NATURAL POLYMERS

FIBROUS PROTEINS I

COLLAGEN

Dr. Ladislav Pospíšil

Time schedule

LECTURE	SUBJECT
1	Introduction to the subject – Structure & Terminology of nature polymers, literature
2	Derivatives of acids – natural resins, drying oils, shellac
3	Waxes
4	Plant (vegetable) gums, Polyterpene – natural rubber (extracting, processing and modification), Taraxacum_kok-saghyz
5	Polyphenol – lignin, humic acids
6	Polysaccharides I – starch
7	Polysaccharides II – celullose
8	Protein fibres I
9	Protein fibres II
10	Casein, whey, protein of eggs
11	Identification of natural polymers
	Laboratory methods of natural polymers' evaluation

1. Repeating of the Basic Terminology related to PROTEINS (Lecture 8)
2. FIBROUS PROTEINS
3. Manufacture of the Gelatine and Animal Glue
4. Tanning industry
   - Skin (Hide) versus Leather
   - Tanning Procedure
5. Leather and Conservator – Restorer

1. Repeating of the Basic Terminology related to PROTEINS (Lecture 8)

Structure Hierarchie of Peptides and Proteins

- **Primary structure** – the Amino acids Sequence of the Protein
- **Secondary structure** – No covalent Interactions in the Backbone of the one Polypeptide (Protein) Chain, usually the near Parte of the Backbone (α – Helix and/or β – Sheet)
- **Tertiary structure** – various Interactions between the Backbones of more then the one Polypeptide (Protein) Chain of Chains or remote NO neighbouring) Segments of one Chain
- **Quaternary structure** – Interactions between the Chain Bundles, between the **Tertiary structures**

Tertiary & Quaternary Structures – we give attention to this in the next Lesson!

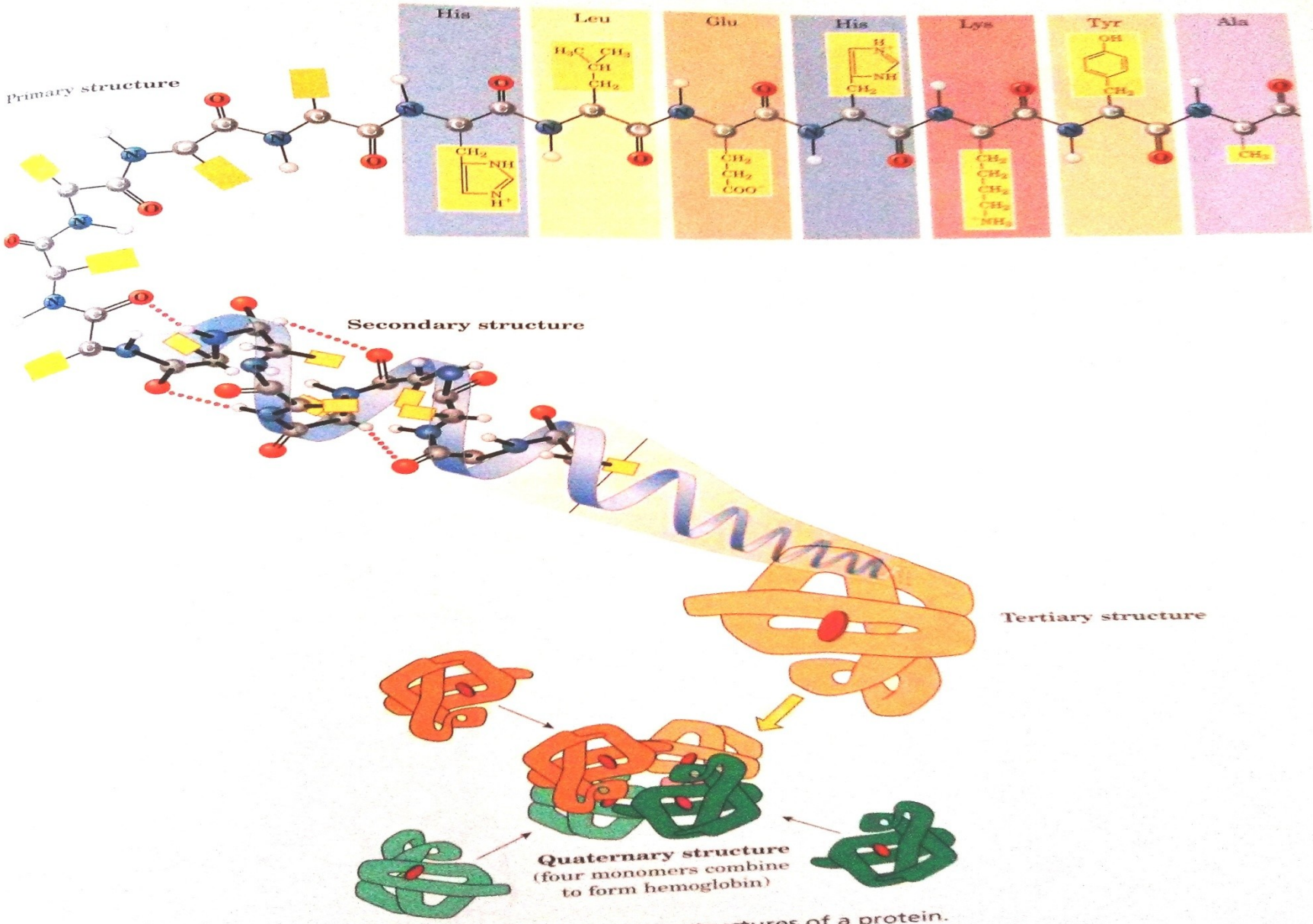
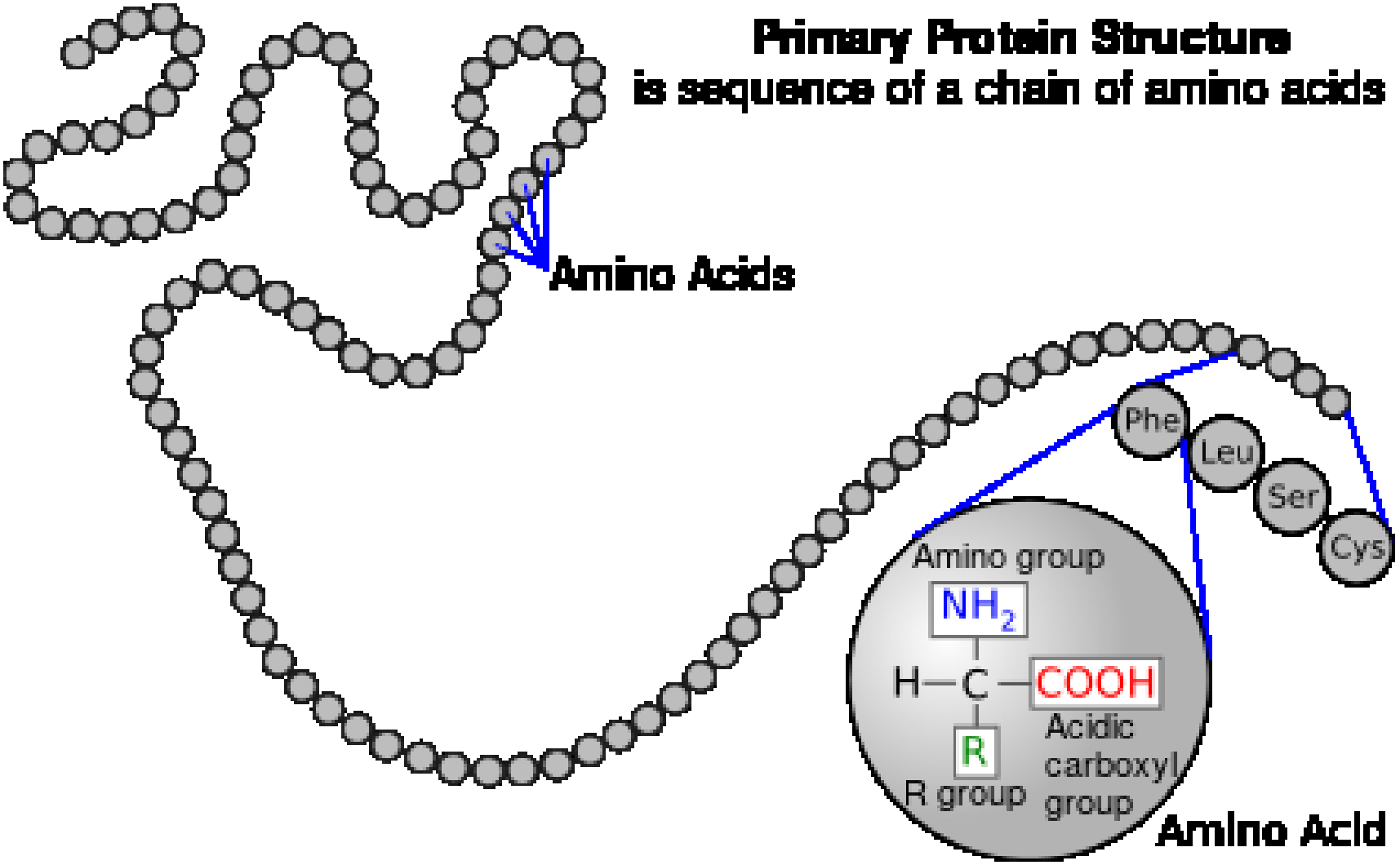
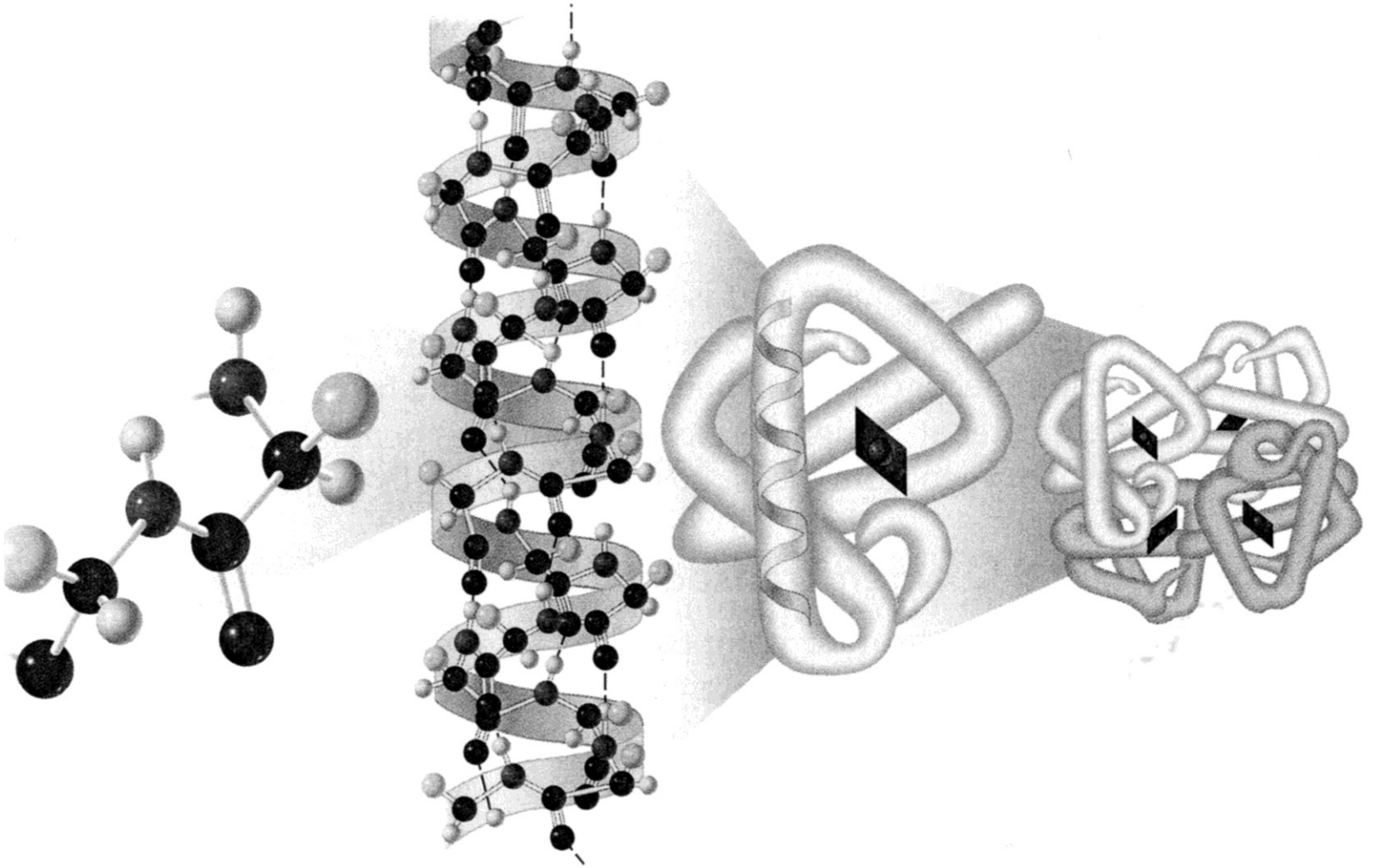


FIGURE 22.21 Primary, secondary, tertiary, and quaternary structures of a protein.

PRIMARY STRUKTURE of Proteine I



PROTEIN SECONDARY STRUCTURE I



PROTEIN SECONDARY STRUCTURE II A

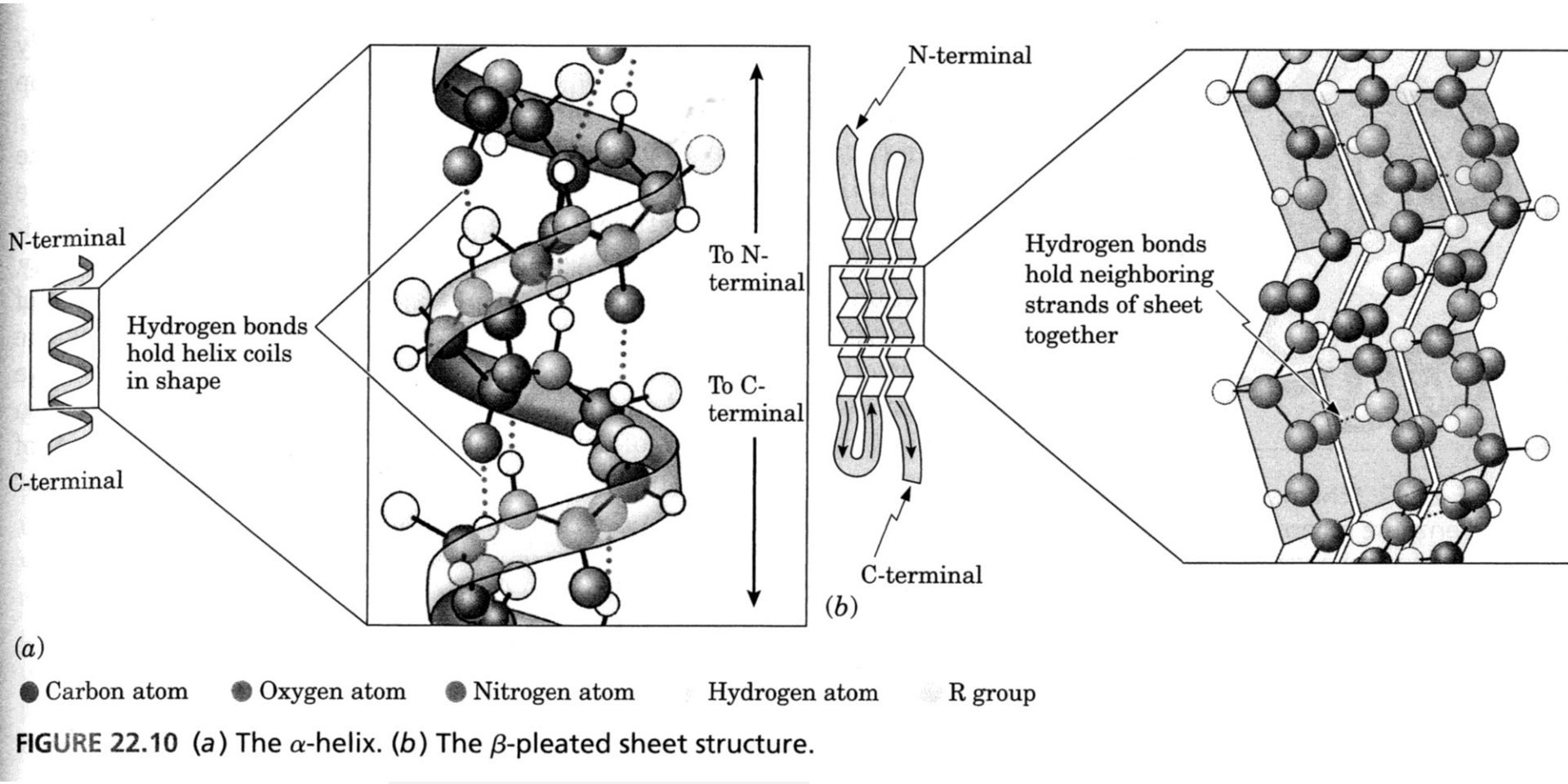


FIGURE 22.10 (*a*) The α-helix. (*b*) The β-pleated sheet structure.

Left-handed Helix

Proteins dividing – the Occurrence of other Components in Macromolecule accordingly

- **Simple protein** – they are broken by Hydrolysis to Amino acids only
- **Compound protein** – they are broken by Hydrolysis to Amino acids, Saccharides, Fats, …
  - LIPOPROTEINE (Fats)
  - GLYKOPROTEINE (Saccharides)
  - FOSFOPROTEINE (Phosphate groups > **KASEIN**)
  - CHROMOPEROTEINE (Colorants, e.g. Haemoglobin, Melamine)

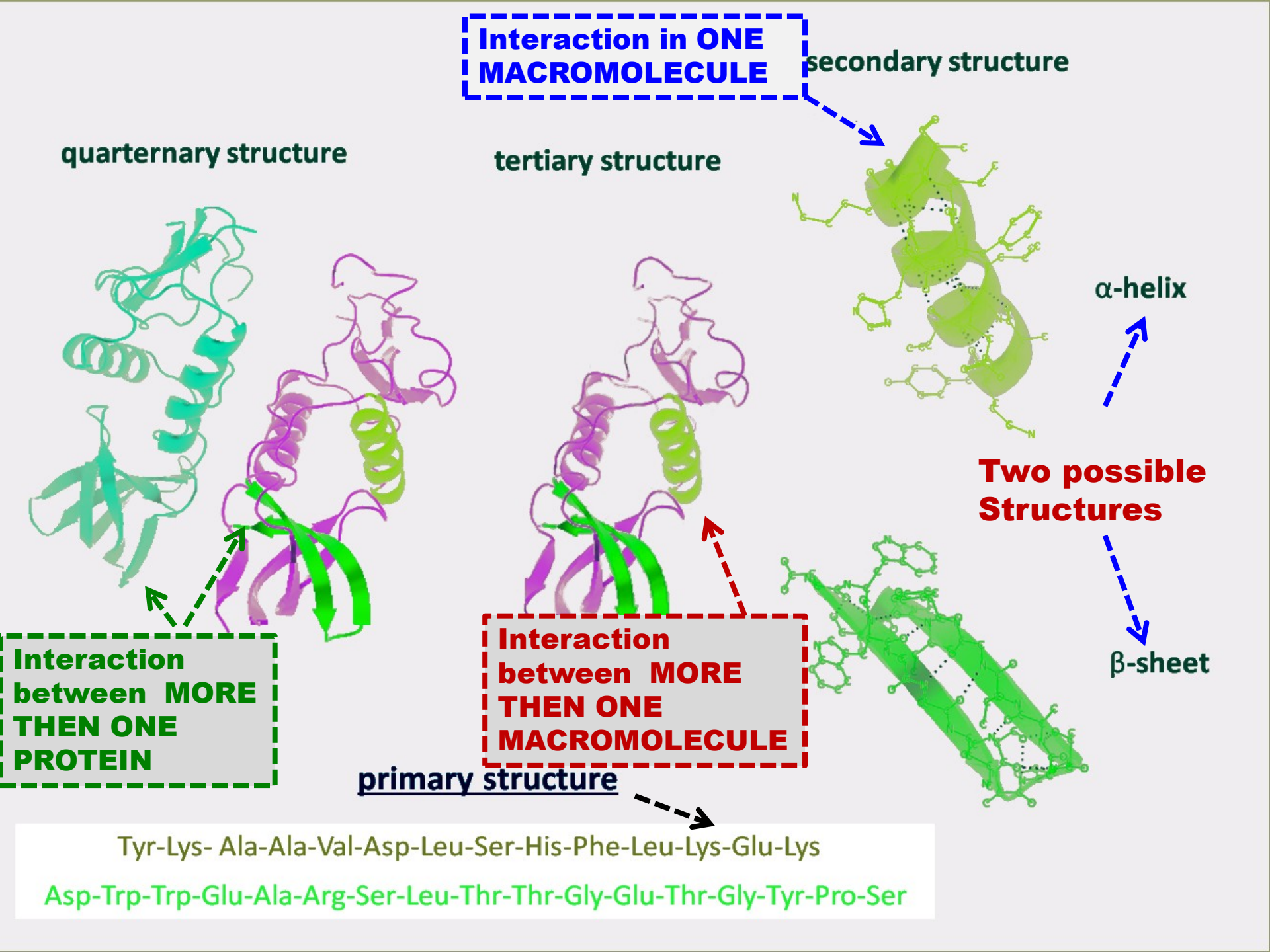
Proteins dividing – Macromolecules' Solubility in Water of accordingly

- **SOLUBLE (SFEROPROTEINE)**
  - HEAT > COAGULATION
  - Albumin > **Egg white**
  - Glutelin > **Glutelin from Wheat**
- **UNSOLUBLE (SKLEROPROREINE)**
  - Keratin α and β
  - Collagen

Proteins dividing – Macromolecules' Shape and Supermolecular Structure accordingly

- **FIBRILAR** > natural/genuine Silk, Hair, animal Hair, Muscles, fibrous connective Tissue
- **GLOBULAR** > ENZYM, Egg white, Milk white, INSULIN, …

2. FIBROUS PROTEINS


Interaction in ONE MACROMOLECULE
secondary structure
quarternary structure
tertiary structure
α-helix
Two possible Structures
β-sheet
Interaction between MORE THEN ONE PROTEIN
Interaction between MORE THEN ONE MACROMOLECULE
primary structure
Tyr-Lys- Ala-Ala-Val-Asp-Leu-Ser-His-Phe-Leu-Lys-Glu-Lys
Asp-Trp-Trp-Glu-Ala-Arg-Ser-Leu-Thr-Thr-Gly-Glu-Thr-Gly-Tyr-Pro-Ser

Interaction between MORE THEN ONE MACROMOLECULE

Functional proteins have four levels of structural organization:

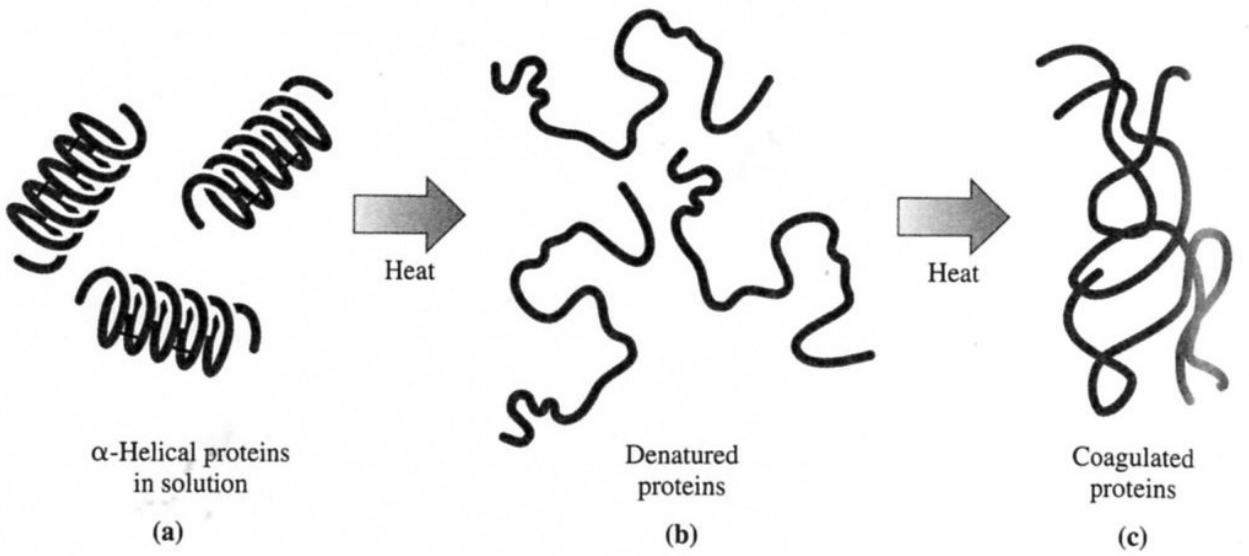
1) Primary Structure : the linear structure of amino acids in the polypeptide chain

2) Secondary Structure : hydrogen bonds between peptide group chains in an alpha helix or beta

3) Tertiary Structure : three-dimensional structure of alpha helixes and beta helixes folded

4) Quaternary Structure : three-dimensional structure of multiple polypeptides and how they fit together

DENATURATION and COAGULATION of Proteines

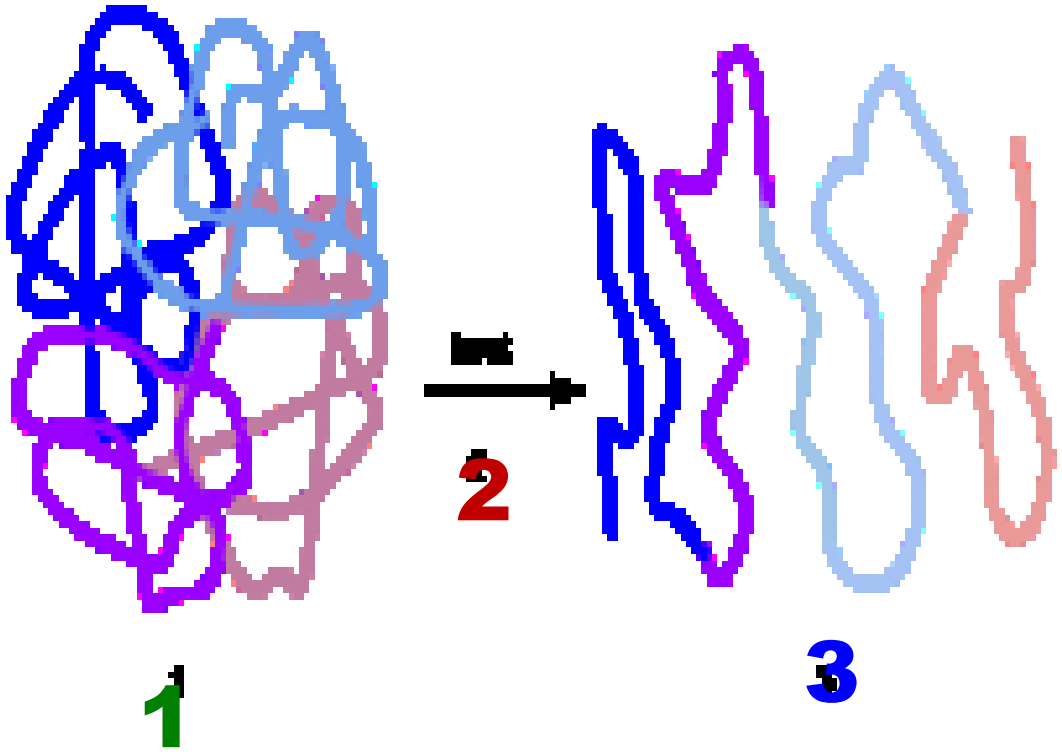


DENATURATION IS THE DISINTEGRATION OF THE PROTEIN STRUCTURE

COAGULATION is the Formation of the UNSOLUBLE Protein Form from the initially SOLUBLE Protein Form by Influence of the Physical Factors, e.g. Heat (e.g. Egg White at Cooking Egg) or by Influence of the Chemical Reagents.

COAGULATION is one of the Forms of DENATURATION

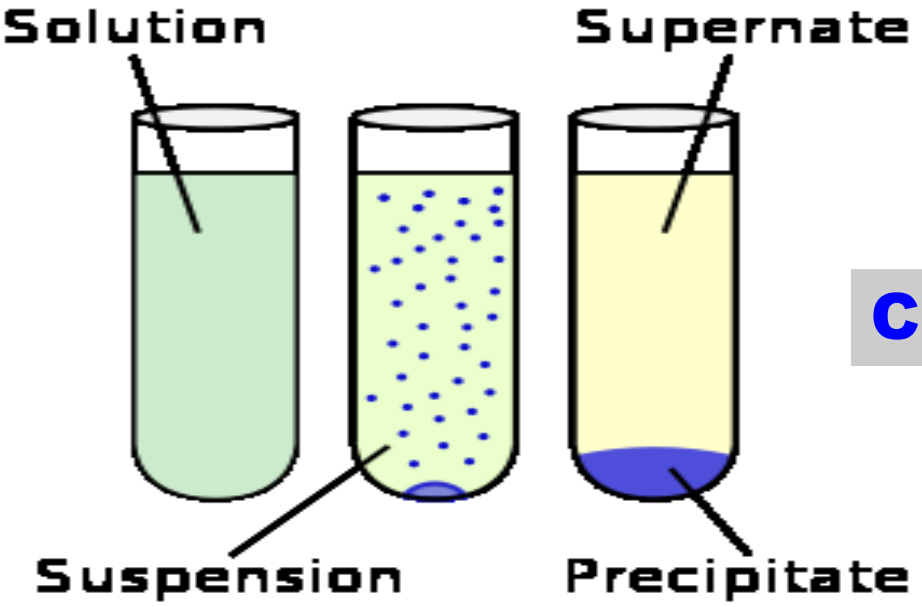
COAGULATION BY HEAT IS USUALLY IRREVERSIBLE

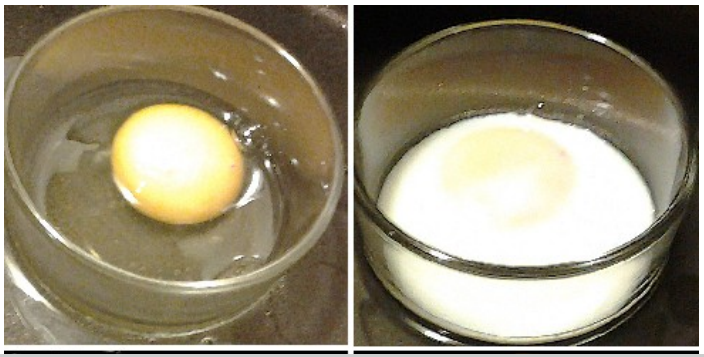


Process of Denaturation:

1) Functional proteins showing a quaternary structure
2) When heat is applied it alters the intramolecular bonds of the Proteins'
3) Unfolding of the polypeptides (Proteins)

Denaturation is a process in which proteins or nucleic acids lose the quaternary structure, tertiary structure and secondary structure which is present in their native state, by application of some external stress or compound such as a strong acid or base, a concentrated inorganic salt, an organic solvent (e.g., alcohol or chloroform), radiation or heat.[3] If proteins in a living cell are denatured, this results in disruption of cell activity and possibly cell death. Denatured proteins can exhibit a wide range of characteristics, from loss of solubility to communal aggregation





COAGULATION BY HEAT

ANALOGY with paper Clips

IUPAC definition

Process of partial or total alteration of the native secondary, and/or tertiary, and/or quaternary structures of proteins or nucleic acids resulting in a loss of *bioactivity*.

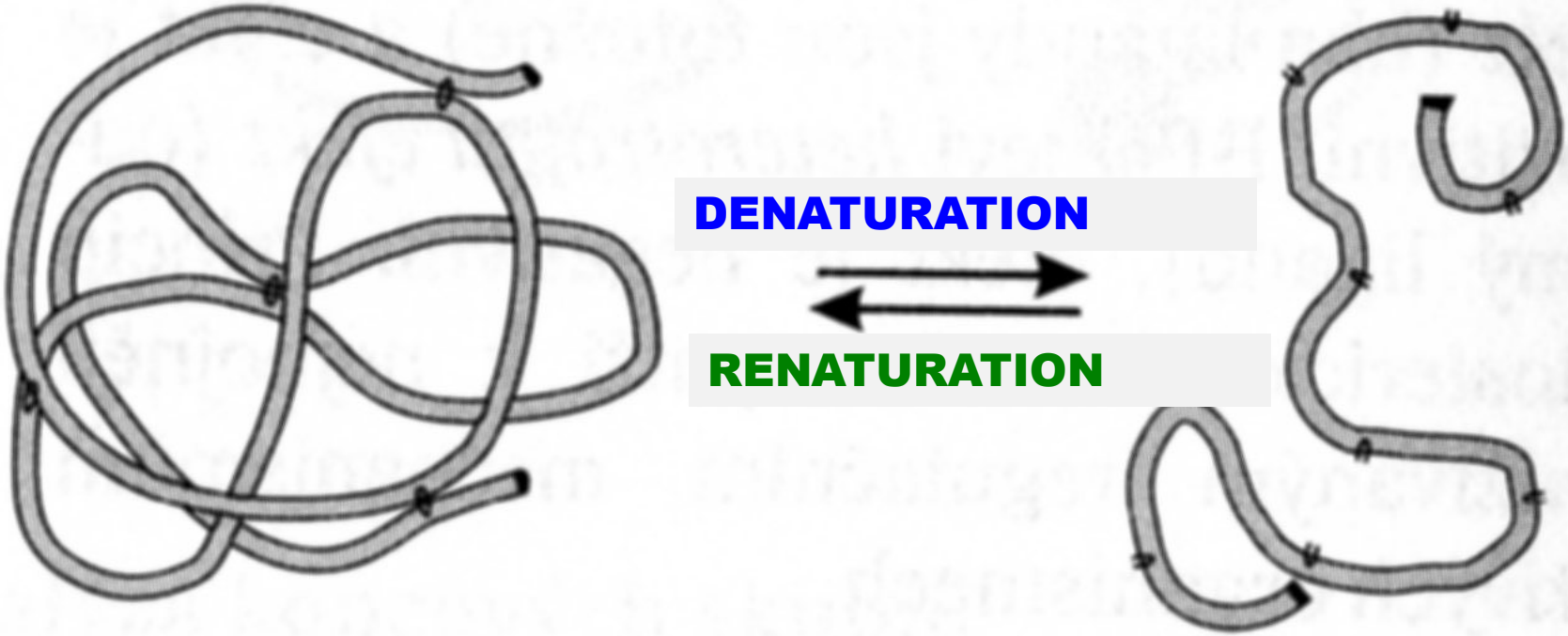
***Note 1*:**

Denaturation can occur when proteins and nucleic acids are subjected to elevated temperature or to extremes of pH, or to nonphysiological concentrations of salt, organic solvents, urea, or other chemical agents.

***Note 2*:**

An *enzyme* loses its catalytic activity when it is denaturized.[2]

DENATURATION *CAN BE* *REVERSIBLE* > RENATURATION



REVERSIBLE **DENATURATION (=RENATURATION) of the Globular Protein with marked –S-S- Bonds**

Reversibility and irreversibility

In very few cases, Denaturation is reversible (the proteins can regain their native state when the denaturing influence is removed). This process can be called Renaturation.

This understanding has led to the **notion** that all the information needed for proteins to assume their native state was encoded in the primary structure of the protein, and hence in the DNA that codes for the protein, the so-called "Anfinsen's thermodynamic hypothesis".

One example of Renaturation is that an egg white can be uncooked **using vitamin C or sodium borohydride (VERY CURIOUS PROCEDURE).**

EXAMPLES Reversibility and Irreversibility COAGULATION

REVERSIBLE

- GELLATINE (it is COLLAGEN with lower MW)
- Temperature Increasing > Dissolving > SOL
- Temperature Decreasing > Solidification > GEL
- TEMPERATURE OF COAGULATION = TEMPERATURE, at which (*at given Concentration*) COAGULATION occurs, it is Transition SOL >> GEL occurs by Temperature Decreasing

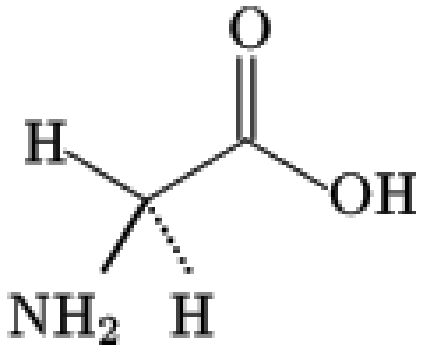
IRREVERSIBLE

- EGG WHITE
- Temperature Increasing (over approx. 60 °C) > Solidification > Transition SOL >> GEL
- TEMPERATURE OF COAGULATION = TEMPERATURE, at which (*at given Concentration*) COAGULATION occurs, it is Transition SOL >> GEL occurs by Temperature Increasing

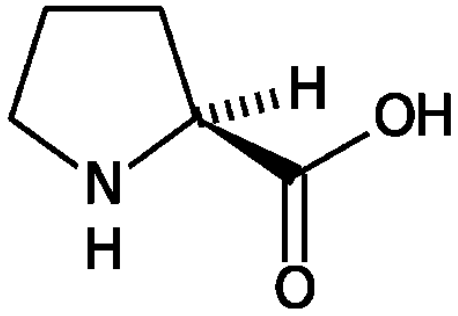
COLLAGEN as the EXAMPLE of the FIBROUS PROTEINS

- SKIN, BONES, CARTILAGE, TENDON, VASCULAR WALLS, CORNEA, …
- Glycine 27 % w/w, Proline 15 % w/w, Sequnce $(GLY\text{-}X\text{-}Y)_n$
- 15 Types of **COLLAGENS** have been described up to now, they are different as to the Occurence and the Abundance of the particular Aminoacids
- TROPOCOLLAGEN –THREE mutually coiled Backbones
- TROPOCOLLAGEN > SELF ARRANGEMENT into COLLAGEN FIBRILS > Crosslinking via Hydrogen Bonds > **COLLAGEN** FIBERS > **BUNDLES of FIBERS**
- DEGRADATION of COLLAGEN by ENZYME COLLAGENASE > SKIN AGEIN

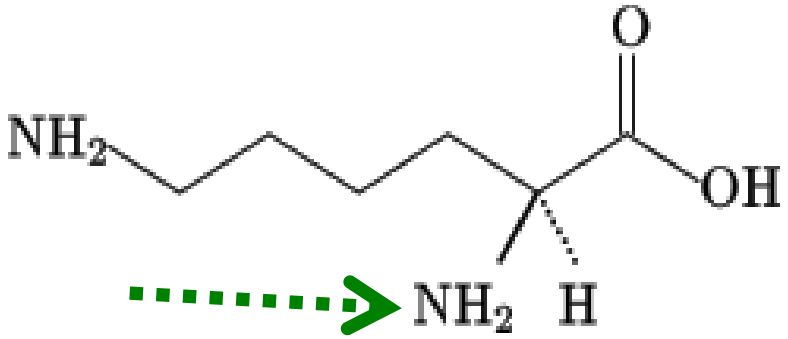
GLYCINE (Gly, G)



PROLINE (Pro, P)



LYSINE (Lys, L)



This Group of the Amino acids is important for the Hydrogen Bonds between the COLLAGEN Molecules

PRIMARY STRUCTURE Proteins II – COLLAGEN as the EXAMPLE (Numbers of particular Amino acids in the Macromolecules are given bellow)

AMK	Typ I		Typ II	Typ III	Typ IV	Typ V	
	alfa1	alfa2				A	B
3-Hyp	1,0	0,0	2,0	—	11	2,5	2,9
4-Hyp	96	86	99	125	130	109	109
Asp	46	44	42	42	51	51	50
Thr	20	20	20	13	23	26	19
Ser	42	43	27	39	37	31	26
Glu	74	66	89	71	84	84	91
Pro	129	113	121	107	61	97	118
Gly	330	336	333	350	310	319	322
Ala	112	102	100	96	33	52	46
Val	20	32	18	14	29	27	18
Gys 1	-	-	-	2	8	-	-
Met	8	6	9	8	10	11	8
Ile	6	16	9	13	30	16	19
Leu	18	32	26	22	54	35	39
Tyr	2	2	1	3	6	18	2,1
Xhe	12	10	13	8	27	14	12
Hyl	4,3	8	20	30	10	18	20
Lys	30	22	2	6	10	11	7,3
Arg	49	51	51	46	33	68	50

COLLAGEN OCCURENCE IN HUMAN BODY

Type	Molecular Composition	OCCURENCE
I	[alfa1(I)]2alfa2	SKIN, BONES, CARTILAGE, TENDON, VASCULAR WALLS, CORNEA
II	[alfa1(II)]3	Hyalin CARTILAGE
III	[alfa1(III)]3	The same as the Type I, formely called Retikuline
IV	[alfa1(IV)]3	Basal Membranes
V		Neoplasm etc.
VI		Interstitial Tissues
VII		Epithel Tissues
VIII		Some Endotel Cells
IX		CARTILAGE together with Type II
X		Part of the Hypertrofic and Mineralised CARTILAGE s
XI		CARTILAGE
XII		OCCURENCE together with Types I and III

Amino acids Sequence alfa1 Chain of the Skin Collagen (N-terminal and C-terminal Regions are given separately)

Glu-Met-Ser-Tyr-Gly-Tyr-Asp-Glu-Lys-Ser-Ala-Gly-Val-Ser-Val-Pro–
Gly-Pro-Met-Gly-Pro-Ser-Gly-Pro-Arg-Gly-Leu-Hyp-Gly-Pro-Hyp-Gly-Ala-Hyp-Gly-Pro-Gln-Gly-Phe-Gln-Gly-Pro-Hyp-
Gly-Glu-Hyp-Gly-Glu-Hyp-Gly-Ala-Ser-Gly-Pro-Met-Gly-Pro-Arg-Gly-Pro-Hyp-Gly-Pro-Hyp-Gly-Lys-Asn-Gly-Asp-Asp-
Gly-Glu-Ala-Gly-Lys-Pro-Gly-Arg-Hyp-Gly-Gln-Arg-Gly-Pro-Hyp-Gly-Pro-Gln-Gly-Ala-Arg-Gly-Leu-Hyp-Gly-Thr-AJa-
Gly-Leu-Hyp-Gly-Met-Hyl-Gly-His-Arg-Gly-Phe-Ser-Gly-Leu-Asp-Gly-Ala-Lys-Gly-Asn-Thr-Gly-Pro-AIa-Gly-Pro-Lys-
Gly-Glu-Hyp-Gly-Ser-Hyp-Gly-Glx-Asx-Gly-Ala-Hyp-Gly-Gln-Met-Gly-Pro-Arg-Gly-Leu-Hyp-Gly-Glu-Arg-Gly-Arg-Hyp-
Gly-Pro-Hyp-Gly-Ser-Ala-Gly-Ala-Arg-Gly-Asp-Asp-Gly-Ala-Val-Gly-Ala-Ala-Gly-Pro-Hyp-Gly-Pro-Thr-Gly-Pro-Thr-
Gly-Pro-Hyp-Gly-Phe-Hyp-Gly-Ala-Ala-Gly-Ala-Lys-Gly-Glu-Ala-Gly-Pro-Gln-Gly-Ala-Arg-Gly-Ser-Glu-Gly-Pro-Gln-
Gly-Val-Arg-Gly-Glu-Hyp-Gly-Pro-Hyp-Gly-Pro-Ala-Gly-Ala-Ala-Gly-Pro-Ala-Gly-Asn-Hyp-Gly-Ala-Asp-Gly-Gln-Hyp-
Gly-Ala-Lys-Gly-Ala-Asn-Gly-Ala-Hyp-Gly-Ile-Ala-Gly-Ala-Hyp-Gly-Phe-Hyp-Gly-Ala-Arg-Gly-Pro-Scr-Gly-Pro-Gln-
Gly-Pro-Ser-Gly-Ala-Hyp-Gly-Pro-Lys-Gly-Asn-Ser-Gly-Glu-Hyp-Gly-Ala-Hyp-Gly-Asn-Lys-Gly-Asp-Thr-Gly-Ala-Lys-
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Gly-Pro-Hyp-Gly-Pro-Ser-Gly-Asn-Ala-Gly-Pro-Hyp-Gly-Pro-Hyp-Gly-Pro-Ala-Gly-Lys-Glu-Gly-Ser-Lys-Gly-Pro-Arg-
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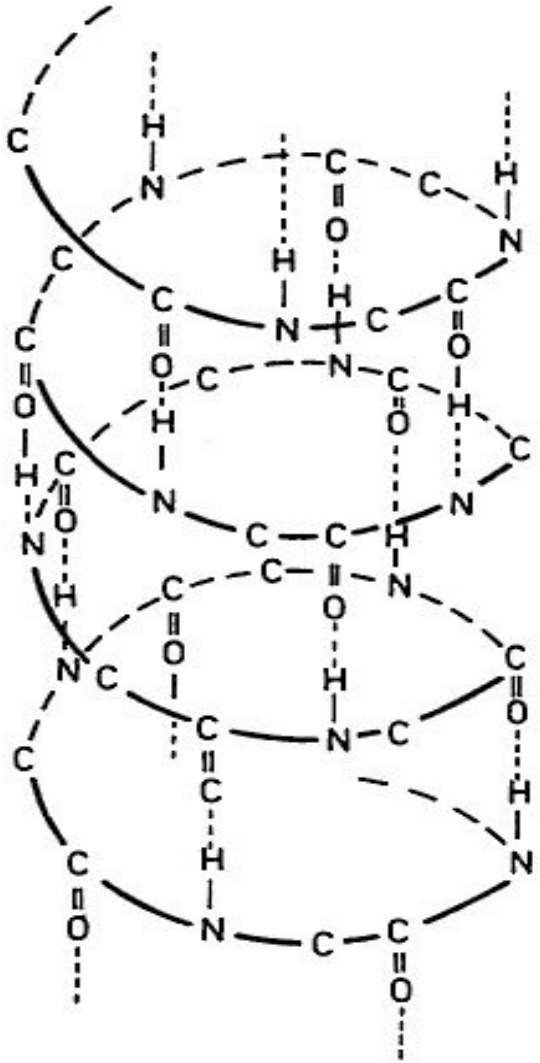
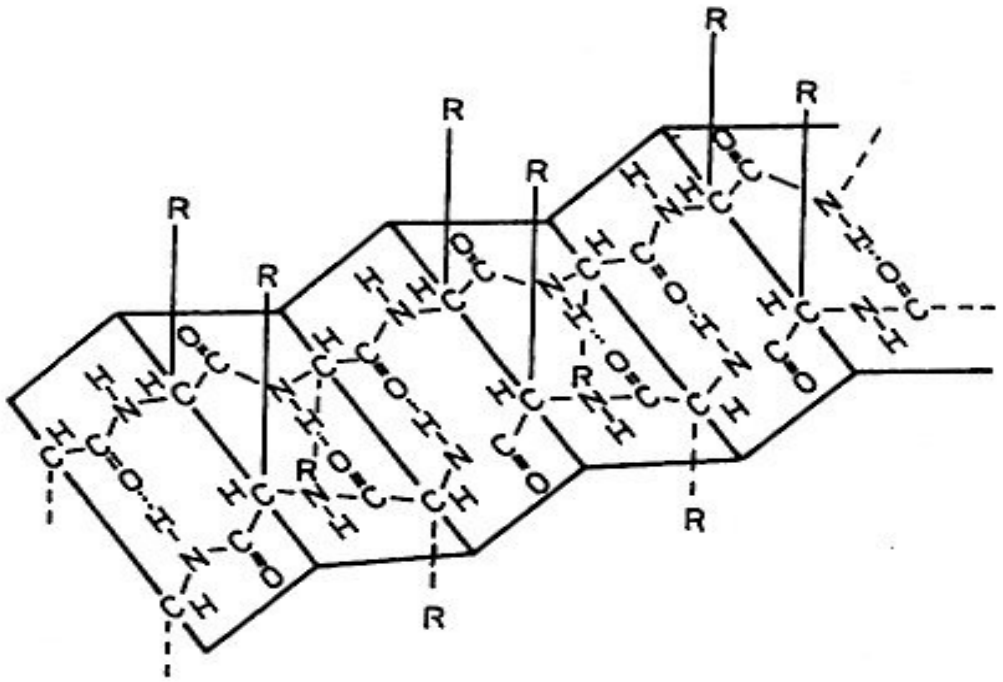
Gly-Leu-Asn-Gly-Leu-Hyp-Gly-Pro-Ile-Gly-Hyp-Hyp-Gly-Pro-Arg-Gly-Arg-Thr-Gly-Asp-Ala-Gly-Pro-Ala-Gíy-Pro-Hyp-
Gly-Pro-Hyp-Gly-Pro-Hyp-Gly-Pro-Hyp-Gly-Pro-Pro-
Ser-Gly-Gly-Tyr-Asp-Leu-Ser-Phe-Leu-Pro-Gln-Pro-Pro-Gln-Glu-Lys-Ala-His-Asp-Gly-Gly-Arg-Tyr-Tyr

PROTEIN SECONDARY STRUCTURE II B

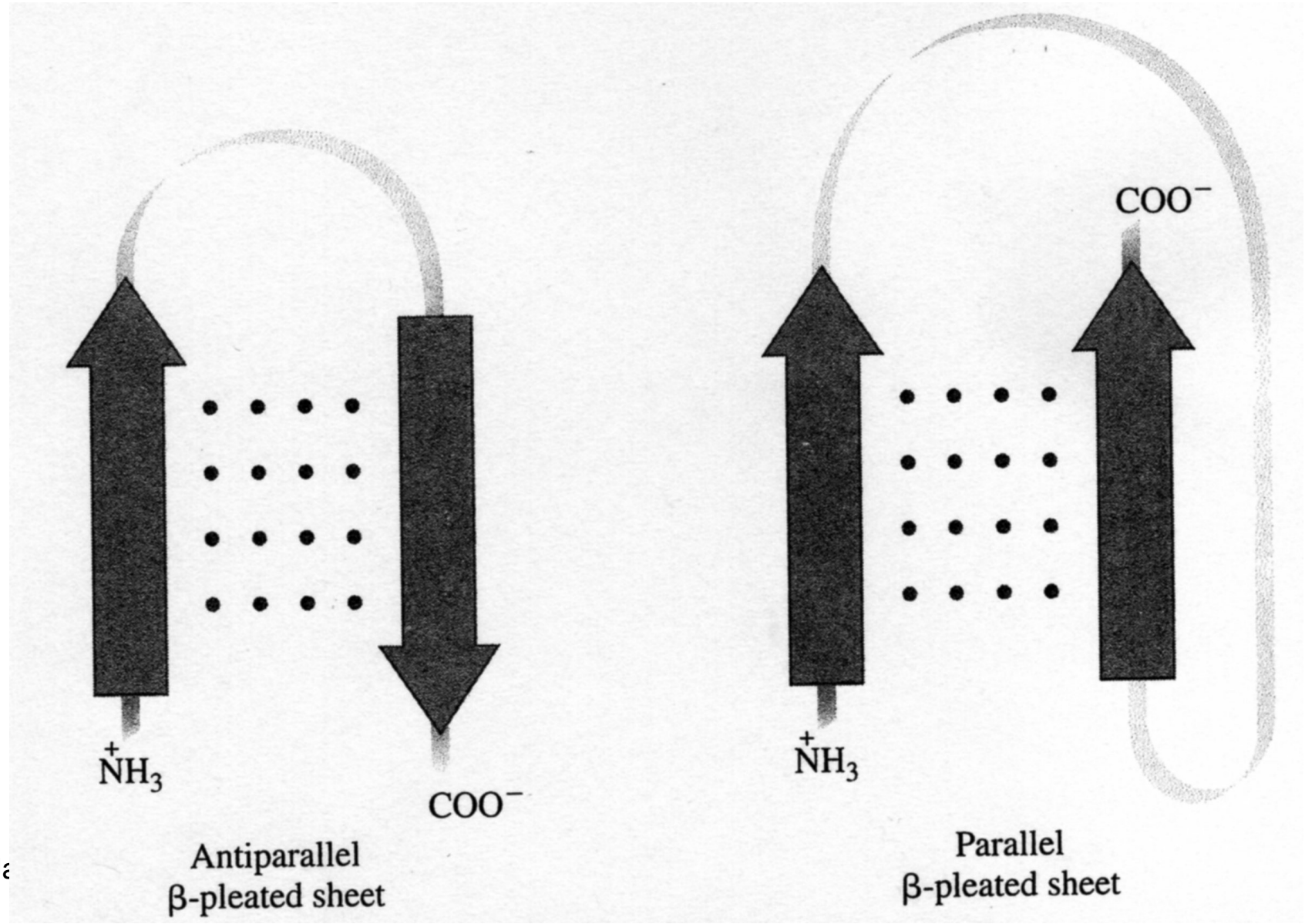




β **Folded Sheet**

◄ **Left-handed α Helix**

β Folded Sheet



SECONDARY STRUCTURE of Proteins II
COLLAGEN as the EXAMPLE

The Conformation of the SECONDARY STRUCTURE (α-Helix, β- Sheet, Statistic Coil) has the Influence on the IR Wave Number of the Amide Group Peak in the IR Spectrum.

IR Spectrum (Wave Number) of the Amide Bond (10^2 m^{-1})		
Structure	**Amide I**	**Amide II**
α – Helix	1650 1652	1515 1546
β–Structure	1630 1645	1530 1550
Statistic Coil	1656	1535

It is a bit unusual Wave Number Unit, but it is right!
It is the same Figure as the usual Unit cm^{-1}.
COLLAGEN is the α-Helix.

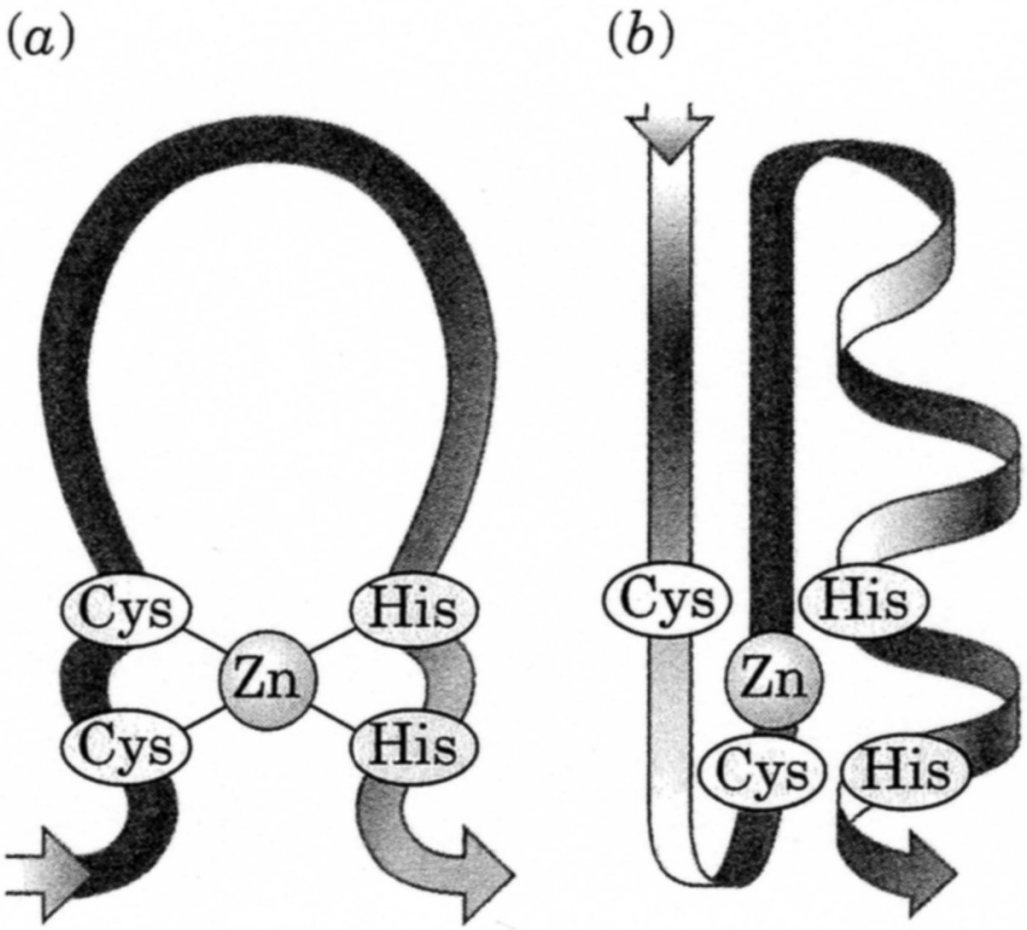


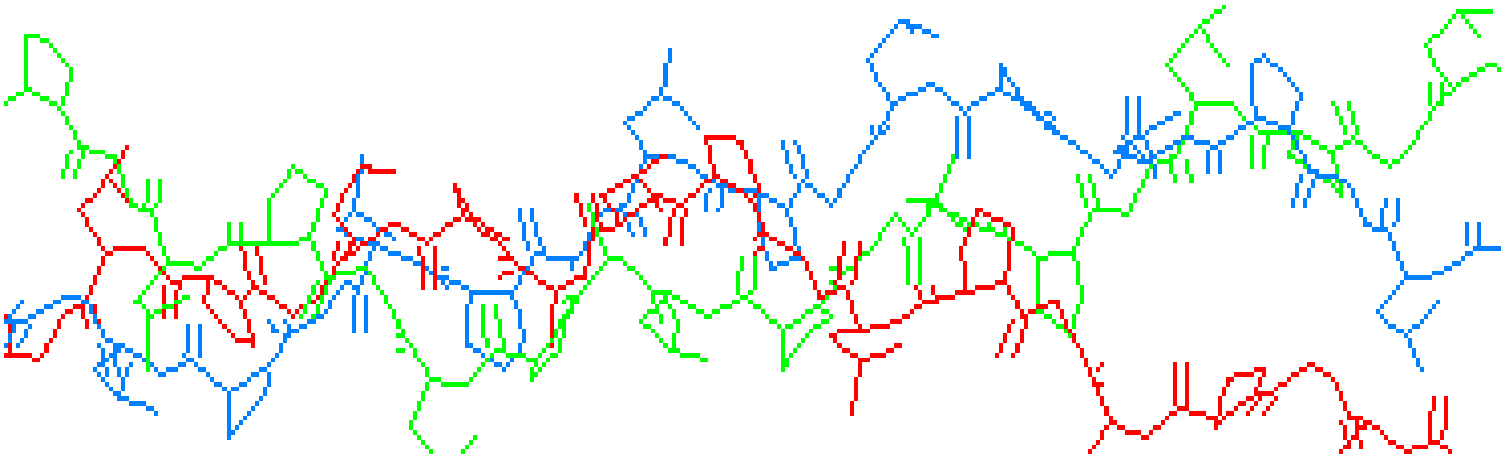
FIGURE 22.18 Cys_2His_2 zinc finger. (*a*) The coordination between zinc and cysteine and histidine residues. (*b*) The secondary structure.

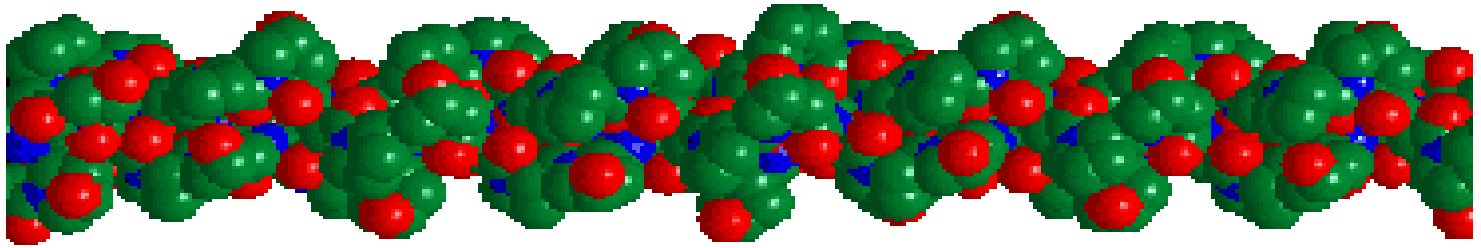
These Interactions are in the ONE MOLECULE, so it is SECONDARY STRUCTURE

TERTIARY STRUCTURE of Proteins II

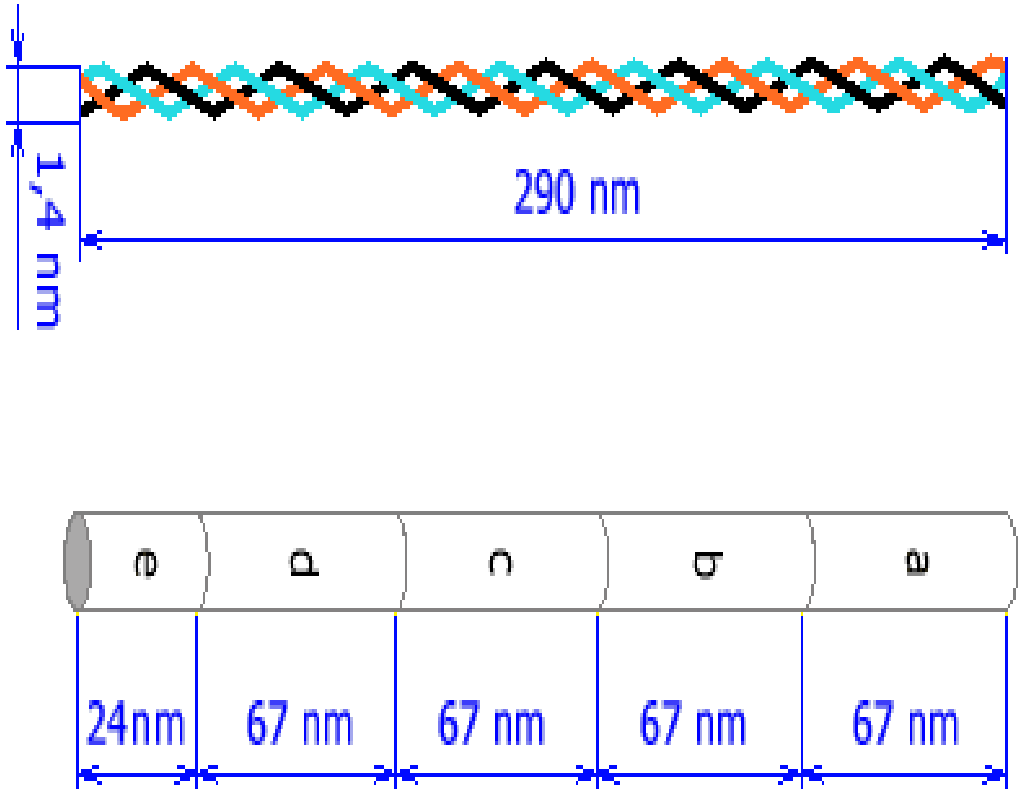
COLLAGEN as the EXAMPLE

three Coils arranged to the next combined coiled Chain





TERTIARY STRUCTURE of Proteins II
COLLAGEN as the EXAMPLE
three Coils arranged to the next combined coiled Chain



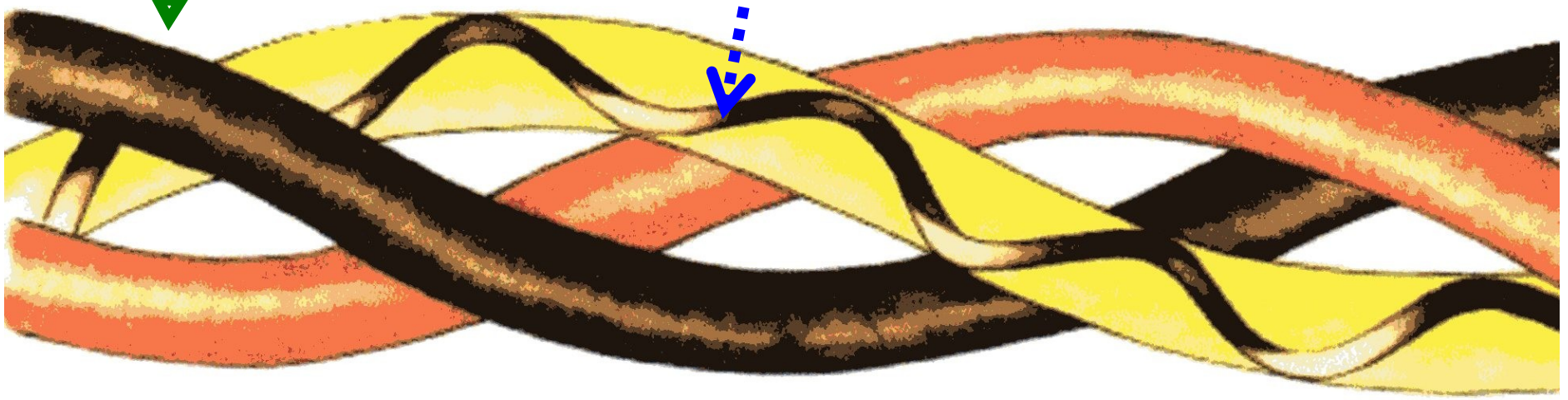
Schematic representation of the COLLAGEN Triple helix Molecule. The marked Intervals on the right (a, b, c, d) are 67 nm, what is the Distance over which are the particular Molecules in the Triple helix shifted each to another .

TERTIARY STRUCTURE of Proteins II

COLLAGEN as the EXAMPLE

three Coils arranged to the next combined coiled Chain

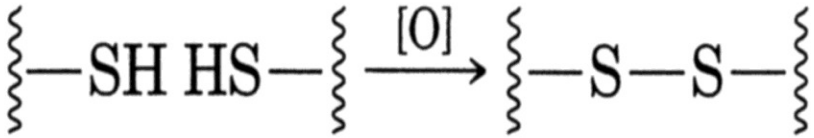
SECUNDARY STRUCTURE



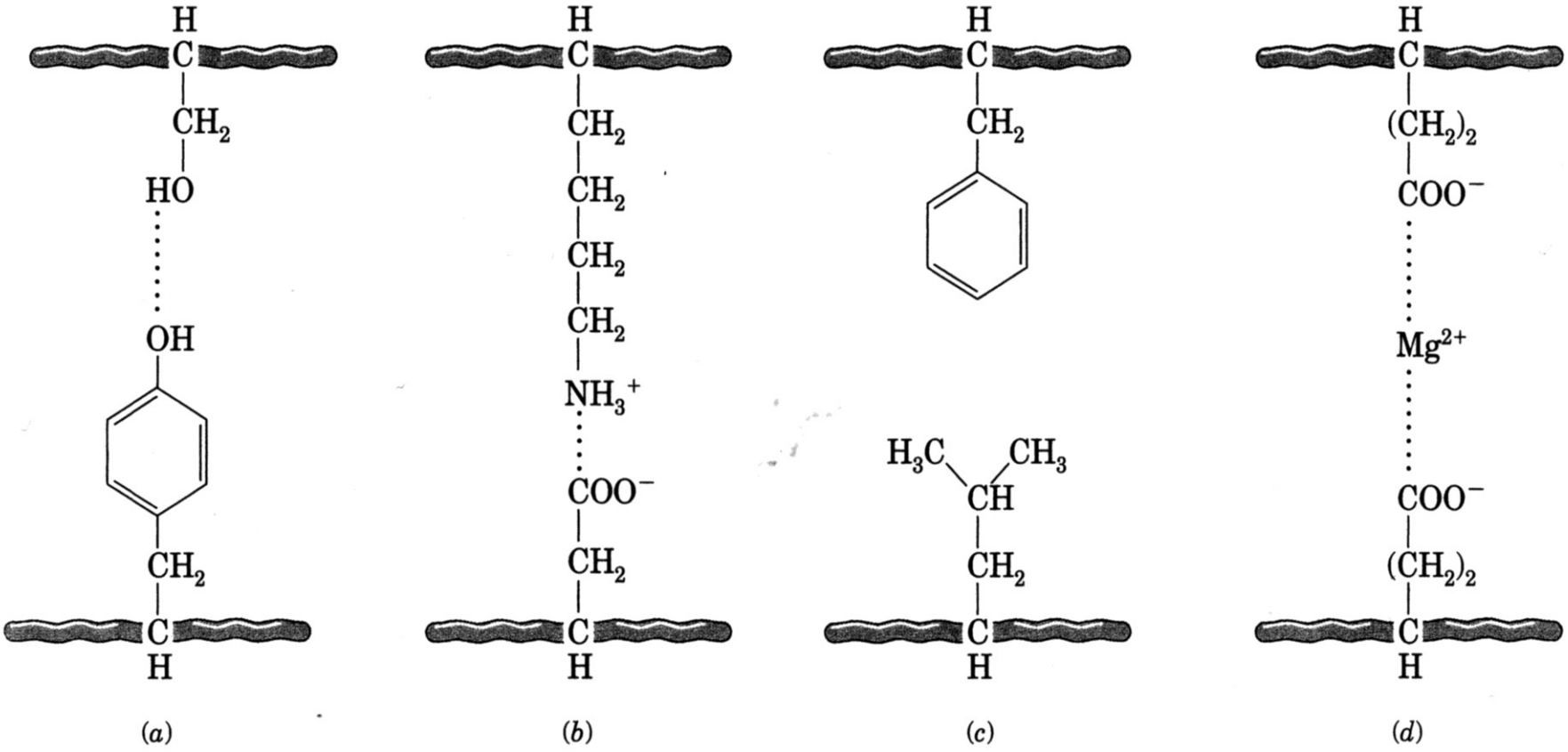
What Is the Tertiary Structure of a Protein?

The **tertiary structure** of a protein is the three-dimensional arrangement of every atom in the molecule. Unlike the secondary structure, it includes interactions of the side chains, and not just the peptide backbone. In general. tertiarv structures are stabilized five wavs:

1. **Covalent Bonds** The covalent bond most often involved in stabilization of the tertiary structure of proteins is the disulfide bond. In Section 22.4, we noted that the amino acid cysteine is easily converted to the dimer cystine. When a cysteine residue is in one chain and another cysteine residue is in another chain (or in another part of the same chain), formation of a disulfide bond provides a covalent linkage that binds together the two chains or the two parts of the same chain:



TERTIARY STRUCTURE Proteins V



Noncovalent interactions that stabilize the tertiary and quaternary structures of proteins: (*a*) hydrogen bonding, (*b*) salt bridge (electrostatic interaction), (*c*) hydrophobic interaction, and (*d*) metal ion coordination.

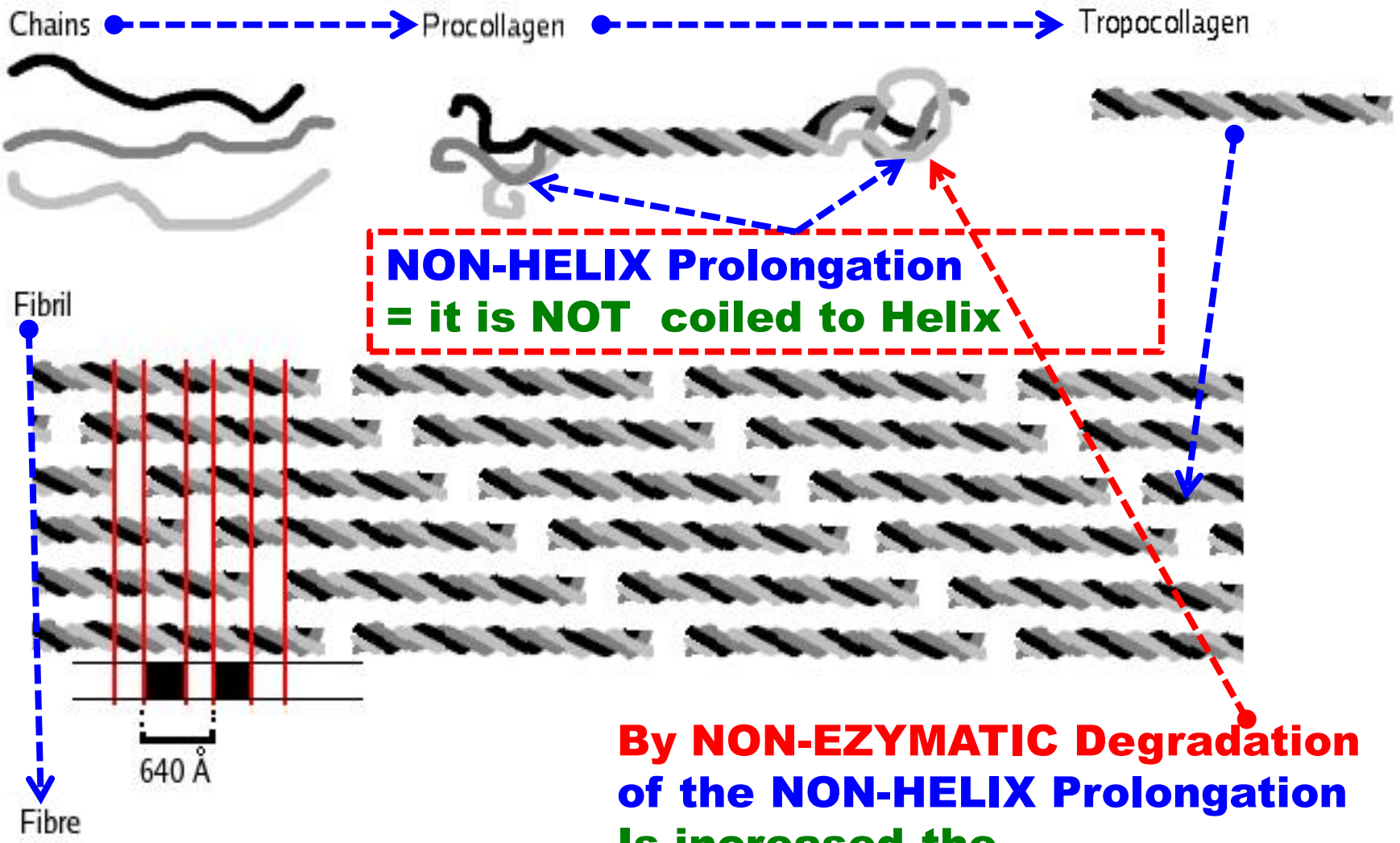
2. **Hydrogen Bonding** In Section 22.9, we saw that secondary structures are stabilized by hydrogen bonding between backbone —C═O and —N—H groups. Tertiary structures are stabilized by hydrogen bonding between polar groups on side chains or between side chains and the peptide backbone [Figure 22.19(*a*)].

3. **Salt Bridges** Salt bridges, also called electrostatic attractions, occur between two amino acids with ionized side chains—that is, between an acidic amino acid ($-COO^-$) and a basic amino acid ($-NH_3^+$ or $=NH_2^+$) side chain. The two are held together by simple ion–ion attraction [Figure 22.19(*b*)].

4. **Hydrophobic Interactions** In aqueous solution, globular proteins usually turn their polar groups outward, toward the aqueous solvent, and their nonpolar groups inward, away from the water molecules. The nonpolar groups prefer to interact with each other, excluding water from these regions. The result is a series of hydrophobic interactions (see Section 21.1) [Figure 22.19(*c*)]. Although this type of interaction is weaker than hydrogen bonding or salt bridges, it usually acts over large surface areas, so that the interactions are collectively strong enough to stabilize a loop or some other tertiary structure formation.

5. Metal Ion Coordination Two side chains with the same charge would normally repel each other, but they can also be linked via a metal ion. For example, two glutamic acid side chains ($—COO^-$) would both be attracted to a magnesium ion (Mg^{2+}), forming a bridge. This is one reason the human body requires certain trace minerals—they are necessary components of proteins [Figure 22.19(*d*)].

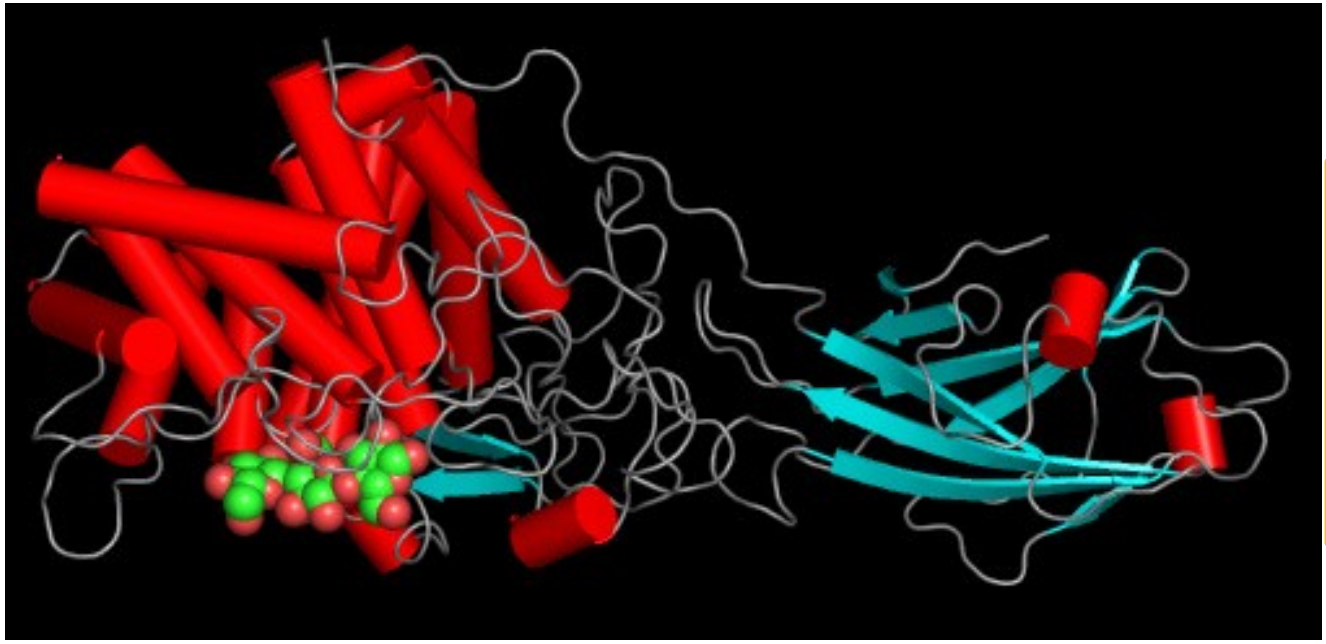
Synthesis of collagen



By NON-EZYMATIC Degradation of the NON-HELIX Prolongation Is increased the BIOCOMPATIBILY, it is the ANTIGENITY is suppressed

QUOTERNARY STRUCTURE Proteins I
CELULASE as the EXAMPLE

The Interaction between the folded up Fibrous Structures coiled to the Helix as the TERTIARY STRUCTURE already.
An Example – **COLLAGEN -** they are **PARALEL BUNDLES of TERTIARY STRUCTURE**. It is called **ASSOCIATES ARISING** sometimes.
It is typical for the **ENZYME**, where they are not **PARALEL BUNDLES,** but they are **GLOBULAR STRUCTURES**.

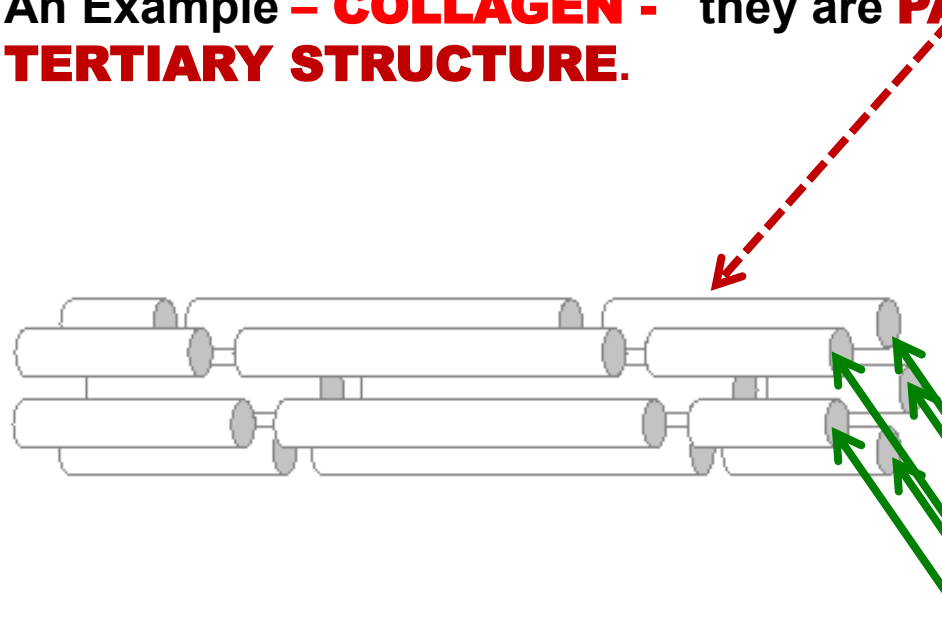


One Enzyme from the „CELULASE“ Group

QUOTERNARY STRUCTURE Proteins I COLLAGEN as the EXAMPLE

The Interaction between the folded up Fibrous Structures coiled to the Helix as the TERTIARY STRUCTURE already.

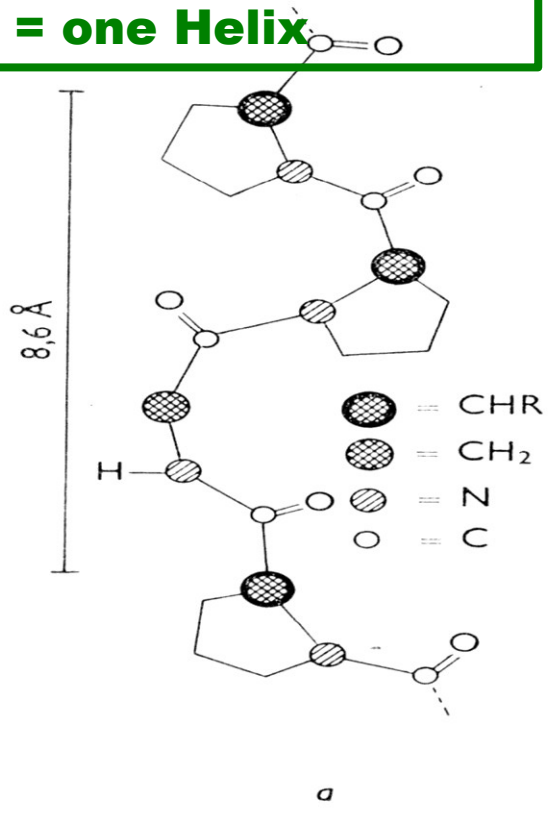
An Example – COLLAGEN - they are PARALEL BUNDLES of TERTIARY STRUCTURE.

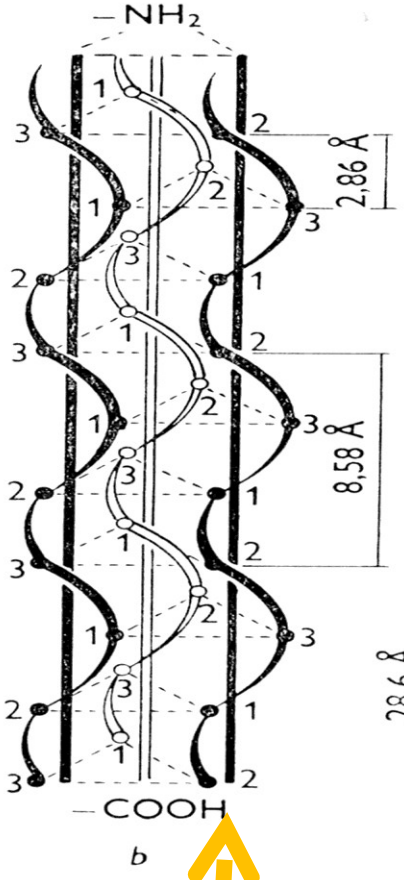


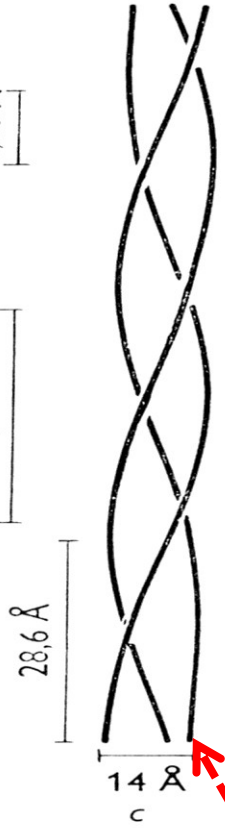
The Microfibril Model created as the Consequence of Interaction polar an d hydrophobic Side Chains. Five Tropocollag Molecules are shifted each to another 4 nm here and they are creating a Cylinder Structure so.

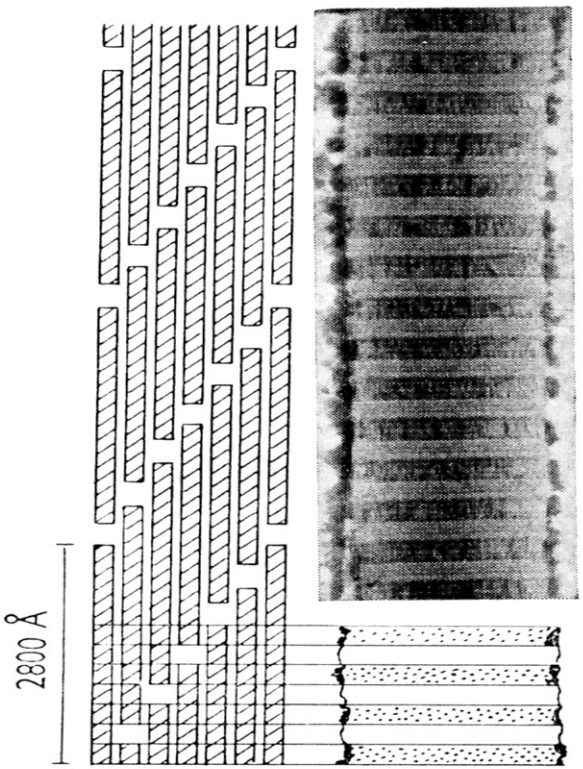
COLLAGEN – Hierarchy of the Structures

SECONDARY
= one Helix









QUOTERNARY

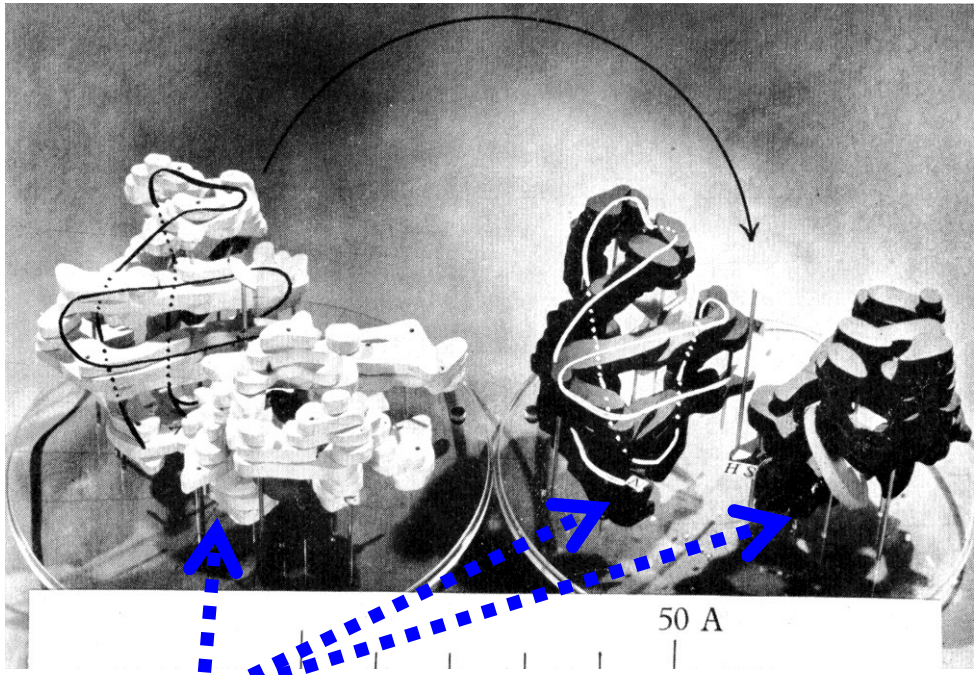
TERTIARY – two different Representations

THREE HELIXES

Left Picture – SIMPLIFIED

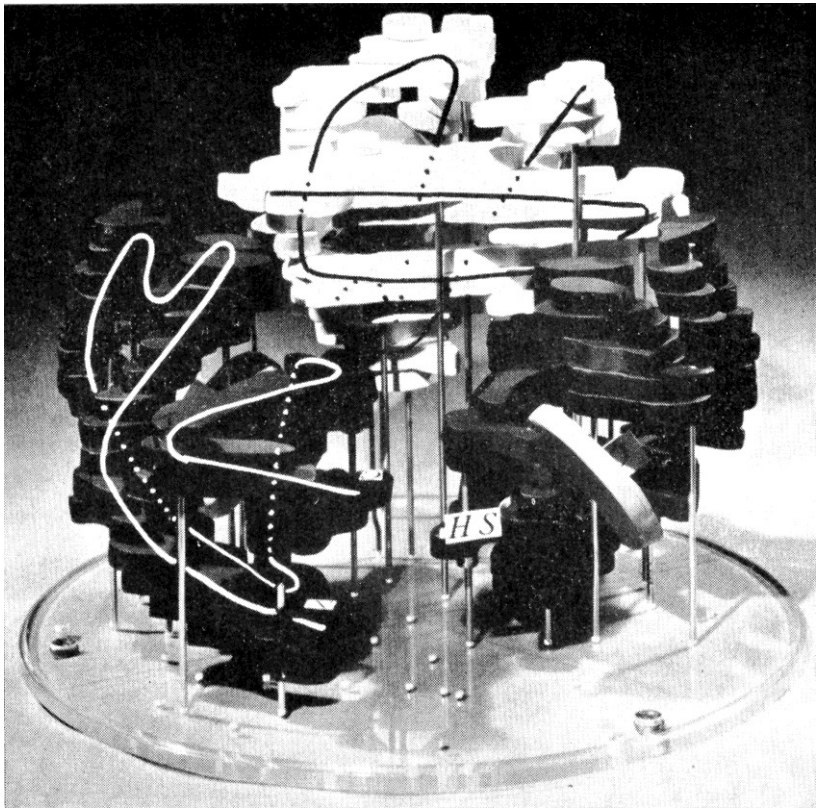
RIGHT PICTURE - REALITY

The EXAMPLE of Creation of the QUOTERNARY STRUCTURE - *HEMOGLOBINE*



Three Parts - Tertiary Structures, which create the QUOTERNARY STRUCTURE

QUOTERNARY STRUCTURE

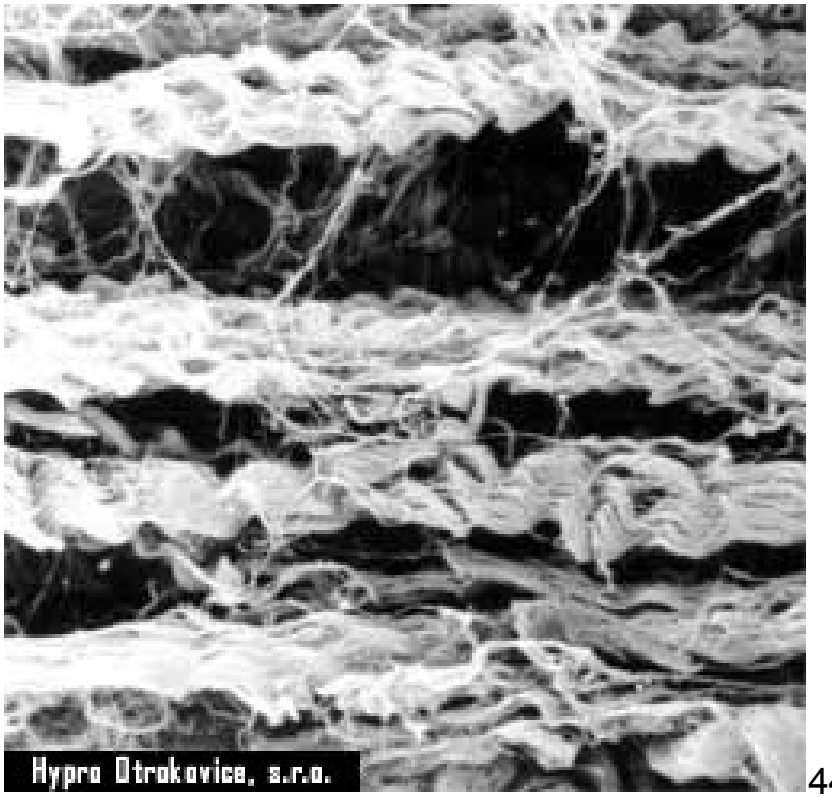


QUOTERNARY STRUCTURE
Proteinů III COLLAGEN as the EXAMPLE

The Interaction between the folded up Fibrous Structures coiled to the Helix as the TERTIARY STRUCTURE already.

An Example – **COLLAGEN -** they are **PARALEL BUNDLES of TERTIARY STRUCTURE**.

These **PARALEL BUNDLES of TERTIARY STRUCTURE** form **QUOTERNARY STRUCTURE**



COLLAGEN - PARALEL BUNDLES of TERTIARY STRUCTURE

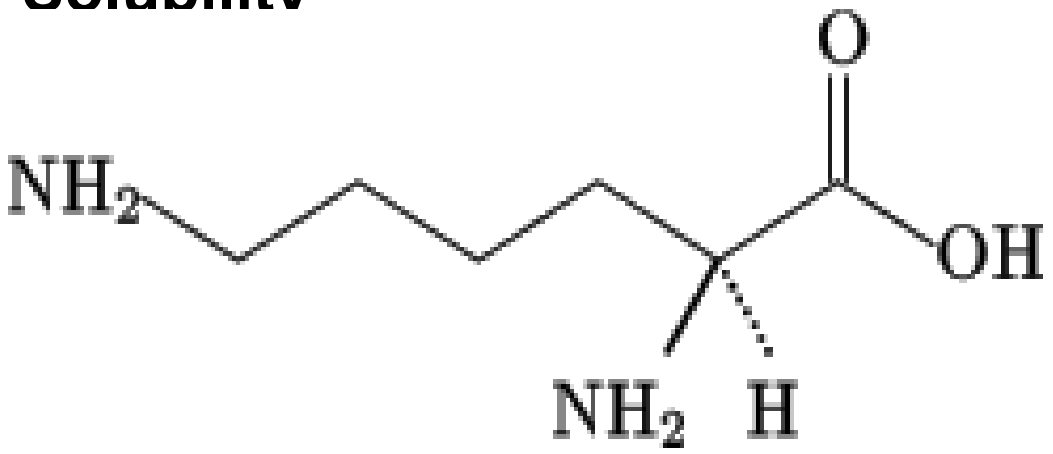
An Overview of Protein Structure and Function

- *Primary Structure:* The primary structure of the protein is the amino acid sequence of the protein. The primary structure results from the formation of covalent peptide bonds between amino acids. Peptide bonds are amide bonds formed between the carboxylate group of one amino acid and the amino group of another.
- *Secondary Structure:* As the protein chain grows, numerous opportunities for noncovalent interactions in the backbone of the polypeptide chain become available. These cause the chain to fold and orient itself in a variety of conformational arrangements. The secondary level of structure includes the α-helix and the β-pleated sheet, which are the result of hydrogen bonding between the amide hydrogens and carbonyl oxygens of the peptide bonds. Different portions of the chain may be involved in different types of secondary structure arrangements; some regions might be α-helix and others might be a β-pleated sheet.
- *Tertiary Structure:* When we discuss tertiary structure, we are interested in the overall folding of the entire chain. In other words, we are concerned with the further folding of the secondary structure. Are the two ends of the chain close together or far apart? What general shape is involved? Both noncovalent interactions between the R groups of the amino acids and covalent disulfide bridges play a role in determining the tertiary structure. The noncovalent interactions include hydrogen bonding, ionic bonding, and van der Waals forces (London dispersion forces and dipole-dipole attractions).
- *Quaternary Structure:* Like tertiary structure, quaternary structure is concerned with the topological, spatial arrangements of two or more peptide chains with respect to each other. How is one chain oriented with respect to another? What is the overall shape of the final functional protein?

COLLAGEN – Cross Bonds IN VIVO

- The Bonds are based on the Reactions of the **Oxidised – HN_2 in Lysine** with **–OH** Group and Reactions between arisen Groups
- Reactions are mainly INTRAMOLECULAR, but also INTERMOLECULAR
- There is less **Cross Bonds in so called YOUNG** COLLAGEN > Higher Solubility
- There is more **Cross Bonds in so called OLD** COLLAGEN > Lower Solubilitv

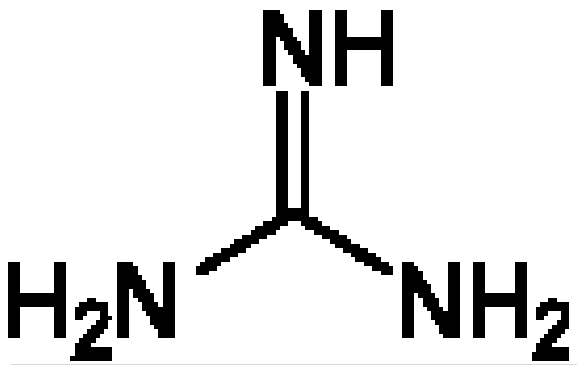
- **Lysine**



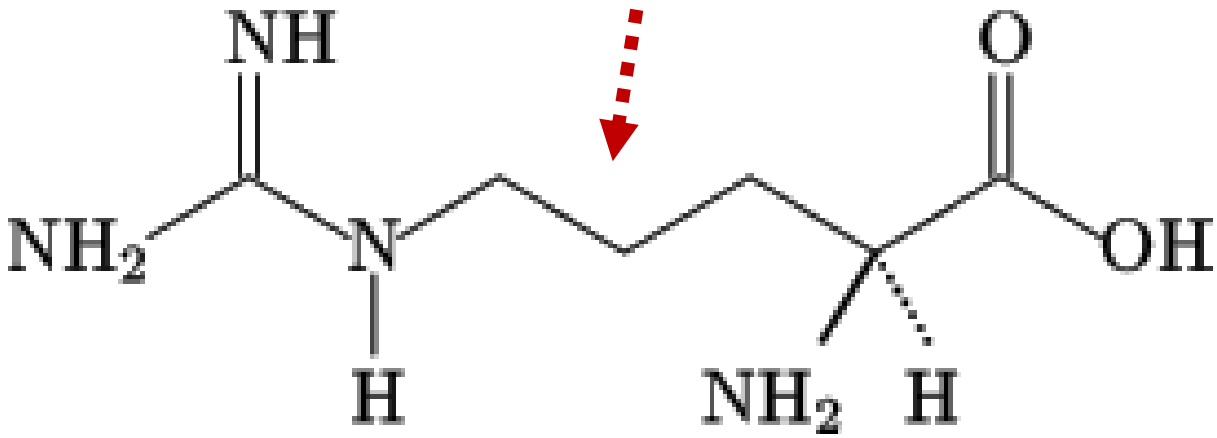
COLLAGEN – REACTIONS IN VITRO 1

- **ACYLATION using acetanhydride**
- **Esterification (various reagents)**
- **Deamination (-NH_2 > -OH)**
- **Deguanidisation**

(Removing the End from the Arginine and cleavage $H_2N – CO – NH_2$)

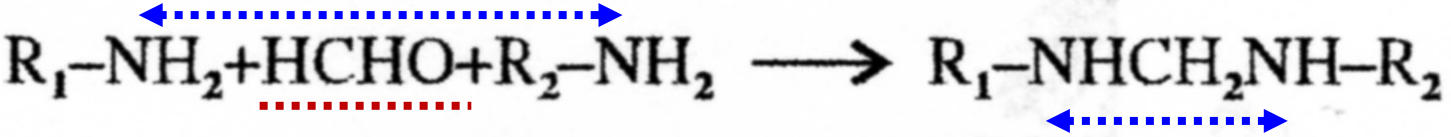


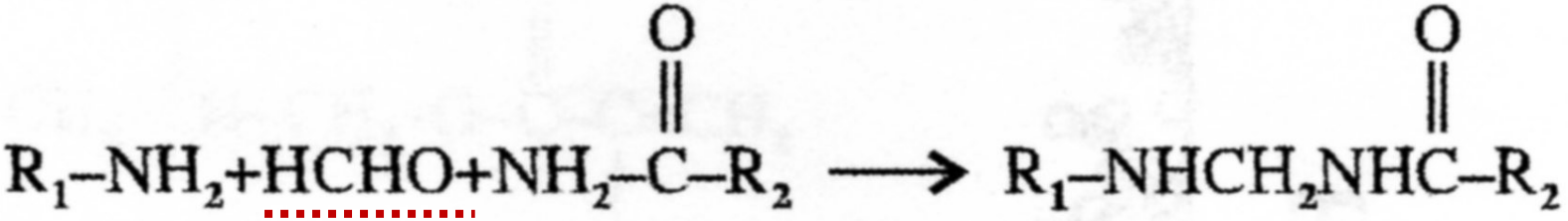
guanidin

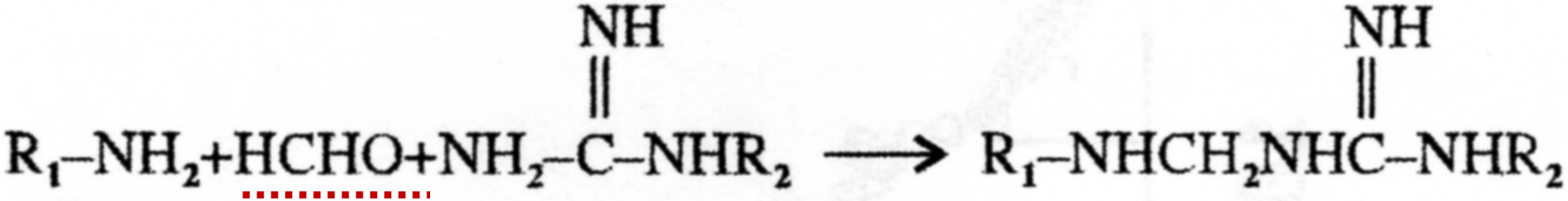


COLLAGEN – REACTIONS IN VITRO 2

- **Aldehydic Condensation**
- **Oxidation by Periodate >**
  5-hydroxylysine > ALDEHYDE > REACTION







COLLAGEN – Reaction with Synthetic Polymers IN VITRO 1

Effort to enhance the COMPATIBILITY of the NATURAL & SYNTHETIC POLYMERS

COLLAGEN – Hierarchy of Structures & USE

Structure Level	Branch of Use/Products
Fibres' Tissue	Leather, Wounds' Covering, Skin Implants, Vessels Substitutes,
Fibres	Casing for Meat Products, Packaging Films, Membranes, Powder for Wounds' Covering, Capsule for Medicines
Fibrils	Biocompatible Plastic or Ceramic Composites for Surgery
Macromolecules	Native Collagen, Derivatives of Collagen for Medicine & Cosmetic Use
Polypeptides	Animal Glue, Gelatine, Hydrolysate of Collagen, Capsule for Medicines, Thickener, Animal feed …

COLLAGEN – Medicine Use 1

COLLAGEN Medicine Preparations – Sort and Use accordingly

Products	Use
Catgut	Surgery Sewing, Wounds' Covering, Wounds' Covering
Fibres – • From the Solutions • From the Suspensions • From the Membranes	Skin Implants, Haemostatics, Vessels Substitutes,
Collagen Sheets – • Genuine skin • Membranes • Nonwoven Textiles • Foams • Sponge	Implants, Fillers for Implants
Vessels/Organs	Connective tissues healing ("Reparations"), Tissues' Glue
Collagen Powder, Glue, Sprays, Face Powder	Face Skin Care

- **Resorption is controlled by chromium salts Crosslinking at Tanning > slower Biodegradation (Resorption) in living Organism**
- **UNCROSSLINKED by chromium salts Crosslinking at Tanning > FASTER Biodegradation (Resorption) in living Organism**

The Discussion is on stream now, if the COLLAGEN degradated Chains are harmful for the Humans Organism or not.

It is sought in some Sources now, that COLLAGEN „CATGUT“ is allowed for the Veterinary use only.

There are not any such bans for „CATGUT“ at the Internet pages of the „CATGUT“ Suppliers up to now (year 2018).

Catgut Chromic Suture | Catgut suture

- **Catgut or gut suture is an absorbable suture usually manufactured from the intestine of sheep or goat.**
- **Catgut suture are composed of highly purified connective tissue derived from either beef or sheep intestines. The membrane is chemically treated and slender strands are woven together to form a suture. The grinding process creates a strand of uniform diameter. The suture strand is then further polished to achieve maximum smoothness, for reliability and strength.**
- **Catgut suture are available in the form of plain catgut or chromic catgut.**
- **Plain catgut is usually having shorter absorption periods and is absorbed more rapidly in infected areas.**
- **The percentage of collagen in the catgut suture often determines the quality of the suture. Higher percentages of collagen allow for: superior tensile strength, longer absorption times, and lower reactions in vivo. Plain catgut is available in ivory colour.**

Catgut Chromic Suture | Catgut suture

- **Chromic catgut is treated with chromium salt solution to resist body enzymes and slower the absorption process thus supporting the wound for longer periods.**
- **Chromic gut is chromicised before it is spun into strands. This allows control over the amount of chromic content for an even absorption rate.**
- **The chromic content not only increases the tensile strength, but also reduces tissue irritation.**
- **Catgut sutures are sterilized by a sterilizing fluid containing EO (ethylenoxide), distilled water and isopropyl alcohol.**

Distinctive Characteristics of Catgut Chromic Sutures

- **Absorption** within 60-90 days for chromic suture and 60-70 days for plain gut suture.
- Allows for **smooth passage** through tissue.
- Packed in IPA to retain memory & increase pliability.
- Uniform chrome content provides required wound support and absorption.
- Catgut suture are available from U.S.P Sizes 5-0 to 2
- **General closure**, Ophthalmic, Orthopaedics, Obstetrics/Gynaecology and Gastro-intestinal Tract Surgery.

COLLAGEN – Solubility 1

Some cosmetics include soluble or **hydrolyzed collagen**. In this case, the collagen molecules have been broken down into much smaller fragments, which are able to penetrate the skin's surface.

COLLAGEN SOLUBILITY DEPENDS ON:

- **MW > hydrolysable Types are more soluble, even in the Water,**
- **pH,**
- **.....**

COLLAGEN – Solubility 2

ISOELECTRIC POINT of COLLAGEN: approx. pH7

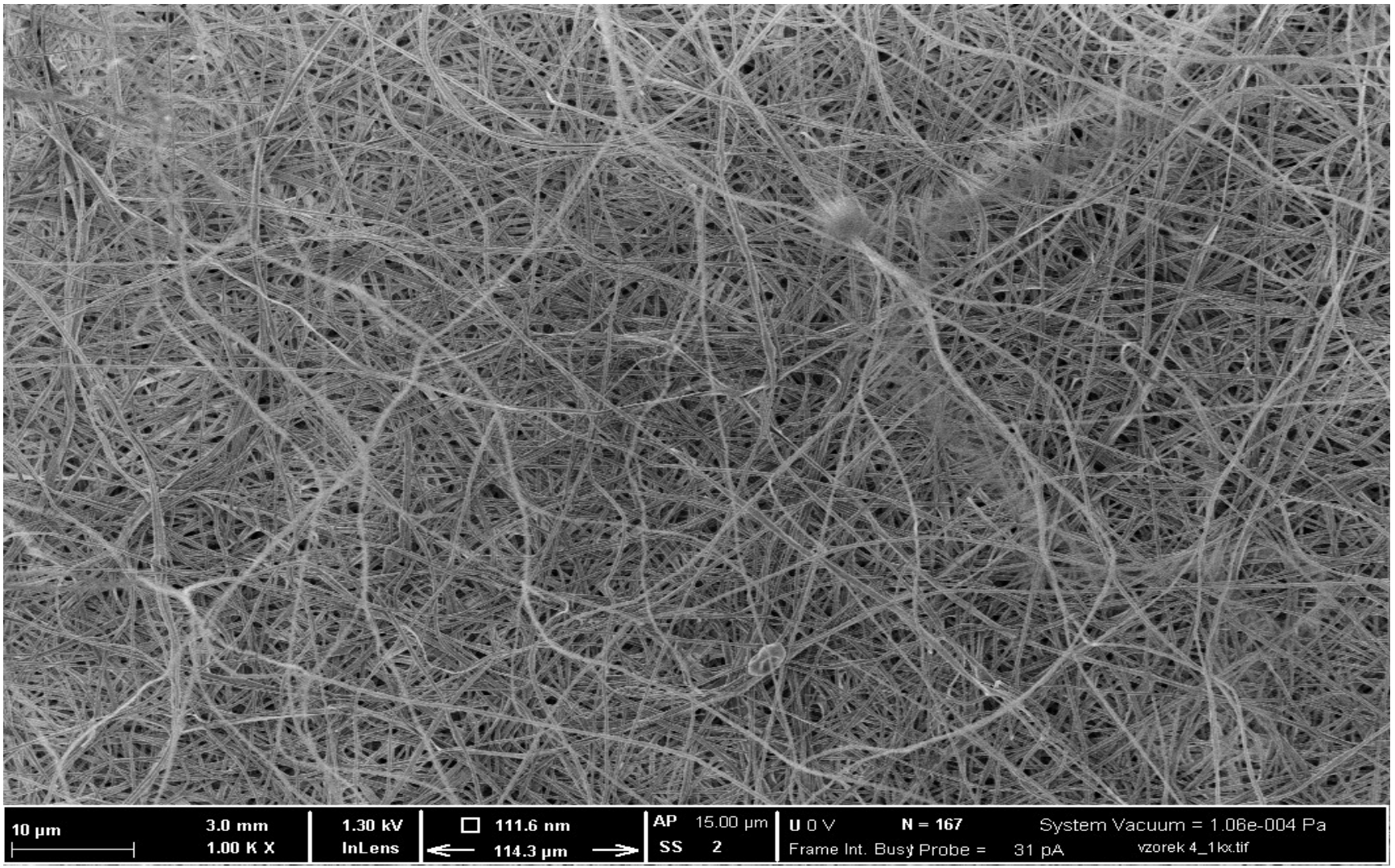
SOL → 28 °C → GEL

GEL → 34 °C → SOL

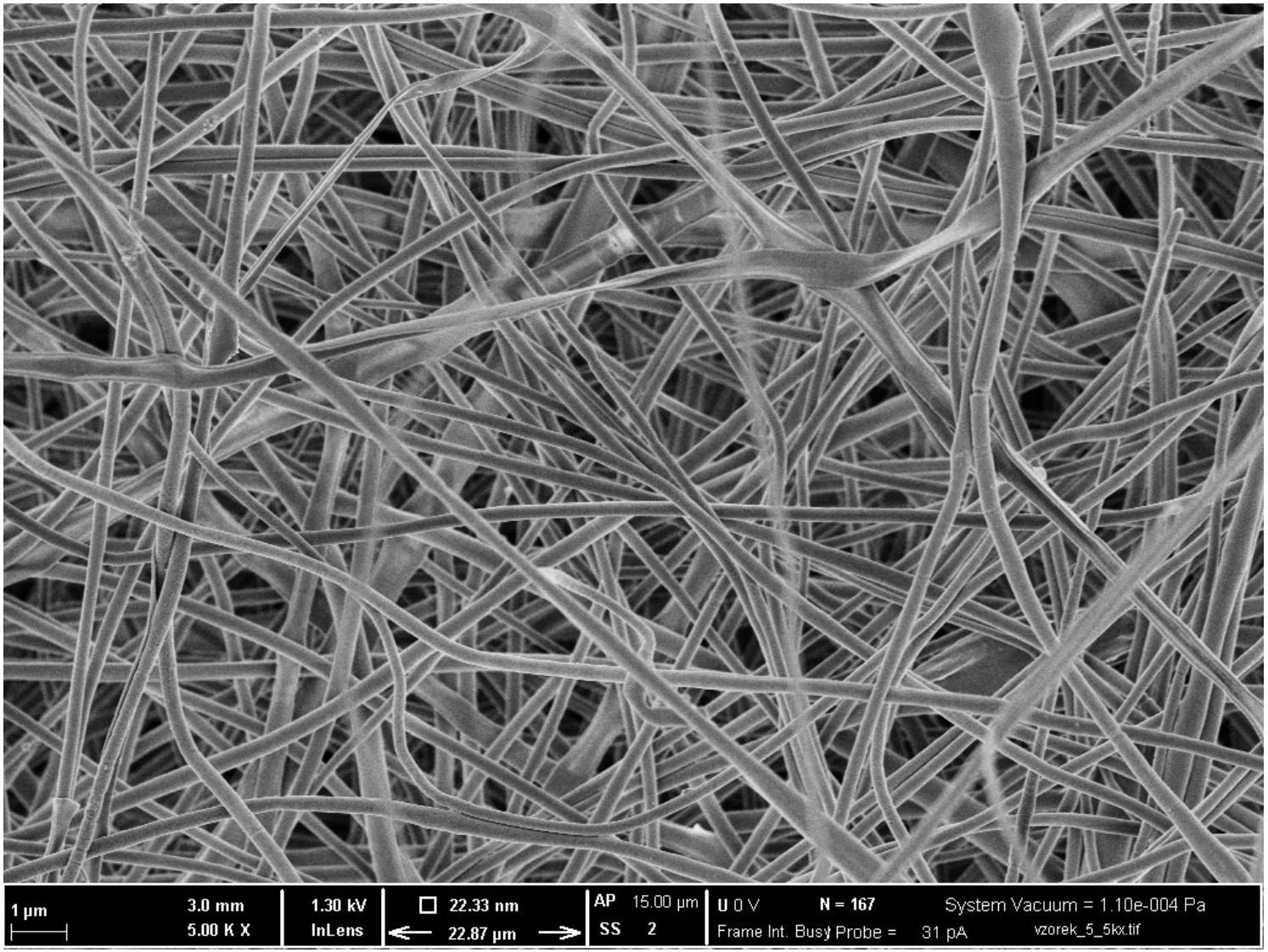
Transition SOL ⟷ GEL is NOT the one POINT CHARACTERISTIC, it is NOT Reversible Process!

It is called „INVERS PROCESS“, because some Changes in the Macromolecular Conformations can occur during this Process.

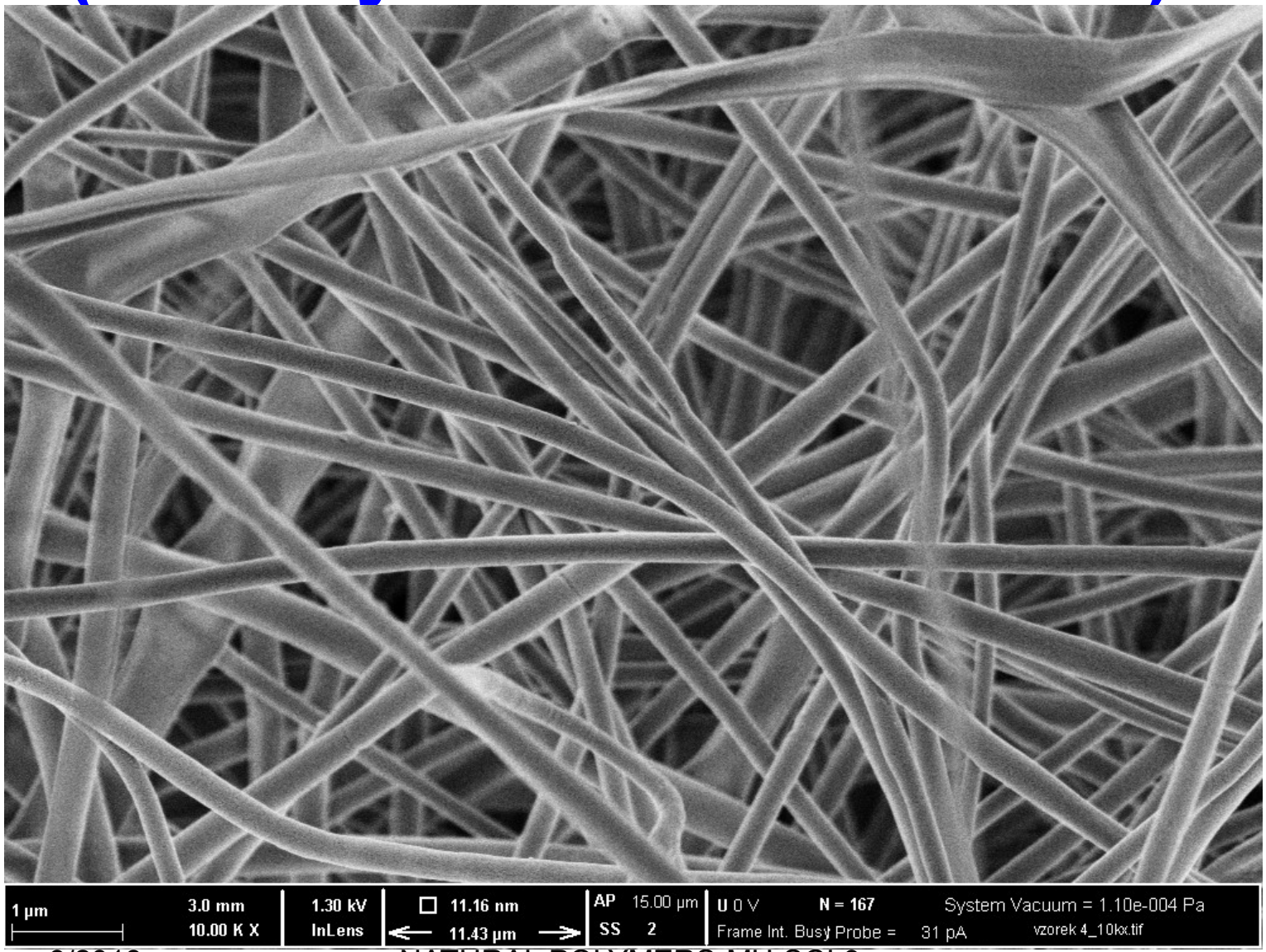
COLLAGEN – Nanofibres 1
(Courtesy of the CONTIPRO Ltd.)



COLLAGEN – Nanofibres 2
(Courtesy of the CONTIPRO Ltd.)



COLLAGEN – Nanofibres 3
(Courtesy of the CONTIPRO Ltd.)



COLLAGEN – Prices on the World Market Grades for Cosmetic, Water-soluble

US $12 - 18 / Kilogram
100% Soluble In Water Solubility Hydrolyzed Collagen For Hair
Supply Ability: 300 Ton/Tons per Month Fish Collagen

Country of Origin: China (PRC)

US $12 - 18 / Kilogram
FOOD GRADE
Supply Ability: 300 Ton/Tons per Month Fish Collagen

Country of Origin: China (PRC)

COLLAGEN as the Basic Substance for Chemical Reactions

The Reaction point is the Amino-group (-NH_2)

ARTICLE (e.g.):

Synthesis and properties of some* GELATIN *derivatives with substituents at the amino groups

J. Chem. Techn. and Biotechnology, 15, (1965), 479

- **If possible – POLYMERANALOGIC CHANGES (REACTIONS), it is without changes of the MW,**
- **They were mostly the Modifications for Photographic Emulsions > PATENTS,**
- **The Substrates for Cultivation of the Cells' Cultures are the main Subject now (Patents, Publications etc.)**

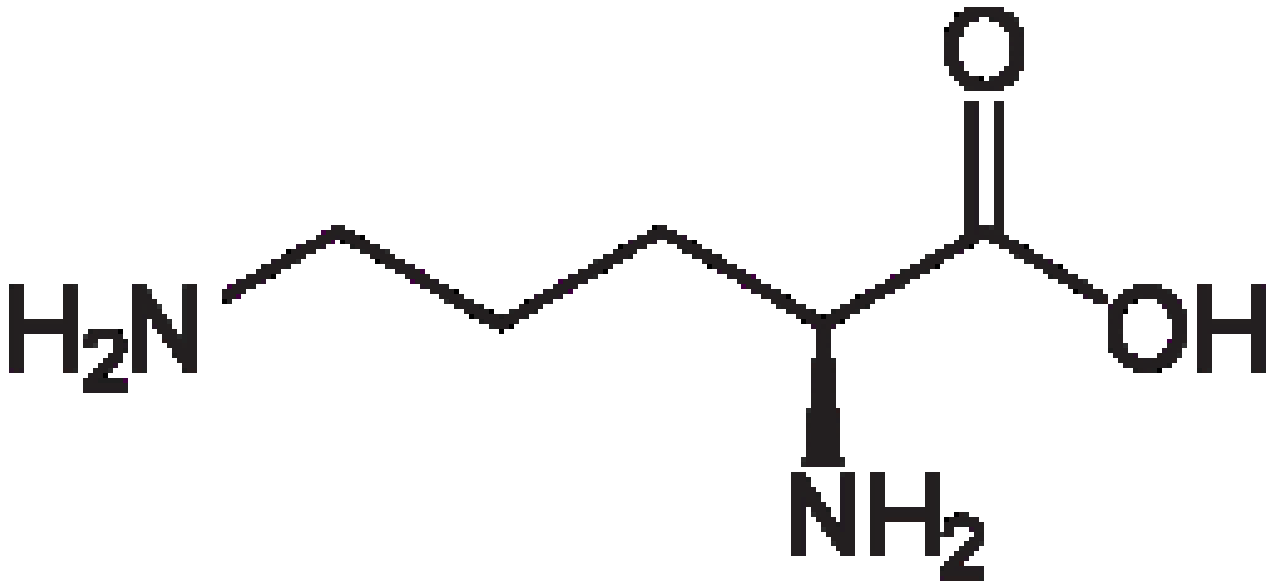
COLLAGEN as the Basic Substance for Chemical Reactions

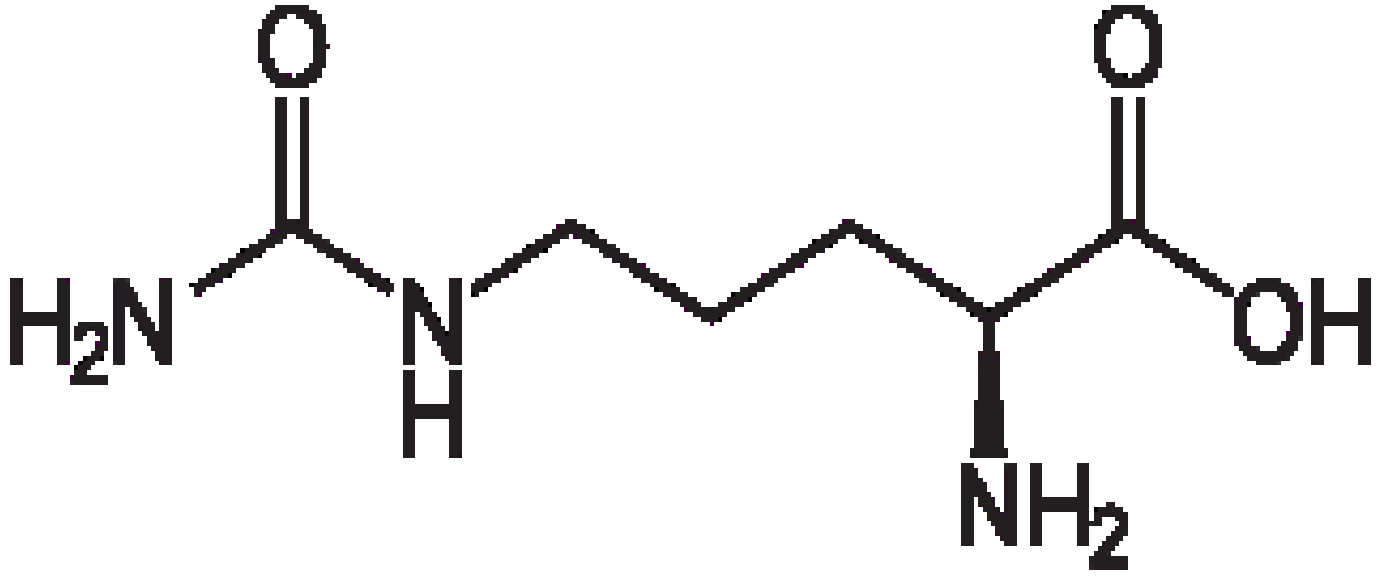
Chemical Modification (*see Slide No. 47 also*)

Reaction with monofunctional Reagents

- **Acylation** (acetanhydride + amino-groups)
- **Esterification** (dimethylsulphate, anhydrous methanol) – the Swelling Curve is changing, Structure and Insolubility is remained
- **Deaminace** (Mixture of Sodium Nitrite and Ice acetic acid) – Amino-groups → Hydroxyl Groups; the Swelling Curve is changing
- **Deguanidinace** – Arginine → Ornithine + Urea or Citrulline + Ammonium

Ornitine





Citrulline

COLLAGEN as the Basic Substance for Chemical Reactions

Chemical Modification

Reactions going to Crosslinked Gel Forming

- Higher Flexibility, Resistance to Enzymes Proteases, lower Swelling Degree → better Product
- **Aldehyde Condensation** (Glutaraldehyde forms Bridges) → Double *Schiff Base* – higher Resistance to to Acid or Basic High temperature Hydrolysis
- **Oxidation by Periodate** (5-hydroxyproline → Aldehyde → + amino Groups of Lysines →Network → higher Mechanical Strength

COLLAGEN as the Basic Substance for Chemical Reactions

Chemical Modification

Reaction with Synthetic Polymers (Surface Immobilisation)

Covering of the Porous **COPOLYMER** of Polyethylene with **Acrylic acid** by **COLLAGEN** bond to the Surface by the Covalent Bond amide Bond (between Carboxylic Groups of the Acrylic acid and the Amino groups of the **COLLAGEN** Molecule) → INCREASING OF THE IMPLANTATES' BIOCOMPACTIBITY

AN EXAMPLE

The Covering of the Vessels Substitutes knitted from PET Fibres

COLLAGEN Medical Applications 1

- **Catgut Chromic Suture** – Resorption is controlled by the Chromium Salts crosslinking
- Split leather, Nonwoven Textiles, Fixed Foams → Radiation-Chemical Sterilisation → Wounds' & Burns Covering
- Spray **Bandage on Wounds** (**COLLAGEN Powder**)
- **Blood Vessel Reparation, Tendon Replacement**
- **Cells' Cultures Growing** *in vitro*
- .....

COLLAGEN Medical Applications 2

- **COLLAGEN** inside covered Teflon hose > **Blood Vessel Replacement**
- Combination with Calcium Phosphate → Bones and Tooth Replacement
- Injection of the Microcapsules impregnated with effective Substance – Wound Healing
- **COLLAGEN** Glue in Surgery
- ……

COLLAGEN based Articular Preparation NUTRITION & REGENERATION of the Articular Cartilage

ORLING Company (Czech Republic) accordingly:

Pure native Collagen is as the Rope coiled from the Three Fibres, which is non-specifically split during Digestion. The Results are split Chains, which are of various Length whenever.

Hydrolysed Collagen is called Collagen Peptides. If the Collagen is split by Enzymes COLLAGENASE, the pure native Collagen is changed to the Collagen Peptides of the same Length every time.

The Biological Activity of the Pure native Collagen and of the Collagen Peptides is different, what was proved by many scientific Studies.

3. Manufacture of GELATIN & ANIMAL GLUE

TANEX Company, Czech Republic

The Pictures were taken during Students' Excursion to this Glue Plant Company

Manufacture of GELATIN & ANIMAL GLUE

The Raw Materials are not looking too atractive 1!

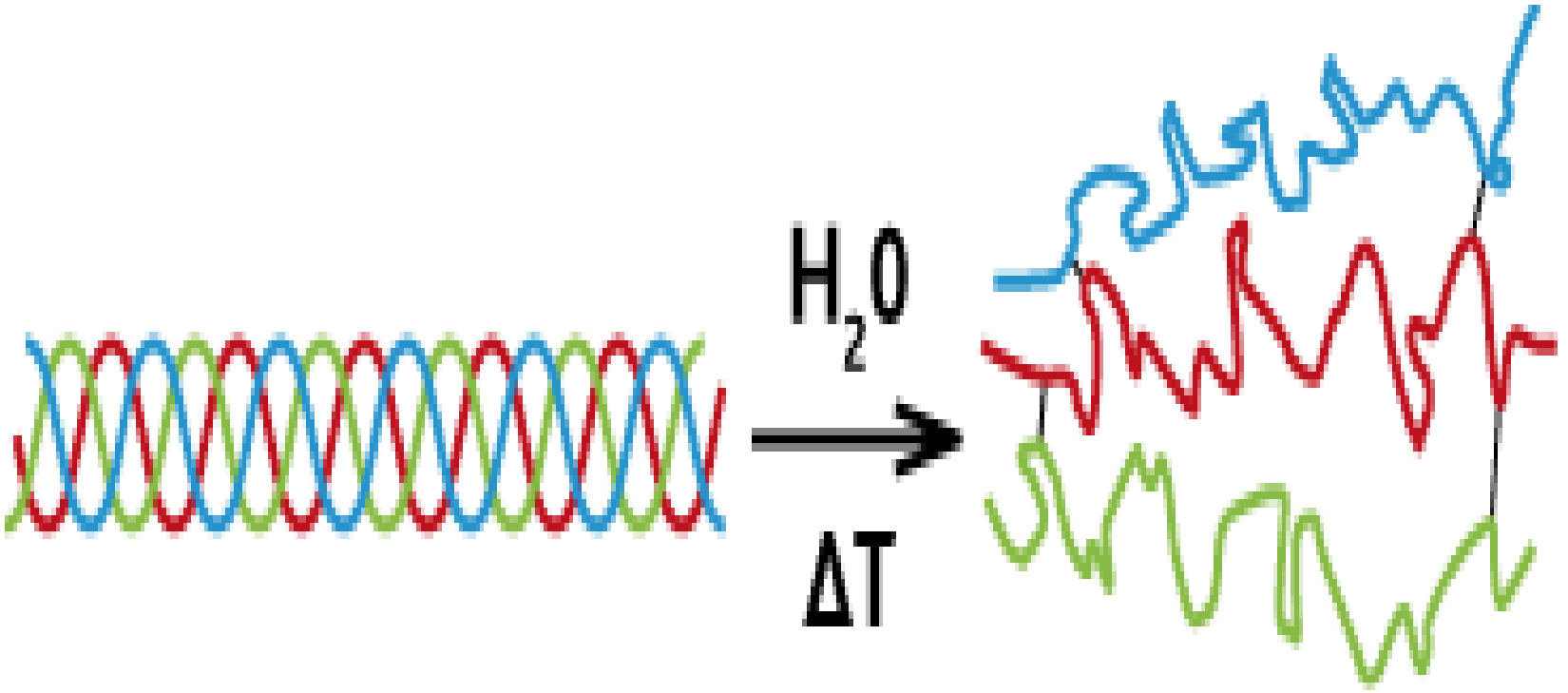
Abattoir by-products from the Pig livestock
Skin, Ears etc.

Manufacture of GELATIN & ANIMAL GLUE

The Raw Materials are not looking too atractive 2!

Abattoir by-products from the Pig livestock
Skin, Ears etc.

Manufacture of GELATIN & ANIMAL GLUE
BASIC SCHEMA



COLLAGEN → **GELATIN**

Example of the GELATIN & Animal Glue Making 1

Parameters	Sequence of Leaching (Lixiviating)			
	1	2	3	4
Product	Edible & Photographic GELATIN	GELATIN 1. Quality	GELATIN 2. Quality	Technical GELATIN & Animal Glue
Temperature (°C)	50 – 55	60 – 65	70 – 75	85 - 100
Time (hours)	5			
pH	5 - 7			
Concentration (% w/w)	3 - 8	3 - 8	10	> 12

Better Raw Material & Mild Manufacturing Conditions > GELATIN

Worse Raw Material & „Rough“ Manufacturing Conditions > Animal Glue

Example of the GELATIN & Animal Glue Making 2

Parameters	Sequence of Leaching (Lixiviating)			
	1	2	3	4
Product	Edible & Photographic GELATIN	GELATIN 1. Quality	GELATIN 1. Quality	Technical GELATIN & Animal Glue
Temperature (°C)	60 – 65	80 – 85	95 – 100	100
Time (hours)	8			
pH	5 - 7			
Concentration (% w/w)	8			

Raw Materials:

1. Abattoir by-products from the Pig livestock (Animal Glue)
2. Bones from Abattoir (Bone Glue)
3. Wastes from the Tannery (Animal Glue)

Manufacture of GELATIN & ANIMAL GLUE

TECHNOLOGICAL STEPS

1. Washing of the Fleshings (Removing of the Preservatives, usually $Ca(OH)_2$) and acidification to pH = 6,2 – 6,5 using HCl or H_2SO_4
2. „Cooking“ of the GELALTIN & GLUE > transformation of the COLLAGEN to so called GLUTINE SOLUTION (NOT GLUTENE!) in several Stages, up to the Rest only about 2 – 5 % w/w of the original Charge
3. Filtration to remove the Impurities
4. Preservation and Bleaching by SO_2 or H_2O_2
5. Thickening in the Evaporator
6. Chilling and Forming
7. Drying to the Water Content 12 – 15 % w/w
8. Cutting to small Cubes od Milling to Powder

Manufacture of GELATIN & ANIMAL GLUE

TECHNOLOGICAL WASTES and their USE

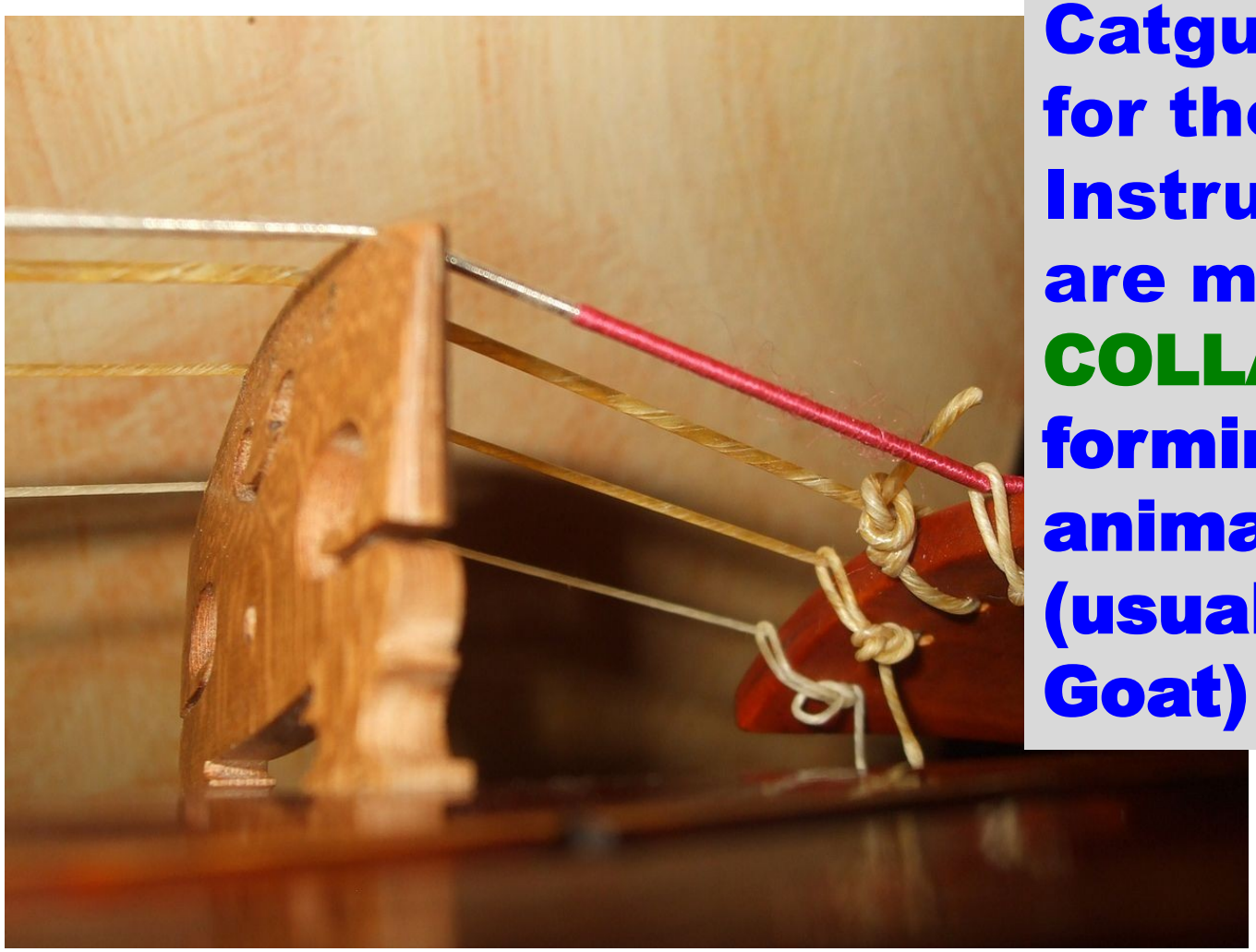
1. **Skin Fat** > Refining (Purification) > Sale or use for the SOAK Manufacture
2. **Impurities filtered out** > Biogas or Burning or approved waste Dump

Bone & Animal Glue and GELATIN In the Work of the CONSERVATOR – RESTORER I

- Wood Gluing,
- Book Binding,
- Paints binding Agent,
- Undercoat (Primer, Primer Coat) for Painting
- Binding Agent for the Nonwoven Textile

- Photographic Plates and Films (**FORMELY**)
- Casting Moulds
- Undercoat for the Contact Gilding (Covering by very thin Gold Foils)

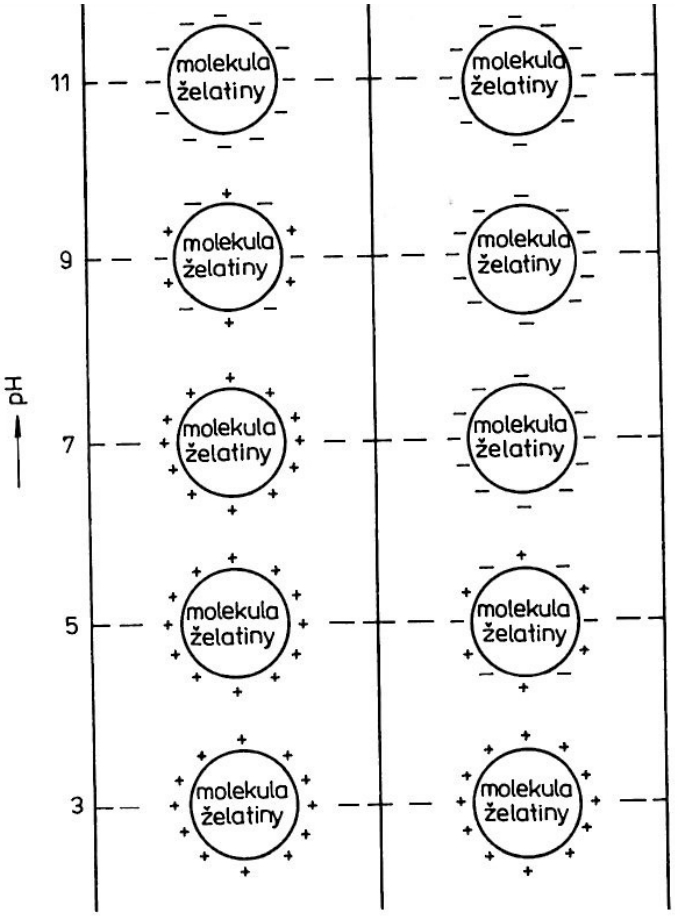
COLLAGEN and CONSERVATOR – RESTORER

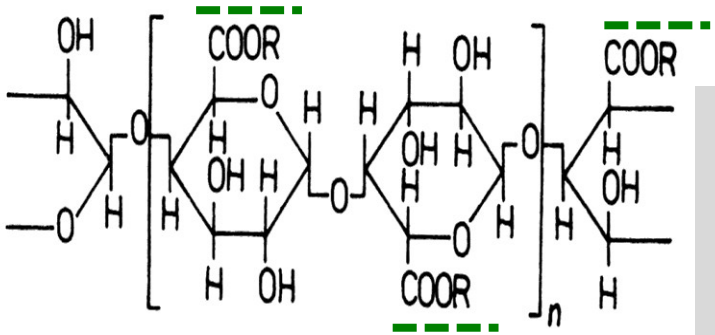


Catgut (STRINGS) for the Bowed Instruments are made of COLLAGEN forming the animal's gut (usually Sheep or Goat)

GELATIN - Clarifying of Wine and fruit Juice

Clarifying = the Measure to remove the Substances forming Wine lees (Haze)





The Structure Formula of PECTIN R = H or $-CH_3$

Isoelectric Point of GELATIN the Manufacturing Technology accordingly:

- **On the Left – acid catalysis**
- **On the Right – basic catalysis**

The main Haze forming Substance in Wine is PEKTIN

From the QUALITY POINT OF WIEV, the LOWEST MW HAS:

GLUE FOR THE Wall House Painting

For the Interior Painting is added go get for the Painting Cohesion Because having the lowest MW, it is the most Degradated COLLAGEN, it is soluble even at room Temperature in the cold Water

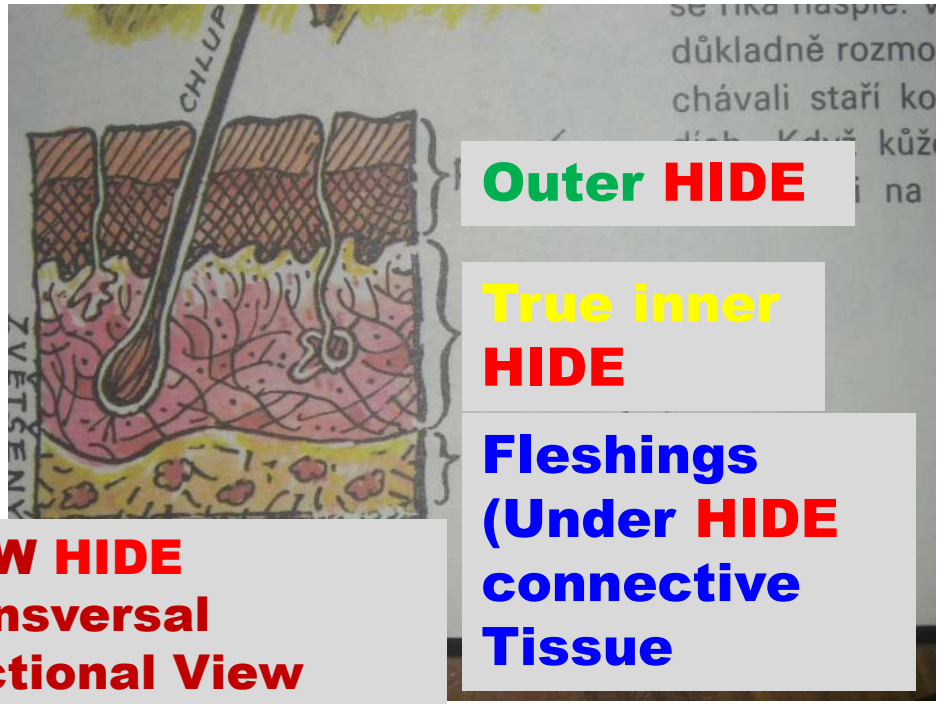
3. Tannery

3.1 HIDE versus Leather

3.2 Tanning of HIDE technology

3. 1 Skin versus Leather

HIDE versus Leather



Outer HIDE

True inner HIDE

Fleshings (Under HIDE connective Tissue

RAW HIDE transversal sectional View

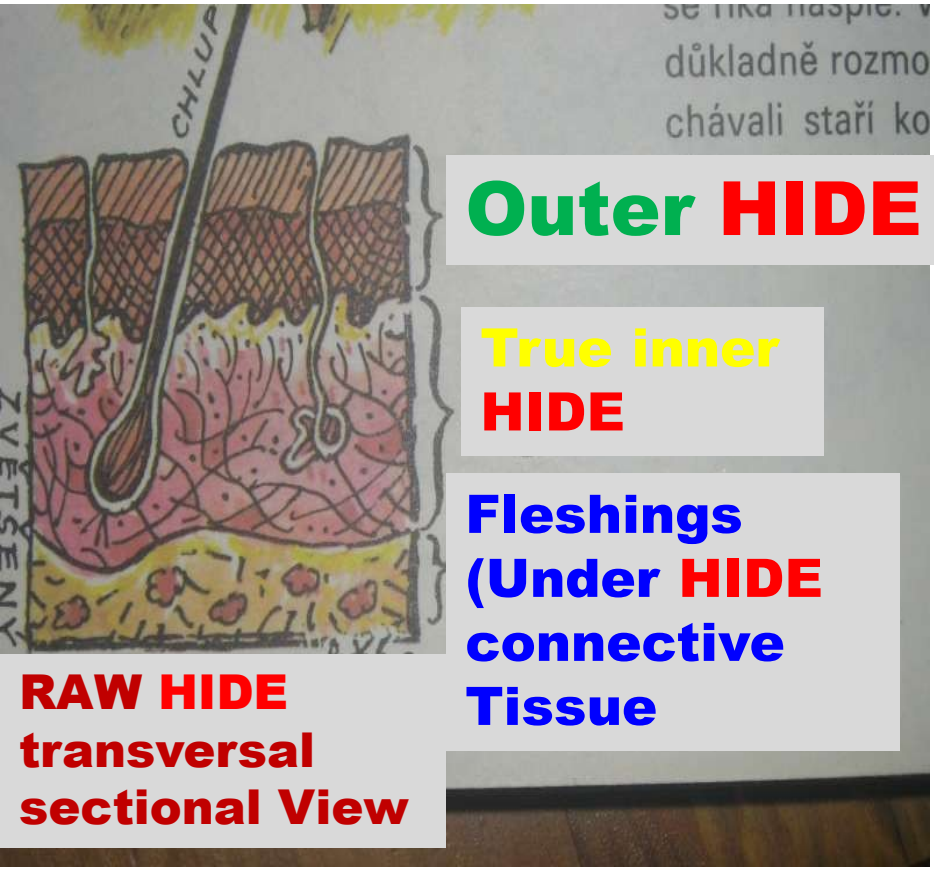
HIDE = it is that, what is taken after the Slaughter of the Animal

LEATHER = it is that, what is gained after Tanning of the HIDE

True Inner HIDE is the principal Part of the the HIDE for the Manufacture of the LEATHER

Outer HIDE & Fleshings (Under HIDE connective Tissue are removed during Processing of the HIDE to LEATHER

HIDE COMPOSITION - CROSSCUT



- Proteins
  - FIBROUS (Collagen)
  - Globularní (e.g. Albumine, they are removed before Tanning)
- Fats
- Water
- Inorganic Substances

Processing of the HIDE to LEATHER = HIDE TANNING

HIDE – Composition of the True Inner HIDE

- **COLLAGEN**
  - FIBRES FORM **THREEDIMENSIONAL STRUCTURE**
- **Outer HIDE = PAPILLARY LAYER**
- **True Inner HIDE = RETIKULARY LAYER**
- **Ratio of the PAPILARY LAYER versus RETIKULAR LAYER Thicknesses determines the HIDE QUALITY and is different for various Animals**

CONTRACTION TEMPERATURE
HIDE VERSUS LEATHER

COLLAGEN CONTRACTION TEMPERATURE for various Tanning Procedures	
Tanning Procedures	**CONTRACTION TEMPERATURE (°C)**
COLLAGEN without Tanning	**58 – 68**
Fur Tawing	**49 – 63**
Formaldehyde Tanning	**63 – 73**
Tannine Tanning	**70 – 87**
Basic Tanning by Aluminium Salts	**74 – 81**
Basic Tanning by Chromium Salts	**77 - 100**

CONTRACTION TEMPERATURE is:
Release of the Hydrogen Bonds between COLLAGEN FIBERS caused by the increased Temperature and the CONTRACTION (SHINKAGE) so released Fibres

This Process is IRREVERSIBLE

HIDE VERSUS LEATHER

- **LEATHER** is more resistant against to Microorganisms in the humid Conditions
- **LEATHER** has the higher chemical Resistance and lower Swelling in the Water
- **LEATHER** has better and advantageous mechanical Properties and is soft and pliable if it is Dry
- **LEATHER** has higher **CONTRACTION TEMPERATURE**

3.2 Technology Steps at HIDE Tanning

Technology Steps at HIDE Tanning

1. **Preservation**
2. **Soaking (Liming)**
3. **Leaching (Unhairing and scudding)** in the Solution Sodium Sulfite and Lime ($Ca(OH)_2$) to remove Fur and the so called depilated raw Hide.
4. **Piling of Hides**
5. **Scraping** – connective Tissue and Muscles Rest are cut off
6. **Decalcification of Clearings(Deliming)** –see 3, Removing of the Ca Salts
7. **Bating** & **Pickling**– Preparation for **Tanning**
8. **Tanning**
9. **Greasing**

COLLAGEN – Cross Bonds IN VITRO 1

COLLAGEN modification Reactions are used in transformation of the HIDE to LEATHER and GELATIN Modification.

Irreversible Process occurs during forming of the Cross Bonds between COLLAGEN Molecules. So called TANNING AGENTS are necessary for this CROSSLINKING of the COLLAGENs Molecules. TANNING AGENTS are the di or tri or multifunctional Substances.

The HIDE is changed (transformed) to LEATHER by Reaction of COLLAGENs Molecules with these TANNING AGENTS.

The chemical and physical Changes occurred during TANNING.

COLLAGEN – Cross Bonds IN VITRO 2 are the CHEMICAL BASIS of the Tanning of the HIDE to LEATHER

a) **INORGANIC COMPOUNDS** – coordination Compound (Complex) based on the Cr, Al, Zr, Fe etc. And heteropolyacids of the Si, P, W, Mo

b) **ORGANIC COMPOUNDS** – Tannins of the Plant Origin, Aldehydes, Chinons, Syntanes, Fats, Sulfochlorites, some synthetic Polymers

Tanning by heavy Metals, Aldehydes, Chinons is possible to compare with the **Cross Bonds IN VIVO.** The most important are Salts of Cr and Zr, Tannins of the Plant Origin, Aldehydes, Syntanes.

Tanning of the HIDE to LEATHER

HIDE Tanning is the CHEMICAL PROCESS, when functional Groups of the C**OLLAGEN** react with **Tanning Substance (Reagent)**

Tanning Substances (Reagents)

1. **TANNINS (HYDROLYSABLE OR CONDENSED) > Vegetable Tanning**
2. **POTASSIUM ALUMINIUM SULPHATE**
3. **CHROMIUM COMPOUNDS**

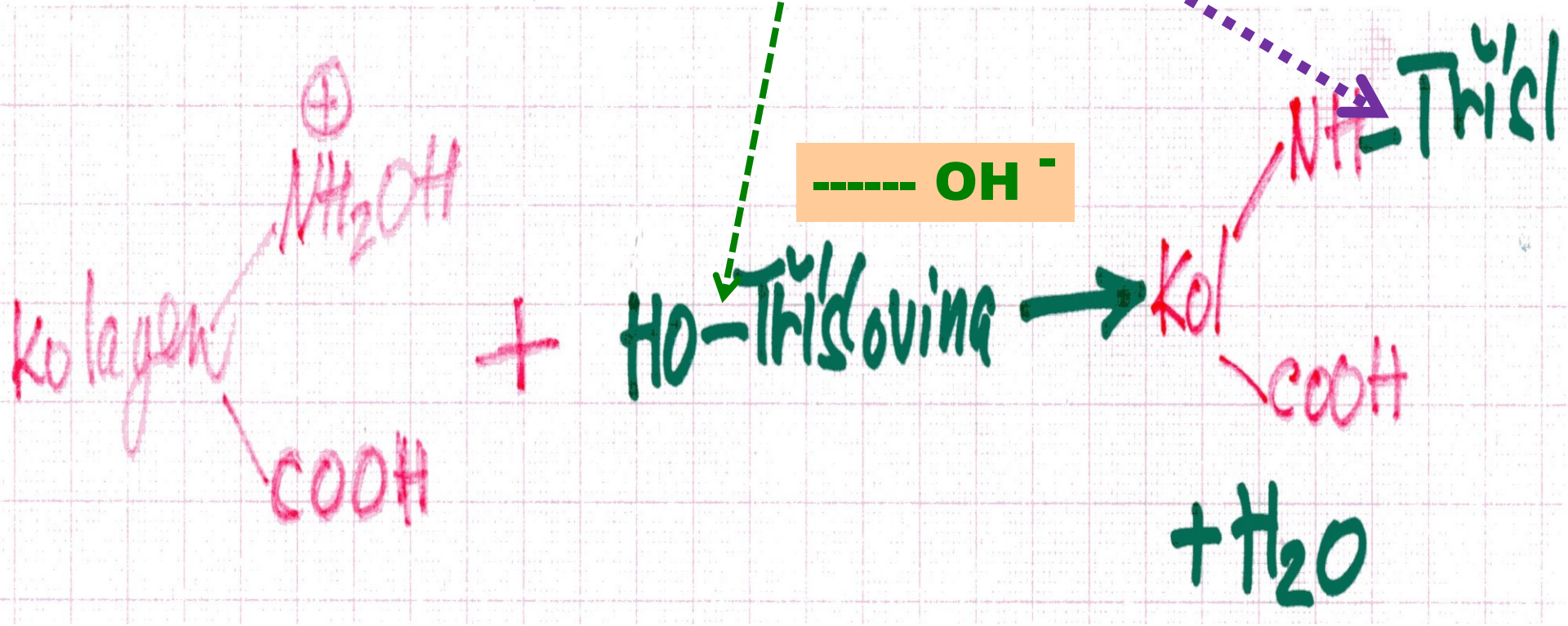
Tanning of the HIDE to LEATHER Similarity with Vulcanisation of the RUBBER to VULCANISED RUBBER

Tanning of the HIDE is the CHEMICAL REACTION, when react **COLLAGENs** functional Group with the **Tanning Substances** and forms **NETWORK BETWEEN MACROMOLECULES OF COLLAGEN**

VULCANISATION of Rubber to VULCANISED RUBBER is the CHEMICAL REACTION called VULCANISATION which forms NETWORK BETWEEN MACROMOLECULES OF POLYIZOPRENE

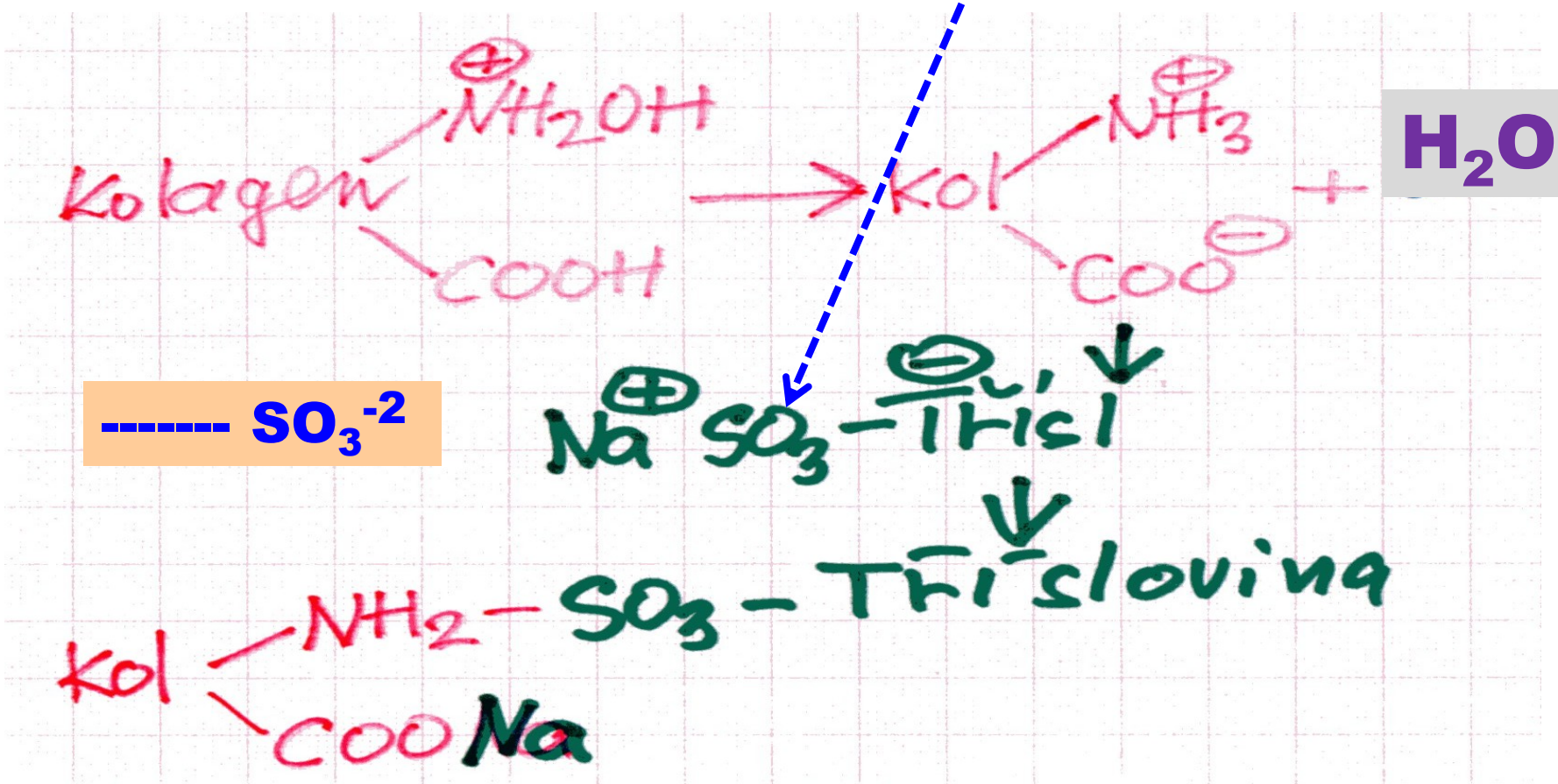
Vegetable Tanning of the HIDE to LEATHER 1 A

Basis is the Interaction of –OH or SO_3^{-2} Groups of Tannin with side Chain Groups on the COLLAGEN Chain forming the COVALENTN or HYDROGEN BOND

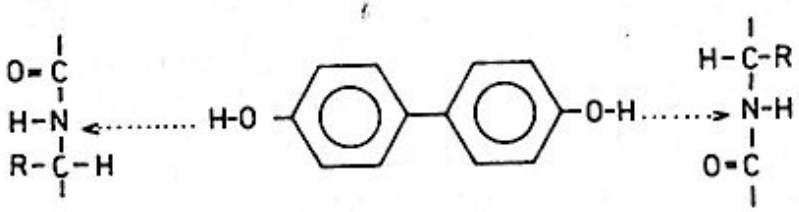


Vegetable Tanning of the HIDE to LEATHER 1 B

Basis is the Interaction of –OH or SO_3^{-2} Groups of Tannin with side Chain Groups on the COLLAGEN Chain forming the COVALENTN or HYDROGEN BOND



Vegetable Tanning of the HIDE to LEATHER 2



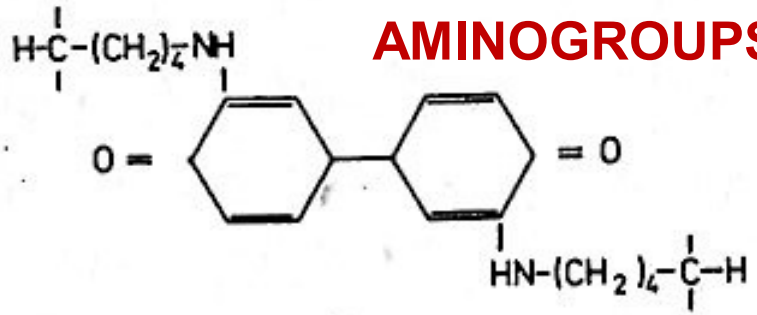
HYDROGEN BOND
WITH IMIDOGROUPS

COLLAGEN

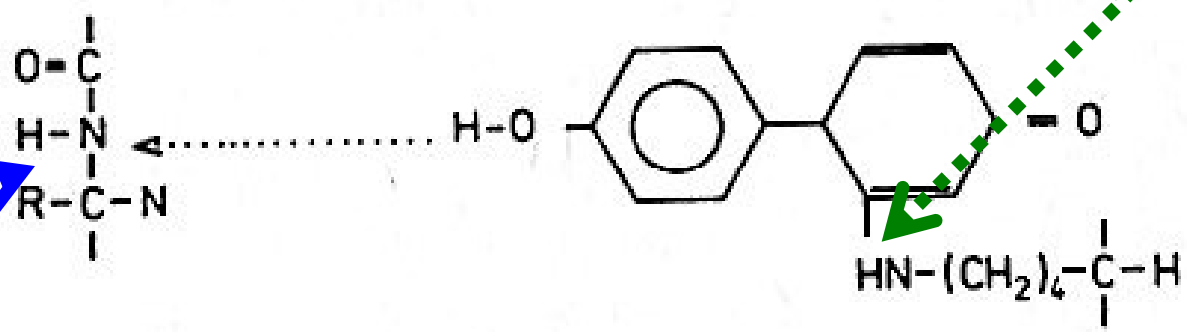
TANNIN

COLLAGEN

COVALENT BOND of
CHINONE to
AMINOGROUPS

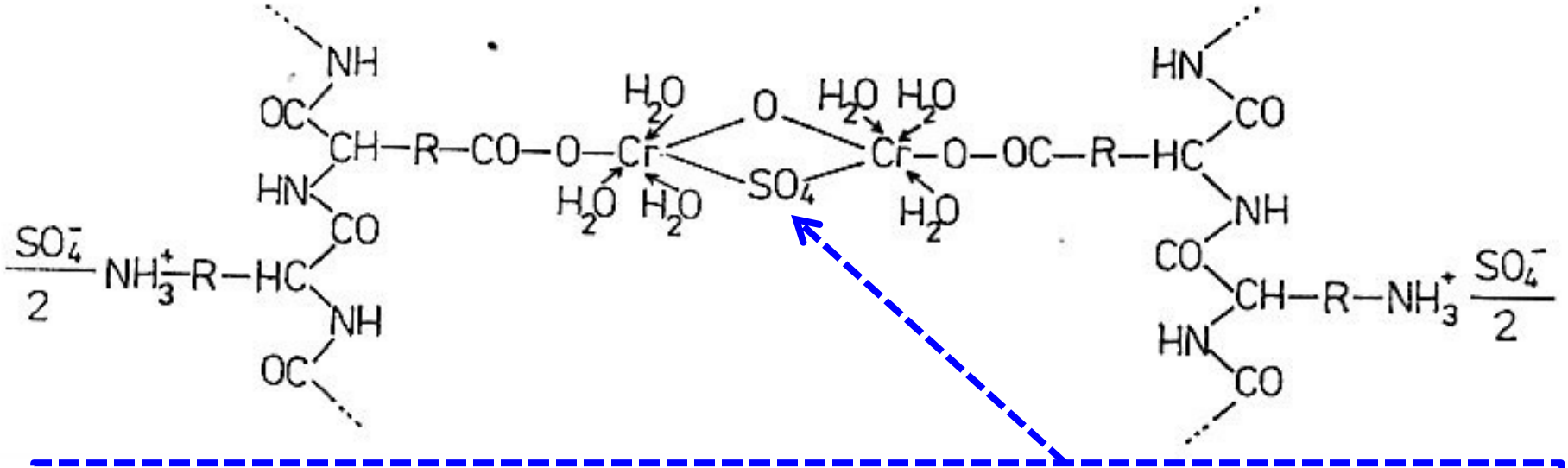


COVALENT BOND of
CHINONE to
AMINOGROUPS +
HYDROGEN BOND
WITH
IMIDOGROUPS

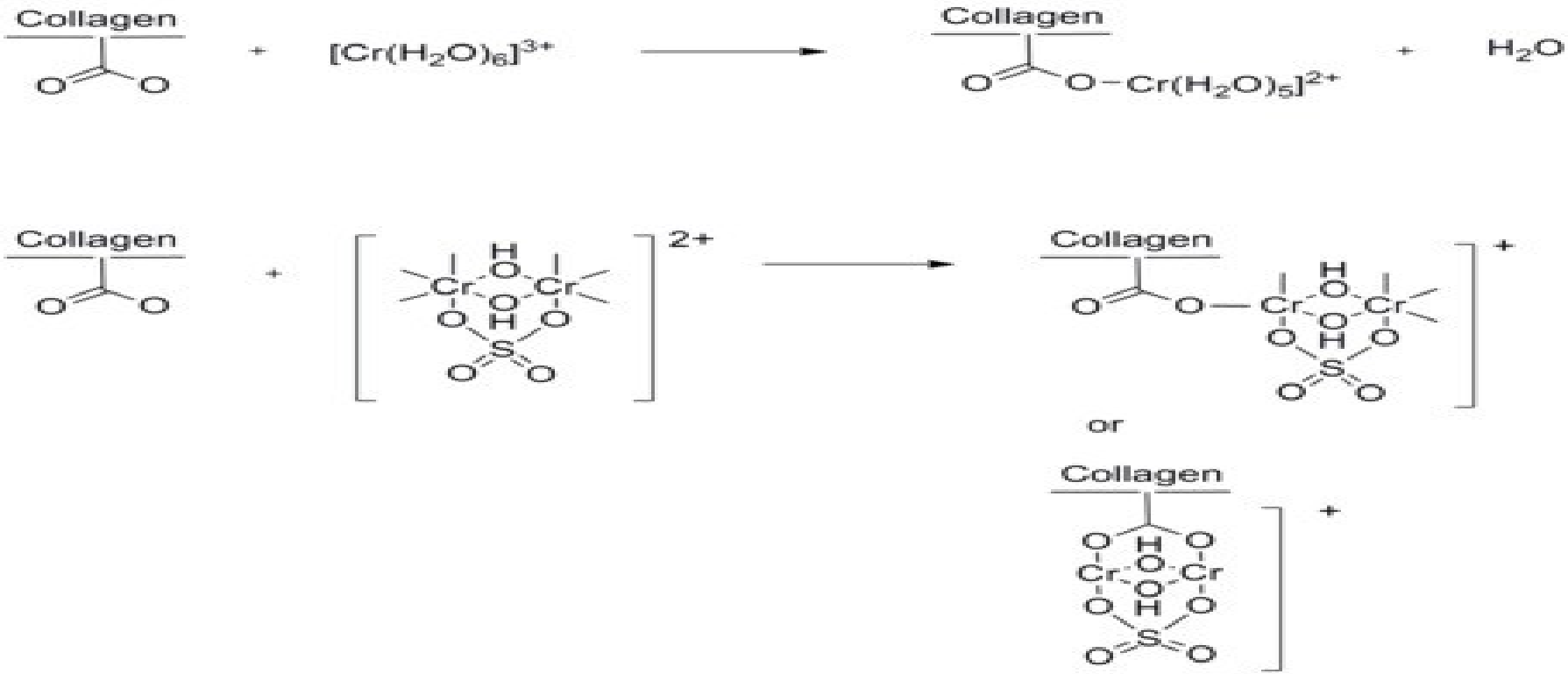


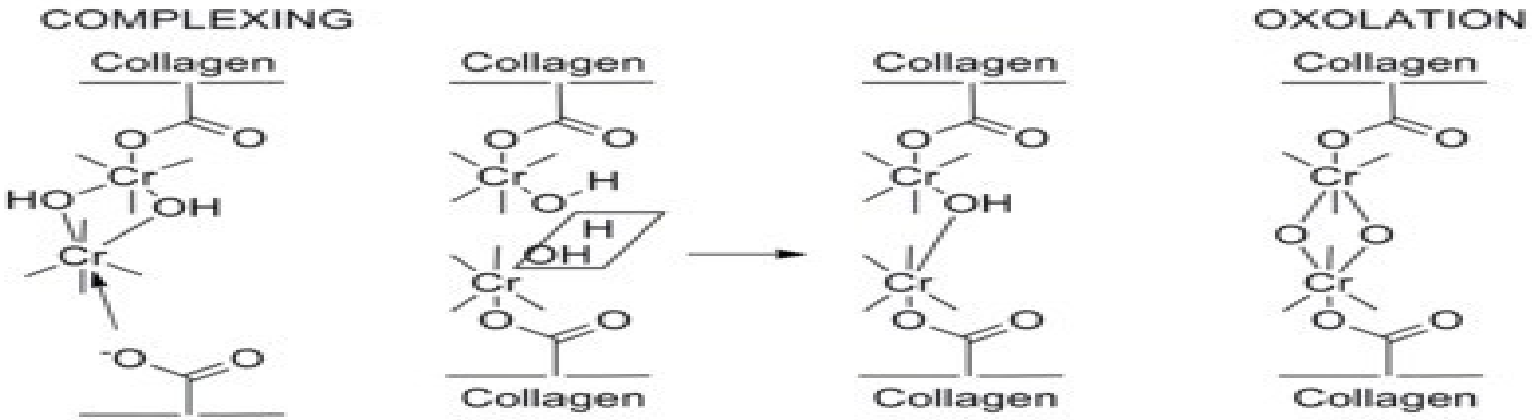
CHROMIUM TANNING HIDE to LEATHER 1

Basis is Interaction of COLLAGEN Group –COOH via Forming CHROMIUM COMPLEX COMPOUND



Chromium(III) sulfate ($[Cr(H_2O)_6]_2(SO_4)_3$) **has long been regarded as the most efficient and effective tanning agent.**


Collagen
$[Cr(H_2O)_6]^{3+}$
Collagen
$O-Cr(H_2O)_5]^{2+}$
H_2O
Collagen
2+
Collagen
or
Collagen


COMPLEXING
Collagen
Collagen
Collagen
Collagen
Collagen
OXOLATION
Collagen
Collagen