Lecture 6:
Secondary Structures of Polypeptides

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Regular Secondary Structures:

- **helices (right-handed, left handed)**
- **β-structures (strands → sheet, parallel versus anti-parallel)**
- **polyproline helices (no hydrogen bonding!)**

Irregular Secondary Structures:

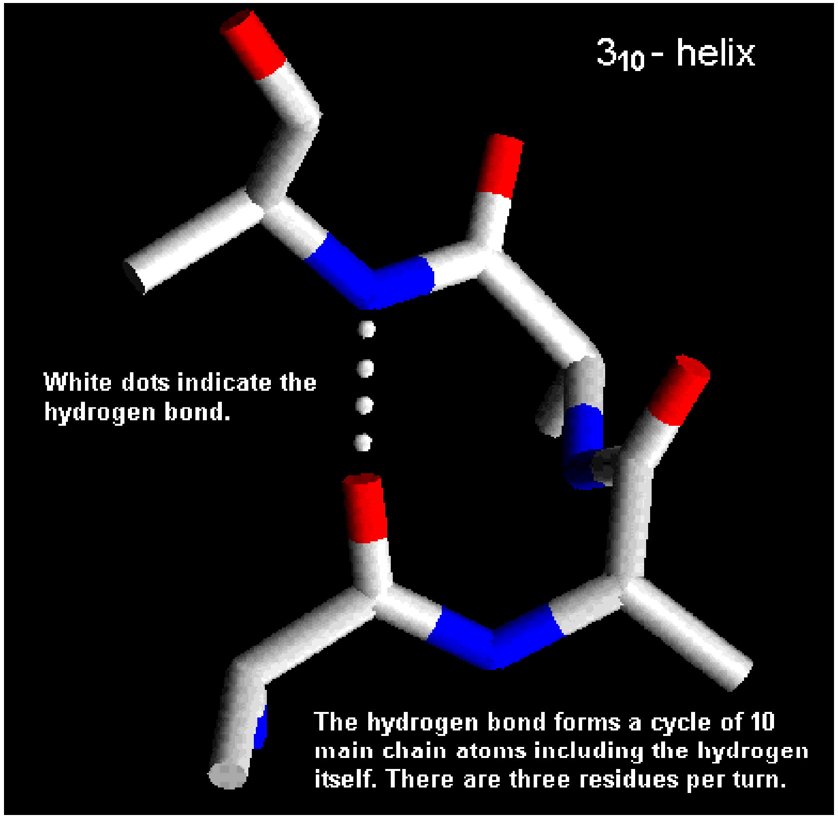
- **β-turns**
- **β-bulges**

Experimental determination of the secondary structure:

- **X-ray spectroscopy**
- **NMR spectroscopy**
- **circular dichroism (CD) spectroscopy**

Types of helices (R versus L-handed):

- i → i+2 – 2_7 helix
- i → i+3 – 3_{10} helix →
- i → i+4 – 4_{13} helix (α-helix)
- i → i+5 – 5_{16} helix (π-helix)



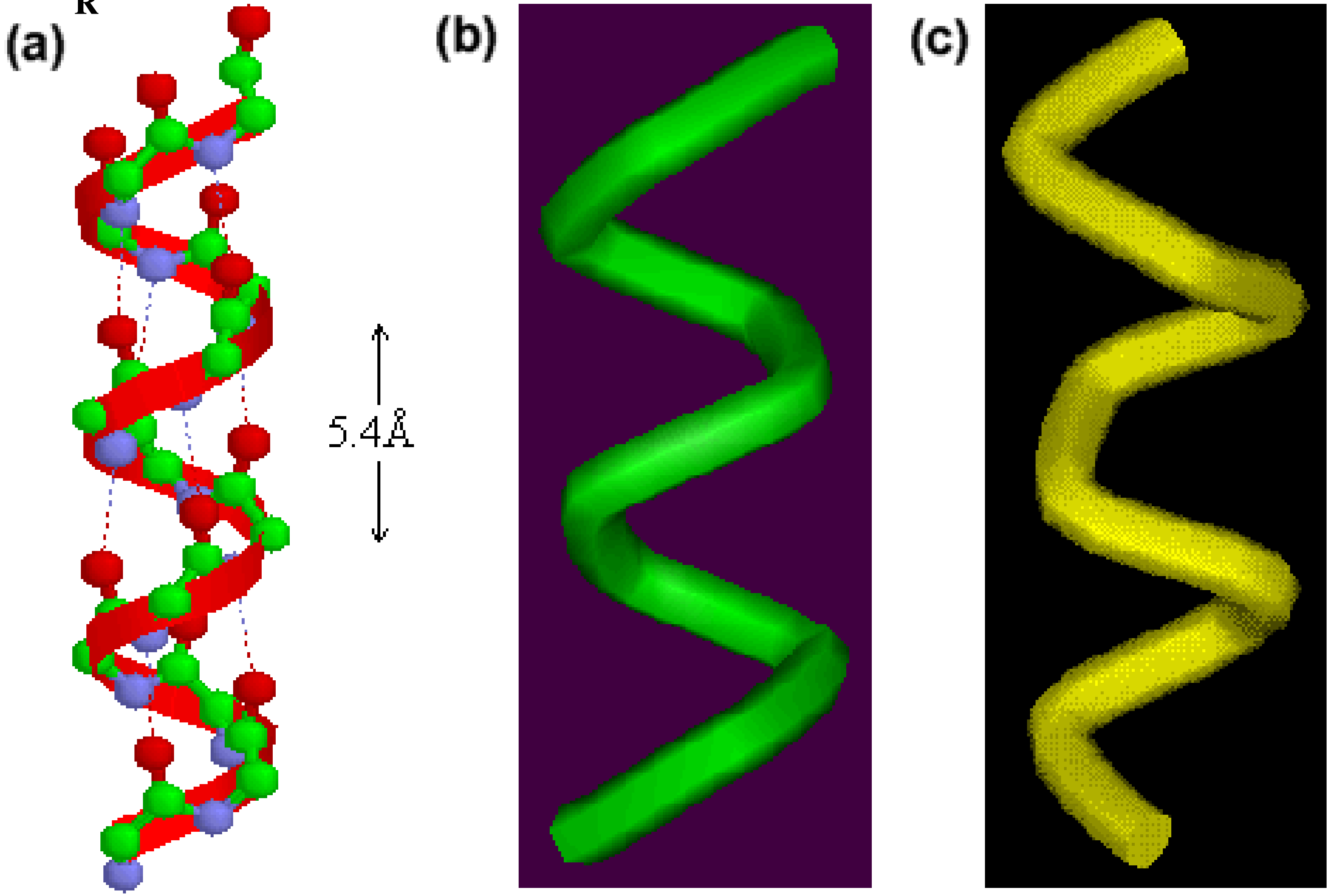
- **hydrogen bond pattern:**

C'O– group of i-th amino acid is H-bonded to the NH– group of the (i+n)-th amino acid

- **the R-handed α-helix = α_R-helix MOST abundant**

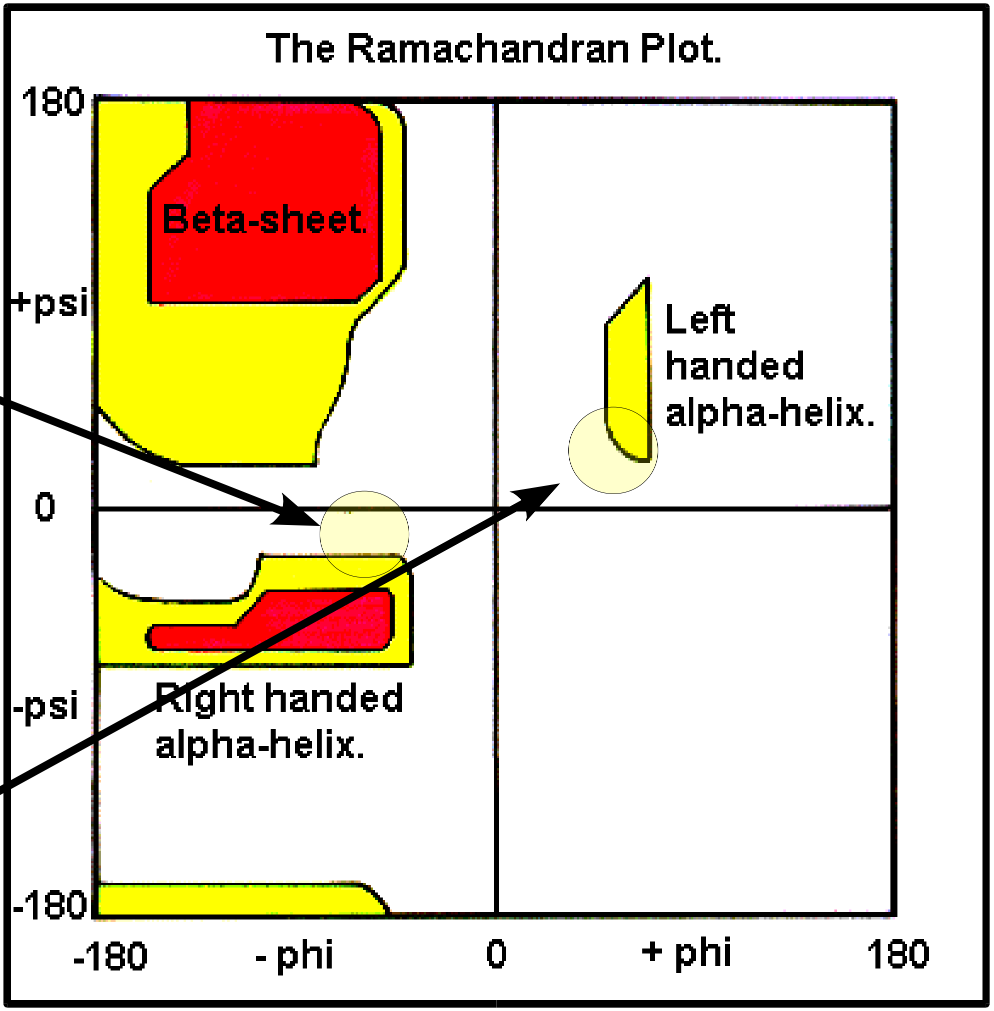
R-handed ↔clock-wise movement → away from the observer

α_R-helix **R** **L**



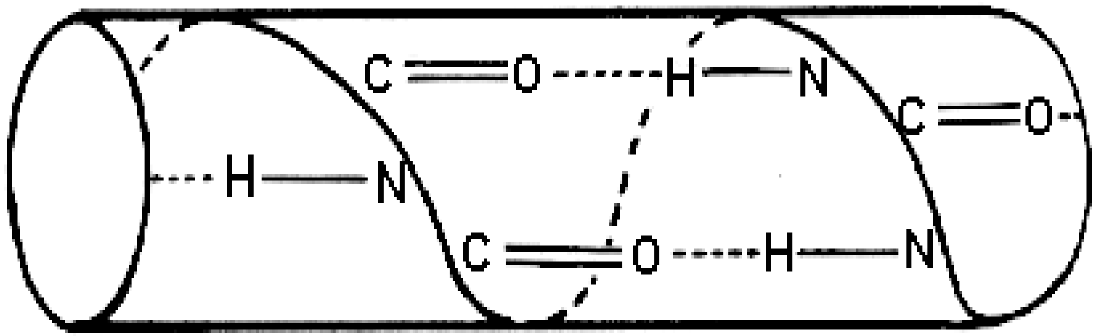
Helix-types present in proteins:

- α_R-helix
- α_L-helix
- short fragments (3-4 aa) of R-handed 3_{10} helix



- L-handed 3_{10} helix (Gly)

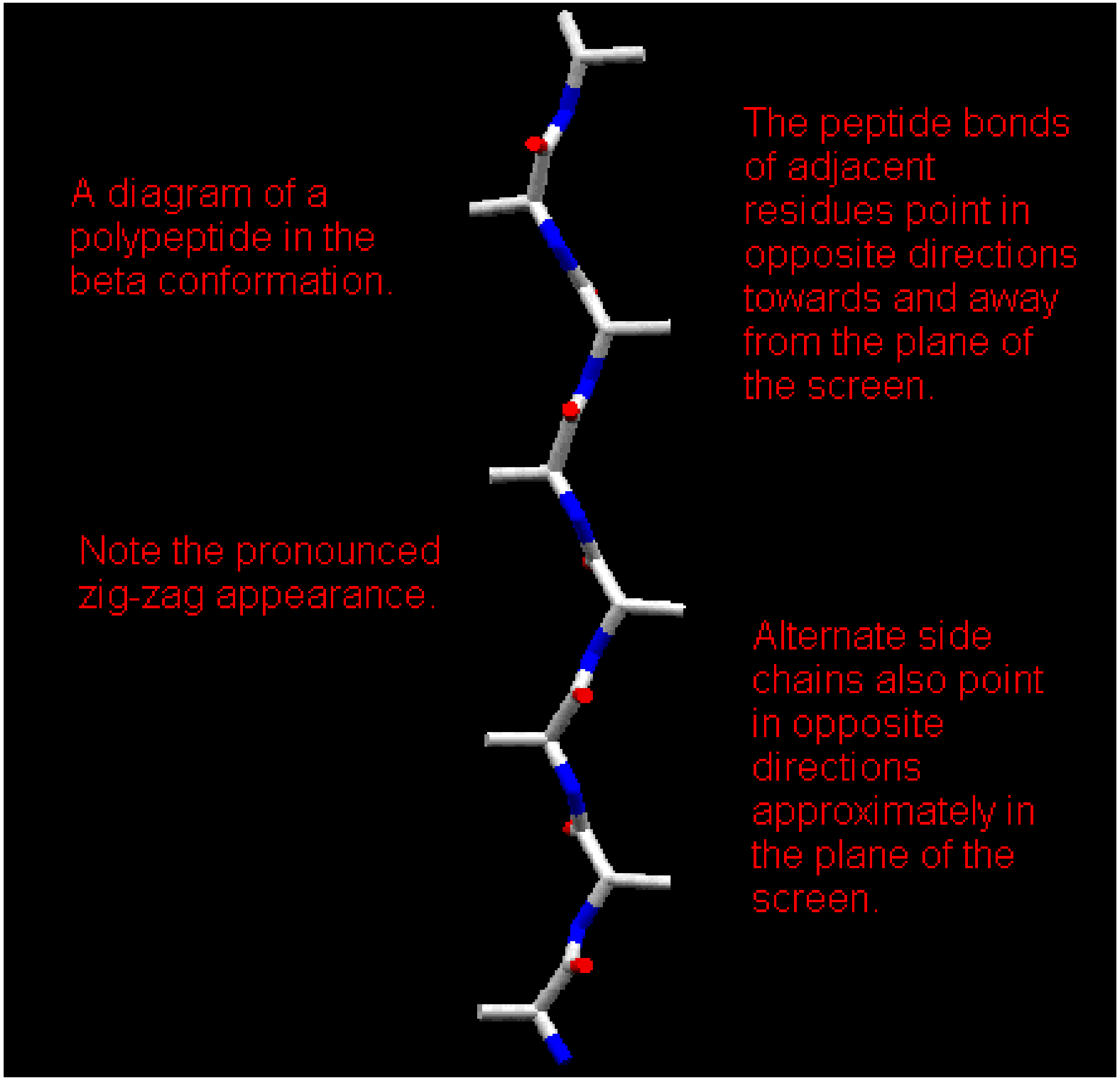
Toilet roll representation of the main chain hydrogen bonding in an alpha-helix.



electropositive
N-terminus
$+\frac{1}{2}e_0$

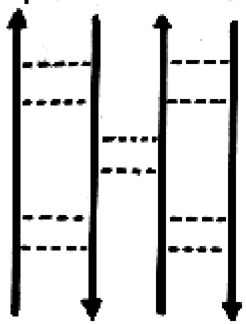
electronegative
C-terminus
$-\frac{1}{2}e_0$

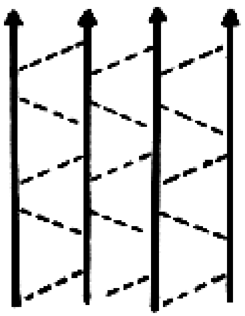
β-strand or extended peptide conformation



β-Sheet Geometry

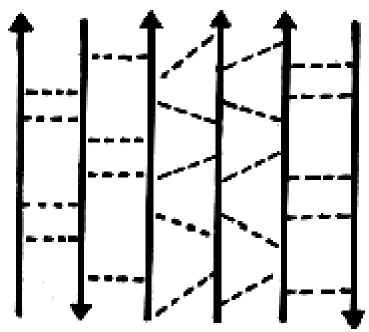
Antiparallel beta-sheet



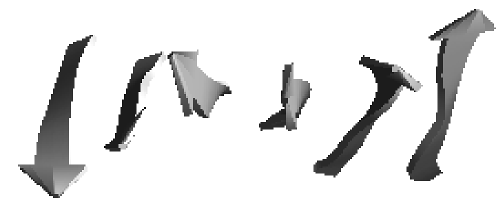


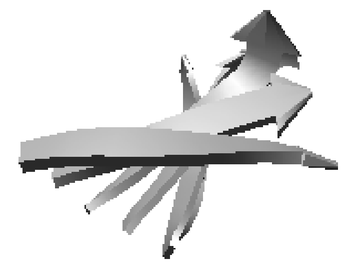
Parallel beta-sheet

The different types of beta-sheet. Dashed lines indicate main chain hydrogen bonds.

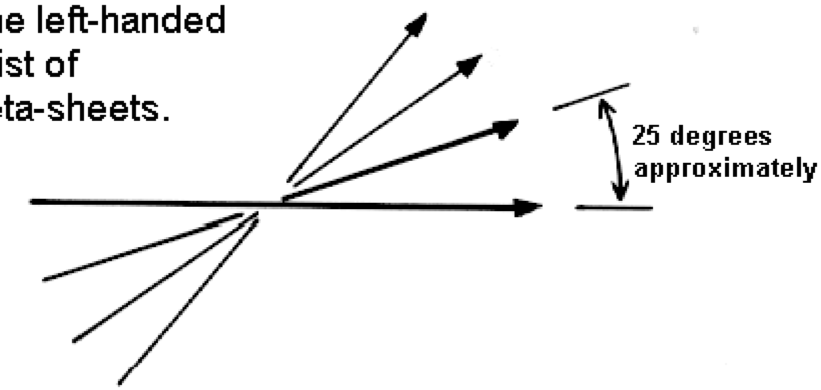


Mixed beta-sheet



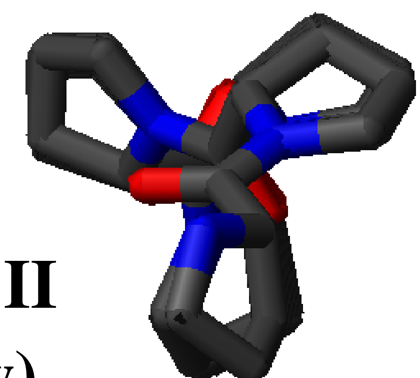


The left-handed twist of beta-sheets.

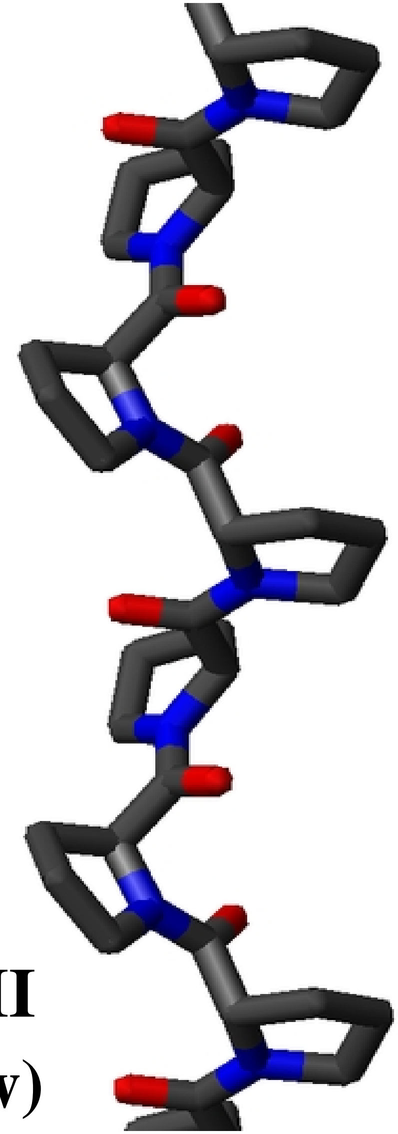


Polyproline helices

- two types, Poly-Pro I and Poly-Pro II
- NO H-bonding
- Poly-Pro II more frequent
- Poly-Pro I includes *cis* conformations (much denser)

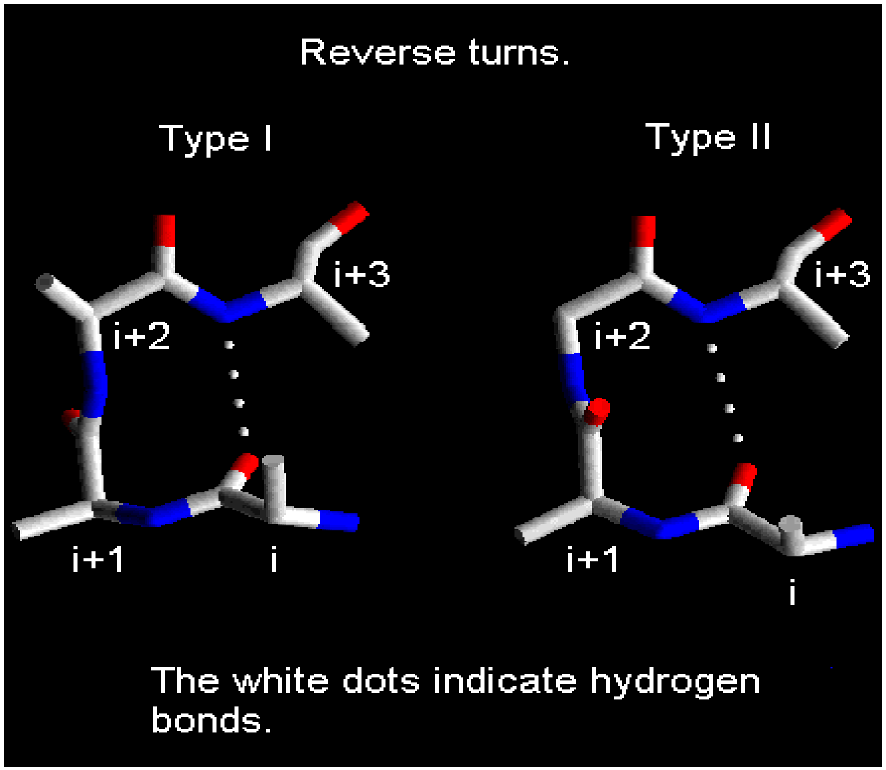


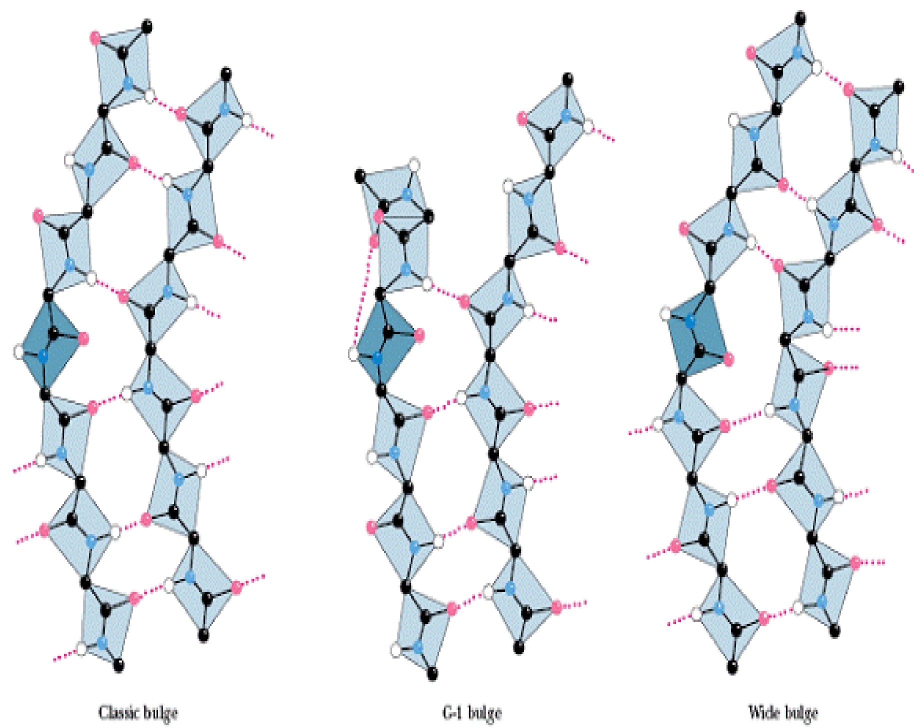
Poly-Pro II (top view)



Poly-Pro II (side view)

Irregular Secondary Structures: β-Turns and β-Bulges





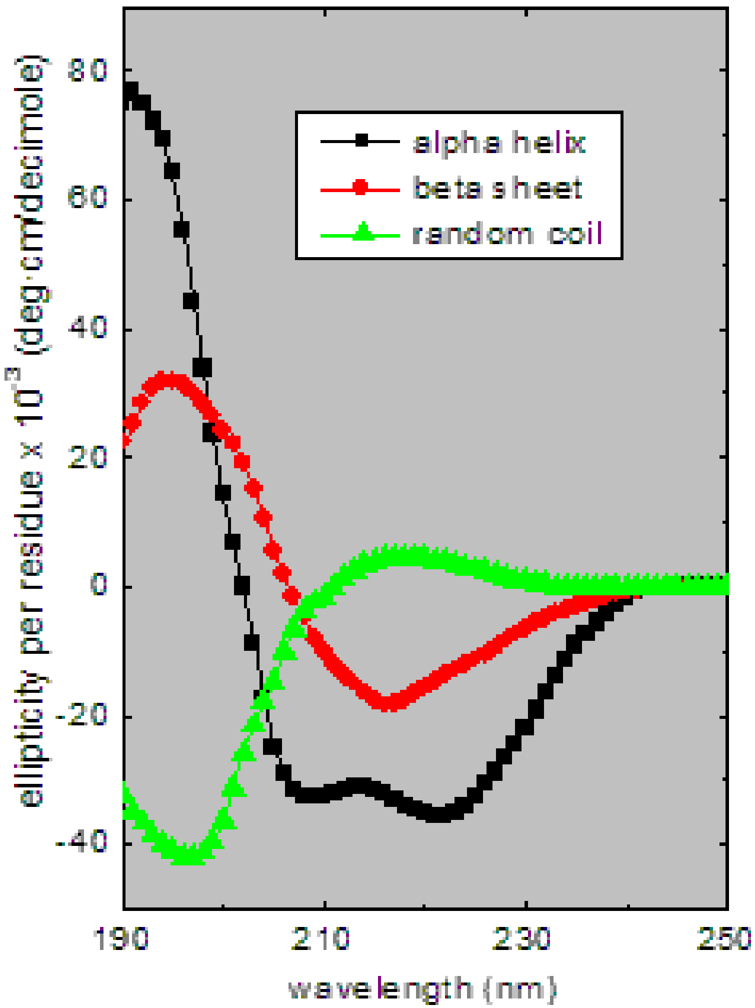
Experimental Determination of the Secondary Structure

Circular dichroism (CD) spectroscopy:

- **no *a priori* knowledge on the protein structure needed**
- **output: average amounts of α-helical and β-sheet structure**
- **linearly polarized light in → elliptically polarized out (clockwise & counter-clockwise polarized light have different absorption, caused by helices of different Handedness)**
- **far UV region (190-240nm): asymmetry in peptide group environment → secondary structure**
- **near UV region (250-280nm): asymmetry in aromatic group environment → tertiary structure**

Far UV Spectrum for Secondary Structure Determination

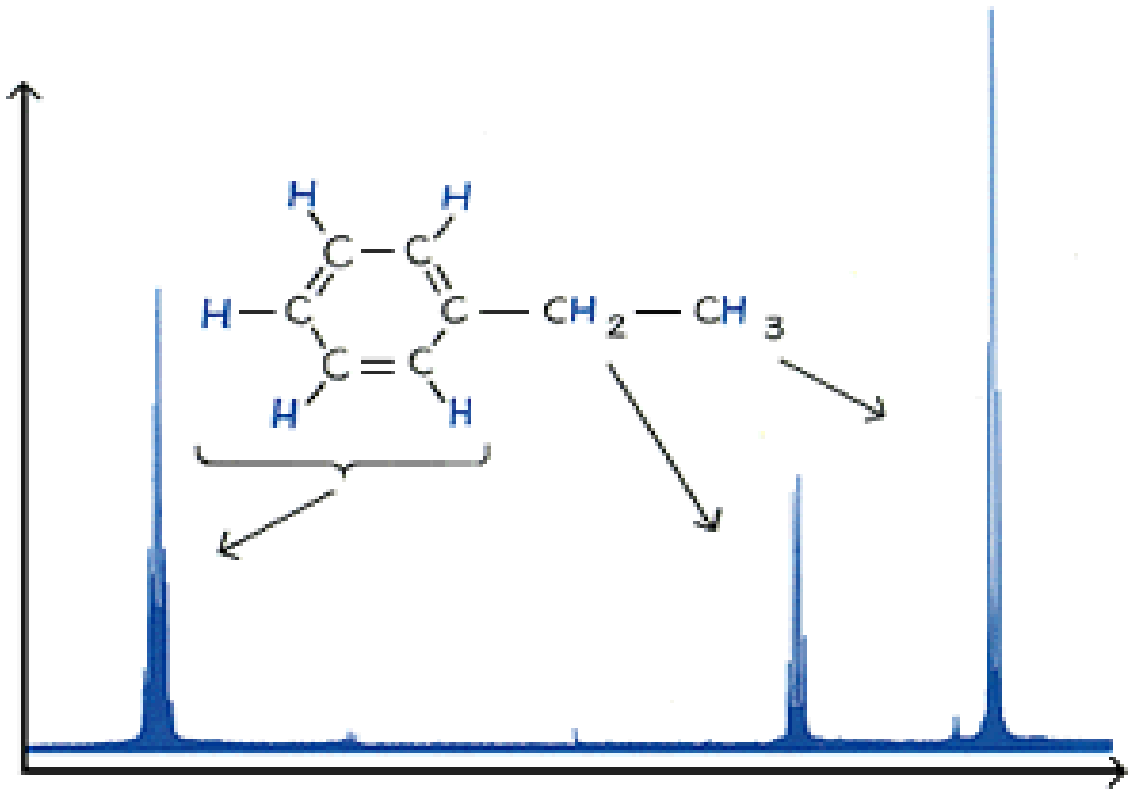
- the chromophore: the peptide bond
- charateristic shapes and magnitudes of the CD spectrum for α-helix, β-sheet, & random coil →
- 20–200 µl of solution with 1 mg/ml to 50 µg/ml protein, buffer with LOW absorbance in the far UV spectrum
- results in average values of the secondary structures



NMR Spectroscopy

- **application of radio—waves to excite magnetic moments of nuclei aligned in a strong magnetic field**
- **the total number of protons & neutrons need to be odd: ^{1}H, ^{13}C, & ^{15}N**
- **detects closely positioned H-atom nuclei (4—5 Å)**
- **resonance at a radio-frequency typical of the nucleus (in a given magnetic field), modified by neighboring chemical bonds**
- **excitation propagates to the neighboring nucleus giving rise to a cross—peak, which demonstrates proximity of the two nuclei**

Example of a simple NMR spectrum:



A proton NMR spectrum of a solution containing a simple organic compound, ethyl benzene. Each group of signals corresponds to protons in a different part of the molecule.