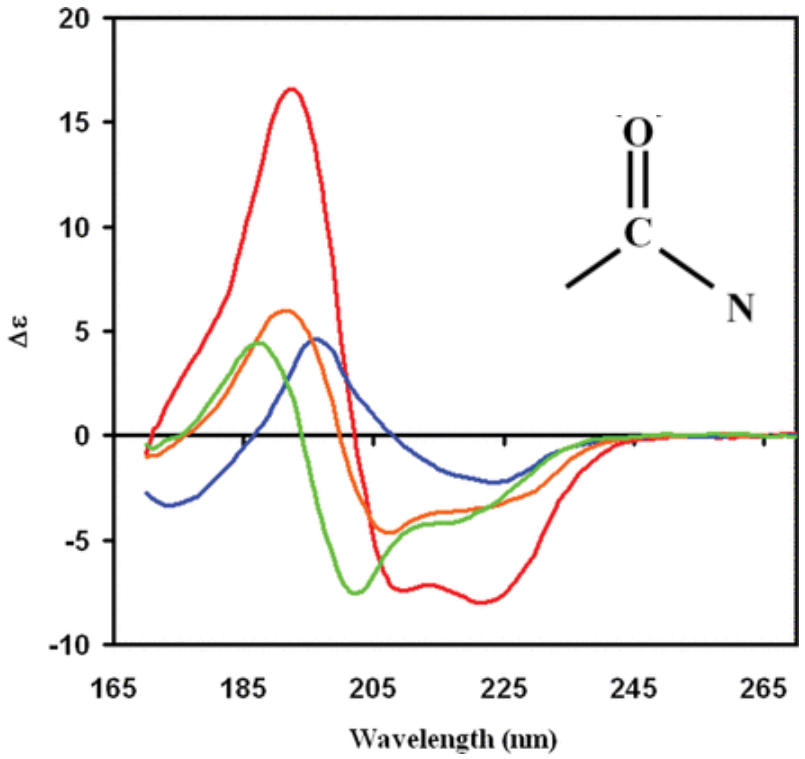
Structural information



Hemoglobin: http://pdb101.rcsb.org/motm/41

Hb spectra: Y. Sugita et al. *J. Biol. Chem.* 246, 383 (1971)

Global conformation determination



- Compare folds of wild type and mutated proteins
- Secondary structure change from binding partner
- Stability with temperature and pH
- Folding and reaction kinetics

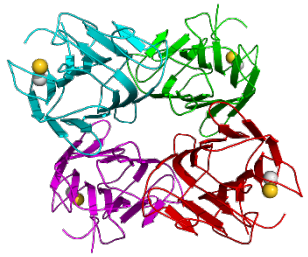
Linear combination of secondary structure motifs

Empirical secondary structure determination:

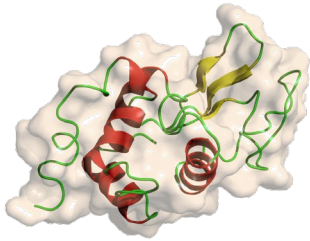
$$\mathrm{CD}(\lambda) = a\,\mathrm{CD}_{\alpha} + b\,\mathrm{CD}\beta\mathrm{s} + c\,\mathrm{CD}\beta\mathrm{t} + d\,\mathrm{CDo}$$

Mostly α-helix protein: myoglobin

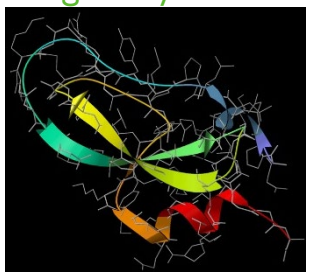
Mostly β-sheet protein: concanavalin A



Mixed helix/sheet protein: lysozyme



Irregularly structured protein: aprotin



Figures: http://nptel.ac.in/courses/102103044/module2/lec9/1.html, Proteins: Wikipedia

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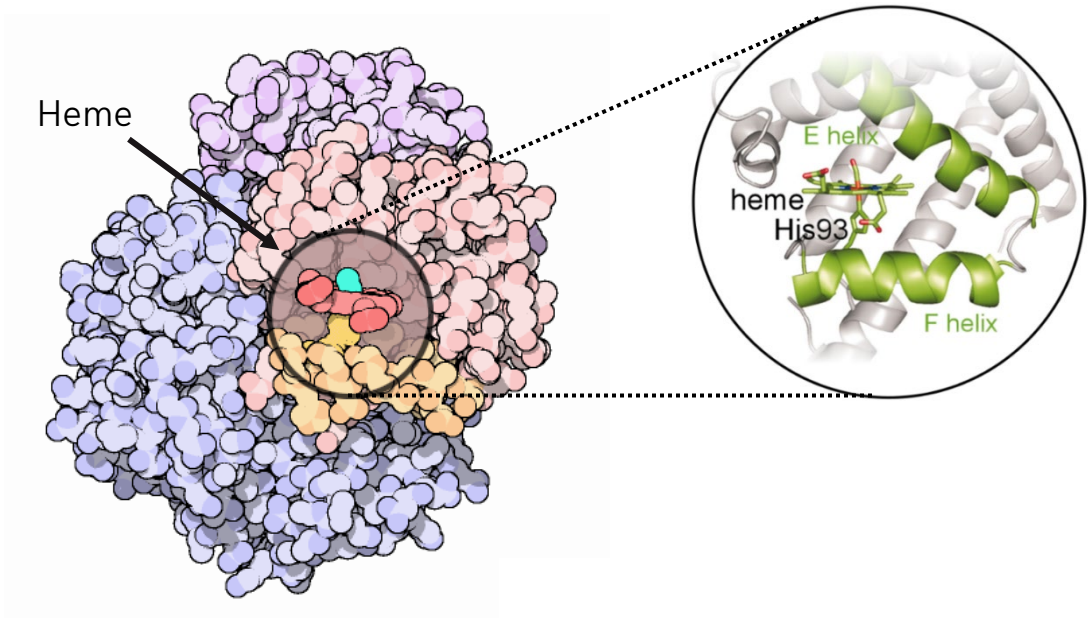
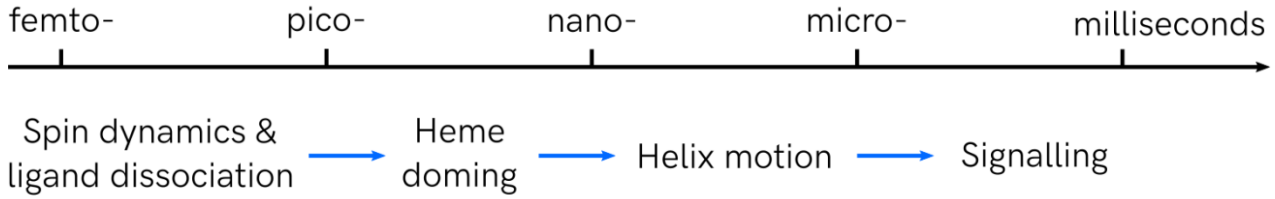
Electronic circular dichroism

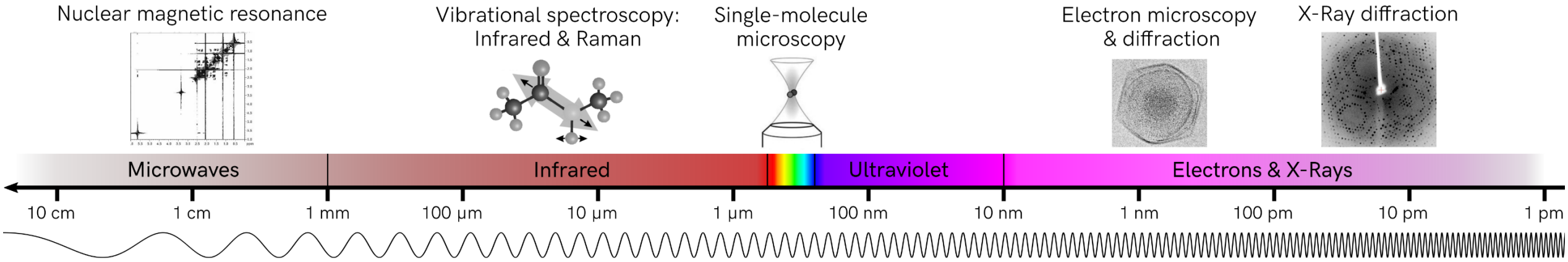
Part IV:
Ultrafast ECD





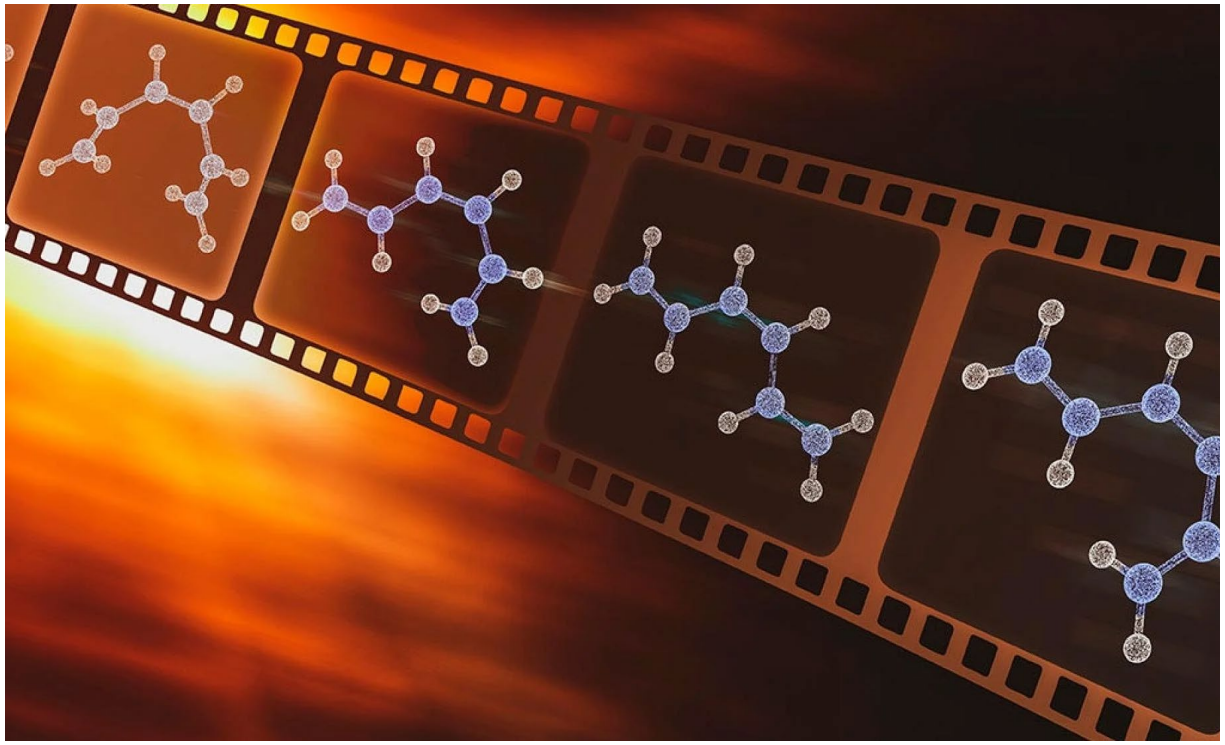
Wishlist for (bio-)chemical dynamics

- Track molecular motion in real-time
- Electronic & structural information
- Femto- to millisecond resolution
- Solution phase samples



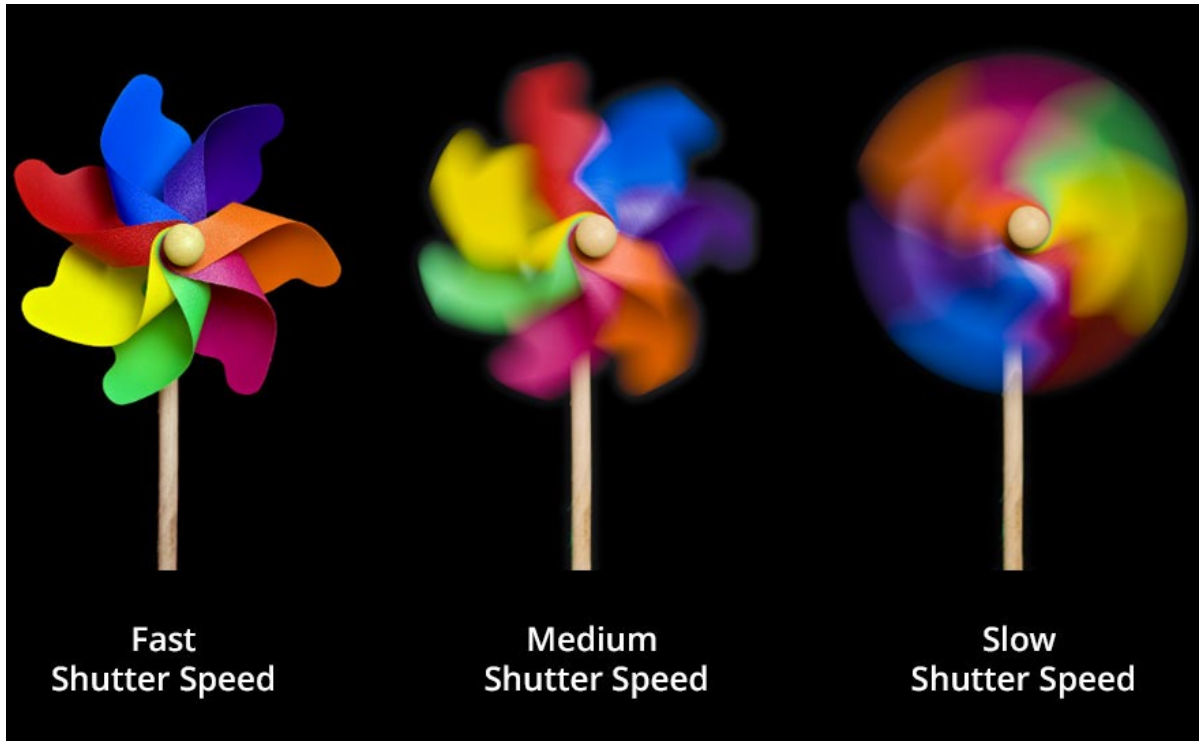
Hemoglobin: http://pdb101.rcsb.org/motm/41; bottom figures: en.wikipedia.org; binding pocket: A. Sato et al. *PNAS* 104, 9627 (2007)

Snapshots of molecular dynamics



Chemical bonds vibrate and break
on the pico- and femtosecond scales

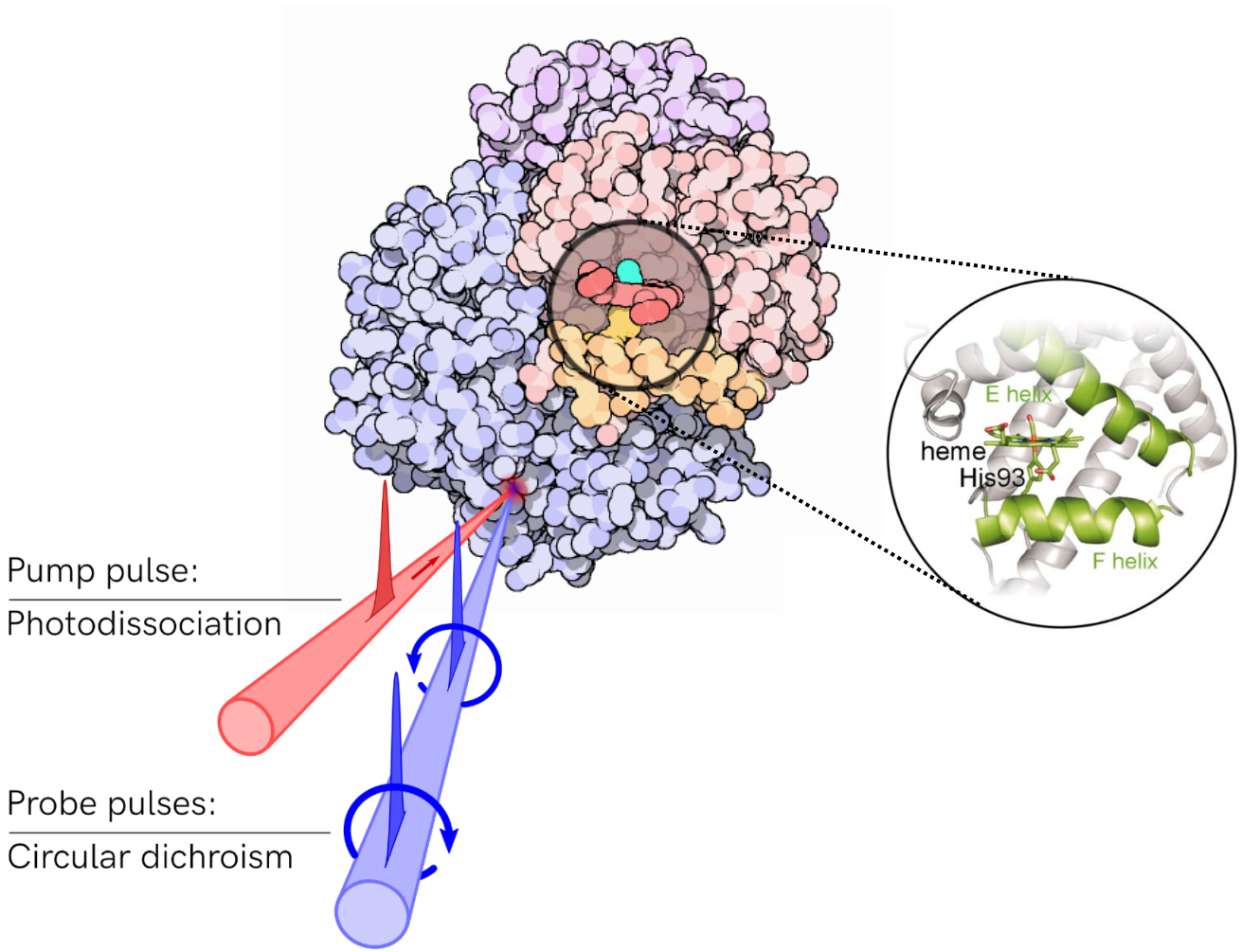
Time-resolved measurements



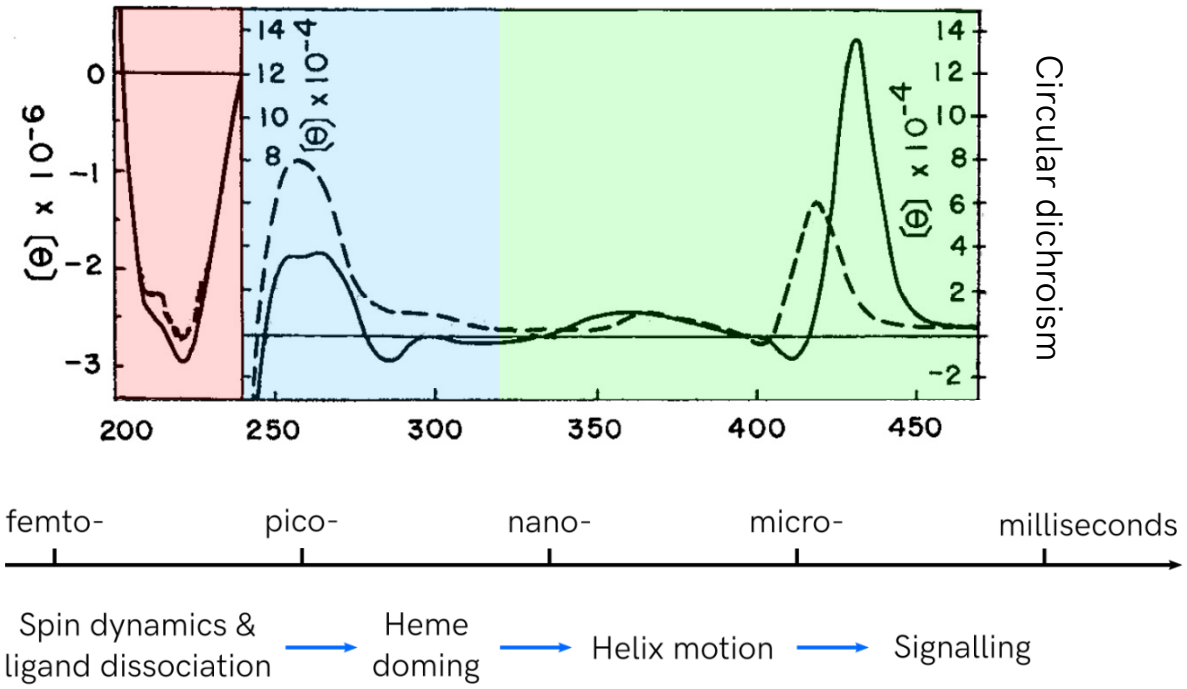
The speed of the measurement must be faster
than the recorded process

Molecular movies: https://www6.slac.stanford.edu/news/2019-10-07-what-molecular-movie

Pump-probe scheme

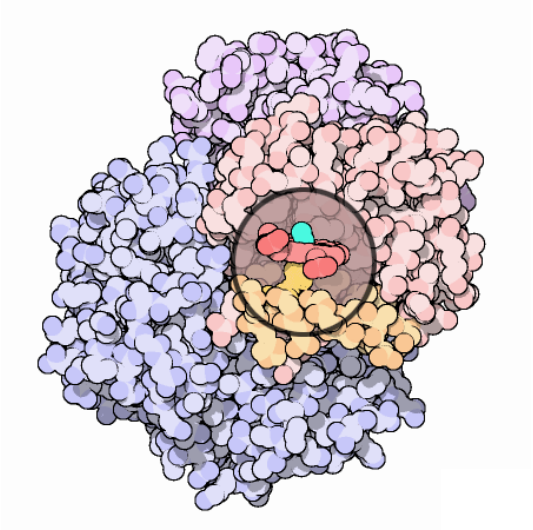


Ultrafast ECD measurements



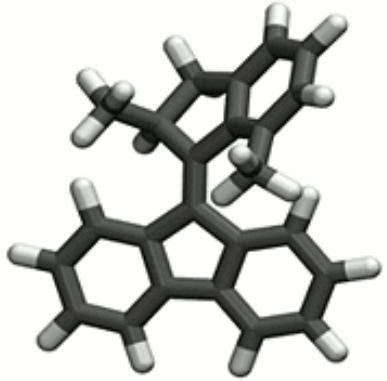
Reviews: P. Changenet & F. Hache, *Eur. Phys. J. Spec. Top.* (2022), M. Oppermann, *Chimia* 78, 45 (2023)

Structural marker



Structural dynamics
in solution

Chiral excited states



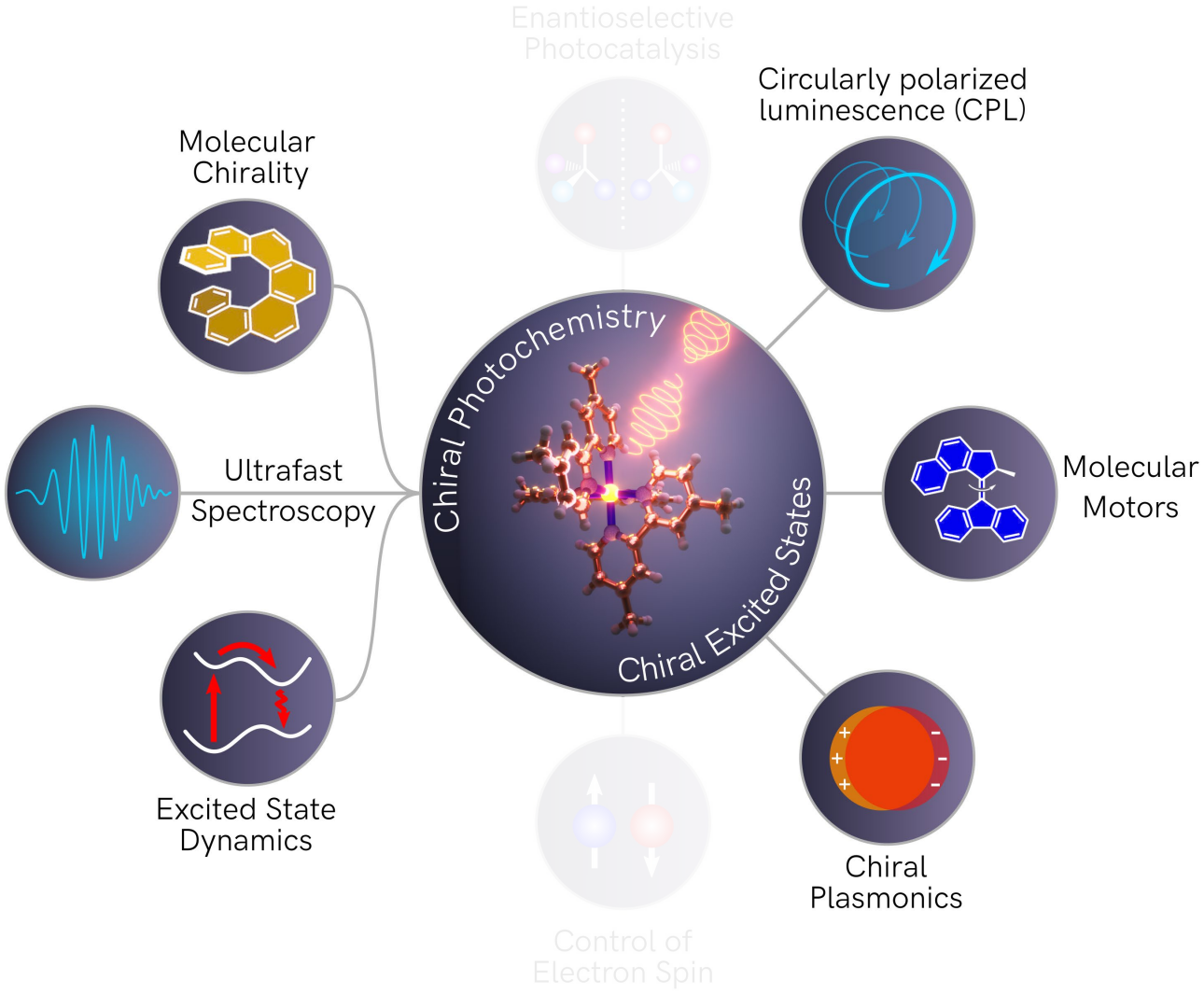
Stereo-controlled
photochemistry

Ultrafast electronic circular dichroism

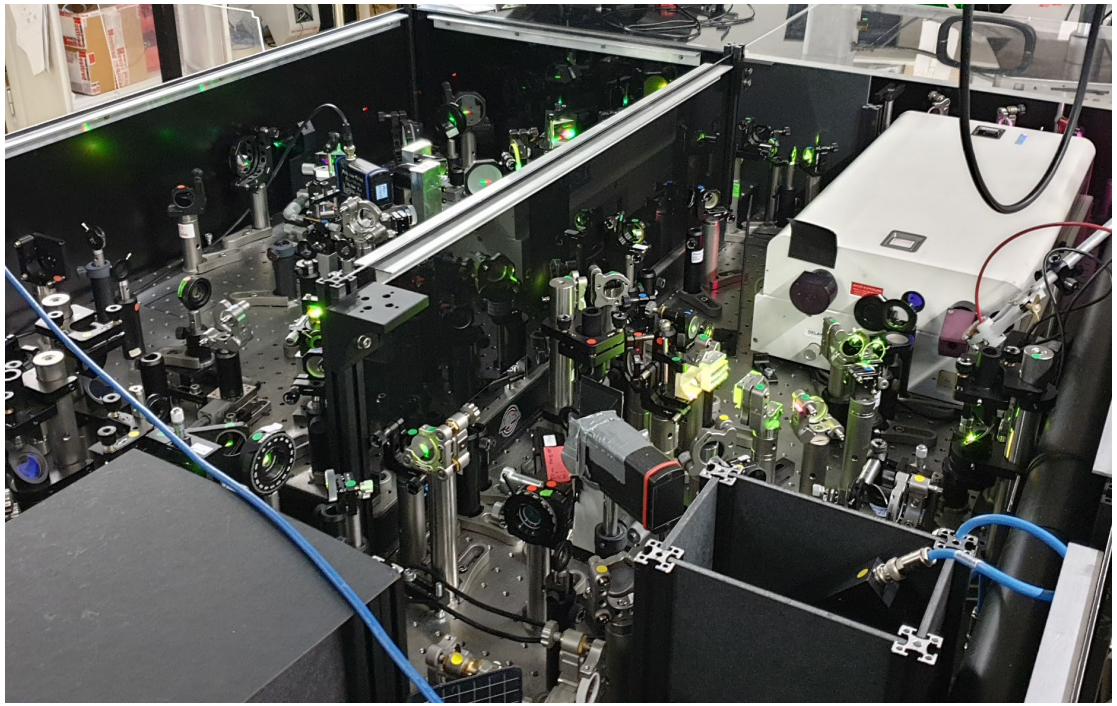
Hemoglobin animation: http://pdb101.rcsb.org/motm/41

Molecular motor: adapted from University of Groningen

Chiral photochemistry



Ultrafast (chiral) spectroscopy



New laser sources and spectroscopy instruments

Small molecule chirality for applications: J.S. Brandt et al., Nat. Rev. Chem. 1, 0045 (2017), CP LED review: F. Furlan et al., Nat. Photon. (2024)