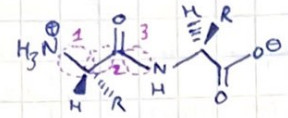
PEPTIDE MAIN CHAIN (BACKBONE) CONFORMATION



In the single bonds of peptide chains, there are certain constraints to the bond rotation which the molecule must adhere to:

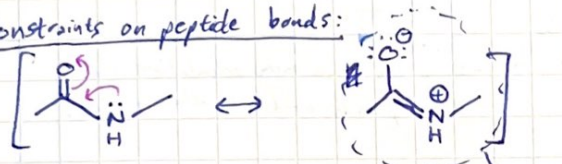
In a polypeptide, there are three types of single bonds:

1 N – Cα single bond

2 Cα – C(=O) single bond

3 C(=O) – N (peptide) single bond

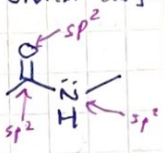
Constraints on peptide bonds:

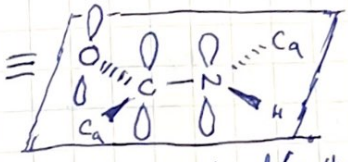


There is a resonance structure present for peptide bonds, and therefore there is a partial negative charge on the oxygen and a partial positive charge on the nitrogen. This confers a partial double bond characteristic to the peptide bond.

∴ NO FREE ROTATION BETWEEN PEPTIDE BONDS!

Orbital diagram:



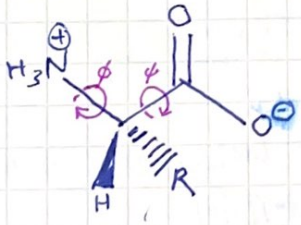


The p orbitals are aligned (so that e⁻ may be shared)

In order for this to happen, all 6 atoms depicted must be in the same plane!

∴ ALL PEPTIDE BONDS ARE PLANAR!

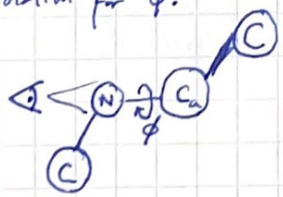
Constraints on main chain conformations



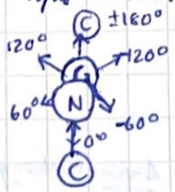
The N – Cα and Cα – C(=O) bonds may freely rotate to an extent. The angles of rotation for the bonds are denoted by φ and ψ respectively.

Determining the value of φ

We use reference bonds to tell the value of rotation for φ:



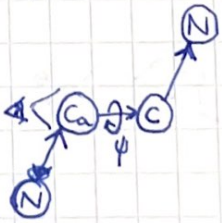
The vertex is always the N superimposed on the alpha carbon:



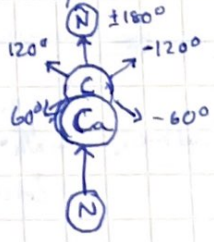
If the value of φ = 180, then the ~~molecules~~ atoms are all in the same plane.

Determining the value of ψ

We use the following reference bonds to tell the value of rotation for ψ:



The vertex is always the Cα superimposed on the carboxy carbon.



ϕ and ψ angles, continued

→ Theoretically, a 360° range of conformations is possible (−180° to +180°)
- However, not all values are equally likely, and some conformations are forbidden.

Ramachandran plot
- Shows all possible conformations of Cα (ϕ, ψ combinations)
- Look for steric clash
- Shows forbidden ϕ, ψ angle pairs that cause steric clash.

PROTEIN FOLDING

→ The protein's function depends on the 3D structure of the protein
→ The 3D structure of a protein is determined by amino acid sequence (primary structure)
→ Folded proteins are stabilized by non covalent interactions
- Folded proteins are not static, and can often undergo small conformational changes which may impact the function of the protein.

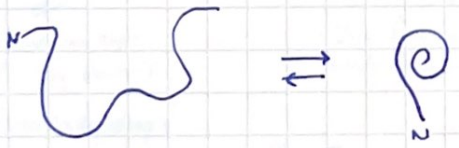
Tertiary protein structure
→ Common theme – nonpolar residues are buried inside the amino acid.
- Polar or charged amino acids typically are on the outside of the protein.
→ The protein interior is very tightly packed and mostly consists of nonpolar side chains with very little water.
→ Why are folded, organized protein structures more entropically favorable than unfolded proteins?
- Nonpolar side chains constrict the possible conformations of water. When nonpolar side chains are hidden from water, entropy of the system increases.

The thermodynamics of protein folding:

protein	$\Delta G°$	$\Delta H°$	$T\Delta S°$	K_{eq} ($= \frac{[folded]}{[unfolded]}$)
RNase A	-44	-280	-236	5.5×10^7
trypsin	-63.7	-275	-211	1.5×10^{11}
myoglobin	-45.4	-6.2	+37.2	4.2×10^7

Note that some protein folding is dependent on $\Delta H°$ more than $T\Delta S°$, whereas other protein folding interactions are the other way around.

The thermodynamics of protein folding
→ Recall that enthalpy (ΔH) is the energy (heat) released when bonds form, or the heat (energy) absorbed when bonds break.



Unfolded protein
- Flexible
- Many conformations
- Higher conf. entropy

Folded protein
- Single conformation
- Rigid
- Low entropy "conformational entropy"

If a protein folds, then $\Delta S_{conformational} < 0$ (disfavors folding!)
- So why do proteins fold?
- Ans: Hydrophobic effect:

$$\Delta S_{hydrophobic} > 0.$$

Reasons why polypeptide chains fold even if $\Delta S_{conformational} < 0$:
→ Hydrophobic effect: $\Delta S_{hydrophobic} > 0$
→ Vander Walls bond formation:
- Strong dependence on distance
- Tightly packed nonpolar groups confer a negative ΔH value: that is, $\Delta H_{vdw} < 0$, which favors folding
→ The energetics of burying a charged group:
- Break bonds to water, without forming new bonds
- Recall example with Na^+ and Cl^- dissolving
- Dehydration of a charged side chain from water has a very positive ΔG.
- $\Delta H >> 0$, ∴ $\Delta G >> 0$
- However, if there are opposing charges inside the structure, a buried salt bridge may be formed.
  - Formation of ionic bonds, $\Delta G° < 0$.

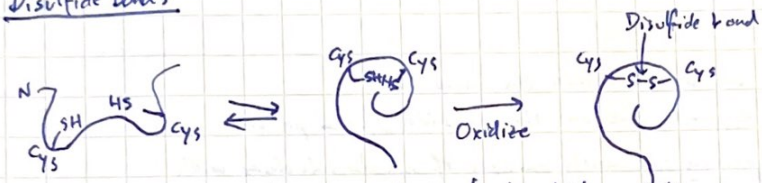
Hydrogen Bonding in Tertiary Protein Structure

→ Overall, forming H-bonds in proteins favors folding.
1) Polar groups exposed to the environment (on surface of protein), $\Delta H \approx 0$, $\Delta G \approx 0$
2) Buried polar groups can be H-bonded to another polar group
   - H bonds to water break, other H-bonds form - $\Delta H \approx 0$.
   - Water is released when groups are buried inside protein: $\Delta S > 0$.
→ Polar groups help to determine specific folded structure: polar groups stick together

Salt Bridges

→ Exposed salt bridges are weaker as they are solvated in water, which has a high dielectric constant.
- On the other hand, buried salt bridges are stronger, as they are buried inside the protein.
- Note that certain charged amino acid residues may be in their uncharged form inside the protein.
  ex.) Instead of Asp - COO^- the R group is Asp - COOH
  or Lys - NH_2 instead of Lys - NH_3^+
  or His H instead of His - H^+ etc.
- Buried salt bridges are rare in proteins.

Disulfide Bonds



Type of covalent bond:
- Only kind of covalent bond in protein.

→ Disulfide bonds are covalent bonds which stabilize some proteins.
→ Disulfide bonds are typically found in extracellular proteins:
- High concentration of reducing agents inside cells prevent many disulfide bonds from forming in an intracellular environment.

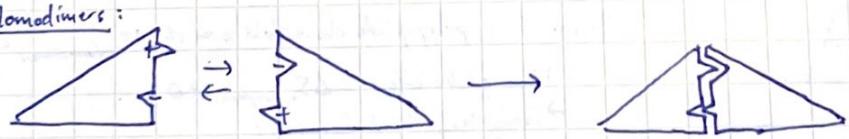
~~Domains in Protein Structure~~

→ Different domains in a protein may confer different functions.

Quaternary structure of proteins

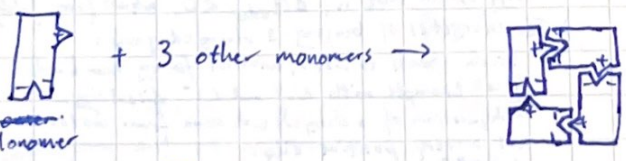
Protein - Protein interactions can be due to:
- Shape
- Chemical complementarity:

Homodimers:



A homodimer

Tetramers:



~~Monomer~~
Monomer

A homotetramer

Ex.) Hemoglobin is a tetramer.
Specifically, it is a heterotetramer composed of 2 α-chains and 2 β-chains.

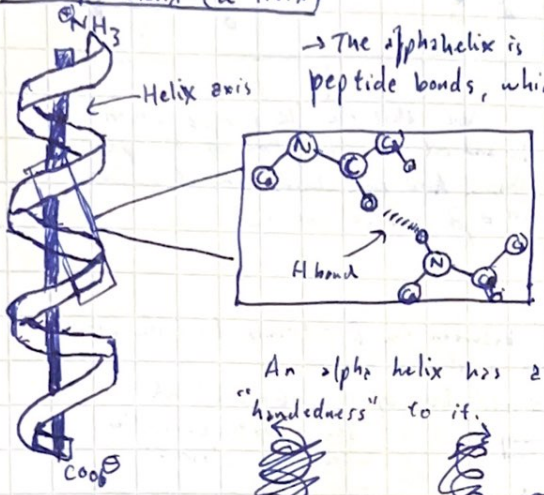
WHEN THE MONOMER IS SUS

Secondary Structures of Proteins

→ There are two types of secondary structures in proteins: the alpha helix (α) and the beta pleated sheet (β).

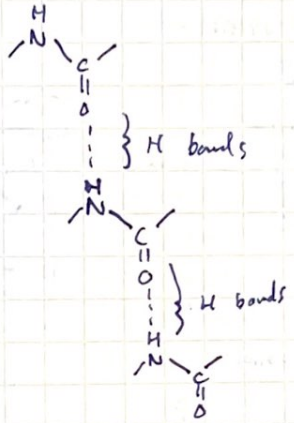
Other smaller types of structures:
- β-strand (one single strand of β-pleated sheets)
- β-turns

The Alpha Helix (α-Helix)



→ The alpha helix is held together by hydrogen bond interactions between peptide bonds, which are in the same plane.

- Since peptide bonds are all in the same plane, in an alpha helix, the bonds will be oriented the same, with oxygens pointing down and nitrogens facing up:



The Φ and Ψ angles between the bonds (amino acid residues) of the α-helix are quite predictable.

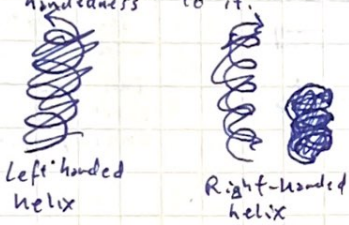
They are usually as such:

$\Phi = -57^{\circ}$

$\Psi = -47^{\circ}$

Which sits in the bottom left to middle left portion of the Ramachandran plot.

An alpha helix has a "handedness" to it.

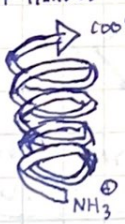


Use the right hand rule to determine the handedness of a helix.

Left handed helix:

View from top: winds up clockwise

Right handed helix:



View from top: winds up counterclockwise.

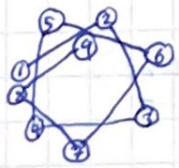
- The alpha helix is always right handed as found in proteins.
- The most common form of DNA in the cell also exists as a right handed helix.
- There are 3.6 amino acid residues in one helical turn (about 5.4 Å in length)
  - This is why for side chain interactions i and i+4 is the most common (followed by i+3)

How many

The R-group branching off the α-helix does not affect the structure of the helix much, as the side chains are not directly involved in the structure of the α-helix

Therefore, α-helices may be amphipathic.

Helical wheel view:

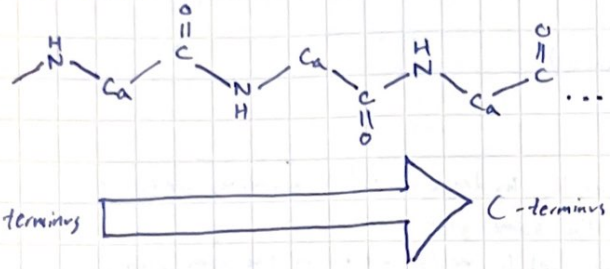


etc.

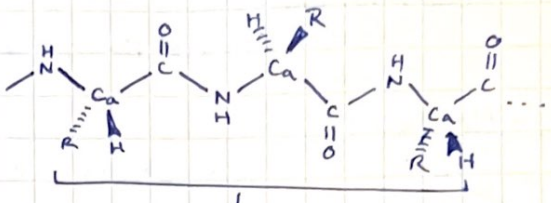
One side may be structured with polar side chains while the other side may contain more non polar side chains.

The β-strand and the β-pleated sheets

A beta-strand:

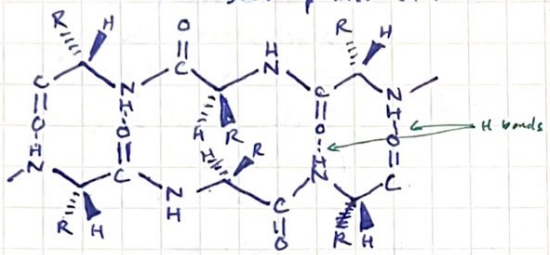


Recall the stereospecific structure of a polypeptide



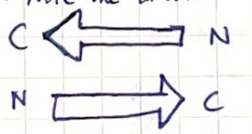
Note that the R groups alternate in and out of the plane of the page. This is how side chains are structured in a β-pleated sheet.

Beta strands to beta pleated sheets:



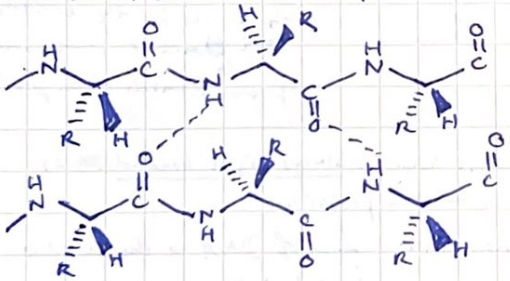
→ The H-bonds between the β-strands keep the strands together to form a pleated sheet.

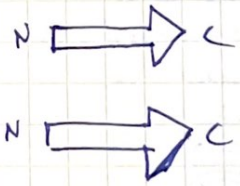
→ Note the directionality of each beta strand:



- They oppose each other when they alternate.
- This kind of β-pleated sheet is known as anti-parallel.

Beta sheets could also be parallel:





Parallel β-strand.

Both types of beta pleated sheets are similar in structural stability.

In the beta pleated sheet, the side chains can stick out with each other and interact
- The space between the side chains is usually not enough to allow water in

In the Ramachandran plot, the β-sheets of antiparallel and parallel β-sheets are typically found in the top left quadrant.

Beta-turns:

→ Usually found on the surface of proteins, turns 180° in 4 residues.

→ There are two types of β-turns:

- One type of β-turn has an H-bond between aa #1 and #4
- Residue two in this β-turn has a ϕ angle of −60°, and this second residue is often proline.
  - The proline prevents the C–N bond from rotating, trapping it in a single conformation
  - The proline confines ϕ to between −20 and −120°.

Type 1 β-turn:

→ Another type of β-turn typically has
- Glycine as the 3rd residue
- There is a glycine at this residue, giving the β turn a ϕ and ψ angle of ∓90 and ±20.

PROPERTIES OF THE ALPHA HELIX

The effects of side chains on formation & stability of an alpha helix:

→ How likely is it for each amino acid to form a part of an alpha helix?
- Using a helical propensity table, we can determine the propensity of a certain amino acid to take up an α-helical conformation:

AA	$\Delta\Delta G^\circ$ (kJ/mol)	AA	$\Delta\Delta G^\circ$ (kJ/mol)
Ala	0	Leu	0.79
Arg	0.3	Lys	0.63
Asn	3	Met	0.88
Asp	2.5	Phe	2.0
Cys	3	Pro	>4
Gln	1.3	Ser	2.2
Glu	1.4	Thr	2.4
Gly	4.6	Tyr	2.0
His	2.6	Trp	2.0
Ile	1.4	Val	2.1

- In this table, a larger number will indicate a lower helical propensity. Therefore, AAs such as Alanine and Arginine are the most likely to be found in an α-helix, whereas proline and glycine are found very rarely in α-helices.
- The experiment to determine the $\Delta\Delta G^\circ$ values used the change in one amino acid residue of a string of amino acids of all one residue (for example, Ala_{10}), and the measurement of ΔG° for the Ala_{10} and +Leu chains:

Ala_{10}:

$^{\oplus}H_3H-Ala_2Ala_2Ala_2Ala_2Ala_2Ala_2Ala_2Ala_2Ala_2Ala_2-COO^{\ominus}$

Unfolded (in a random coil)

$K = \frac{[helix]}{[coil]}$ $\Delta G^\circ_{(Ala_{10})} = -RT\ln(K)$

↓ Change to Leucine

$^{\oplus}H_3H-Ala_2Ala_2Ala_2Leu\,Ala_2Ala_2Ala_2Ala_2Ala_2Ala_2-COO^{\ominus}$

$K = \frac{[helix]}{[coil]}$ $\Delta G^\circ_{(+Leu)} = -RT\ln(K)$

$\Delta\Delta G^\circ_{(Leu)} = \Delta G^\circ_{(+Leu)} - \Delta G^\circ_{(Ala_{10})}$

~~Stability of α-helical side chains:~~

How do side chains interact in an α-helix?

Review of non-covalent interactions:

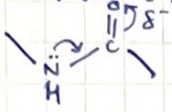
→ Hydrogen bonds
- Depends on distance (donor → acceptor ≤ 3Å)
- Depends on orientation

→ Ionic bonds in water
- Weak (H_2O has high dielectric constant)
- Salt bridges are typically 4–5 Å apart
- Orientation not important

→ Van der Waals
- Focus on carbon atoms
- Atoms must be very close: C→C ≅ 4–5 Å
- Orientation not important.

Alpha Helix Dipole:

Recall that there is a resonance structure for a peptide bond:



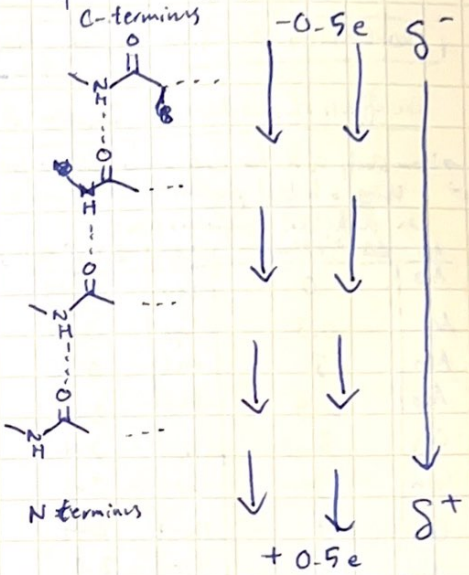
which gives a partial negative charge on the carbonyl oxygen, and a partial positive charge on the ~~hydrogen for nitrogen~~ hydrogen*

*the hydrogen actually contains a positive charge rather than the nitrogen here

In the α-helix, the peptide dipoles line up to give the helix a large overall dipole, with a net positive at the N-terminus and a net negative at the C terminus.

- Often this charge interacts with other biomolecules in enzymatic reactions.

The dipole is oriented toward the N-terminus and away from the C-terminus:



Charged residues in α-helices:

There are four main charged residues that contribute to the overall structure of a protein: Asp and Glu (negatively charged) and Lys and Arg (negatively charged).

When analyses of α-helices are done, one can find that Asp and Glu are more commonly found on the N-terminus, whereas Lys and Arg are more commonly found on the C-terminus.

The reasoning here is mainly due to the entropically favorable interaction between the negatively charged residues and the positively charged N-terminus and vice versa.

This stabilizes the helix.

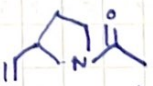
α-helix capping

α-helixes are typically capped by residues with a low helical propensity. Residues such as Serine, Threonine, Histidine, Asparagine, Aspartate (most of which are uncharged polar amino acids) can actually stabilize an amino acid by capping it.

→ The C and N terminus of the α-helix want to form H-bonds. However, if the terminus projects toward the interior of the protein, where there is a much less polar environment, there should be a polar residue to help stabilize it.

- Note however that if any of these uncharged polar residues are inside the α-helix itself, this destabilizes the helix due to their high $\Delta\Delta G^\circ$ value.

Addendum - Proline in an α-helix & Glycine

→ Proline removes a hydrogen from the peptide bond:  , which causes problems for an α-helix which is mainly stabilized by H bonds. Proline destabilizes an α-helix

→ Glycine has a very high conformational entropy, therefore $\Delta S_{conformational} \ll 0$, which disfavors folding and destabilizes the α-helix.