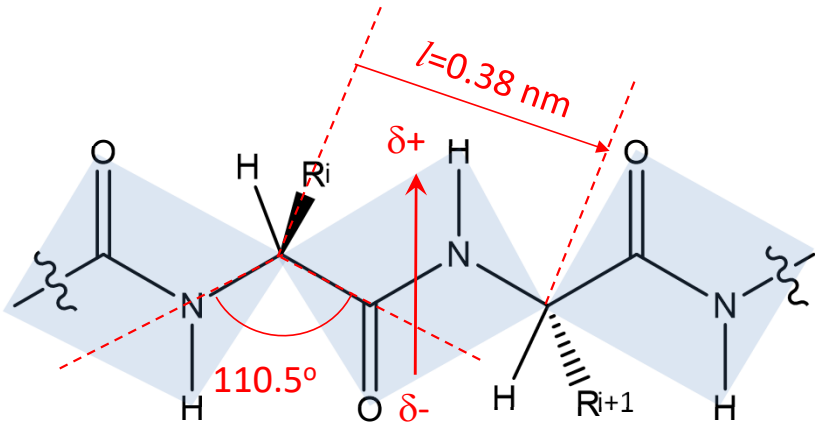
Molecular details: The peptide unit revisited



amino acid i i+1

Dipole moment of 3.5 Debeye

bond length and angles are described by **harmonic potentials**

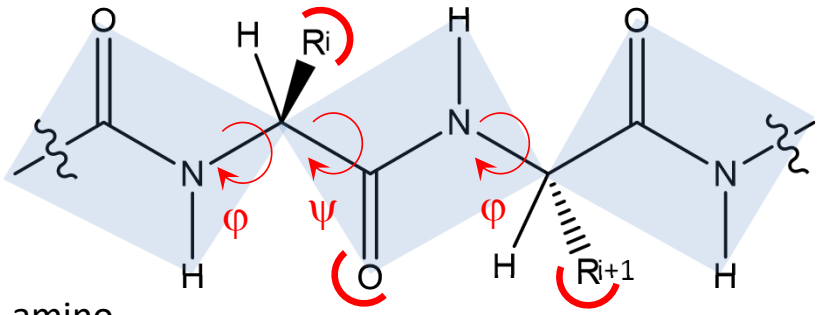
$$U(x) = \frac{1}{2}\phi\left(x - x_0\right)^2$$

force constant for bond flexing (C-C):
2761 kJ / Å^2 / mol

displacement of 0.05 Å more than kT → bond lengths are constant

For bond angles: 10x smaller

Molecular details: The peptide unit revisited



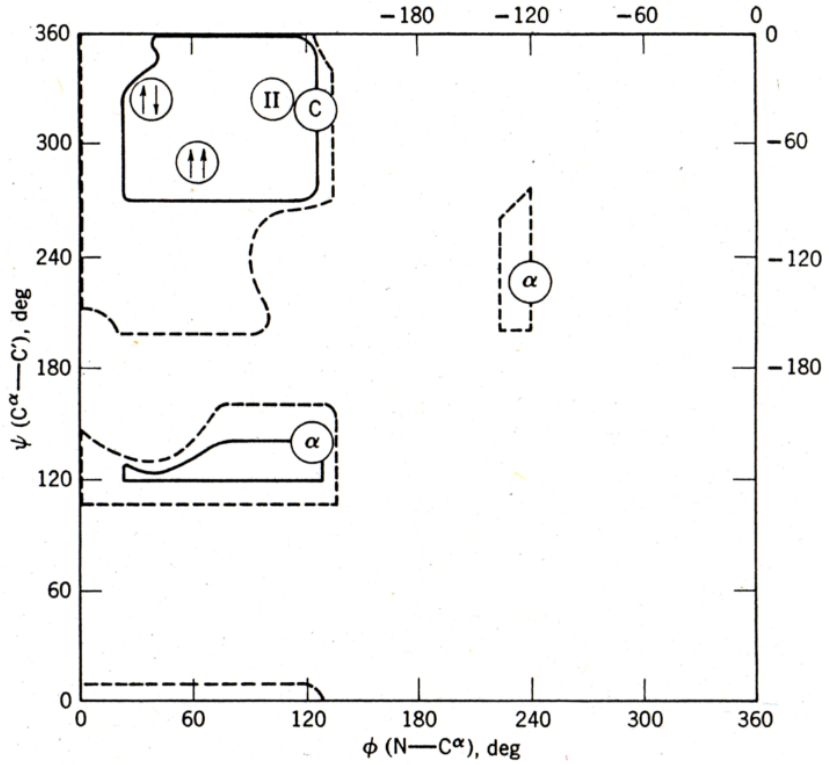
Torsion angles

Per amino acid, two rotatable bond in backbone

Complex potential

Rotation leads rapidly to steric interactions → restrictions in the allowed angles

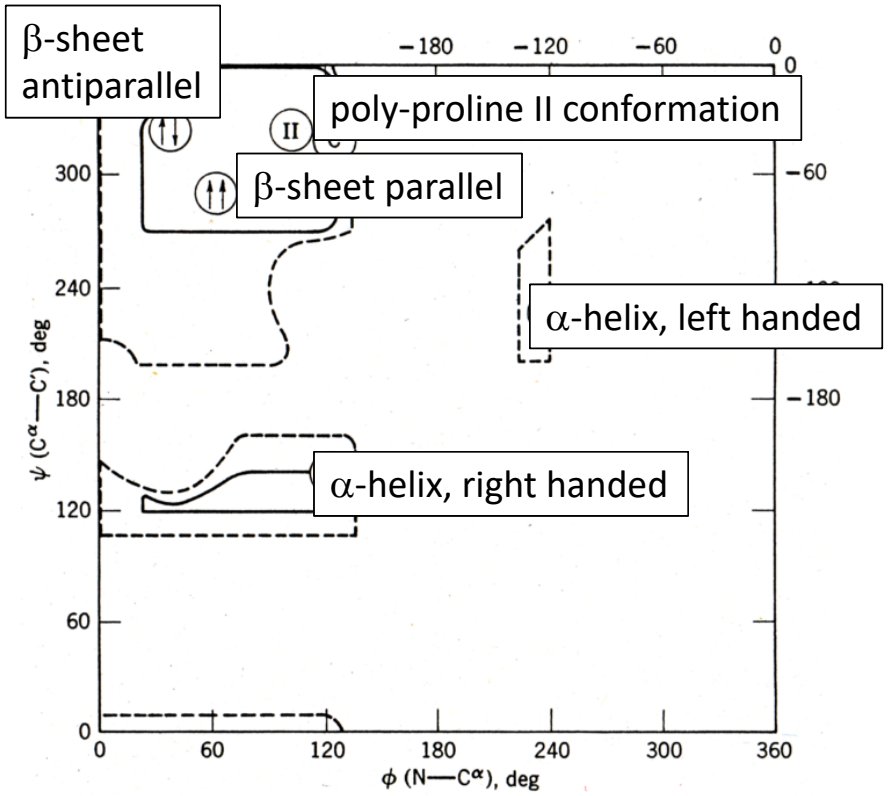
The Ramachandran steric map



G. N. Ramachandran
1922-2001

Mapped allowed angles based solely on **steric** grounds

The Ramachandran steric map



G. N. Ramachandran
1922-2001

Mapped allowed angles based solely on **steric** grounds

Conformational energy maps

What are the configurational distributions of **real peptide chains**?

Recalculation of the φ,ψ-maps using an energy function:

torsion angle potential

$$V(\varphi,\psi) = \frac{V_{\varphi}^{0}}{2}(1-\cos 3\varphi) + \frac{V_{\psi}^{0}}{2}(1-\cos 3\psi)$$

$$+ \sum_{k,l} E_{k,l}(\varphi,\psi) + E_C$$

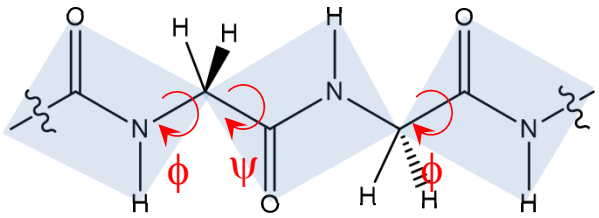
Coulomb interactions (charges and dipoles)

sterics and VdW interactions

$$E_{kl} = A_{kl} / r_{kl}^{12} - B_{kl} / r_{kl}^{6}$$

Paul John Flory
1910-1985
1974 Nobel Prize

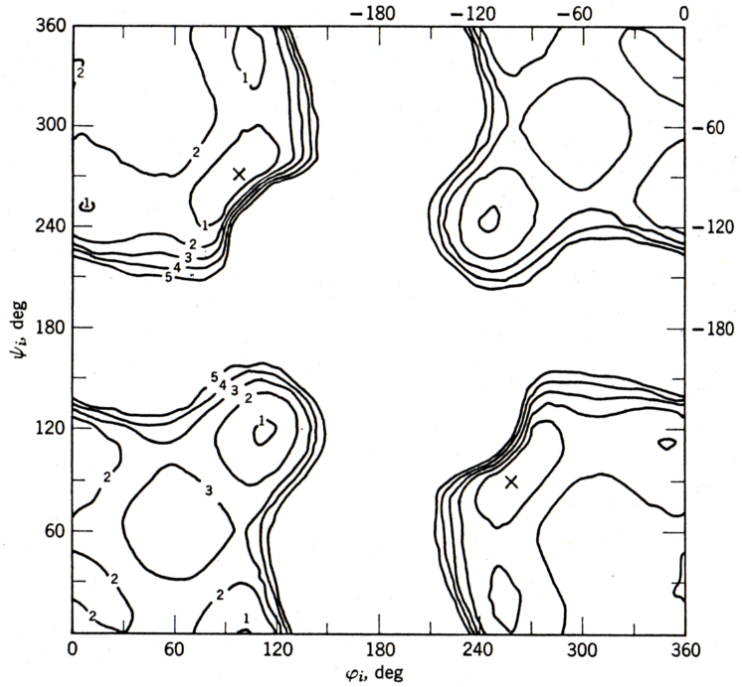
The glycine-glycine dipeptide



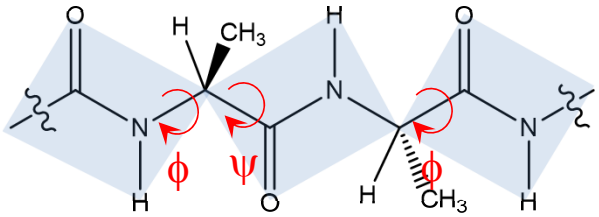
Glycine has **no sidechain**

large accessible conformational space, closest to ideal chain

high chain entropy



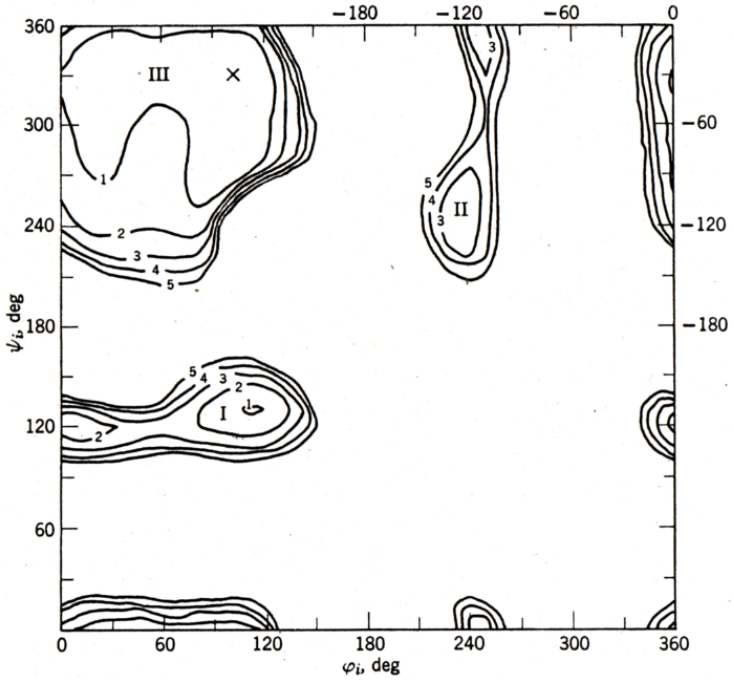
The alanine-alanine dipeptide



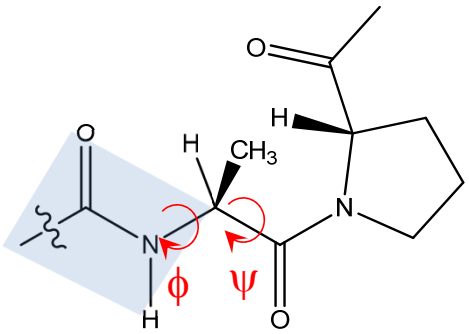
Alanine has a **small sidechain**

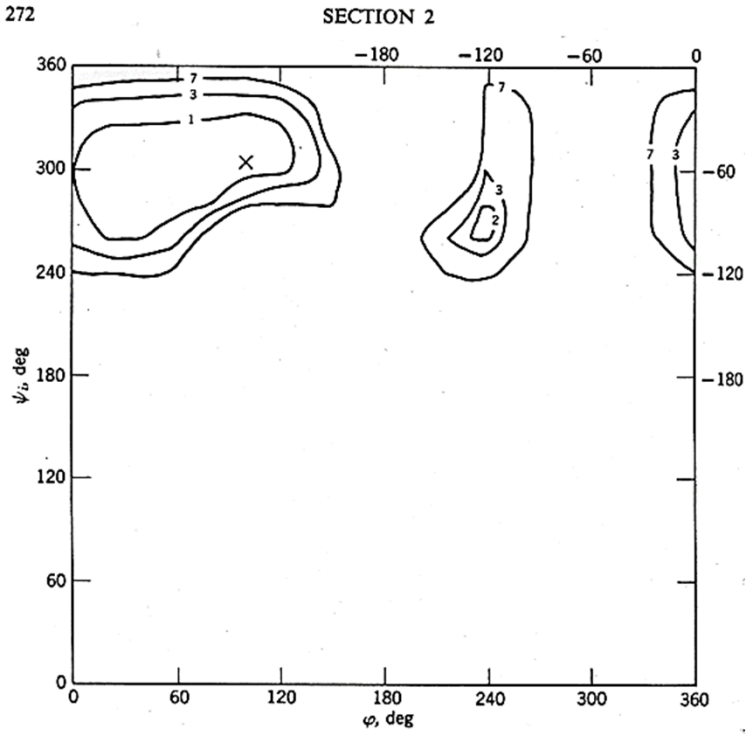
similar to Ramachandran map

β-branched amino acids are more restricted



The alanine-proline dipeptide





Proline has a **seriously restricted** conformational space

increases chain stiffness, but decreases chain entropy, as lower number of conformations are allowed

Chain stiffness & the characteristic ratio

To account for the **increased chain stiffness** due to torsion angle restriction

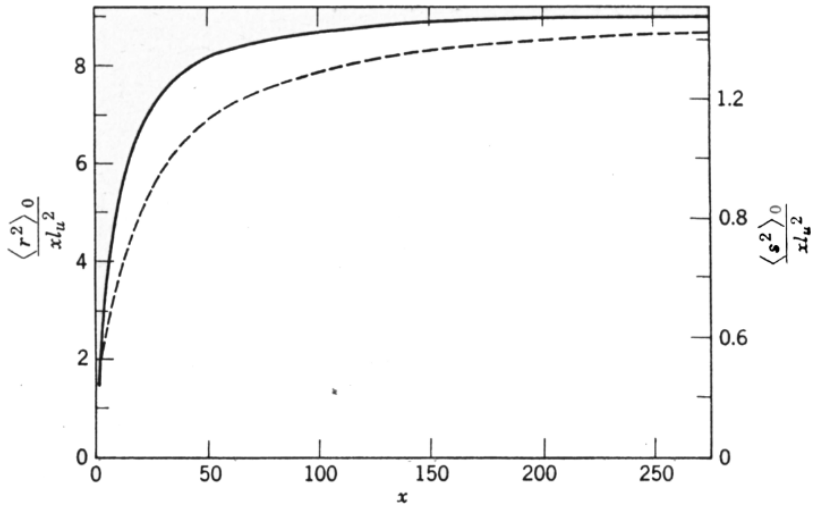
Flory introduced the **characteristic ratio**: $\left\langle R^2 \right\rangle = CNl^2$

$$C = \frac{\left\langle R^2 \right\rangle}{Nl^2}$$

C is dependent on the lenght of the polymer

for long polymers, C adopts a **limiting value**:

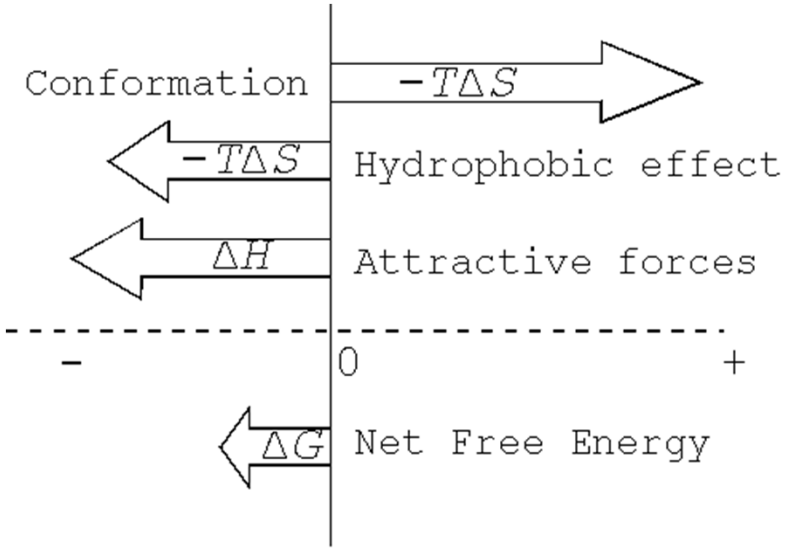
	C_∞
Gly	2.16
Ala	9.27
Pro	116



Summary - Energies

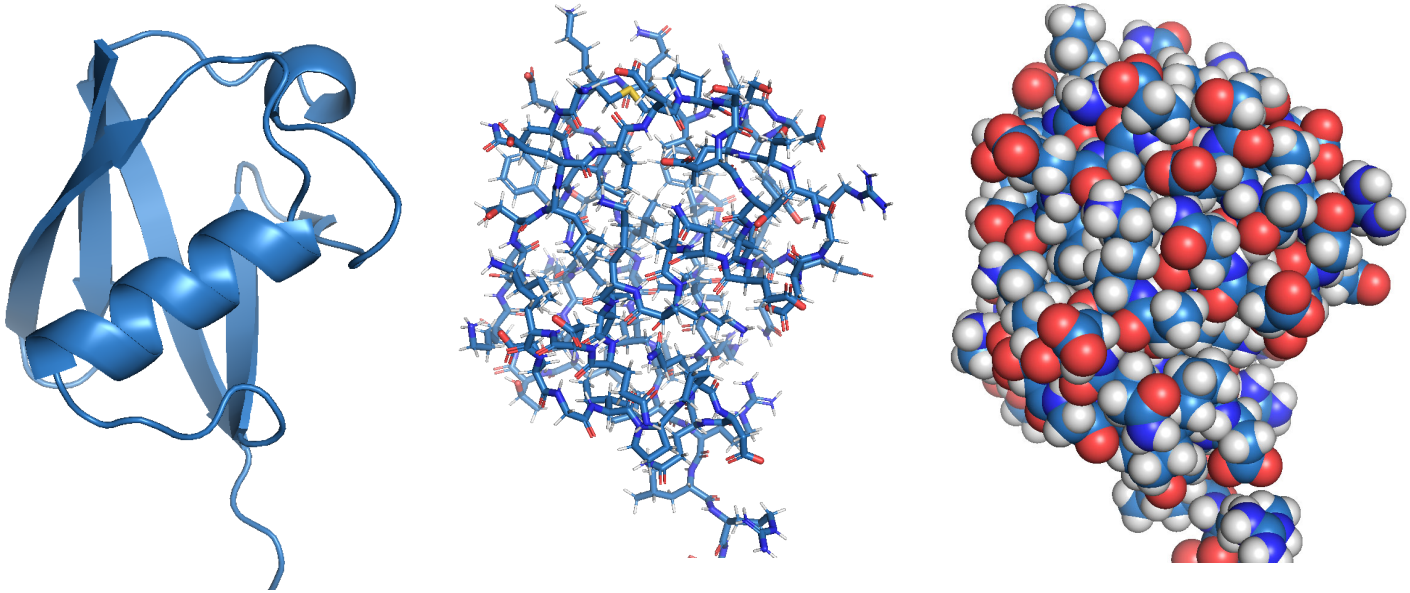
We have investigated all **energetic contributions to protein stability**

We have tried to characterize a ground state for protein folding – **the unfolded state**



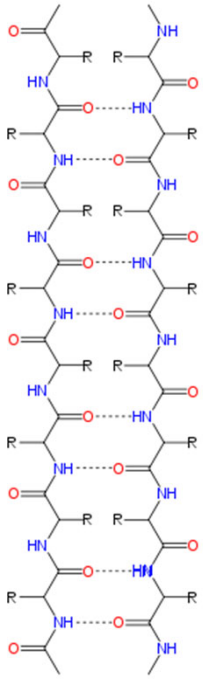
What about protein structure?

Proteins – Secondary structure

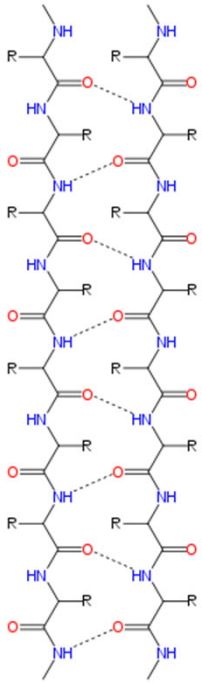


The beta-sheet

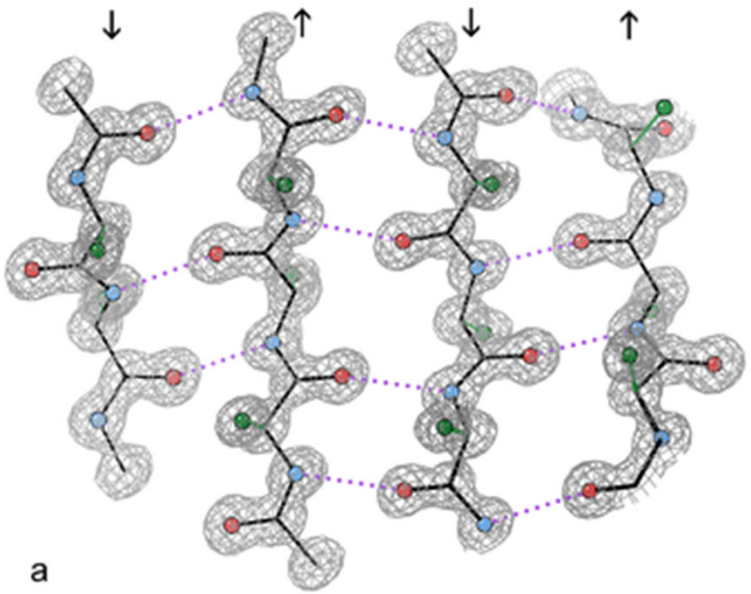
anti-parallel



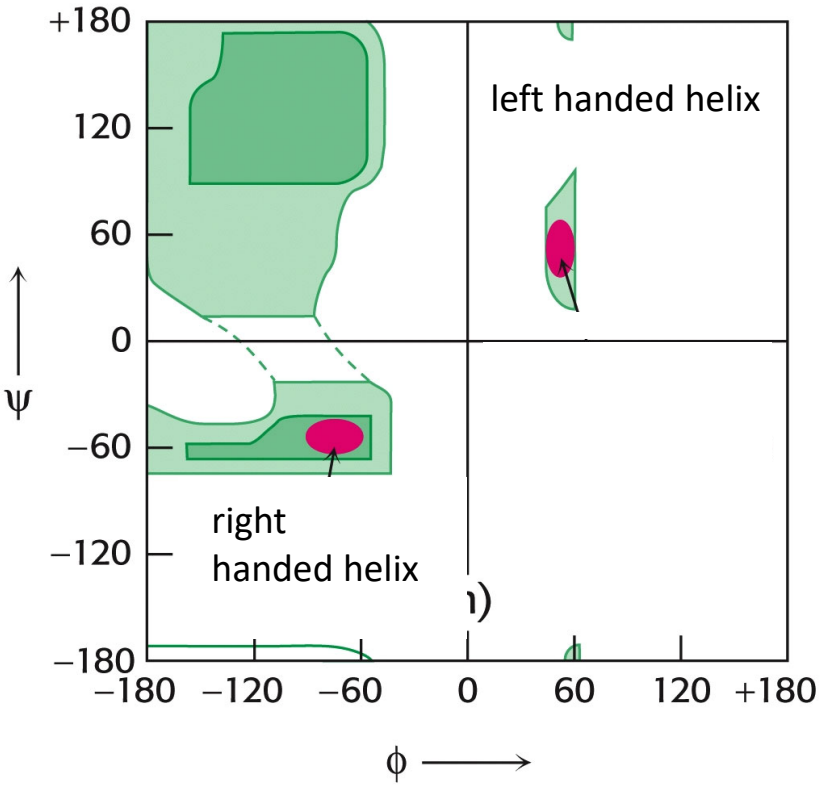
parallel

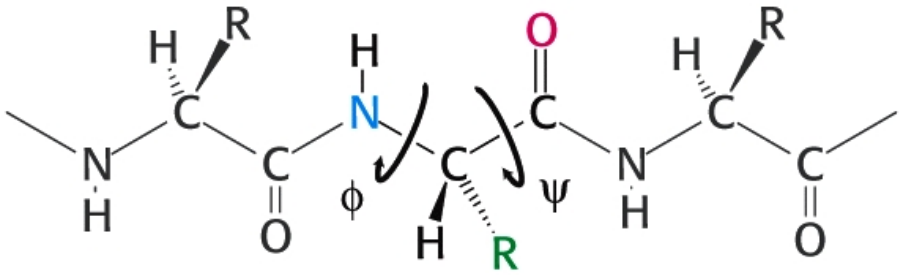


X-ray structure of beta-sheet in catalase



The alpha-helix

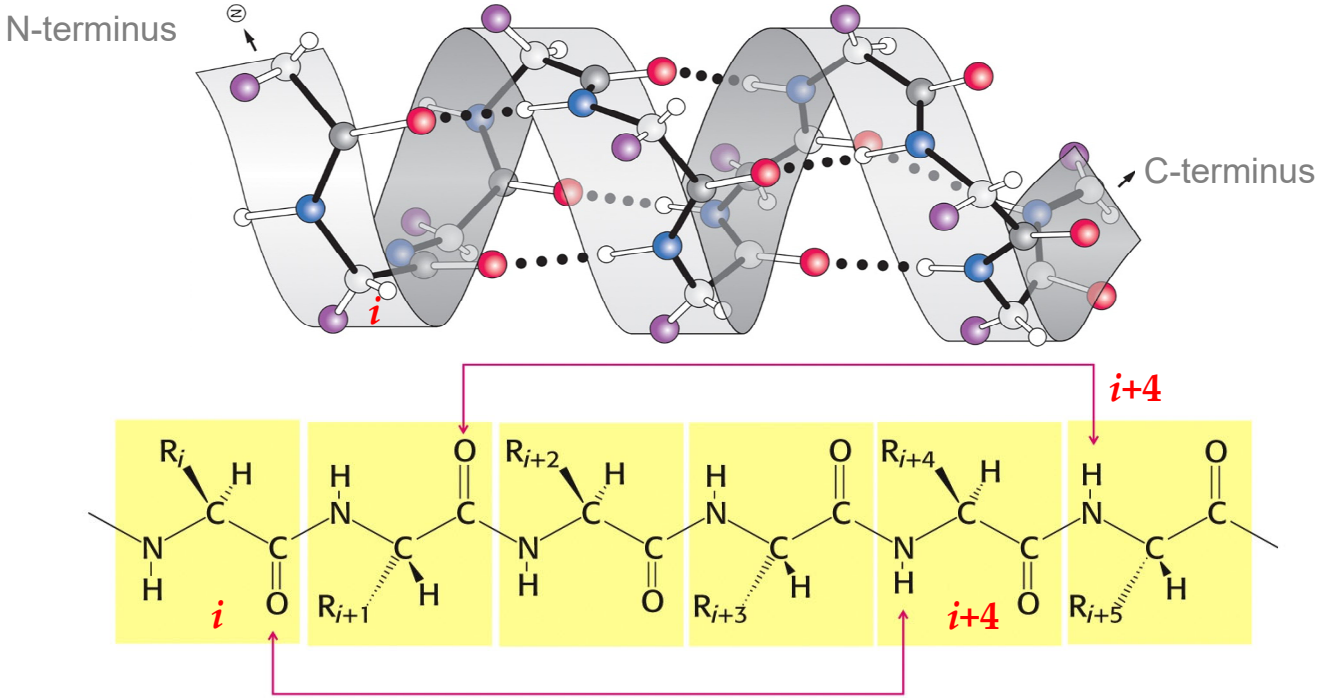




Both right- and left-handed helices are allowed.

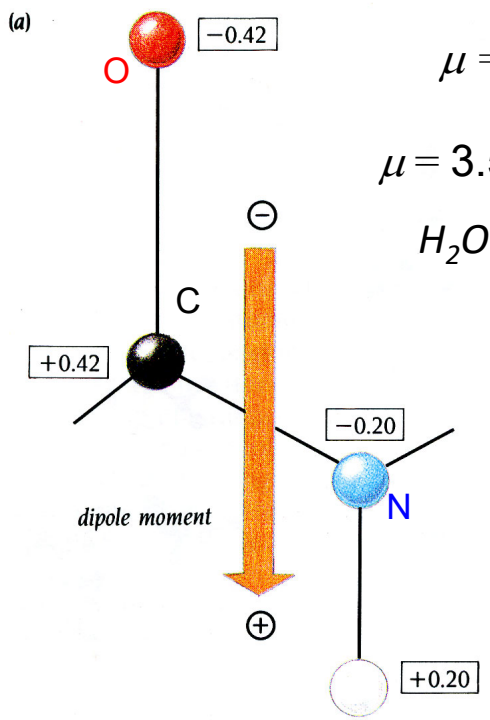
In proteins most helices are right-handed.

Hydrogen-bonding scheme for the α-helix



C=O of aa ***i*** -> **H-bond** with N-H of aa ***i*+4**

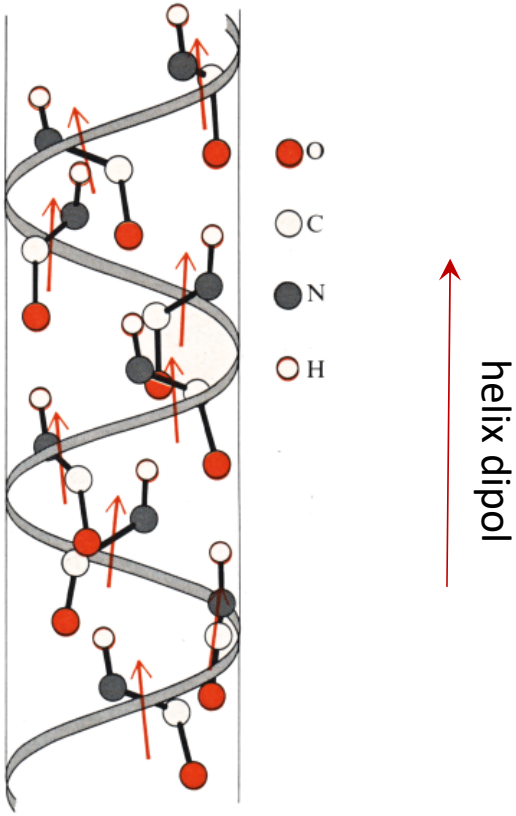
Helices are electrical dipoles made up of peptide bond dipoles



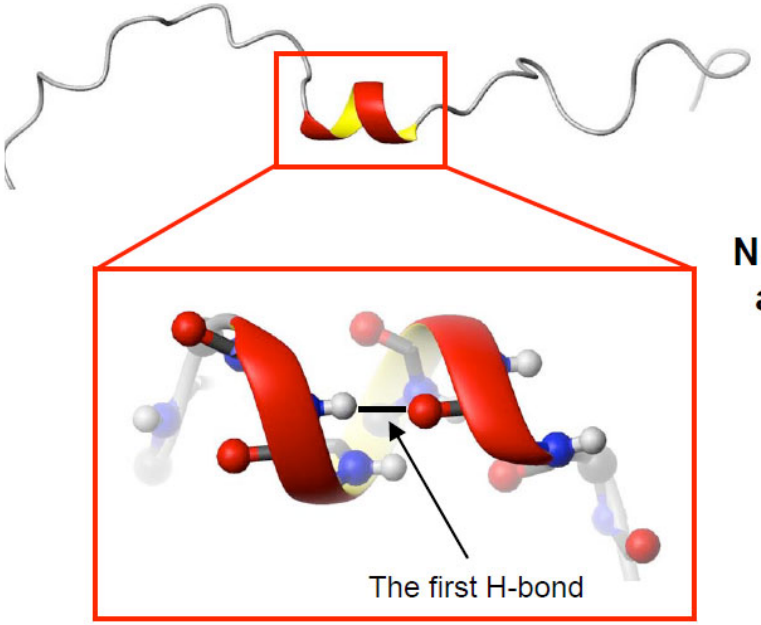
$\mu = z\, d$

$\mu = 3.5$ Debye

H_2O 1.85 D



Nucleation, the first H-bond



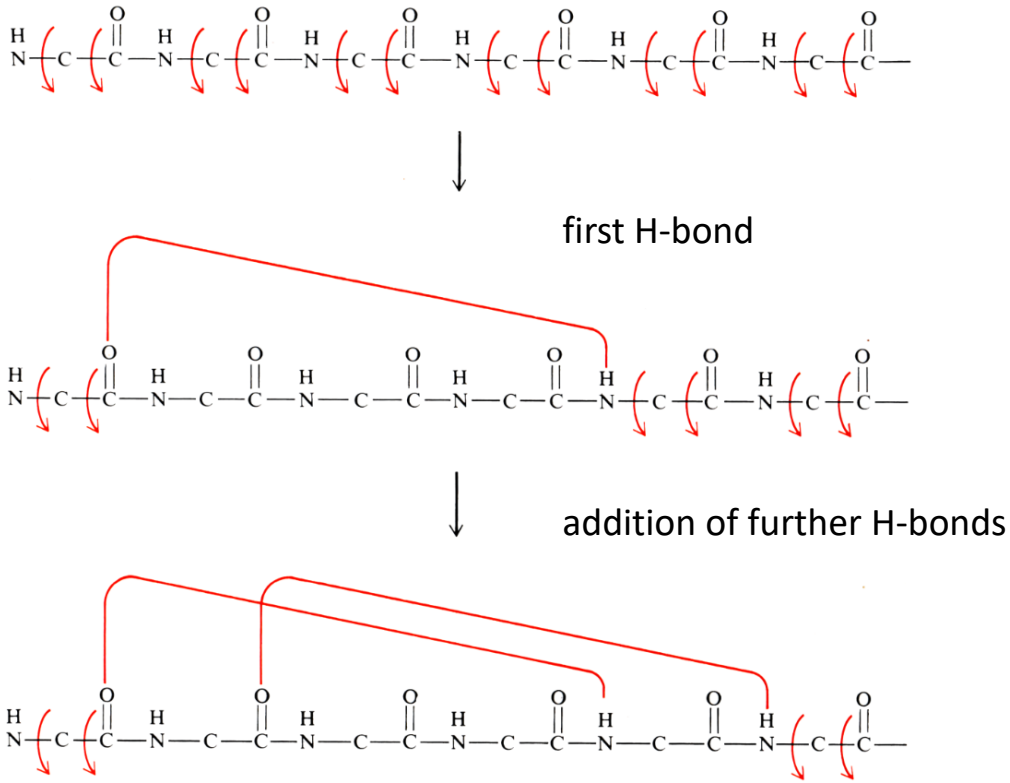
Nucleation of alpha helix

the conformations of 3 consecutive residues have to be fixed without proper H-bond compensation

high entropic cost associated

nucleation is rare and disfavored

Mechanism of helix formation



What is the secondary structure of a protein?

Sequence:

MQIFV**K**TLTG**K**TITLEVEPSDTIENV**K**A**K**IQ
D**K**EGIPPDQQRLIFAG**K**QLEDGRTLSDYNI
Q**K**ESTLHLVLRLRGG

- measure the secondary structure of a protein? → **Circular dichroism**
- predict the secondary structure by sequence? → **Statistical thermodynamics**

Absorption processes

Definition of light absorption:

a photon of energy

is absorbed by a molecule:

This results in:

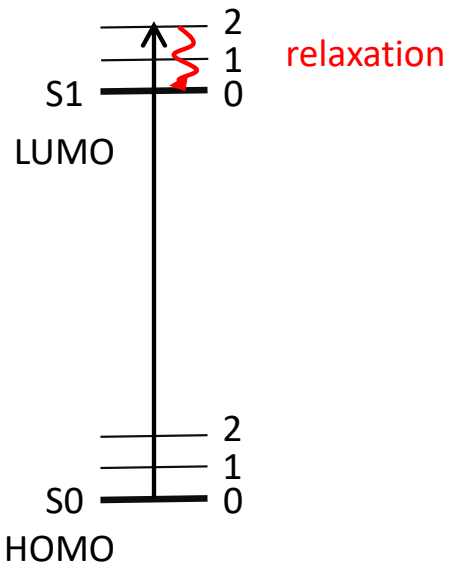
Electron from a *binding* or *non-binding* MO switches to an *anti-binding* MO with higher energy than the ground state

The residence time in the HOMO is very short (ns)

Fate of the excited state:

- internal conversion: heat
- emission of a photon: fluroescence, phosophorescence

Simple Jablonski diagram:



Beer Lambert's Law & Absorption Spectra of Aromatic Amino Acids

absorption

$$A = \log \frac{I_0}{I} = \varepsilon l c$$

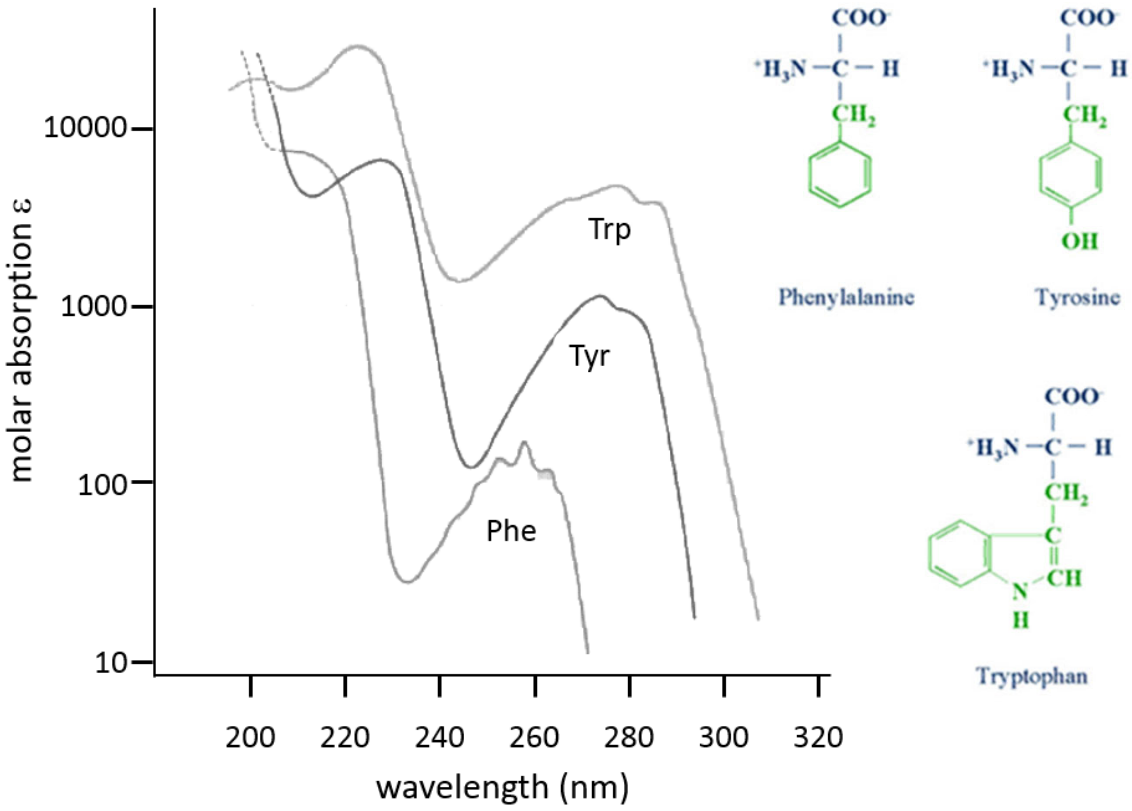
$$A = \varepsilon l c$$

with $\varepsilon = \sigma N_a 10^{-3} \log e$

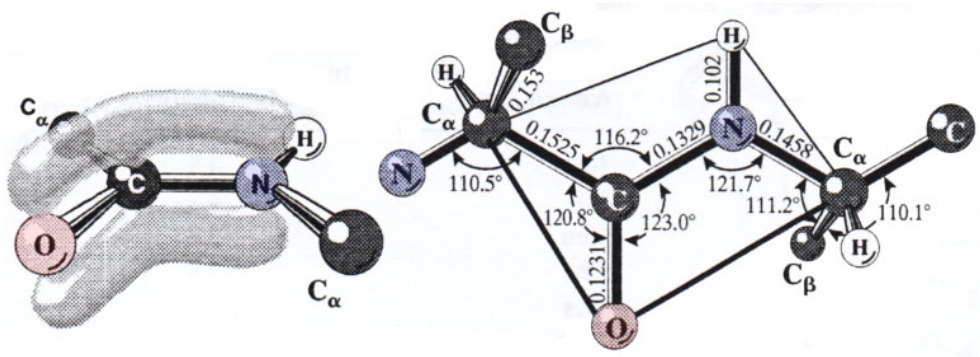
Units: $M^{-1}cm^{-1}$

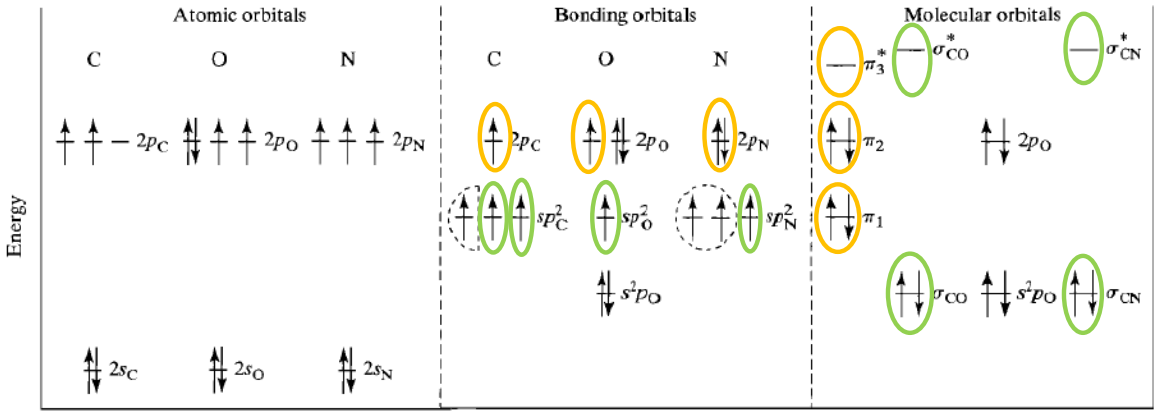
The extinction coefficient is wavelength dependent

$$A(\lambda) = \varepsilon(\lambda) l c$$



CD spectrosocopy: Optical properties of peptide bonds

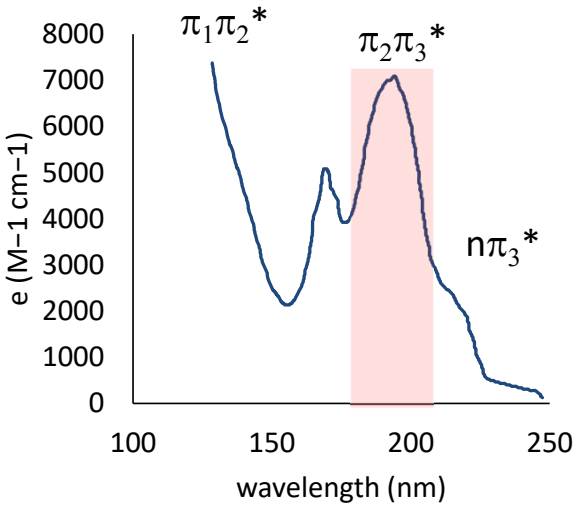




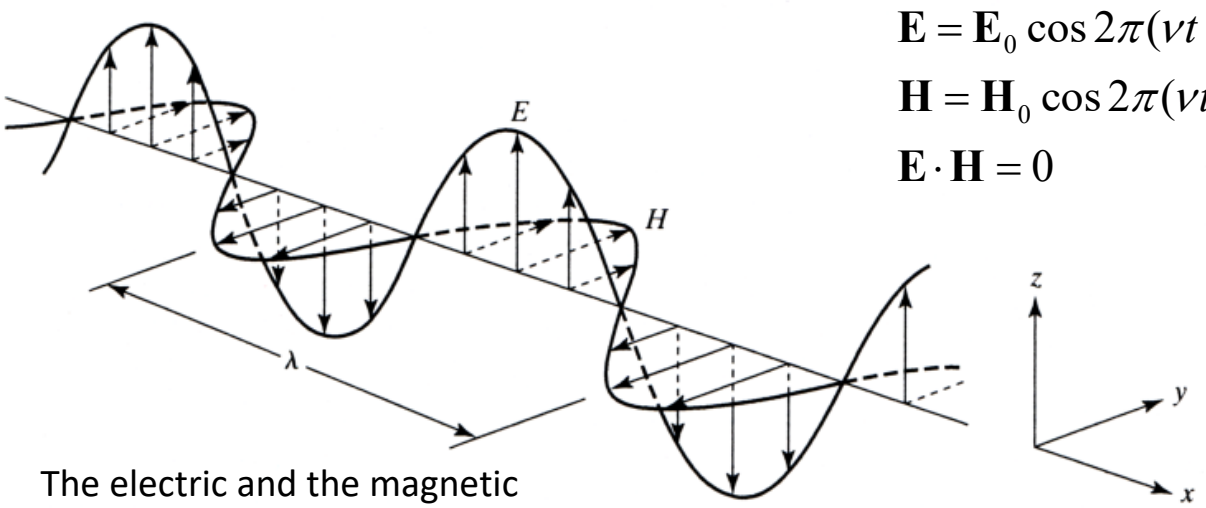
proteins are optically active

peptide bond absorption:

- π – π* transition
- absorption band around 190 nm
- absorption properties influenced by asymmetric environment



Polarization of light



$$\mathbf{E} = \mathbf{E}_0 \cos 2\pi(\nu t - x/\lambda)$$

$$\mathbf{H} = \mathbf{H}_0 \cos 2\pi(\nu t - x/\lambda)$$

$$\mathbf{E} \cdot \mathbf{H} = 0$$

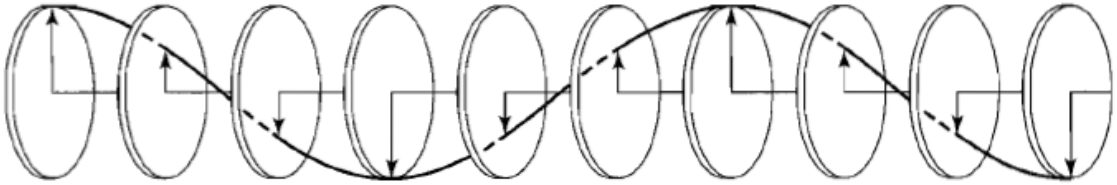
The electric and the magnetic field vector are orthogonal, and are orthogonal respective to the direction

Polarization: Defined as the plane of oscillation of the electric field vector

Individual photons are always polarized

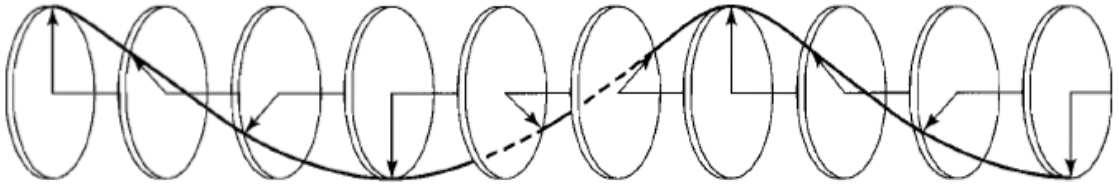
Linear and circular polarized light

Linear polarized light



E vector lies in one defined plane

Circular polarized light

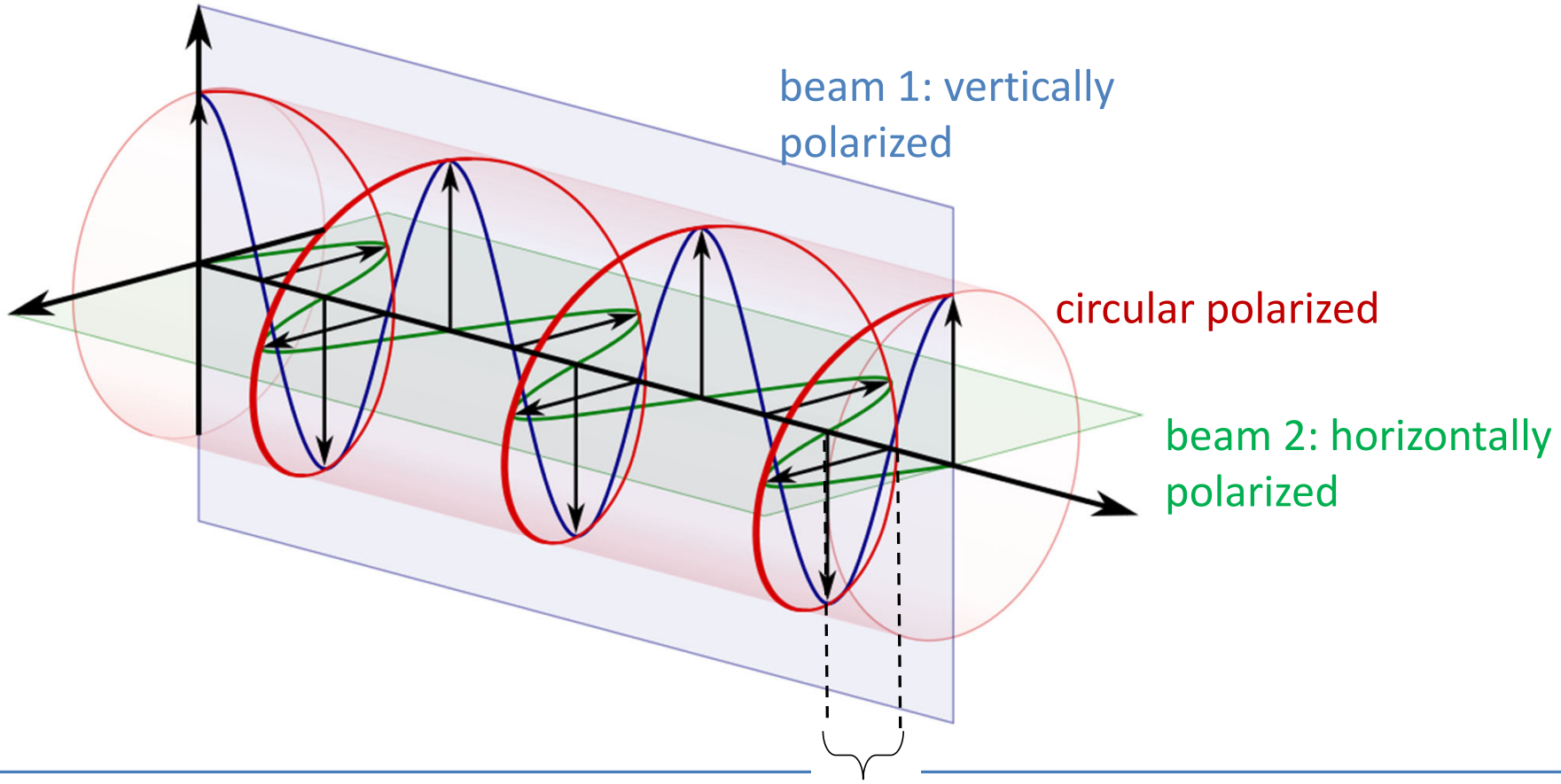


Elliptic polarized light (E II and E _I different length)

E vector describes a helix in space

helix can be right- or left-handed

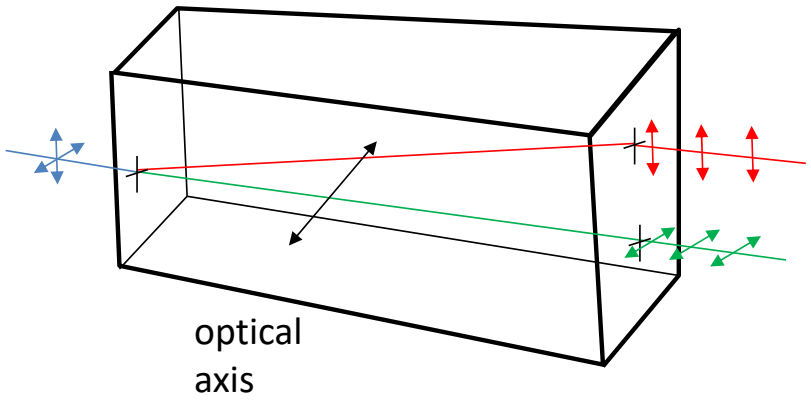
Circular polarized light: Superposition of orthogonally polarized beams with π/2 phase shift

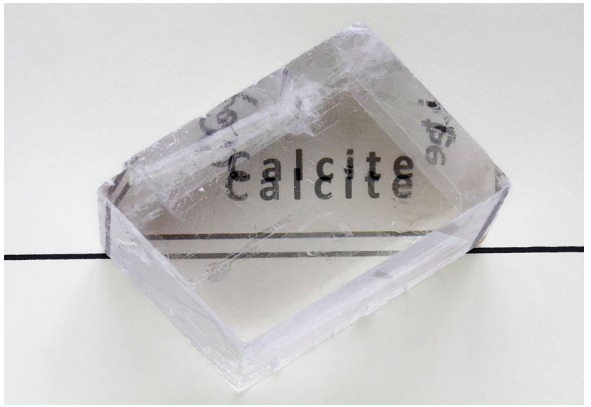


Production of circular polarized light

Birefringence:

Property of crystals: Different refractive indices for differently polarized light





Example: Calcite ($CaCO_3$)

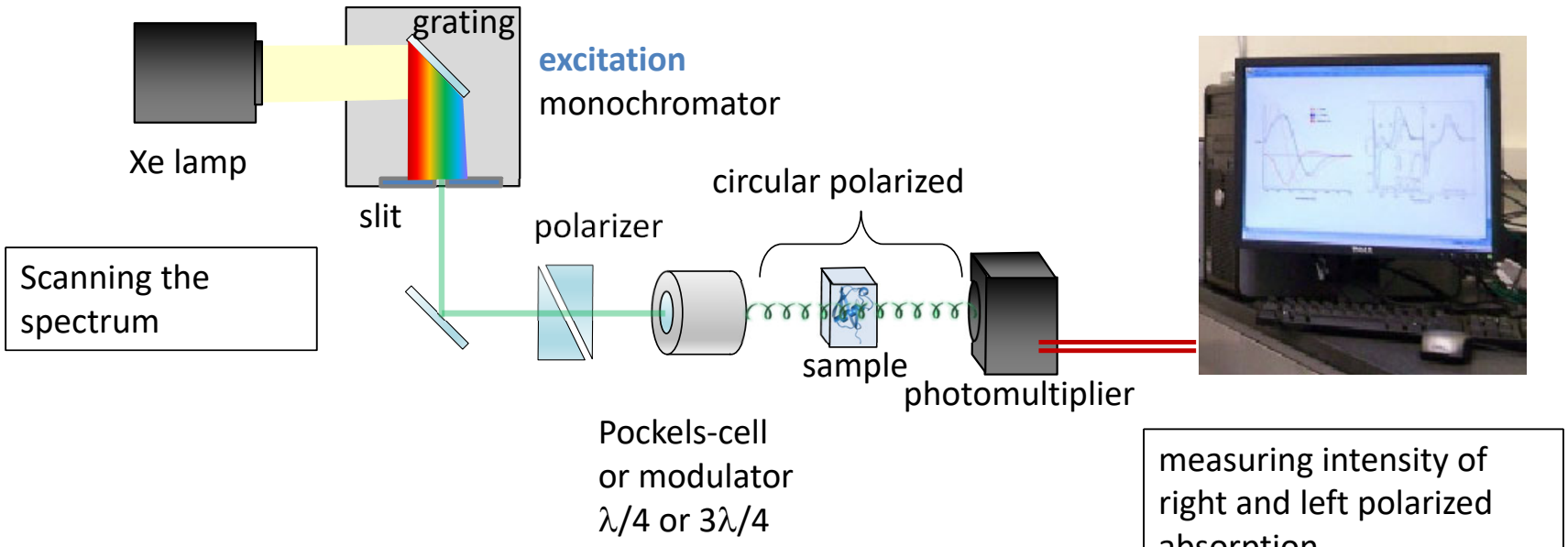
→ cont on bb.

Interaction of circular polarized light with optically active compounds: CD Spectroscopy

Optically active compounds in solution exhibit birefringence if irradiated with circular polarized light **dependent if the light is right-polarized (E_+) or left-polarized (E_-)**

- The solution has different extinction coefficients ε_+ and ε_- for right- and left-polarized light.
- This difference in absorption $\Delta\varepsilon$ is called the **circular dichroism (CD)** and can be measured for every wavelength
- An absorption spectrum specific for circular polarized light is measured similar to a UV spectrum
- Between λ = 180-250 nm information about protein secondary structure is obtained (**far-UV CD**)
- Between l = 250-300 nm a fingerprint for every folded protein is determined (**near-UV CD**)

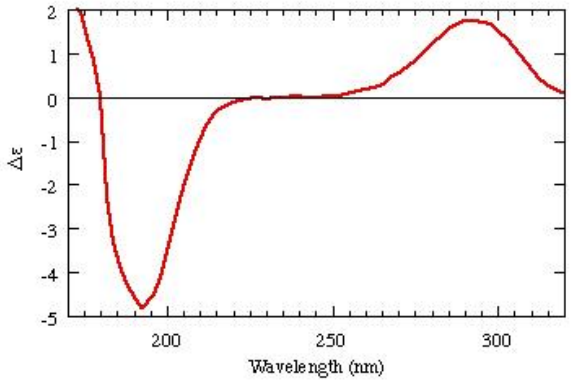
Measuring a CD-Spectrum



CD measurements

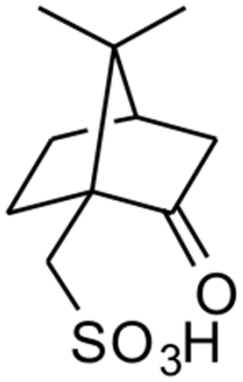
Difference between right- and left-polarized absorption:

Ellipticity: $$\psi_{obs} = \frac{(E_+ - E_-)}{(E_+ + E_-)}$$



Example: d-10-camphorsulfonic acid in water

Extinction coefficient	[cm^{-1} M^{-1}]	**Molar ellipticity** [deg cm^2 $dmol^{-1}$]	
ε_{285} (UV absorption)	34.5		
$(\varepsilon_L-\varepsilon_R)_{290.5}$	2.36 x 10^{-3}	$[\theta]_{290.5}$	7.8
$(\varepsilon_L-\varepsilon_R)_{192.5}$	-4.72 x 10-3	$[\theta]_{192.5}$	-15.6
$(\varepsilon_L-\varepsilon_R)_{290.5}/(\varepsilon_L-\varepsilon_R)_{192.5}$	-2.0	$[\theta]_{290.5}/[\theta]_{192.5}$	-2.0



Units of CD spectroscopy

De -> all necessary information.

historical reasons:
molar ellipticity

$$[\theta]_{\lambda}^{T} = \frac{100 \cdot \psi_{obs}}{c \cdot d}$$

(grad cm^2 dmol^{-1})
with
c: molar concentration
d: pathlength (cm)

$$\psi_{obs} = \frac{(E_+ - E_-)}{(E_+ + E_-)}$$

$$[\theta]_{\lambda}^{T} = 3300 \cdot \Delta\varepsilon$$

proteins:
average molecular weight

$$M_0 = \frac{\text{MW protein}}{\text{No. amino acids}}$$

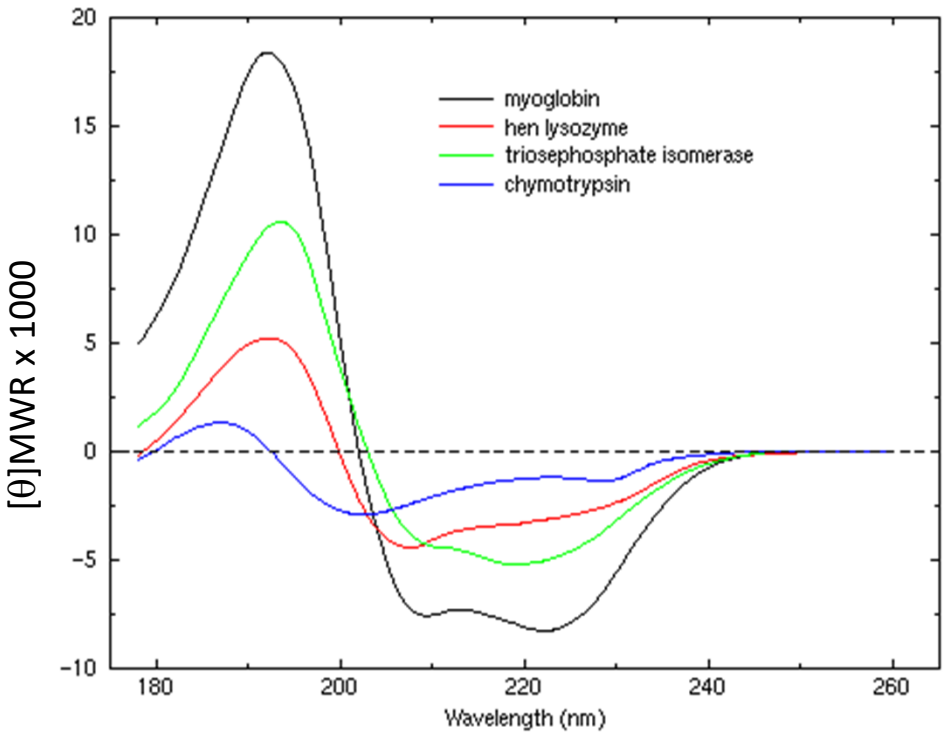
average ellipticity per amino acid:

$$[\theta]_{MRW} = \frac{100 \cdot \psi_{obs}}{c \cdot d \cdot N_{\text{aminoacid}}}$$

(grad cm^2 dmol^{-1})

Far-UV CD spectroscopy in proteins

CD spectra of different proteins



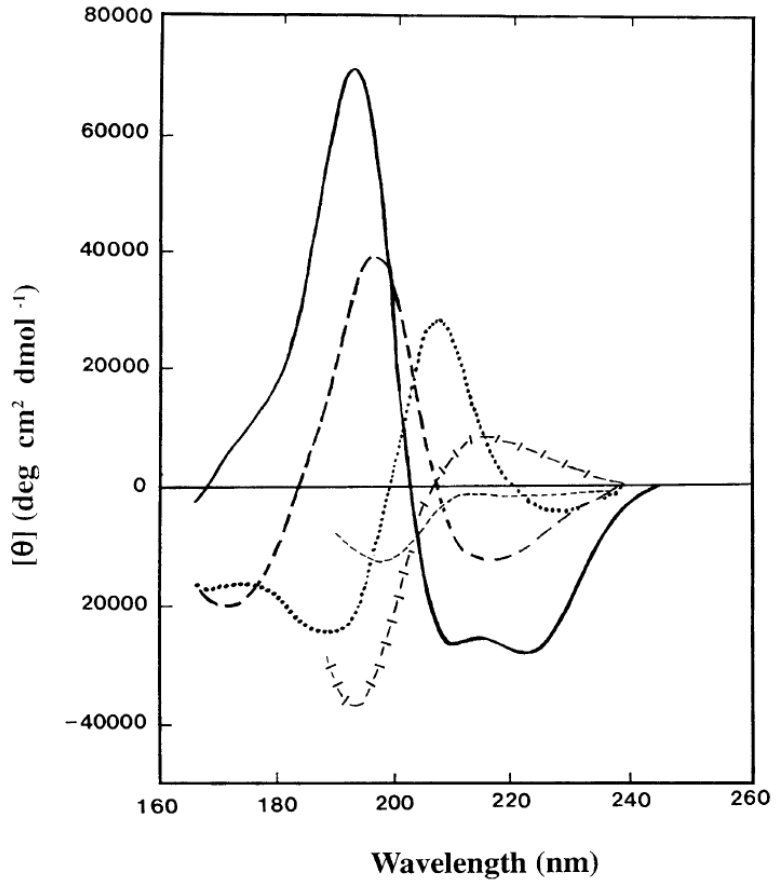
chymotrypsin

myoglobin

lysozyme

triosephosphate isomerase

Far-UV Basis spectra of secondary structure



basis spectra of secondary structure elements

- Solid line: α-helix
- long dashed line: anti-parallel β-sheet
- dotted line: type I β-turn
- cross dashed line: extended 3_1-helix or poly Pro II helix
- short dashed line: irregular structure

Kelly et al.
BBA 1751, p 119, 2005

Determining the amount of secondary structure from a Far-UV CD spectrum

Conformation	CD-properties	
	nm	$[\theta]_{MRW}$
a-helix	193	+73000
	208	-35000
	222	-38000
b-sheet	198	+50000
	217	-8000
collagen	198	-50000
	220	+6000
random coil	200	-15000
	198	-20000

estimate amount of secondary structure:

$$[\theta]_{MRW} = X_{coil}[\theta]_{coil} + X_{\beta}[\theta]_{\beta} + X_{\alpha-helix}[\theta]_{\alpha-helix}$$

with X being the fraction of residues in the indicated state

α-helix content determined by Far-UV CD

Conformation	CD-properties	
	nm	$[\theta]_{MRW}$
a-helix	193	+73000
	208	-35000
	222	-38000
b-sheet	198	+50000
	217	-8000
collagen	198	-50000
	220	+6000
random coil	200	-15000
	198	-20000

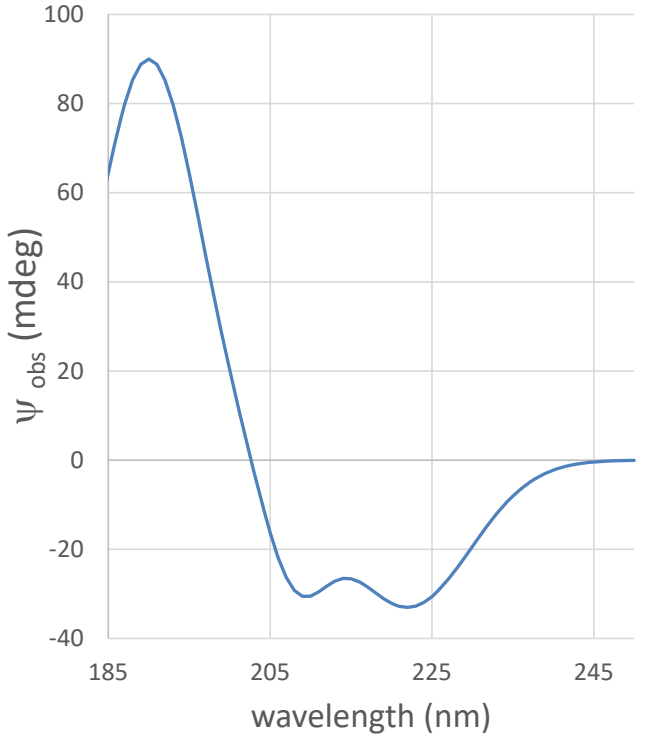
at 208 nm, the helix content can be calculated directly:

$$X_{a-helix} = \frac{[\theta]_{208} + 4000}{-31000}$$

Greenfield & Fasman, Biochemistry 8, 1969

Quiz

- you determine a CD spectrum for a small, alpha-helical protein (153 amino acids, MW 16950 Da).
- At a concentration of 0.17 mg/ml and 1mm cuvette length, you measure a the following data:
- What is the % of helix for this protein?



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Sequence:

MQIFV**K**TLTG**K**TITLEVEPSDTIENV**K**A**K**IQ
D**K**EGIPPDQQRLIFAG**K**QLEDGRTLSDYNI
Q**K**ESTLHLVLRLRGG

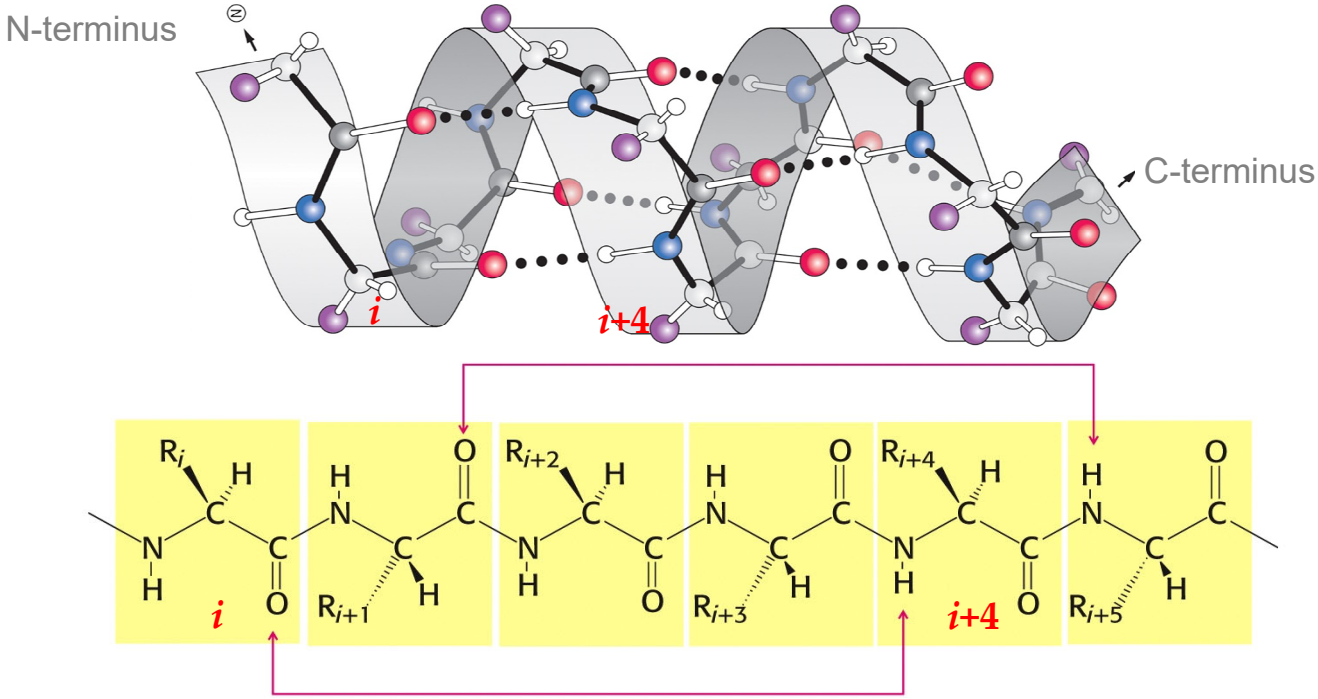
- Can we measure the secondary structure of a protein sample?

→ Circular dichroism measurement

- Can we predict the secondary structure of a protein by the sequence?

→ Statistical thermodynamics

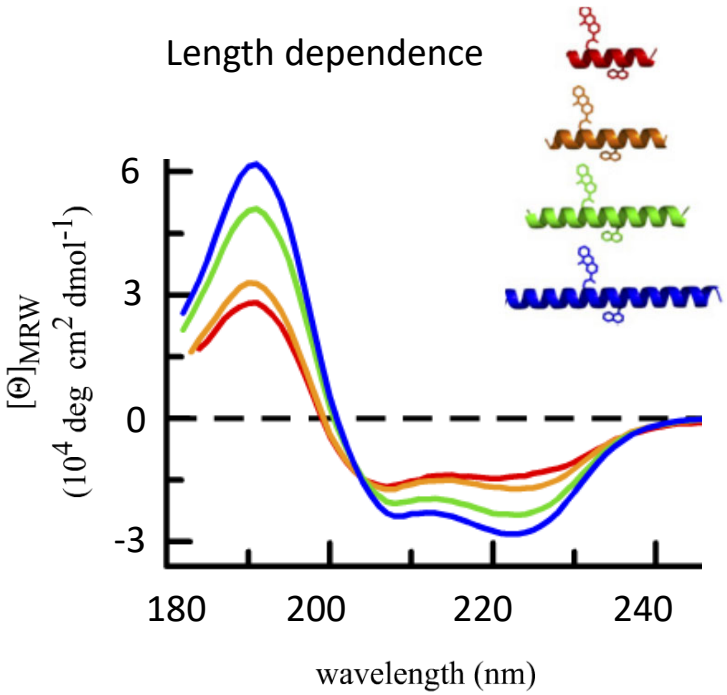
Hydrogen-bonding scheme for the α-helix



In the α-helix, the C=O group of amino acid ***i*** forms a hydrogen bond with the N-H group of residue ***i+4***

bb simple thermodynamic model

Helicity increases with increasing peptide length



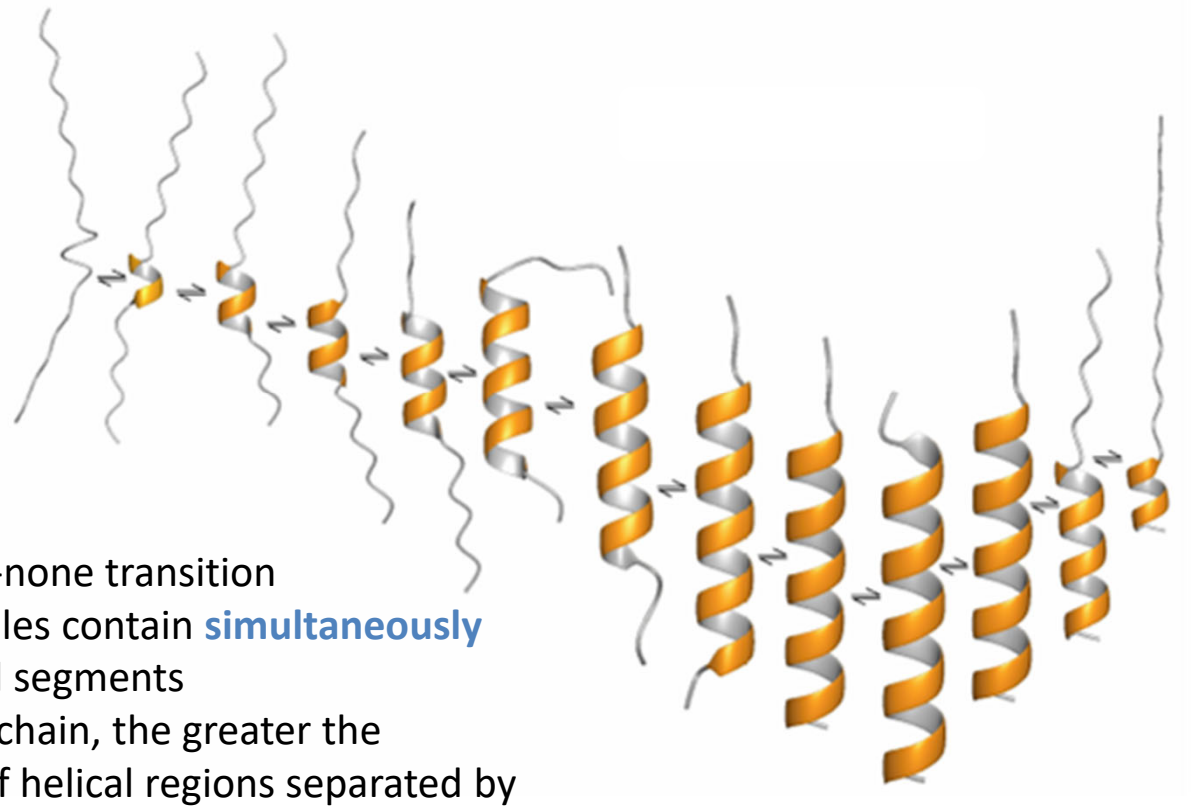
Intensity of CD bands increase with increasing α-helix length

This is due to coupled oscillators in a long helix

Thus, for a helix of given length, the maximal ellipticity at 208 nm can be approximated by:

$$[\theta]_{208,helix} = -36000\left(1 - 3.5/n\right)$$

helix – coil transition



- **not** an all-or-none transition
- most molecules contain **simultaneously** helix and coil segments
- the longer a chain, the greater the probability of helical regions separated by coil