Molecular Biophysics

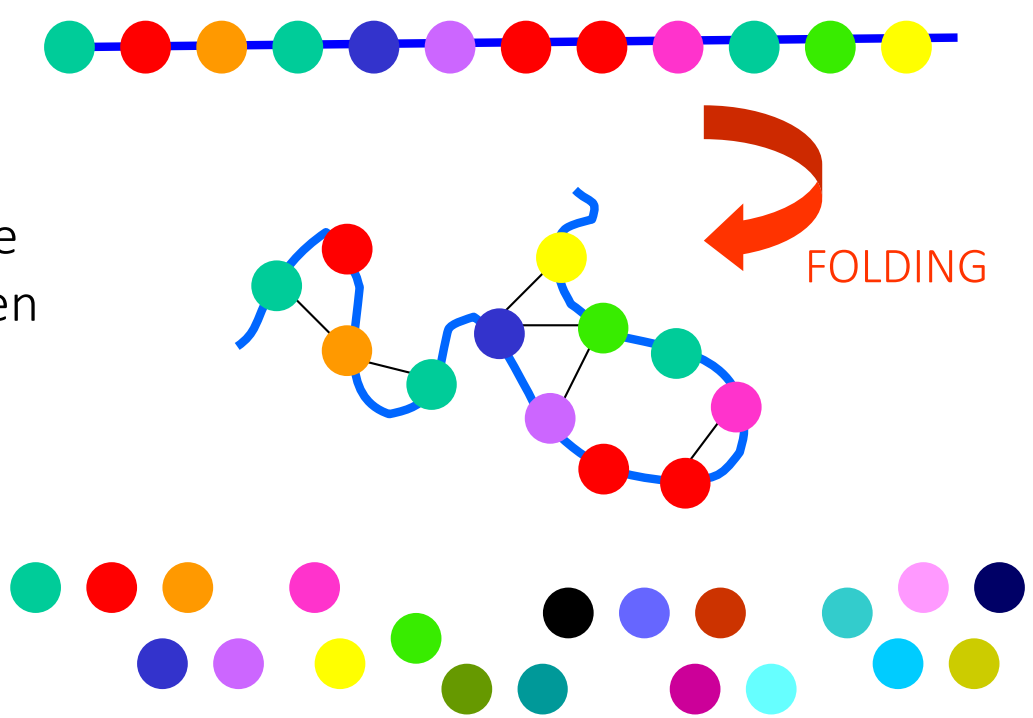
Amino acids and proteins

Proteins

Proteins are linear chains of amino acids.

These chains fold in 3D due to the non-covalent interactions between regions of the linear sequence

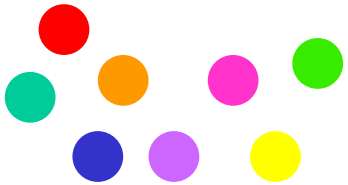
There are 20 different types of amino acid, each with different physico-chemical properties.



- FUNCTION DEPENDS ON 3D STRUCTURE
- 3D STRUCTURE DEPENDS ON SEQUENCE
- SEQUENCE IS DETERMINED GENETICALLY

Overview of protein architecture

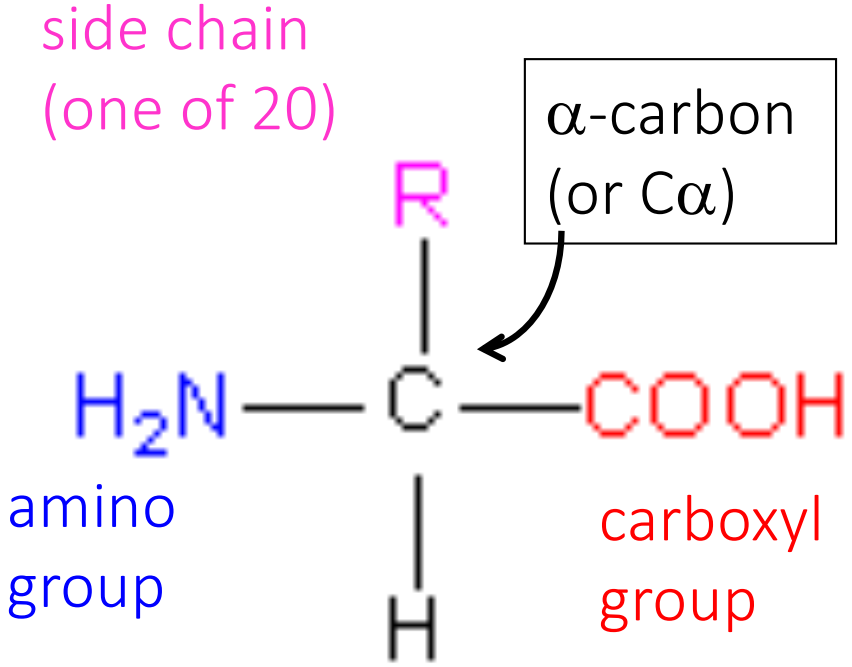
1) structure and chemistry of amino acids



2) how amino acids are linked together through peptide bonds to form a polypeptide chain

3) how the polypeptide chain folds in 3D
   - the Ramachandran plot
   - secondary structure elements (α-helix and β-sheet)
   - how secondary structure elements pack together

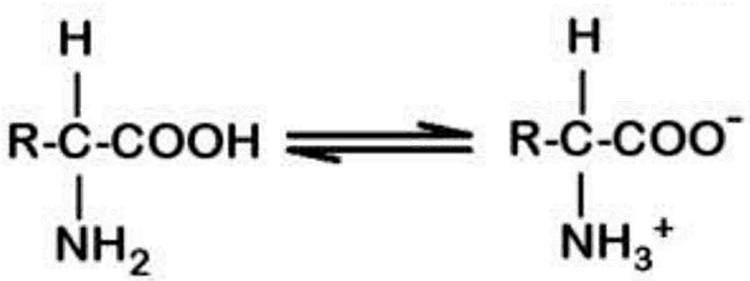
Structure of amino acids



At neutral pH:

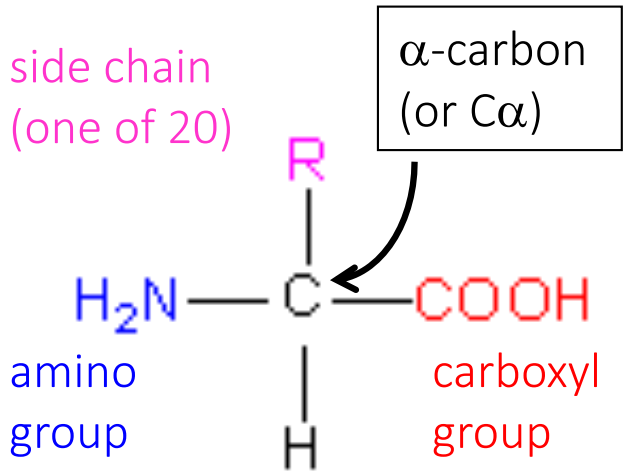
NH_2 is a base —— NH_3^+

COOH is an acid —— COO^-

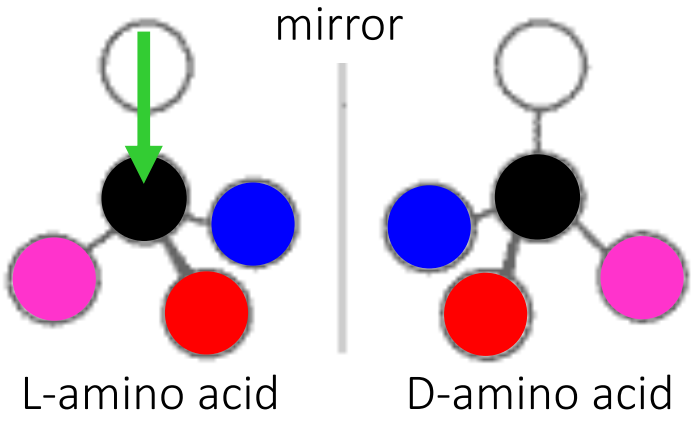


zwitterion
(dipolar form)

Structure of amino acids

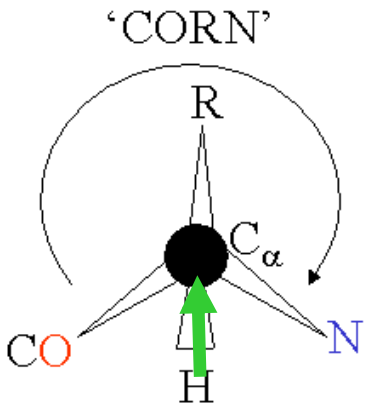


The Cα is an asymmetric carbon (bound to 4 different groups) and therefore is a chiral centre.
Two configurations (stereoisomers) are possible, which are one the mirror image of the other:

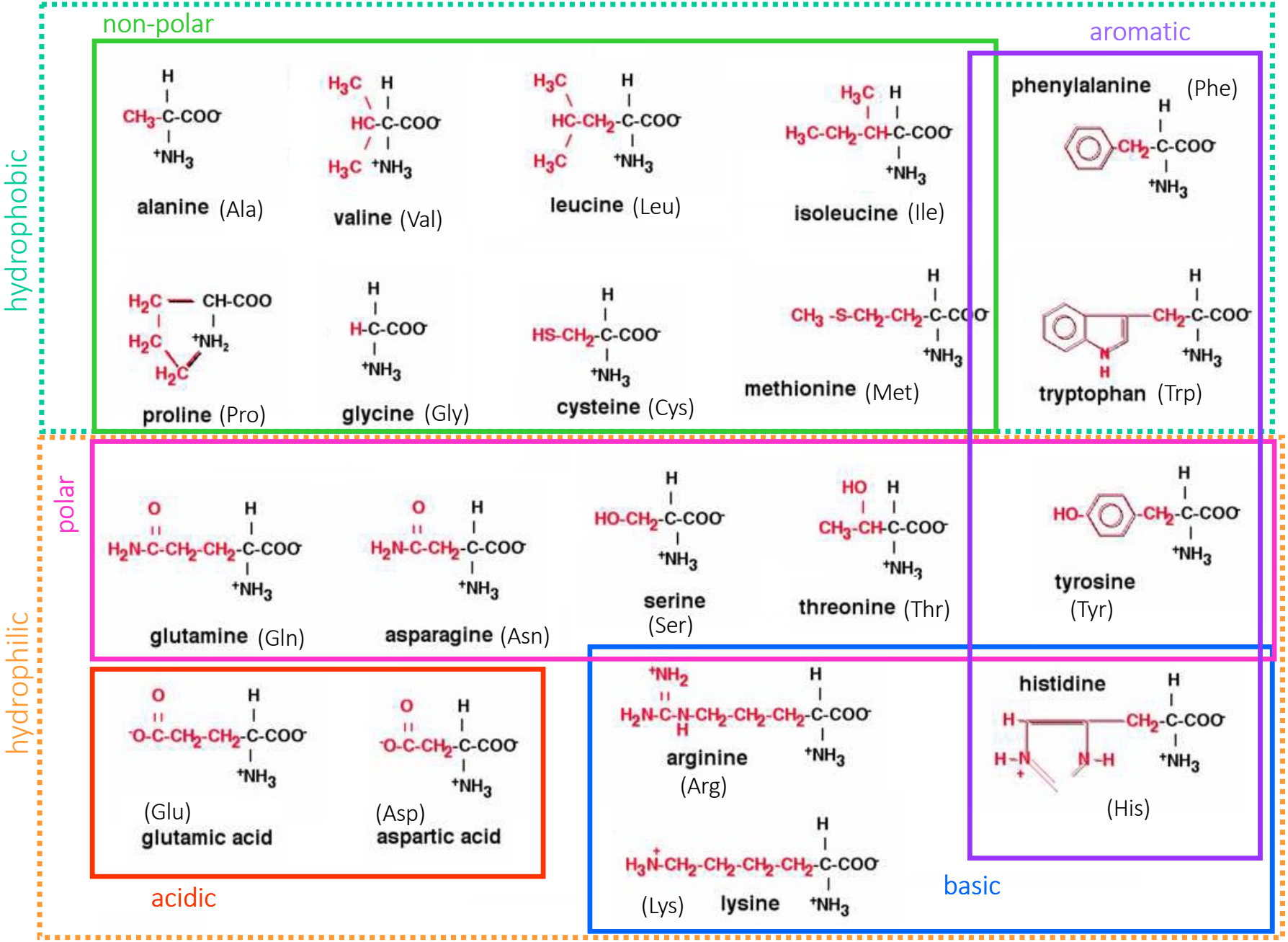


all amino acids in proteins are L!!

CORN rule:
looking down the H-Cα bond for an L amino acid we read the groups CO-R-N clockwise



The 20 amino acids:



Properties of amino-acid side chains

R varies in

- shape
- size
- charge
- hydrophobicity
- reactivity

Hydrophobic amino acids: insoluble or slightly soluble in water

(side chains made of C, H, S - atoms with similar electronegativity)

avoid water by coalescing into oily droplets - the same forces causes hydrophobic aa to pack together in the interior of proteins, away from acqueous solutions.

Hydrophylic amino acids: soluble in water

(side chains contains atoms such as N and O, which can make HB)

- polar
- basic
- acidic

Charged side chains

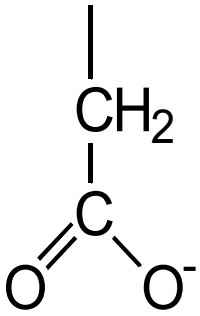
at neutral pH

negative charge

positive charge

Aspartic acid

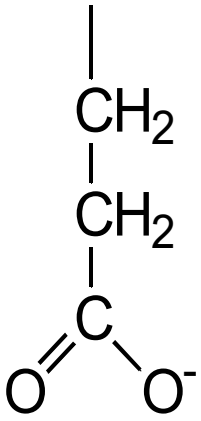
(Asp or D)



pK_a=3.9

Glutamic acid

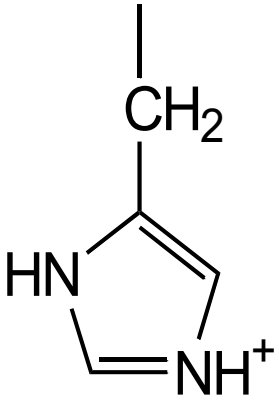
(Glu or E)



pK_a=4.2

Histidine

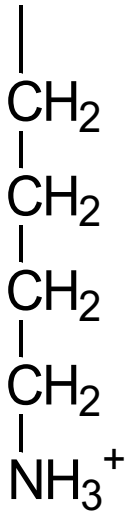
(His or H)



pK_a=6.1

Lysine

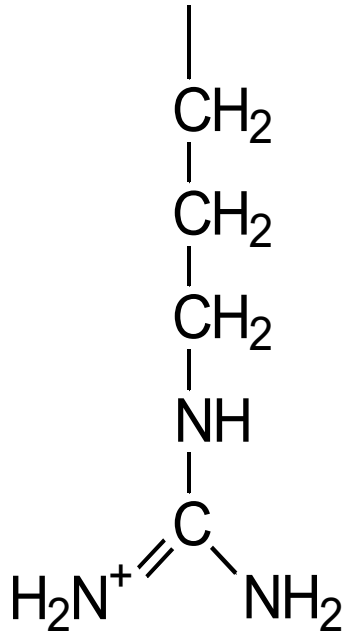
(Lys or K)



pK_a=10.8

Arginine

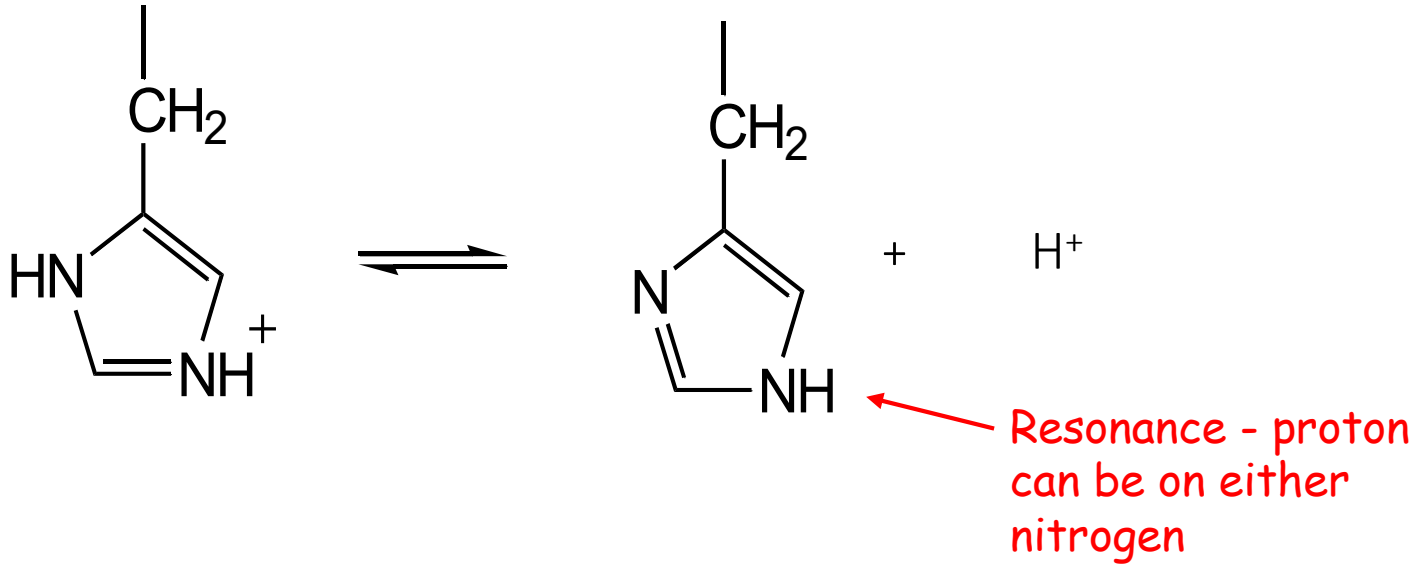
(Arg or R)



pK_a=12.5

Histidine

The side chain of a histidine residue is uncharged at high pH

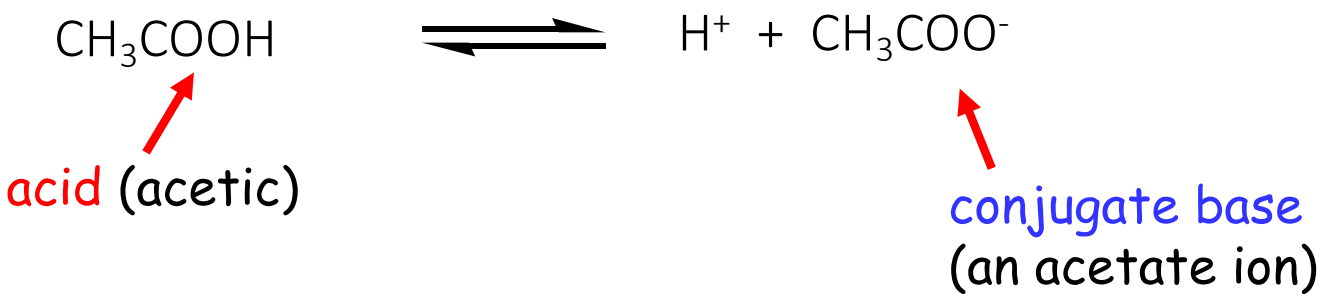


$pK_a = 6.1$

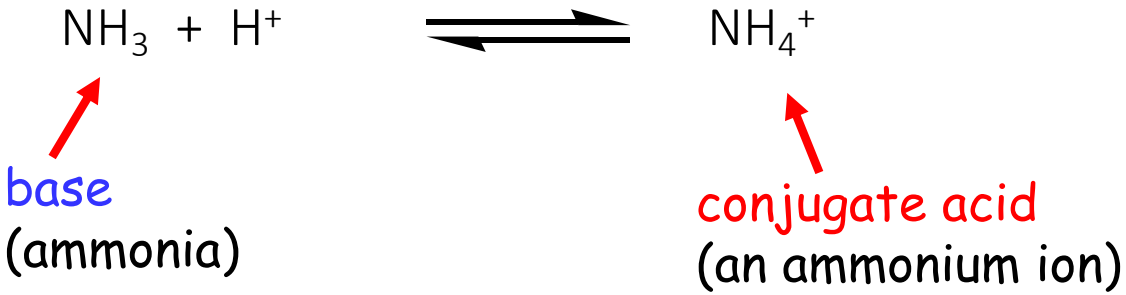
Charge on His residue in a protein at neutral pH will depend on the local environment - small shifts of pH or local environment can change the charge of a His (important in enzyme mechanism)

Acids and bases

- an acid is a proton (H^+) donor



- a base is a proton acceptor



pK_a

For an acid:

$$HA \rightleftharpoons H^+ + A^-$$

and the dissociation constant is

$$K_a = \frac{[H^+][A^-]}{[HA]}$$

K is an equilibrium constant (in this case an acid dissociation constant)

where [X] indicates the molar concentration of X

We define:

$$pK_a = -\log_{10}(K_a)$$

eg $K_a = 10^{-9}$ then $pK_a = 9$

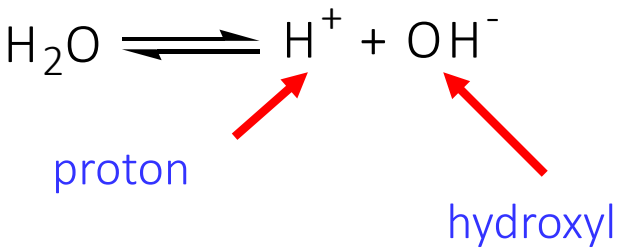
More dissociated → more equilibrium to right → larger K_a → smaller pK_a

Smaller pK_a → strong acid / weak conjugate base

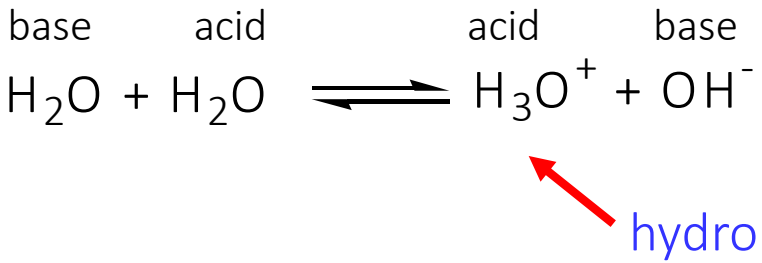
Larger pK_a → weak acid / strong conjugate base

Dissociation of water

Water has a small tendency to dissociate:



In reality, protons are not free in solution and it is more correct to write:



but we can ignore this and only consider protons (H^+).

For pure water: $[H^+] = [OH^-] = \sqrt{10^{-14}} = 10^{-7} M$ ie .1 μM

pH

The acidity of a solution is measured on a "pH" scale where:

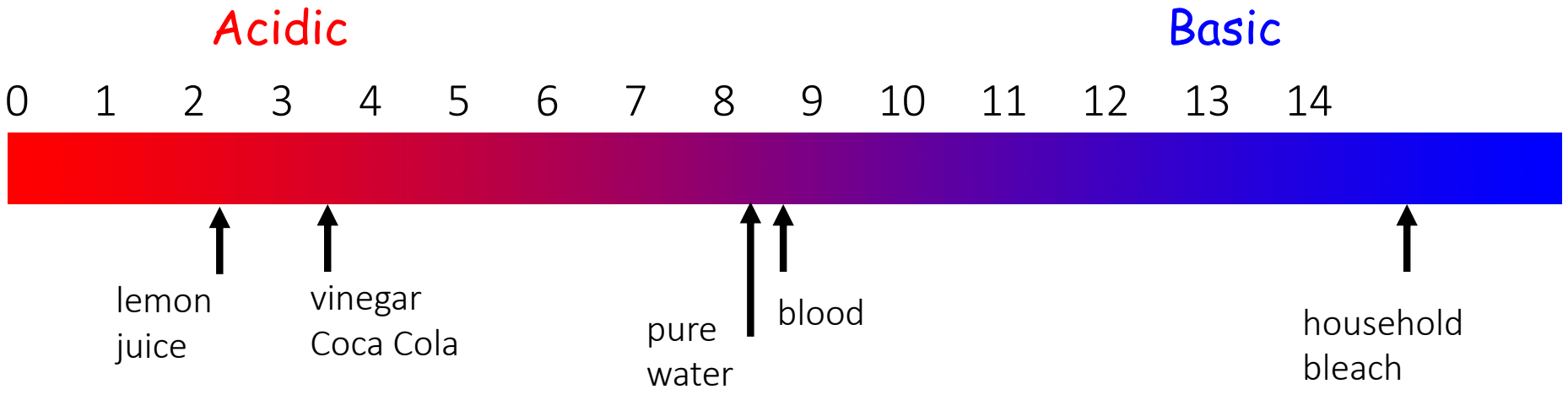
$$pH = -\log_{10}[H^+]$$

For pure water $[H^+] = 10^{-7}$ M and thus the pH = $-\log(10^{-7}) = 7$

pH < 7.0 then [H+] > [OH-] → solution is acidic

pH > 7.0 then [H+] < [OH-] → solution is basic (or alkaline)

Practical pH range is between 0 and 14:



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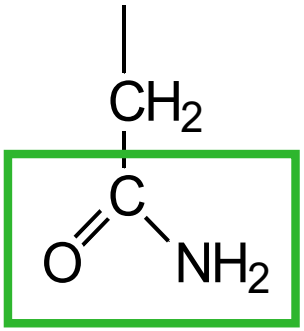
The interior of a cell is kept close to neutrality by the presence of buffers: weal acid and bases that can release or take up protons near pH 7, keeping the environment of the cell relatively constant under a variety of conditions.

Uncharged polar side chains

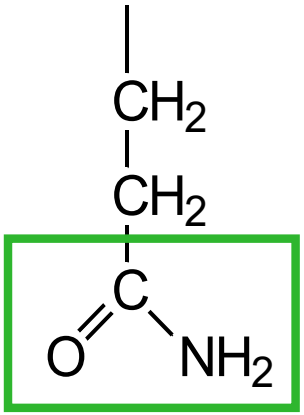
Asparagine

(Asn or N)



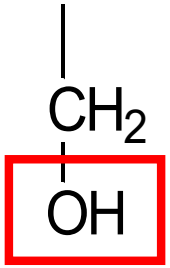
Glutamine

(Gln or Q)



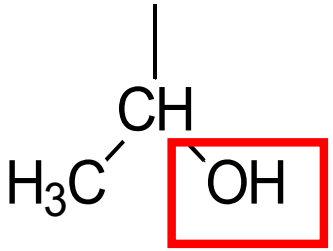
Serine

(Ser or S)



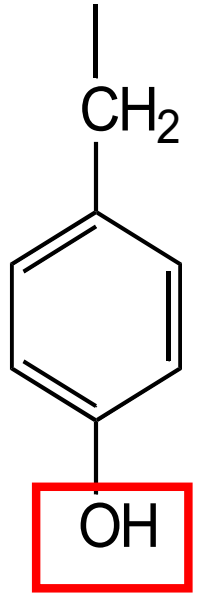
Threonine

(Thr or T)



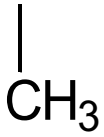
Tyrosine

(Tyr or Y)

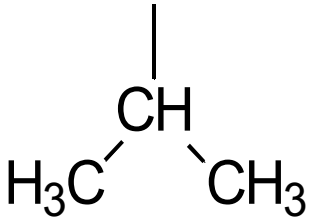


Non-polar side chains

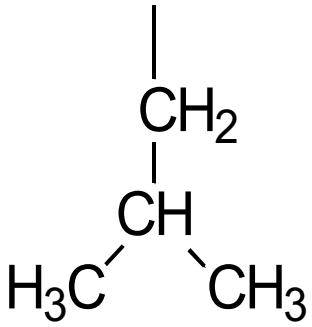
Alanine
(Ala or A)



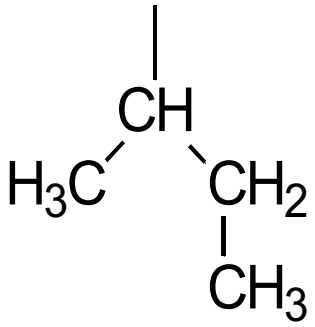
Valine
(Val or V)



Leucine
(Leu or L)



Isoleucine
(Ile or I)

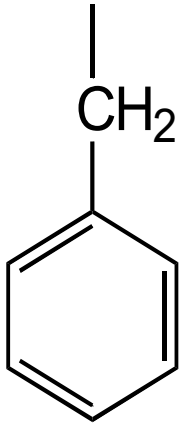


SMALL

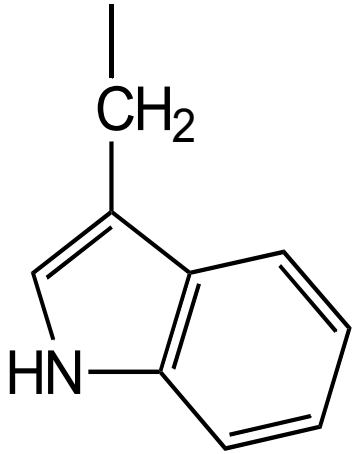
Glycine
(Gly or G)

Glycine is the smallest amino acid: has no side chain and is not chiral

Phenylalanine
(Phe or F)



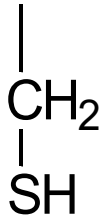
Tryptophan
(Trp or W)



LARGE

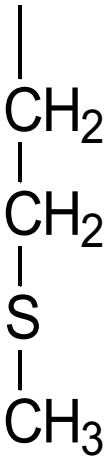
Remaining non-polar side chains

Cysteine
(Cys or C)



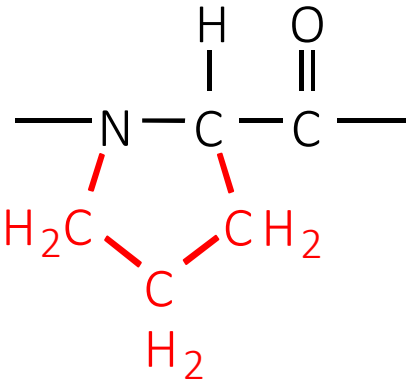
Cysteine residues can form disulphide bonds.

Methionine
(Met or M)



Proteins usually start with a Met

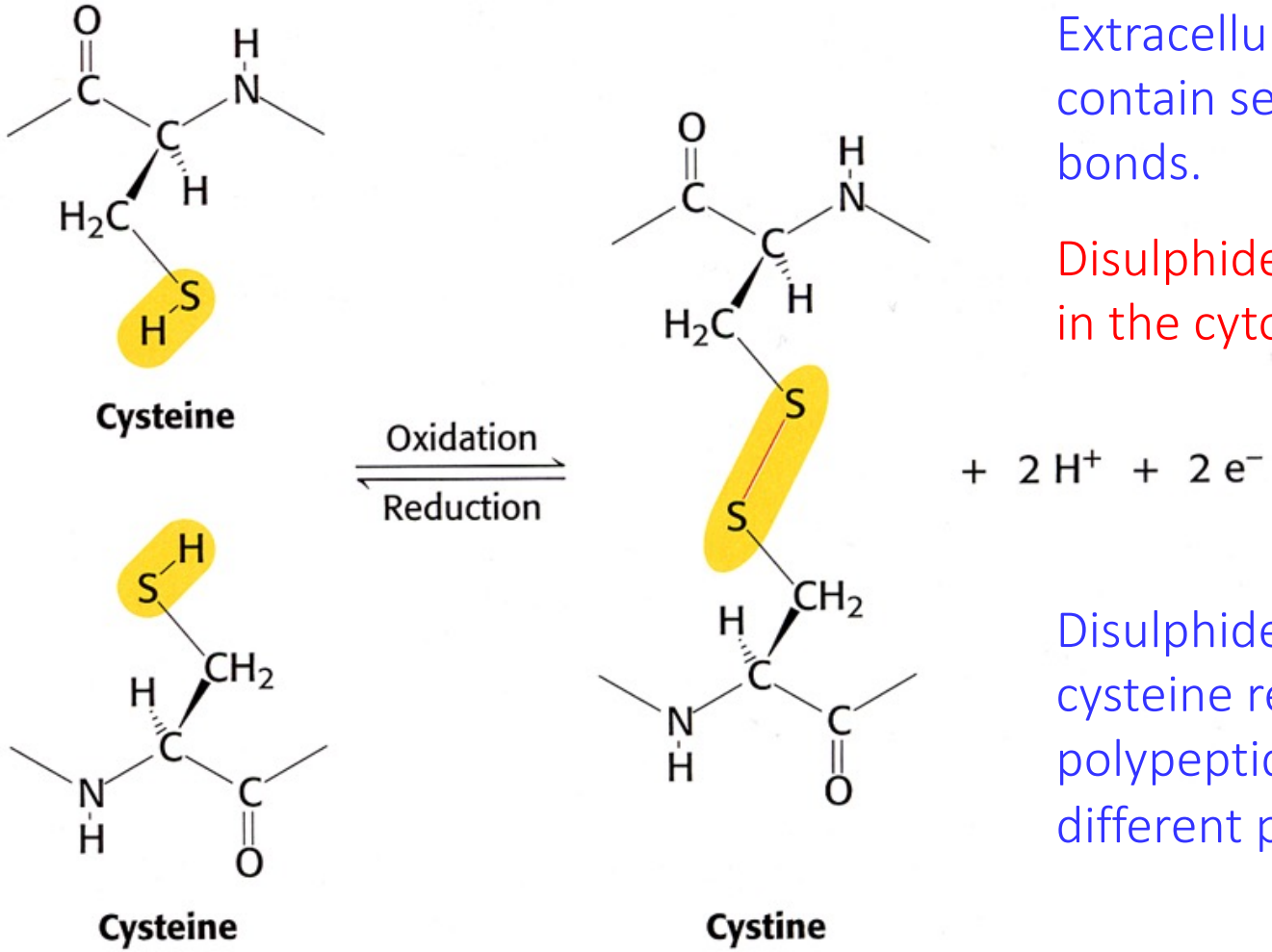
Proline
(Pro or P)



The side chain is covalently bonded to the main chain nitrogen. This locks the conformation around the N-Cα bond – reducing flexibility of the polypeptide chain.

Disulphide bonds

A disulphide bond can form between two cysteine residues in proteins.



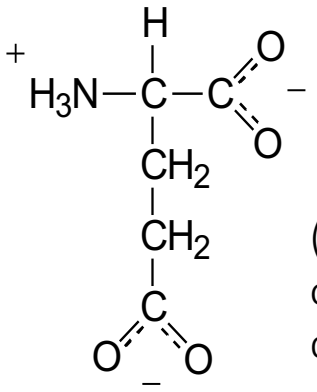
Extracellular proteins often contain several disulphide bonds.

Disulphide bonds do not form in the cytosol.

Disulphide bonds can link cysteine residues within a single polypeptide chain or on different polypeptide chains.

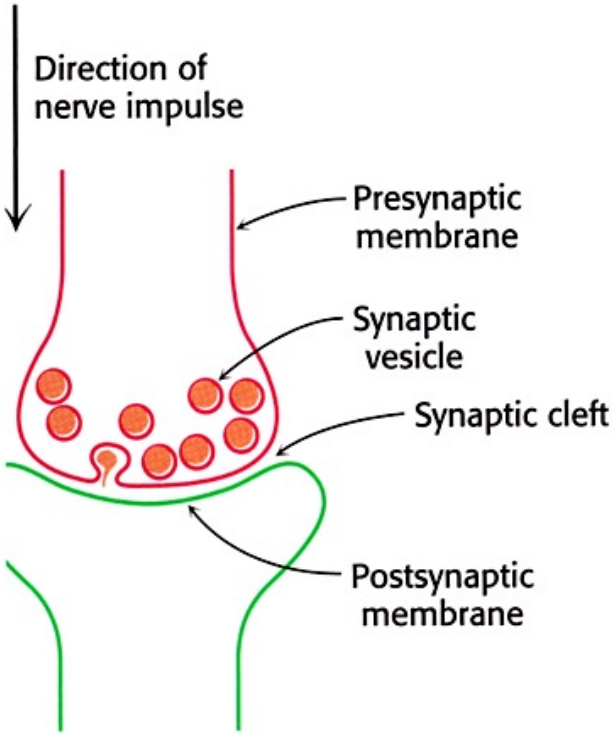
Amino acids as neurotransmitters

Glutamate is an excitatory neurotransmitters

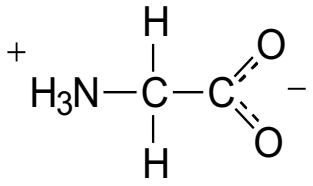


Glutamate

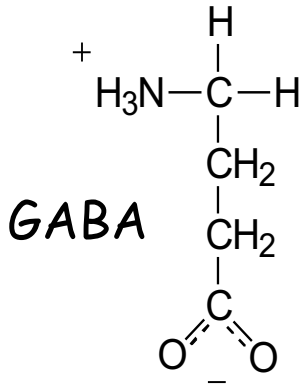
(release of glutamate leads to the opening of cation channels and the depolarisation of the membrane)



Glycine and γ-aminobutyric acid (GABA) are inhibitory neurotransmitters



Glycine



GABA

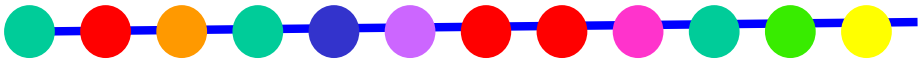
(release of glycine or GABA leads to the opening of Cl^- channels)

formed from glutamate by loss of carboxyl group

Overview of protein architecture

1) structure and chemistry of amino acids

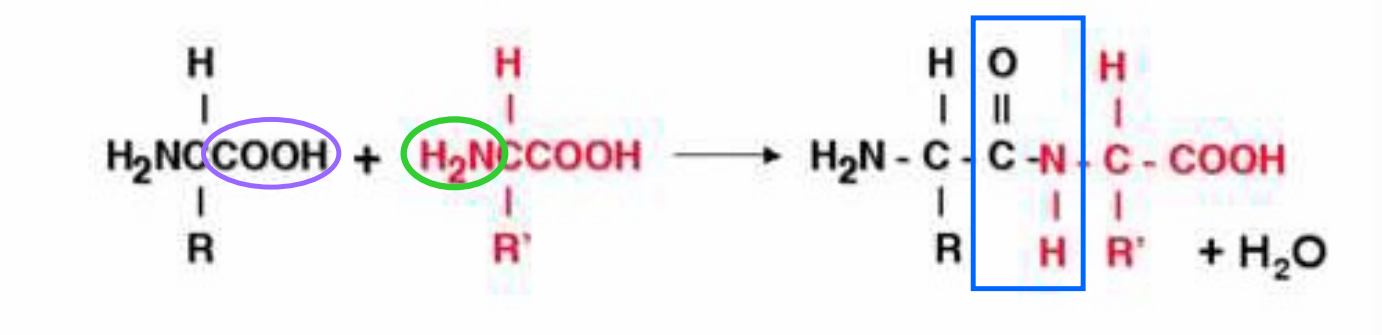
2) how amino acids are linked together through peptide bonds to form a polypeptide chain



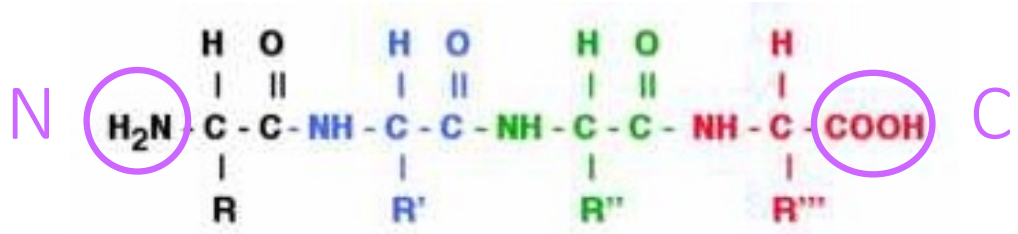
3) how the polypeptide chain folds in 3D
   - the Ramachandran plot
   - secondary structure elements (α-helix and β-sheet)
   - how secondary structure elements pack together

The peptide bond

The amino acids of a protein are joined together through a covalent bond between the carboxyl group of one aa and the amino group of the next aa (peptide bond).



This produce a chain of amino acids which is asymmetric: on one end there is a free NH_2 group (N terminus) and at the other end a free COOH (C terminus).



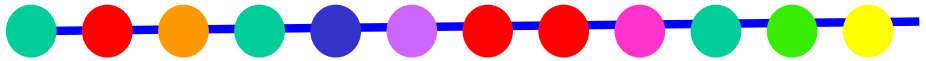
arg	ala	phe	gly
R	A	F	G

A peptide/protein sequence is always given from the N to the C terminus (here RAFG).

Primary structure

the linear sequence of amino acids

- the sequence is always written N⊢→C
- each protein has a unique and defined sequence, which is genetically determined
- a typical protein contains 100-1000 aa
- sequencing=determining the number and order of the aa in the chain



In 1953 Saenger sequenced insulin (Nobel price); now it is more common to sequence the corresponding gene.

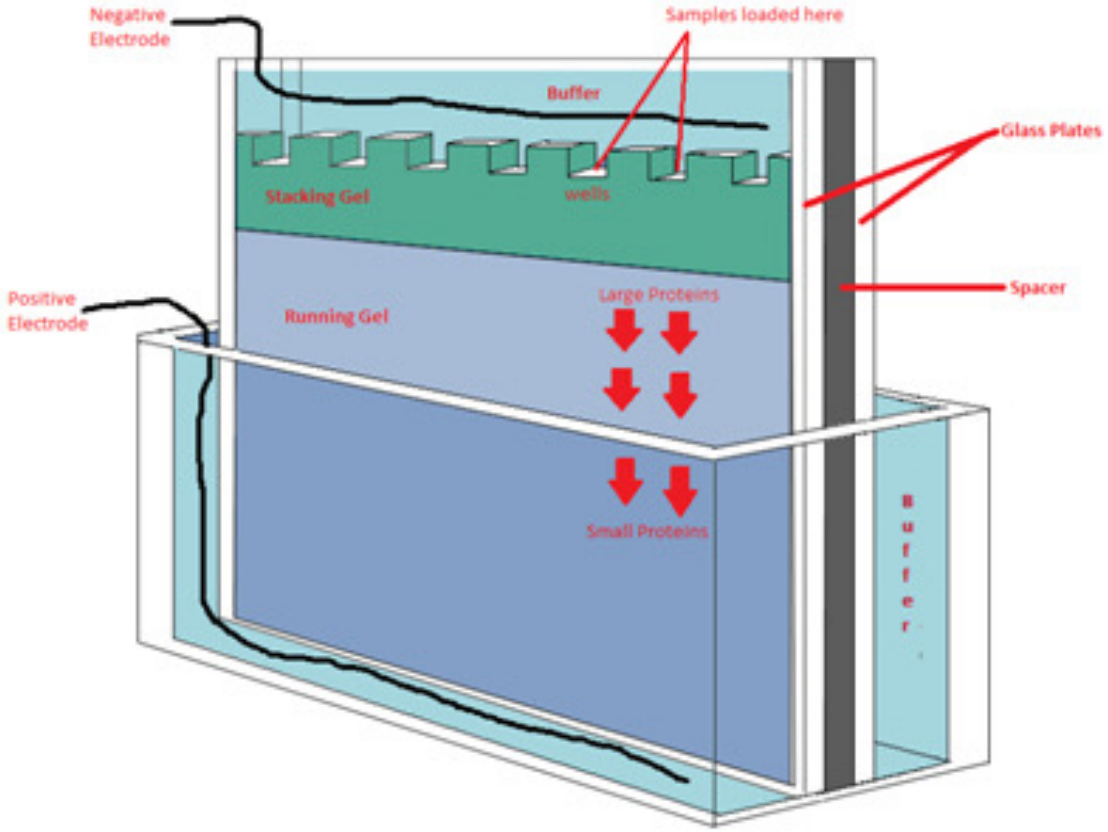
We can guess the function of an unknown protein if it shows sequence similarity to a protein of known function.

Often we know the sequence of the same protein from different organisms: these are more and more different the more the organisms have diverged in evolution.
Proteins evolve by changing (little by little) their aminoacid sequence

Mass of a protein

1D –SDS-PAGE

Sodium Dodecyl Sulphate - PolyAcrylamide Gel Electrophoresis



Concentration of acrylamide (%)	Protein size (kDa)
5	36-200
7.5	24-200
10	14-200
15	14-60

a method that separates protein by molecular weight over a range of about 10 to 300 kilodaltons (kDa). Samples are weighed and dissolved in sodium dodecyl sulfate (SDS). SDS is a negatively charged detergent that has both hydrophilic and hydrophobic regions. SDS likes to bind to proteins (1.4 g SDS/1 g protein) and to be in water. This SDS- protein-water interaction allows water insoluble proteins to dissolve in water, and to dissolve protein mixtures. Proteins are completely denatured. When an electric field is applied, the negative charge of the SDS causes the proteins to move through a clear acrylamide matrix toward the positive electrode. This matrix has holes in it that sieve out the proteins by molecular weight. Large proteins move more slowly through the matrix than the smaller proteins thereby separating proteins by molecular weight.

1 Da = 1 g/mol

Average mol. weigth of 1 aminoacid: 110 Da

Noncovalent bonds and folding

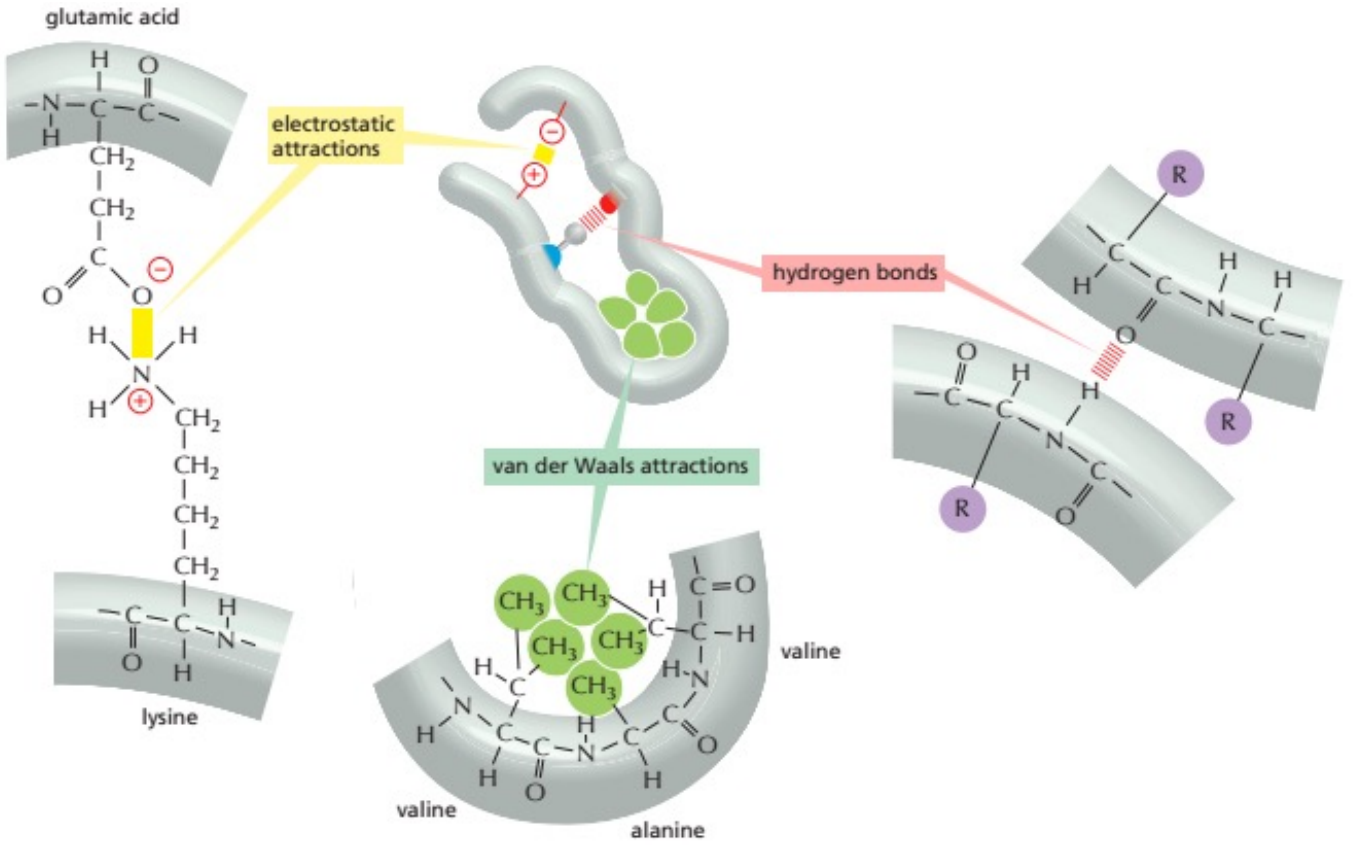
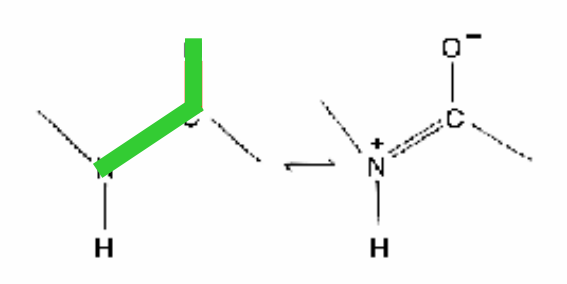
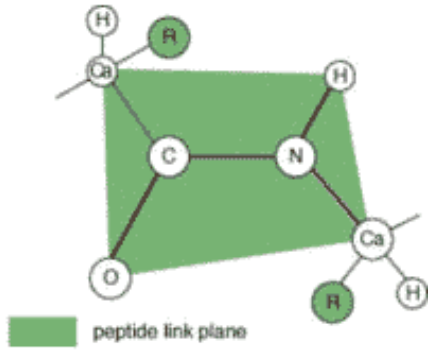


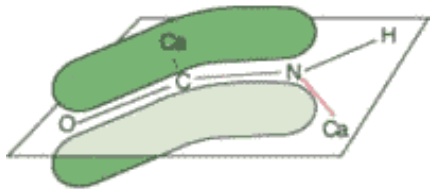
Figure 3–4 Three types of noncovalent bonds help proteins fold. Although a single one of these bonds is quite weak, many of them act together to create a strong bonding arrangement, as in the example shown. As in the previous figure, R is used as a general designation for an amino acid side chain.

Planarity of the peptide bond



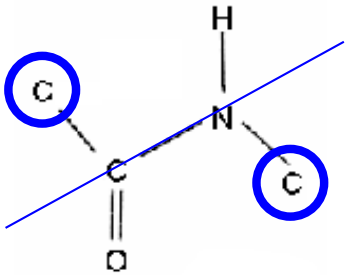
Partial double bond character of the N-C bond leads to restricted rotation
the region NH-CO is planar:

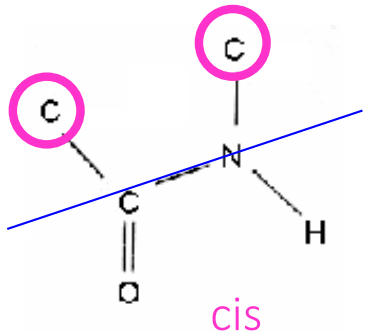




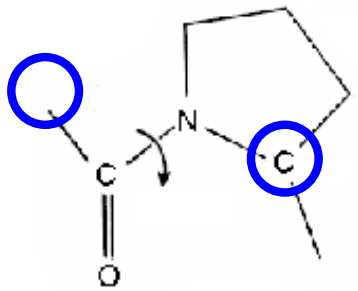
delocalisation of the π electrons over the entire peptide bond, rather than simply over the C=O bond

The peptide bond can assume a trans or a cis conformation: the trans form is favoured 1000:1.

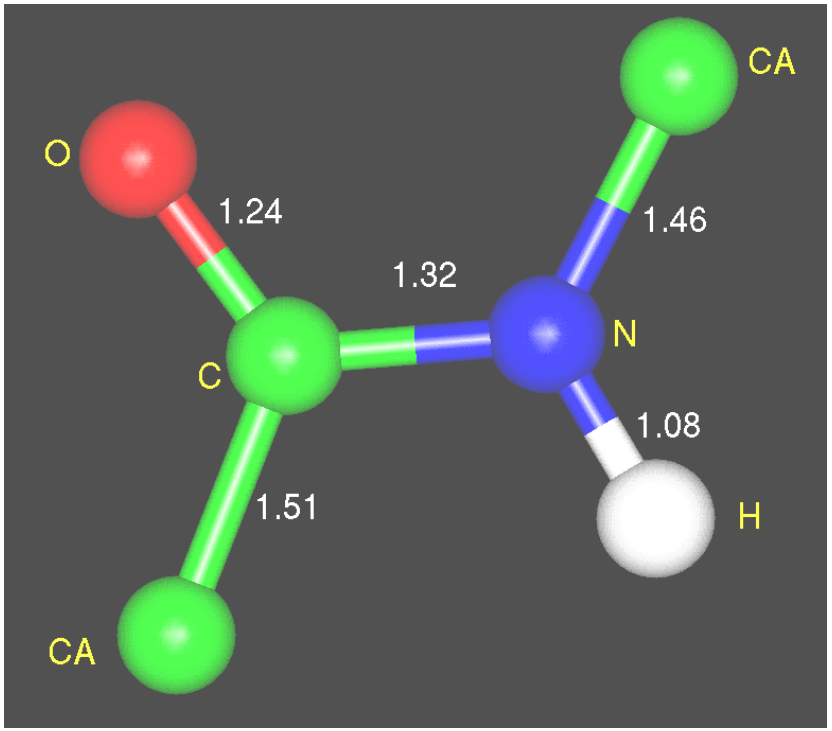

trans


cis

In the case of prolines, the trans form is only favoured 15:1


trans
Proline

The ideal peptide



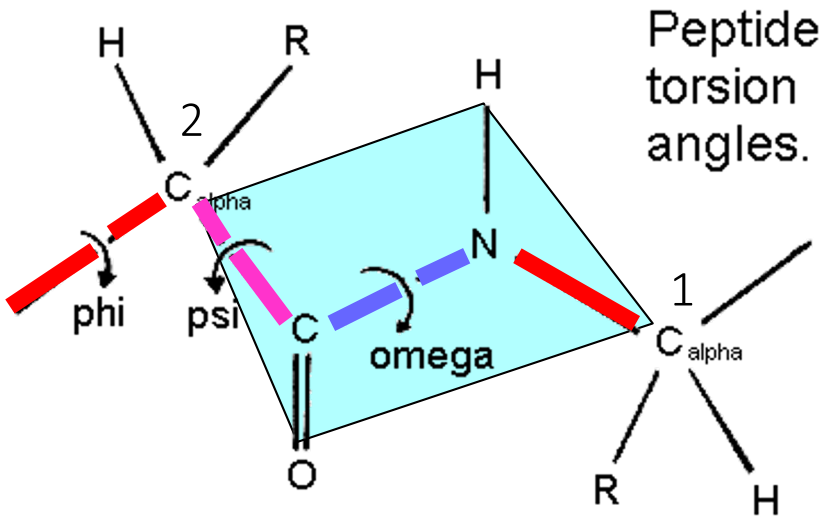
C-N single bond ~ 1.48 Å

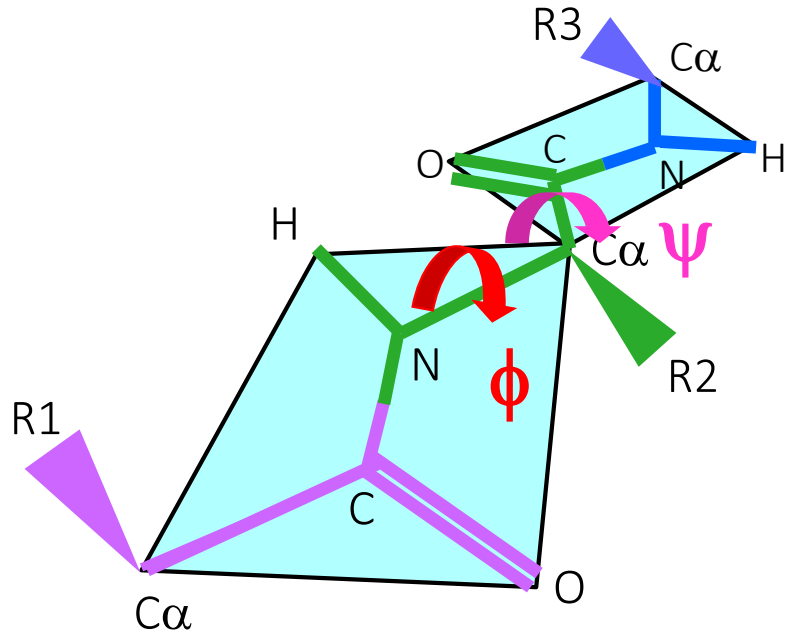
C=O double bond ~ 1.20 Å

peptide bond C-N = 1.32 Å (i.e. shorter than a single bond due to partial double bond character) while C=O bond is slightly longer

Peptide bond	Average length	Single Bond	Average length	Hydrogen Bond	Average (±0.3)
Cα – C	1.51 (Å)	C - C	1.54 (Å)	O-H --- O-H	2.8 (Å)
C - N	1.32 (Å)	C - N	1.48 (Å)	N-H --- O=C	2.9 (Å)
N - Cα	1.46 (Å)	C - O	1.43 (Å)	O-H --- O=C	2.8 (Å)

The torsion angles ψ and ϕ





omega (**ω**) = rotation around C-N bond
not allowed because of resonance,
therefore ω=180° (for trans)

planar region

phi (**ϕ**)= free rotation around Cα-N bond

psi (**ψ**)= free rotation around Cα-C bond

The main chain conformation is defined by the sequence of the (ψ,ϕ) angles: the list of the (ψ,ϕ) for each amino acid dictate the fold of the polypeptide chain, i.e. the 3D structure of the protein

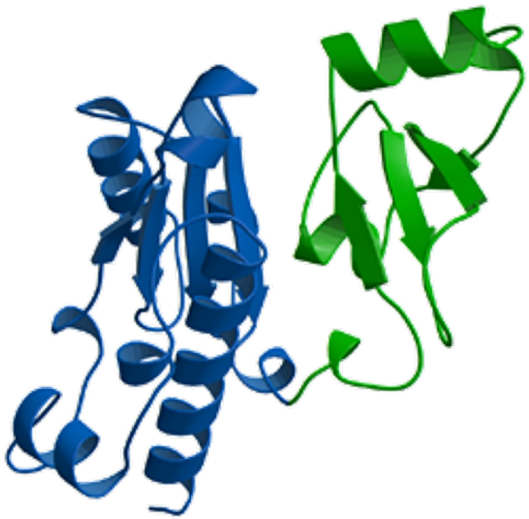
Overview of protein architecture

1) structure and chemistry of amino acids

2) how amino acids are linked together through peptide bonds to form a polypeptide chain

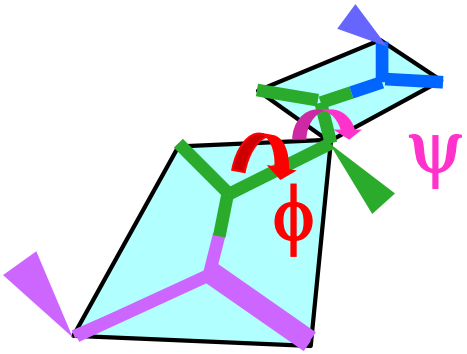
3) how the polypeptide chain folds in 3D:

   - the Ramachandran plot
   - secondary structure elements (α-helix and β-sheet)
   - how secondary structure elements pack together



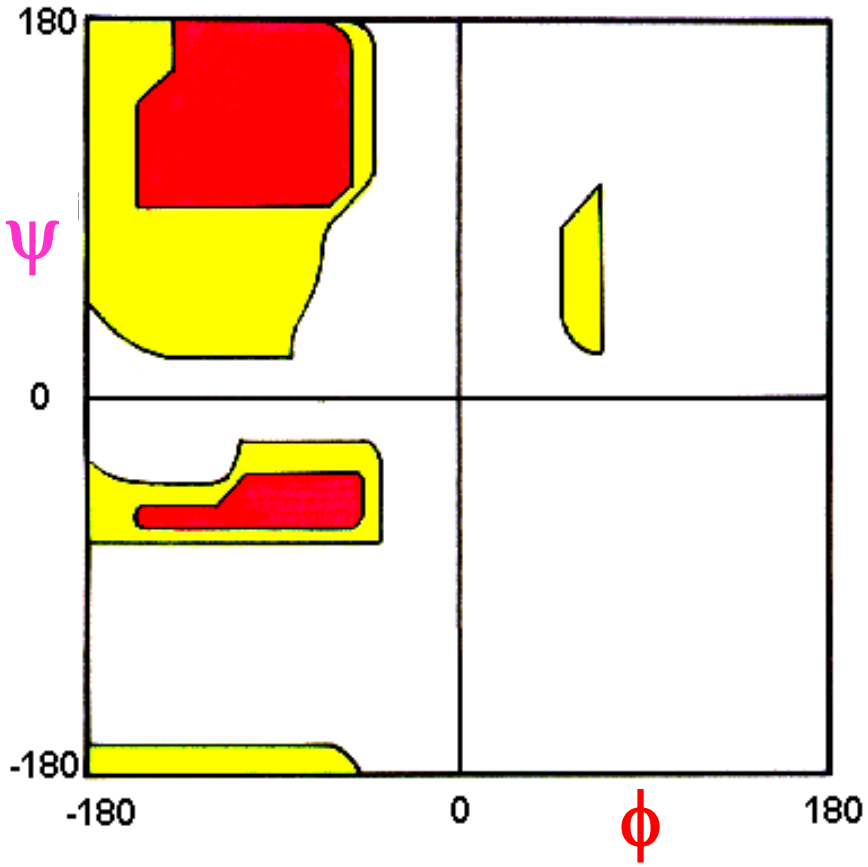
The Ramachandran plot

Because of steric clashes, only certain combinations of torsion angles are allowed: we can plot these allowed combinations in the (ψ,ϕ) plane - this is called the Ramachandran plot.

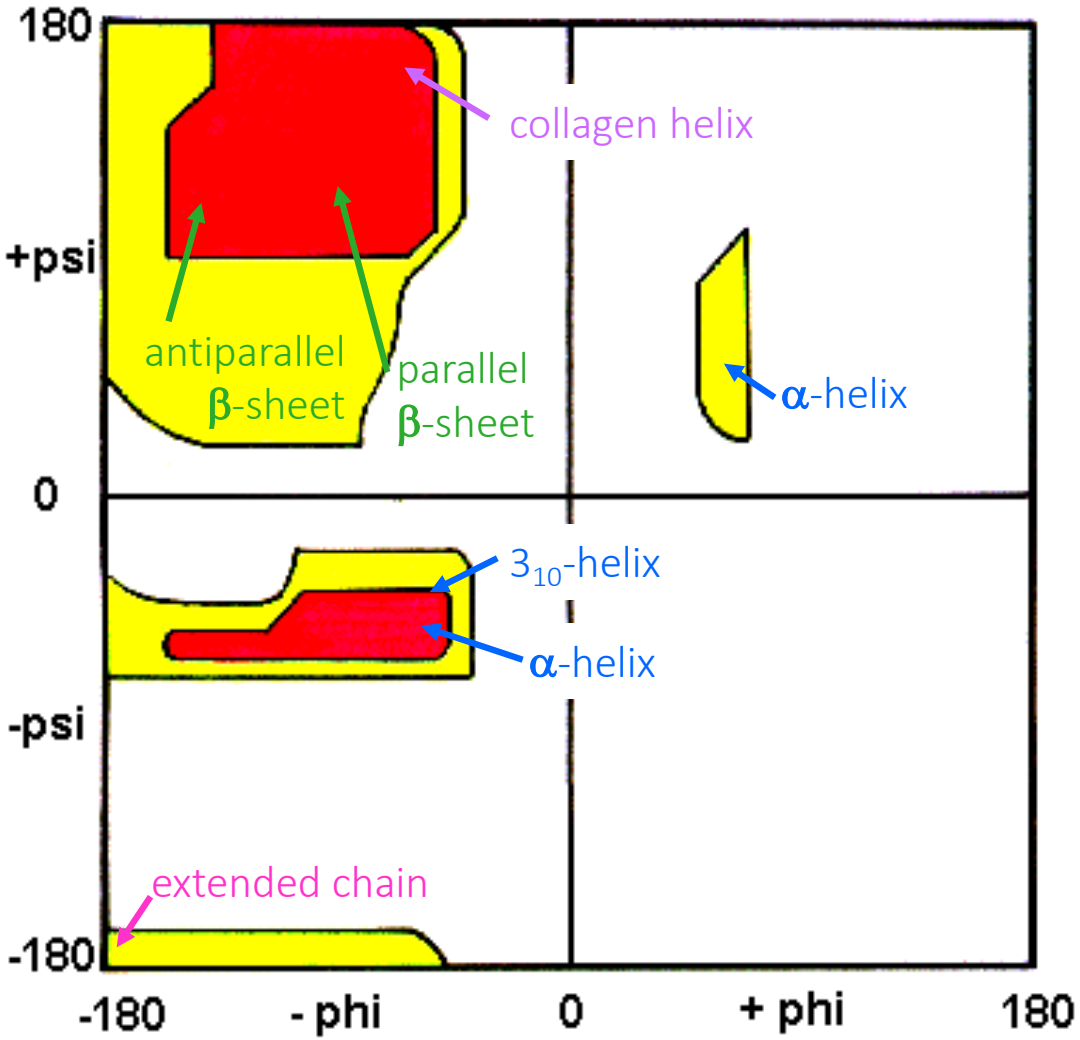


favorable regions for all aa

allowed regions for all aa



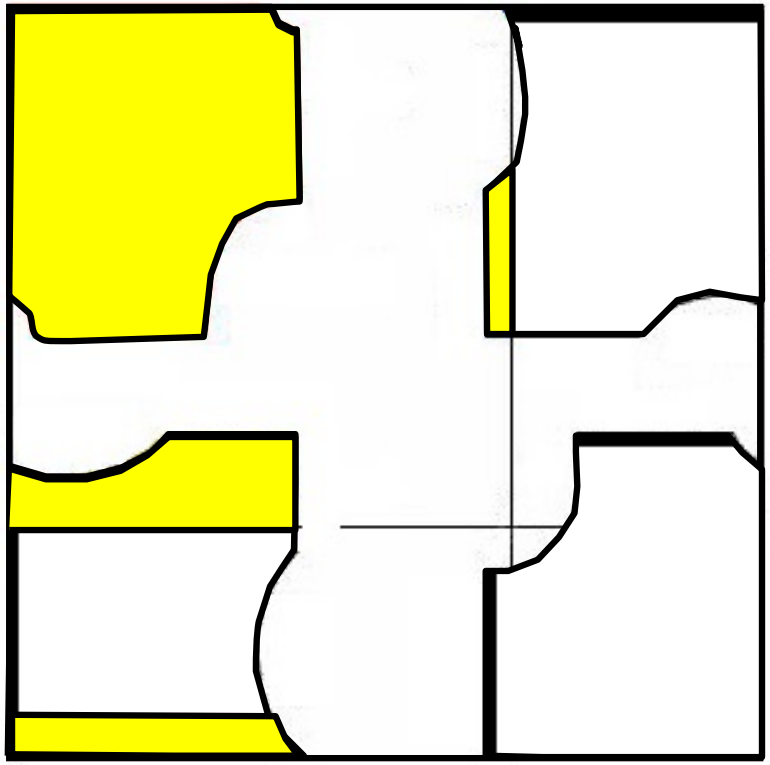
The Ramachandran plot: secondary structure elements



The main chain conformation is defined by the sequence of the (ψ,ϕ) angles: the list of the (ψ,ϕ) for each amino acid dictate the folding of the polypeptide chain, i.e. the 3D structure of the protein

Therefore secondary structure elements will be associated with specific average values of ψ, ϕ and therefore with specific regions of the Ramachandran plot.

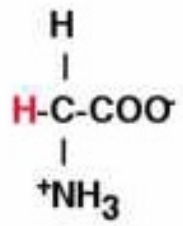
The Ramachandran plot: glycine residues



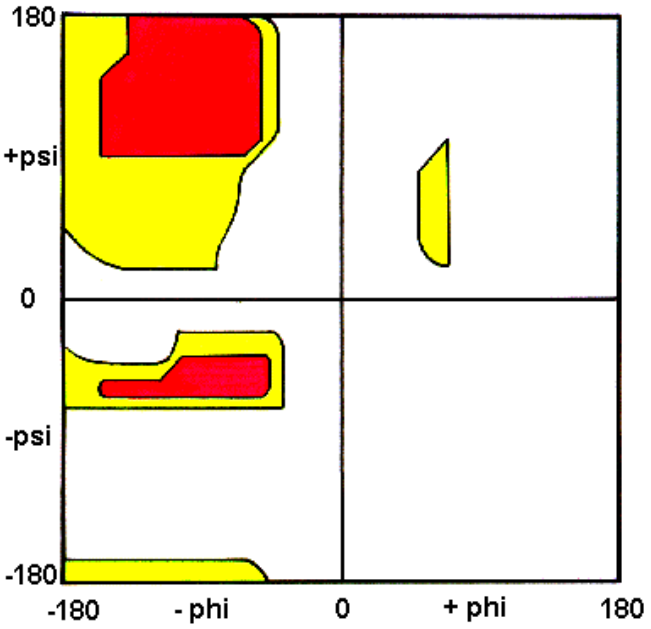
regions allowed only for glyine

regions allowed only for all aa

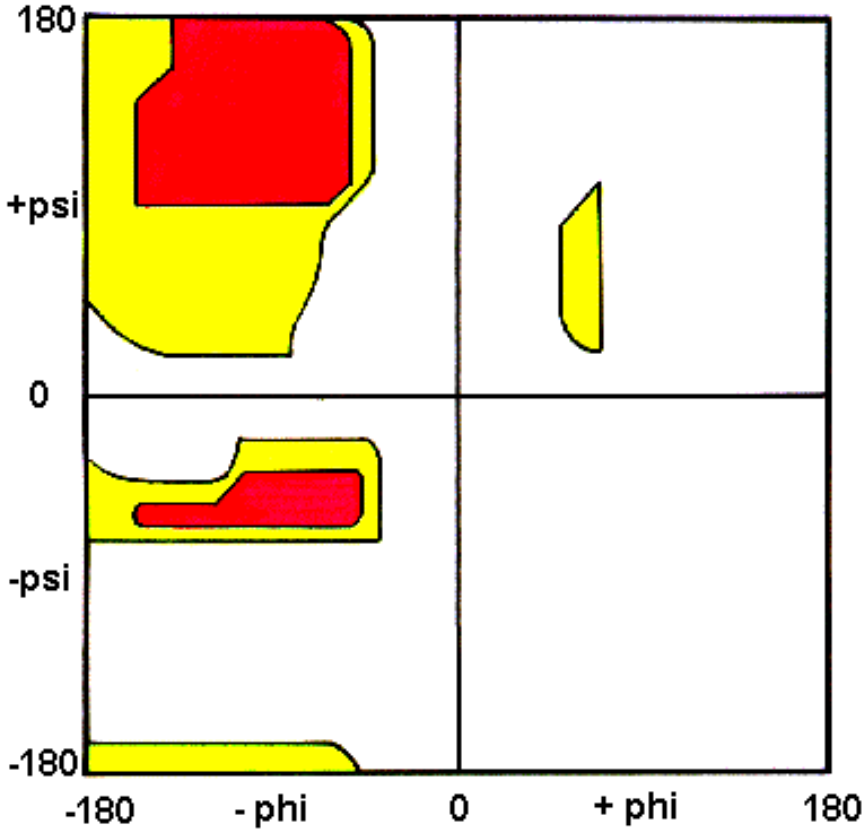
glycine



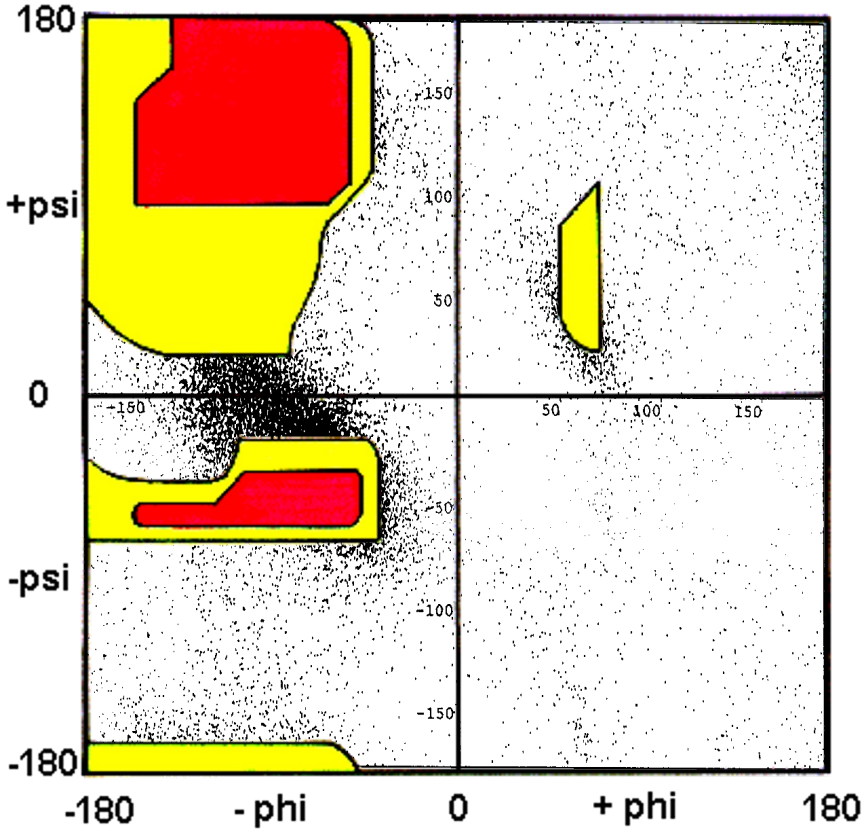
side chain=H (very small, hardly any steric hindrance)



The Ramachandran plot:in real life



Here the allowed regions are derived from
a theoretical analysis of the steric constraints



This is the statistical distribution observed
for many protein (excluding Gly and Pro).

There can be occasional outliers, but they should be rare (placing a residue in an unfavourable conformation has an energetic cost)

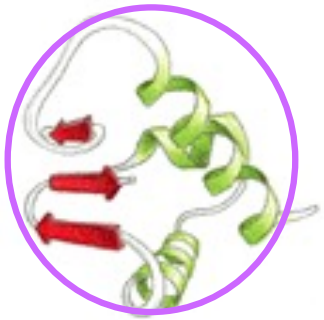
Protein architecture

Secondary structure

local organisation of the polypeptide chain

α-helix

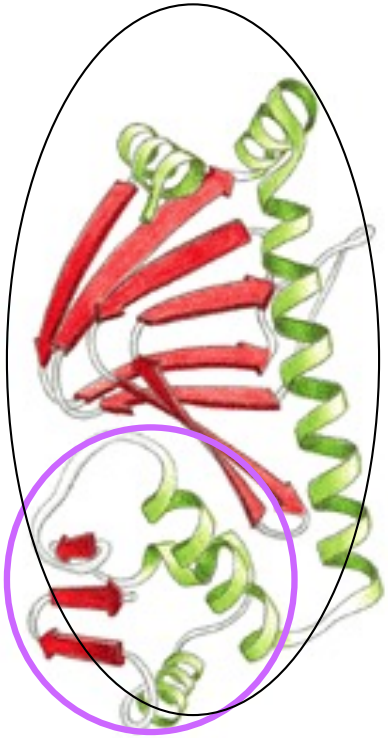
β-sheet



domain

Tertiary structure

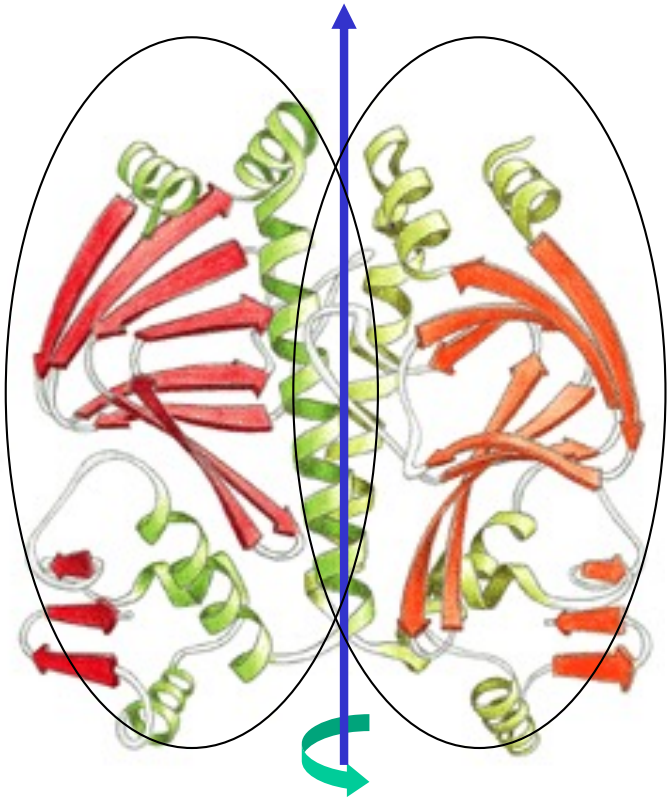
how the secondary structure elements pack together to give a 3D structure



monomer (or subunit)

Quaternary structure

the number and relative position of the subunits in a multimeric protein



dimer (α2)

Secondary structure

Local organisation of the polypeptide chain:

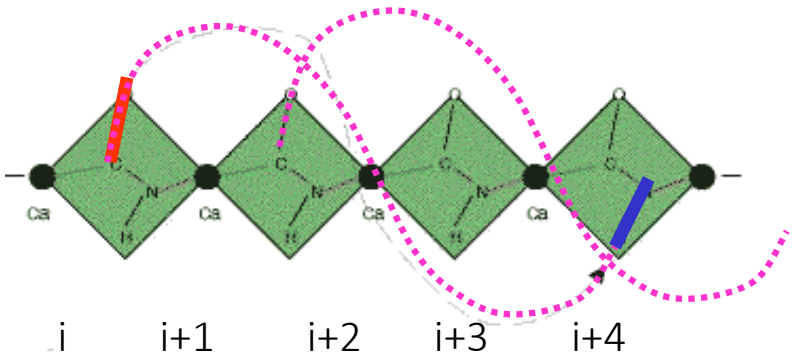
- coil
- α-helix
- 3_{10}-helix
- β-sheet
- β-turns

both α-helices and β-sheets are held together by main-chain ⟼ main-chain hydrogen bonds

Linus Pauling and Robert Corey (~1940)

- carried out X-ray diffraction studies of the structure of amino acids and small peptides to obtain a set of standard bond lengths and bond angles
- using these data by modelling they proposed two periodic structures: the α-helix and the β-sheet. Only 20 years later their predictions could be confirmed by experiments

The α-helix

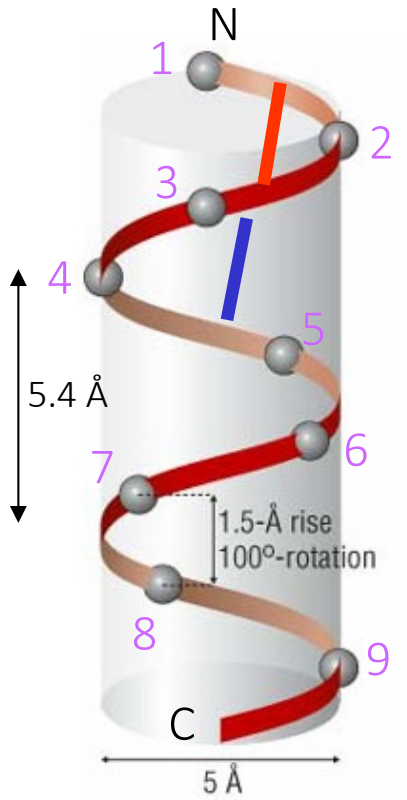


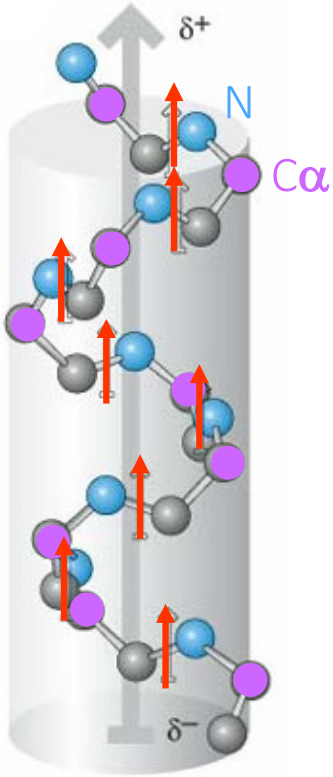
H-bonding pattern $CO_i \mapsto NH_{i+4}$
(local interactions)

all main-chain CO and NH are bonded

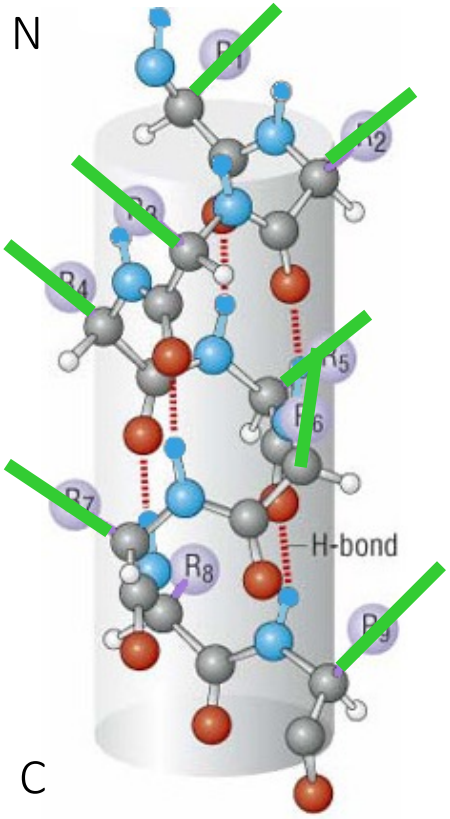
3.6 amino acids per turn;
1.5 Å rise per amino acid
$\mapsto$ 5.4 Å pitch

each peptide bond has a small dipole moment; in a helix all peptide bonds point in the same direction and generate a dipole pointing towards N





The α-helix

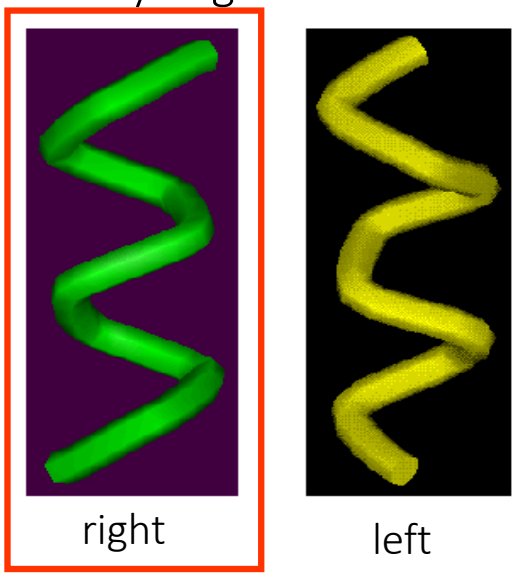


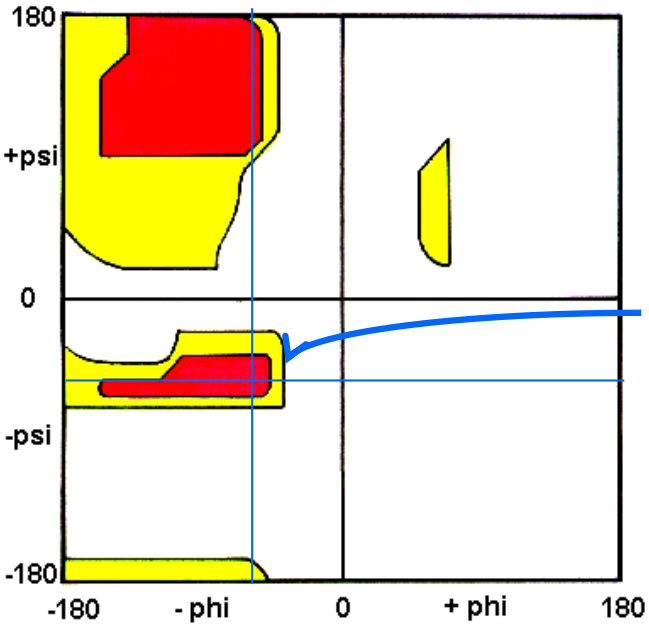
- nitrogen
- oxygen
- carbon
- R=side chain

rod-like structure with side chains extending outside

if the helix is oriented so that it goes from N (top) to C (bottom), the side chains point upwards

always right-handed





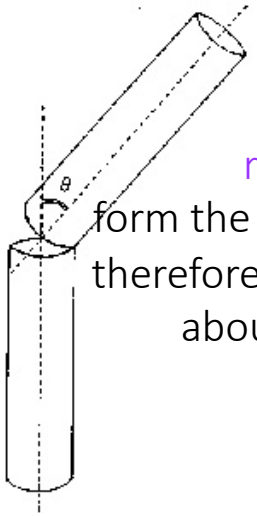
Ramachandran plot:
$(\psi,\phi) \sim (-60^\circ, -60^\circ)$

right-handed helix

can accommodate all residues except proline

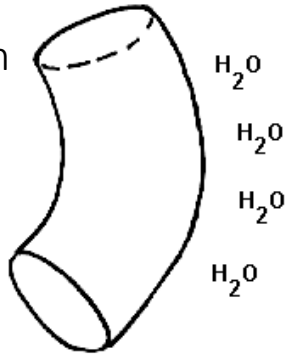
The α-helix

Distortions in α-helices:



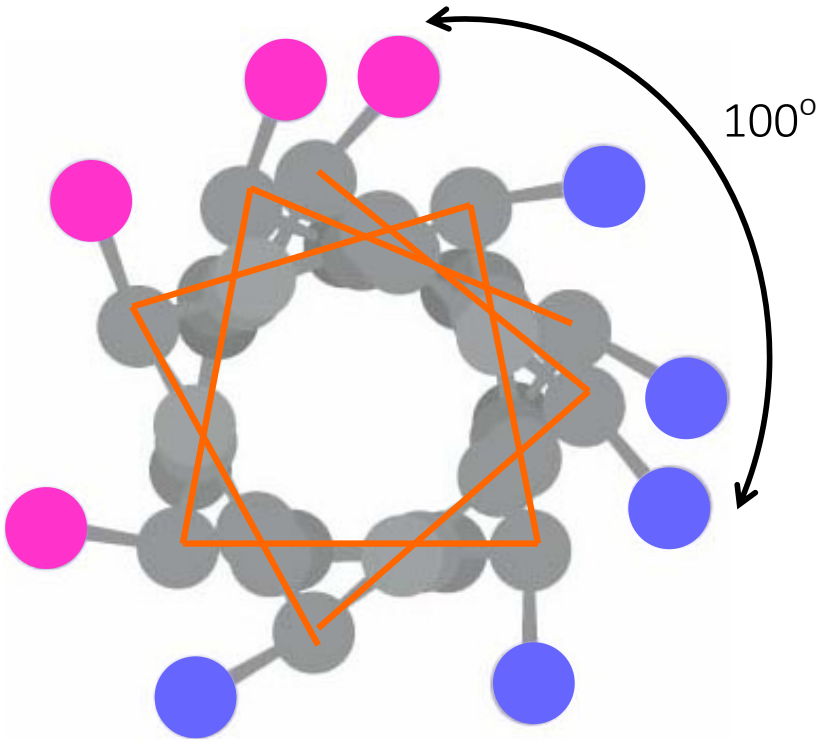
proline residues cannot form the correct HB and therefore bend the helix about 20° (Pro only occur in long helices)

helices exposed to solvent are often slightly bent away from the solvent region because exposed C=O tend to form HB with water molecules as well.



The helical wheel:

projecting down the helical axis

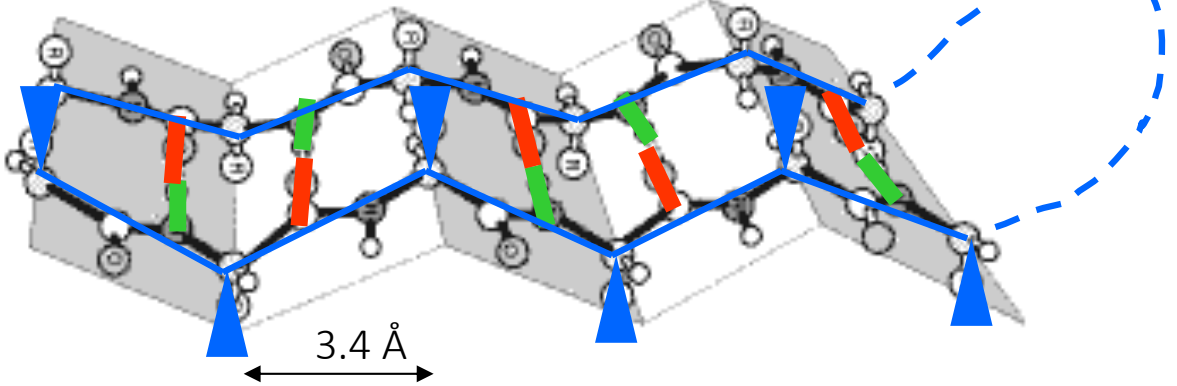


for a partially exposed helix:

hydrophobic residues

hydrophilic residues

The β-sheet



anything between 2 and few hundreds amino acids

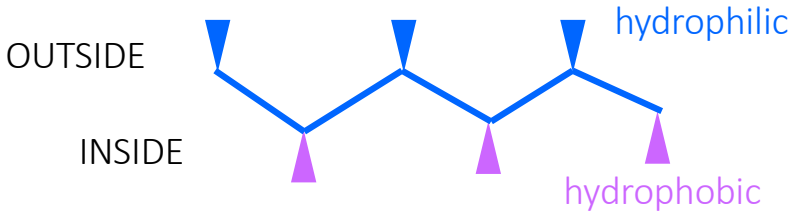
β-strand

β-strand
(zig-zag)

β-sheet

the polypeptide is almost fully extended (3.4 A per residue)

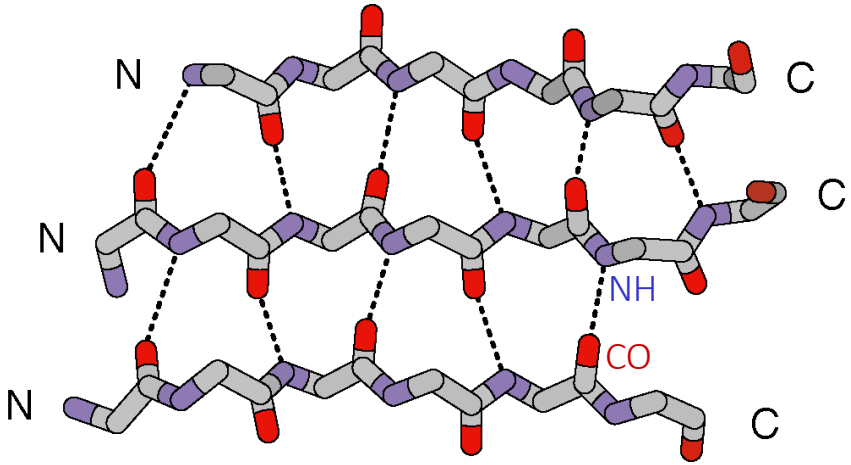
side chains points alternatively up and down

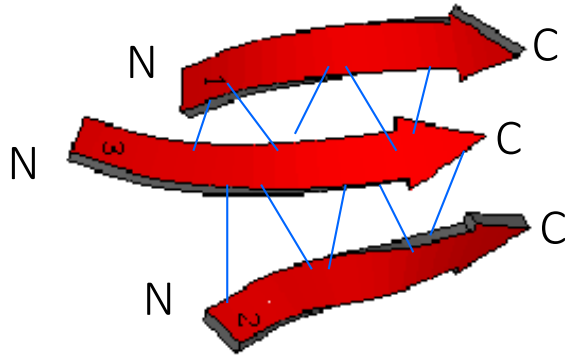


stabilised by main-chain:main-chain NH/CO hydrogen bonds between adjacent strands; contrary to the α-helix these are H bonds between NH/CO groups far apart in the amino-acid sequence

The β-sheet

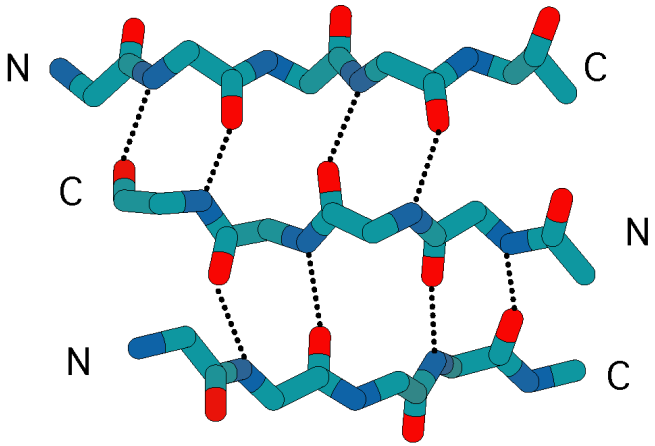
can be parallel (strands run in the same direction)

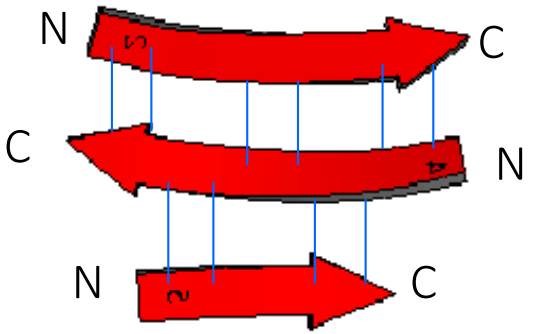




hydrogen bonding pattern

or antiparallel (strands run in opposite direction)





We often have mixed β-sheet, with some strands parallel and some antiparallel.

Tertiary structure:

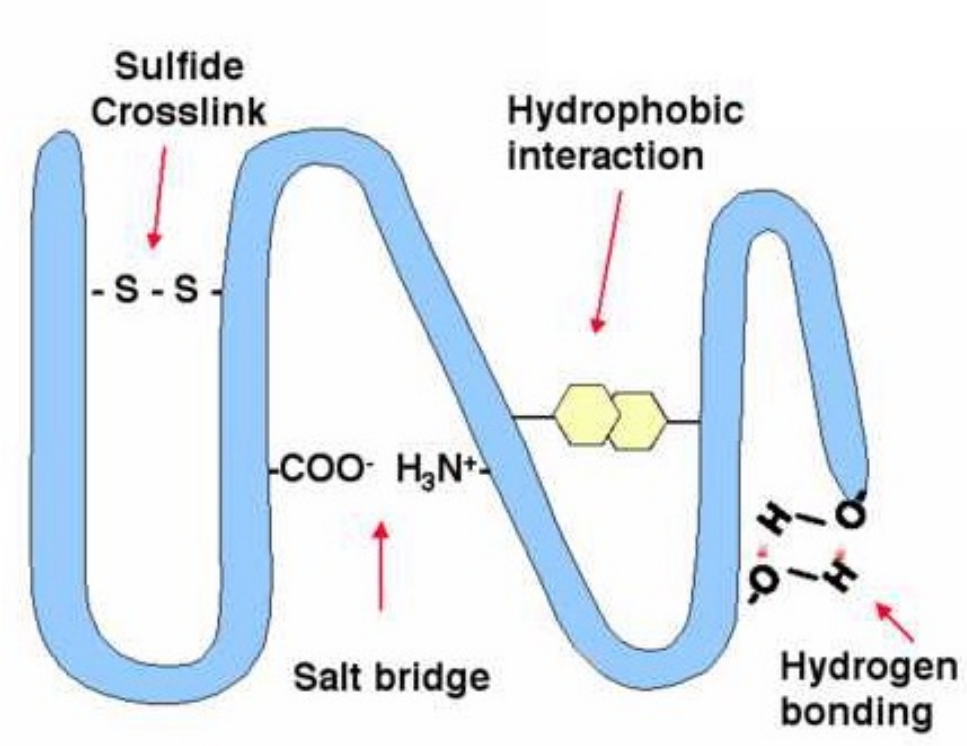
how the secondary structure elements pack together to give a 3D structure

3D structures are held together by “hydrophobic forces” and hydrogen bonds

hydrophobic side chains tend to cluster together in the interior of the protein

polar and charged amino acids interact with each other through hydrogen bonds and ionic interactions or gather on the outside of the protein where they can interact with water molecules

in some proteins S-S bonds and metal ions help to stabilise the 3D structure

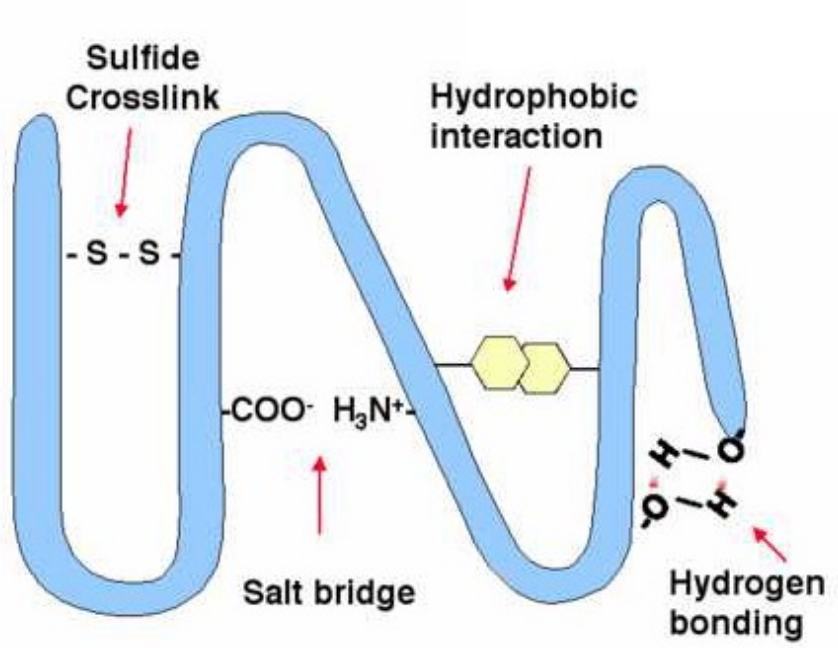


Tertiary structure:

All proteins have a well defined structure. A randomly arranged polypeptide has no biological activity

The function of a protein depends on the structure.

Proteins with similar sequences have similar structures (and similar functions), but not always the opposite is true: proteins with very different sequences can adopt similar conformations!

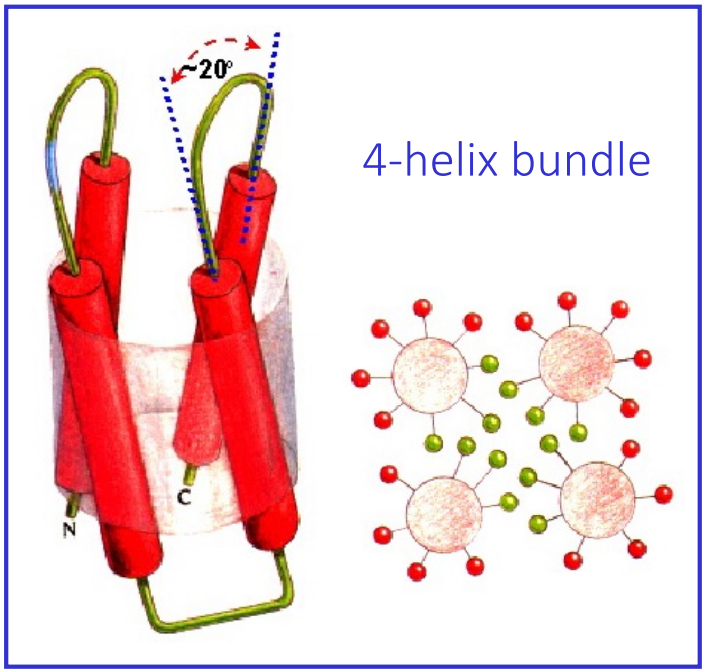


The structure is more conserved than the sequence.

Tertiary structure:
motifs in protein structures

Secondary structure elements are often connected to form structural motifs, i.e some specific geometric arrangements that occur often in protein structures; some of these motifs may be associated with certain functions, others have no specific biological function.

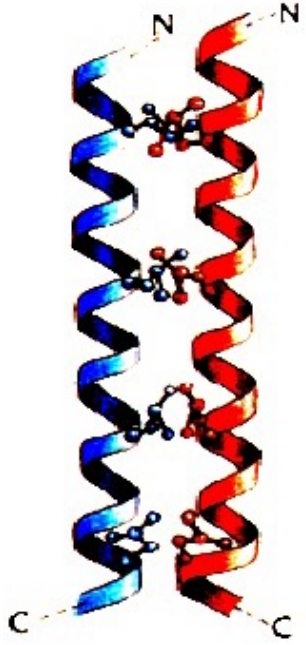
It is difficult to systematically list and classify all the motifs - here are examples of some of the common ones:



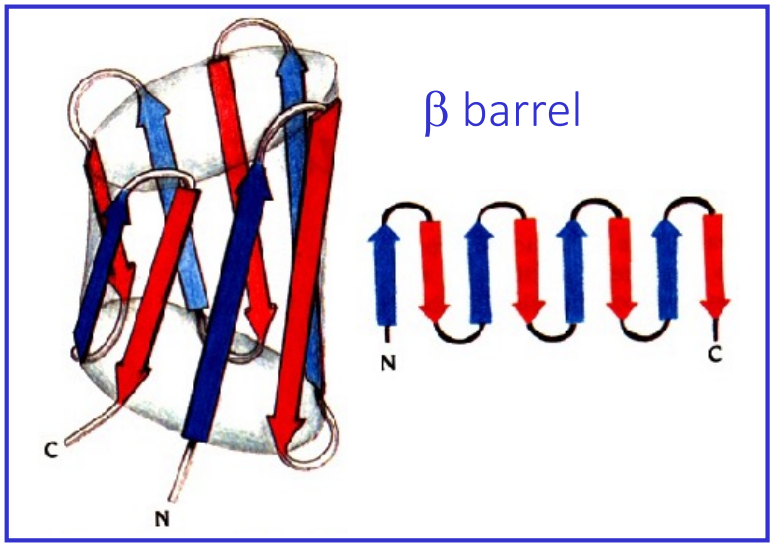
4-helix bundle

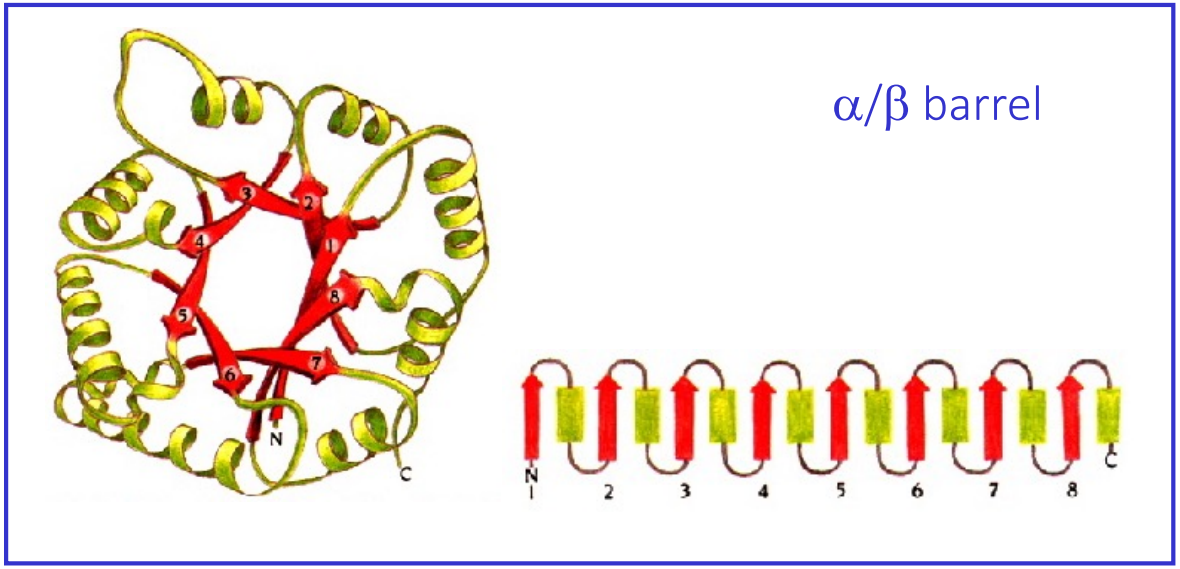
Leu zipper

a leucine residue repeated every 7 residues.



Tertiary structure:
motifs in protein structures



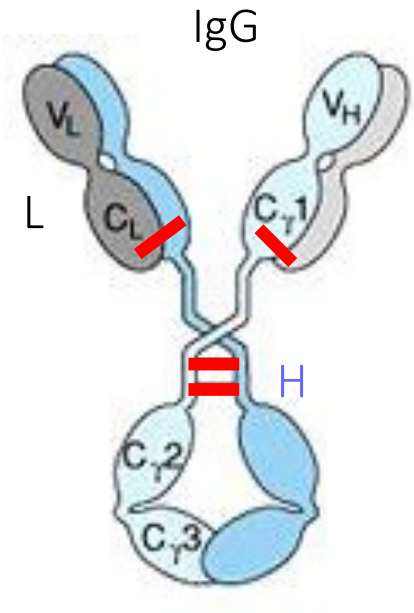


.... and many,
many more!!!!

Quaternary structure:

how subunits aggregate to form multimeric proteins

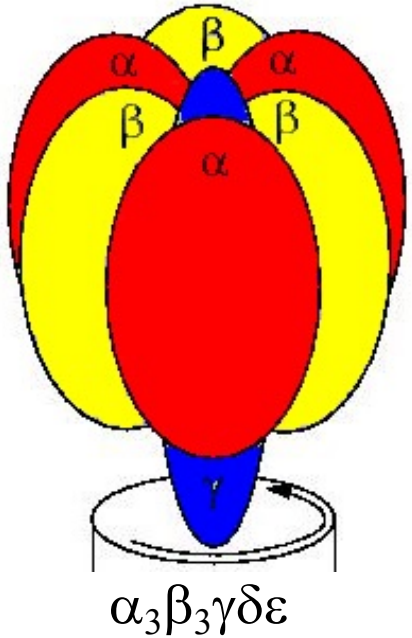
Covalently-linked polypeptide chains



For example an antibody is formed by two copies of a heavy chain H (in blue) and two copies of a light chain (in grey) connected by disulphide bridges

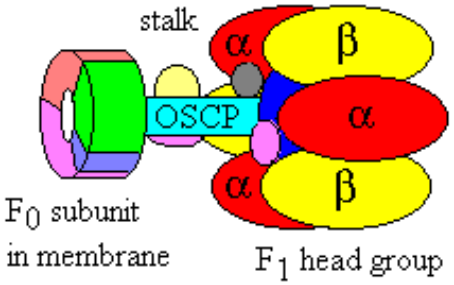
S-S bridges

Hetero-multimers: different polypeptides aggregating together to form a unit.



An example is the F1 head of the ATP synthase which is formed by 3 α subunits, 3 β subunit and one each of γ, ε, δ subunits.

The entire molecule is even more complex, with a transmembrane portion as well:

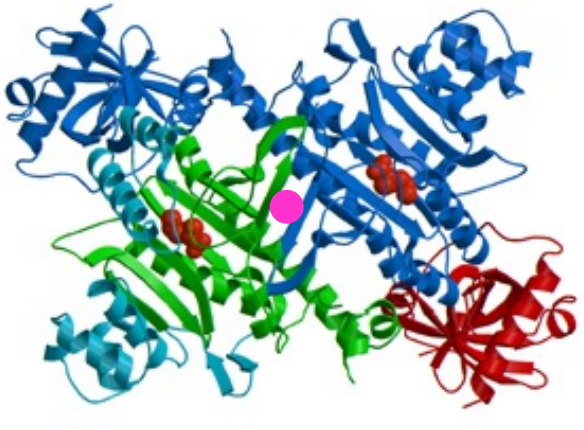


Quaternary structure:

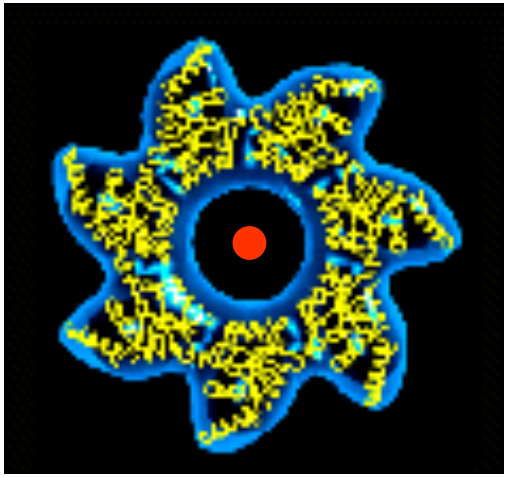
how subunits aggregate to form multimeric proteins

Homo-multimers: multiple copies of the same polypeptide associating non-covalently.

Such complexes usually exhibit rotational symmetry about one or more axes, forming dimers, trimers, tetramers, pentamers, hexamers, octamers, decamers, dodecamers, (or even tetradecamers in the case of the chaperonin GroEL).



Lysyl-tRNA synthetase:
● 2-fold axis



GROEL:
7-fold axis ●
72 symmetry

Quaternary structure:

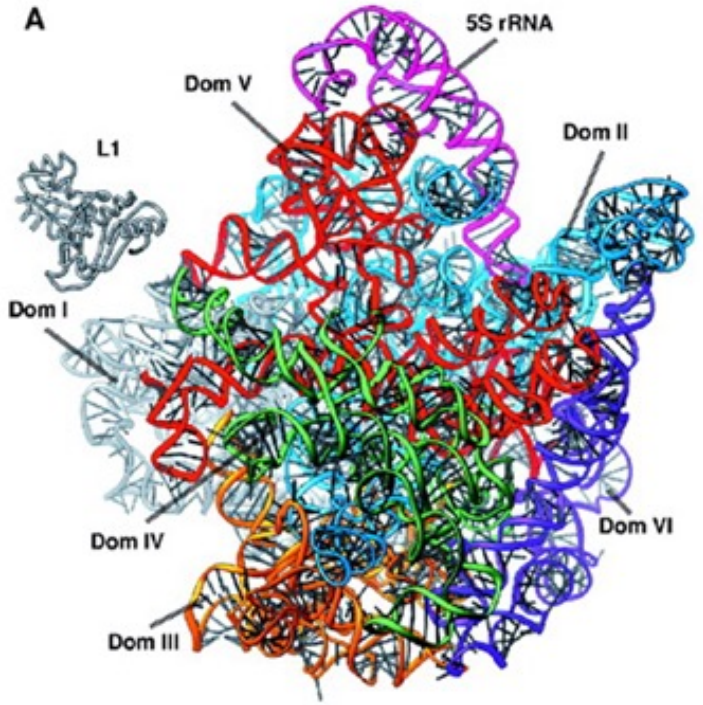
how subunits aggregate to form multimeric proteins

Larger Structures

The molecular machinery of the cell and indeed of assemblies of cells, rely on components made from multimeric assemblies of proteins, nucleic acids, and sugars. A few examples include :

- Viruses
- Microtubules
- Flagellae
- Ribosomes
- Histones

Here is the 3D structure of the large subunit of the ribosome



Fibrous proteins

Triple helix in collagen - next

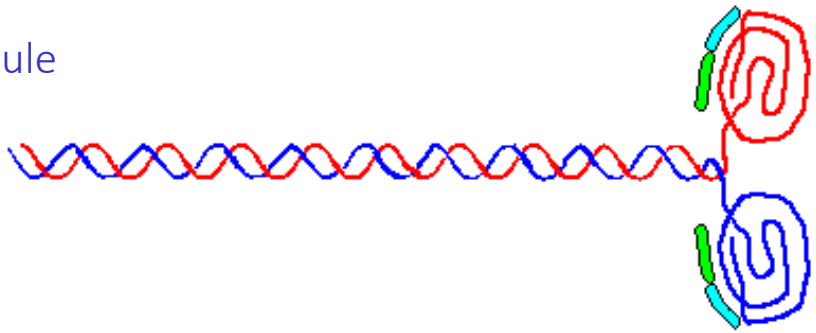
Coiled-coil α-helices present in keratin and myosin:

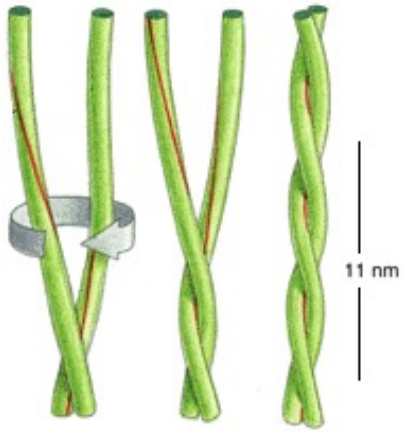
two a-helices twisted around each other to form a left handed coiled coil (7 residues repeat)

Example:a myosin molecule

heavy chains

light chains





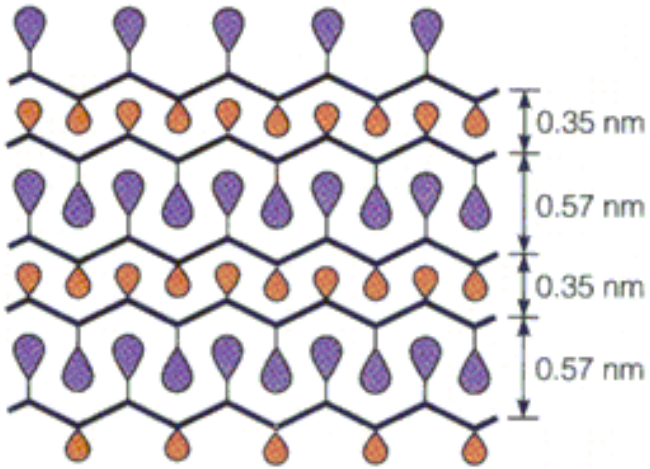
coiled-coil helices

β-sheets in amyloid fibres, spider webs and silk

antiparallel β-sheet whose chains extend parallel to the fibre axis

Ala or Ser

Gly

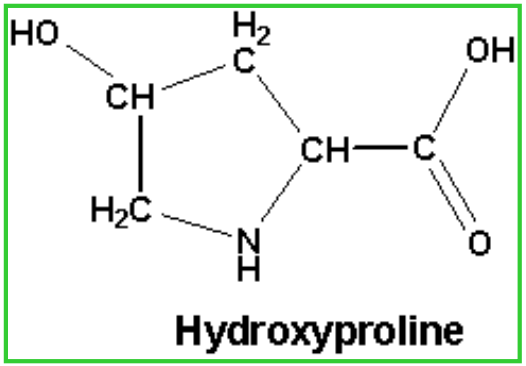


Fibrous proteins: the collagen helix

Collagens are family structural proteins forming the tendons and the extracellular matrix. Bones and teeth are made by adding mineral crystals to collagen.

Collagen is composed of three chains wound together in a triple helix.

Each chain is very long and consists of a repeating sequence of three amino acids: every 3rd amino acid is a glycine that fits in the interior of the triple helix; many of the remaining positions contain prolines and hydroxyprolines:



The enzyme that modifies a proline into hydroxyproline requires vitamin C; lack of vitamin C causes scurvy.

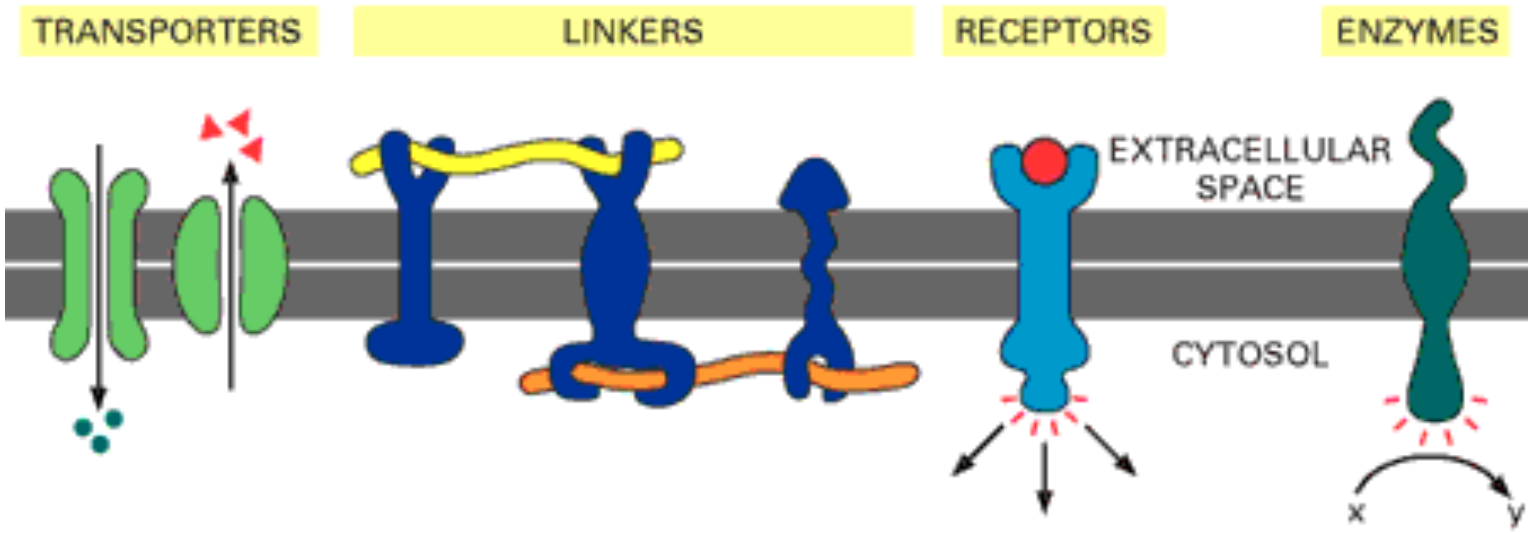
There are other non-standard aa (such as hydroxylysines) which are used to crosslink the chains.

Membrane proteins: biological roles

Membrane proteins are defined as proteins that sit in the lipid bilayer: they perform very different biological roles:

- pumps
- channels
- receptors
- cell-to-cell adhesion

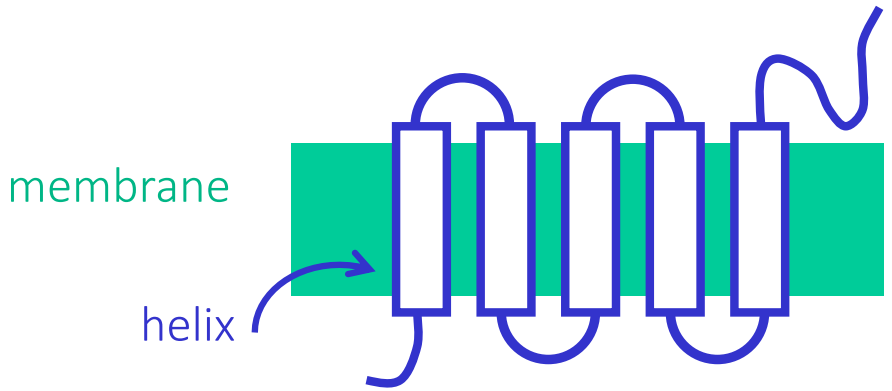
control the flow of chemicals and information between the inside and the outside of the cell and mediate communication between different cells.



Structures of membrane proteins

Less is known about the 3D structure of membrane proteins since in general they are much more difficult to crystallise than soluble proteins.

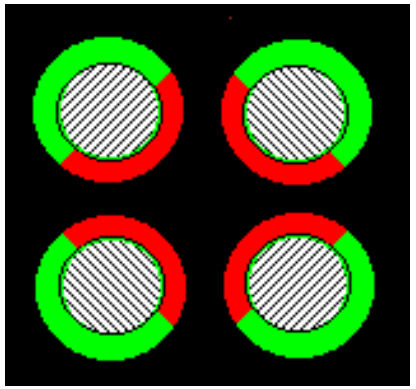
They are often built of α-helices spanning the membrane; but some are built of extended β-barrels (such as porins)



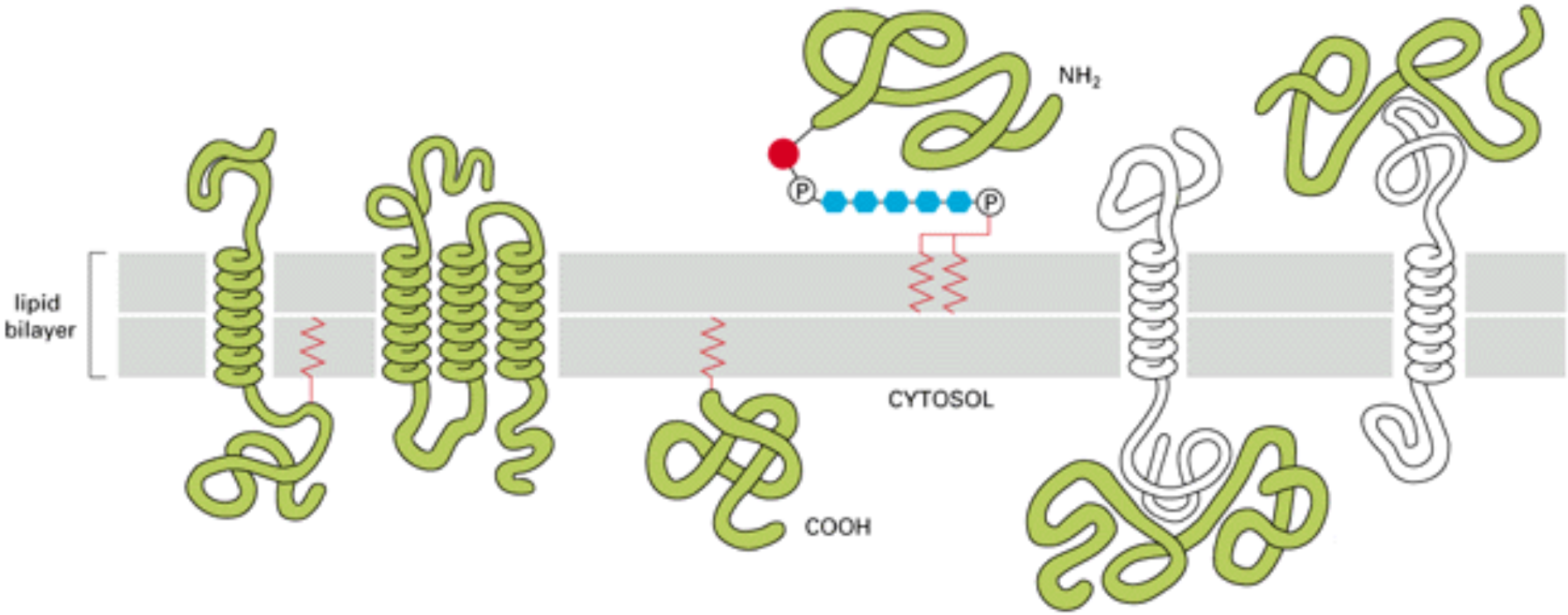
Contrary to soluble proteins, the hydrophobic residues will be on the outside, where they will interact with the chains of the lipids, while hydrophilic side chains will cluster inside

hydrophilic

hydrophobic



Membrane proteins associate with the lipid bilayer in various ways:

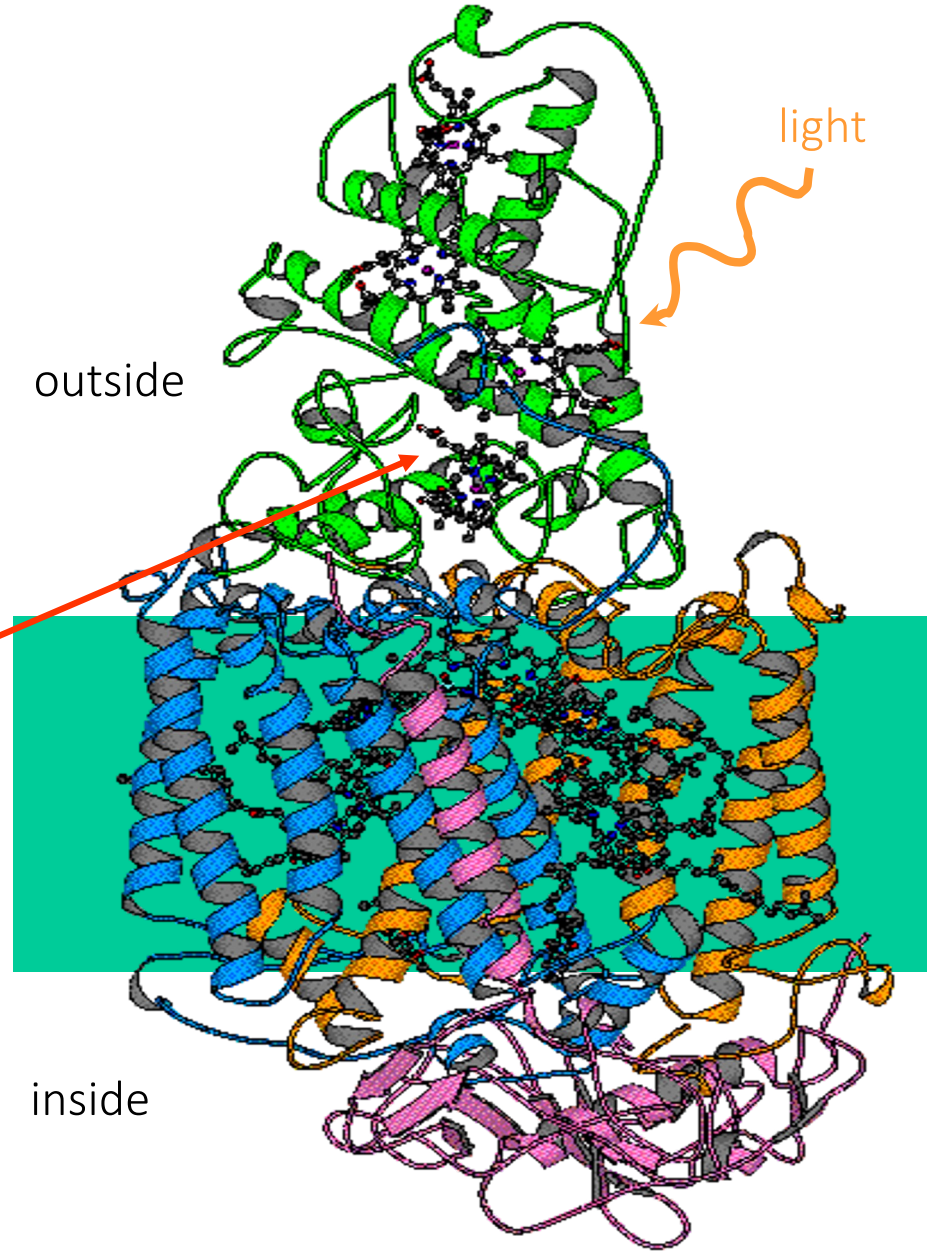


Examples of membrane proteins: the photosynthetic reaction centre

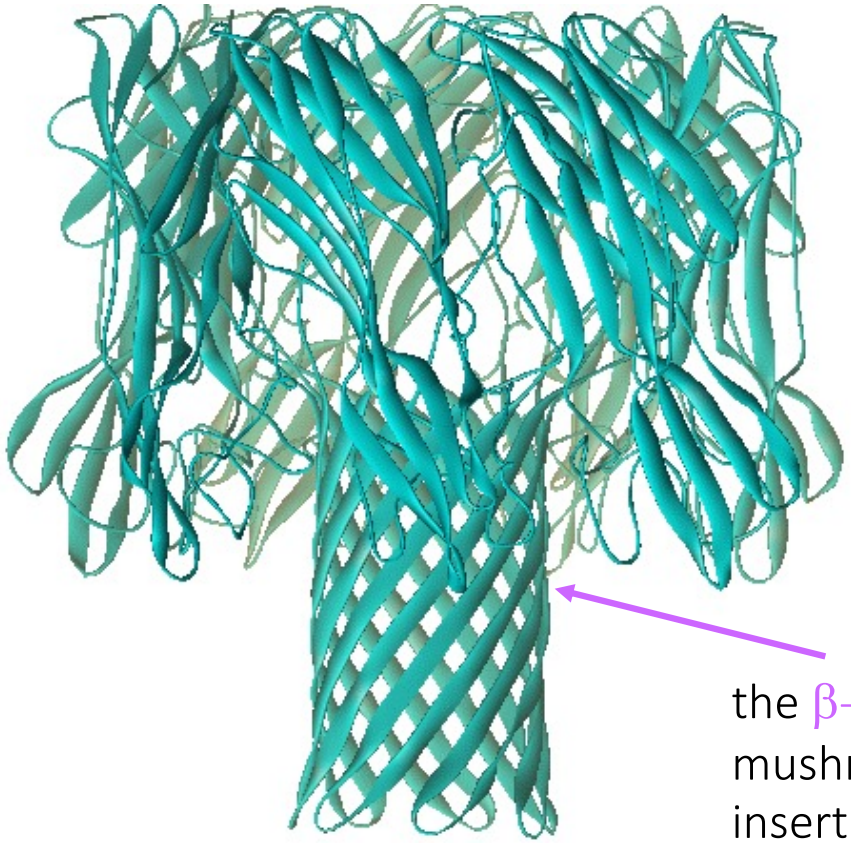
Structure determined by R. Huber, H. Mitchell & H. Deisenhofer (Nobel prize 1988)

Found in the membranes of chloroplasts and in photosynthetic bacteria; convert energy from the sun into electrical and chemical energy.

Contains a lot of pigments (such as chlorophylls, quinons, carotenoids, etc...) to capture photons

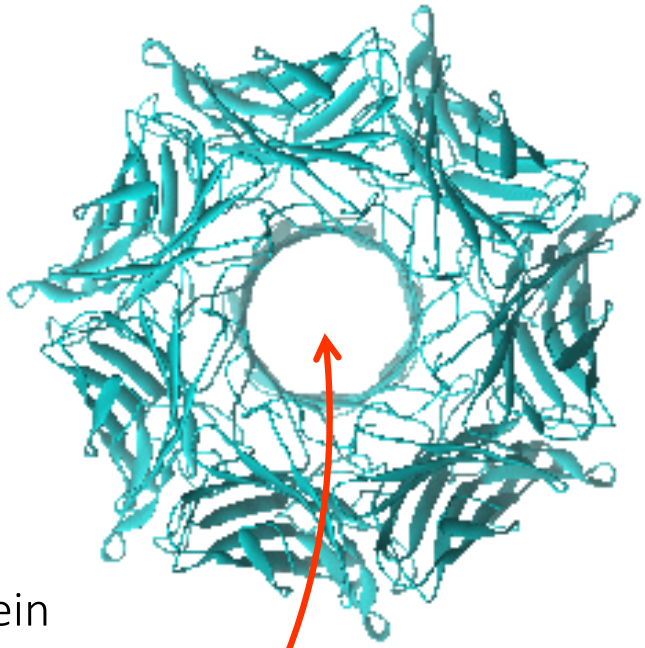


Examples of membrane proteins: the bacterial toxin a-hemolysin



seen from the side

seen from the top

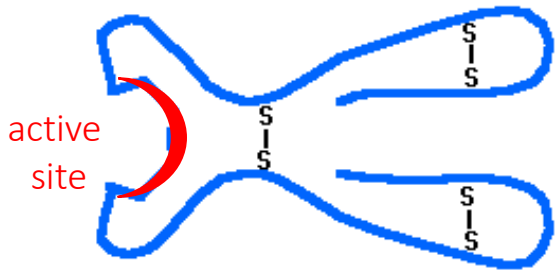


the β-barrel stalk of this mushroom shaped protein insert across the cell membrane and causes lethal permeability changes due to the central pore

Denaturation

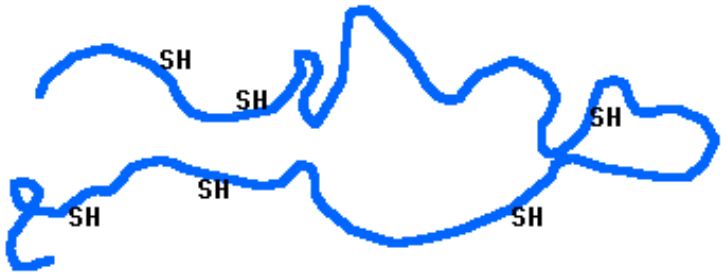
Many proteins can be unfolded and refolded:

FOLDED, active protein



urea, pH, high T

UNFOLDED, denatured protein



refold = regain biological activity

unfold = loose biological activity

When a protein contains disulphide bridges (S-S)
we also need to reduce them to SH to unfold.

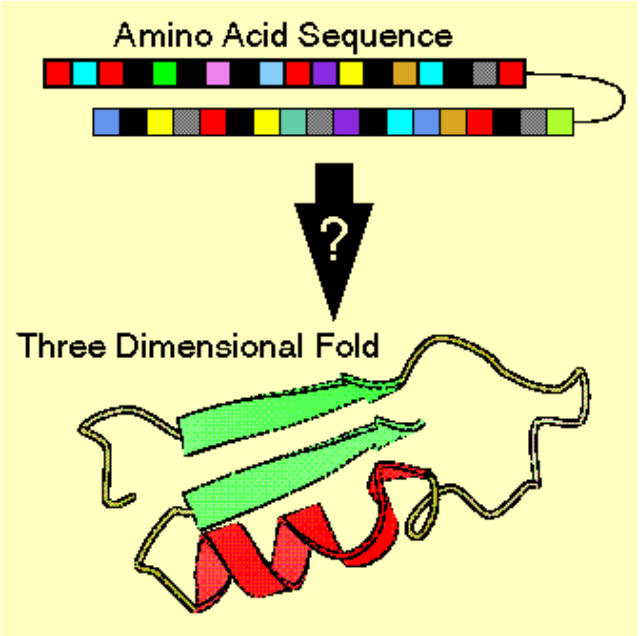
It does not work for all proteins - some proteins, once unfolded cannot be easily refolded again.

How do proteins fold in the cell?

The amino-acid sequence specify the 3D structure, which is (probably?) the energy minimum for that particular sequence...

BUT how does a protein reach the correct three-dimensional fold?

by trying out all the possible conformations?



- consider the number of possible conformations of a chain of 100 amino acids
- assume each amino acid can have only 3 different conformations
- 3^{100} = 5×10^{47} possible different conformations
- if it took only 0.1 psec (10^{-13} sec) to try each possibility, it still would take 1.6×10^{27} years to find the minimum of energy!

➡ There must be a 'folding pathway'!!!

first forming local structures quickly, then packing them together

The “folding problem”

Experimental approach

Studying experimentally how folding of a particular protein occur in vitro by using techniques like NMR which can detect the presence of secondary structure elements in a partially unfolded protein (trying to determine the ‘folding pathway’)

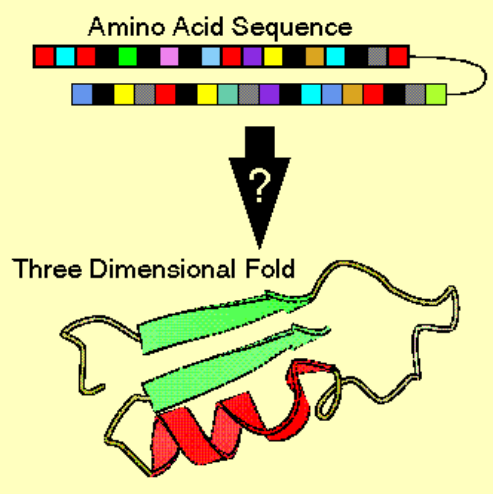
Studying experimentally how folding occur in the cell: some proteins fold by themselves, others require the help of other proteins called chaperones.

Theoretical approach

Using bio-informatics to predict the 3D structure from the amino-acid sequence. The sequence dictate the fold, but we are not very good at going from the sequence to the structure!

Problems?

- poor energy functions and parameters
- complexity
- treatment of solvent



Molecular evolution

Proteins evolve by changing little by little their amino-acid sequence

Changes are due to random mutations in the gene that code for that protein

- some mutations disrupt the structure and/or function of the protein and are eliminated by the selective pressure
- some mutations are 'neutral' and therefore allowed
- some (rare) mutations improve the functionality of the protein or change the function in a way that is advantageous for the cell

→ evolution will select the favourable mutations

A lot of small changes occurring in all protein sequences accumulate with time and are responsible for the variety of living forms we see.

By comparing amino-acid sequences of proteins we can build evolutionary trees:

- key residues (structurally or functionally) are usually conserved
- other residues are usually very similar in organisms that have diverged recently but more and more diverse in distantly related organisms

Summary

Amino acids: structure and properties

The peptide bond: polarity of the bond, concept of primary sequence, planarity and torsion angles, the Ramachandran plot.

Secondary structure: the α-helix and the β-sheet.

Tertiary structure and motifs.

Quaternary structure and multimeric proteins.

Fibrous proteins: coiled-coil, amyloid fibers, collagen triple helix.

Membrane proteins.

The folding problem.