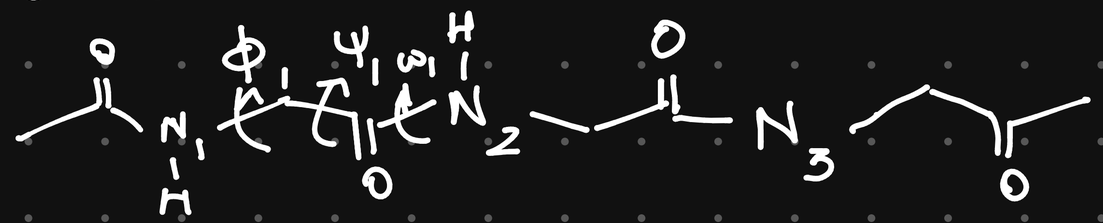
Structural Biology

Topics to be covered :

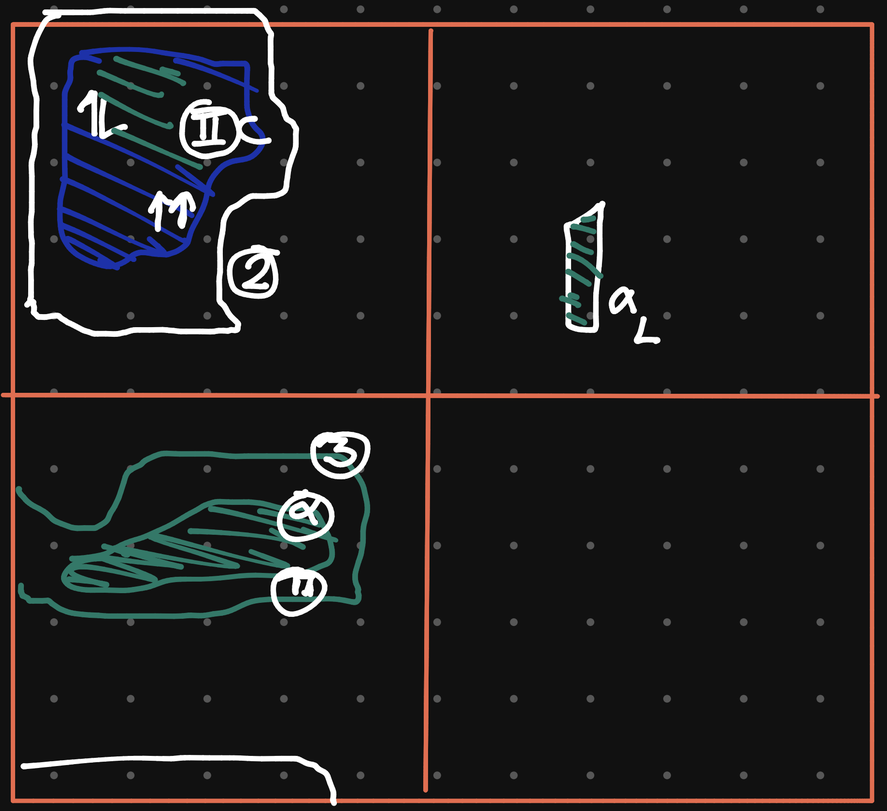
(A) Basics



Dihedral angles : $\Phi_1 = K(C_0 - N_1 - C_\alpha' - C_1)$, $\Psi_1 = K(N_1 - C_1^\alpha - C_1 - N_2)$

$$\omega_1 = K(C_1^\alpha - C_1 - N_2 - C_2)$$

Ramachandran Map



2° Str.	ϕ	ψ
α_R	-60	-50
α_L	60	50
↑↓	-140	135
↑↑	-120	115
π	-60	70
3_{10}	-50	-30
2.2_7	-80	60

Proteins are complex molecules essential for various biological functions. They are made up of long chains of amino acids that fold into specific three-dimensional structures.

1. Levels of Protein Structure :
   - Primary Structure : This is the linear sequence of amino acids in the

polypeptide chain. The specific order of amino acids determines the protein's identity and function.

- Secondary Structure: This refers to local folding patterns within the protein, primarily due to hydrogen bonding between backbone atoms. Common secondary structures include:
  - Alpha helices: Coiled structures stabilized by hydrogen bonds.
  - Beta sheets: Sheet-like structures formed by hydrogen bonds between different segments of the polypeptide chain.
- **Tertiary Structure**: This is the overall three-dimensional shape of a single polypeptide chain. It is formed by interactions between various side chains (R groups) of the amino acids, including hydrophobic interactions, ionic bonds, hydrogen bonds, and disulfide bridges.
- **Quaternary Structure**: Some proteins consist of more than one polypeptide chain. The quaternary structure refers to the arrangement and interaction of these multiple polypeptide subunits.

2. **Factors Influencing Protein Folding**:
   - **Hydrophobic and Hydrophilic Interactions**: Nonpolar side chains tend to be buried

inside the protein structure, away from water, while polar side chains are often on the protein surface.
    - **Chaperones**: Proteins that assist in the proper folding of other proteins, helping prevent aggregation and misfolding.
    - **Post-Translational Modifications**: Chemical modifications after synthesis (like phosphorylation or glycosylation) can affect protein shape and function.

3. **Protein Stability**:
    - Proteins achieve stability through various interactions, including hydrogen bonds, ionic bonds, van der Waals forces, and disulfide bonds. Changes in temperature or pH can disrupt these interactions, leading to denaturation.

4. **Techniques to Determine Protein Structure**:
    - **X-ray Crystallography**: A method to determine the atomic structure of proteins by crystallizing them and analyzing the diffraction pattern of X-rays.
    - **Nuclear Magnetic Resonance (NMR) Spectroscopy**: Used to study the structure of proteins in solution by observing the magnetic properties of nuclei.

- **Cryo-Electron Microscopy**: A technique that visualizes proteins at low temperatures, allowing for the observation of large complexes and membrane proteins.

5. **Importance of 3D Structure**:
   - The specific 3D shape of a protein is crucial for its function. Enzymes, for example, have active sites formed by their unique shapes that allow them to bind to specific substrates.

NMR

any isotope (→ odd atomic / mass no.) with magnetic moment μ, and spin angular momentum, (I):

$$\mu = \hbar \gamma I \qquad (\gamma = \text{gyromagnetic ratio})$$

For spin $1/2$ nuclei, nuclear charge distribution is spherically symmetric

⇒ nucleus ⟷ bar magnet ; interactions are purely magnetic

→ high resolution, higher sensitivity spectra → hence used in NMR

NMR-active nuclei → ^{13}C, ^{15}N, ^{1}H, ^{19}F, ^{31}P ; NMR-silent nuclei : ^{12}C, ^{32}S

Principles of NMR

Energy of interaction (associated with μ, B_0) : $E = -\gamma \hbar m_I B_0 \Rightarrow \Delta E = -\gamma \hbar B_0 \Delta m_I = \gamma \hbar B_0$

$\Delta E = h\nu = \hbar\omega = \hbar\gamma B_0$ $\quad \Rightarrow \gamma = \frac{\mu}{P}$ → magnetic dipole moment

↓ P (principle component of angular mom)

Larmor frequency

(NMR absorption freq)

So, for two energy states α and β, population distribution is given by Boltzmann eq^n :

$$\frac{N_\beta}{N_\alpha} = e^{-\Delta E/kBT}$$

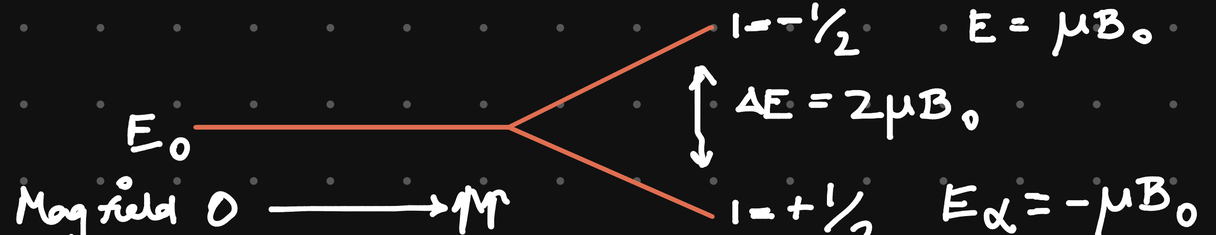
$\downarrow -1/2 \rightarrow E = +\frac{1}{2}\hbar\gamma B_0$

$\uparrow 1/2 \rightarrow E = -\frac{1}{2}\hbar\gamma B_0$

$\Delta E = \hbar\gamma B_0$

magnetic fields of the spinning nuclei will align either with or against ext. field

Photon with right amount of energy can be absorbed → proton flip



How is NMR studied ?

↳ recording (radiofrequency electromagnetic radiation) interaction (nuclei of molecules placed in strong mag. field)

↳ Zeeman effect

NMR → immersion of nuclei in magnetic field → matching EMR frequency with frequency of precession
→ energy absorption

Larmor frequency

due to torque $\mu \times B_0$

If radiofrequency field is now applied in a direction $\perp$ to $\vec{B}$ and frequency = Larmor frequency, energy absorption occurs

↓

sudden flip of nucleus from lower energy orientation (precession in direction of external field) to higher energy orbit (opp. direction)

↓

relaxation to lower energy state through **spin lattice relaxation** by transfer of energy to assembly of surrounding molecules (T_1) or to another nucleus (T_2)

↓

impedance in oscillator coil changes due to relaxation | is measured as a signal in the form of a decaying beat pattern

↓

FID (Free induction decay)

If the populations of upper & lower states are equal, then no energy difference b/w two states if nucleus in its ↑↑ & ↑↓ orientations would exist, and no NMR signal would be observed.

↳ But, Boltzmann excess of nuclei in lower energy state → responsible for NMR signal

Peptide NMR

structure determination by two complementary techniques → NMR
→ crystallography

→ NMR limited by high molecular wt.
→ utilises NOE (nuclear Overhauser effect)