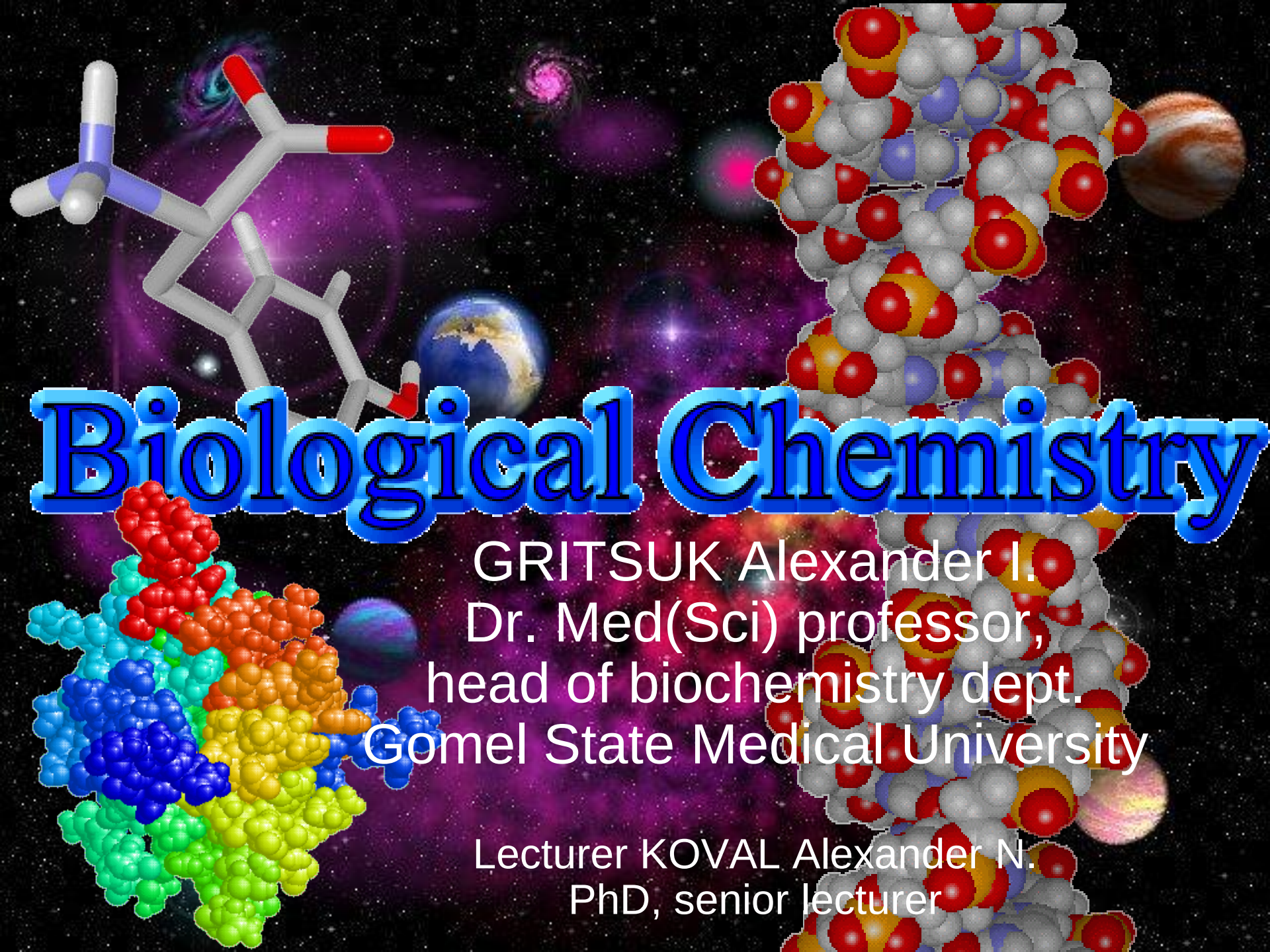


GRITSUK Alexander I.
Dr. Med(Sci) professor,
head of biochemistry dept.
Gomel State Medical University

Lecturer KOVAL Alexander N.
PhD, senior lecturer

Content

- Introduction to biochemistry.
- Historical background.
- Importance of biochemistry for the doctor.
- Protein chemistry.

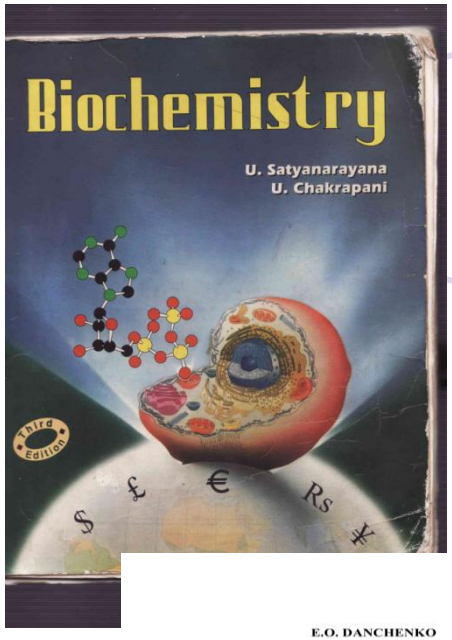
Biochemistry Board foundation

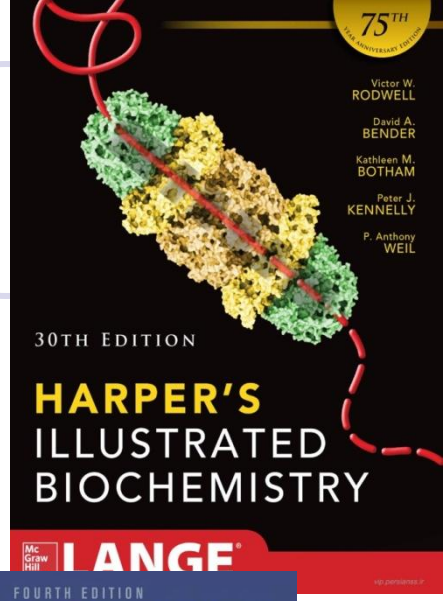
- **Gritsuk Alexander I.**
  Dr. Med (Sci) professor, head of biochemistry dept. (1996-2018)

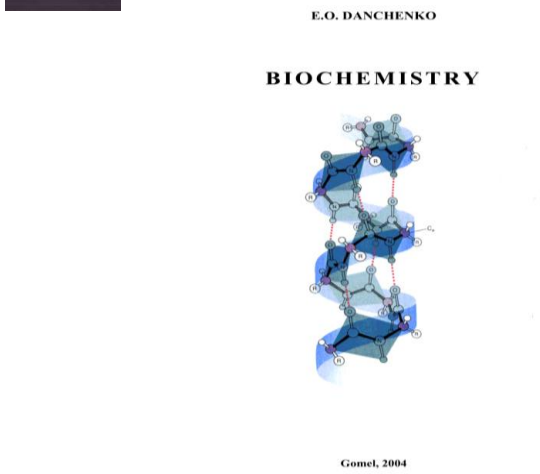
Biochemistry Dept. staff (teachers), 2018-19 acad year

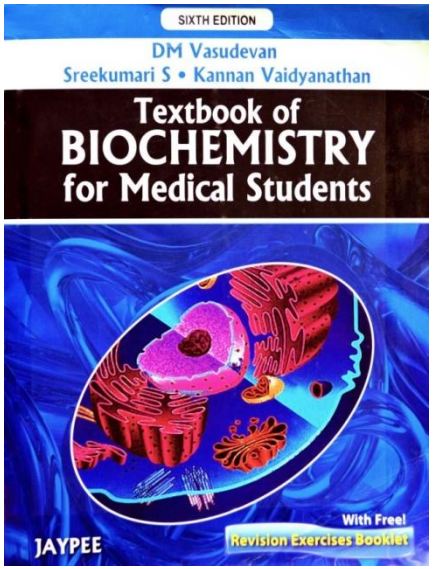
- **Head of tne dept. - Nikitina Irina A.** – Ph.D., lecturer
  - Nikitina Irina A. – Ph.D., head of the department.
  - Yegorenkov Nikolay I. – Dr. Sci, teacher
  - Koval Alexander N. – Ph.D., senior lecturer (english)
    - Gromyko Marina V. – teacher
    - Skrypnikova Lubov' P. – assistant (english)
    - Mazanik Maria Ye. – teacher. (english)
    - Myshkovets Nadezhda S. – assistant.

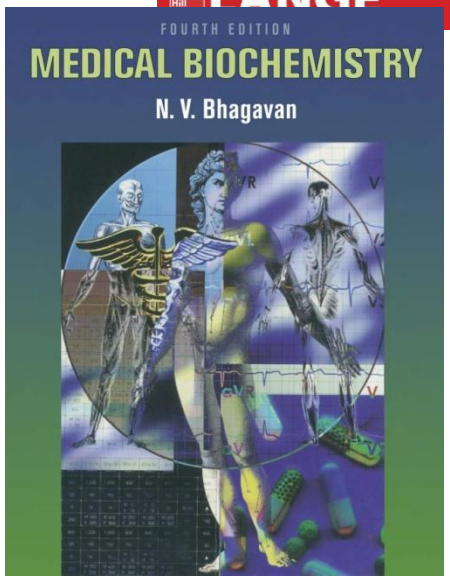
Literature







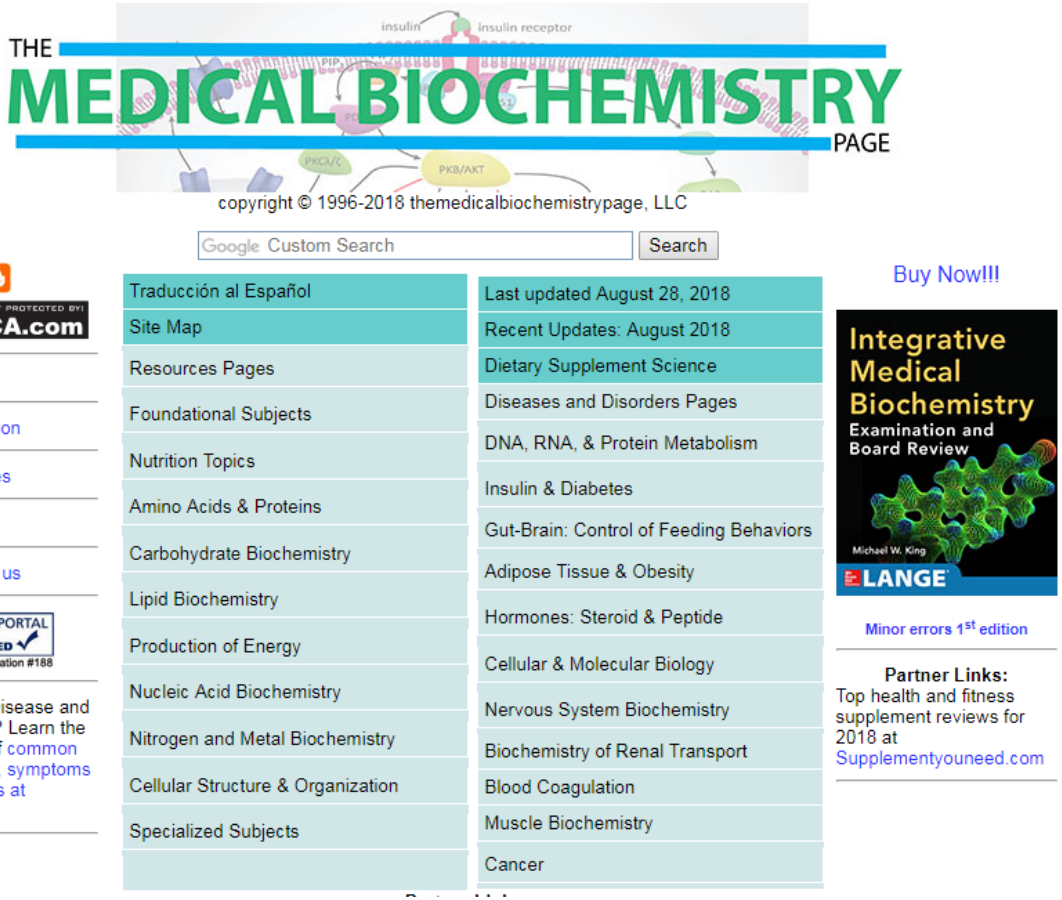




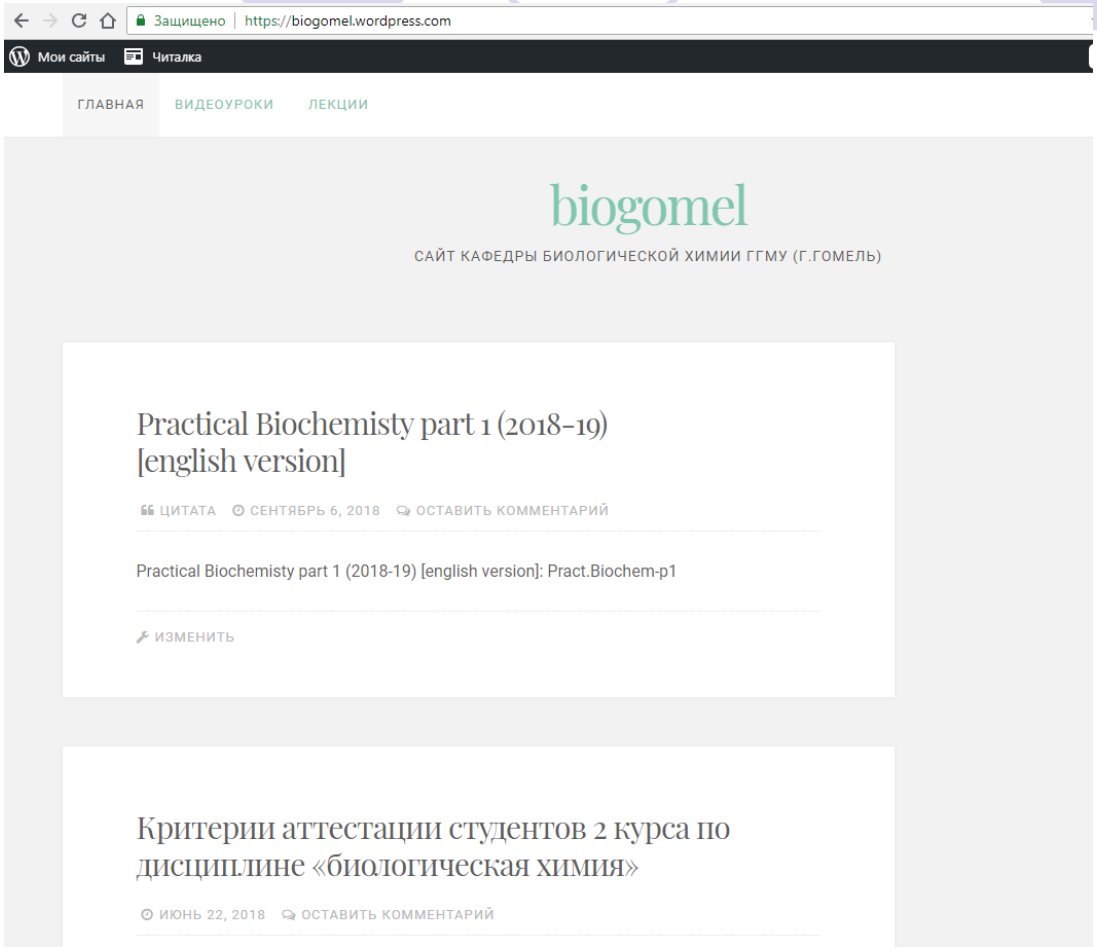
- Recommended textbooks for studying biochemisty.
- Ask in the library.

Online sources

https://themedicalbiochemistrypage.org/index.php

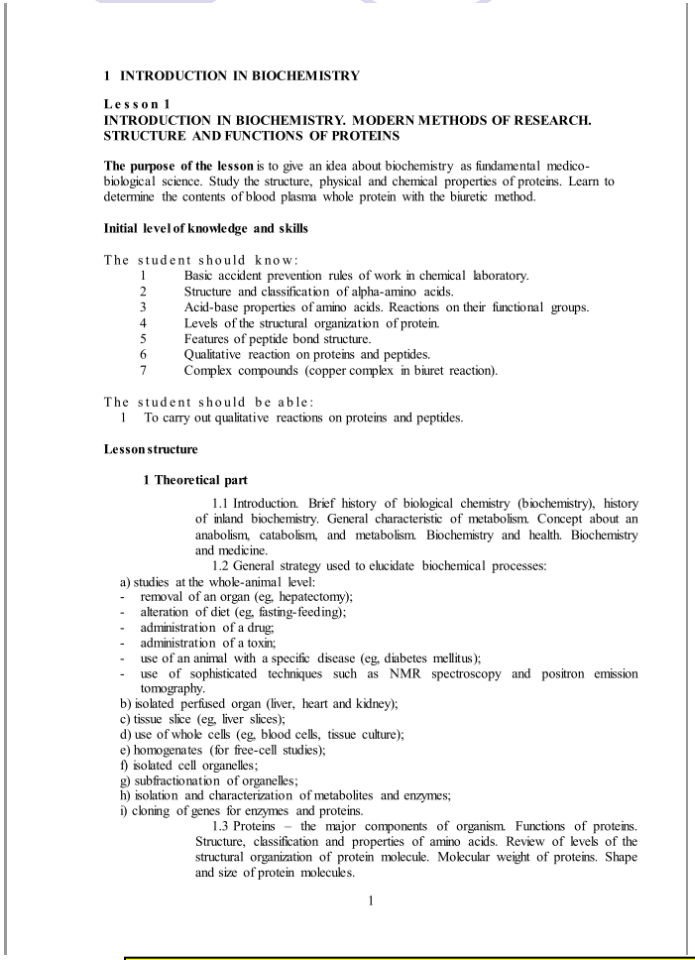


biogomel.wordpress.com



- This is our department's site to help students in studying biochemistry.
- Copy-books, exam questions, lecture notes etc…

The main book for practical classes



1 INTRODUCTION IN BIOCHEMISTRY

Lesson 1
INTRODUCTION IN BIOCHEMISTRY. MODERN METHODS OF RESEARCH. STRUCTURE AND FUNCTIONS OF PROTEINS

The purpose of the lesson is to give an idea about biochemistry as fundamental medico-biological science. Study the structure, physical and chemical properties of proteins. Learn to determine the contents of blood plasma whole protein with the biuretic method.

Initial level of knowledge and skills

The student should know:
1 Basic accident prevention rules of work in chemical laboratory.
2 Structure and classification of alpha-amino acids.
3 Acid-base properties of amino acids. Reactions on their functional groups.
4 Levels of the structural organization of protein.
5 Features of peptide bond structure.
6 Qualitative reaction on proteins and peptides.
7 Complex compounds (copper complex in biuret reaction).

The student should be able:
1 To carry out qualitative reactions on proteins and peptides.

Lesson structure

1 Theoretical part

1.1 Introduction. Brief history of biological chemistry (biochemistry), history of inland biochemistry. General characteristic of metabolism. Concept about an anabolism, catabolism, and metabolism. Biochemistry and health. Biochemistry and medicine.

1.2 General strategy used to elucidate biochemical processes:

a) studies at the whole-animal level:
- removal of an organ (eg, hepatectomy);
- alteration of diet (eg, fasting-feeding);
- administration of a drug;
- administration of a toxin;
- use of an animal with a specific disease (eg, diabetes mellitus);
- use of sophisticated techniques such as NMR spectroscopy and positron emission tomography.

b) isolated perfused organ (liver, heart and kidney);
c) tissue slice (eg, liver slices);
d) use of whole cells (eg, blood cells, tissue culture);
e) homogenates (for free-cell studies);
f) isolated cell organelles;
g) subfractionation of organelles;
h) isolation and characterization of metabolites and enzymes;
i) cloning of genes for enzymes and proteins.

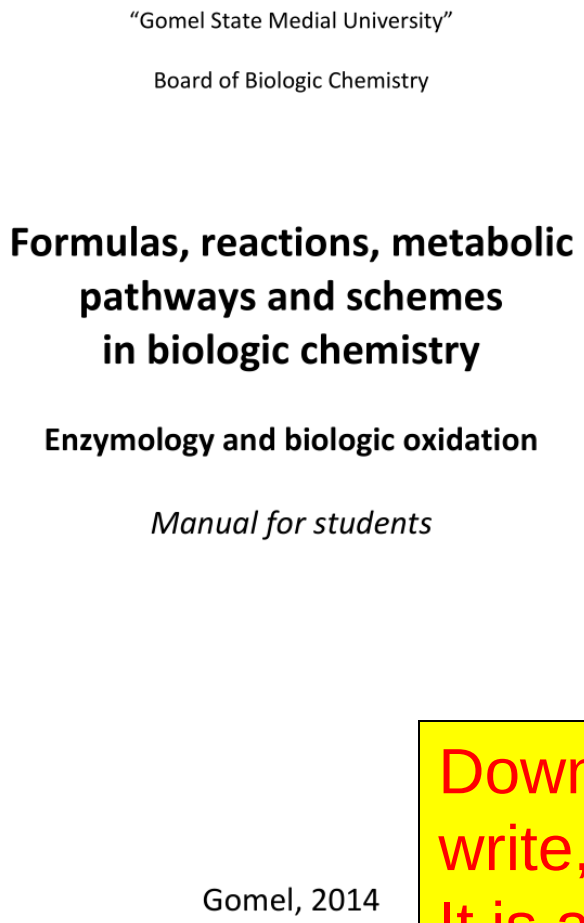
1.3 Proteins – the major components of organism. Functions of proteins. Structure, classification and properties of amino acids. Review of levels of the structural organization of protein molecule. Molecular weight of proteins. Shape and size of protein molecules.

1

- In the practical biochemisty classes you will have
  - theoretical questions,
  - practical part *(unfortunately this year is not accessible…)*
  - also tests.
- Be ready every class to answer!
- The teacher will ask you about the lab. work principles and course of the work.

Download it, print out and bring to every class!

Where from to study the cycles and structures?



- The manual provides the minimal and necessary structures, formulas, cycles and schemes in biochemistry.
- It is like "saving ring" for the students studying biochemistry.

Download it, print out and study, write, revise, practicize regularly!
It is almost impossible to study the cycle in 1 day (proved by some unhappy students)

The road to wisdom?

The road to wisdom? – well, it's plain and simple to express:

Err

and err

and err again

but less

and less

and less.

Piet Hein

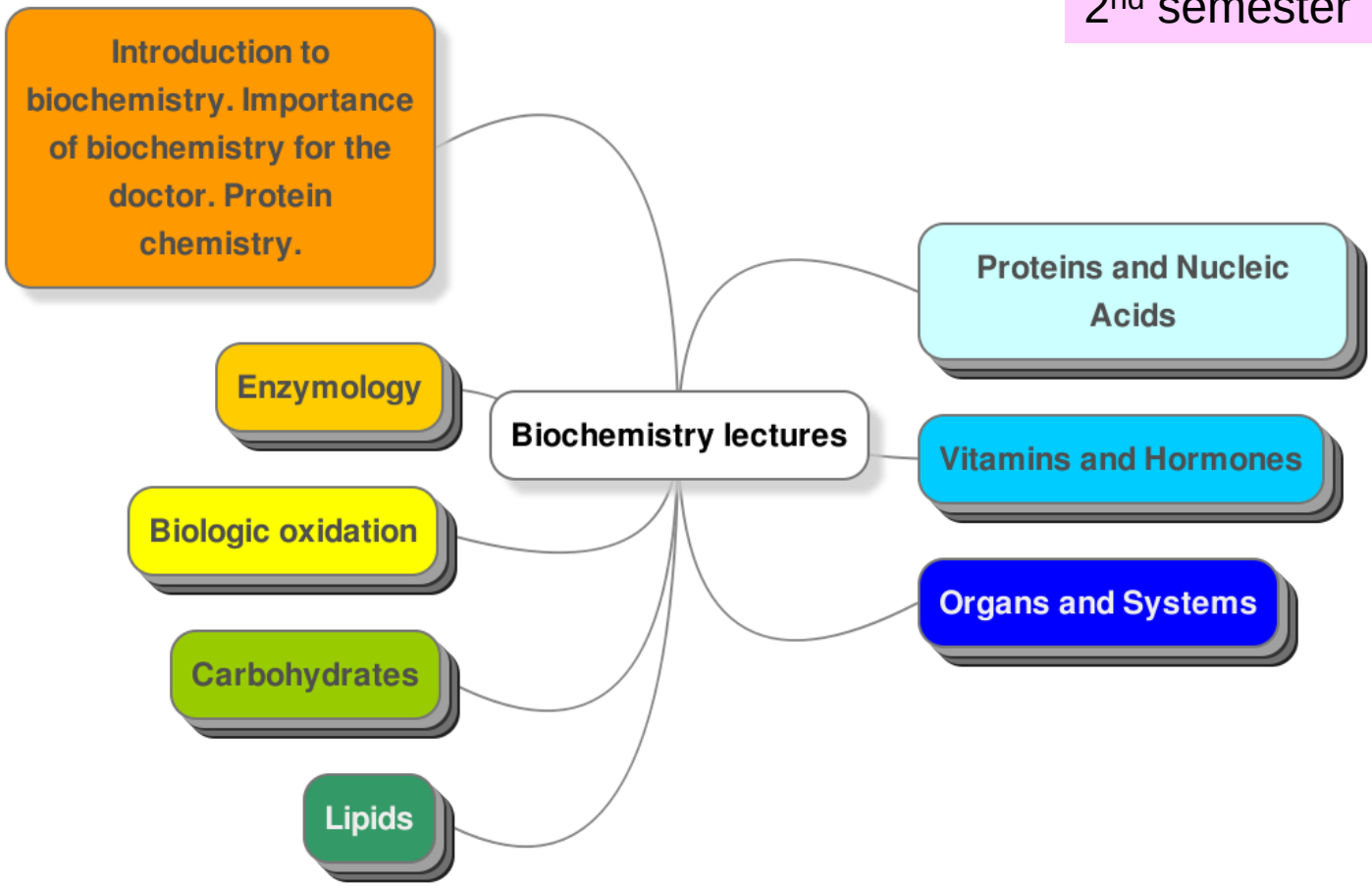
Lecture book

- V.Rajaram sponsored the book “Biochemistry Lectures”

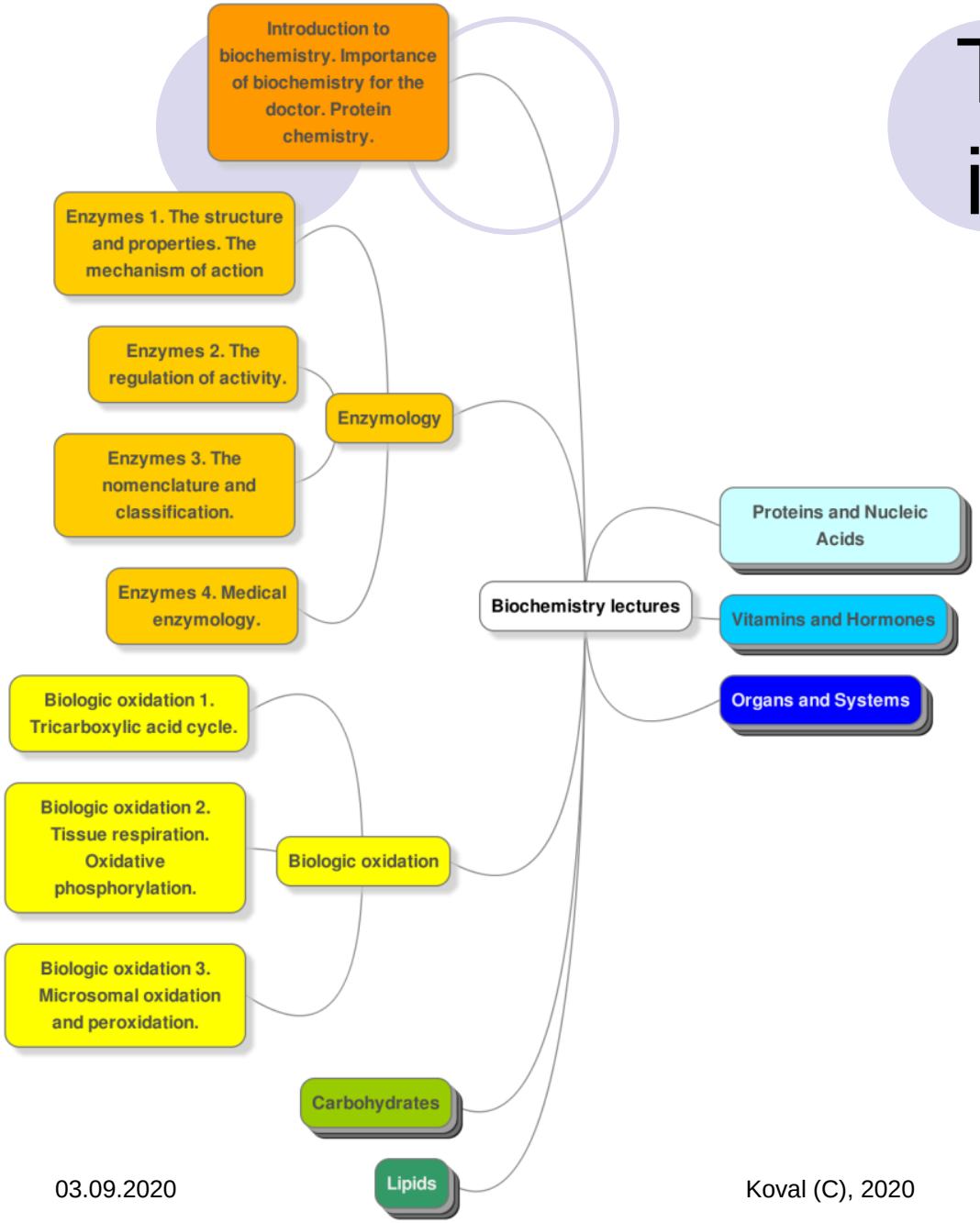
Structure of the Lecture Course

1st semester

2nd semester



The structure of initial 8 lectures



Lecture 1

Introduction
Brief historical background of biochemistry

Introduction to Biochemistry

- **Biochemistry** is the science studying quality and quantitative structure, also pathways, laws, biological and physiological role of transformation of the **substances, energy and information** in living organisms.
  - Biochemistry can be defined as *the science concerned with the chemical basis of life* (Gk *bios* “life”). Biochemistry encompasses large areas of **cell biology,** of **molecular biology,** and of **molecular genetics.**

Historical Background

c. 3500 B.C	People learned to make ***bronze***.
c. 400 B.C	Democritus proposed an ***atomic theory***.
A.D. 600's	***Alchemy*** began to spread from Egypt to the Arabian Peninsula and reached western Europe in the 1100's.
Early 1700's	Georg Ernst Stahl developed the ***phlogiston*** theory.
1766	Henry Cavendish identified ***hydrogen*** as an element.
1770's	Carl Scheele and Joseph Priestley discovered ***oxygen***.
Late 1700's	Antoine Lavoisier stated the ***law of the conservation of mass*** and proposed the ***oxygen theory of combustion***.
1803	John Dalton proposed his ***atomic theory***.
Early 1800's	Jons J. Berzelius calculated accurate ***atomic weights*** for a number of elements.

Historical Background (cont'd)

1828	Friedrich Wöhler made the first synthetic organic substance (urea) from inorganic compounds.
1869	Dmitri Mendeleev and Julius Lothar Meyer – Periodic Law.
1913	Niels Bohr proposed his model of the atom.
1916	Gilbert N. Lewis described electron bonding between atoms.
1950's	Biochemists began to discover how such chemicals as deoxyribonucleic acid (DNA) and ribonucleic acid (RNA) affect heredity.
Early 1980's	Chemists began working to develop a solar-powered device that produces hydrogen fuel by means of the chemical breakdown of water.

Historical Background. Beginnings

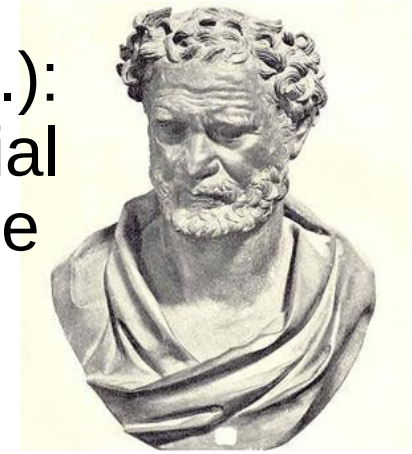
- In prehistoric times, people gradually developed a great deal of practical biological knowledge.
  - In ancient times, people of China, India, and the Middle East accumulated knowledge of plants and animals.
    - they knew how to use numerous plants as medicines or poisons.

Historical Background. Ancient greeks

http://de.wikipedia.org/wiki/Image:Empedokles.jpeg

- Several ancient Greek philosophers developed theories about the basic substances that make up the world.
  - **Empedocles** (400's B.C.) argued that there were 4 primary elements - *air, earth, fire, and water* - combined in various proportions to form all other substances.

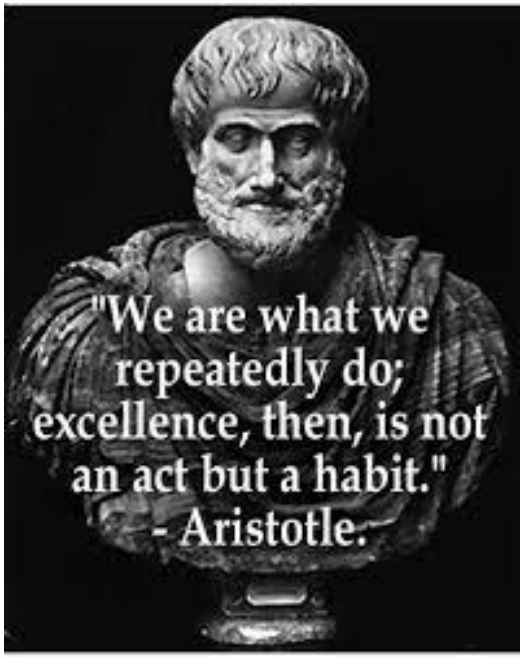
- A greek philosopher **Democritus** (400 B.C.): all matter was composed of a single material that existed in the form of tiny, indestructible units called *atoms*.
  - According to his theory, differences among substances were caused only by differences in the size, shape, and position of their atoms.



http://www.nndb.com/people/790/000087529/democritus-1-sized.jpg

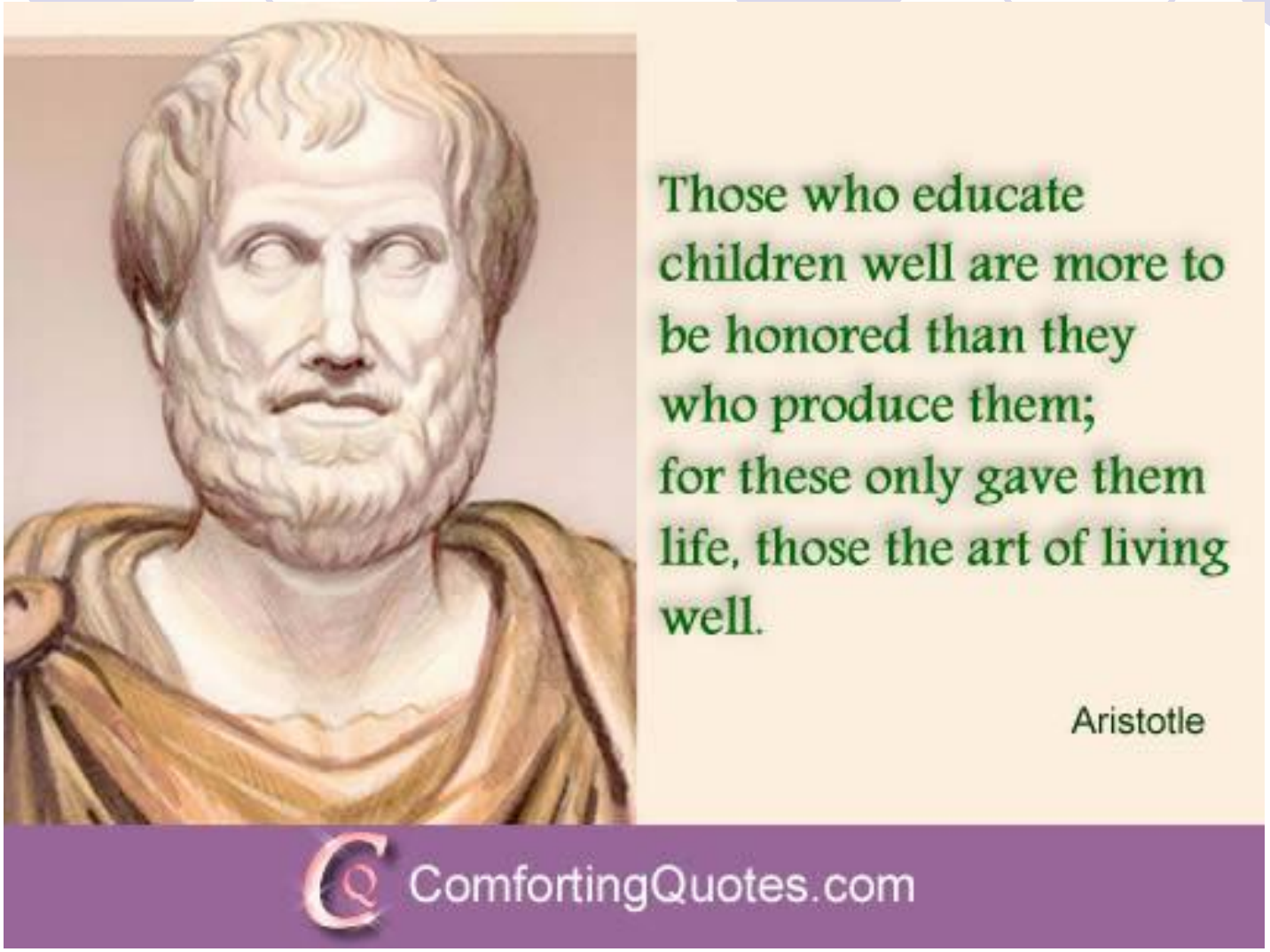
Historical Background. Aristotle

- The Greek philosopher **Aristotle** (300's B.C.): each of the 4 primary elements could be changed into any of the other elements by adding or removing heat and moisture.



  - He stated that such a change - called *transmutation* - occurred whenever a substance was involved in a chemical reaction or changed from one physical state - solid, liquid, or gas - to another.
  - Aristotle believed that water, for example, changed to air when it was heated.

Aristotle's wisdom



Historical Background. Alchemy

- During the first 300 years AD, scholars and craftworkers in Egypt developed a chemical practice that came to be called alchemy.
  - They based their work on Aristotle's theory of the transmutation of elements and tried to change lead and other metals into gold.
- Alchemy began to spread to the Arabian Peninsula in the A.D. 600's and to much of western Europe in the 1100's.
- Until the 1600's, alchemy was a major source of chemical knowledge.

Historical Background. Alchemist's Achievements

- Alchemists failed to produce gold from other materials.
- They did gain wide knowledge of chemical substances, however, and invented many chemical tools and techniques.
  - Alchemists used such equipment as funnels, strainers, balance scales, and crucibles (pots for melting metals).
  - They discovered new ways of producing chemical changes,
  - Also earned to make and use various acids and alcohols.

Historical Background. Iatrochemistry

- Alchemists also searched for a substance that could cure disease and lengthen life.
- During the 1500's, some alchemists and physicians began to apply their knowledge of chemistry to the treatment of disease.

- The medical chemistry of the 1500's and 1600's is called ***iatrochemistry***.
  - The prefix comes from *iatros*, the Greek word for *physician*.
- **Paracels** was the first iatrochemist.
- Iatrochemists made the first studies of the chemical effects of medicines on the human body.

Historical Background. Theories based on experiments

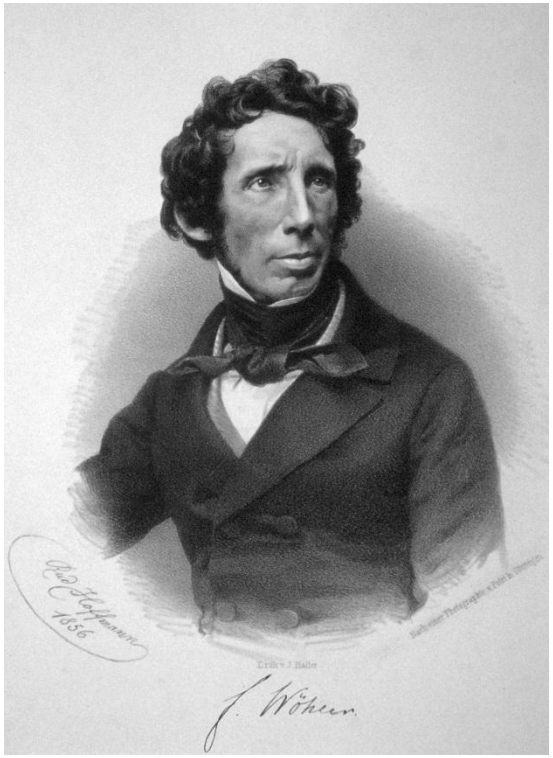
- Robert **Boyle**, an Irish scientist of the 1600's, was one of the first modern chemists.
- He taught that ***theories*** must be supported by careful ***experiments***.
  - Boyle conducted many experiments that showed that air, earth, fire, and water are not true elements.
  - He believed that the best explanation of the properties of matter was provided by an ***atomistic theory*** that described substances as composed of tiny particles in motion.

Historical Background. Vitalism

- Most chemists of the early 1800's believed that organic compounds could be produced only with the aid of a *vital force*, a life force present in plants and animals. That belief is called **vitalism**.

In 1828, a German chemist named Friedrich **Wöhler** mixed two inorganic substances, heated them, and obtained **urea** - an organic compound found in urine.

Wohler thus made the first synthetic organic substance from inorganic materials and proved that a vital force is not necessary for the production of an organic compound!



Historical Background. Empirical period

- The middle of XVII – end of XVIII cc is the **empirical** period of development of an organic chemistry.
  - great Swedish chemist J. Berzelius said it was chemistry of «plant and animal substances».
  - Accumulation of huge actual material
  - No theoretical background.

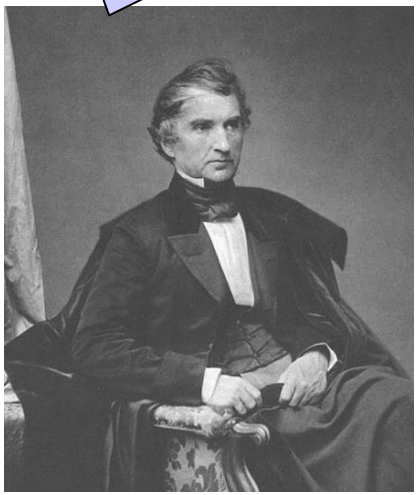
Jöns Jacob Berzelius (1779-1848)

Historical Background. Analytical period

- The next period was named **analytical** (the end XVIII - the middle of XIX centuries).
- Researches on investigation of structure of substances.
  - It was stated that all organic compounds contain carbon.
- Some achievements of this period:
  - In 1839 J. von Liebig has investigated, that meal contains proteins, fats and carbohydrates.
  - In 1845. G. Kolbe synthesized acetic acid
  - In 1854 M. Berthelot synthesized fats.
  - In 1861 A. M. Butlerov synthesized carbohydrates.

The portraits of the chemist of the analytical period

Proteins, fats and carbohydrates

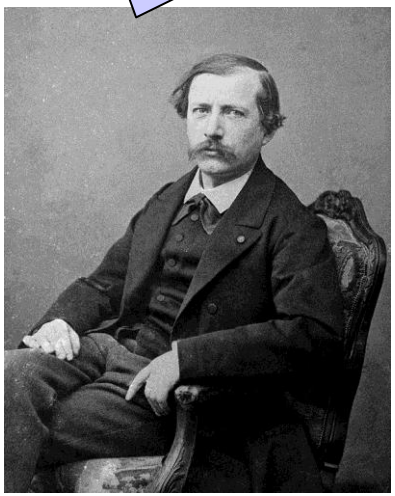


Justus von Liebig (1803-1873)

Acetic acid

Adolph Wilhelm Hermann Kolbe (1818-1884)

Fats



Marcellin Berthelot (1827 - 1907)

Carbohydrates

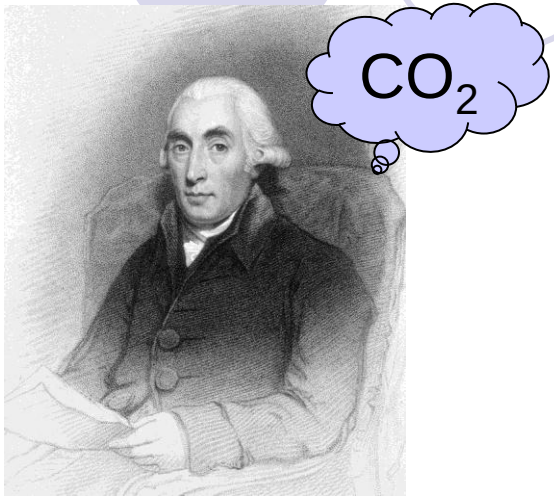
Alexander M. Butlerov (1828-1886)

Historical Background. Phlogiston Theory

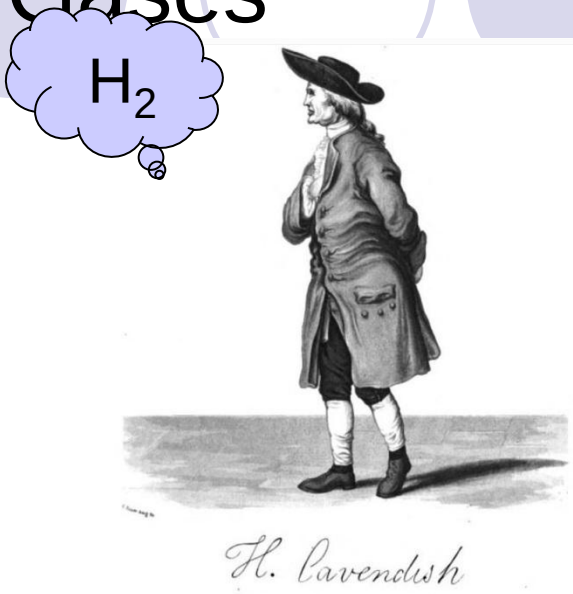
- It was developed in the early 1700's by a German chemist and physician named Georg Ernst **Stahl**.

- All flammable materials contained a substance called ***phlogiston***.
  - Materials gave off phlogiston as they burned. Air absorbed the phlogiston. Plants, in turn, removed phlogiston from the air, accumulate it and burned when dry.
  - The phlogiston theory explained the results of a variety of experiments, was widely accepted and led to many findings in chemistry.

Historical Background. Isolation and Studying of Gases



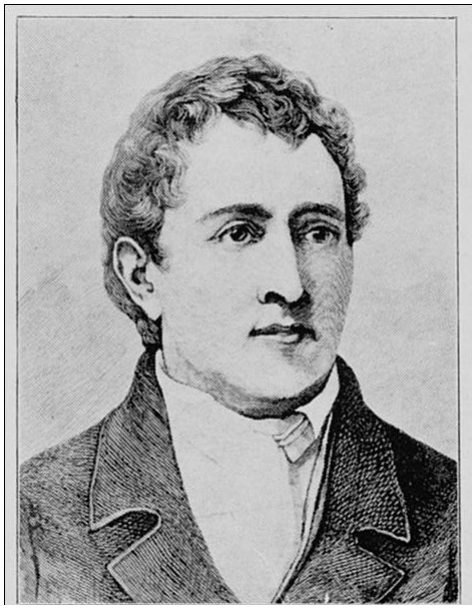
Joseph Black
(1728 – 1799)



Henry Cavendish
(1730-1810)

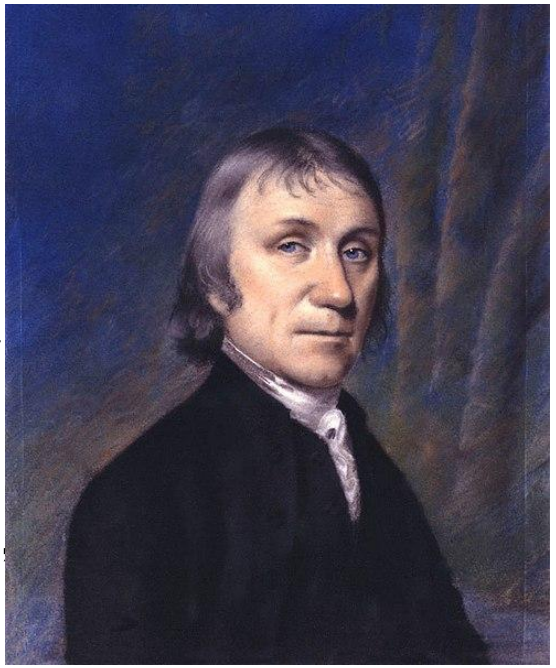
- Chemists of the middle and late 1700's developed ways to isolate and study gases.
  - 1750's Joseph **Black** identified carbon dioxide (CO_2).
  - In 1766 Henry **Cavendish**, discovered important properties of hydrogen.
    - He believed it was pure phlogiston.

Two scientist who discovered oxygen



Carl Wilhelm Scheele
(1742-1786)

- Oxygen was discovered independently by the Swedish chemist Carl **Scheele** in the early 1770's and the English chemist Joseph **Priestley** in 1774.
  - Wood burns stronger in oxygen than in air. Thus, Priestley believed oxygen could absorb great quantities of phlogiston.
  - He called oxygen *dephlogisticated air* (air without phlogiston).



Joseph Priestley
(1733-1804)

Historical Background. Lavoisier's Contributions

- Antoine **Lavoisier**, a French chemist, revolutionized chemistry in the late 1700's.
  - He repeated many of the experiments of earlier chemists but interpreted the results far differently.
  - Lavoisier paid particular attention to the weight of the ingredients involved in chemical reactions and of the products that resulted.
  - He found that the weight of the products of combustion equals that of the original ingredients.
  - His discovery became known as the law of the conservation of mass (or matter).
- Also Mikhail **Lomonosov** (Russia) independently formulated the same law.

Antoine-Laurent de Lavoisier (1743-1794)

Mikhail Lomonosov
(1711-1765)

Mikhail Lomonosov

- Mikhail Lomonosov was a Russian polymath, scientist and writer, who made important contributions to literature, education, and science.
  - Among his discoveries were the atmosphere of Venus and the law of conservation of mass in chemical reactions.
  - His spheres of science were natural science, chemistry, physics, mineralogy, history, art, philology, optical devices and others.
  - Lomonosov was also a poet and influenced the formation of the modern Russian literary language.

From: https://en.wikipedia.org/wiki/Mikhail_Lomonosov

Historical Background. Biochemistry in Russia

- 1847 – A. I. Hodnev – the first physiological chemistry textbook
- 1864 – A. Ya. Danilevsky – the first physiological chemistry board at Kazan university.
- 1891 – M. V. Nentsky – the first biochemical laboratory at the Institute of experimental medicine (St. Petersburg).
- 1880 – N. I. Lunin – vitamin investigation.
- 1896 – A. N. Bakh – theory of peroxidation.
- 1899 – I. P. Pavlov, N. P. Shepovalnikov – proenzyme investigation.
- 1903 – M. S. Tsvet – chromatography technique investigation.
- 1912 – V. I. Palladin – proposed the theory of biological oxidation.

Historical Background. Prominent Russian Biochemists

- A. I. Oparin
  - Life origin theory
- Academician V. A. Engelgardt,
  - in 1959 г. Founded Institute of molecular biology at USSR Academy of Sciences, investigated oxidative phosphorilation, mechanochemistry of muscle, carbohydrate metabolism etc.
- Academician Yu. A. Ovchinnikov – membrane biology investigations.
- Academician A. S. Spirin – molecular mechanisms of protein synthesis.
- Academician V. P. Skulachev – bioenergetics investigations.

Historical Background. Prominent Russian Biochemists (cont'd)

- **Belorussian Biochemists**
- Acad. Yu. M. Ostrovsky – vitamins investigations. (Institute of Biochemistry AS RB, Grodno).
- **Ukrainian Biochemists**
- Acad. A. V. Palladin – neurochemistry and vitamins investigations,
  - Also protein and lipid metabolism, age biochemistry.

The Subject and Tasks of Biochemistry

1. Knowledge of molecular mechanisms of physiological, genetic and immunological processes of vital activity in norm and pathology and the influence of numerous factors on the organism.
2. Perfection of the methods of prophylaxis, diagnosis and treatment of diseases.
3. Development of the new medicines for normalizing of metabolic processes.
4. Development of the rational healthy lifestyle with balanced nutrition on the scientific base.

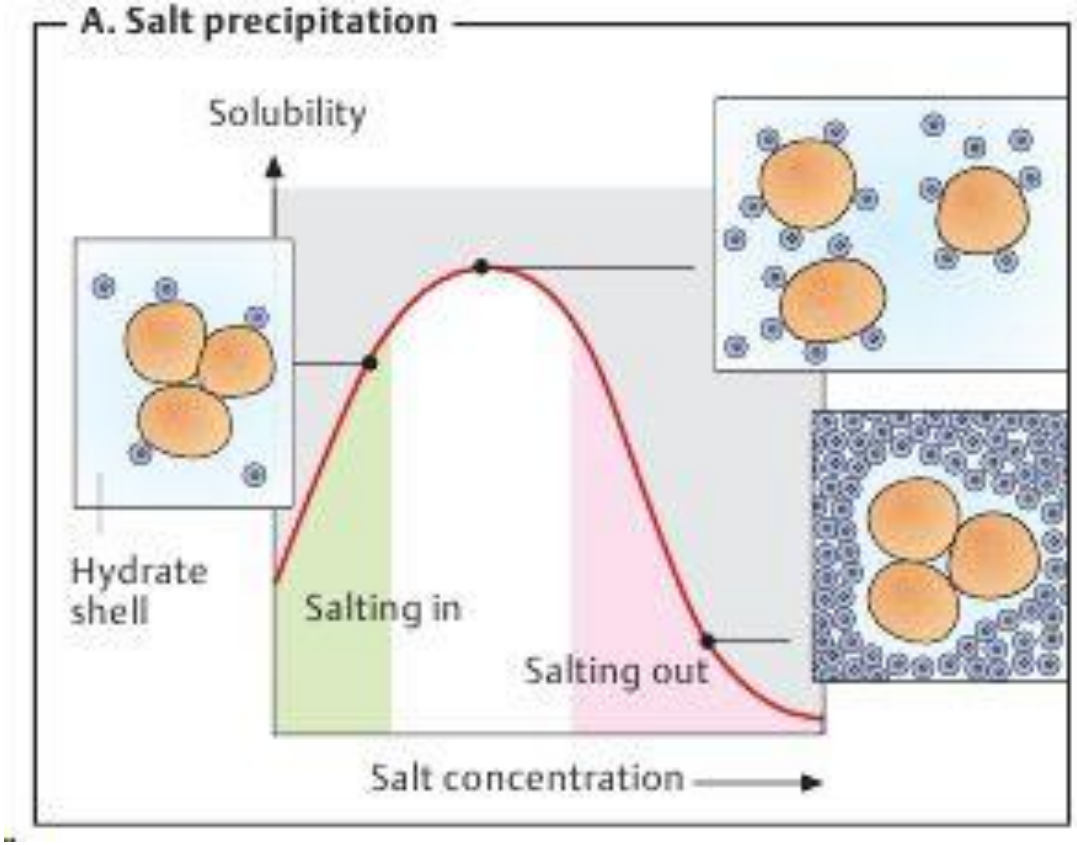
Three Directions in Biochemistry

- **Static biochemistry** – researches qualitative and quantitative chemical composition of living organisms.
- **Dynamic biochemistry** – studies transformations of substances, energy and information in the living organisms.
- **Functional biochemistry** – studies the chemical bases of functions of tissues, organs, organ systems and interorgan relationships.

The Principal Methods and Preparations Used in Biochemical Laboratories (by Marry et al.)

- Methods for Separating and Purifying Biomolecules
- Methods for Determining Biomolecular Structures
- Preparations for Studying Biochemical Processes

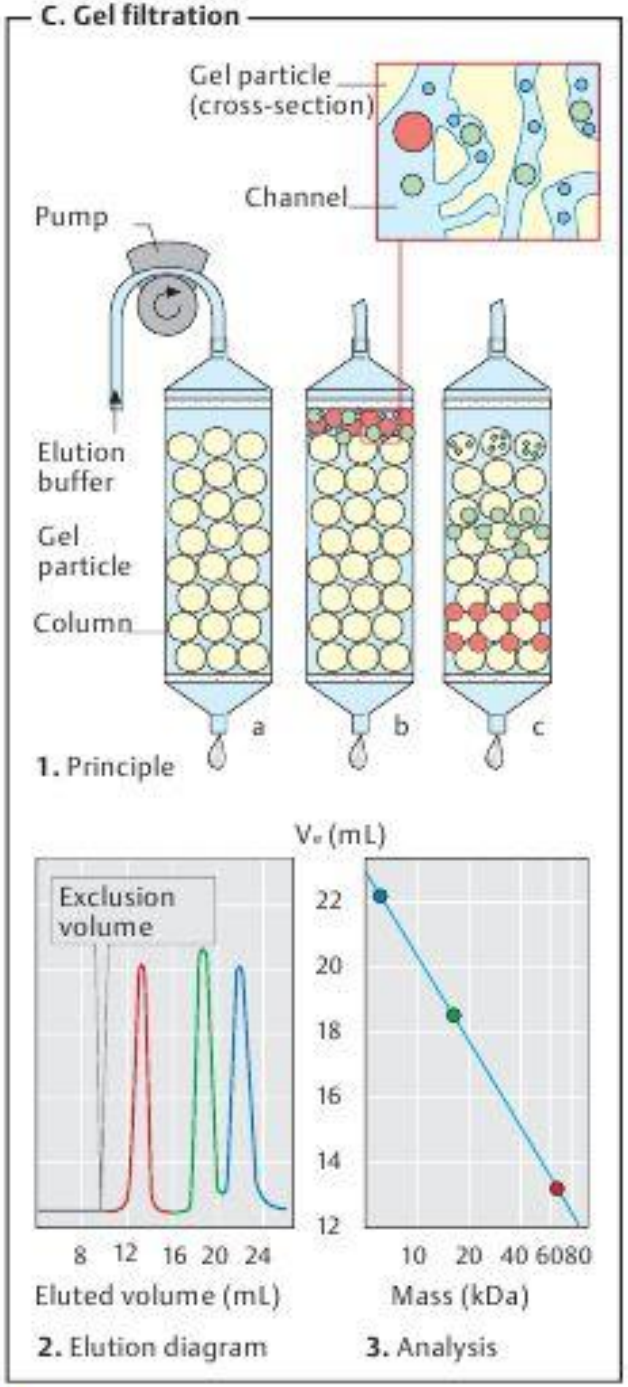
Methods for Separating and Purifying Biomolecules



- **Salt fractionation** (eg, precipitation of proteins with ammonium sulfate)
- **Ultracentrifugation**

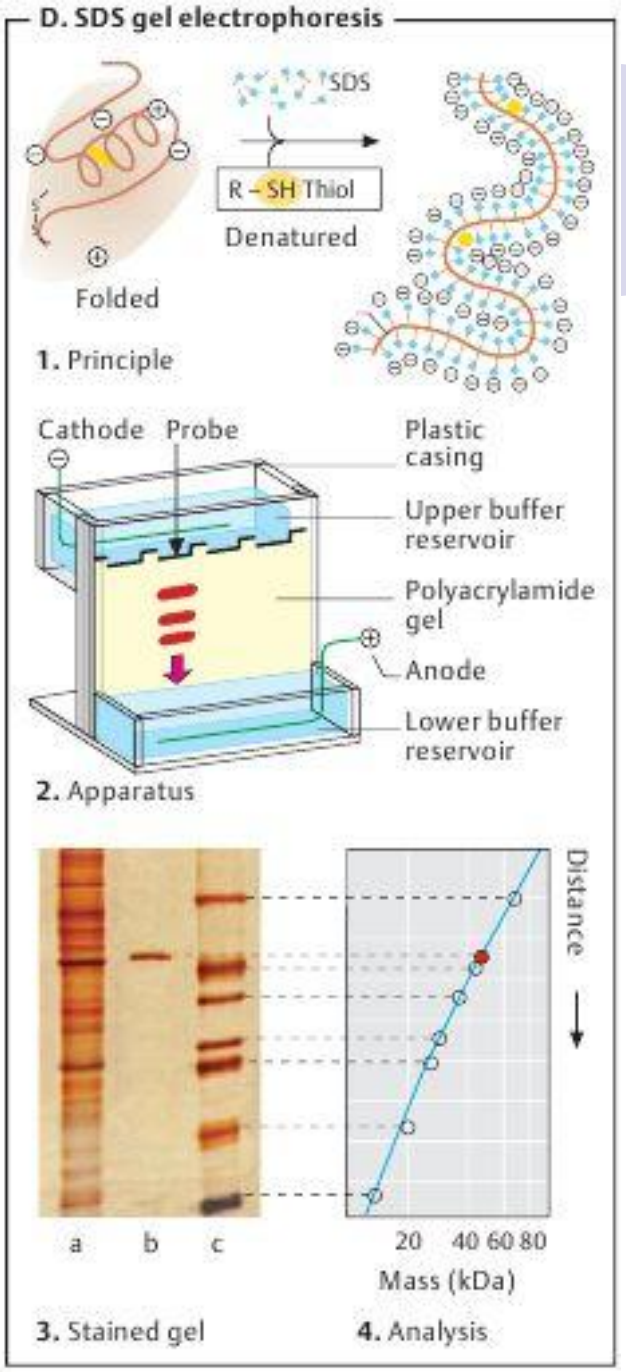
Gel filtration

- **Chromatography**: Paper; ion exchange; affinity; thin-layer; gas-liquid; high-pressure liquid; gel filtration.



Electrophoresis

- **Electrophoresis**: Paper; high-voltage; agarose; cellulose acetate; starch gel; polyacrylamide gel; ***SDS-polyacrylamide gel***



Methods for Determining Biomolecular Structures

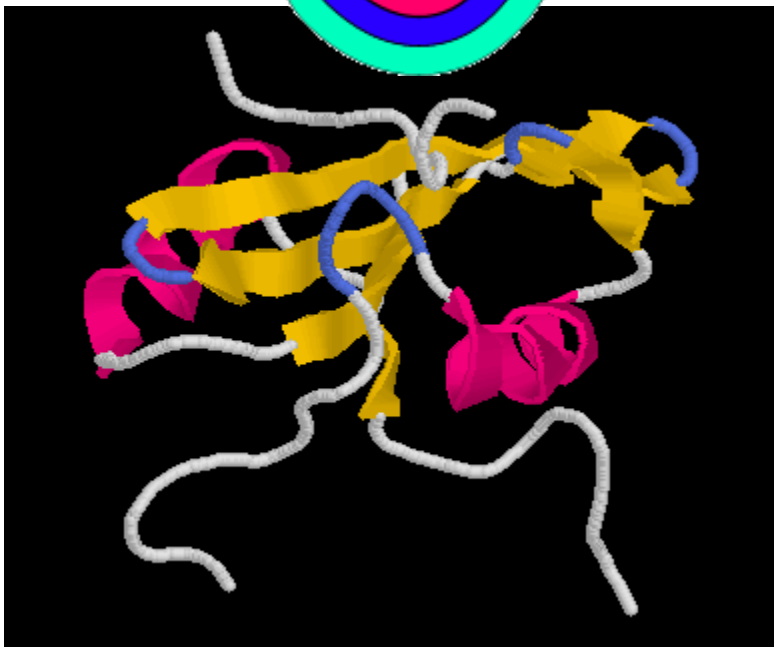
- **Elemental analysis**
- UV, visible, infrared, and NMR **spectroscopy**
- Use of **acid or alkaline hydrolysis to degrade** the biomolecule under study into its basic constituents
- Use of a battery of **enzymes** of known specificity **to degrade** the biomolecule under study (eg, proteases, nucleases, glycosidases)
- **Mass spectrometry**
- Specific **sequencing methods** (eg, for proteins and nucleic acids)
- **X-ray crystallography**

Preparations for Studying Biochemical Processes

- Whole animal (includes transgenic animals and animals with gene knockouts)
- Isolated perfused organ
- Tissue slice
- Whole cells
- Homogenate
- Isolated cell organelles
- Subfractionation of organelles
- Purified metabolites and enzymes
- Isolated genes (including polymerase chain reaction and site-directed mutagenesis)

Protein Chemistry

Proteins – are high molecular compounds, polipeptides, formed by co-polymerization of 20 proteinogenic aminoacids (AA)



Example: Phospholipase C, PLC (E.C.3.1.4.11)

Proteins

- **Proteins** are large organic compounds made of amino acids arranged in a linear chain and joined together by peptide bonds between the carboxyl and amino groups of adjacent amino acid residues.

- The sequence of amino acids in a protein is defined by a *gene* and encoded in the *genetic code*.
  - Although this genetic code specifies 20 "standard" amino acids plus *selenocysteine* and - in certain *archaea* - *pyrrolysine*, the residues in a protein are sometimes chemically altered in post-translational modification: either before the protein can function in the cell, or as part of control mechanisms.
- Proteins can also work together to achieve a particular function, and they often associate to form stable complexes.
  - *From http://www.wikipedia.org*

20 Common Aminoacids

- **These amino acids can be classified as:**
  - Non polar (hydrophobic)
  - Polar (hydrophilic)
    - Neutral (non-charged)
    - Charged
      - Negative (asp glu)
      - Positive (arg, his, lys)

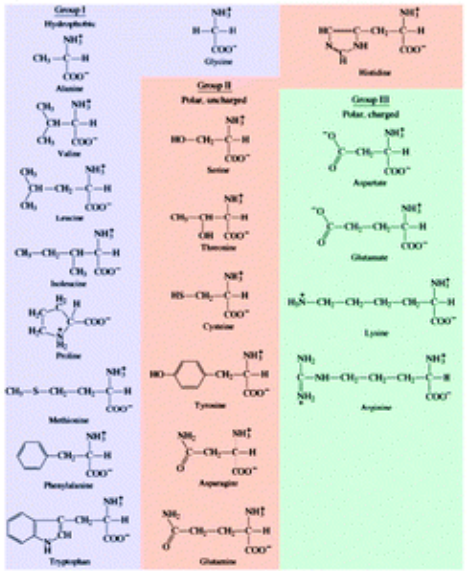
- Depending on the structure of the radical we can also find:
  - Cyclic
    - Aromatic
    - Non aromatic (heterocyclic)
  - Acyclic
    - Aliphatic
  - Sulfur-containing (met, cys)
  - Imino acid (pro)
- By physiological value
  - Non-essential
  - Essential

Characteristics of Amino Acids

- Based upon the nature of their R groups, amino acids can be categorized as:
  - hydrophobic (nonpolar)
  - polar, uncharged
  - polar, charged

Table 3.3
Classification of the amino acids based on side chain reactivity and polarity at pH 7.4

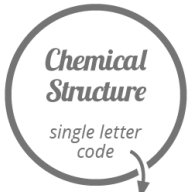
Group I, Hydrophobic	Group II, Polar, Uncharged	Group III, Polar, Charged
Gly	Ser	Asp
Ala	Thr	Glu
Val	Cys	Lys
Leu	Tyr	Arg
Ile	Asn	
Pro	Gln	
Met	His	
Phe		
Trp		



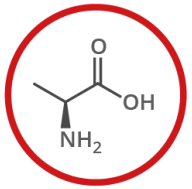
A GUIDE TO THE TWENTY COMMON AMINO ACIDS

AMINO ACIDS ARE THE BUILDING BLOCKS OF PROTEINS IN LIVING ORGANISMS. THERE ARE OVER 500 AMINO ACIDS FOUND IN NATURE - HOWEVER, THE HUMAN GENETIC CODE ONLY DIRECTLY ENCODES 20. 'ESSENTIAL' AMINO ACIDS MUST BE OBTAINED FROM THE DIET, WHILST NON-ESSENTIAL AMINO ACIDS CAN BE SYNTHESISED IN THE BODY.

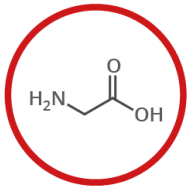
Chart Key: ALIPHATIC · AROMATIC · ACIDIC · BASIC · HYDROXYLIC · SULFUR-CONTAINING · AMIDIC · NON-ESSENTIAL · ESSENTIAL



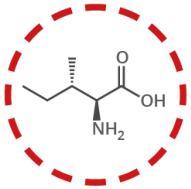
NAME (A)
three letter code
DNA codons



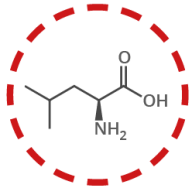
ALANINE (A)
Ala
GCT, GCC, GCA, GCG



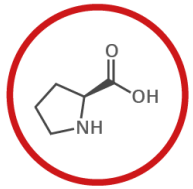
GLYCINE (G)
Gly
GGT, GGC, GGA, GGG



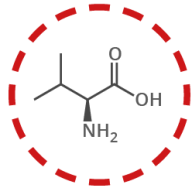
ISOLEUCINE (I)
Ile
ATT, ATC, ATA



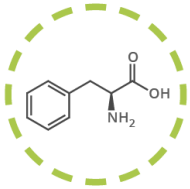
LEUCINE (L)
Leu
CTT, CTC, CTA, CTG, TTA, TTG



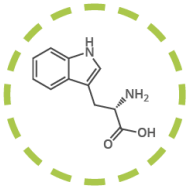
PROLINE (P)
Pro
CCT, CCC, CCA, CCG



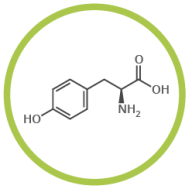
VALINE (V)
Val
GTT, GTC, GTA, GTG



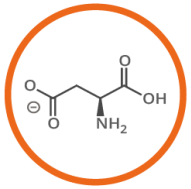
PHENYLALANINE (F)
Phe
TTT, TTC



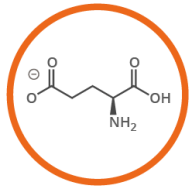
TRYPTOPHAN (W)
Trp
TGG



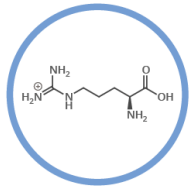
TYROSINE (Y)
Tyr
TAT, TAC



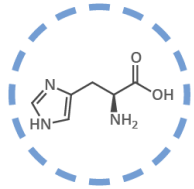
ASPARTIC ACID (D)
Asp
GAT, GAC



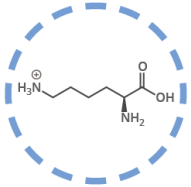
GLUTAMIC ACID (E)
Glu
GAA, GAG



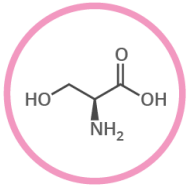
ARGININE (R)
Arg
CGT, CGC, CGA, CGG, AGA, AGG



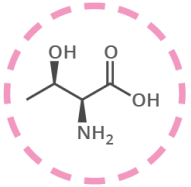
HISTIDINE (H)
His
CAT, CAC



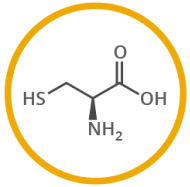
LYSINE (K)
Lys
AAA, AAG



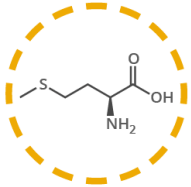
SERINE (S)
Ser
TCT, TCC, TCA, TCG, AGT, AGC



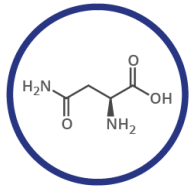
THREONINE (T)
Thr
ACT, ACC, ACA, ACG



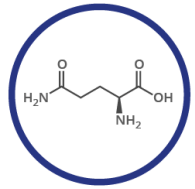
CYSTEINE (C)
Cys
TGT, TGC



METHIONINE (M)
Met
ATG



ASPARAGINE (N)
Asn
AAT, AAC



GLUTAMINE (Q)
Gln
CAA, CAG

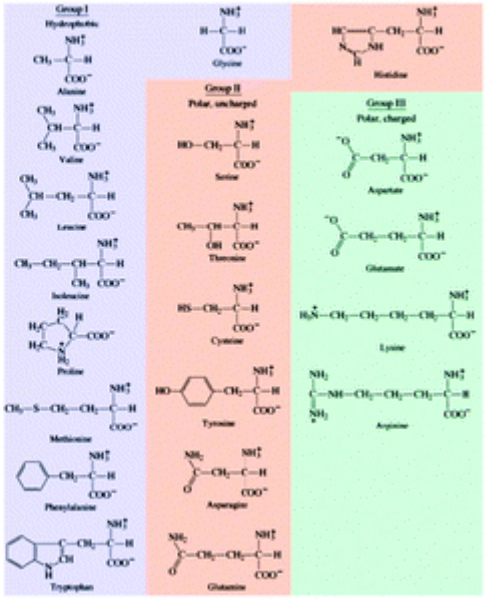
Note: This chart only shows those amino acids for which the human genetic code directly codes for. Selenocysteine is often referred to as the 21st amino acid, but is encoded in a special manner. In some cases, distinguishing between asparagine/aspartic acid and glutamine/glutamic acid is difficult. In these cases, the codes asx (B) and glx (Z) are respectively used.

Characteristics of Amino Acids

- Based upon the nature of their R groups, amino acids can be categorized as:
  - hydrophobic (nonpolar)
  - polar, uncharged
  - polar, charged

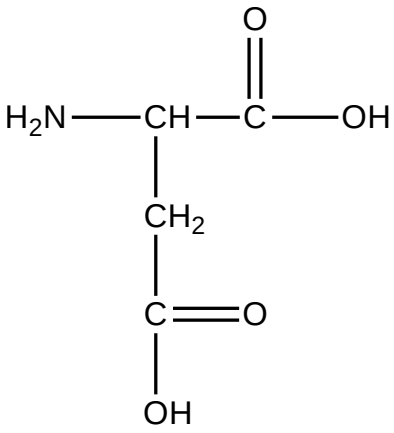
Table 3.3
Classification of the amino acids based on side chain reactivity and polarity at pH 7.4

Group I, Hydrophobic	Group II, Polar, Uncharged	Group III, Polar, Charged
Gly	Ser	Asp
Ala	Thr	Glu
Val	Cys	Lys
Leu	Tyr	Arg
Ile	Asn	
Pro	Gln	
Met	His	
Phe		
Trp		



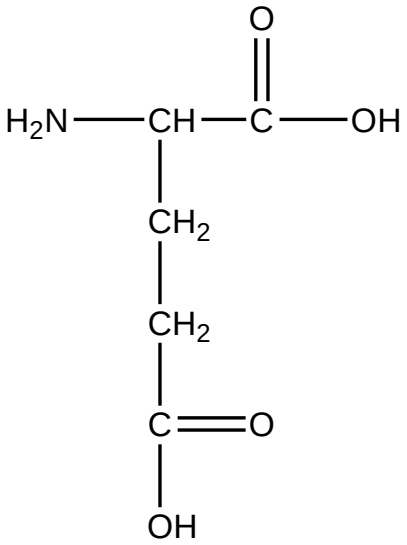
Negative Charged Aminoacids

Aspartic acid



Asp, D

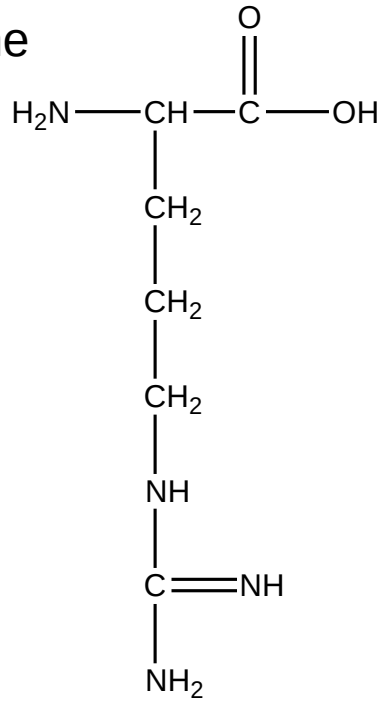
Glutamic acid



Glu, E

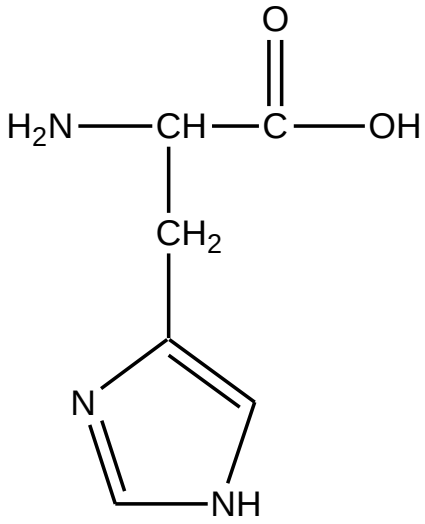
Positive Charged Aminoacids

Arginine



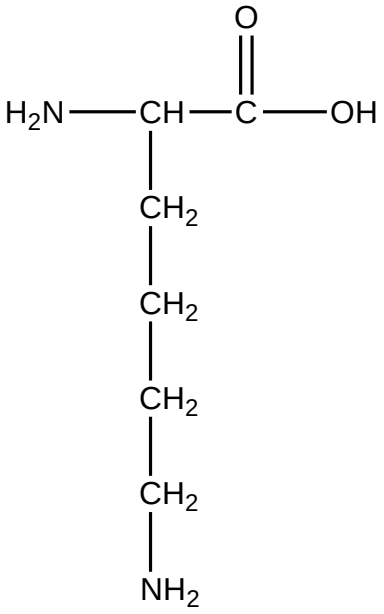
Arg, R

Histidine



His, H

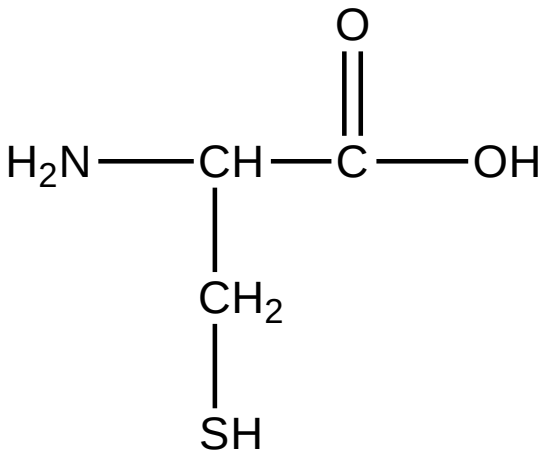
Lysine



Lys, K

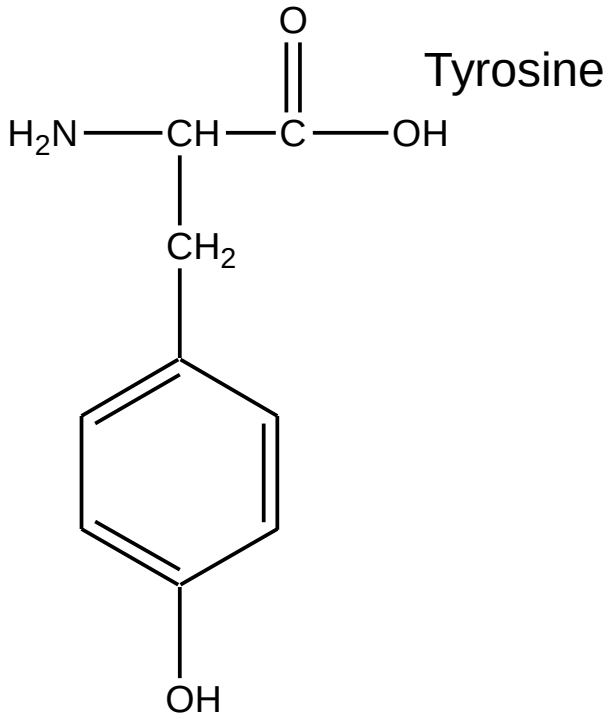
Polar Aminoacids, Wich can be Charged Negative

Cysteine



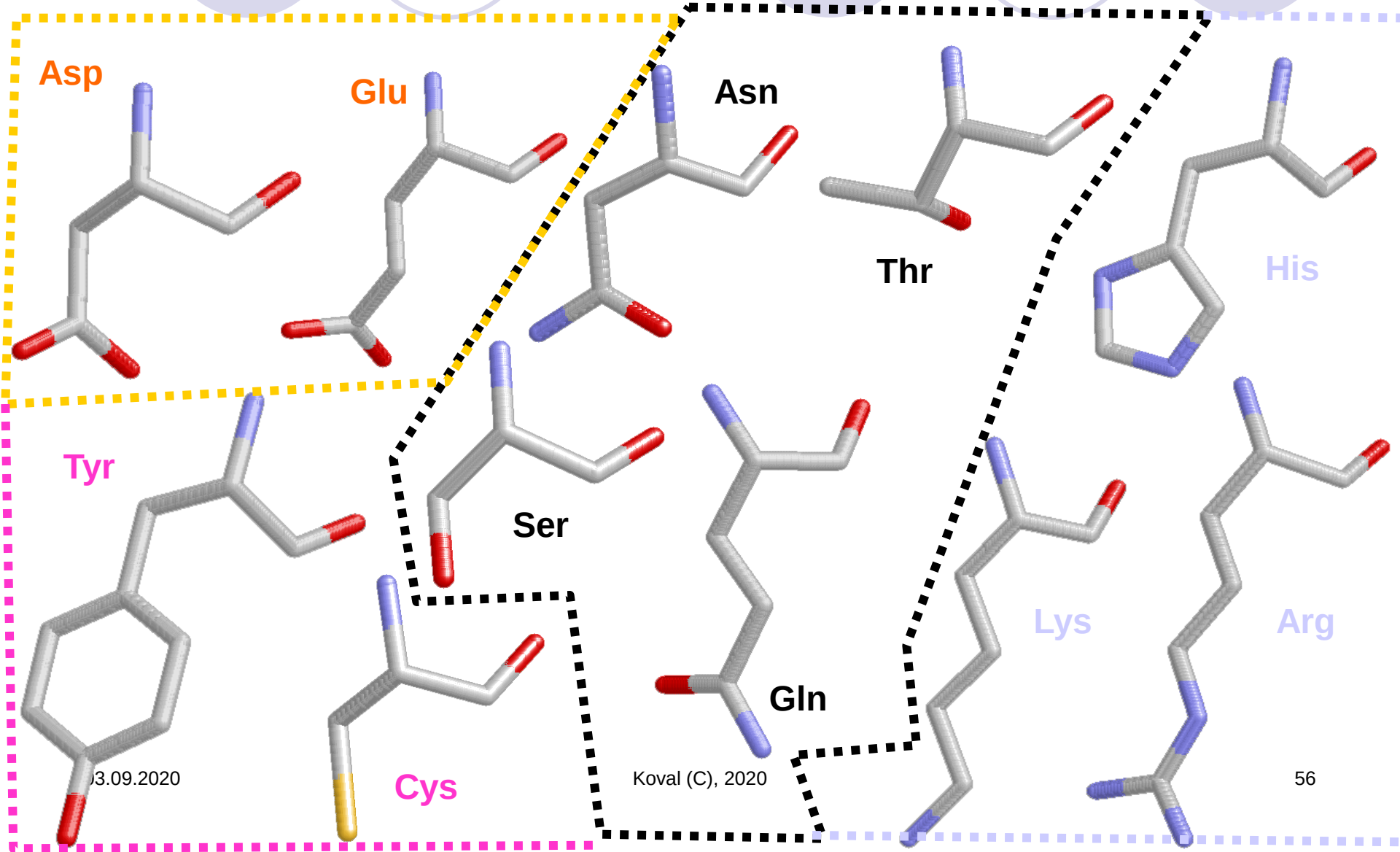
Cys, C

Tyrosine



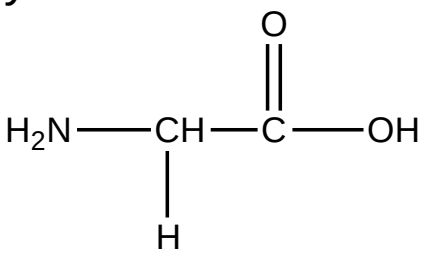
Tyr, Y

3D Models of 11 Polar Amino Acids

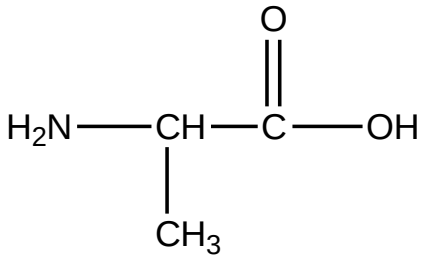


Aminoacids with Hydrophobic Radicals (5 Aliphatic)

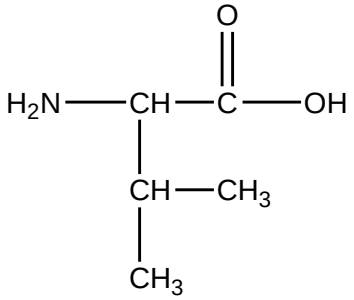
Gly

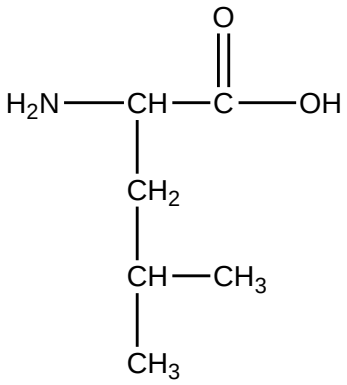


Ala

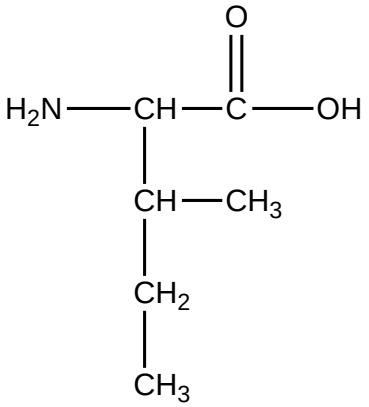


Val



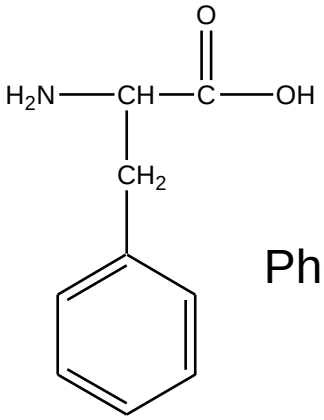


Leu

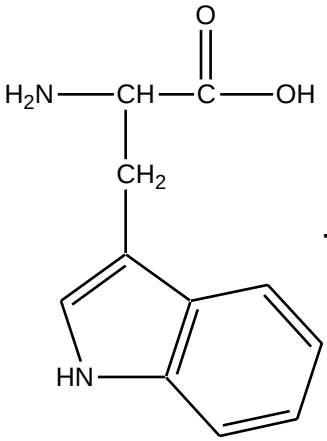


Ile

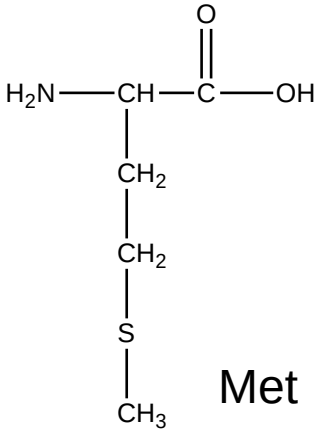
Aminoacids with Hydrophobic Radicals (4 Rest)



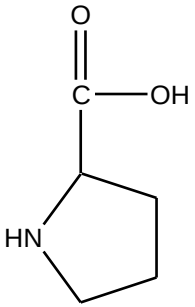
Phe



Trp



Met



Pro

- Phenylalanine – also Tyr & triptophan (Trp) – aromatic AAs.
- Methionine (Met) & Cys – sulfur containing AAs.
- Proline (Pro) – the only **imino**acid.

Heuristic idea, E.Fischer

1. Proteins consists from α-AA.

   (From the net hydrolytic products of protein cleavage the AA are the main product. All other substances are secondary products*).*
2. AA in the proteins of animals are in L-form.
3. Protein molecule is the fairly linear polymer.
4. α-AA form linear polymer by the formation of the peptide bond between the carboxyl and amino group.

Structural Organization of Protein Molecule

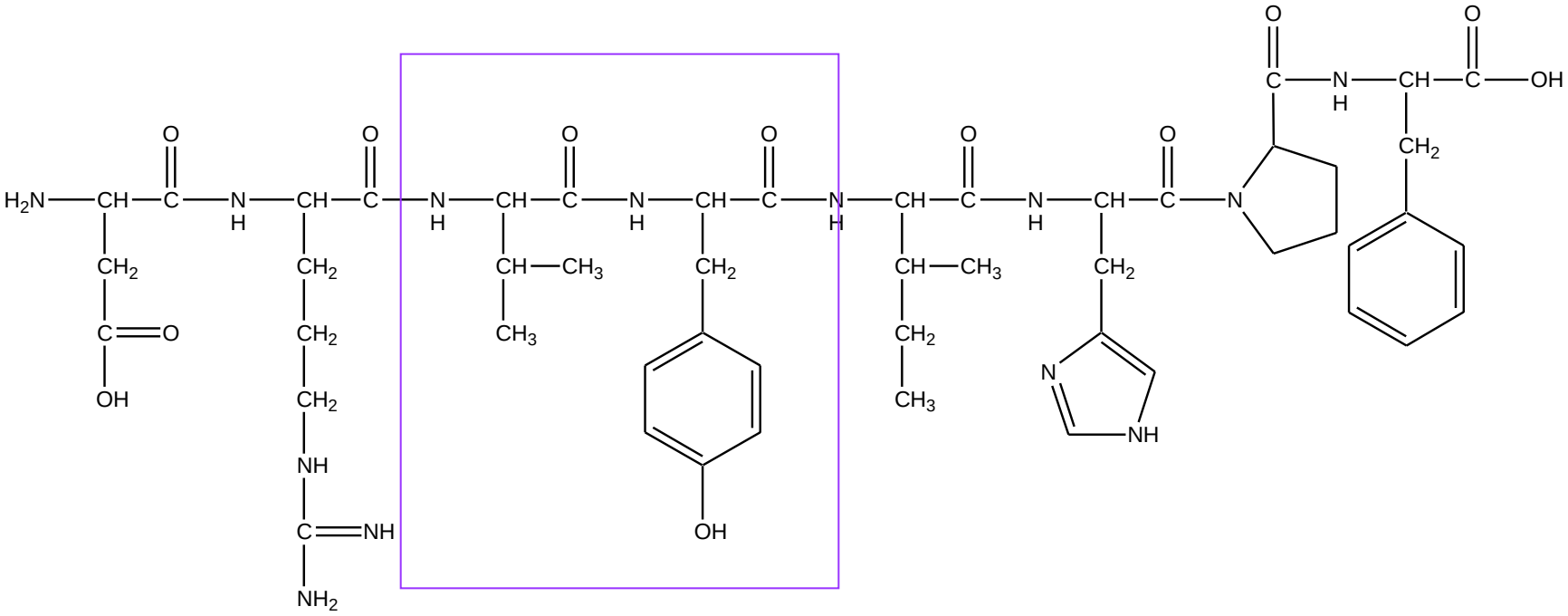
- There are 4 principal levels of the structural organization of the protein molecule (by K.Linderstroem-Lang) :
  - Primary
  - Secondary
  - Tertiary
  - Quaternary

Structural Organization of Proteins

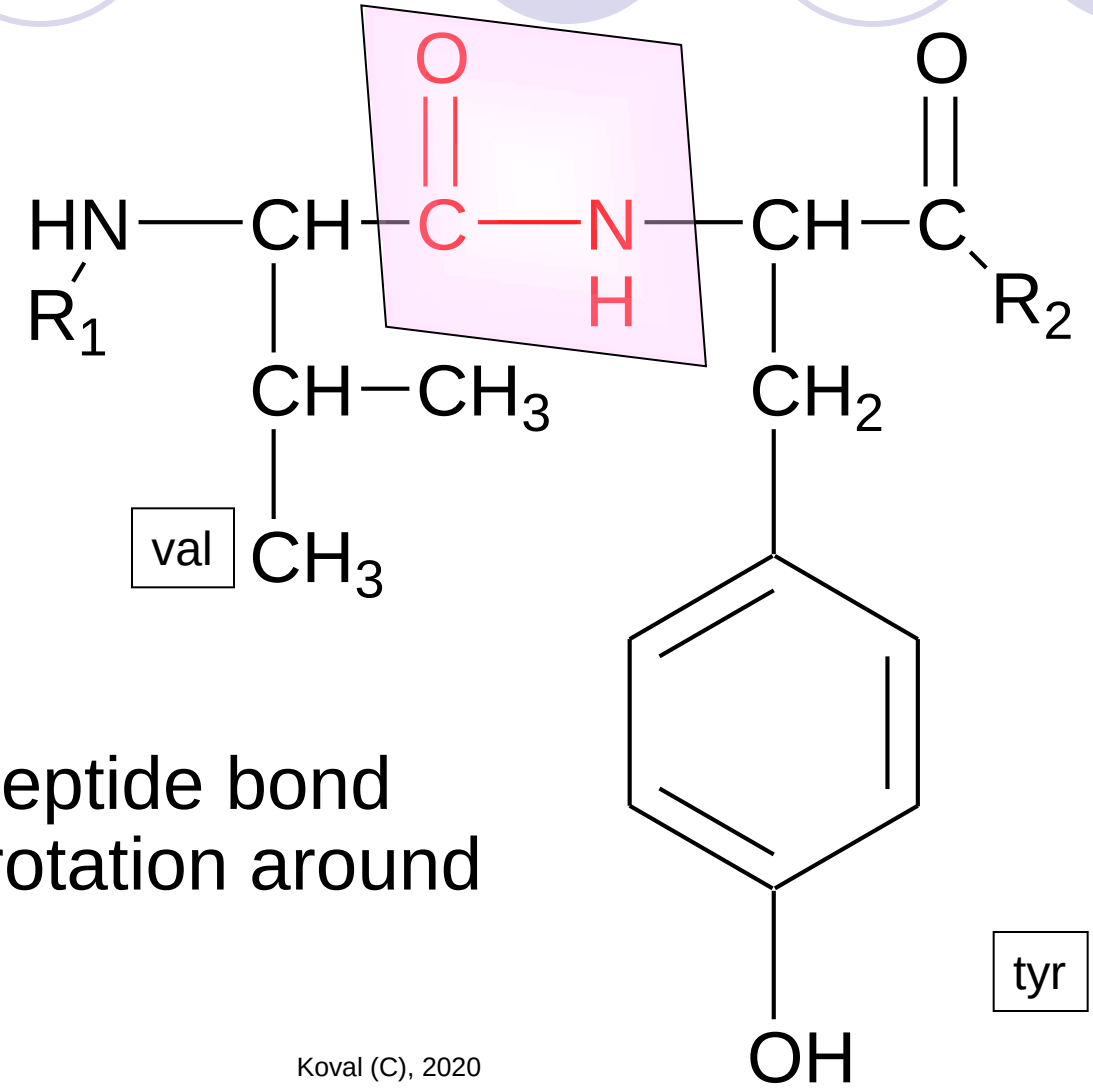
- **Primary structure**: the amino acid sequence.
- **Secondary structure**: regularly repeating local structures stabilized by hydrogen bonds. The most common examples are the alpha helix and beta sheet. Because secondary structures are local, many regions of different secondary structure can be present in the same protein molecule.
- **Tertiary structure**: the overall shape of a single protein molecule; the spatial relationship of the secondary structures to one another. Tertiary structure is generally stabilized by nonlocal interactions, most commonly the formation of a hydrophobic core, but also through salt bridges, hydrogen bonds, disulfide bonds, and even post-translational modifications. The term "tertiary structure" is often used as synonymous with the term fold.
- **Quaternary structure**: the shape or structure that results from the interaction of more than one protein molecule, usually called protein subunits in this context, which function as part of the larger assembly or protein complex.

Example of Primary Structure:
angiotensin-2, hypertensive peptide

H_2N-asp-arg-val-tyr-ile-his-pro-phe-COOH

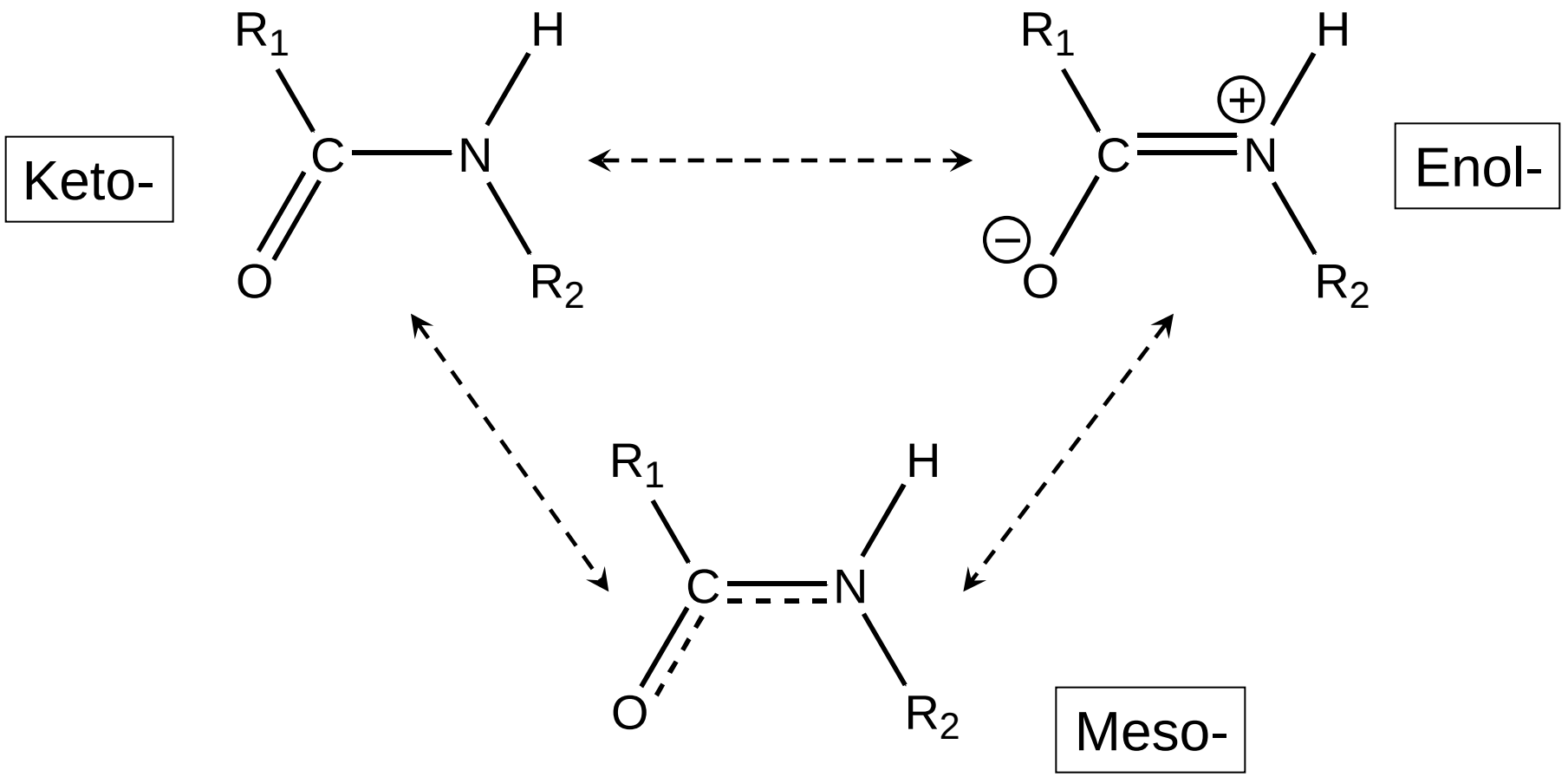


Peptide Bond Properties



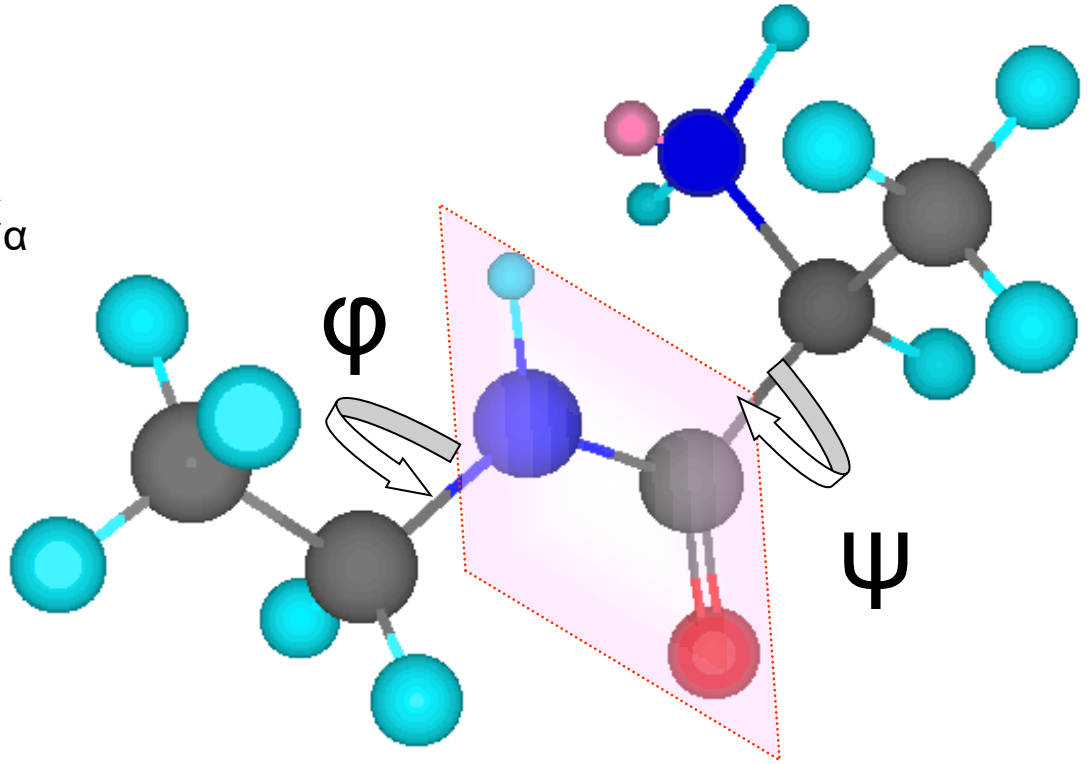
- Complanar peptide bond restricts the rotation around C-N

Mesomery of Peptide Bond

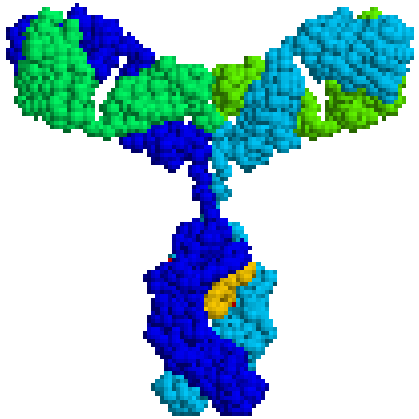


Polypeptide chain conformation

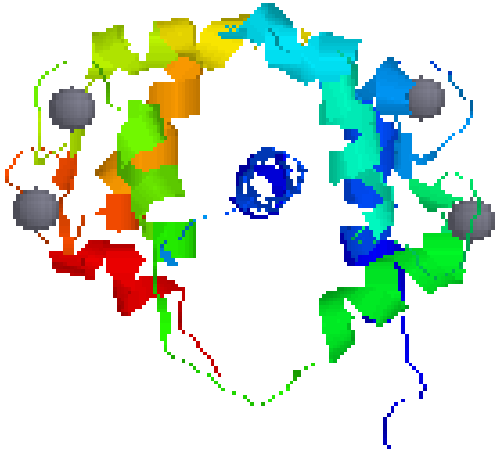
- Actually, peptide bond is coplanar. So rotation is possible by other bonds.
- Angle φ («phi») is characterizing the rotation around N-C_α bond, i.e., to the preceding peptide bond.
- Angle ψ («psi») is characterizing the rotation around the bond C_α-C, i.e., following bond.



Example of Protein Molecules

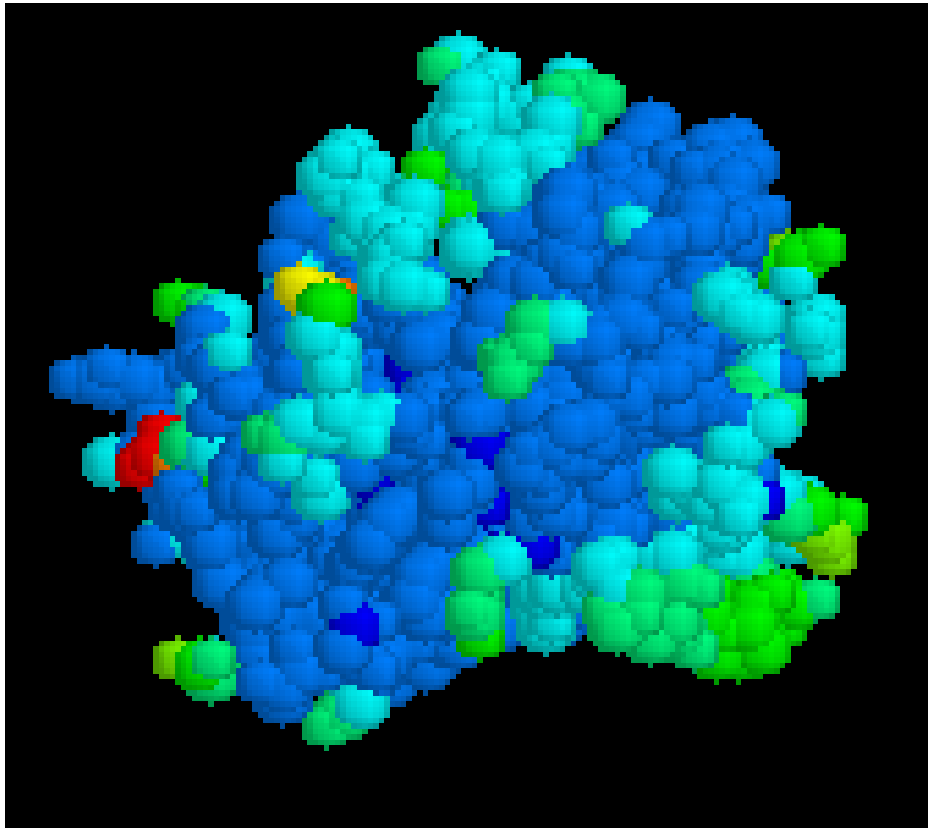


Immunoglobulin

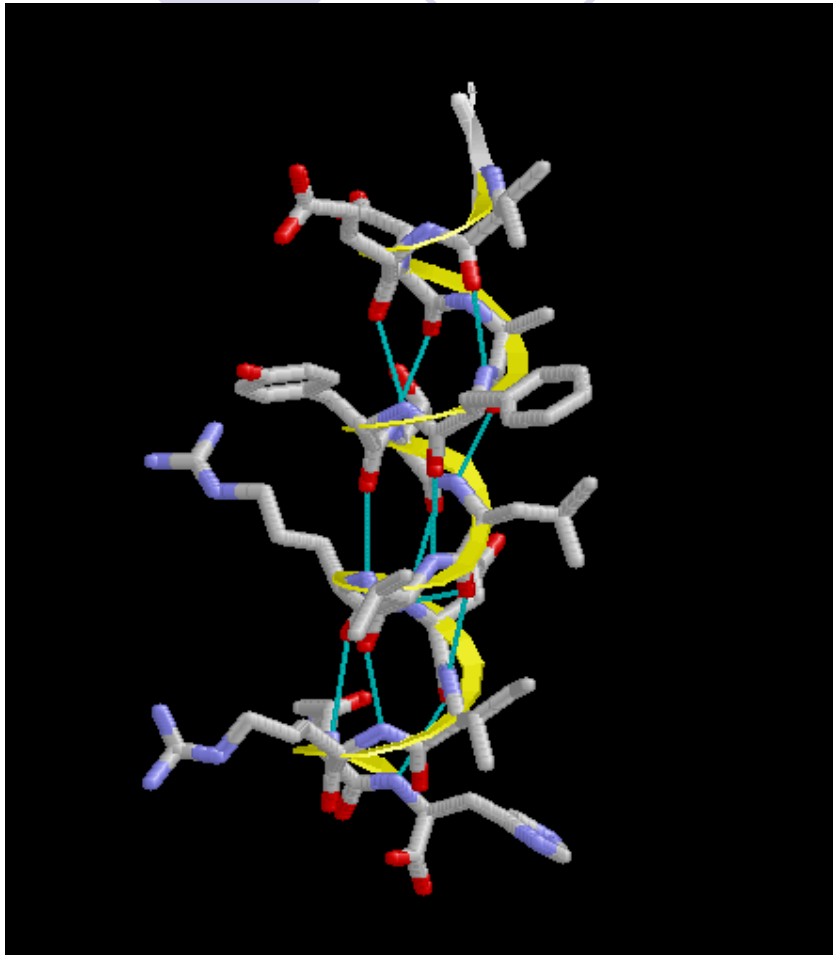


Calcium-binding protein

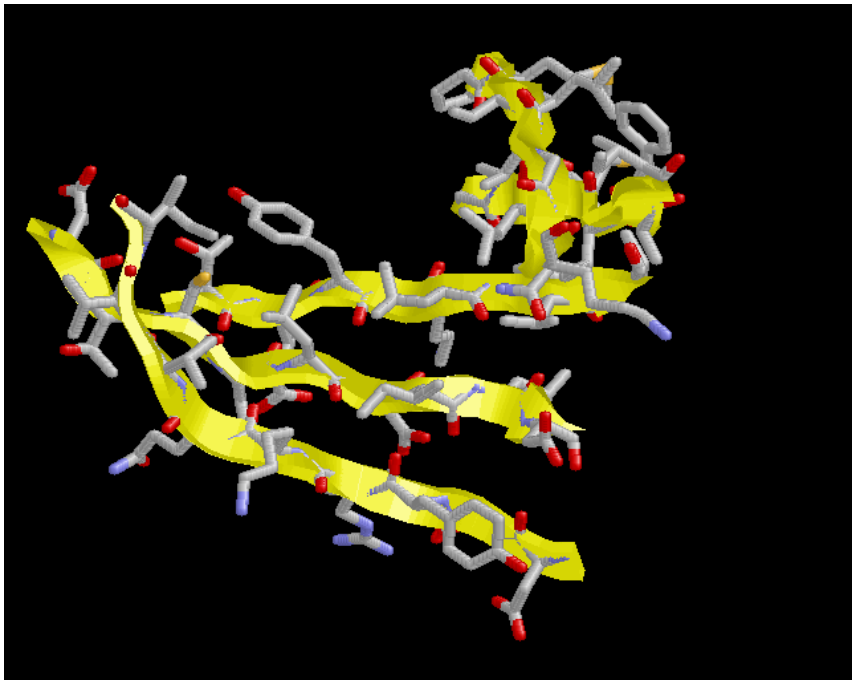
Dynamics of Protein Molecule



Secondary Structure(2-D, space)

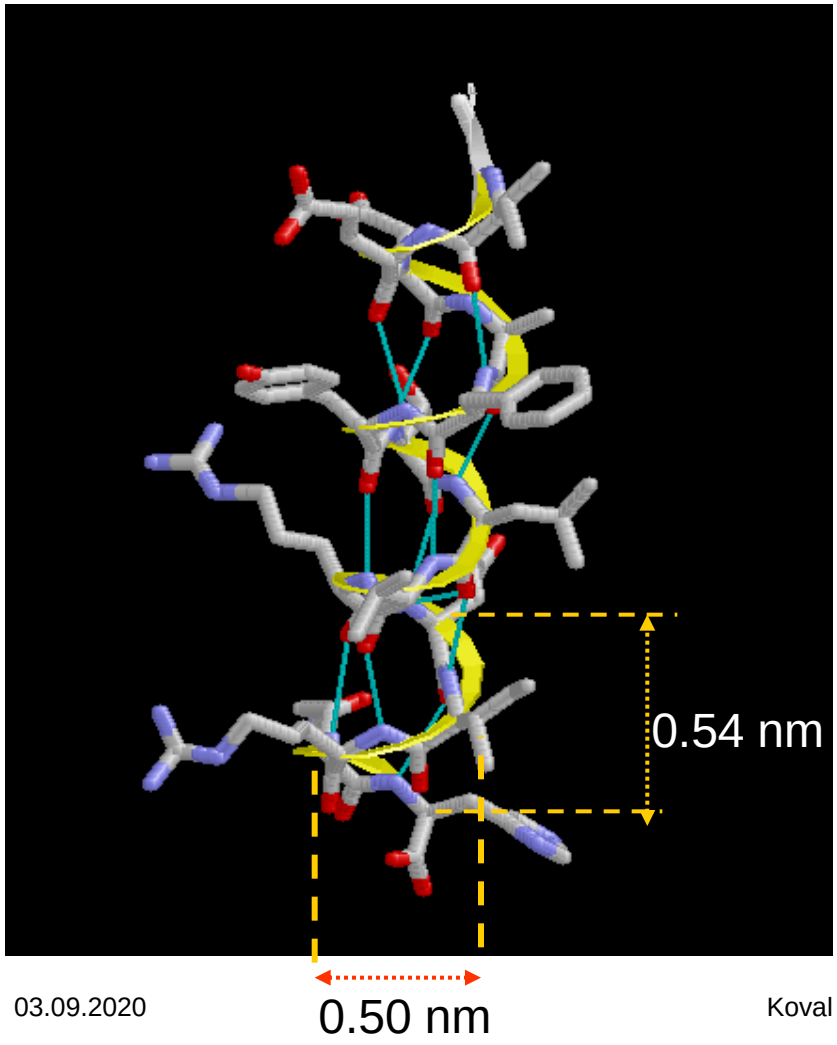


α-helix



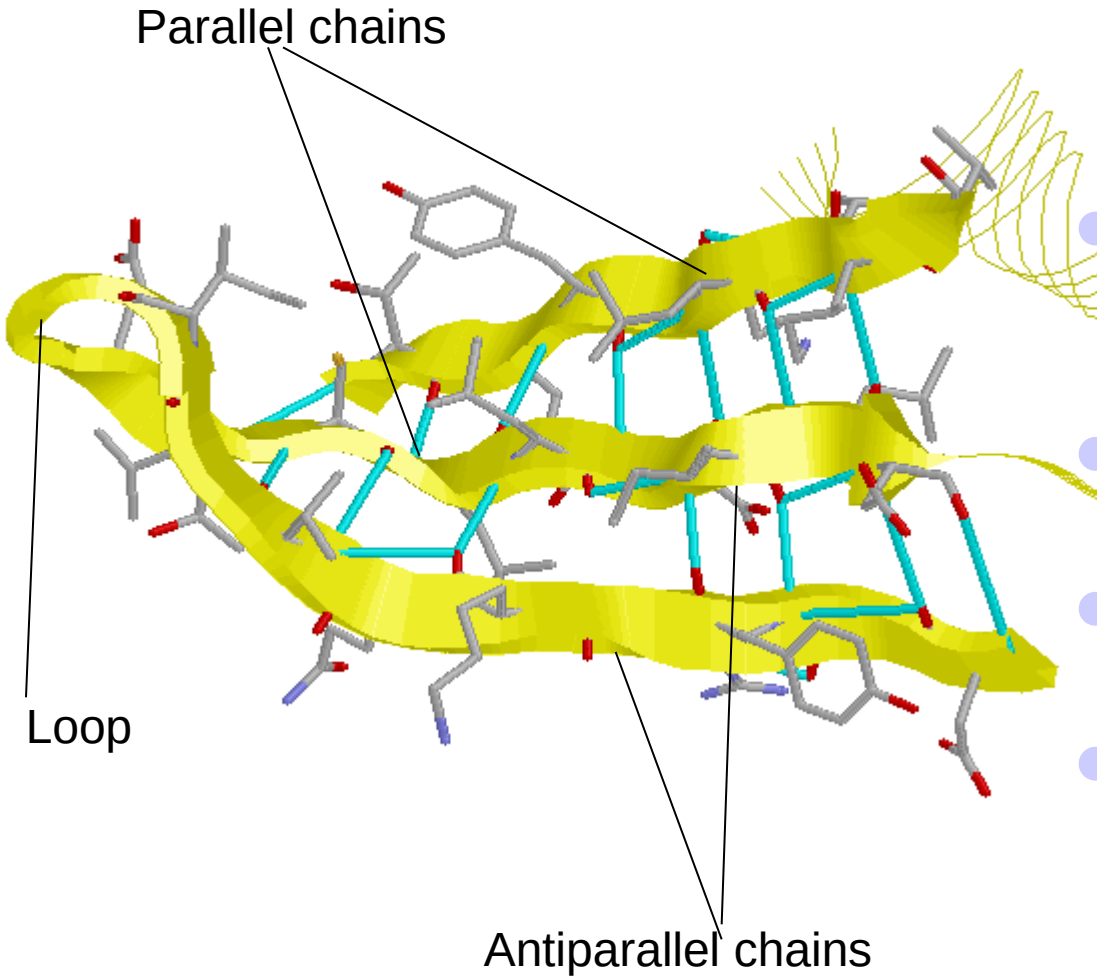
β-structure

Properties of α-helix



- Pitch 0.54 nm
  - 3.6 AA res./turn,
  - 13 atoms),
- Diameter 0.50 nm,
- Is stabilized by hydrogen bonds between n-th CO group and (*n*+4)-th NH_2-group.

Beta-structure properties



- Polypeptide chains interacts by hydrogen bonds of peptide groups.
- Hydrogen bonds are in the plane.
- AA radicals – above and below the plane.
- Parallel chains and antiparallel chains.

Other Variants of Secondary Structure

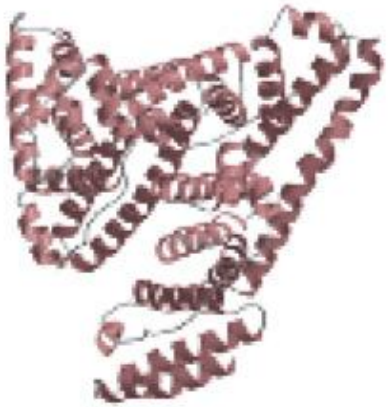
- Besides α-helix there are also:
  - 3_{10}-helix (3 AA res./turn, 10 atoms) – more convoluted,
  - π-helix (4.4 AA res/turn, 16 atoms) – more loose,
  - α_{II}-helix (4 AA res./turn, 14 atoms) – loose.
  - Collagen helix – zigzag, left-handed, extended.
    - There are every 1/3 AA is glycin, 1/5 – prolin and hydroxyprolin, rare - oxylyzine.
- Also may be:
  - *Loops* (changing the direction of chains in beta-structures),
  - *Non-structured regions*.

Structures proportion in proteins

- α-proteins:
  - Myoglobin, hemoglobin, paramyosin, α-keratine.
- β-proteins :
  - Concanavallin A (plant *lectines*), superoxide dismutase, silk fibroin, spider silk.
- α+β- proteins (one part of the chain are α-helices, other – β-sheets) – rare:
  - termolysine (bact.),
- α/β- proteins (α- and β- structures alternate) – most often:
  - Phosphoglycerate kinase, flavodoxin.
- no α,β (no helices and sheets):
  - ferredoxin (bact.)

All α-proteins

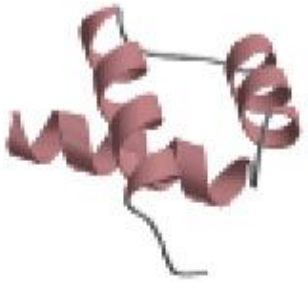
All α



1AO6
Serum albumin
Serum albumin
Serum albumin
Serum albumin
Human (*Homo sapiens*)

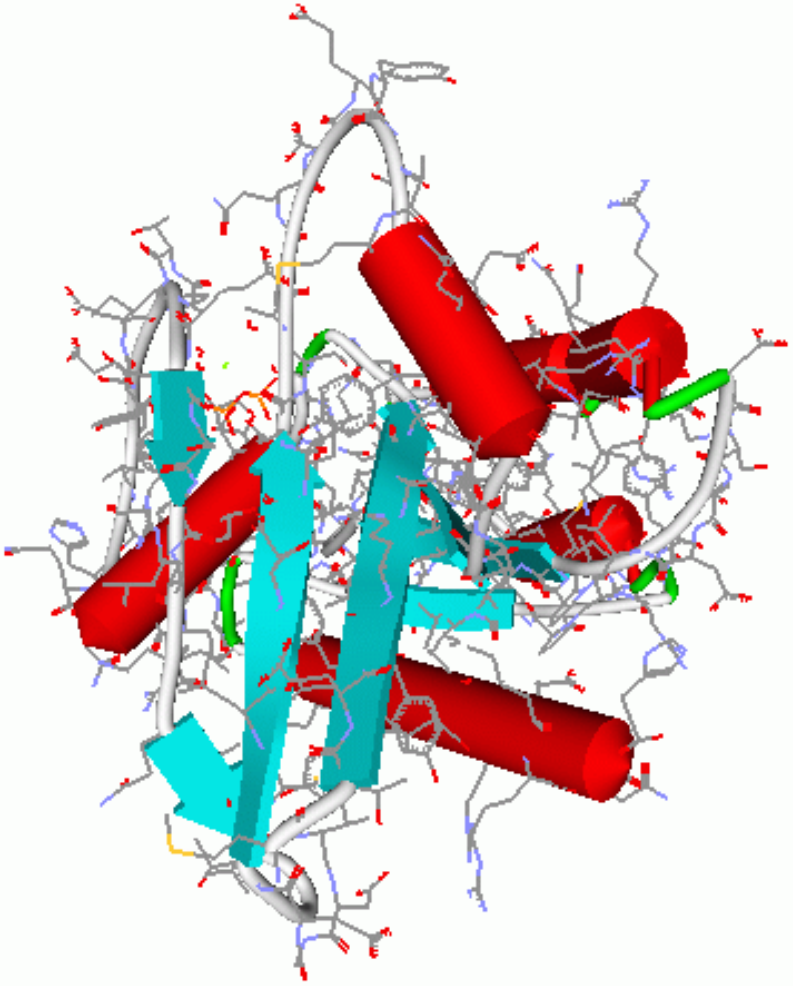
1BCF
Ferritin-like
Ferritin-like
Ferritin
Bacterioferritin (cytochrome b_1)
Escherichia coli

1GAI
α/α toroid
Six-hairpin glycosyltransferase
Glucoamylase
Glucoamylase
Aspergillus awamori, variant x100



1ENH
DNA/RNA-binding 3-helical bundle
Homeodomain-like
Homeodomain
engrailed Homeodomain
Drosophila melanogaster

Tertiary structure



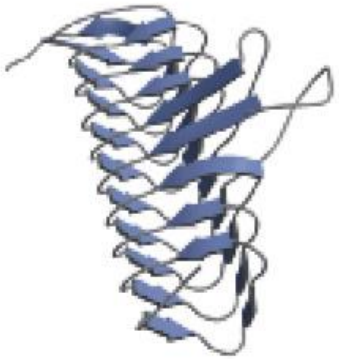
ONCOGENE PROTEIN
(C-H-RAS P21 PROTEIN)

- The term “tertiary structure” refers to the entire three dimensional conformation of a polypeptide.
  Bonds:
  - **covalent** (disulfide, pseudopeptide);
  - **non-covalent** (electrostatic, hydrogene, hydrophobic interactions).
- Protein **folding** is realized and controlled by specific proteins – *shaperones* and *shaperonines* (aka *heat shock proteins*, *hsp*).

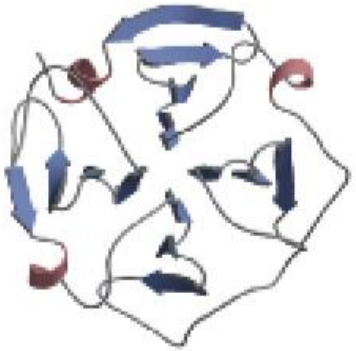
All β-proteins

All β

1HOE
α-Amylase inhibitor tendamistat
α-Amylase inhibitor tendamistat
α-Amylase inhibitor tendamistat
α-Amylase inhibitor tendamistat
Streptomyces tendae

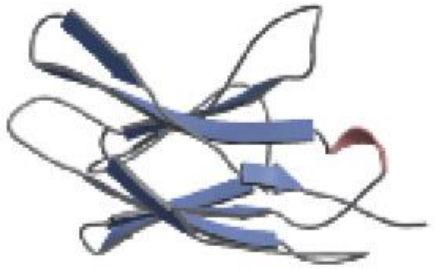


1LXA
Single-stranded left-handed β helix
Trimeric LpxA-like enzymes
UDP *N*-acetylglucosamine acyltransferase
UDP *N*-acetylglucosamine acyltransferase
Escherichia coli



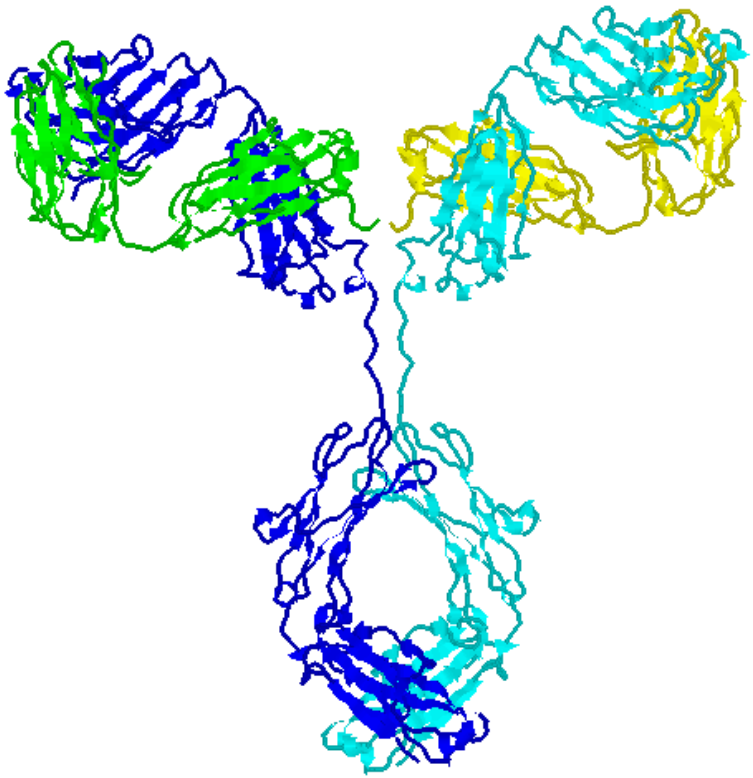
1PEX
Four-bladed β propeller
Hemopexin-like domain
Hemopexin-like domain
Collagenase-3 (MMP-13), carboxyl-terminal domain
Human (*Homo sapiens*)

1JPC
β-Prism II
α-D-Mannose-specific plant lectins
α-D-Mannose-specific plant lectins
Lectin (agglutinin)
Snowdrop (*Galanthus nivalis*)



1CD8
Immunoglobulin-like β sandwich
Immunoglobulin
V set domains (antibody variable domain-like)
CD8
Human (*Homo sapiens*)

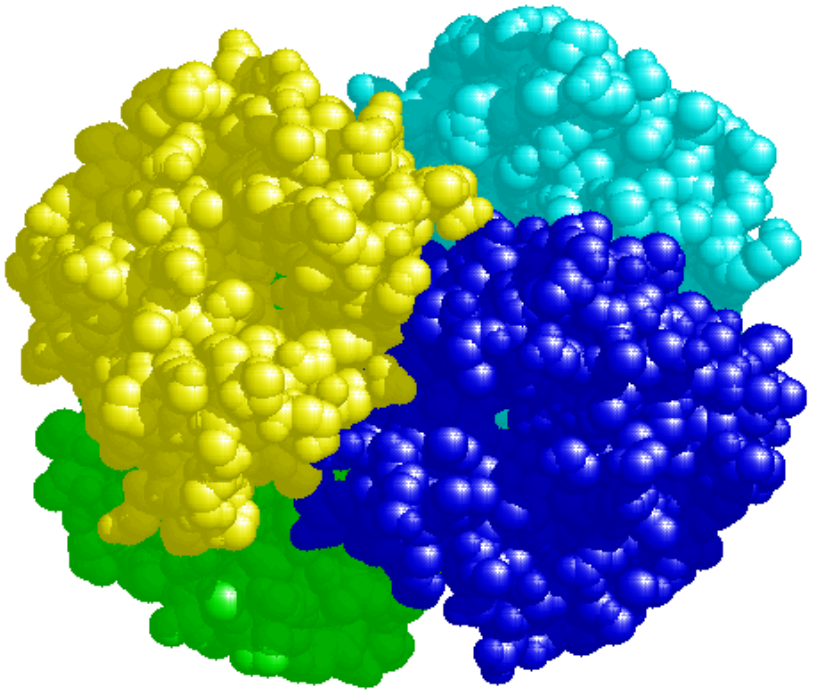
Domain Protein Organization



HUMAN IGG1

- A **domain** is a section of protein structure sufficient to perform a particular chemical or physical task such as binding of a substrate or other ligand.
- There are 12 domains in immunoglobuline G_1 (IgG_1):
  - 2 light chainds contain 2 domains (V_L, C_L)
  - 2 heavy chains contain 4 domains (V_H, C_{H1}, C_{H2}, C_{H3}).
- Other domains may anchor a protein to a membrane or interact with a regulatory molecule that modulates its function.

Quaternary Structure



Hemoglobine A_1 is a tetrameic protein

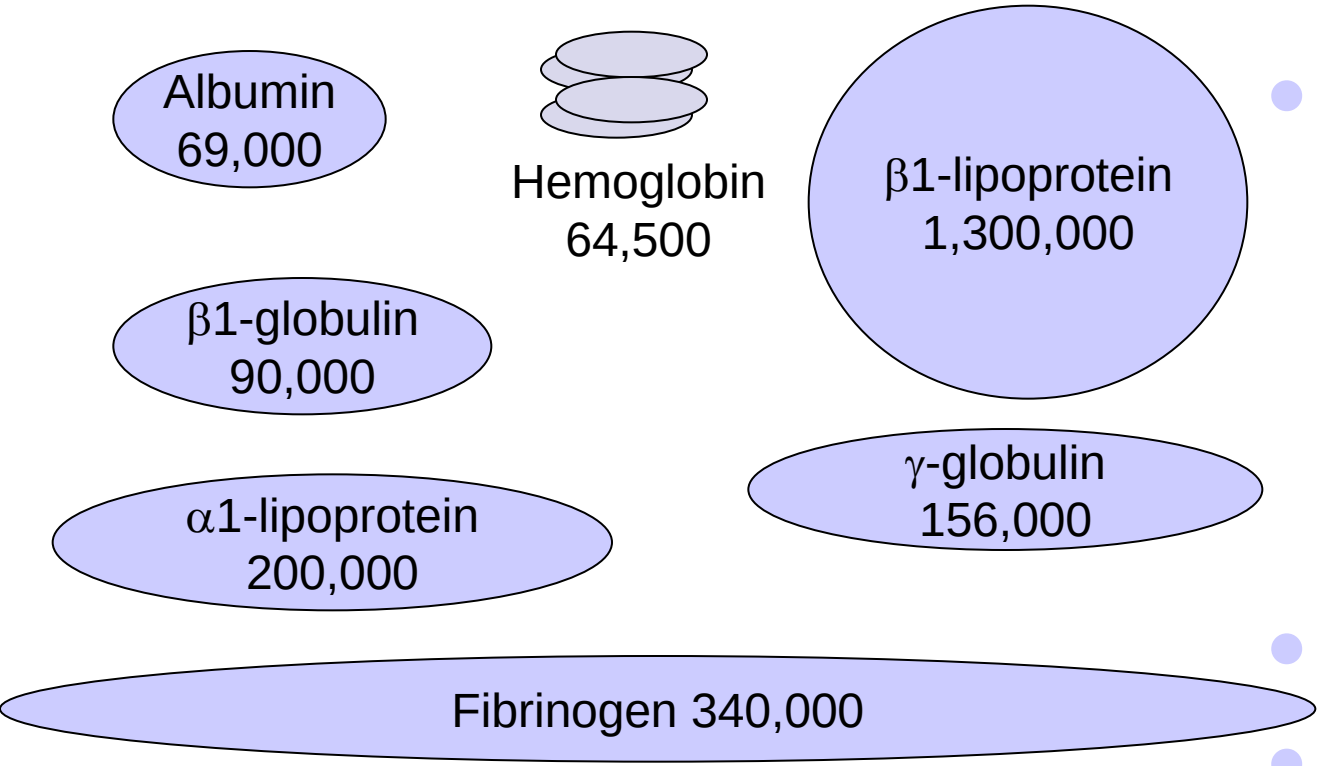
- **Quaternary structure** defines the polypeptide composition of a protein and, for an oligomeric protein, the spatial relationships between its subunits or protomers.
  - **Bonds:** hydrogen bonds, hydrophogic interactions.
  - **Monomeric** proteins consist of a single polypeptide chain.
  - **Dimeric** proteins contain two polypeptide chains.
  - **Homodimers** contain two copies of the same polypeptide chain, while in a heterodimer the polypeptides differ. Greek letters (α, β, γ etc) are used to distinguish different subunits of a **heterooligomeric** protein, and subscripts indicate the number of each subunit type.

For example, α4 designates a homotetrameric protein, and α2β2γ a protein with five subunits of three different types.

Fifth Level of Protein Molecule Organization

- Sometimes the fifth level is marked – metabolon. i.e. the complex of enzymes, which catalyze certain metabolic pathway (e.g. Krebs cycle).
- Also:
  - Pyruvate dehydrogenase complex;
  - α-ketoglutarate dehydrogenase comples;
  - Palmitoyl-synthase complex.

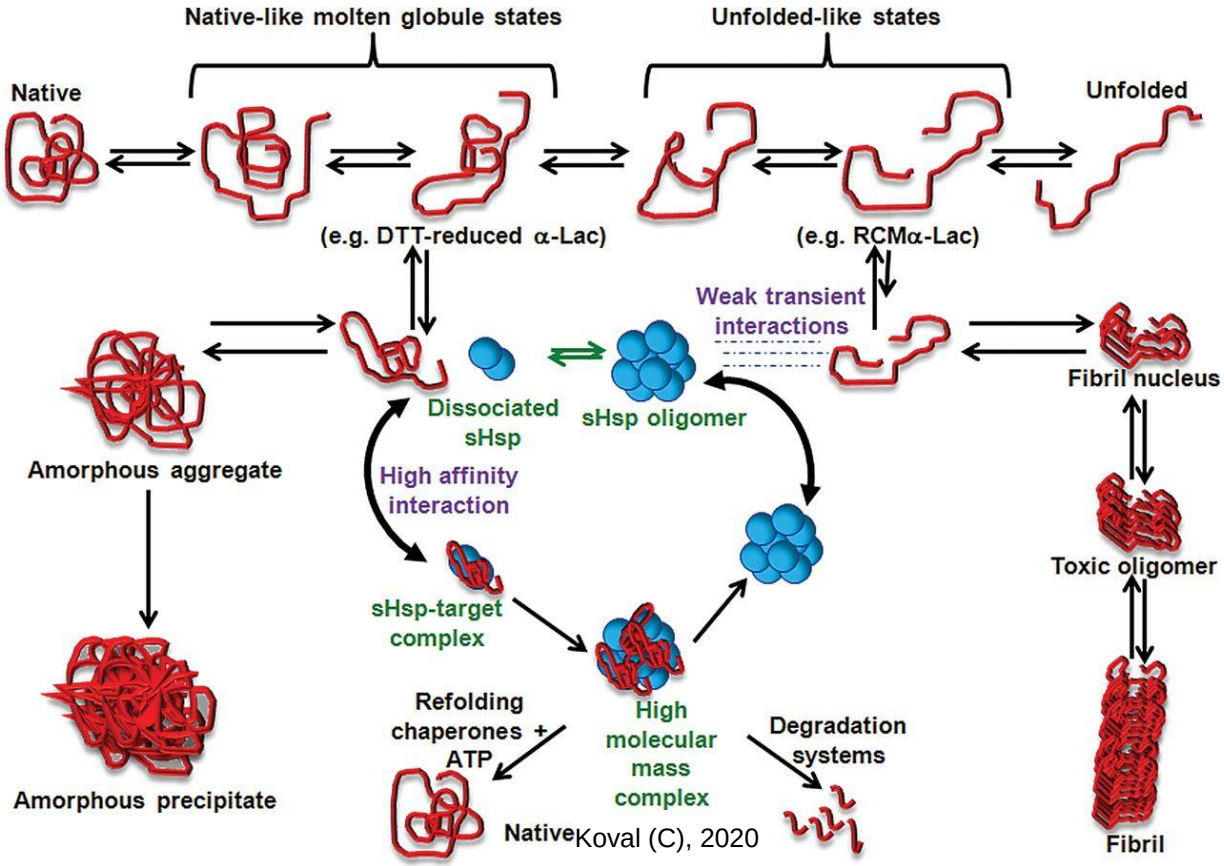
Relative Dimensions and Approximate Molecular Masses of Protein Molecules in the Blood



- By shape:
  - **Globular** (albumin, ribonuclease, myoglobin, hemoglobin).
    - Spheric, ellipsoid, elongated.
  - **Fibrillar** (keratins, fibroin, F-actine, tropomyosin).
    - fibrous.
- By size - 2,5 to 300 nm.
- By weight – 13 000 to 500 000 Da (dalton).

STATE OF THE PROTEIN IN THE LIVING CELL

Up to 60% of the proteins in the living cell are in the **intrinsically unstructured (natively unfolded)** state



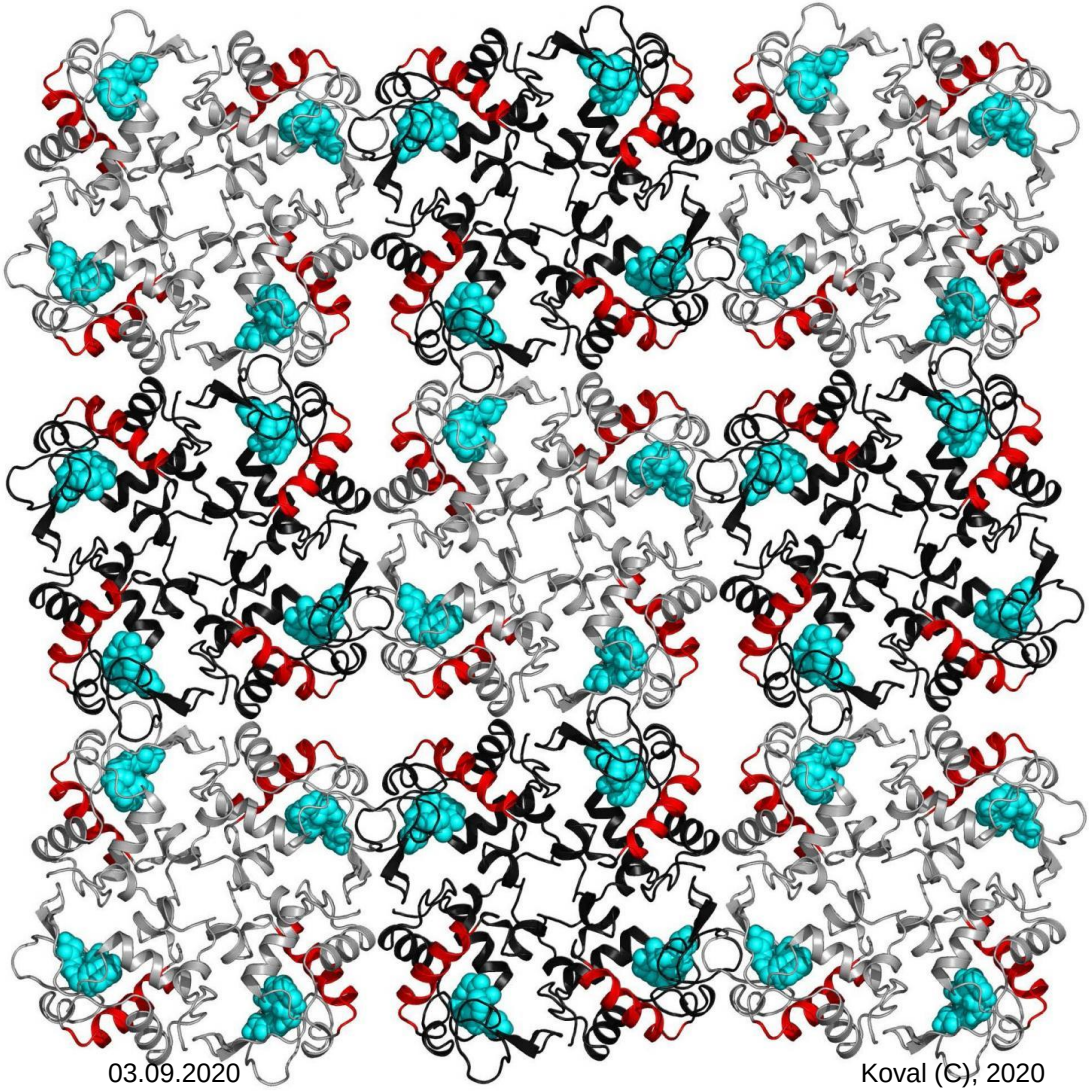
General plan of protein molecule

("molten globule" model)

- There are hydrophobic amino acids in the center of the molecule.
- Hydrophilic amino acids are in the periphery.
- As a result in the aqueous medium the protein surface is charged, and the hydrate layer is formed.

Actually: the protein surface is mosaic-like; hydrophilic and hydrophobic zones are alternating.

Protein as aperiodic crystal



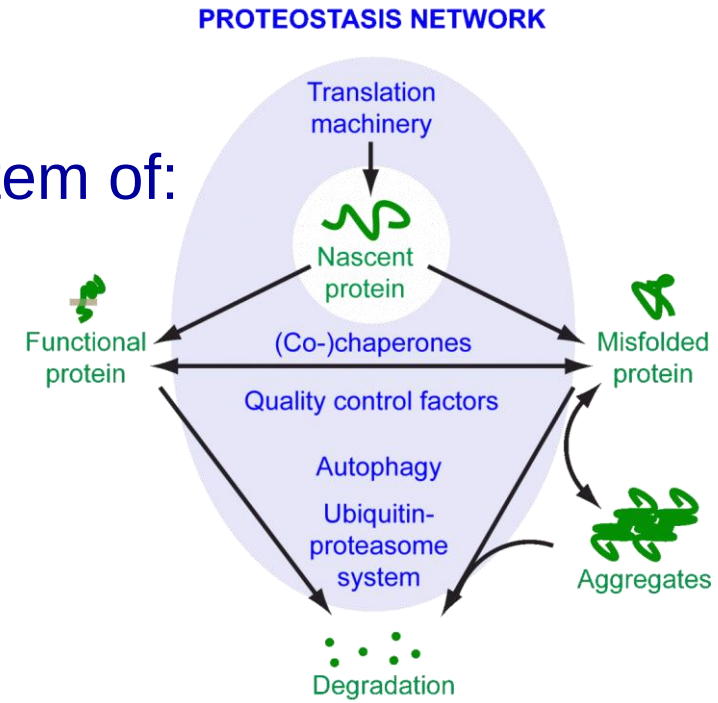
- Proteins are structurally highly ordered.
- There are repeating elements.
- Strictly determined structure.

Protein folding

- **FOLDING** – the process whereby an unorganized polypeptide acquires a specific three-dimensional structure.
  - is facilitated by **heat shock proteins (hsp),** or molecular **chaperones**, **chaperonines**, co-chaperones etc.
- The heat-shock proteins are classified, according to their size, in three classes:
  - **Hsp60, Hsp70, and Hsp90.**

Proteostasis

- Homeostatic system to maintain the functionally active tertiary structure of the protein, preventing its aggregation.
  - It is provided by a proteostatic network (PN) - a system of:
    - chaperones,
    - chaperonins,
    - cochaperones,
    - proteases
    - and other proteins.



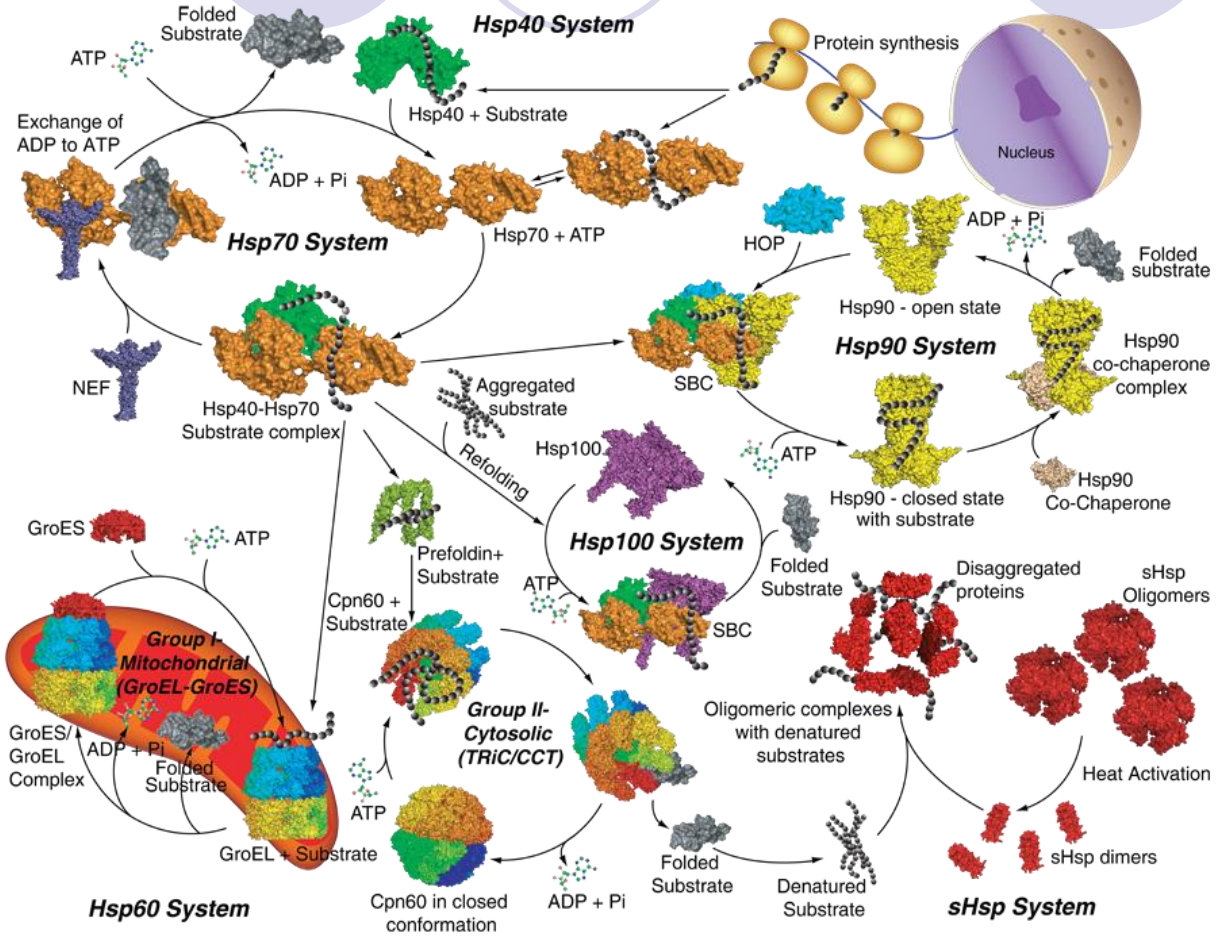
https://kampmannlab.ucsf.edu/proteostasis-network

Proteostatic net in mammalian cells

Consisnts of ~ 1300 various proteins which participate in:

- Protein biogenesis (~**400**),
- Proteins conformation maintainance (~**300**),
- Protein degradation (~700),
- The cells are of the various proteostasis properties, so the cells have various stress sensitivity, and protein aggregation damage.

HSP classification and role

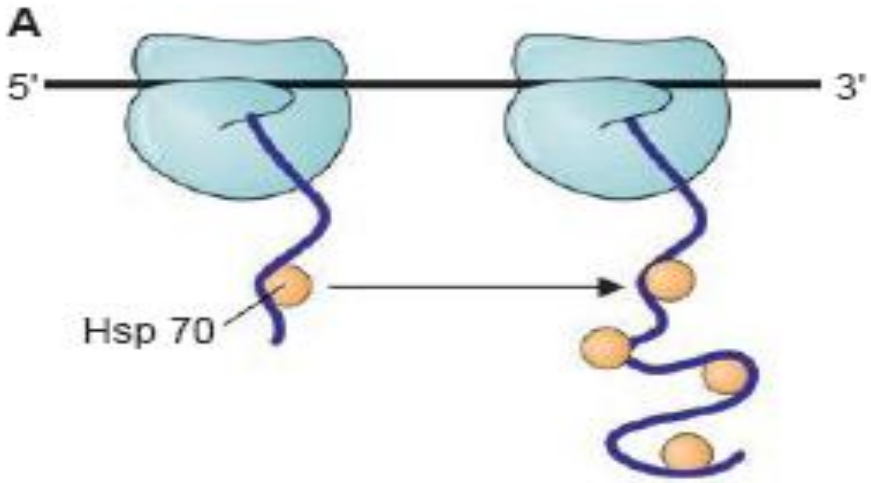


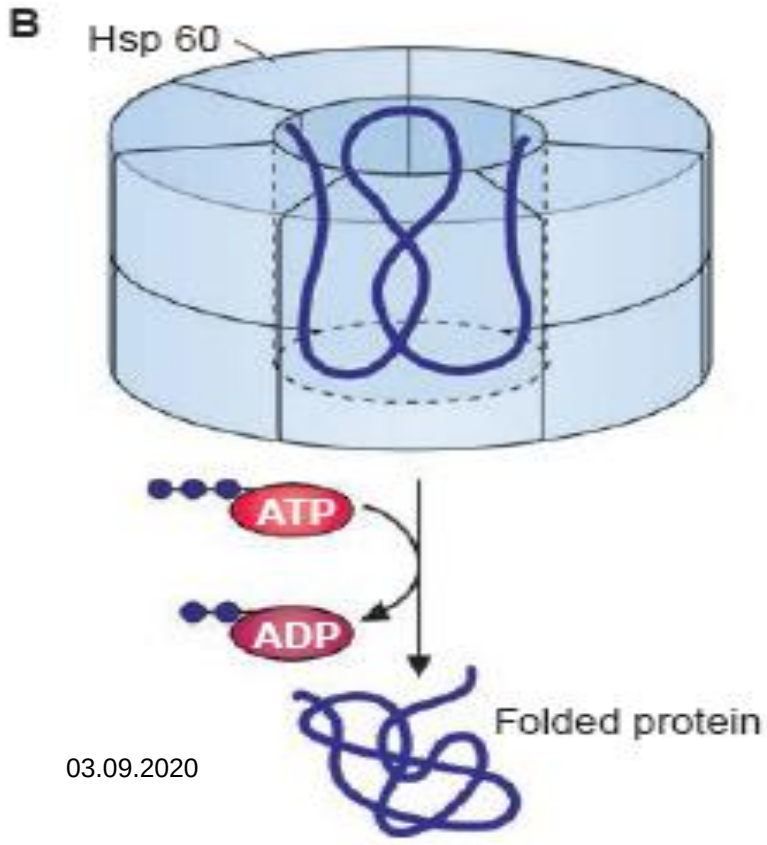
Six major Heat Shock Proteins (HSPs):

1. sHsp (HSP20),
2. Hsp40 (J-Proteins),
3. Hsp70 (DnaK/Ssa),
4. Hsp60 (Chaperonin),
5. Hsp90 (HtpG) and
6. Hsp100 (Clp).

http://pdslab.biochem.iisc.ernet.in/hspir/index.php

The role of HSP in biosynthesis and folding of proteins





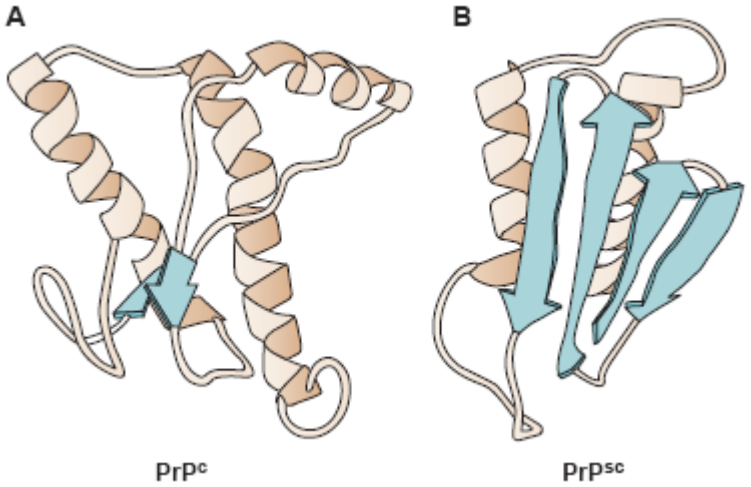
A. HSP 70 prevents protein misfolding during the biosynthesis.

B. HSP 60 (chaperonines) protein folding is ATP-driven.

Protein folding pathology *(misfolding)*

- Alzheimer disease
- Parkinsonism
- α1-antitripsin deficiency
- Prion diseases (*prion - proteonaceous infectious particle*) (kuru, Creutzfeld-Jacob disease, “mad cow” disease).

The conformation of prion protein in norm (A) and pahology (B)



Is coded by the same gene as for the normal analog, but the protein have different conformations:

- PrP^{C} (*Prion protein cellular*) - Normal protein has many α-helices,
- PrP^{Sc} (*Prion protein scrapie*) – pathological prion, has many β-sheets,
  - Proteolytically resistant: therefore accumulated in the cell.

Alzheimer's Disease

- Progressive senile dementia with complete loss of cognitive and intellectual abilities.
- Amyloid is a product of pathological partial proteolysis of APP (amyloid precursor protein).
- Refolding or misfolding of endogenous protein from human brain tissue.
- Accumulation of amyloid causes conformational rearrangement of soluble highly α-helix state to the state of rich β-layers prone to autoaggregation.
- Apo E is a potential mediator of conformational transformation.

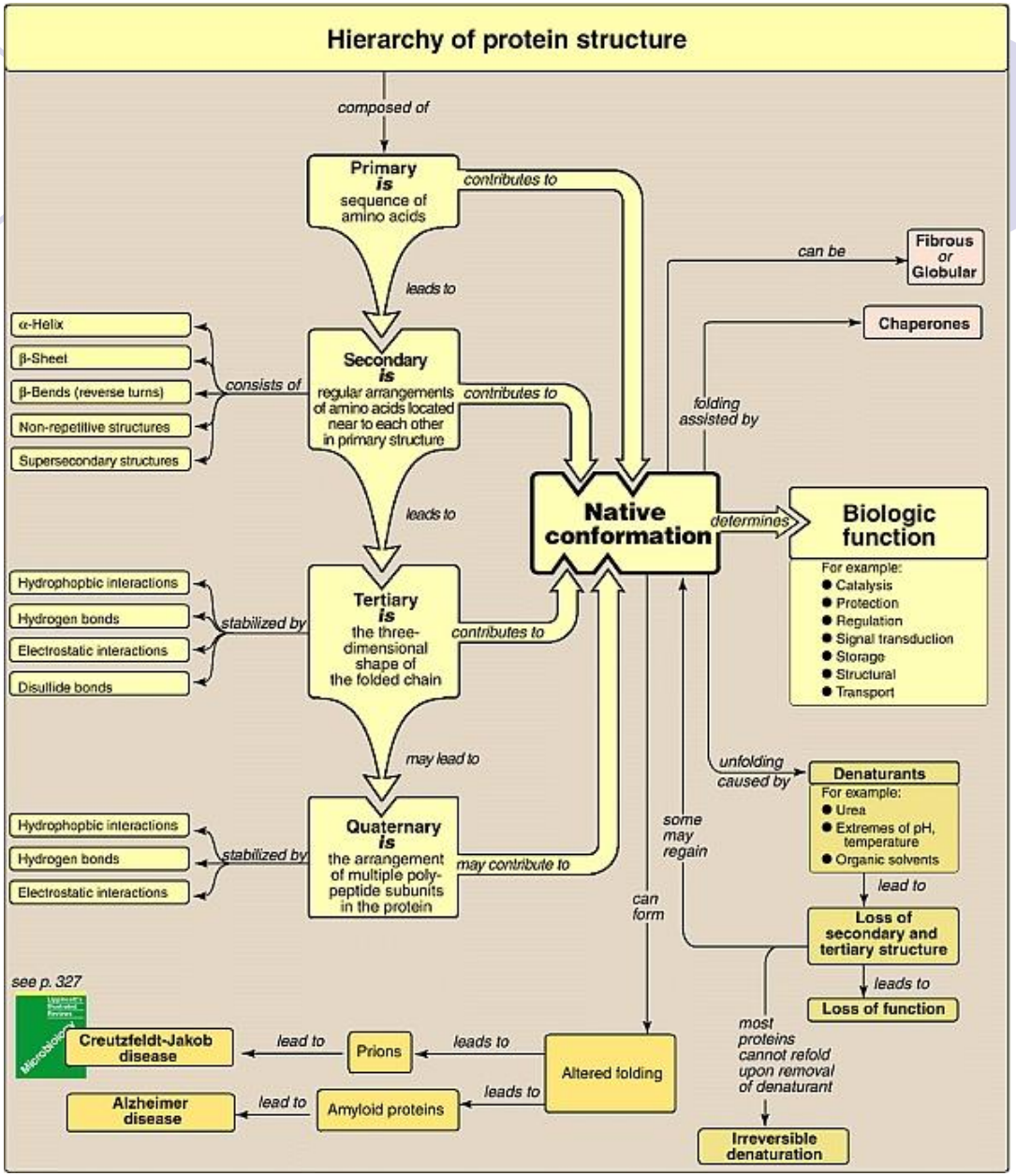
Pathology of Protein Folding

- The primary structure of the protein guarantees a stable conformation, but some hydrophilic proteins can change it and lose solubility, forming insoluble aggregates of white color - **amyloid** stained with **iodine**.

Causes:

- overproduction of protein,
- increased protein breakdown,
- the formation of insoluble decay products, changing its conformation,
- getting into the cell of proteins, changing the conformation of its protein.
- point mutations in protein structure.

Conclusion


Hierarchy of protein structure
composed of
Primary
is
sequence of amino acids
contributes to
leads to
can be
Fibrous or Globular
Chaperones
folding assisted by
α-Helix
β-Sheet
β-Bends (reverse turns)
Non-repetitive structures
Supersecondary structures
consists of
Secondary
is
regular arrangements of amino acids located near to each other in primary structure
contributes to
leads to
Native conformation
determines
Biologic function
For example:
Catalysis
Protection
Regulation
Signal transduction
Storage
Structural
Transport
Hydrophobic interactions
Hydrogen bonds
Electrostatic interactions
Disulfide bonds
stabilized by
Tertiary
is
the three-dimensional shape of the folded chain
contributes to
may lead to
unfolding caused by
Denaturants
For example:
Urea
Extremes of pH, temperature
Organic solvents
lead to
Loss of secondary and tertiary structure
leads to
Loss of function
some may regain
can form
Hydrophobic interactions
Hydrogen bonds
Electrostatic interactions
stabilized by
Quaternary
is
the arrangement of multiple polypeptide subunits in the protein
may contribute to
see p. 327
Creutzfeldt-Jakob disease
lead to
Prions
leads to
Altered folding
Alzheimer disease
lead to
Amyloid proteins
leads to
most proteins cannot refold upon removal of denaturant
Irreversible denaturation

The End of the Lecture
«Introduction to Biochemistry»

The next lecture is «Enzymes 1. The Structure and Properties»