

School of Industrial and Information Engineering
Course 096125 (095857)
Introduction to Green and Sustainable Chemistry

 POLITECNICO DI MILANO



Protein Based Biopolymers.

Prof. Attilio Citterio

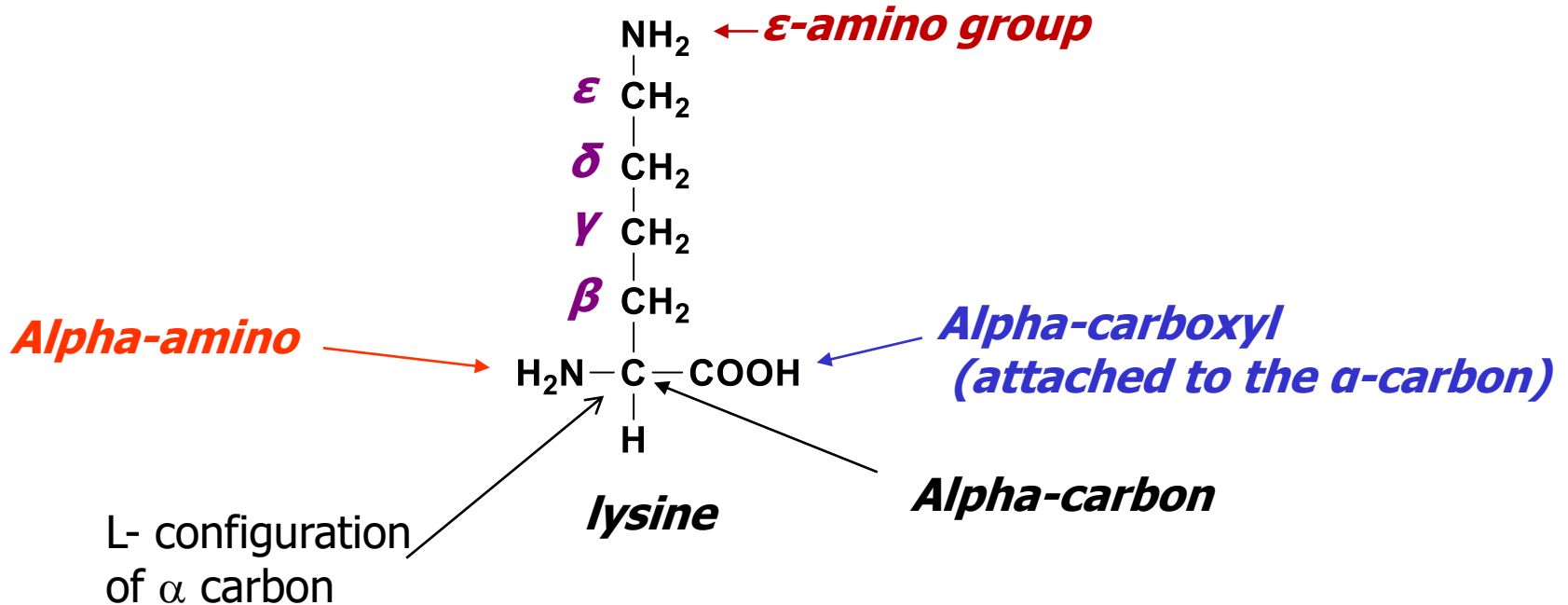
Dipartimento CMIC “Giulio Natta”

<https://iscamapweb.chem.polimi.it/citterio/it/education/course-topics/>



Numbering (lettering) Amino Acids.

- Proteins are built up by amino acids that are linked by peptide bonds to form a polypeptide chain.

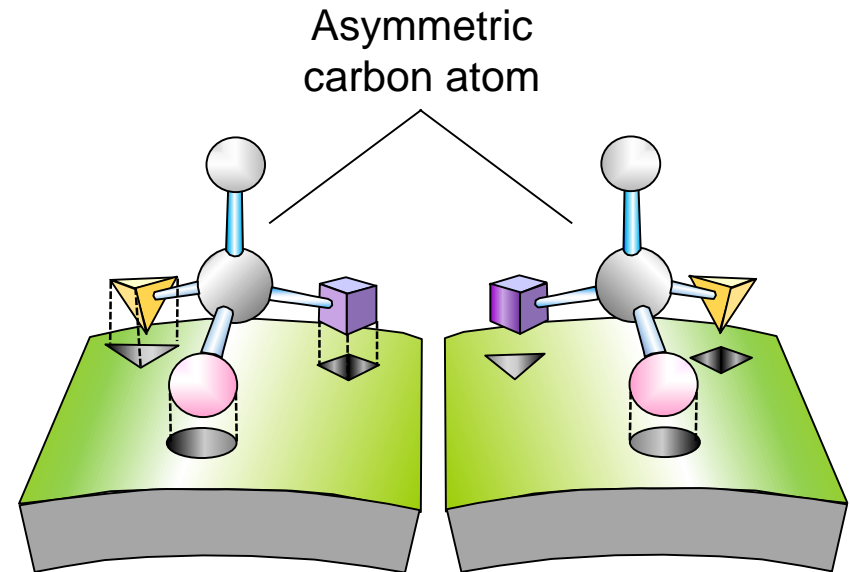


- An amino acid has several structural components:
 - A central carbon atom (C_α) is attached to
 - an amino group (NH_2),
 - a carboxyl group (COOH),
 - a hydrogen atom (H),
 - a side chain (R).



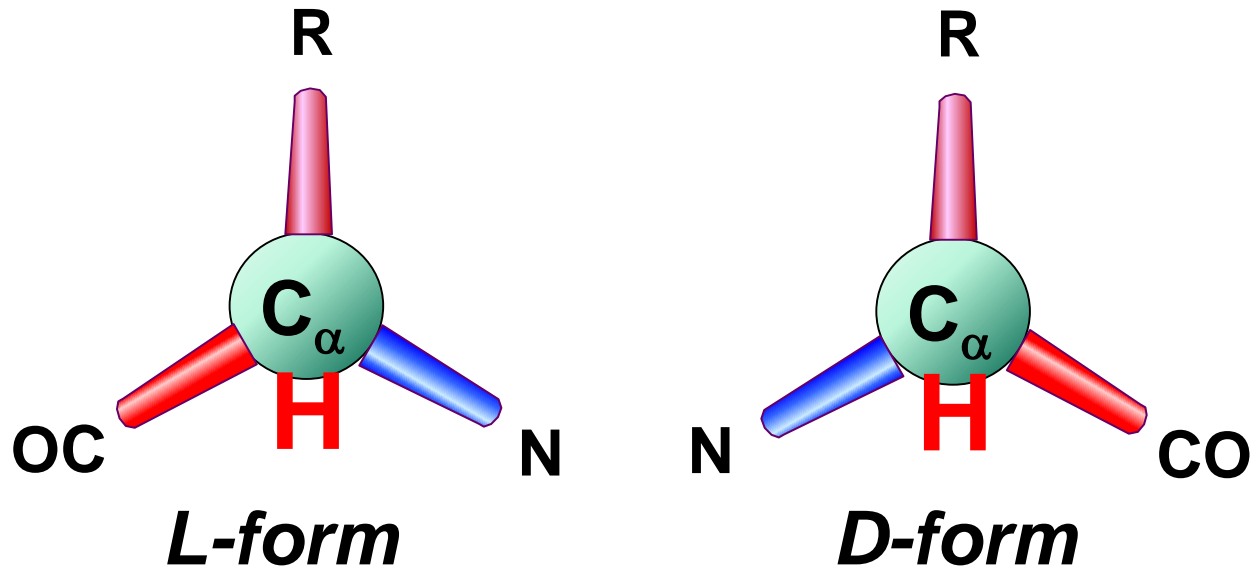
Amino Acids in 3 Dimensions.

- Asymmetric carbon (4 different groups attached)
- Stereoisomers
- Rotate polarized light
- Optical isomers
- Non-superimposable
- Mirror images
- **L and D forms**
 - **Natural: only L configuration**





The “Handedness” of Amino Acids.



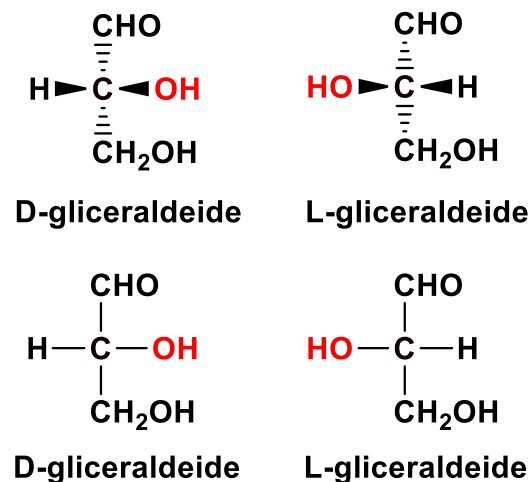
- Looking down the H- C_α bond from the hydrogen atom, the L-form has CO, R, and N substituents from C_α going in a clockwise direction. For the L-form the groups read CORN in the clockwise direction.
- All a.a. except Gly (R = H) have a chiral center
- All a.a. incorporated into proteins by organisms are in the L-form.



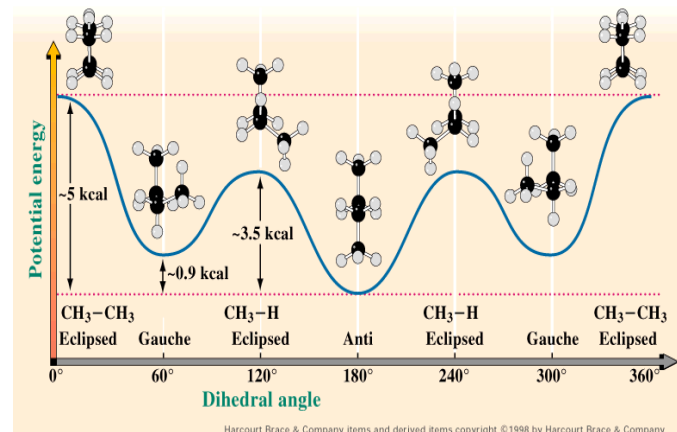
Configuration vs. Conformation.

- **Configuration** = different geometries due to orientation in space
 - *cis* vs. *trans* (planar peptide bond)
 - D vs. L; R vs. S (chiral amino acids)
 - You can not move from one configuration to another without breaking bonds.

- **Conformation** = alternating atom arrangement derived from molecular motion around a single bond
 - Chair conformation vs. boat conformation in cyclohexane
 - You can convert one conformation to another.

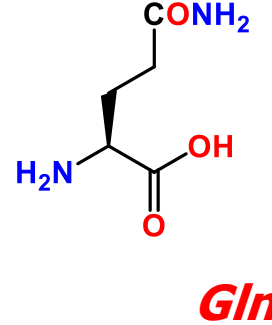
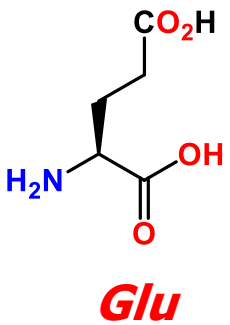
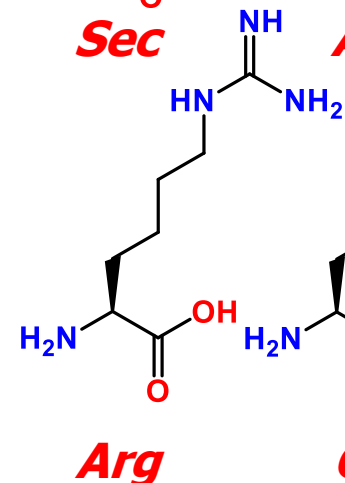
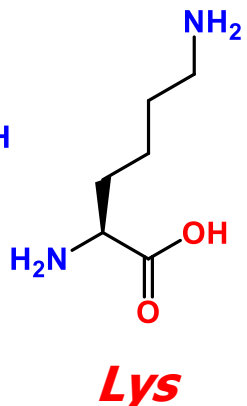
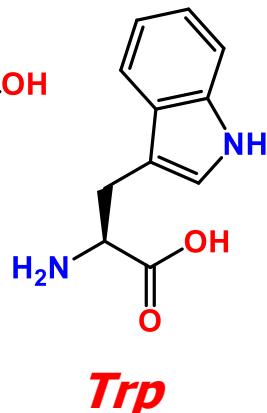
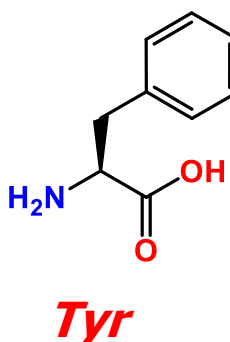
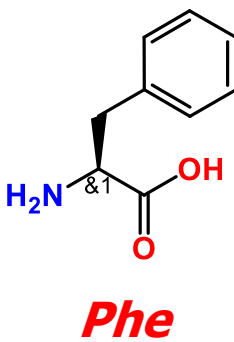
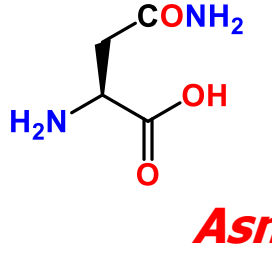
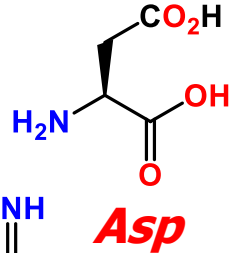
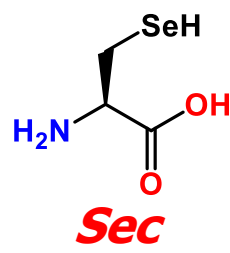
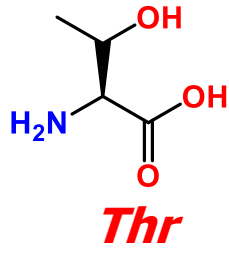
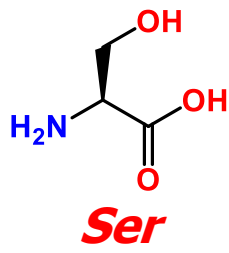
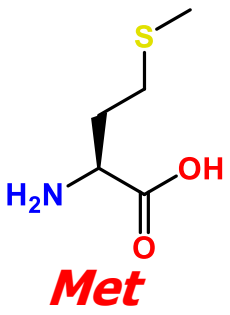
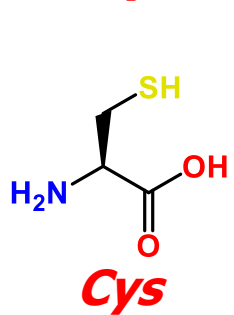
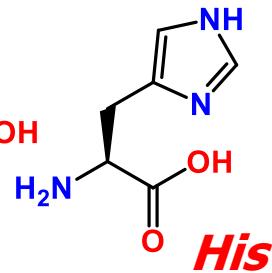
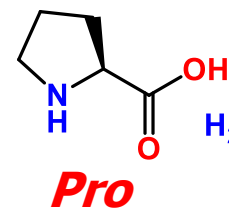
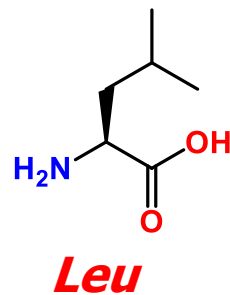
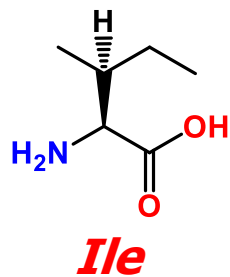
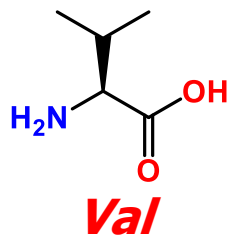
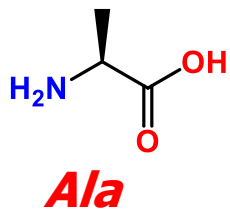
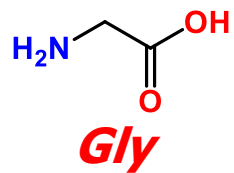


PE diagram of butane as a function of dihedral angle





Natural Amino Acids (20).





The Twenty Amino Acids Found in Proteins (1).

1) Amino acids with **positive/negative** charged hydrophilic side chains

Positive \oplus

Negative \ominus

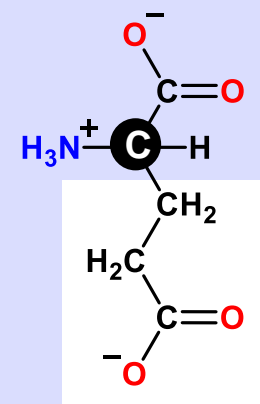
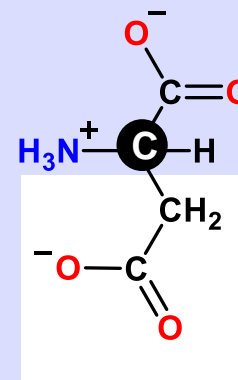
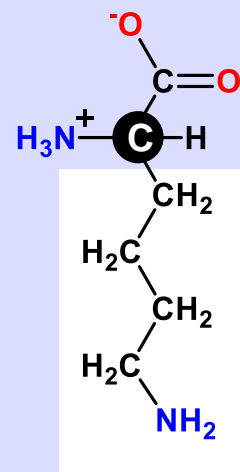
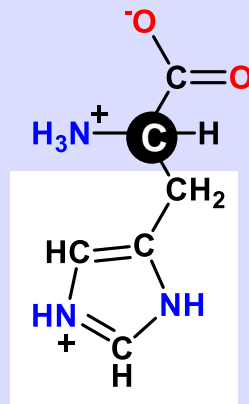
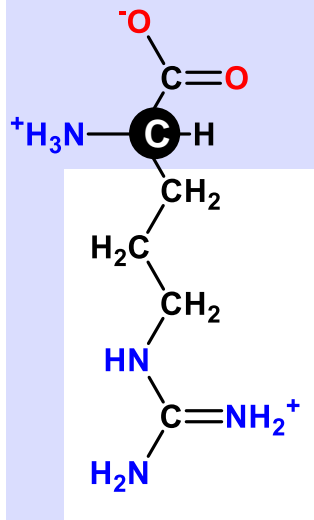
Arginine
(Arg) (R)

Histidine
(His) (H)

Lysine
(Lys) (K)

Aspartic acid
(Asp) (D)

Glutamic acid
(Glu) (E)

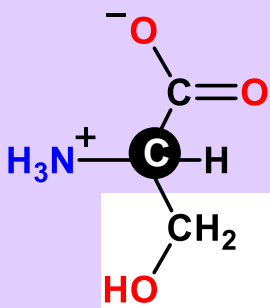




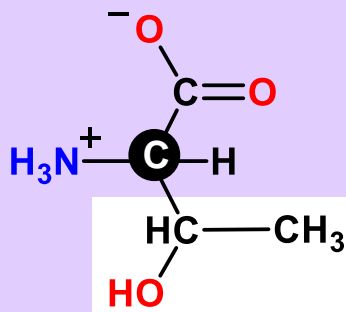
The Twenty Amino Acids Found in Proteins (2).

2) Amino acids with polar (hydrophilic) uncharged side chains

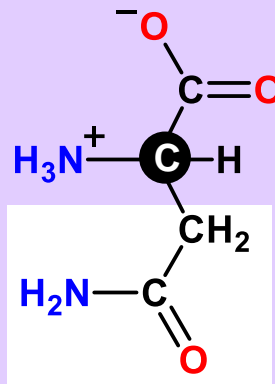
Serine
(Ser) (S)



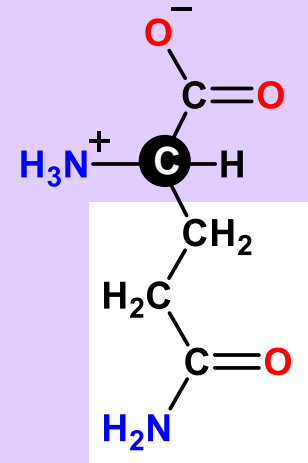
Threonine
(Thr) (T)



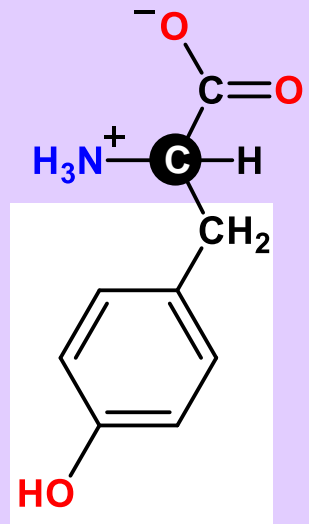
Asparagine
(Asn) (N)



Glutamine
(Gln) (Q)

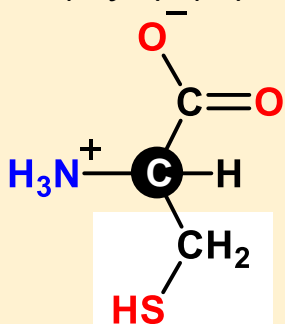


Tyrosine
(Tyr) (Y)

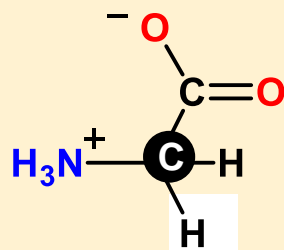


2') Special cases

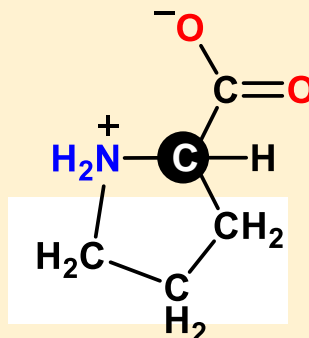
Cysteine
(Cys) (C)



Glycine
(Gly) (G)



Proline
(Pro) (P)

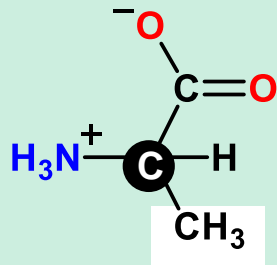




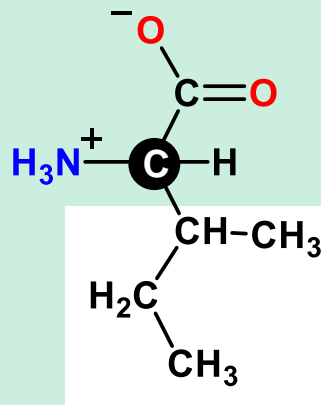
The Twenty Amino Acids Found in Proteins (3).

3) Amino acids with nonpolar (hydrophobic) side chains

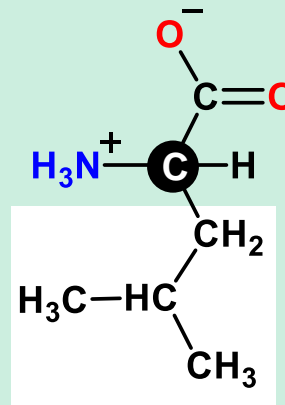
Alanine
(Ala) (A)



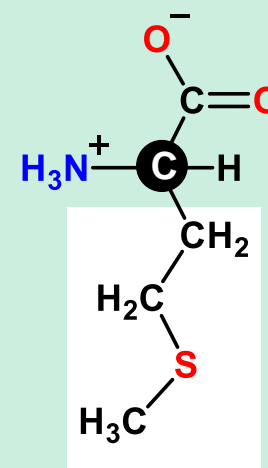
Isoleucine
(Ile) (I)



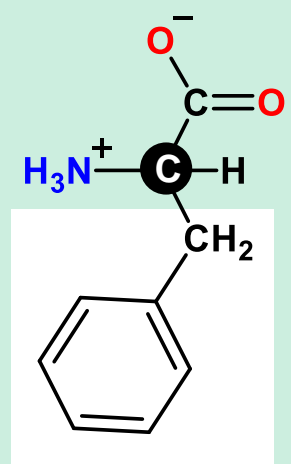
Leucine
(Leu) (LN)



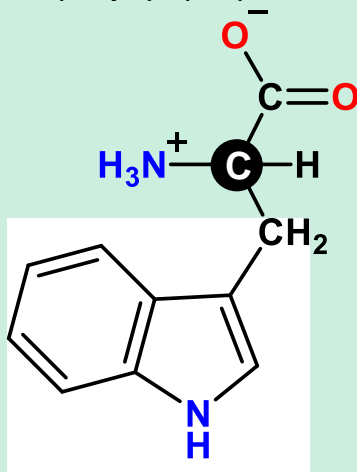
Methionine
(Met) (M)



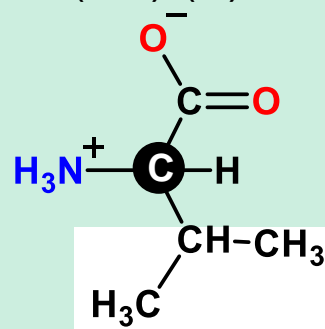
Phenylalanine
(Phe) (F)



Tryptophan
(Trp) (W)



Valine
(Val) (V)





Classes of Amino Acids.

- Nonpolar (NP), non-interactive
 - ala, val, leu, ile, pro, trp, phe, met
- Nonpolar (overall), interactive (I) group
 - cys, tyr
- Polar (P)
 - gly, ser, thr, asn, gln (gly is non-interactive)
- Acidic (A)
 - asp, glu
- Basic (B)
 - lys, arg, his



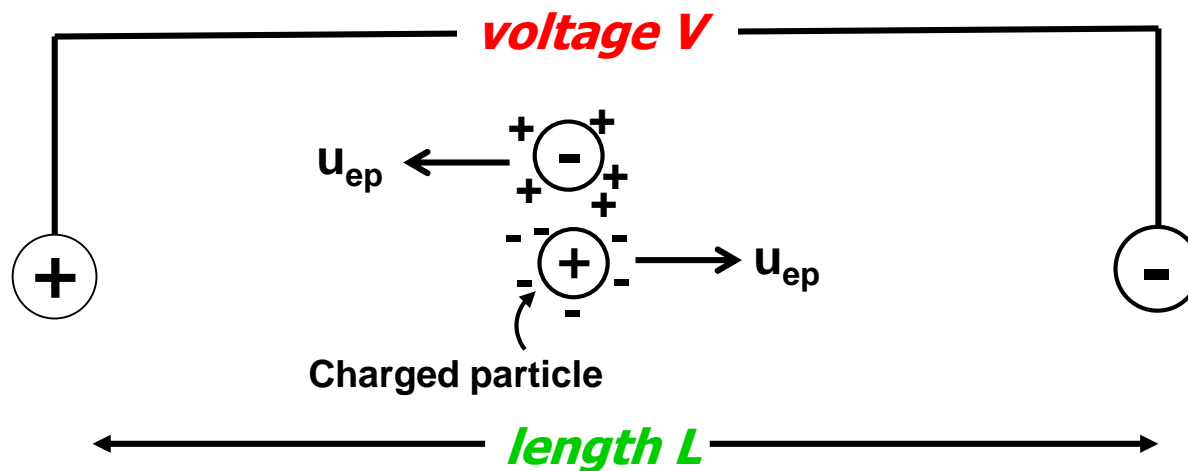
pK_a Values of Natural Amino Acids.

AMINO ACID	α - Carboxy	α - Amino	Side Chain Unit
Gly	2.34	9.60	
Ala	2.34	9.69	
Val	2.32	9.62	
Leu	2.36	9.68	
Ile	2.36	9.68	
Ser	2.21	9.15	
Thr	2.63	10.43	
Met	2.28	9.21	
Phe	1.83	9.13	
Trp	2.38	9.39	
Asn	2.02	8.80	
Gln	2.17	9.13	
Pro	1.99	10.6	
Asp	2.09	9.82	3.86
Glu	2.19	9.67	4.25
His	1.82	9.17	6.00
Lys	2.18	8.95	10.53
Arg	2.17	9.04	12.48
Cys	1.71	10.78	8.33
Tyr	2.20	9.11	10.07



Isoelectric Point.

- The net charge on an amino acid or peptide changes as the pH is changed.
- **Isoelectric Point (pI)** - The pH at which the net charge on an amino acid or peptide chain is zero.
- Electrophoresis - A method of separating charged species by causing them to migrate toward a positive or negative electrode.
 - Positive ions move toward the negative electrode
 - Negative ions move toward the positive electrode.



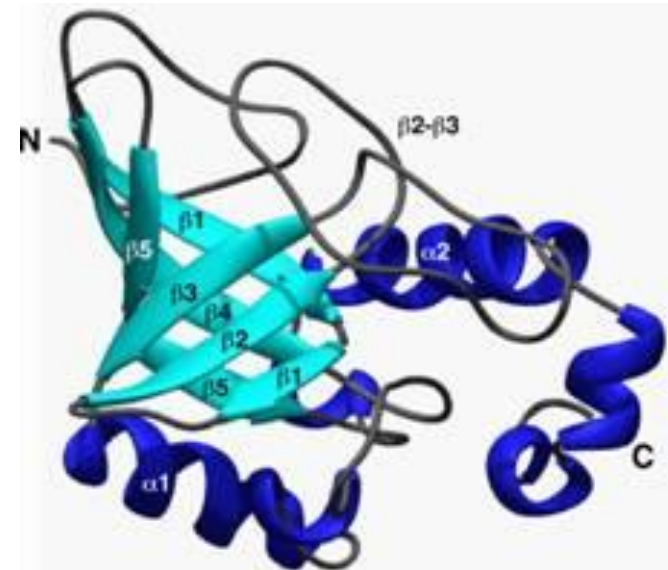
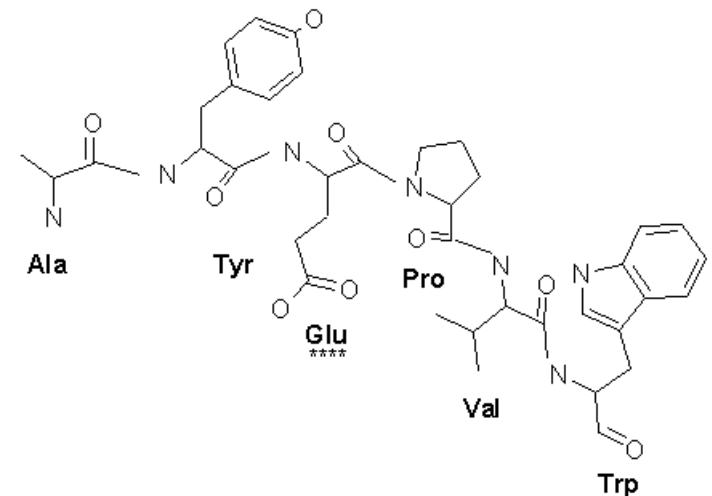
$$u_{ep} = \mu_{ep} \frac{V}{L}$$

V : applied voltage / V
 μ_{ep} : electrophoretic mobility / $m^2/(s V)$



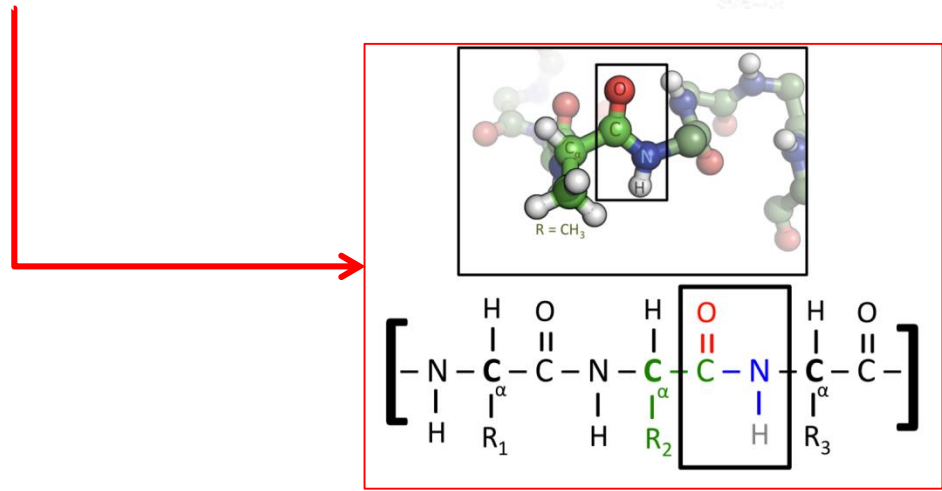
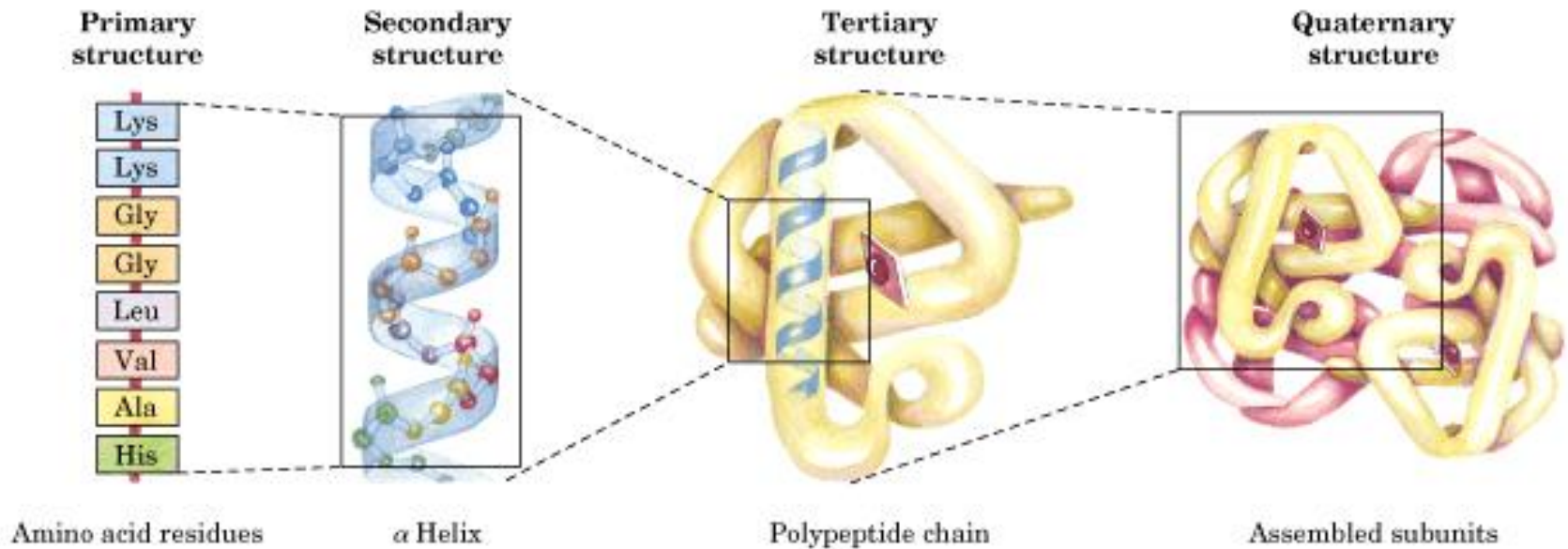
Peptides, Polypeptides and Proteins.

- **peptide** = a condensation product of amino acids
 - dipeptide = 2 aa, etc
 - oligopeptide = up to 20 aa
 - polypeptide = > 20 aa
- **protein** = a functional polypeptide with a biological role
 - sometimes contains non-polypeptide portions as well.
 - very small “proteins” are often hormones





The Four Levels of Protein Structure.





Levels of Protein Structure – I.

□ primary structure (1°)

- the sequence of amino acids with modifications, including additions of other units **covalently**

□ secondary structure (2°)

- **patterned large scale** H-bonding involving the **backbone** components of the chain

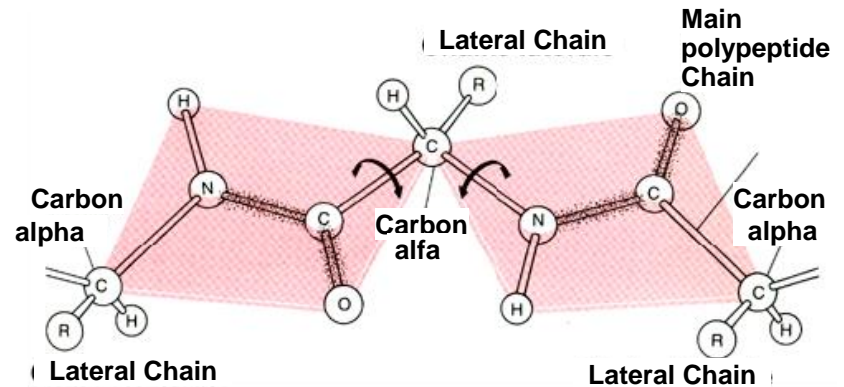
➤ domains and motifs

- an intermediate type of terms meant to describe a certain region of a protein having certain structural features

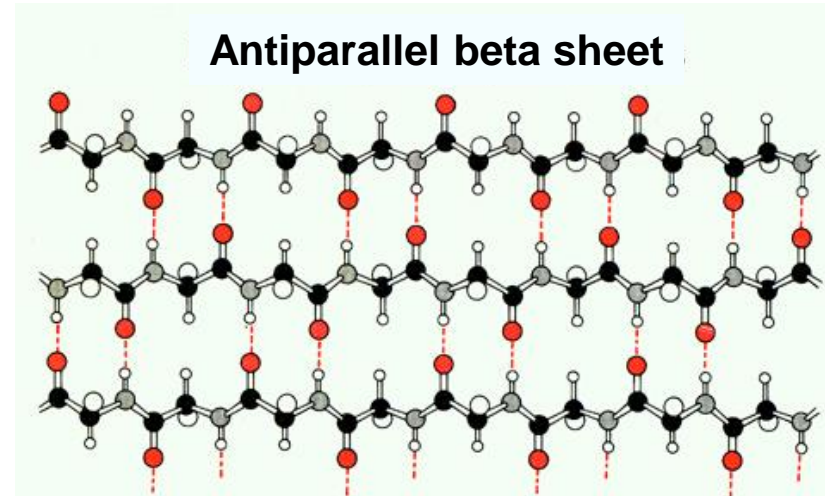
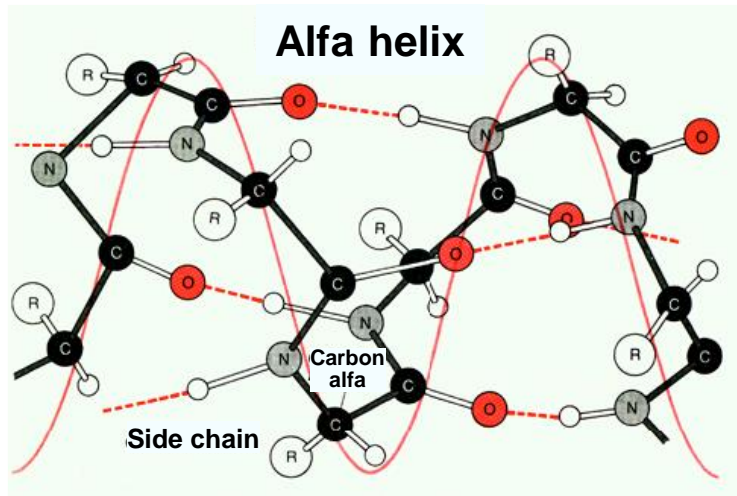


Tridimensional Structure of Proteins.

Peptide chain
= primary structure



Secondary structure





Denaturation of Proteins.



Cooking denatures proteins

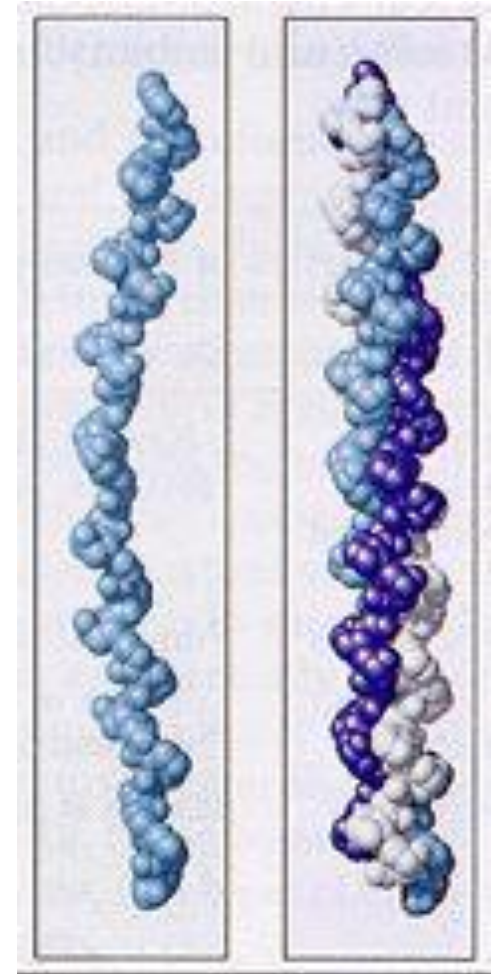
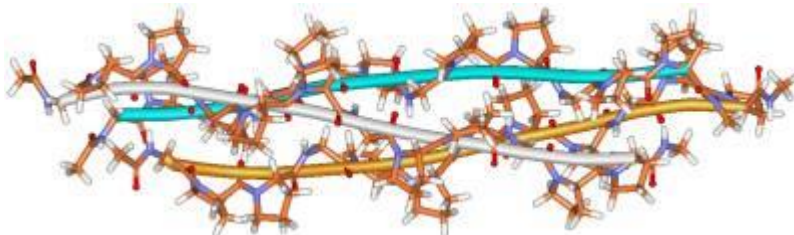
- Changes that occur to an egg white when is cooked
- Protein modify irreversibly their native tridimensional structure and can liberate the species they bind (i.e. biotin and iron) for digestion.

Other conditions (acidity, radiation, radicals, some chemicals,) produce similar irreversible denaturation processes.



Secondary Structure of a Protein: Collagen.

- Triple Helix - Three coiled polypeptide chains intertwined to form a rope
 - **Structural** function (**connective tissue**)
 - H-bond is perpendicular to chains
 - H-bonds are **inter**-molecular
 - H-bond is between glycine donor N-H and proline (hydroxyproline) C=O acceptor
 - alternating H-bonds between chains
 - one H-bond per one unit of triple helix
 - It represent 25-30% of all human proteins.





Amino Acid Composition of Fibrous Proteins.

	a-Keratin (Wool)	Fibroin (Silk)	Collagen (Tendon)	Elastin (Aorta)
Gly	8.1	44.6	32.7	32.3
Ala	5.0	29.4	12.0	23.0
Ser	10.2	12.2	3.4	1.3
Glu + Gln	12.1	1.0	7.7	2.1
Cys	11.2	0	0	tr.
Pro	7.5	0.3	22.1	10.7
Arg	7.2	0.5	5.0	0.6
Leu	6.9	0.5	2.1	5.1
Thr	6.5	0.9	1.6	1.6
Asp + Asn	6.0	1.3	4.5	0.9
Val	5.1	2.2	1.8	12.1
Tyr	4.2	5.2	0.4	1.7
Ile	2.8	0.7	0.9	1.9
Phe	2.5	0.5	1.2	3.2
His	0.7	0.2	0.3	tr.
Met	0.5	0	0.7	tr.
Trp	1.2	0.2	0	tr.



Levels of Protein Structure (2).

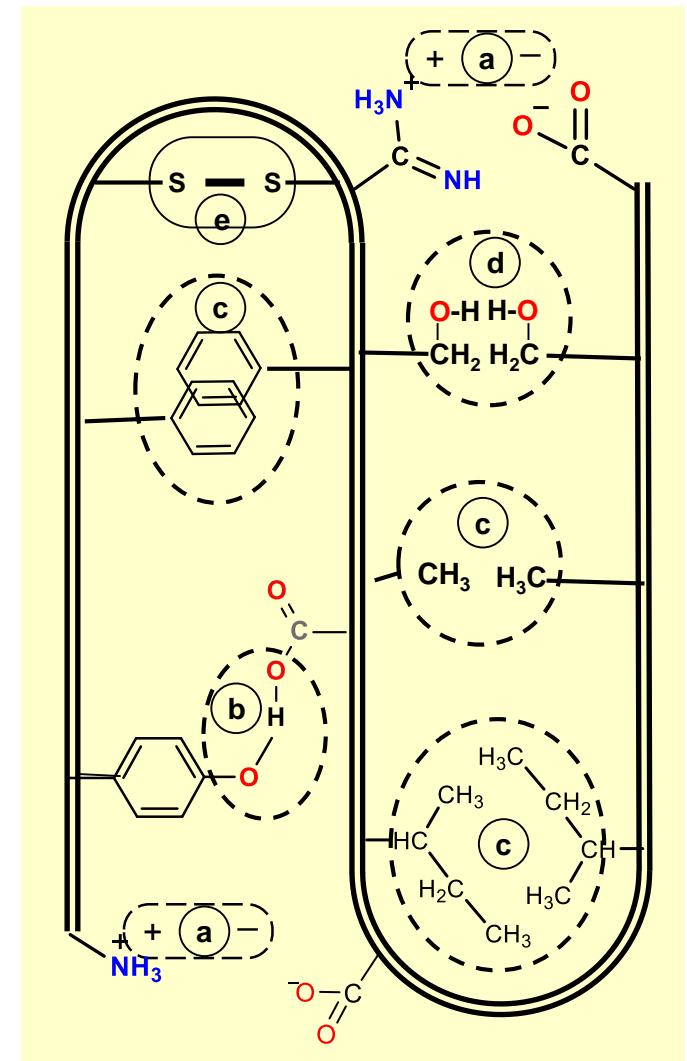
- **tertiary structure (3°)**
 - other attraction and reactions within a single polypeptide chain

- **quaternary structure (4°)**
 - associations of polypeptide chains with:
 - a) other polypeptides
 - b) other biopolymers
 - c) with small (organic) molecules
 - d) with small (inorganic) usually metal ions



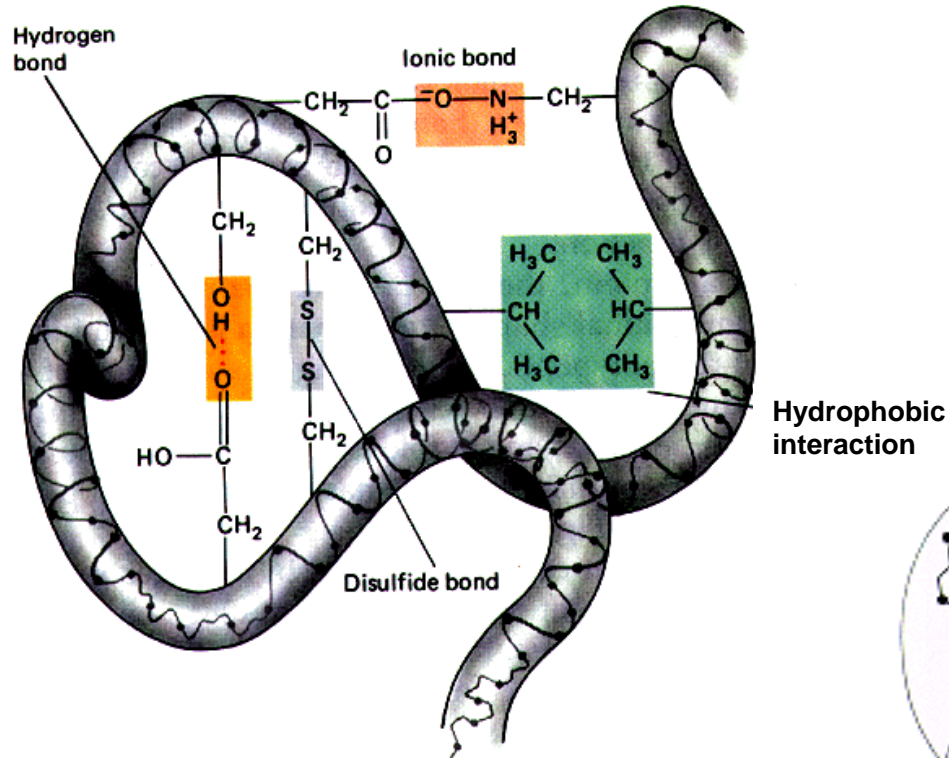
Tertiary Structure of a Protein.

- ❑ Five classes of tertiary structure:
 - Salt linkages (a)
 - Side chain H-bonding (b)
 - Hydrophobic force (attraction) (c)
 - Dipole-dipole interaction (d)
 - Disulfide linkages (e)
- ❑ All involve interactions between side chains of amino acids in chain.
- ❑ Disulfide linkages are covalent bonds; others are weak attractions (exception are ionic bonds).

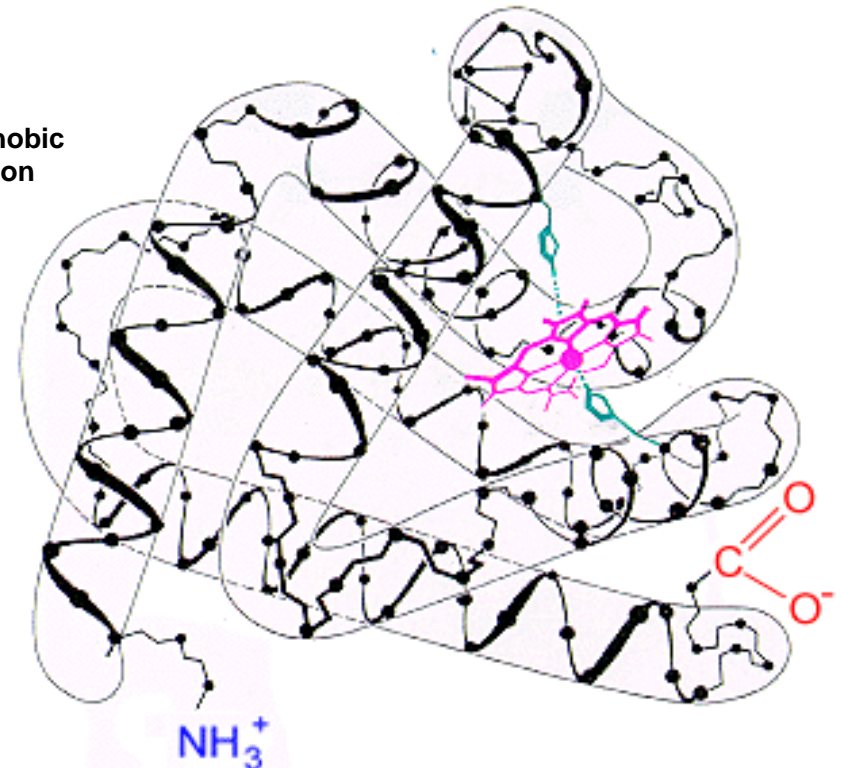




Tertiary Structure of Proteins (2).



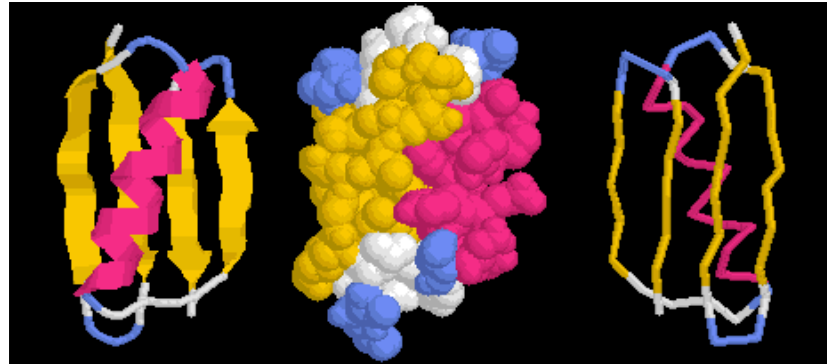
Myoglobin - a tertiary structure





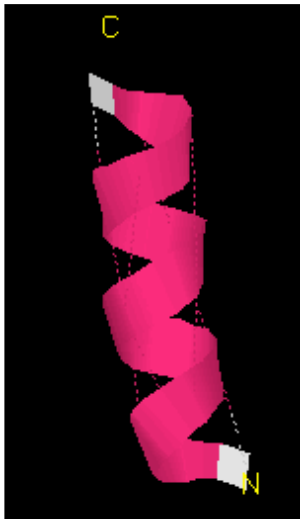
Alternative Visualization of Proteins.

Ribbons



Lines

α helix

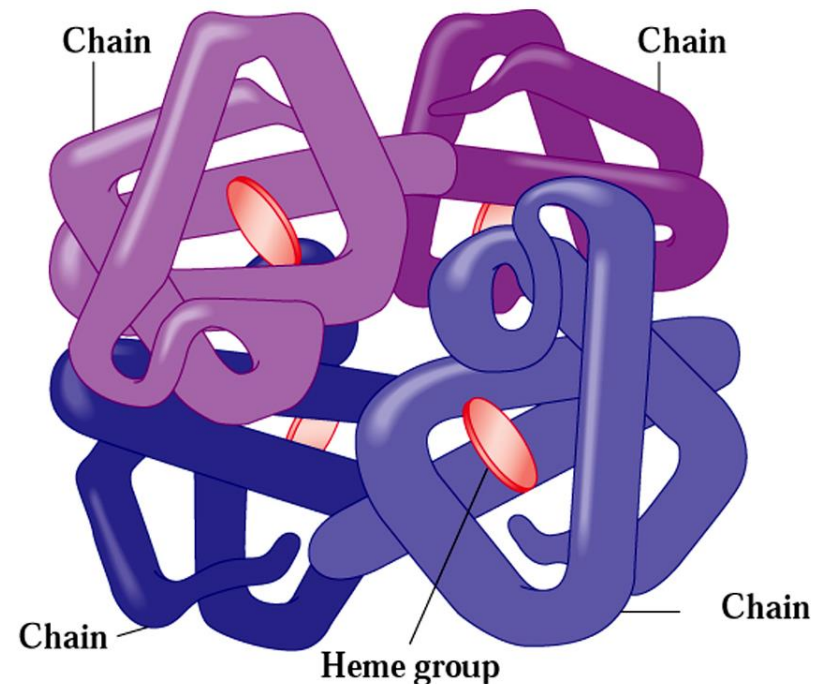


β sheets
and hair
connectors



Quaternary Structure in Proteins.

- The **quaternary structure** contains two or more tertiary subunits (protein chains)
- Held together by same interactions as tertiary structure
- **Hemoglobin** contains four chains
- The heme group in each subunit picks up oxygen for transport in the blood to the tissues





Variability in Protein Organization.

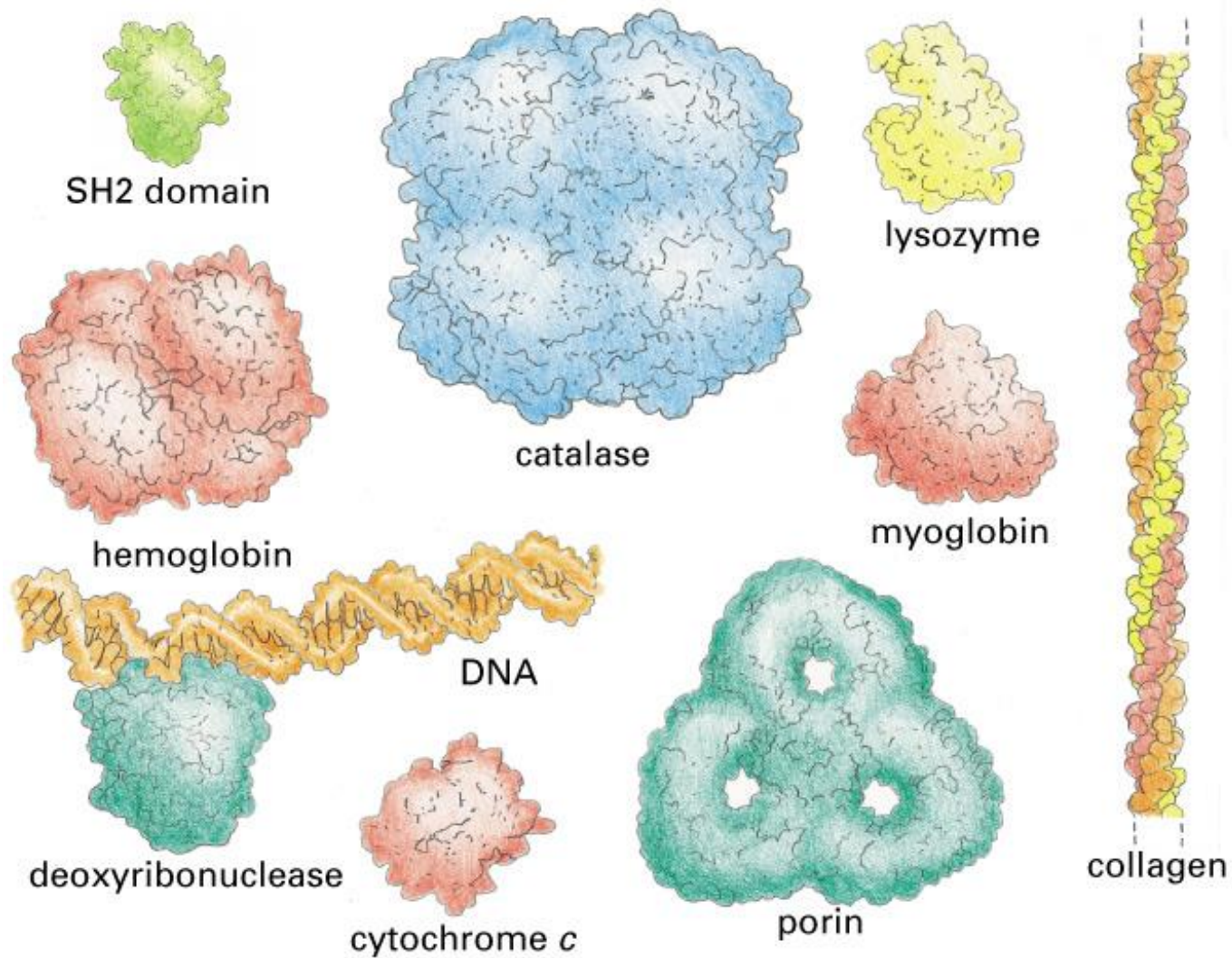


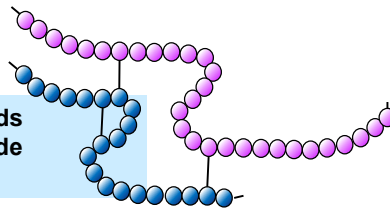
Figure 3-24 part 1 of 2. Molecular Biology of the Cell, 4th Edition.



Summary of Structural Levels in Proteins.

PRIMARY STRUCTURE

The sequence of amino acids present in a protein's peptide chain or chains



SECONDARY STRUCTURE

The regularly repeating ordered spatial arrangements of amino acids near each other in the protein chain, which result from hydrogen bonds between carbonyl oxygen atoms and amino hydrogen atoms.

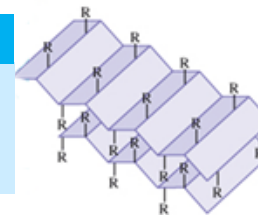
Alpha Helix

Hydrogen bonds between every fourth amino acids



Beta Pleated Sheet

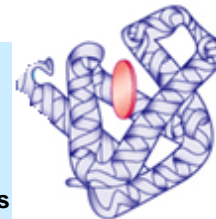
Hydrogen bonds between two side-by-side chains, or a single chain that is folded back on itself.



TERTIARY STRUCTURE

The overall three-dimensional shape that results from the attractive forces between amino acid side chains (R groups) that are not near each other in the protein chain

- Disulfide bonds
- Electrostatic interaction
- Hydrogen bonds
- Hydrophobic Interactions



QUATERNARY STRUCTURE

The three-dimensional shape of a protein consisting of two or more independent peptide chains, which results from noncovalent interactions between R groups

- Electrostatic interaction
- Hydrogen bonds
- Hydrophobic Interactions

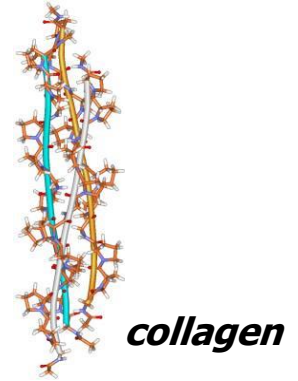
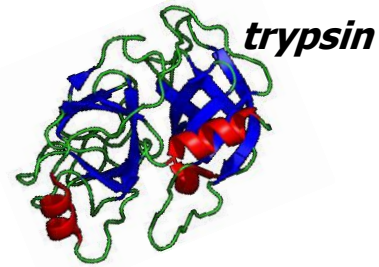




Protein Classification.

➤ Classification by shape

- globular
- fibrous



➤ Classification by function

- Structural



- Signaling pathways



- Metabolic



- Transportation



➤ Classification by what else is present

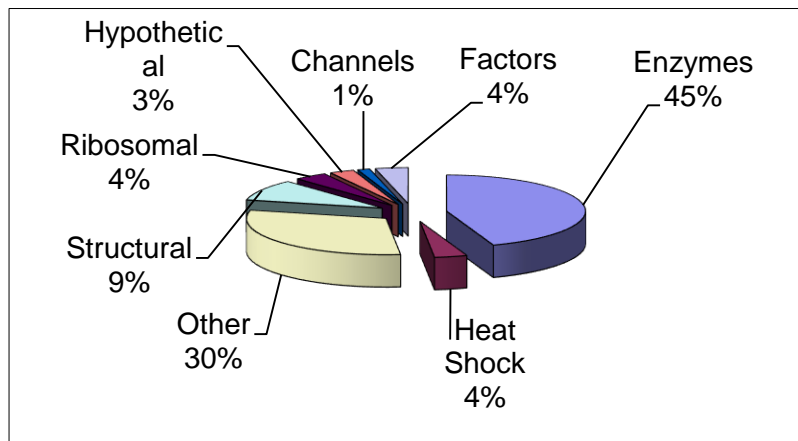
- derivative proteins



Protein Classification by Function.

➤ Classification:

- Enzymes
- Structural proteins
- Defense proteins
- Transport proteins
- Storage proteins
- Effector proteins
 - ✓ hormones, reg. proteins, toxins

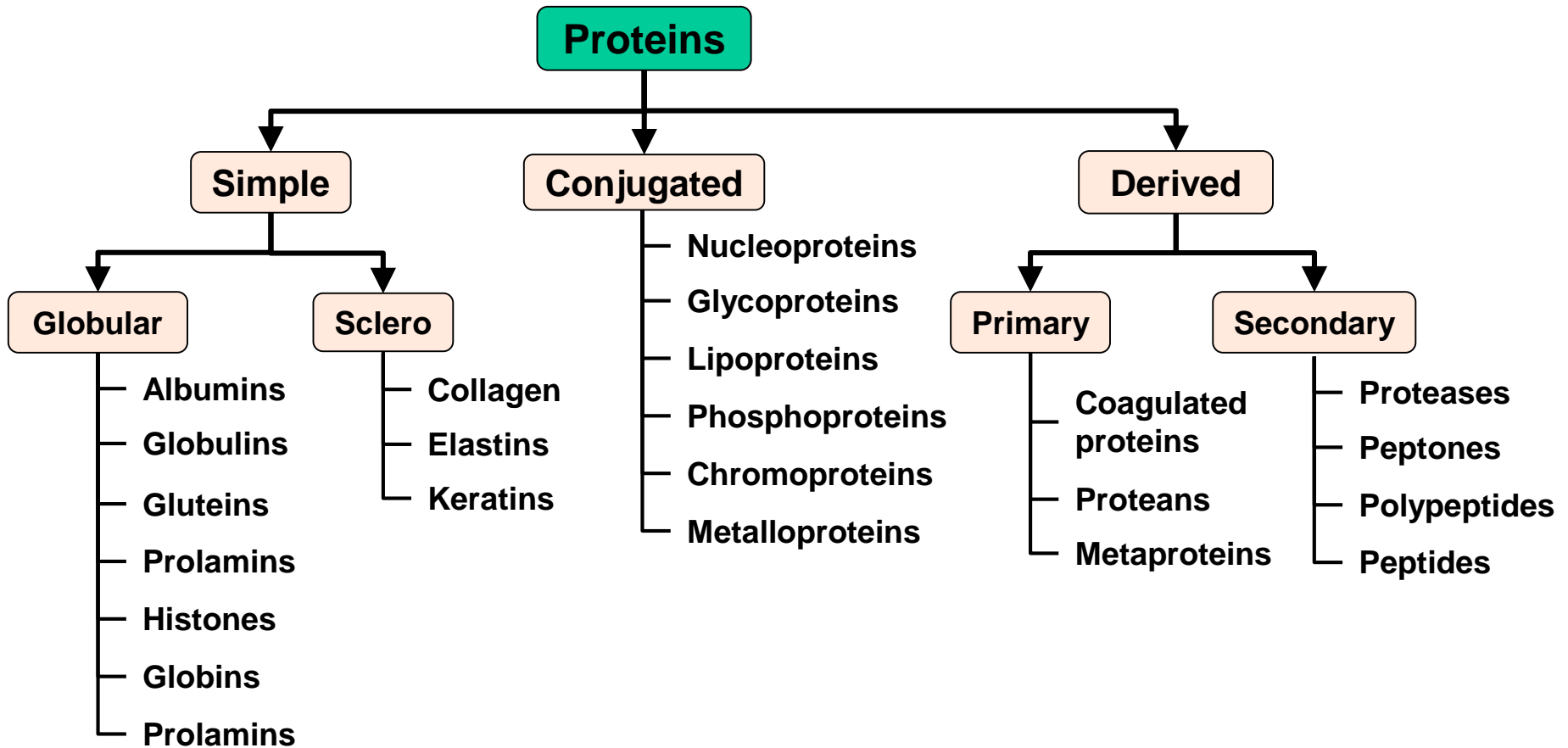


➤ Functions of protein:

- Provide structural and mechanical support
- Maintain body tissues
- Functions as enzymes and hormones
- Help maintain acid-base balance
- Transport nutrients
- Assist the immune system
- Serve as a source of energy when necessary
- Serve for cell movement and transport.



Classification of Proteins.





Derivative Proteins.

- Glycoproteins
- Lipoproteins
- Nucleoproteins
- Conjugated proteins (holoproteins)
 - protein portion = apoprotein
 - attached small groups = organic/inorganic pieces
 - heme, flavin, metals, phosphate

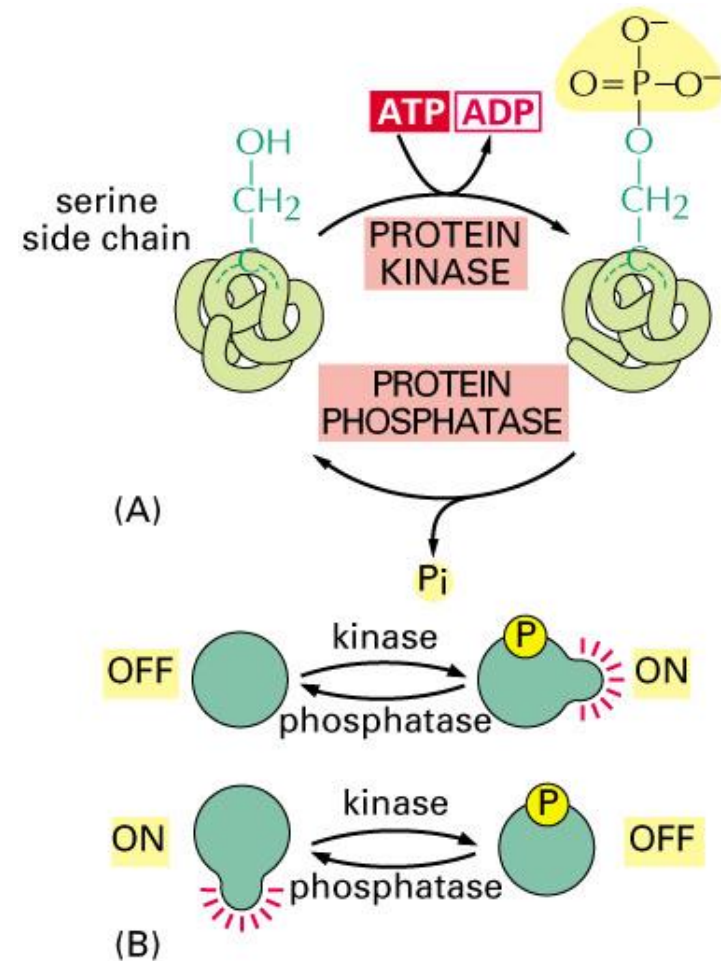
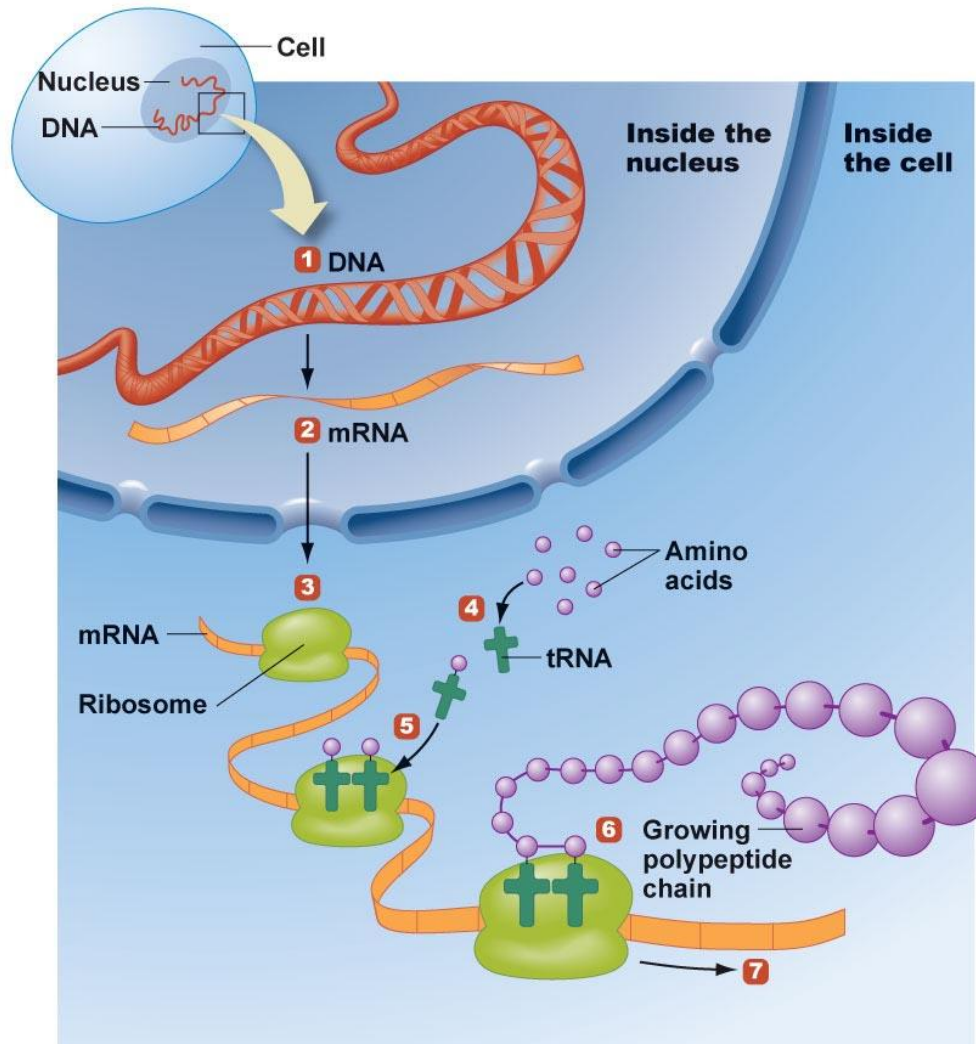


Figure 3-63. Molecular Biology of the Cell, 4th Edition.



Protein Synthesis in Cell.



1 Each strand of DNA holds the code to create specific proteins. Because the DNA can't leave the nucleus of the cell, a copy of the code, called messenger RNA (mRNA), is made. This is called transcription.

2 The mRNA takes this information outside the nucleus and brings it to the ribosome.

3 The ribosome moves along the mRNA, reading the code. This is the phase called translation.

4 Another type of RNA called transfer RNA (tRNA) collects the specific amino acids that are needed to make the protein. There are 20 different tRNAs, one for each amino acid.

5 The tRNA brings the amino acid to the ribosome.

6 The ribosome then builds a chain of amino acids (the protein) in the proper sequence, based on the code in the mRNA, called elongation.

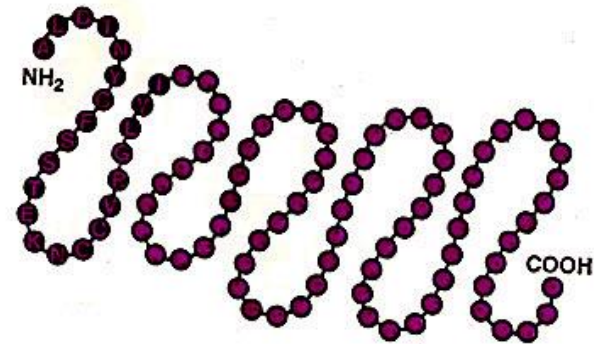
7 The ribosome continues to move down the mRNA strand until all the appropriate amino acids are added and the protein is complete.

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PROTEINS – Definitions.

Apoprotein – amino acids only.



Cofactors – small organic (e.g., vitamins, ATP, NAD, FAD) or inorganic molecules (particularly metal ions) that are required for activity; can be **loosely** bound (coenzymes) or **tightly** bound (prosthetic groups).

Prosthetic group – **tightly** bound group (e.g., heme) to apoprotein.

Holoprotein – **active** protein with cofactors and prosthetic groups attached.



COFACTORS.

- ✓ They may participate directly in catalytic processes or carry other small molecules; binding to proteins may be weak or strong
- ✓ are required in small quantities, may have to be supplied in diet and are either water or fat soluble

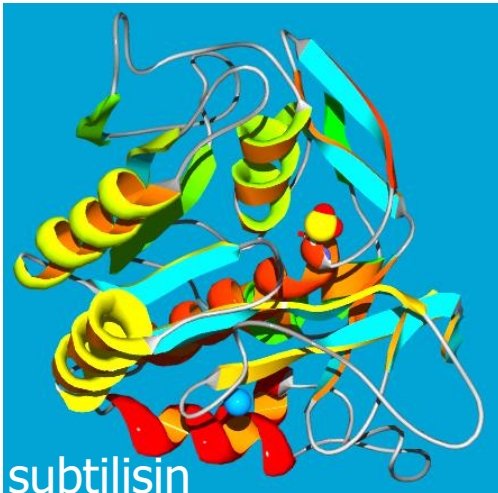
Functions (see slides on secondary metabolites)

- metal ions maintain protein conformation through electrostatic interactions (METAL LIGATION)
- prosthetic groups like heme may bind to active site and change the conformation to control bonding
- may accept a substrate during reaction
 - *common bridging ligands*
 O^{2-} , OH^- , $-CH_2S^-$, S^{2-} , $-CH_2CO_2^-$, imidazole
 - *exogenous terminal ligands are also often bound to metals*
 - H_2O , OH^- , O^{2-} , HS^- , S^{2-}

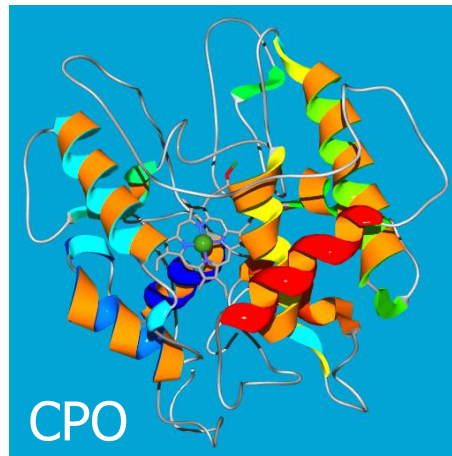


Enzymes.

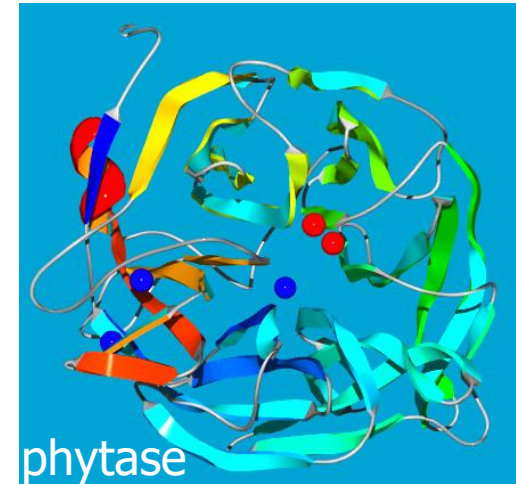
Produced by living organisms, are compounds of proteic nature with **catalytic properties**. These catalysts are both efficient and highly specific for an individual chemical reaction which involves the synthesis, degradation or alteration of a compound. In these reactions, where molecules are reduced, oxidized, transposed, or assembled, cofactors are frequently involved. Some enzymes are modified covalently by phosphorylation, glycosylation, and other processes.



Promotes the proteolysis of a peptide bond. .



Chloroperoxidase catalyzes several oxidations of organic substrates.



Catalyzes the hydrolysis of phytic acid.



Proteins and Bio-transport: Types of Movement in Living Organisms.

1. **Continuous movement:** occurs inside each cell of the living organism cells for the continuity of its vital activities, such as cytoplasmic streaming.
2. **Positional movement:** occurs in some organs of the living organism, peristalsis movement in the intestines of vertebrates.
3. **Total movement:** By which the living organism can move from a place to another in order to search for food or a mate or to avoid some dangers, It leads to the spread of the animal in nature and as the means of movement was strong and fast, the circle of animal spread increases.

Motion has evolved in a variety of structured systems (or organs) by using very similar molecules, i.e. special proteins generally named **molecular motors**



Molecular Motors.

Most forms of movement in living world are powered by tiny protein machines. Among the best known are motors that use sophisticated intramolecular amplification mechanisms to take nanometer steps along protein tracks in the cytoplasm.

Kinesin and Dynein.

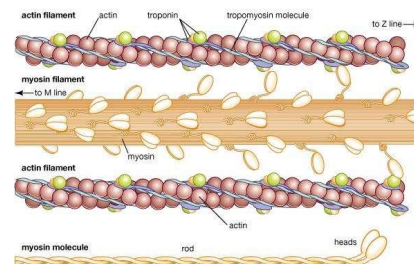
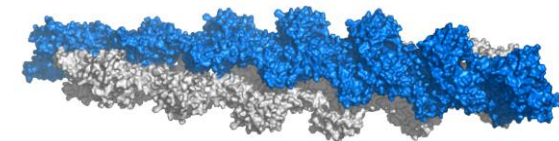
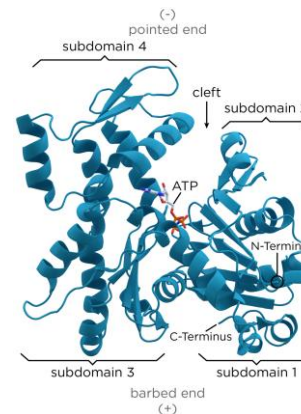
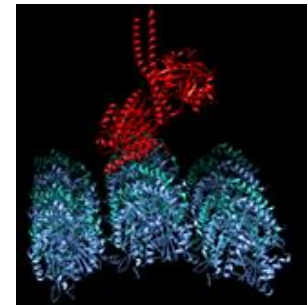
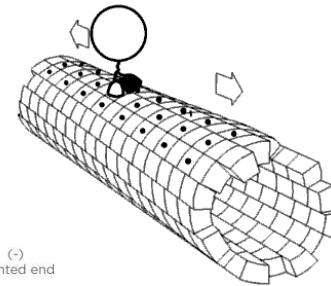
Kinesin and dynein are the molecular motors responsible for transport along microtubules.

Actin.

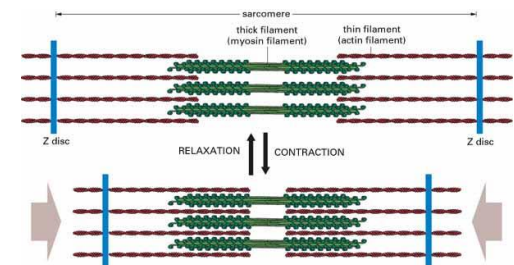
Actin is a family of globular multi-functional proteins that form micro-filaments in all eukaryotic cells.

Myosins.

Myosins are ATPases that generate force for movement of actin filaments.



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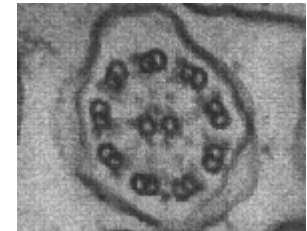
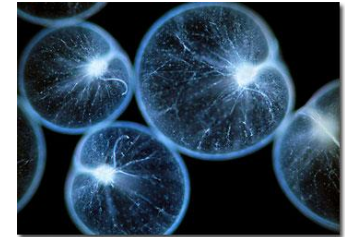




Proteins in Locomotion in Living Organisms.

Locomotion in the **higher unicellular organism** is carried out by:

1. **Amoeboid (= Pseudopodial) Movement:**
2. **Flagellar Movement:** long, sheathed cylinder containing microtubules in a 9+2 arrangement:
 - covered by an extension of the cell membrane
 - 10X thicker than prokaryotic flagella
 - function in motility
3. **Ciliary Movement:** similar in overall structure to flagella, but shorter and more numerous found only on a single group of protozoa and certain animal cells



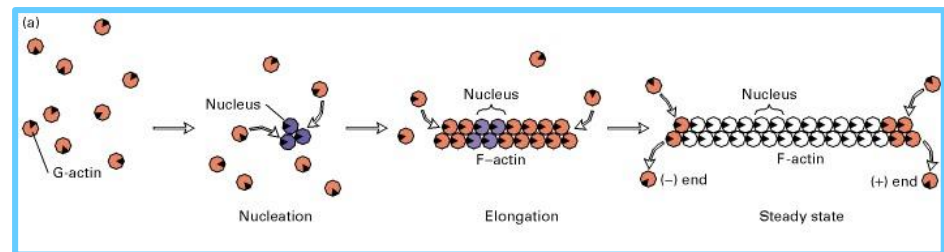
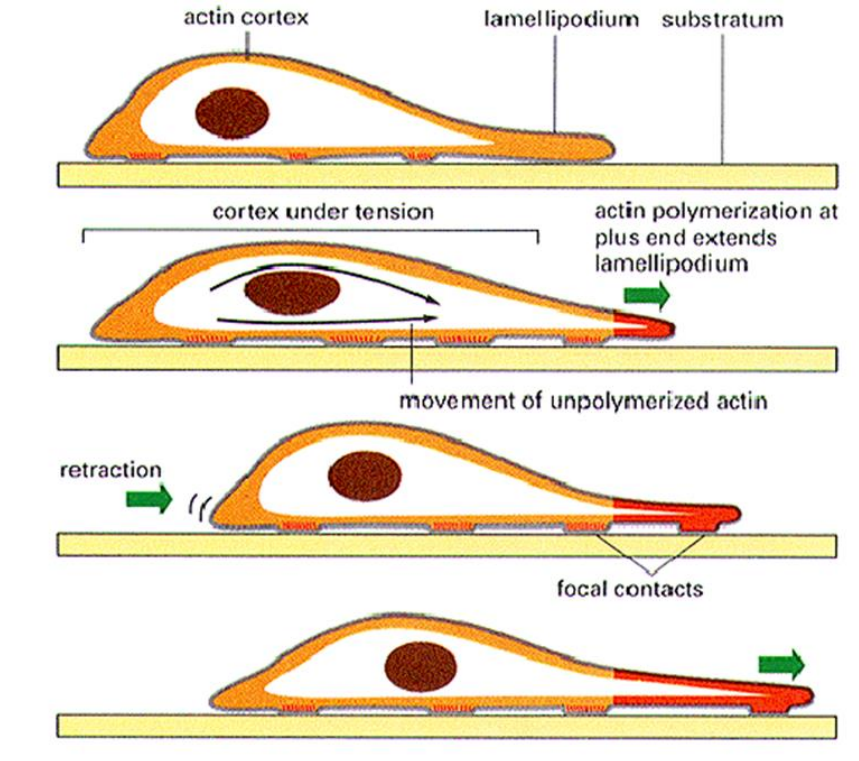
The **multicellular organism** like humans and other members of Animal kingdom uses locomotory organs such as legs, hands, fins, etc. to move in the surrounding

4. **Muscular Movement:**



Amoeboid Movement.

- **Cytoplasmic extension**
 - **Actin** (45 kDalton) polymerization thrusts leading edge of cell cytoplasm forward
- **Adhesion**
 - Leading edge adheres to surface
- **Retraction**
 - Interaction between actin and myosin
 - ATP hydrolysis



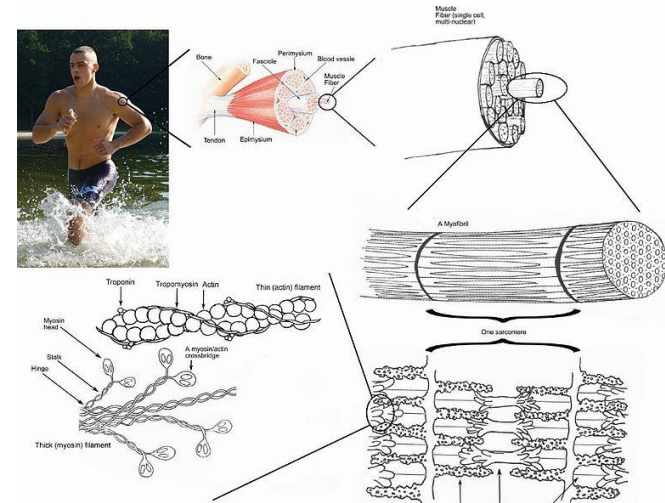


Muscular Movement.

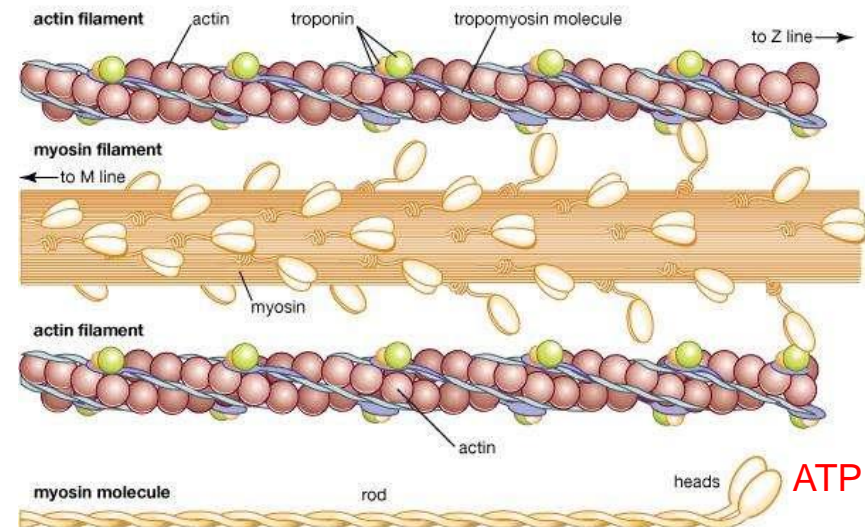
Myosin is a motor protein that generates the force in a muscle contraction. It consists of a head and a tail region. Together, the tails of approximately three hundred myosin molecules form the shaft of the thick filament. The myosin heads of these molecules project outward toward the thin filaments like the oars of a rowboat.

Actin Molecules and Thin Filaments

Actin is a spherical protein that forms, among other things, the thin filament in muscle cells. Thin filaments are composed of two long chains of these actin molecules that are twisted around one another. Each actin molecule has a myosin-binding site where a myosin head can bind.



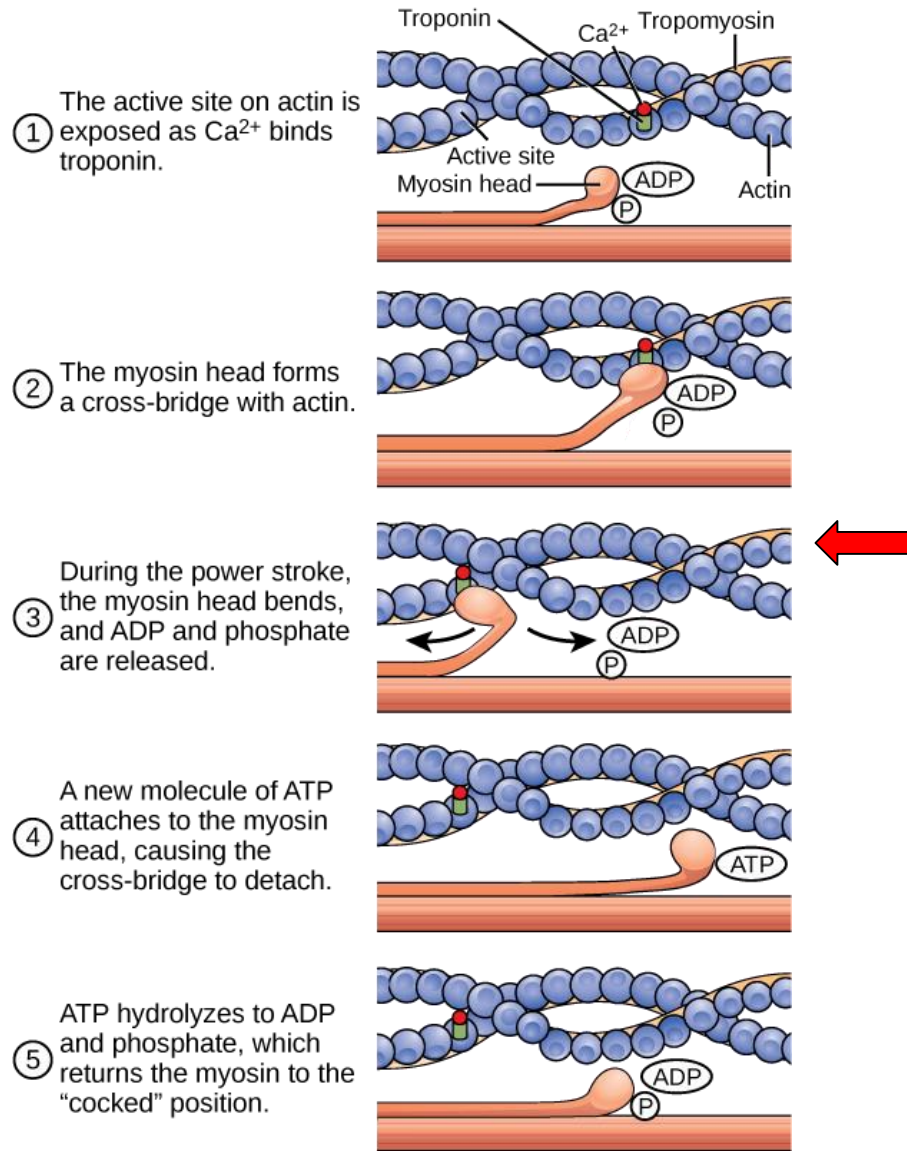
Actin-Myosin interaction.



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The Cross-bridge Muscle Contraction Cycle, which is Triggered by Ca^{2+} and ATP



The motion of muscle shortening occurs as **myosin** heads bind to **actin** and pull the **actin** inwards. (net energy balance 15-35%).

This action requires energy, which is provided by **ATP**.

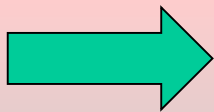
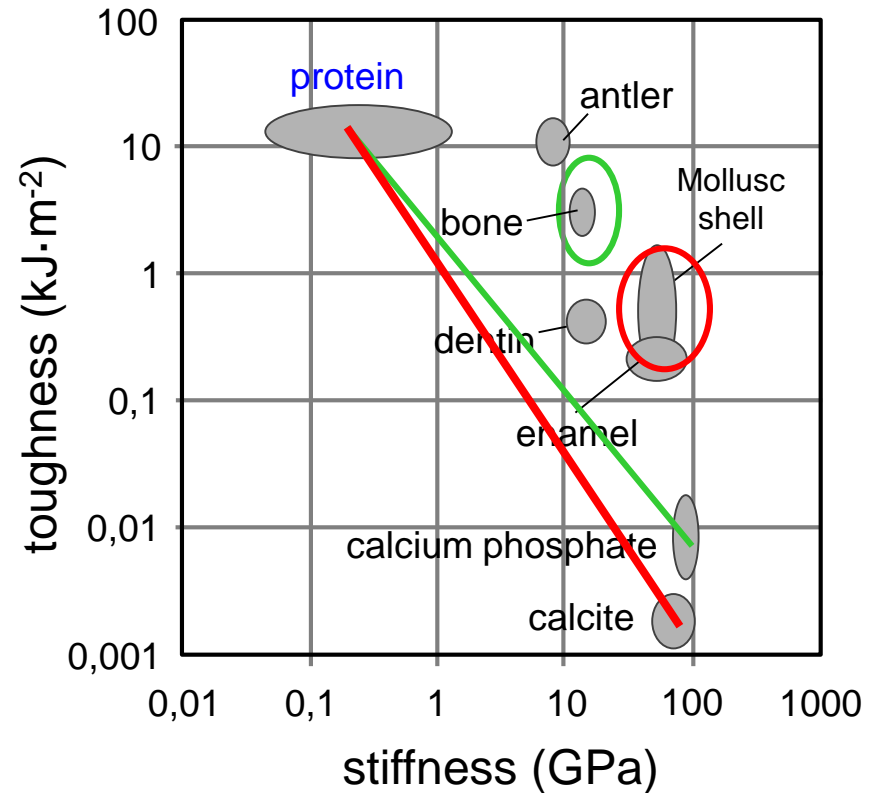
Myosin binds to **actin** at a binding site on the globular **actin** protein. **Myosin** has another binding site for ATP at which enzymatic activity hydrolyzes ATP to ADP, releasing an inorganic phosphate molecule and energy. **Tropomyosin** and **Troponin** are regulatory proteins: the first blocks myosin, the second activate via **Ca²⁺** the contraction.



Proteins as Binder in Composites with Inorganics.

Plot of stiffness against toughness

- Direct line:
Expected area of the composite
- Circle:
Actual position of the composite



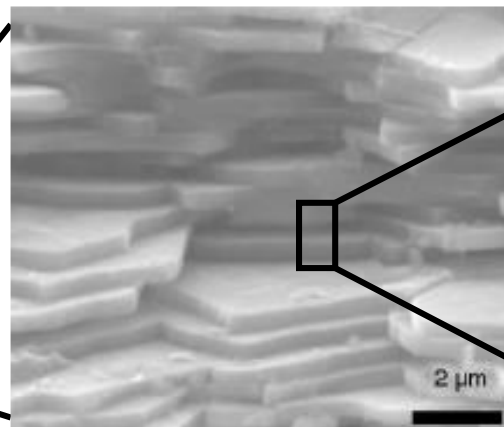
Synergistic Effect: 1+1>2

P. Fratzl, H.S. Gupta, E.P. Paschalis, P. Roschger, J. Mater. Chem. 2004, 14, 2115 – 2123

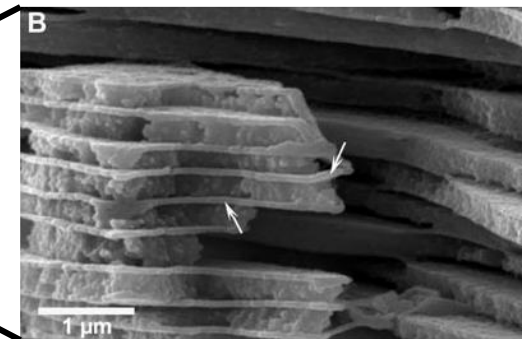


Structure and Properties of Nacre Composite.

	Nacre	Steel
E-Module	80 GPa/m ²	210 MPa/m ²
Tensile strength	800 MPa/m ²	150 MPa/m ²
Compression strength	450 MPa/m ²	500 MPa/m ²



Matrix of β -chitin - proteins

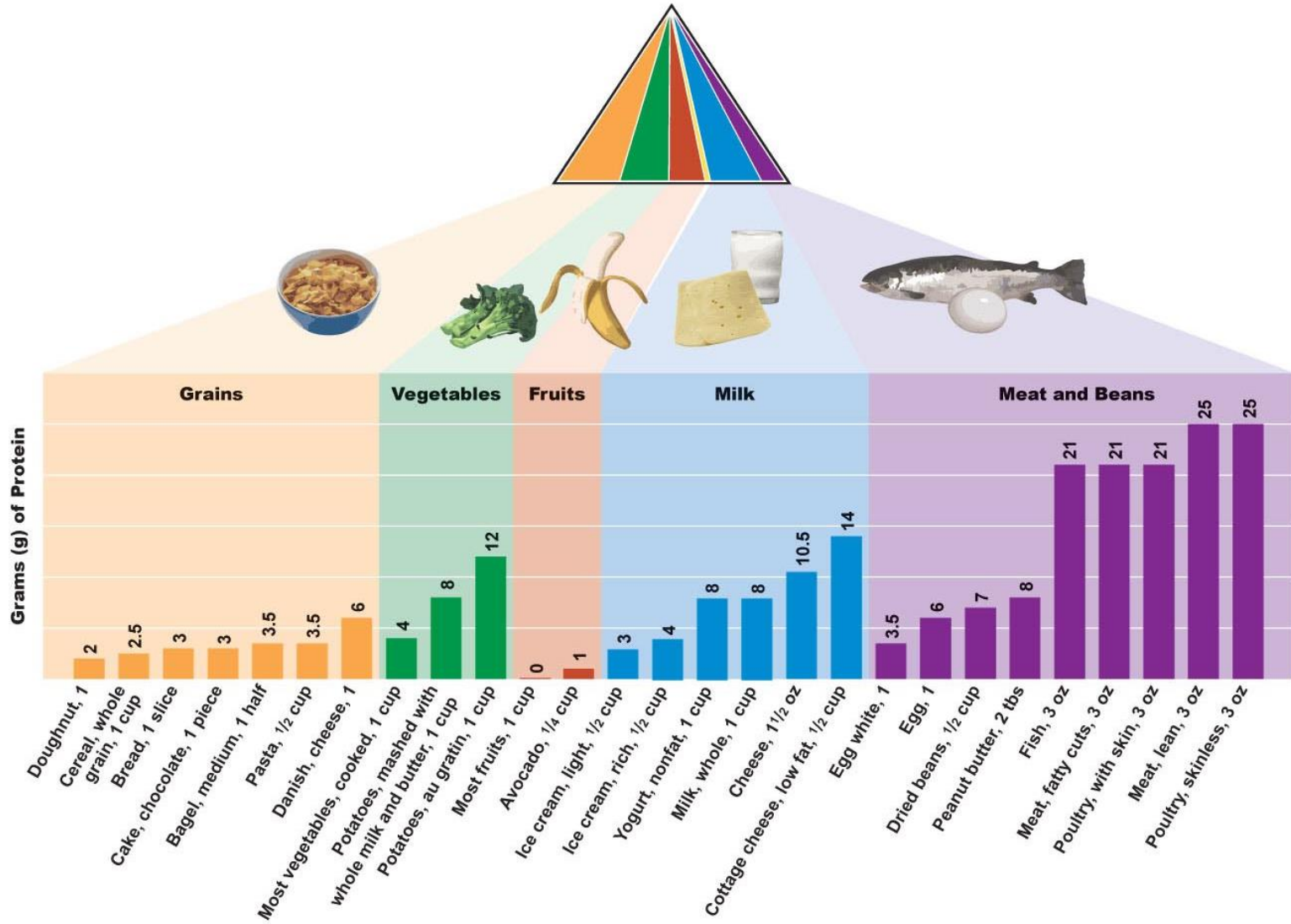


Hexagonal aragonite platelets



Main Use of Proteins as Food.

Best Sources of Proteins for Human Diet.



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Digestibility of Proteins by Humans.

Digestibility of a protein varies from food to food.

The amino acids from animal sources are more easily digested:

- Animal sources: 90+% digested and absorbed
- Legumes: \approx 80%-90% digested and absorbed
- Grains and other plant foods: \approx 70%-90% digested

High-quality proteins

Dietary proteins containing all of the essential amino acids in relatively the same amounts that human beings require (may also contain nonessential amino acids).

Limiting Amino Acids

- Is an essential amino acid present in dietary protein in a small amount
- Thereby limiting the body's ability to build protein
- Lack of availability will slow protein synthesis
- When the limiting amino acids are available again cells resume their normal protein synthesis.



Protein Based Biopolymers (Natural and Bio-derived).

Soy Protein

Soybeans
agricultural surplus

40% of protein:
mainly globulins



P- γ -GA

Bacteria
homopolymers

Synthetic Polypeptides

Tailor made synth.
peptides

chemical synthesis:

1. Merrifield synth.
2. N-carboxyanhydrides

biological synthesis:

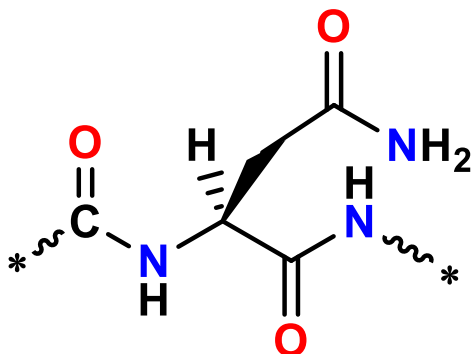
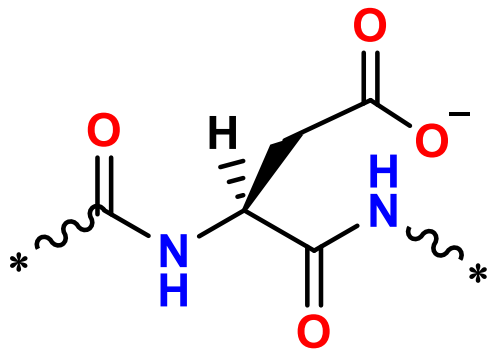
Gene expression



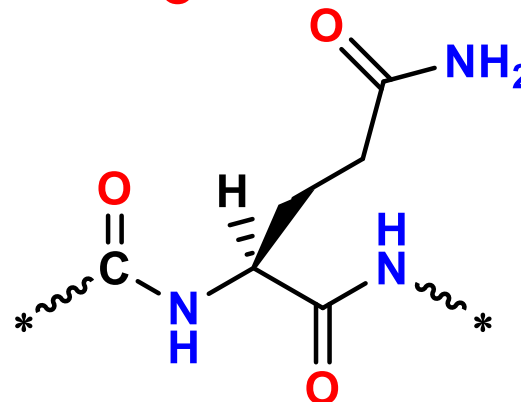
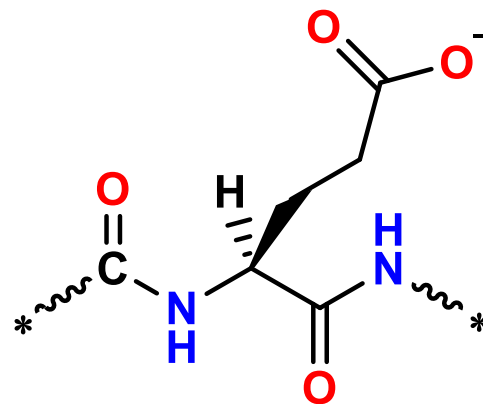
Soy Proteins.

Composition: (main constituents)

aspartic acid + asparagine
11.3 %



glutamic acid + glutamine
17.2 %





Soy Protein Isolation.

Soy is increasing in popularity because:

- High-quality protein source
- Low in saturated fat
- Contains isoflavones
- Phytoestrogens

Recovery of soy protein concentrate occurs, after oil extraction, by:

1. Oil extraction → margarine production

Recovered soy protein concentrate by:

2. Alkali extraction 90%
3. Aqueous alcohol leaching > 65%
4. Acid leaching > 65%
5. Moist heat denaturing > 65%

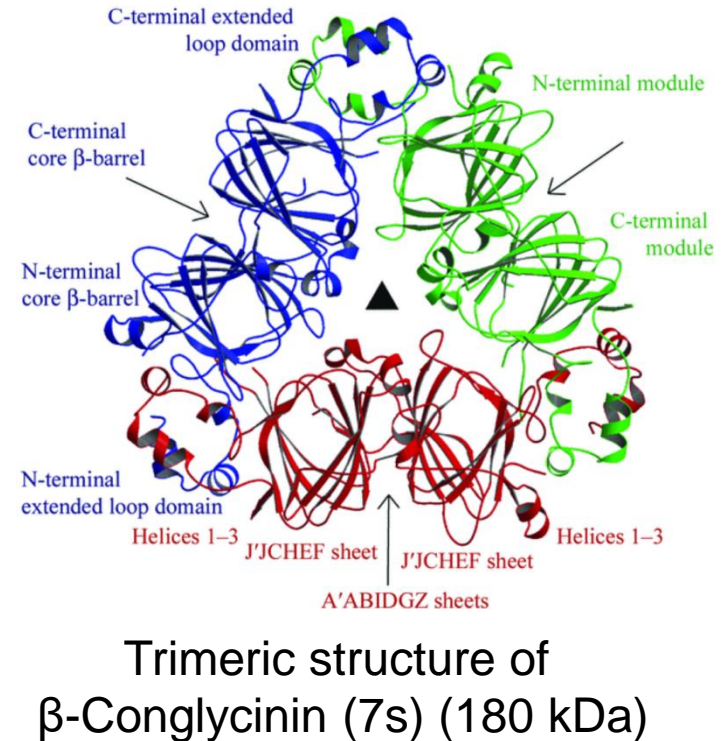
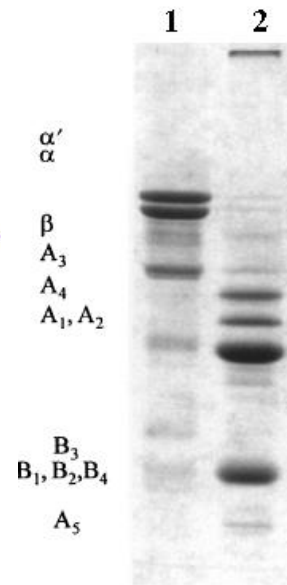
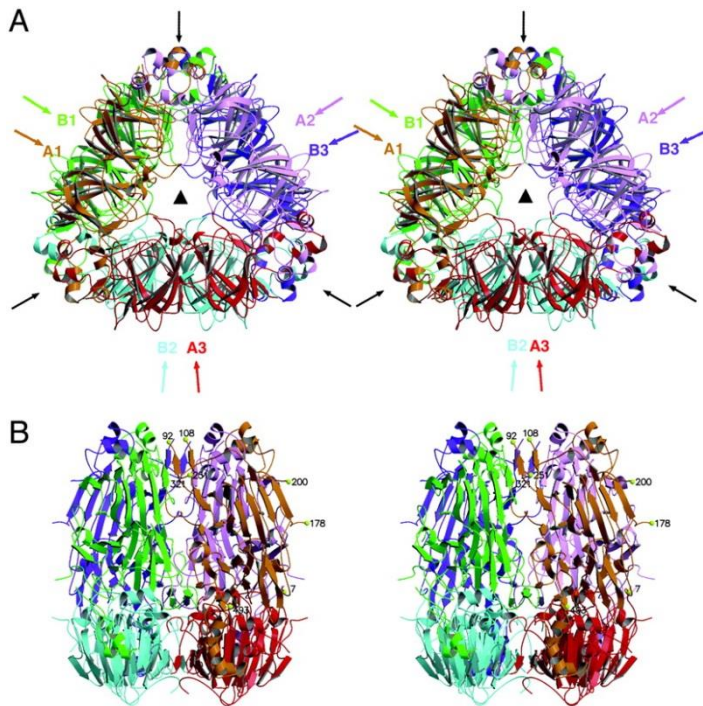
Soy protein concentrate: 1.1 – 1.5 \$/kg



Soy Protein for Plastics.

The main proteins in soybeans are two storage proteins:

1. β -Conglycinin (7s) and
2. Glycinin globulins (11s)



Structure of Glycinin hexamer (11s) (320 kDa) orange (A1), pink (A2), red (A3), green (B1), cyan (B2), and dark blue (B3)



Soy Protein for Plastics (2).

- ❖ Filler in petroleum-based plastics
 - enhances biodegradability
- ❖ Processing from melt (extrusion, injection moulding, blow moulding, compression moulding)
 - compression-moulded soy proteins: brittle
 - use of plasticizers (water, PVA, EG, PG, glycerol)
 - potential addition of fillers (cellulose from ramie or hemp)
- ❖ Cross linking with formaldehyde or glutaraldehyde
 - The main investigated materials (with reinforcing fibres) used for internal parts of cars.
- ❖ Composites with inorganic materials and nanoparticles.



Application of Soy Proteins.

Historically: 1940s: strong interest in use of soy-based plastics
plastics: car-parts
fibres: textile application

After WWII: Trend reversed by more cheaply produced petroleum based polymers

Currently Only 0.5 % of soy protein used in industrial products

- Coating of paper

Future

soy mixed with starch	mouldable plastics
films O ₂ /UV blocker	packaging agricultural mulch films
foams	insulation replacement of styrofoam

Rakesh Kumar et al. *Industrial Crops and Products* **16**, 155–172, 2002.

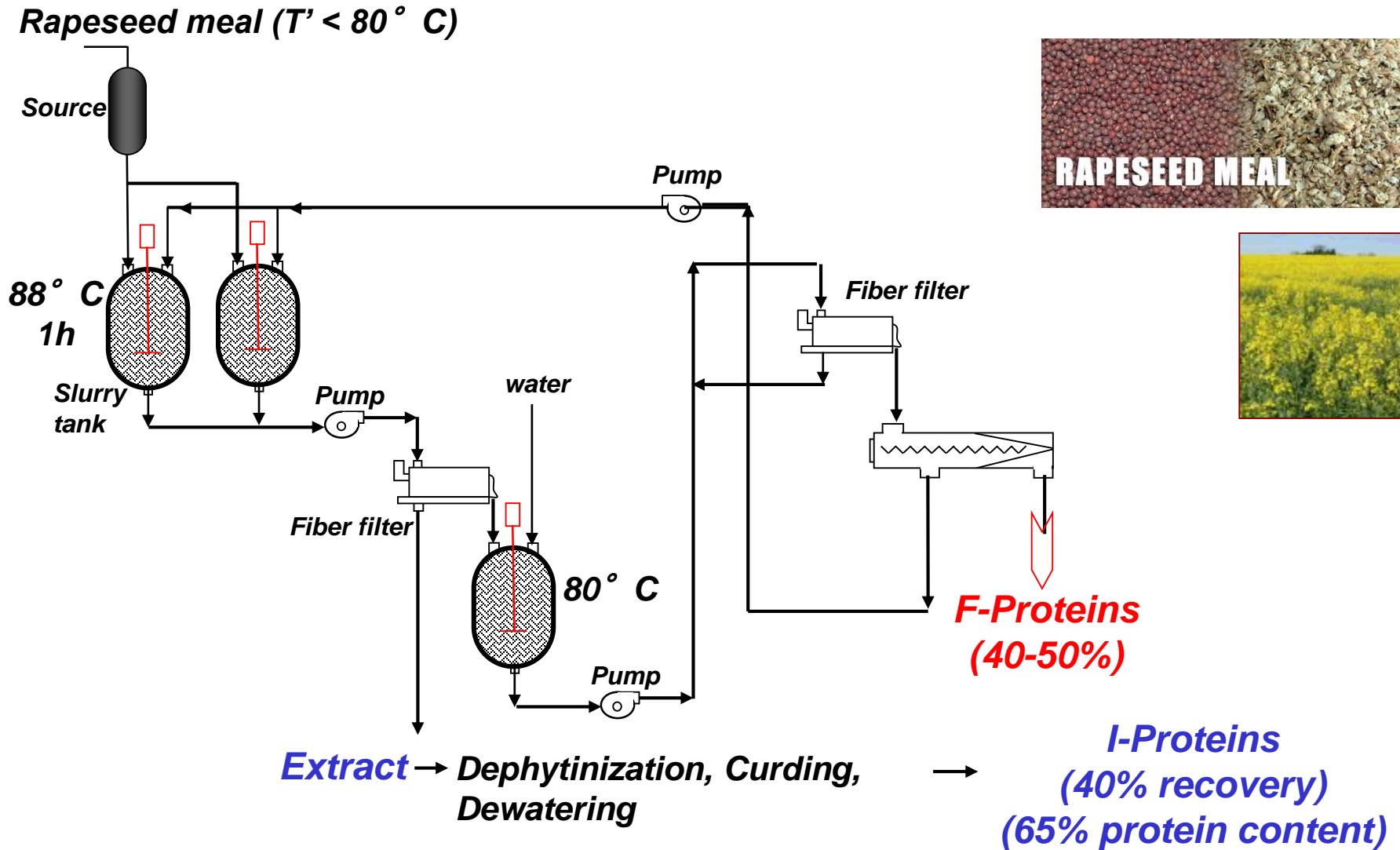


Proteins from Rapeseed Meal (10% moisture basis) - Typical Chemical Composition

Component	Average	Amino acid	Average %
Moisture (%)	10.0	Alanine	1.53
Crude protein (N x 6.25;%)	35.0	Arginine	2.12
Rumen bypass protein (%)	35.0	Aspartate	2.55
Oil (%)	3.5	Cysteine	0.94
Linoleic acid (%)	0.6	Glutamate	6.43
Ash	6.1	Glycine	1.75
Crude fibre (%)	12.0	Histidine	1.13
Tannins (%)	1.5	Isoleucine	1.41
Sinapine (%)	1.0	Leucine	2.39
Phytic acid (%)	4.0	Lysine	2.02
Glucosinolates (µmoles/g)	16	Methionine	0.77
		Methionine + cysteine	1.71
		Phenylalanine	1.54
		Proline	2.23
		Serine	1.64
		Threonine	1.50
		Tryptophan	0.46
		Tyrosine	1.05
		Valine	1.71

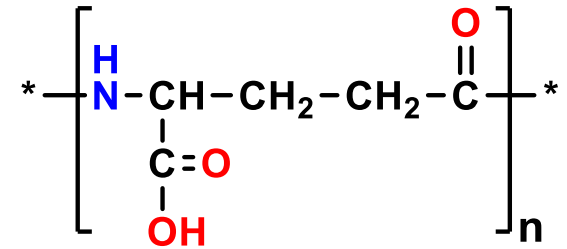
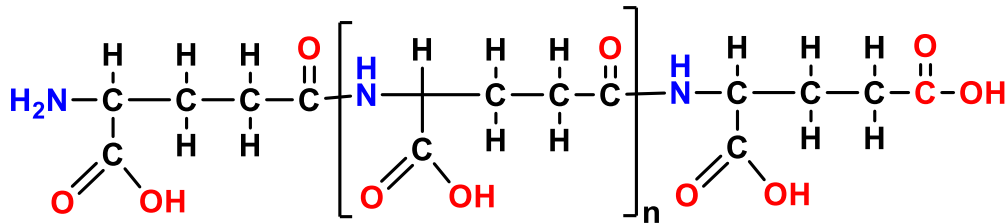


Process to Recover Proteins from Rapeseed Meal.





Natural Homopolymer Protein: Poly- γ -Glutamic Acid.



Extracellular polymer excreted
by *Bacillus* bacteria

Acid extraction \longrightarrow
polymer precipitation in alcohol

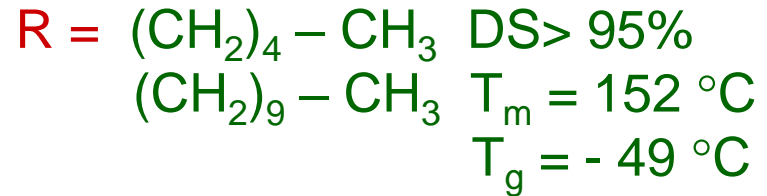
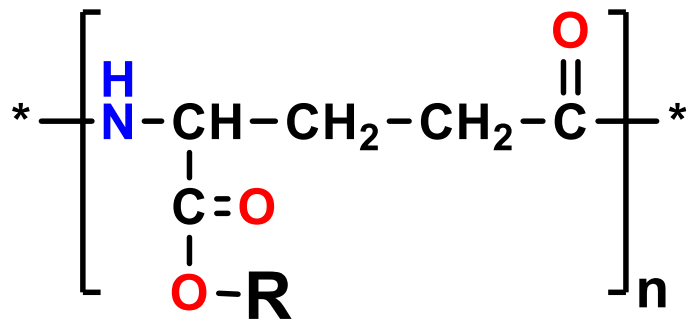


All aqueous
processes

MW = 300,000 – 500,000 Dalton



Poly- γ -Glutamic Acid – Chemistry.



Esterification

→ reduce water-solubility

→ induce meltability

Owing to its biodegradable, non-toxic and non-immunogenic properties, it has been used successfully in the food, medical and wastewater industries

Shah et al. Polym. Preprints 1992, 33(2) 488; US Patent 5,378,807

Ogunleye A, Bhat A, Irorere VU, Hill D, Williams C, Radecka I. Microbiology. 2015 Jan;161(Pt 1):1-17



Poly- γ -Glutamic acid – Application.

Water solubility

rheological modifiers
water treatment
super absorbents
polymeric emulsifiers
detergents

} Replace p-(acrylic acid, p-(acrylamide)
PVA, PEG

Biomedical application:

blood plasma extenders
stimuli responsive “smart” polymers
hydrogels

Currently: limited commercial supplier



Synthesis of Polypeptides and Biotechnological Proteins.

Proteins = biological macromolecules

Conventional materials: silk, wool

Chemically-derived polypeptides:

- polyamides with a large number of repeat units and a multitude of sequences

Biotechnological Proteins: natural but produced via engineered organism

Tailoring

Necessitates sequence control



Synthetic Polypeptides Classification

Fiber-forming

Spider silk:

High strength and elongation

Elastomers

arterial wall material:

> one billion cycles of extension/relaxation in human lifetime without evidence of fatigue or hysteresis

Structure of prim.

repeat unit

$(\text{Val-Pro-Gly-Val-Gly})_n$

$n = 11$

Adhesives

glue:

from animal bone
= collagen
mussels attached to (submarine) surface via 3-protein cement



Fiber Forming Polypeptides.

	Elongation [%]	Energy to break [$\times 10^3$ J/kg]
Spider silk *	10 – 39	120
Bx	15 – 55	70
Steel	8	2
Kevlar	4	30

* *Nephila clavipes*, dragline



Commercial Application of Synthetic Polypeptides.

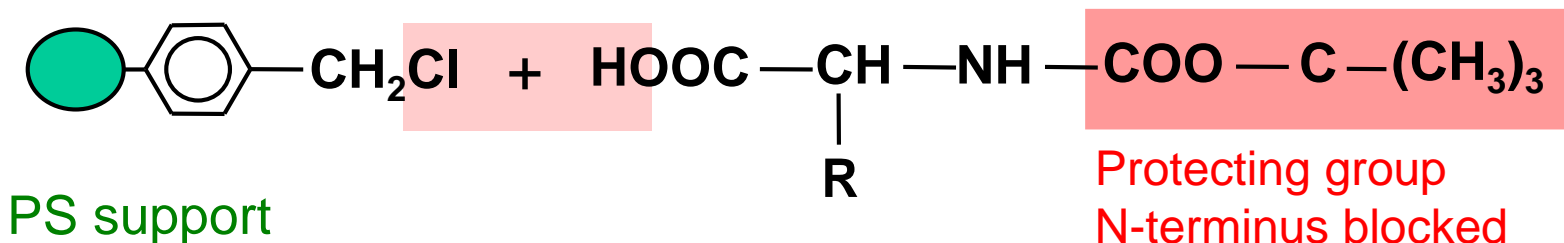
Fiber-forming	Elastomers	Adhesives
Biomedical	Biomedical	Metal recovery
Bioerodible sutures	Artificial elastin proven to be fully	from waste stream (Fe(II), Fe(III))
Tissue regeneration	biocompatible	dissociation const. for complex
High strength engineering application		with mussel protein 10^{39} M^{-1} under-water application



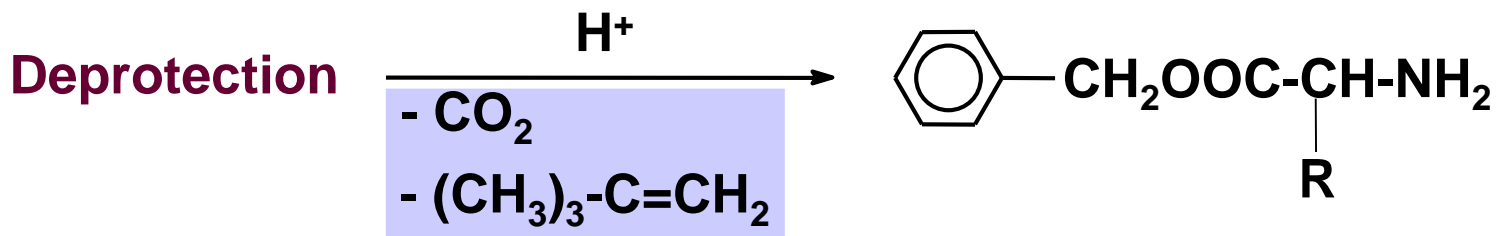
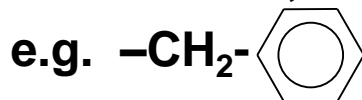
Synthesis of Polypeptides.

1. Chemistry

1.1 Polycondensation: Merrifield Synthesis

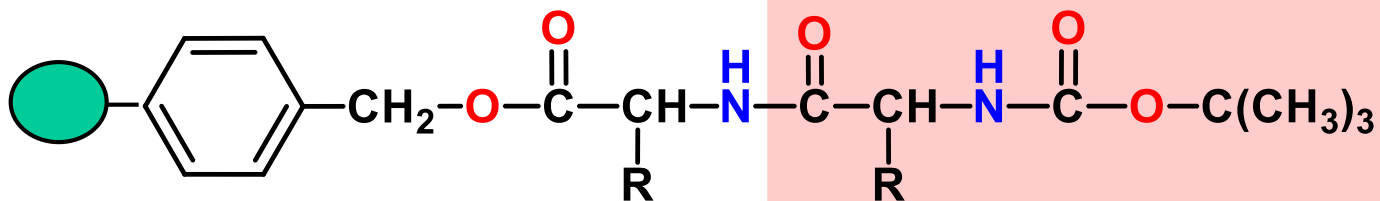
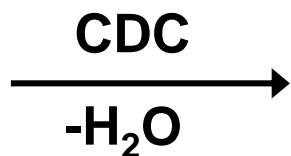
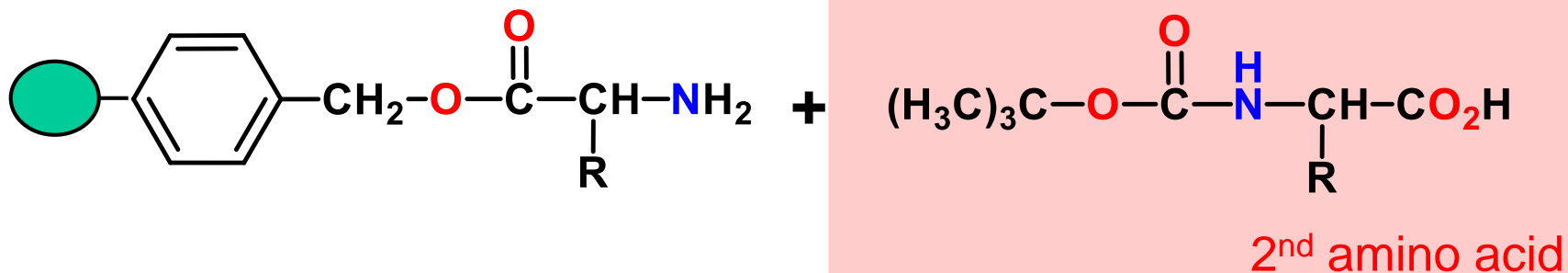


if R = COOH, needs protecting group





Synthesis of Polypeptides (2).

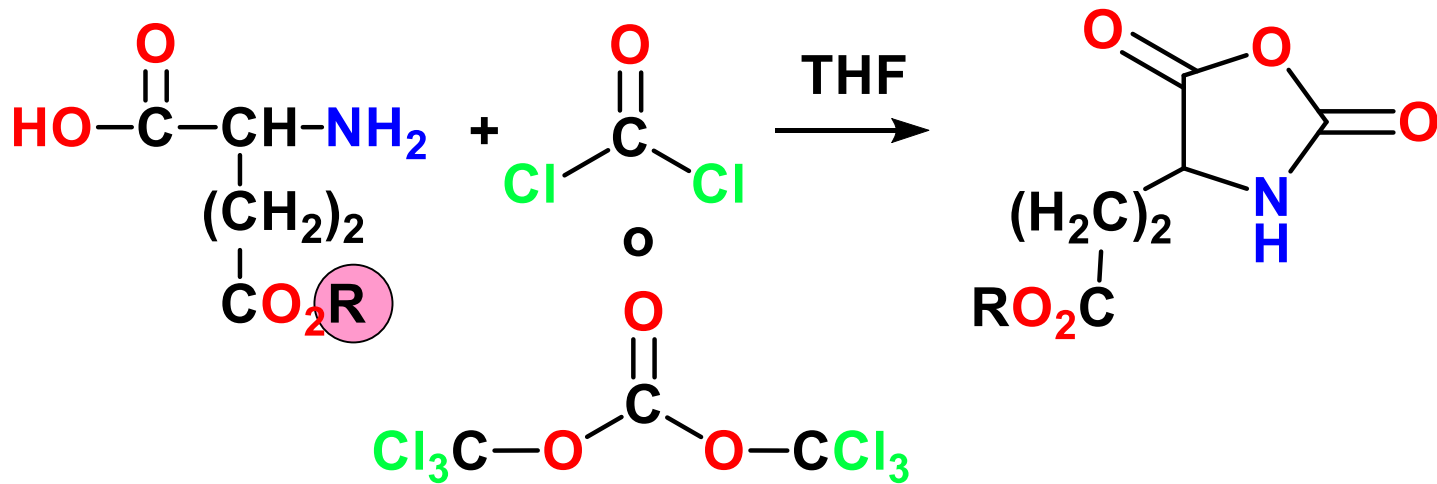




Synthesis of Polypeptides (3).

1.2. Polymerization of N-carboxyanhydrides

Example: poly(glutamic acid) synthesis

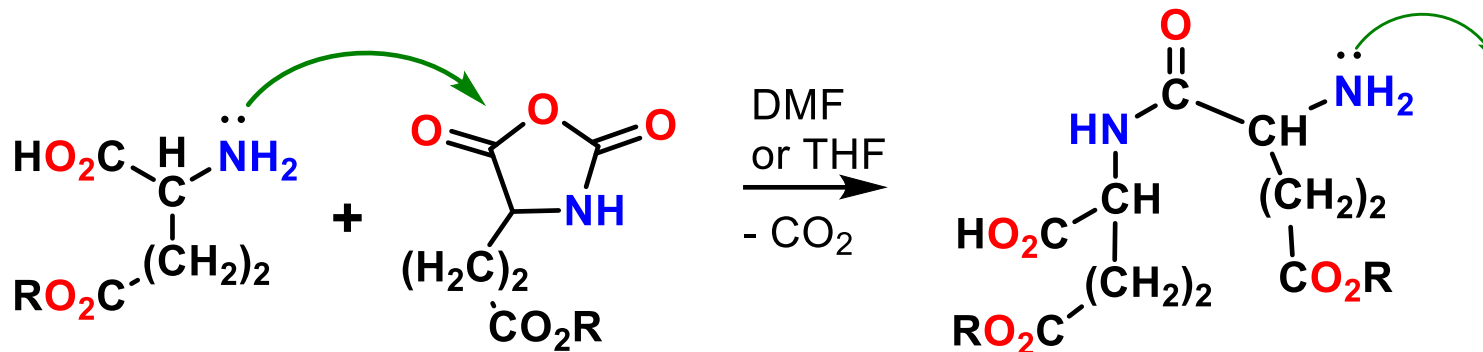


Phosgenation reaction

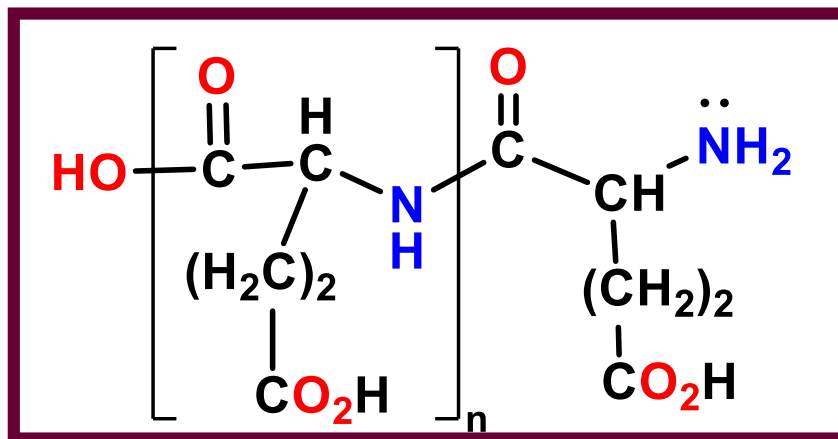
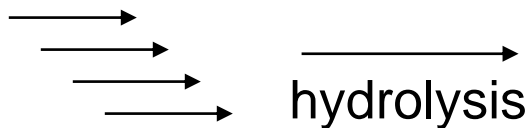
Reaction product:
N-carboxyanhydride - NCA



Synthesis of Polypeptides (4).



Attacks next **NCA**

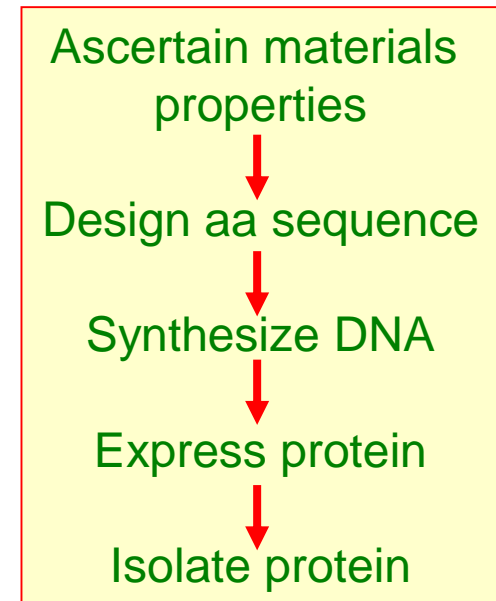




Synthesis of Polypeptides (5).

2. Biosynthetic Production

- Complete tailoring of sequences
- Rather fault-free



Genetic Design: determine aa sequence

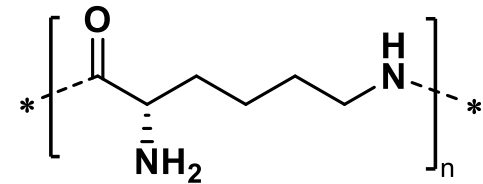
each natural aa encoded by 3 consecutive nucleotides
most aa specified by more than one codon



Biosynthetic Production of Polylysine

ϵ -Polylysine is a homopolymer of L-lysine, an essential amino acid. ϵ -Polylysine differs from usual proteins in that the amide linkage is not between the alpha-amino and carboxyl groups as typical of peptide bonds, but is between the ϵ -amino and carboxyl group. ϵ -Polylysine is produced from aerobic bacterial fermentation by *Streptomyces albulusi* into a medium at concentrations of up to 4–5 g/L. The bacterium strain 346 was first isolated from Japanese soil. A more active mutant (4 times more active) was later isolated).

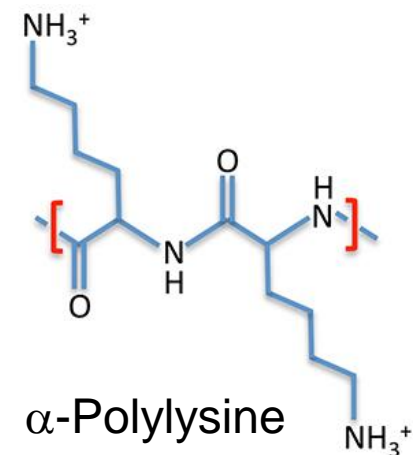
ϵ -Polylysine molecules are cationic, surface active agents due to their positively charged amino groups in water. They have hydrophobic methylene groups on the inside and hydrophilic carboxyl and amino groups on the outside of the molecule in polar solutions. Cationic surface-active compounds generally inhibit the proliferation of microorganisms and ϵ -Polylysine is an effective antimicrobial by growth inhibition studies with yeast, fungi and bacteria.



ϵ -Polylysine

Poly[imino[(2S)-2-amino-1-oxo-1,6-hexanediyl]

α -Polylysine is synthetically produced by a basic **polycondensation** reaction.



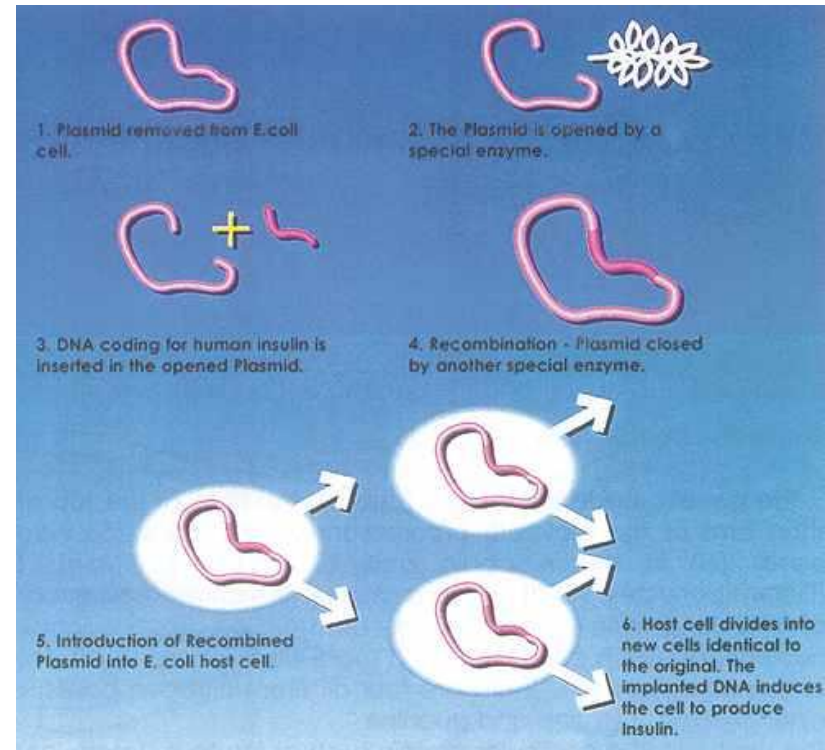
α -Polylysine



Biosynthetic Production of Proteins: Recombinant DNA Technology.

Insulin polypeptide (chemically identical to its naturally produced counterpart) was produced by inserting the insulin gene into a suitable vector, the E. coli bacterial cell via the Recombinant DNA technology. This method (summarized on the right) is a more reliable and sustainable than extracting and purifying the abattoir by-product or recovering insulin from other animals.

The biosynthesis of polypeptides/proteins with controlled presentation of functional groups in multiple positions, coupled with their subsequent chemical modification with biologically relevant ligands, permit the production of well-defined, bioactive macromolecules useful as pharma drugs or enzymes.



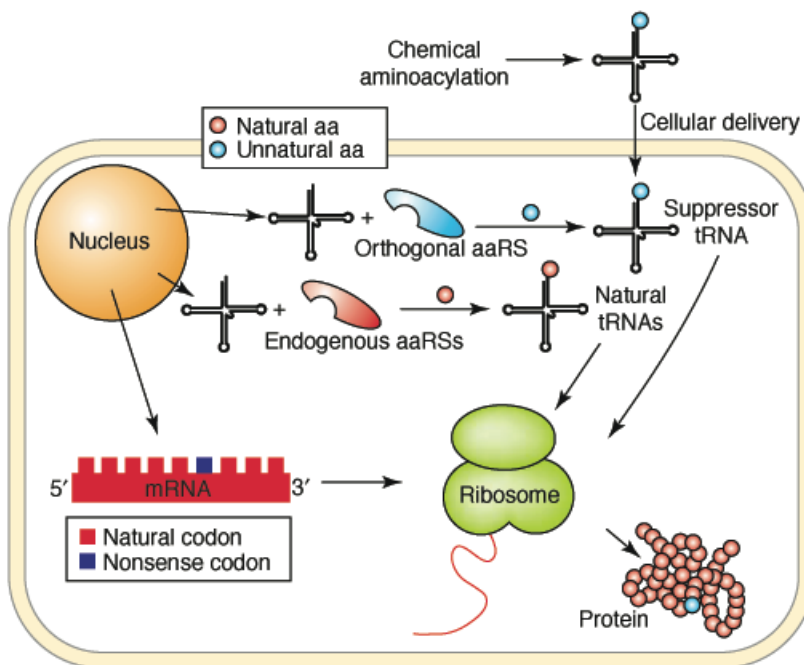
An overview of the recombination process.

Source: Novo - Nordisk promotional brochure, p 6.



Expanded Genetic Code

Some natural aminoacyl-tRNAs are tolerated by the ribosome, others are not. In addition, the unnatural aminoacyl-tRNA must be efficiently transported into the cytoplasm when it is added to the growth medium or biosynthesized by the host, and it must be stable in the presence of endogenous metabolic enzymes:



T/BS

Peter Schultz's Laboratory has used genetic manipulation to engineer a bacteria able to synthesize p-aminophenylalanine (35 previous slide) and incorporate it into proteins. To do this, a mutant aminoacyl-tRNA synthetase capable of loading this amino acid onto a mutant suppressor tRNA was evolved.

J. Am. Chem. Soc. 2003 Jan 29;125(4):935-9.