Theory of CD over time.

magenta vector resulting from a right (clockwise) and left (anticlockwise) vectors

• Beam of polarized light (viewed end-on)

• Beam of unpolarized light (viewed end-on)

Let there be light

On the other hand

• Decompose the magenta vector in two green vectors of equal intensity.
• Change in intensity of the magenta electric field vector causes these two green vectors to rotate in opposite directions round the circumference.
• A beam of polarized light is described by the set of each of the two green vectors along the original plane polarized light direction.
• a plane polarized light can be seen as resulting from a right (clockwise) and left (anticlockwise) circularly polarized beams of light.

Theoretical Studies of Protein Circular Dichroism

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Outline

• Light + dichroism
• Electronic structure:
  – 1
  – 2
  – Many
• Applications
• Charge transfer (deep-UV)

To put it plainly

• plane polarized light - an oscillation of the electric field vector intensity in a plane that has the same direction of the beam (red light).
• the intensity of the electric field vector (magenta) varies from a positive maximum (t1) to a negative minimum (t3) over time.
Refractive index
- Passage of light through any medium is characterized by medium’s refractive index, \( \eta \)
- Optically active compounds have different refractive indices for left and right circularly polarized light
  - \( \eta_L \neq \eta_R \)
- Leads to a rotation of the plane of polarization

Dichroism – two colours (Gk.)
- Linear polarized light
  - superposition of opposite circular polarized light of equal amplitude and phase.
- different absorption of the left- and right-hand polarized component
  - ellipticity (CD)
  - optical rotation (OR)
- Actual effect is minute

Ellipticity
- The ratio of the minor to the major axis of the ellipse is the tangent of the angle \( \theta \)(blue). This angle is called ellipticity; angle OR is called optical rotation.
  - \( \theta = 32.98 \Delta \varepsilon \)
- To remove the dependence on cuvette path length and solute concentration, use molar ellipticity, defined as:
  - \( [\theta] = 100 \frac{\theta}{C l} \)
  - where \( C \) is the solute molarity and \( l \) the cuvette path length. The factor 100 as \( l \) in cm.
- Dimensions of molar ellipticity are:
  - 100 deg dm\(^3\) mol\(^{-1}\) cm\(^{-1}\) = 100 deg cm\(^2\) 1000 mol\(^{-1}\)
  - cm\(^2\) = deg cm\(^2\) 10 mol\(^{-1}\) = deg cm\(^2\) dmol\(^{-1}\)

Circular Dichroism (CD)
- Traditional electronic absorption spectroscopy
- Selection rule: electric dipole allowed and magnetic dipole forbidden
  or magnetic dipole allowed and electric dipole forbidden
- Intensity (oscillator strength, \( f \)) \( \propto \mu^2 \)
- Circular dichroism – differential absorption of left and right circularly polarised light
- Selection rule: transitions are electric and magnetic dipole allowed
- Intensity (rotational strength, \( R \)) \( \propto \mu m \)
- Electric dipole allowed = translation of charge
- Magnetic dipole allowed = rotation of charge
- Translation + rotation = helix
Amide electronic structure

\[ \sigma^* \quad \pi^* \]

\[ n \quad \pi_{ab} \]

\[ n' \quad \pi_n \]

\[ \pi^* \quad \sigma^* \]

\[ n \quad \pi_{ab} \]

\[ n' \quad \pi_n \]

\[ \pi\pi^* \text{ transition @ 220 nm} \]

\[ \sigma^* \quad \pi^* \]

\[ n \quad \pi_{ab} \]

\[ n' \quad \pi_n \]

\[ \pi\pi^* \text{ transition @ 190 nm} \]

\[ \sigma^* \quad \pi^* \]

\[ n \quad \pi_{ab} \]

\[ n' \quad \pi_n \]

\[ \pi\pi^* \text{ transition} \]

- Electric dipole allowed
- Electric transition moment
- Translation of charge

\[ \mu \]

Molecular orbital diagram

\[ \text{monoamides} \]

\[ \text{Gas phase} \quad \text{Current} \quad \text{Condensed phase} \]

\[ \sigma^* \quad \pi^* \quad \sigma^* \quad \pi^* \]

\[ n \quad \pi_{ab} \quad n \quad \pi_{ab} \]

\[ n' \quad \pi_n \quad n' \quad \pi_n \]

\[ \pi_n \quad \pi_n \]

\[ \text{Rydberg} \quad \pi^* \quad \pi^* \]

\[ \text{Pre-1996} \quad \text{Current} \quad \text{Condensed phase} \]

\[ E_{\text{ex}} + V_{12} \quad \pi^* \quad \pi^* \]

\[ \text{Molecular orbital diagrams} \]

\[ \text{Monomer 1} \quad \text{Dimer} \quad \text{Monomer 2} \]

\[ \text{Monomer} \]
Diamide Hamiltonian matrix

\[
H = \begin{pmatrix}
E_{1}\pi^{*}\pi^{*}& V_{11}^{\pi^{*}\pi^{*}}& V_{12}^{\pi^{*}\pi^{*}}& V_{12}^{\pi^{*}\pi^{*}} \\
V_{11}^{\pi^{*}\pi^{*}}& E_{2}^{\pi^{*}\pi^{*}}& V_{12}^{\pi^{*}\pi^{*}}& V_{22}^{\pi^{*}\pi^{*}} \\
V_{21}^{\pi^{*}\pi^{*}}& V_{21}^{\pi^{*}\pi^{*}}& E_{2}^{\pi^{*}\pi^{*}}& V_{22}^{\pi^{*}\pi^{*}} \\
V_{21}^{\pi^{*}\pi^{*}}& V_{21}^{\pi^{*}\pi^{*}}& V_{22}^{\pi^{*}\pi^{*}}& E_{2}^{\pi^{*}\pi^{*}}
\end{pmatrix}
\]

Moffitt’s dipole-dipole interaction

\[
V = \int \frac{\rho_{1\pi^{*}} \rho_{2\pi^{*}}}{r_{12}} \approx \sum_{s} \sum_{t} q_{s} q_{t}
\]

\[
V = \frac{\mu_{1} \cdot \mu_{2}}{r^{3}} - \frac{3 (\mu_{1} \cdot r) (\mu_{2} \cdot r)}{r^{5}}
\]

Dimer of interacting monomers

- Hamiltonian: kinetic + potential energies
- \( H = H_{1} + H_{2} + V_{12} \)
- \( V_{12} \) - potential energy of interaction

\[
V = \frac{\mu_{1} \cdot \mu_{2}}{r^{3}} - \frac{3 (\mu_{1} \cdot r) (\mu_{2} \cdot r)}{r^{5}}
\]

\[
\mu \cdot r = |\mu| |r| \cos \theta
\]

\[
|\mu| = \sqrt{r_{\mu}^{2} + r_{\pi}^{2}}
\]

\[
\theta = 0 \quad (parallel) \Rightarrow \cos \theta = 1 \Rightarrow \mu \cdot r = |\mu| |r|
\]

\[
\theta = 90 \quad (perpendicular) \Rightarrow \cos \theta = 0 \Rightarrow \mu \cdot r = 0
\]

Dimer energies & intensities

- \( E_{\pi^{*}} = E_{0} + V_{12} \)
- \( E_{\pi} = E_{0} - V_{12} \)
- \( D_{\pi^{*}} = D_{0} + D_{0} \cos \theta \)
- \( D_{\pi} = D_{0} - D_{0} \cos \theta \)
- \( D_{0} = \mu^{2} \)

Angle between dipoles

\[
\pi^{*} + \pi^{\pi} = E_{\pi^{*}} + V_{12} \quad \pi^{\pi} - V_{12}
\]
Parallel dimers

\[ V = \mu_1 \cdot \mu_2 / r^3 - 3(\mu_1 \cdot r)(\mu_2 \cdot r) / r^5 \]

Stacked dimers

\[ V = \mu_1 \cdot \mu_2 / r^3 - 3(\mu_1 \cdot r)(\mu_2 \cdot r) / r^5 \]

\[ D_z = D_0 + D_0 \cos(0) = 2D_0 \]

\[ D_0 = D_0 - D_0 \cos(0) = 0 \]

Anti-parallel dimers

\[ V = -\mu_1 \cdot \mu_2 / r^3 + 3(\mu_1 \cdot r)(\mu_2 \cdot r) / r^5 \]

\[ V = -2\mu_1 \cdot \mu_2 / r^3 - 2\mu_1 \cdot r = -2D_0 / r^3 \]

\[ D_\perp = D_0 + D_0 \cos(0) = 2D_0 \]

\[ D_\perp = D_0 - D_0 \cos(0) = 0 \]

Orthogonal, stacked dimers

\[ \theta = 90^\circ \]

Orthogonal, in-plane dimers

Angle between dipoles is 90°; angle between dipole and \( r \) is 45°.
Combinations of monomer $\pi\pi^*$ transitions

- In phase combination
  - parallel polarisation
- Out-of-phase combination
  - perpendicular polarisation

Textbook calculation

Idealised $\alpha$-helix (1968)
Reproduced in Cantor & Schimmel

Don’t trust textbooks

Ribbon clesease A, Kurapkat et al. (1997)

The Matrix Method

The Matrix Method forms the framework within which the isolated chromophores interact. Initially, some parameterization of the local transitions that occur within each chromophore is required.

The $a$'th transition on the $i$'th chromophore is then interacted with the $b$'th transition on the $j$'th chromophore in the following manner:

$$V_{i,j} = \int f_i(r_i) f_j(r_j) \, dr_1 \, dr_2$$

$$\approx \sum_i \sum_j \frac{R_{i,j}}{R^{\alpha\alpha}}$$

Diagonalization of the $V$ matrix allows the chromophores to couple, and the resulting eigenvectors and eigenvalues determine the total protein transitions and energies.

Interaction of amide chromophores leads to CD spectrum

Ad $\text{initio}$ ground state charge distribution
(CASSCF calculation with continuum solvent model)
**CD – probe of protein 2° structure**

![Circular dichroism spectra of peptides in the α-helical, β-sheet, and P2 conformations.](image)

**Protein ECD**

- **47 proteins**
- Near-quantitative correlation between calculated & exp. intensity at 222 nm
  - Key wavelength, reflecting helix content
  - Backbone only

**Protein ECD**

- **126 excised helices – only possible in silico**
  - “Cooperativity” – residues in longer helices contribute more per residue to \([\theta]_{222}\)
  - End effect – \(k \approx 3\);
  - Infinite helix \([\theta]_{222} \approx -40,000\)

- Unfolding a helix by fraying ends reduces \([\theta]_{222}\) less than breaking in the middle.

**Protein ECD**

- **Highly helical, e.g. myoglobin**
- **No helix, e.g. plastocyanin**

**Protein ECD**

- **Kemp – peptides with >100% helicity!**
- Previous
  - Ab initio side chains
  - JACS, 1999; JPCB, 2003
  - Angew Chemie, 2001; Biochemistry, 2004

- **Protein ECD**

- **Errors over 30 proteins**
  - Previous
  - Ab initio side chains
**Protein ECD**

Variation of computed $\Delta \beta_{22}$ for different regular helices ($\beta_{12}$, $\alpha$, and $\pi$)

[km $\beta_{22}$ for infinite helix $\sim$ 40,000
Kemp peptides show $\sim$55,000 !?
Exptl conditions (solvent, low T) favour short h-bonds?
Test in silico — build models, compute CD

**Protein near-UV ECD**

CASCC/CASPT2 studies of aromatic side chains $\rightarrow$ parameters for matrix method, which improve accuracy in near-UV

**Protein deep-UV ECD**

CASCC/CASPT2 calculations on dipeptides with 10 important geometries from across the Ramachandran plot

**Charge Transfer**

Origin of band @165 nm?
- $\pi\sigma^*$?
- $n\sigma^*$?
- $n\pi^*$?

Protein CD - Summary

- Semi-quantitative – more to do, but can …
- Probe structure-spectra relationships computationally
  - Helices
  - Conformational dynamics
- Sidechains – near UV
- Charge transfer – deep UV

http://comp.chem.nottingham.ac.uk/dichrocalc


Further reading

- Fasman *Circular dichroism and the conformational analysis of biomolecules*
- Charney *The molecular basis of optical activity*
- Cantor & Schimmel *Biophysical Chemistry Part II Techniques for the study of biological structure and function*