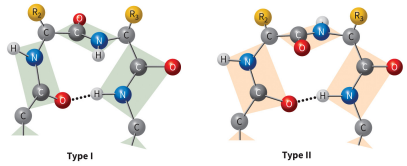
Turns Allow Protein Strand to Change Direction

Also governed by H-bonding—do you see a theme here?



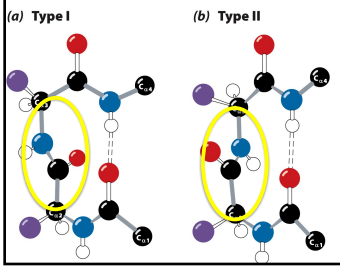
H bonds stabilized by H bonding of backbone – but…

Pro, because of it's cyclic structure and fixed ϕ angle drives the formation of these turns! Don't forget this aa also breaks alpha helix…

Gly is also commonly found in turns—why do you think this is true?

Two Beta Turns

Reverse turns/ bends – four amino acids each stabilized by H bonding from backbone



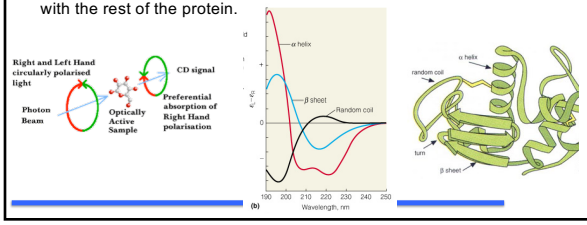
Type I and II differ at peptide bond orientation at turn

Type II angle requires Glycine on second aa position!

Protein Structure – Do all proteins "fold?"

The segments of a protein that are *not helices or sheets* are traditionally referred to as "random coil," athough this term is misleading:

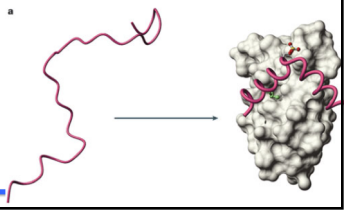
- Most of these segments are neither coiled or random.
- They are usually organized and stable, but don' t conform to any frequently recurring pattern.
- Random coil segments are strongly influenced by side-chain interactions with the rest of the protein.



Intrinsic Disorder

Coupled folding and binding is the process in which an intrinsically disordered protein folds into an ordered structure concomitant with binding to its target. For example, the phosphorylated kinase-inducible domain (pKID) of CREB is unstructured when it is free in solution but it folds on forming a complex with the KID-binding (KIX) domain

Entropy cost of increasing order in ID proteins as the protein transitions from disorder to order – paid for (thermodynamic driving force) is a change in enthalpy of many weak binding forces between proteins



Intrinsic Disorder

Some regions or domains of proteins do not become structured (predictable secondary structure) until binding to another protein or target (lipid, carbohydrate, small molecule, substrate…).

- Identified as "missing electron density in crystal structures – atom positions and backbone Ramachandran angels fluctuate
- Until binding target interacts, ID appears as a random coil
- Breaks some of the structure-function paradigm
- High in Gly, Pro and Ala, low in Cys and Asn
  - Order breaking aa and order promoting aa
  - Often low hydrophobic and high net charge add to charge repulsions and less compact structure

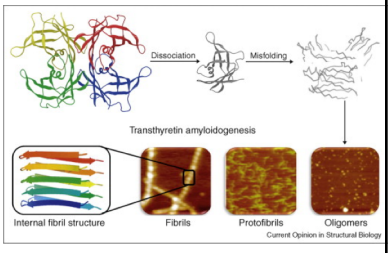
Quaternary Interactions Gone Awry: Amyloidoses

We've covered interactions between already folded subunits.

Unfolded or misfolded monomers can glob together to form aggregate structures, which organized into cross-β-sheets (amyloid)

Amyloidoses are a major health problem in the ageing population (Alzheimer's disease, Systemic amyloidoses, etc.)

Homotetramer TTR dissociates into monomers, misfolds, then aggregates



Connelly, S. et al.Current Opinion in Structural Biology, Volume 20, Issue 1, February 2010, Pages 54–62

Mad Cow Disease

All known prion diseases are fatal. Since the immune system does not recognize prions as foreign, no natural protection develops. Scrapie in sheep was first described during the18th century. It has been transmitted to other animals such as mink and cats, and more recently to cows (mad cow disease or bovine spongiform encephalopathy, BSE) through contaminated feedstuff.

In New Guinea, the Fore-people contracted kuru by eating the brains of deceased people. Creutzfeldt-Jakob Disease (CJD) frequently arises spontaneously, while fatal familial insomnia (FFI) Gerstmann-Sträussler-Scheinker GSS) disease, and 10-15% of CJD are caused by mutations in the gene encoding the prion protein. A new variant CJD, diagnosed in some 20 patients, may have arisen through transmission of BSE to humans.

The Nobel Foundation

BSE - Mad Cow; A protein gone wrong

The prion protein exists in two forms. The normal, protein (PrPc) can change its shape to a harmful, disease-causing form (PrPSc). The conversion from PrPc to PrPSc then proceeds via a chain-reaction. When enough PrPSc proteins have been made they form long filamentous aggregates that gradually damage neuronal tissue. The harmful PrPSc form is very resistant to high temperatures, UV-irradiation and strong degradative enzymes.

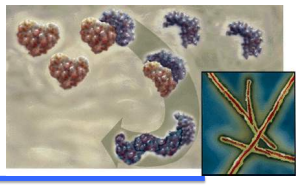
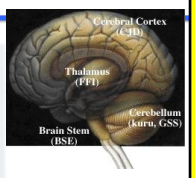


Figure adapted from Fred Cohen, University of California at San Francisco

Prions and protein folding

Prions affect different regions of the brain. A sponge-like appearance results when nerve cells die. Symptoms depend on which region of the brain is affected.



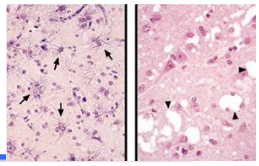
Cerebral cortex -loss of memory and mental acuity (CJD).

Thalamus Damage results in insomnia (FFI).

Cerebellum Damage results in problems to coordinate body movements and difficulties to walk (kuru, GSS).

Brain stem In the mad cow disease (BSE).

A precise diagnosis of a prion disease can only be made upon autopsy. The figures show thin sections of diseased brains. FFI, with typical proliferation of astrocytes, the support cells of the brain, is shown to the left (arrows). CJD, with the characteristic spongiform appearance with vacuoles (arrows) is shown to the right.



Prion diseases arise in three different ways

1. Through horizontal transmission from e.g. a sheep to a cow (BSE).
2. In inherited forms, mutations in the prion gene are transmitted from parent to child.
3. They can arise spontaneously.

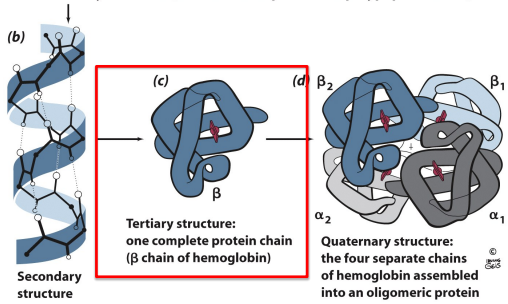
Route of infection

When cows are fed with offals prepared from infected sheep, prions are taken up from the gut and transported along nerve fibers to the brain stem. Here prions accumulate and convert normal prion proteins to the disease-causing form, PrPSc. Years later, BSE results when a sufficient number of nerve cells have become damaged, affecting the behavior of the cows.

Protein Structure – 1°, 2°, 3°, & 4°

(a) – Lys – Ala – His – Gly – Lys – Lys – Val – Leu – Gly - Ala –

Primary structure (amino acid sequence in a polypeptide chain)



Secondary structure (helix)

Tertiary structure: one complete protein chain (β chain of hemoglobin)

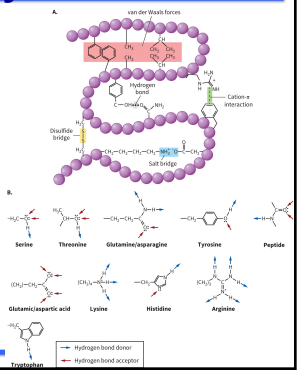
Quaternary structure: the four separate chains of hemoglobin assembled into an oligomeric protein

Figure 6-1
Illustration, Irving Geis. Image from the Irving Geis Collection/Howard Hughes Medical Institute. Rights owned by HHMI. Reproduction by permission only.

Bonding Forces Involved in Tertiary Structure

Forces include

- hydrogen bonding
- London dispersion forces
- dipole–dipole interactions
- salt bridges
- cation–π interactions
- disulfide bonds



Hydrophobic Effect Defined

Hydrophobic effect describes the phenomenon in which hydrophobic groups cluster together.

Incorporate London dispersion and Van Der Waals forces

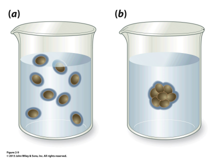
Non-covalent interactions in tertiary structure

Covalent: 490, O-H

Type of Interaction	Strength, kJ/mol	Example	Dependence of Energy on Distance
** **(a) Charge–charge** Longest-range force; nondirectional	$\alpha\ (q^+q^-)/r^2D$	$-NH_3$ … O C O	$1/r$
(b) Charge–dipole Depends on orientation of dipole		$-NH_3$ … q^-O q H H	$1/r^2$
(c) Dipole–dipole Depends on mutual orientation of dipoles	0.5-4	q^-O q H H … q^-O q H H	$1/r^3$
(d) Charge–induced dipole Depends on polarizability of molecule in which dipole is induced		$-NH_3$ … q^- q^+	$1/r^4$
(e) Dipole–induced dipole Depends on polarizability of molecule in which dipole is induced		q^-O H H … q^- q^+	$1/r^5$
** **(f) Dispersion** Involves mutual synchronization of fluctuating charges	<1	q^+ q^- / q^- q^+	$1/r^6$
(g) van der Waals repulsion Occurs when outer electron orbitals overlap			$1/r^{12}$
** **(h) Hydrogen bond** Charge attraction + partial covalent bond	6-20	N—H···O=C Hydrogen bond length	Length of bond fixed

** dipole (b)–(e)

Protein Structure – Hydrophobic effect in tertiary structure



Water adjacent to a non-polar molecule sacrifice rotational and translational freedom to maintain molecular interactions. Thus, non-polar molecules associate to reduce the surface area with aqueous solvent.

How do primary sequences fold into stable three-dimensional structures?

Protein Structure – Folding

Why study how a protein folds:

The structure that a protein adopts is vital to it's chemistry.

Not necessarily true!

Its structure determines which of its amino acids are exposed to carry out the protein's function.

Its structure determines what substrates it can react with.

Levinthal's Paradox

Consider a 100 residue protein. If each residue can take only 3 positions, there are $3^{100} = 5 \times 10^{47}$ possible conformations.

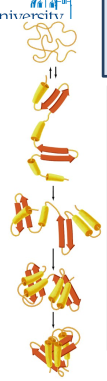
If it takes 10^{-13} s to convert from 1 structure to another, an exhaustive search would take 1.6×10^{27} years!

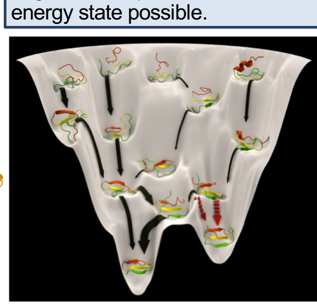
Folding must proceed by progressive stabilization of intermediates.

How can this path be found?

Protein Structure – Folding process

Secondary structure forms, then the protein begins to compact itself until it reaches the lowest energy state possible.





As the peptide samples conformations, stable intermediates direct the folding pathway to an optimized (sometimes the lowest energy) folded state.

Figure 6-41

Protein Structure – Folding process

Levinthal's Paradox

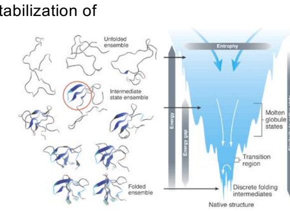
Consider a 100 residue protein. If each residue can take only 3 positions, there are $3^{100} = 5 \times 10^{47}$ possible conformations.

If it takes 10^{-13}s to convert from 1 structure to another, an exhaustive search would take 1.6×10^{27} years!

Folding must proceed by progressive stabilization of intermediates.

How can this path be found?

May not be a single path: may be an energy landscape.



Protein Structure – Folding process

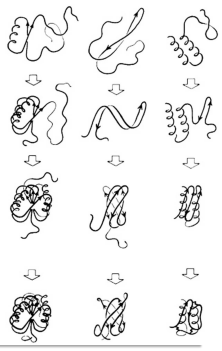
Protein Folding

Steps:

1) The polypeptide collapses in upon itself due to the **hydrophobic effect**

2) An intermediate "molten globule" forms with elements of 2°

3) The backbone rearranges to achieve a stable native conformation (usually electrostatic or H-bonding)

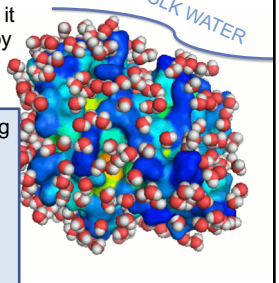


How Does Protein Stability Arise?

We're losing a lot of entropy when a protein folds (floppy to ordered)—how does it overcome that loss (entropy-enthalpy compensation)?

$\Delta G = \Delta H - T\Delta S$

- Intramolecular hydrogen bonding
- Hydrophobic Collapse
- The reduction of surface area accessible to solvent (smaller solvent shell)
- Salt-bridges
- Van der Waals contacts



http://mspc.bii.a-star.edu.sg/tankp/help.htm

Protein Structure – Folding process

Why do proteins fold?

Protein Folding Problem-
Folded state is only slightly more stable than unfolded state

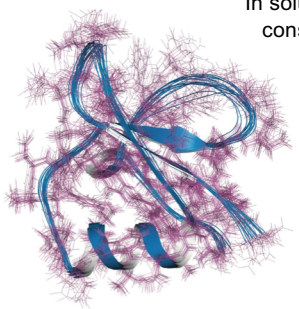
ΔG_{folded} = - 0.1 kcal/mol per AA
For comparison: a H-bond is 1 - 4 kcal/mol

Forces that drive folding:
Water entropy (ΔS)
Non-polar and polar interactions in protein
Disulfide bonds
Total = ~200 kcal/mol

Forces that prevent folding:
Chain entropy (ΔS)
Polar interactions between AAs and water
otal = ~200 kcal/mol

Protein Structure – Molecules in motion

In solution, proteins are constantly in motion

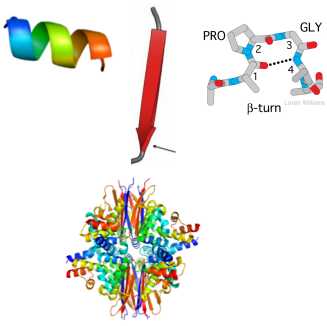


Proteins can undergo ***conformational changes*** that involve the movement of whole sections of protein/side-chains/atoms—can be in response to stimuli (e.g. small molecule substrate)

NMR structure-ensemble that has a variety of structures that fit the data

Components of tertiary structure

secondary structure
⇩
supersecondary structure
⇩
folds
⇩
domains
⇩
fully folded chain



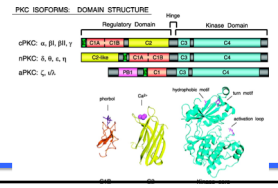
Motifs versus Domains

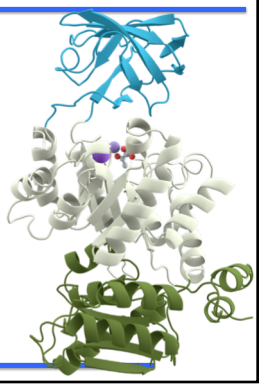
Motifs
- Combinations of secondary structures

Domains
- Pieces of a protein that retain their structure in the absence of the rest of the protein
- A discretely folding structure

Protein Structure – Domains

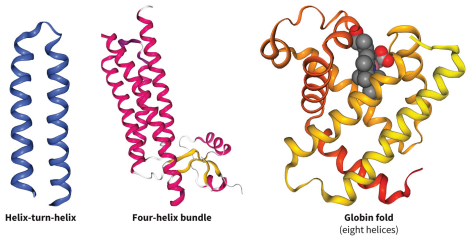
① Combination of Folds, Supersecondary and secondary structures.
② Independently folded, compact units in proteins.
③ Single domain may have a particular function (ie bind small molecule, catalyze rxn).
④ In multi-functional enzymes, each catalytic activity can be on one of several domains.





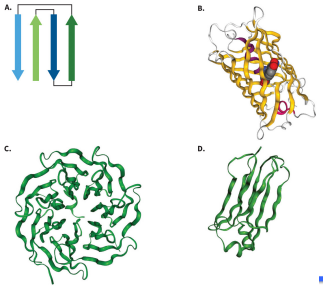
Alpha Structures

Alpha motifs – mostly bundles of organized helical structure.
- Helix plus turns
- Many motifs are found in many proteins – retained through evolution



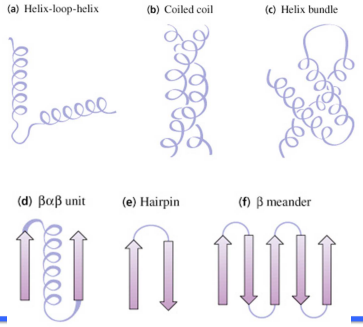
Beta Motifs

Contain mostly β structures
– Include the β barrel, Greek key motif, and β propeller



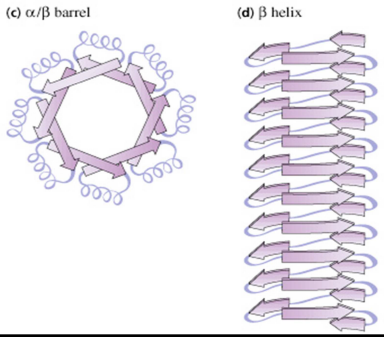
Supersecondary structure (aka motifs)

Common combinations of secondary structural elements



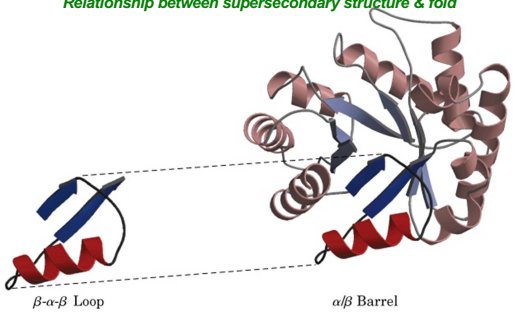
Protein Structure – Folds

Combinations of supersecondary structures



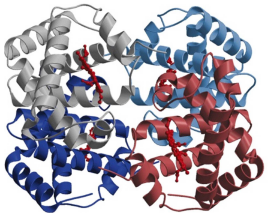
Protein Structure – Folds

Relationship between supersecondary structure & fold



Protein Structure – Quaternary structure

Bringing Protein Chains (Subunits) Together



Hemoglobin is made up of 4 subunits (all bind O_2)

1) Refers to association between tertiary structure of individual polypeptide chains to form a larger complex with defined function – quaternary structure.
2) Each polypeptide chain is called subunits (may be identical or different). Quaternary structure has a defined stoichiometry and arrangement:
   1) Homodimer- Two identical subunits
   2) Heterotrimer- Three different subunits
   3) Homotetramer- Four identical subunits
3) Subunits are held together by many **noncovalent** interactions (hydrophobic, electrostatic, H-bonding, London).
4) Sometimes when have multi-subunit globular proteins, one subunit affects the activity of another subunit (Allosteric Regulation).
5) Quaternary structure can be dependent on concentration of and affinity between subunits.

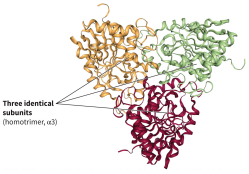
Quaternary Structure

Many proteins exist as multiple polypeptide units:

•Quaternary structure only exists when there are more than one protein subunits involved in a protein

•Subunits are separate genes/proteins which come together with similar or different subunits to form a complete protein

•Homo or hetero proteins
•Monomer, dimer, drimer, tetramer, pentamer, hexamer…
•Also called multimeric proteins or enzymes
•Often involved in cooperativity or allosteric regulation



Quaternary Structure: Driving Forces

The Bad News:

• Considerable entropy loss when subunits come together
• Loss of translational degrees of freedom
• Residues that were able to move at the subunit interface are now restricted

The Great News:

• Increased Van der Waals contacts—but nearly as many are lost with water as are made with the new oligomer
• Increased hydrophobic interactions—the money maker (roughly 100-200kJ/mol)
• Polar interactions at the interface
• Salt bridges/disulfides

Protein Stability

Protein stability is labile – very little energy to denature

•U40 kJ/mol for an avg100 aa protein to denature. H bond breaking takes ~20kJ/mol

Stabilizing factors– global impact of non-covalent and covalent interactions maintaining tertiary and quaternary structure

High Influence

- hydrophobic aa in center of protein keep structure stable
- Large number of van der Waals are lost if denatured and maintain overall structure

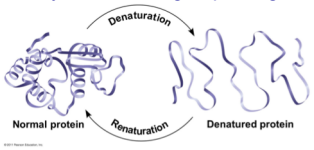
Lower influence

- H bonding – important for structure but the balalnce in native or denatured is same H bonding energy as H bonding will occur with water in denatured state
- salt bridges – entropy and solvation changes offset most of the ionic interactions

Protein Denaturing

Denaturation and Renaturation (sometimes reversible)

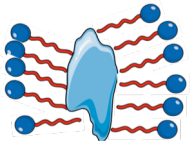
•**Heat** – disrupts or melts the van der Waal and other forces holding protein in native form
•**pH** – both basic and acidic will alter functional group charge decreasing ionic interactions within chain or at surface of protein. Also can cause loss of H bonding potential – consider Carboxyl and Amino group at high and low pH

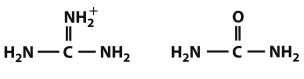


Protein Denaturing

Denaturation and Renaturation (sometimes reversible)

•**Detergents** – hydrophobic, non-polar amino acids will unravel and bind to hydropathic soaps/detergents
•**Chatropic agents**: bind water tightly away from protein.
•**Reducing agents**: reduce Cys- disulfides





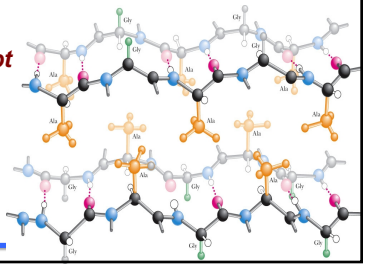
Guanidinium ion **Urea**

Silk

Anti parallel conformations are stronger - alignment of H bonding.

- Often found in silk
- R groups can interact - glycine and alanine

H bonding within 3 residues can disrupt the sheets - causes bend or turn in the chain.



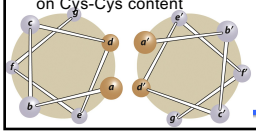
Keratin

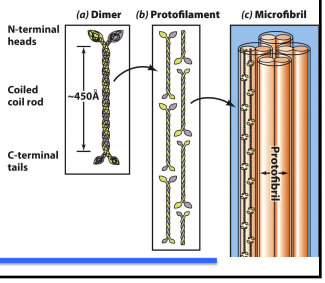
Fibrous protein – makes up most of protein in hair, nails, horns and feathers >30 different keratin genes

- Two classes α-mammals, β – birds and reptiles

Basic unit is two left handed helical wrapped around the other

- Left handed coil-coil
- Top down view shows nonpolar aa allowing hydrophobic interactions
- Hard or soft keratin is based on Cys-Cys content

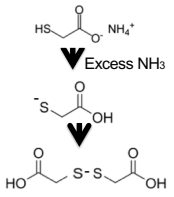


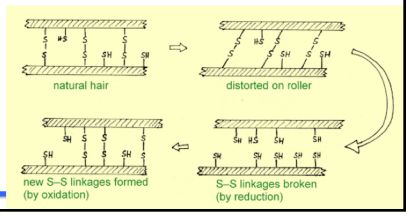


Keratin

Cys rich allows for disulfide bridges between strands and within fibers

- Hair perms use mercaptans to reduce S-S bridges
- Ammonium thioglycolate is perm salt
- Peroxide oxidizes SH back to S-S

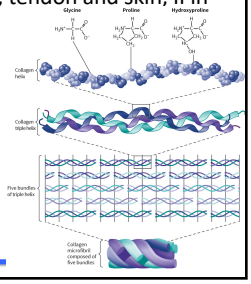




Unique structure of collagen

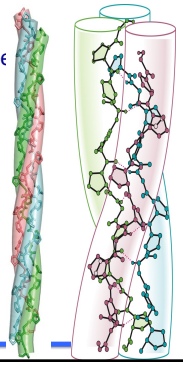
A special helical protein

- biological significance - fibrous, structural component
- Type I collagen is found in bone, tendon and skin, II in cartilage and III in blood vessels
- very different amino acid sequence from alpha helix
- 3 residues / turn
- 1/3 amino acids are glycine Gly X Y



Unique structure of collagen

- Glycine R group face inside others outside
- up to 30% are proline or hydroxyproline - important for maintaining secondary structure
- hydroxyprolines involved in H bonding of three strands together
- helical structure formed by three left handed helices twisted to form a right handed superhelix (gives strength)
- hydrogen bonding between 3 helices
- (thus the glycine)
- covalent bonding of lysine between strands necessary for strength



Fibrous – Components of collagen

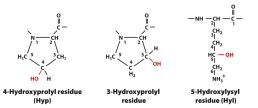
A special helical protein

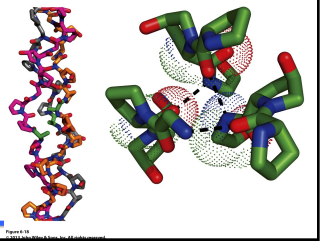
Unconventional amino acid composition: 30% glycine; >20% proline or hydroxyproline.

Helix contains 3 residues/turn (<3.6 res./turn in a-helix).

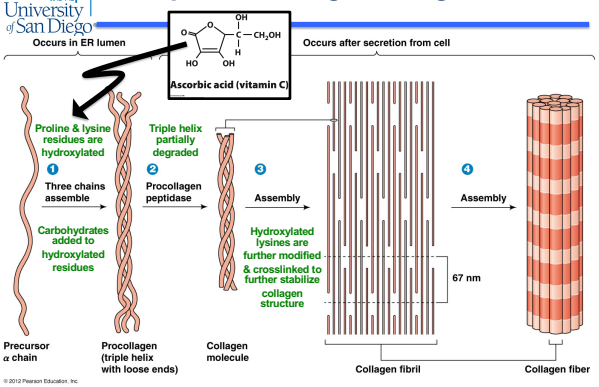
Modification to amino acids provide functional groups that stabilize structure through hydrogen bonding.

Quaternary helical structure formed by three left handed helices twisted to form a right handed superhelix (gives strength).





Steps to forming a collagen fibril

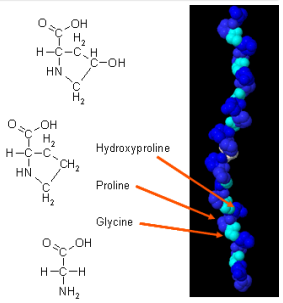


Unique structure of collagen

Hydroxylations on pro are performed by an enzyme called prolyl hydroxylase, which is an enzyme that requires vitamin C as a cofactor in the reaction.

Absence of vitamin C in the diet reduces hydroxylation of pro, and collagen fibres begin to break down and new collagen not formed properly.

Lack of vitamin C causes scurvy because collagen fibres are not formed properly, and this causes skin lesions, weakened gums so teeth fall out etc.



Unique structure of collagen

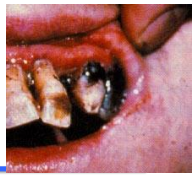
A special helical protein

Equally important is hydroxy-lys catalysed by lysine hydroxylase. Attached to the lys residues are three sugars gal-gal-glu, and these enable H-bonding to occur between triple helices, which is essential for stability of the greater complex that binds fibers together to form a matrix bed to binds cells to the matrix and form a tissue.

Collagen Related Disease

Loss of flexibility with age is likely due to increased amount cross-linked collagen compared to younger tissue

- Scurvy – problems with sea voyages, lack of food other than salted meats.



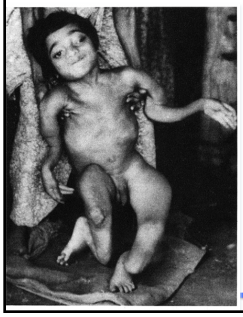
Collagen Related Disease

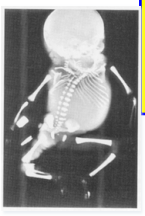
Loss of flexibility with age is likely due to increased amount cross-linked collagen compared to younger tissue

- Scurvy – problems with sea voyages, lack of food other than salted meats.
  - Symptoms include, swollen gums, loose teeth, small black-and-blue spots on the skin, and bleeding from small blood vessels are among the characteristic signs of scurvy.
  - Caused when vitamin C (ascorbic acid) is lost from diet
  - Vit C is needed to keep Iron reduced in the active site of prolyl hydroxylase. This is the enzyme responsible for conversion of proline to hydroxyproline. The H bonding of hydroxyproline is vital for the connective protein's function
  - In 1795, the British Royal Navy provided a daily ration of lime or lemon juice to all its men. English sailors to this day are called "limeys", for lime was the term used at the time for both lemons and limes.

Collagen Related Disease

- Several heritable diseases result from mutations in the collagen





Brittle Bone Disease – results from a Gly-Ala mutation – Consider the consequences of this mutation, both in the protein's triple helix and the strength of the bone!

University of San Diego

Collagen Related Disease

- **Several heritable diseases result from mutations in the collagen**

Marfan's Syndrom and Ehler's-Danlos syndromes - inherited disorder of connective tissue which affects many organ systems, including the skeleton, lungs, eyes, heart and blood vessels. All resulting from various mutation in collagen and other fibril associated proteins, ultimately affecting the structure and molecular interaction.

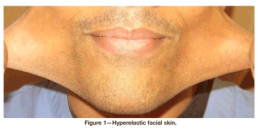


Figure 1—Hyperelastic facial skin.

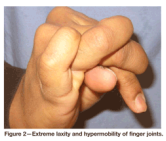


Figure 2—Extreme laxity and hypermobility of finger joints.