Aris Kaksis 2019. year Riga Stradin's University

Bioorganic compounds Proteins

http://aris.gusc.lv/NutritionBioChem/38Olbalt10311Eng.doc

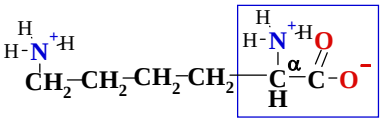
Key terms. Bioorganic compounds L-α-amino acids, peptides, classification, building, functional groups. Optical isomers of L- **α**-amino acids. Opened, branched, cyclic and aromatic carbon chains.

Bioorganic amino acids are carbon-carbon C-C-C-C-C-C chains

Bioorganic amino acids form carbon atoms combinatorics 2,3,4,5,6 of carbon atoms chains linear, branched, cyclic and aromatic.
Carbon atoms form the functional groups as are bound to atoms of oxygen **C**–**O**-, of nitrogen **C**-**N**< and of sulfur **C**–S-.

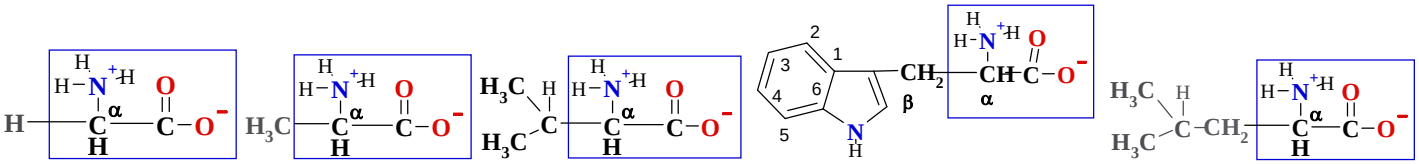
Six carbon **-C-C-C-C-C-C-** chain

L- **α**-lysine



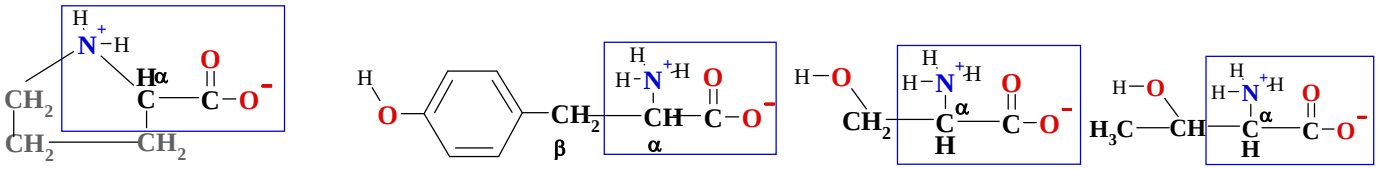
Amino acids carbon atoms chains **-C-C-C-C-C-C-** with branches

Two carbons **-C-C-**; Three **-C-C-C-**; Four **-C-C-C-C-**; **-C-C-C-C-C-** chain & benzoyl ring; Five **-C-C-C-C-C-**



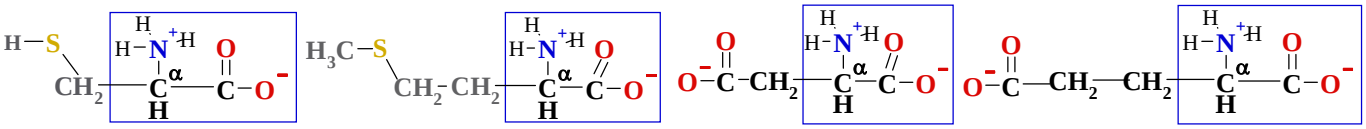
L- **α**-glycine L- **α**-alanine; L- **α**-valine; L- **α**-tryptophan; L- **α**-leucine

Five cyclic-**C-C-C-C-C-**;**-C-C-C-**chain plus phenol-**OH** cycle;**-C-C-C-**chain with-**OH**;**-C-C-C-C-**chain with-**OH**



L- **α**-proline L- **α**-tyrosine; L- **α**-serine L- **α**-threonine

Three-**C-C-C-** on chain--SH; Four-**C-C-C-C-** on chain **CH₃-S-**;**-C-C-C-** with **-C=OO⁻**;**-C-C-C-C-** with **-C=OO⁻**



L- **α**-proline L- **α**-tyrosine; L- **α**-aspartate L- **α**-glutamate

Carbon atoms sequence linear, cyclic and aromatic chains of carbon atoms combinatorics 2, 3, 4, 5, 6 reflect bioorganic amino acids molecular nano motors variation adaptation diversity for fitness to life processes circumstances in organism homeostasis (see 11.page combinations $1{,}9 \cdot 10^{240}$ of 184 amino acids on polypeptide).

Six type functional groups in compounds of carbon atoms with oxygen **C**–**O**-, nitrogen **C**-**N**< and sulfur **C**–S-.

1) Karbonic acids group **-C=OOH** is deprotonated H^++ **-C=OO⁻** negative charge, because physiologic pH is 7,36;

2) Amino group **–NH₂** is protonated **-NH₃⁺** positive charge cation because physiologic pH is 7,36;

3) Hydroxil group **–OH**;. 4) Sulfur hydro group -SH; Methionine **CH₃-S-**;

5) Aliphatic non polar group; 6) Aromatic planar non polar group.

21 L-α-Amino Acids proteins polypeptide

47 protolysis pK_a values and average pK_a **isoelectric point IEP** value

At physiologic pH=7, 36 ±0.01 carboxylic groups **R-COO⁻** negative charged and amino groups **R-NH_3^+** positive charged. For example, glutamic acid pK_a reference to physiologic pH value smaller as physiologic pH: $pK_{aR\text{-}COO^-}$=4.25<7.36, pK_{aCOO^-}=2.19<7.36 and for amine is greater as physiologic pH: 9.67=$pK_{a\text{-}NH3+}$>7.36 .

Table shown constants pK_a of four type parallel protolytic equilibria in each amino acid molecule:

acid ⇔ base $+H^+$;
1. **R-COOH** ⇔**R-COO⁻** $+H^+$;
2. **R-NH_3^+** ⇔**R-NH_2** $+H^+$;
3. **Tyr**-phenol-**OH**⇔**Tyr**-phenol-**O⁻** $+H^+$,
4. Cys-SH ⇔Cys-**S⁻** $+H^+$

Parallel protolytic equilibria number NpKa average isoelectric point and constant pK_a value IEP= pK_a is calculated as

IEP= pK_a= (Σ $pK_{a\ R\ group}$+ $pK_{a\text{-}NH3+}$+ $pK_{a\text{-}COOH}$)/NpK$_a$

In *Ostwald's dilution law*: pH= $\frac{pK_a - \log C}{2}$ =.....

Amino acid and protein at isoelectric point value pH=**IEP** sum of total overall **ion** charge is zero

0—— acidic charge (+)————zero „**0**" charge **IEP**————in basic medium charge minus (-)——→pH scale

-COOH & **-NH_3^+** positive charge .....**-COO^-** & **-NH_3^+**..... charge is negative **-COO^-** & **-NH_2**

Amino Acid	pKa-COOH	pKa-NH3+	pKa R group
Isoleucine	2.36	9.68	
Valine	2.32	9.62	
Leucine	2.36	9.60	
Phenylalanine	1.83	9.13	
Cysteine	1.96	10.28	8.18
Methionine	2.28	9.21	
Alanine	2.34	9.69	
Proline	1.99	10.96	
Glycine	2.34	9.60	
Threonine	2.11	9.62	
Serine	2.21	9.15	
Tryptophan	2.38	9.39	
Tyrosine	2.20	9.11	10.07
Histidine	1.82	9.17	6.00
Aspartate	1.88	9.60	3.65
Glutamate	2.19	9.67	4.25
Asparagine	2.02	8.80	
Glutamine	2.17	9.13	
Lysine	2.18	8.95	10.53
Arginine	2.17	9.04	12.48

Table5.3 Reginald H. Garrett, Charles M. Grishman, **Biochemistry**, University of Virginia 1995

Myoglobin IEP=7,36 is neutral zero „**0**" charged molecule, as IEP=7,36 is equal physiologic pH_{blood}=7,36 1MBO.pdb

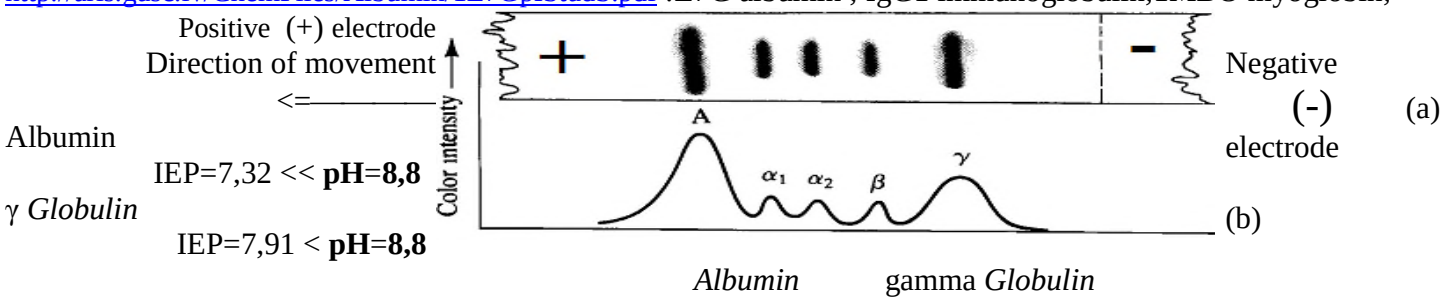
Albumin molecule E7G.pdb 7,32=IEP 7 fatty acids small (–) charge and 7,40=IEP absent 7 fatty acids (+) positive at physiologic pH=7.36, but gamma *Globulin* IgG1.pdb molecule has positive (+) charge, as at physiologic pH=7.36 is greater IEP=7.91.

Iso electric point IEP=pK_a as well protolytic constant pK_a calculates one of side residues R constants sum Σ$pK_{aRside\ residue}$ plus $pK_{aNterminusNH3+}$ and plus $pK_{aCterminusCOO-}$ sum dividing with number NpKa of acidic groups in molecule

IEP=pK_a=(Σ$pK_{aR\ side\ residue}$+$pK_{aNterminus}$+$pK_{aCterminus}$)/NpKa

Figure Separation of serum proteins by **electrophoresis**. a) A sample is applied as a narrow line at the origin. After **electrophoresis** at pH **8.8**, the paper is dried and stained. b) A plot of color intensity of spots. γ *Globulin* slower *Albumin*. **Proteins** move this direction← spot line sample origin at start

http://aris.gusc.lv/ChemFiles/Albumin/1E7GpIStudS.pdf !E7G albumin ; IgG1 immunoglobulin;1MBO myoglobin;



Seleno cysteine, the 21st L-α-Amino Acid

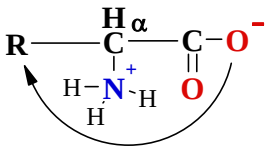
Seleno cysteine is an L-α-amino acid found in a handful of proteins, including certain **peroxidases** and **reductases** where it participates in the catalysis of electron transfer reactions.As its name implies, a selenium **Se** atom replaces the sulfur **S** of its structural analog, cysteine. The pK_3 of seleno cysteine 5.2 is 3 units lower than that of cysteine 8.18. Since seleno cysteine is inserted into polypeptides during translation, it is commonly referred to as the "21st amino acid." However, like the other 20 genetically encoded amino acids, seleno cysteine is specified by a simple three-letter codon **UGA** (see class 16 week Nucleo proteins tRNA 62 codons).

Fisher projections for Santa Barbara University 3D L-α-amino acids

http://aris.gusc.lv/ChemFiles/MCDB108A/tw-amn/aasframes.htm

Harper's Biochemistry Illustrated Table 3-1 on 15-16 page: projection of D-α-amino acids, which Not found in human organism proteins.

D-α-amino acids



CW Clock Wis
Dexter rotational

Fisher projections of L-α-amino acids

CCW Counter Clock Wise
Levos rotational

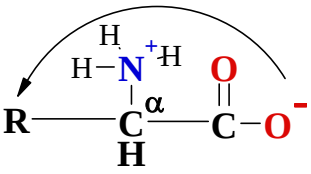


Table The 20 common L-α-amino acids found in protein. Physiologic pH=7.36 .

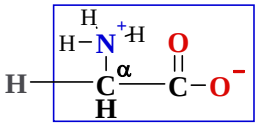
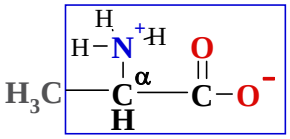
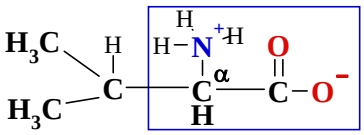
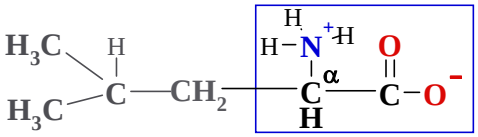
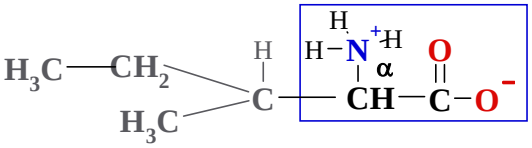
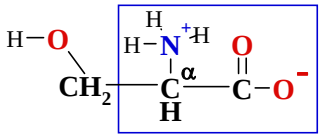
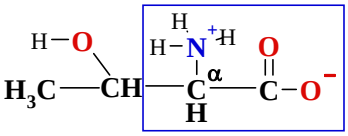
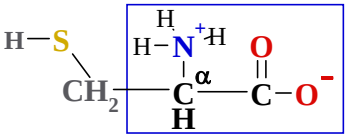
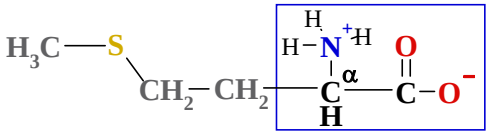
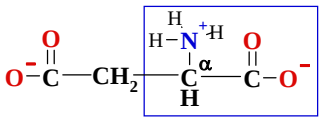
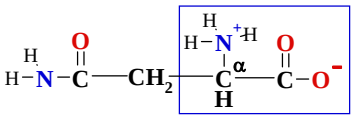
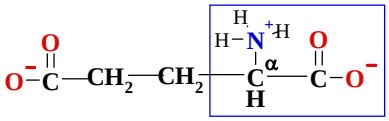
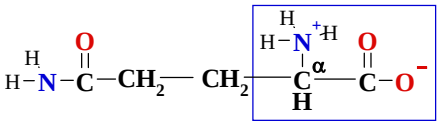
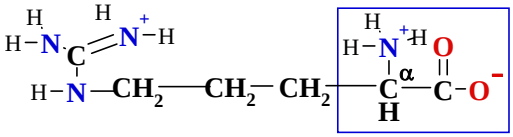
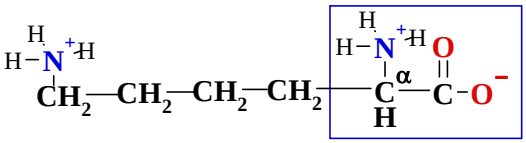
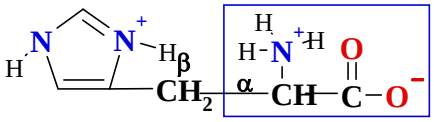
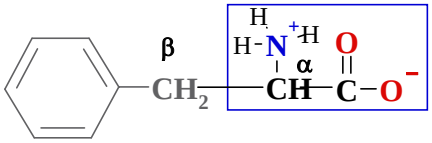
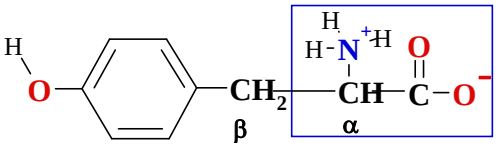
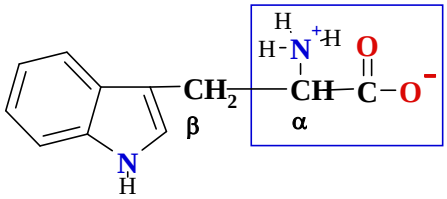
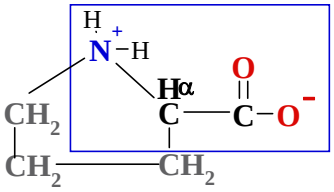
Protein-derived Amino Acids with **aliphatic** side chains left side	**Name**	**Symbol**	**Show Fisher projection Structural Formula**
1	Glycine	Gly [G]	
2	Alanine	Ala [A]	
3	Valine	Val [V]	
4	Leucine	Leu [L]	
5	Isoleucine	Ile [I]	
With side chains containing **hydroxyl (—OH)** groups left side			
6	Serine	Ser [S]	
7	Threonine	Thr [T]	
18	Tyrosine	Tyr [Y]	Shown below ↓.
With side chains containing **Sulfur** atoms **(—S— ; —SH)** left side			
8	Cysteine	Cys [C]	
9	Methionine	Met [M]	

Table The 20 common L-α-amino acids found in protein. . Physiologic pH=7.36 .

	Name	Symbol	Show Fisher projection Structural Formula
With side chains containing **Acidic (—COO⁻)** groups or their **Amides (—CO—NH₂)**			
left side 10	Aspartate Aspartic acid salt	Asp [D]	
11	Asparagine	Asn [N]	
12	Glutamate Glutamic acid salt	Glu [E]	
13	Glutamine	Gln [Q]	
With side chains containing **Basic (—NH$_n^{(+)}$) Groups**			
left side 14	Arginin	Arg [R]	
15	Lysine	Lys [K]	
16	Histidine	His [H]	
Containing **Aromatic** Rings 16	Histidine	His [H]	Shown above ↑
left side 17	Phenylalanine	Phe [F]	
18	Tyrosine	Tyr [Y]	
19	Tryptophan	Trp [W]	
Imino Acid 20	Proline	Pro [P]	

:Hexa peptides from _2s1 to _2s7 of 20 amino acids http://aris.gusc.lv/NutritionBioChem/LW_protein_2s1.pdf

Protein synthesis Primary 1° structure Terms. Poly condensation reaction in ribosomes. Protein primary 1° polypeptide chain begin at **N**-terminal and finish at C-terminal. First amino acid free amino group in water medium protonate $^{+}\mathbf{H_3N}$-AA charge is positive and last amino acid free carboxylic group AA-**COO**$^{-}$ at finish charge is deprotonate negative.

Calculate ΔH_r, ΔS_r, ΔG_r at standard conditions 298,15 K, ionic force 1 M, pH=7,36. Peptide synthesis poly condensation enzyme ribosome reaction of glycine Gly (G) amino acid using table data! Consider reaction is **exoergic** or **endoergic**! glycine + glycine → glycil-glycine dipeptide synthesis

Substance	$\Delta H°_r$, $^{kJ}/_{mol}$	$\Delta S°_r$, $^{J}/_{mol/K}$
Gly$_{aqua}$	−554.56	76.45
Gly- Gly$_{aqua}$	-790.99	-1
H_2O	-285.83	69.9565
I=0 M	**I=0,1 M**	I=0.2 M
-180.13	**-177.07.**	-176.08
-200.55	**-195.65**	-194.07
-213.275	-213.275	213.275

Gly$_{aqua}$ + **Gly**$_{aqua}$ +**Q** +ΔG → $\xrightarrow{Ribosome}$ → **Gly-Gly**$_{aqua}$+ **H_2O**

1. $\Delta H_{reaction} = \Sigma\Delta H°_{products} - \Sigma\Delta H°_{initial\ compounds}$
2. $\Delta S_{reaction} = \Sigma\Delta S°_{products} - \Sigma\Delta S°_{initial\ compounds}$
3. $\Delta G_{reaction} = \Delta H_{reaction} - T\bullet\Delta S_{reaction}$

Ionic force 0 M, 0,1 M, 0,2; M formation $\Delta G°_{H2O}$ =-213,27 $^{kJ}/_{mol}$

Gly$_{aqua}$ formation; $\Delta G_r = \Sigma\Delta G°_{products} - \Sigma\Delta G°_{initial\ compounds}$

Gly- Gly$_{aqua}$; ΔG_r=-200,5-213,275-(2*-180,13)=-53.515..... $^{kJ}/_{mol}$

H_2O;ΔG_r=-195.65-213,275-(2*-177.07)= -54,785..... $^{kJ}/_{mol}$

ΔG_r= -194,07-213,275 (2*-176,08)= -55,185..... $^{kJ}/_{mol}$ **endoergic**.....

$\Delta H_r = \Delta H°_{Gly-\ Gly} + \Delta H°_{H2O} - 2\Delta H°_{Gly}$=..... $^{kJ}/_{mol}$ endothermic.....

=-790.99-285.83-(2*-554.56)=-1076.82+1109.12=32.3..... $^{kJ}/_{mol}$ endothermic.....

$\Delta S_{dispersed} = -\Delta H_r / T$ =-32.3/298.15= -108.335..... $^{J}/_{mol/K}$

$\Delta S_r = \Delta S°_{Gly-\ Gly} + \Delta S°_{H2O} - 2\ \Delta S°_{Gly}$ =78+69.9565-(2*108.2)=147.9565-216.4= - 68.4435..... $^{J}/_{mol/K}$

3. $\Delta S_{total} = \Delta S_r + \Delta S_{dispersed}$=-83,944-108,335= -192,279..... $^{J}/_{mol/K}$

$\Delta G_r = \Delta H_r - T*\Delta S_r$ =32,3-298,15*-0,083944=32,3+25,0279=57,328..... $^{kJ}/_{mol}$ **endoergic**.....

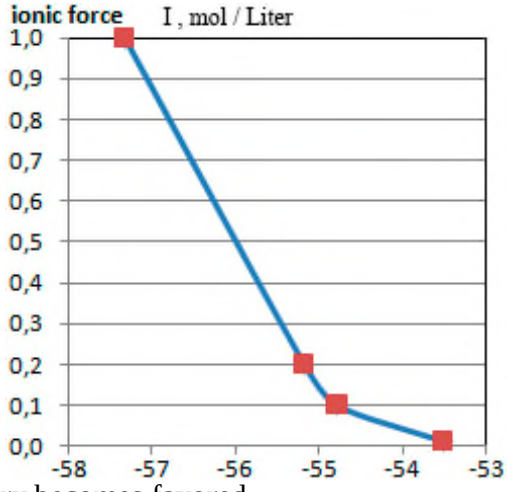
$T\bullet\Delta S_{total}$ =-192,279 $^{J}/_{K/mol}\bullet$298,15 K= **-57,328**..... $^{kJ}/_{mol}$ bound in peptide bond.....TΔS

Total ions concentration sum-ionic force I in measurement is 1 M and unfavored, endoergic reaction ΔG_r=57,328 $^{kJ}/_{mol}$.

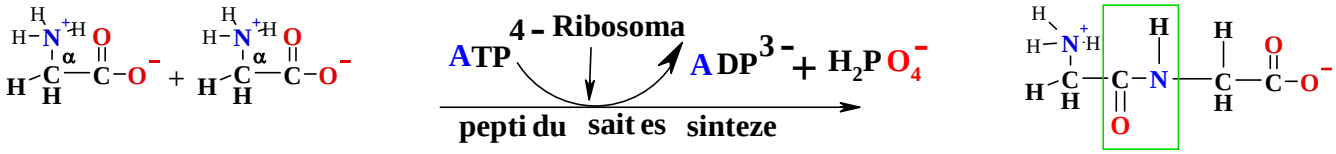
Chem. Phys. CRC, 2010, p.876,882,1220,1223

Free ATP^{4-} energy ΔG_{reac} =+57,3..... $^{kJ}/_{mol}$ transfer and accumulation in peptide bond at protein formation in **ribosomes**: **gly + gly→gly-gly+ H_2O.**

One mol of peptide bonds accumulates free energy ΔG_{reac}=+57,3..... $^{kJ}/_{mol}$. Ribosome join in tandem peptide synthesis with ATP hydrolysis, that transfer free energy -115,71 $^{kJ}/_{mol}$ and store $\Delta G_{reaction}$=+57,3..... $^{kJ}/_{mol}$ in one mol of peptide bonds during the reaction.

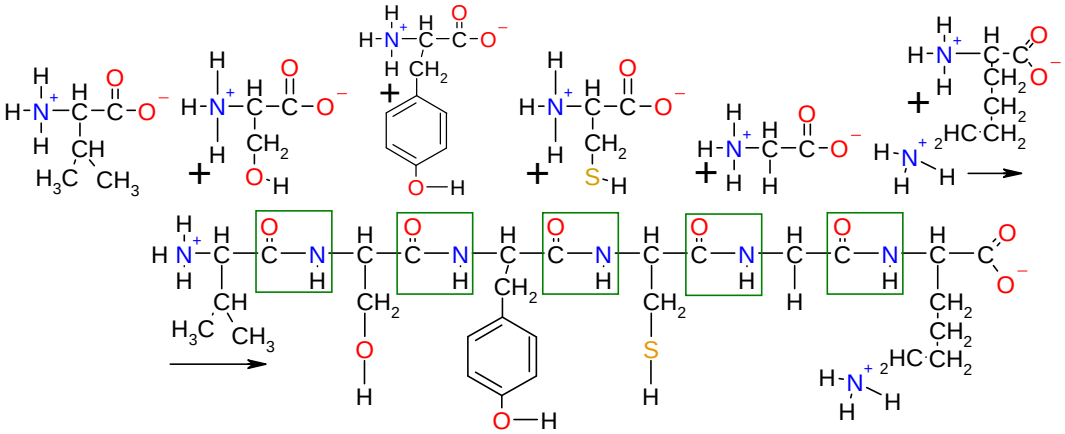


ATP hydrolise is favored ΔG = -115,71 $^{kJ}/_{mol}$ and complex reaction summary becomes favored....., because $\Delta G_{reaction} < 0$ negative, favored..... $\Delta G_{reaction}$ =+57,3 - 115,71= -58,4..... $^{kJ}/_{mol}$:



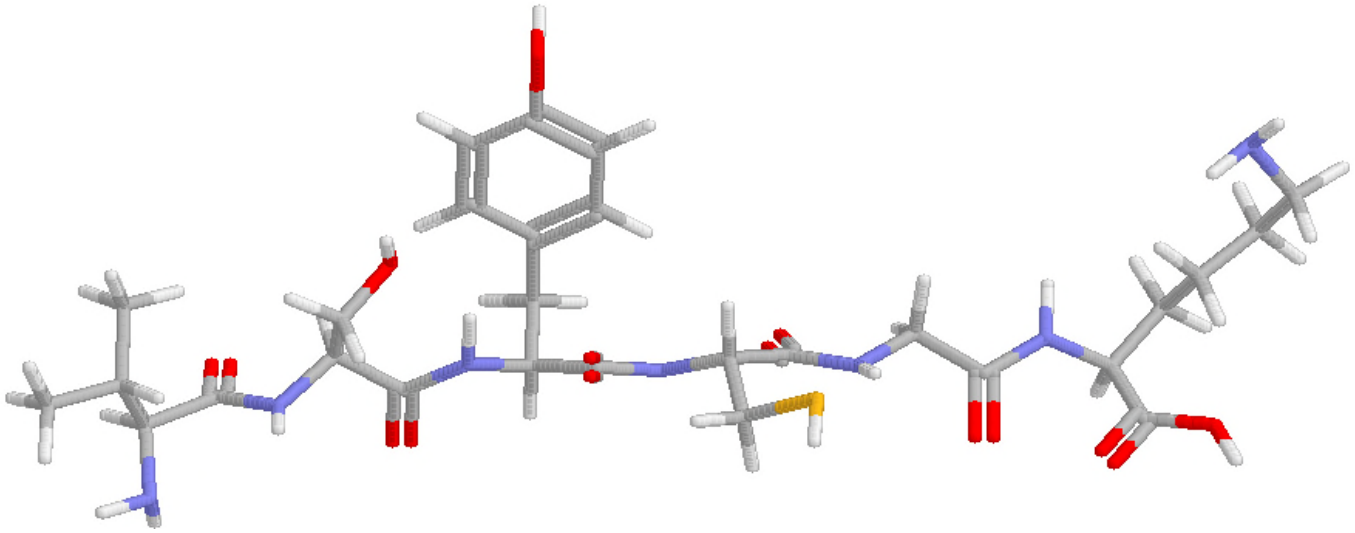
1. Hexa peptide ribosomal synthesis-poly condensation from six amino acids

Starting of N-terminus $^{+}\mathbf{H_3N}$-Val,Ser,Tyr, Cys, Gly, Lys-$\mathbf{COO^-}$ end with C-terminus.



+ 5 H_2O

A63D.tgf

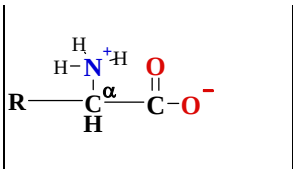


N-terminus amino acid is Val1-Ser2-Tyr3-Cys4-Gly5-Lys6 is C-terminus amino acid.

Amino acid or protein molecules have four type acidic functional groups: **-COOH** neutral, $\mathbf{-NH_3^+}$ positive charged, phenol-**OH** neutral, **-SH** neutral.

Functional acidic groups are involved in four parallel protolytic equilibriums:

Acid	⇔	base	+ proton	amino acid
1. **R-COOH**	⇌	**R-COO$^-$**	+**H$^+$**,	
2. **R-NH$_3^+$**	⇌	**R-NH$_2$**	+ **H$^+$**	
3. **R**-phenol-**OH**	⇌	**R**-phenol-**O$^-$**+**H$^+$**,		
4. **R--SH**	⇌	**R—S$^-$**	+ **H$^+$**	



Blood concentration $[\mathbf{H^+}]=10^{-7,36}$ M at pH=7.36 value .

At physiologic pH 7.36 four type groups exist prevailing as: negative charged **R-COO$^-$**, positive charged amino groups **R-NH$_3^+$** , neutral group of Tyrosine phenol-**OH** and Cysteine sulfur hydrogen **R--SH**.

Parallel net reaction equilibrium constant as well isoelectric point of functional groups for the same molecule IEP=$pK_{netConstant}$ constants sum average is:

$$IEP=pK_{netConstant}=\frac{\sum pKa_{Rgroup}+pKa_{N\,ter\,min\,us\,NH_3^+}+pKa_{C\,ter\,min\,us\,COO^-}}{NpKa}.$$

where NpKa is the acidic functional groups account number in one molecule.

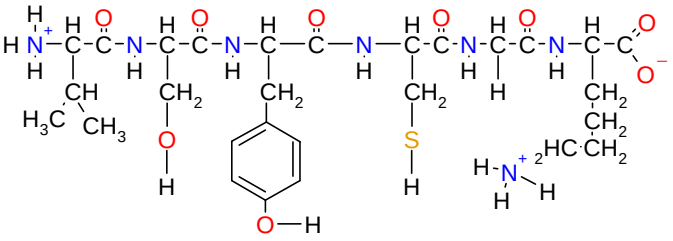
Net *Ostwald's* dilution law: $[\mathbf{H^+}]=\sqrt{K_{netConstant}\bullet C}=10^{-\mathbf{pH}}$ **M** molarity. Hydrogen ion net production amount expressed as pH value: $pH=\frac{\mathbf{pK}_{netConstant}-\log \mathbf{C}}{2}$

2. Calculate net reactions average Equilibria constant IEP=$pK_{netConstant}$ hexa peptide

Val1I N-terminus-Ser2-Tyr3-Cys4-Gly5-C-terminus Lys6, net charge of molecule and pH of hexa peptide solution with concentration C=0.1 M!

Nr	Amino Acid	pK_{COOH}	pKaR $pKNH_3^+$
1	Valine	9,62	.....
2	Serine		.....
3	Tyrosine		10,07
4	Cysteine.		8,18
5	Glycine		.....
6	Lysine	2,18	10,53

Asn1 N-terminus-Met2-Ile3-Trp4-Ala5- C-terminus Lys6



NpKa=5..... 27,87+12,71=40,58

$IEP=pK_{netConstant}=(\Sigma pKa_{Rside\ residue}+ pKa_{NterminalNH3+}+ pKa_{CterminalCOO-})/NpKa=$

=(10.07.....+ 8.18…..+9.62…...+10.53.....+2.18.....) / 5.... = 40.58..../5.... = 8.116....

Underline and determine existing charge: positive (+) or negative (-) or zero "0"!

-COOH & **-NH_3^+** positive charge ...**-COO^-** & **-NH_3^+**...charge is negative **-COO^-** & **-NH_2**

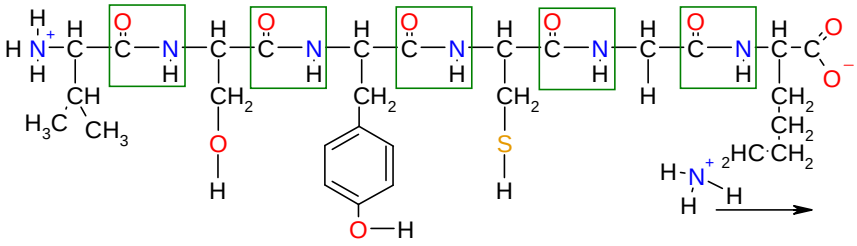
physiologic value of blood pH = 7.36 < 8.116.....= pK_a=IEP

$$pH = \frac{pK_{netConstant} - \log C}{2} = \frac{8{,}116 - \log 0{,}1}{2}$$ =(8,116.....-log0,1.....)/2....=

=(8,116.....+1.....)/2...=9,116...../2.....=4,558...

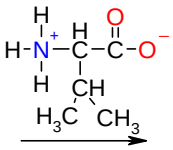
3. Hexa peptide hydrolyse reaction governed by enzyme hydrolase

N-terminus amino acid is Val, Ser, Tyr, Cys, Gly, Lys is C-terminus amino acid by hydrolyse are separated to six free amino acids. In hydrolyse reaction separate six free amino acids and give the names for!



+ 5 H_2O

=>



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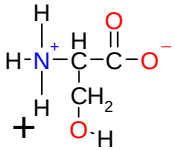

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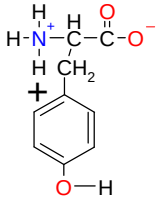
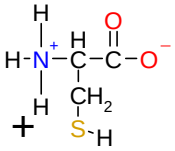


+

.....Valine..... Serine.....Tyrosine.....



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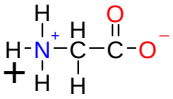

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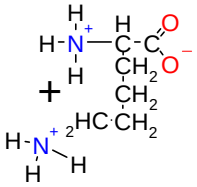


…. Cysteine.....Glycine.....Lysine.....

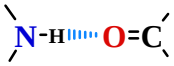
Theoretical concepts and key terms.

Structural stabilization of biomolecules as well proteins supported by five intermolecular forces:

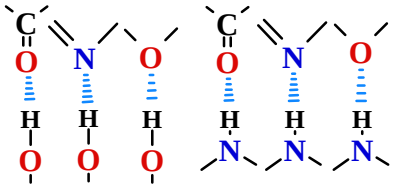
1st hydrogen bonds; 2nd salt bridges; 3rd hydrophobic bonds; 4th coordinative bonds; 5th disulphide bonds.

1st Linus Pauling and Robert Corey in beginning 1939 assumed that in proteins conformations of greatest stability is because:
(1) all atoms in a peptide bond lie in the same plane and
(2) each amide group is hydrogen bond bonded with >N-**H** between the other peptide carbonyl group oxygen **O**=**C**<.
Secondary 2° structures on this bases are alpha **α** helixes and beta **β** sheets folded from primary 1° structure of polypeptide chains.
Hydrogen bond is established between oxygen **O** and nitrogen **N** atoms.

Secondary 2° structure **α** & **β** formed by hydrogen bonds



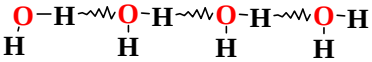
Hydrogen bond acceptor atoms are shown above and hydrogen bond donors below:



hydrogen bond acceptor atom **O** or **N**
↑
H
hydrogen bond donor atom **O** or **N**

Task 1. Hydrogen bonds in secondary 2°, tertiary 3° and quaternary 4° structures

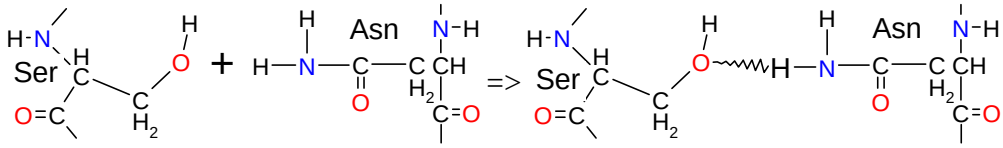
1) Write hydrogen bond formation between four water molecules:

$H_2O+HOH+H_2O+H\text{-}O\text{-}H\Rightarrow$  water tetramer $(H_2O)_4$

2) Bond in protein chains with serine Ser-O-H group and asparagine carbonyl group O=C<NH_2.:

Ser-O-H + O=CNH_2-Asn => Ser-O-H....O=CNH_2-Asn .....

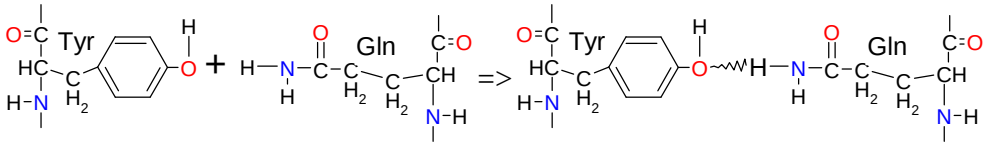
3) Bond in protein chains with serine Ser-O-H and asparagine amide hydrogen H-NHC=O-Asn:



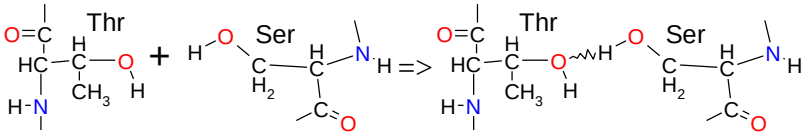
4) Bond in protein chains with tyrosine Tyr-O-H group and glutamine carbonyl group O=C<NH_2:

Tyr-O-H + O=CNH_2-Gln => Tyr-O-H....O=CNH_2-Gln .....

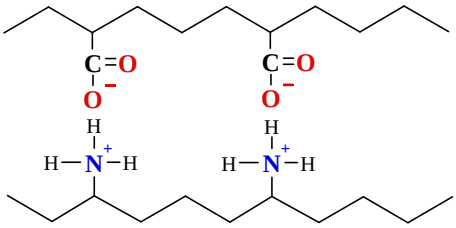
5) Bond in protein chains with tyrosine Tyr-O-H and glutamine amide H-NHC=O-Gln:



6) Hydrogen bond in protein chains with threonine and serine:



2nd Salt bridge-ionic bond forms between negative charged carbonic acid and positive charged ammonium functional groups —COO^-…$H_3{}^+N$—. Salt bridges are forming in polypeptide tertiary 3° and quaternary 4° protein structures to folding secondary structure units of alpha **α** helix or / and beta **β** sheet.



negative charged carboxyl groups

\- -

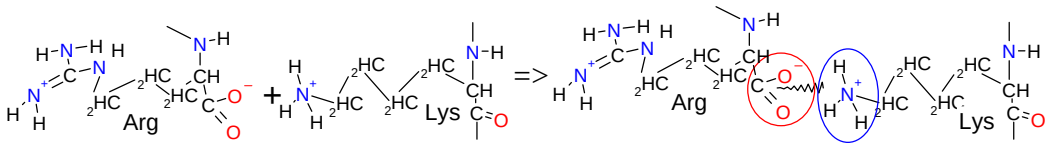
\+ +

positive charged ammonium groups

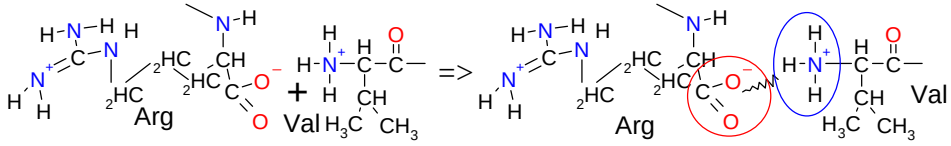
Task 2. At physiological pH=7,36

13th page: http://aris.gusc.lv/NutritionBioChem/38OlbaltEng10311.pdf

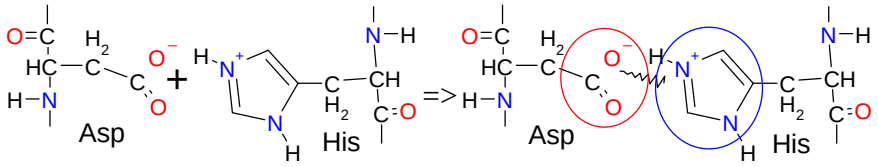
1) Write salt bridge with alpha1 Arg141 C-terminus—COO^-...**$H_3{}^+N$**— Lys127 alpha2:
alpha2 Arg141 C-terminus—COO^-...**$H_3{}^+N$**— Lys127 alpha1



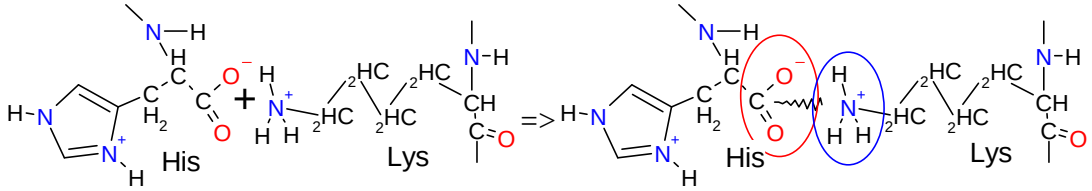
2) Write alpha1 Arg141 C-terminus—COO^-...**$H_3{}^+N$**—Val1 N-terminus alpha2
alpha2 Arg141 C-terminus—COO^-...**$H_3{}^+N$**— Val1 N-terminus alpha1



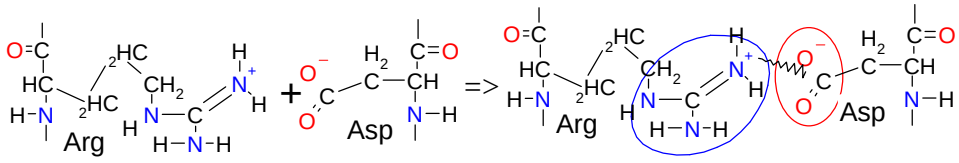
3) Write salt bridge with beta2 Asp94 C-terminus—COO^-...**$H_3{}^+N$**—beta2 His146:
beta1 Asp94 C-terminus—COO^-...**$H_3{}^+N$**—beta1 His146



4) Write salt bridge with beta2 His146 C-terminus—COO^-...**$H_3{}^+N$**—alpha1 Lys40:
beta1 His146 C-terminus—COO^-...**$H_3{}^+N$**—alpha2 Lys40



5) Write salt bridge with alpha2 Arg141—**$NH_3{}^+$**...**^-OOC**—Asp126 alpha1:
alpha1 Arg141—**$NH_3{}^+$**...**^-OOC**—Asp126 alpha2

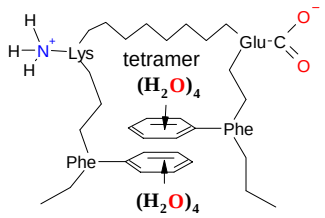


3rd **Hydrophobic <u>bond</u>** forms in water medium. Meeting two protein chains and touching residues of nonpolar amino acids, for example, phenylalanine and leucine or isoleucine, water molecules press together with force, which is ten times stronger as Van der Walls forces. Hydrophobic force influences cooling of heated gelatin water solution, which forms jelly, similar as cooked legs or hade of pig in soup, which after cooling turns into jelly or (*receklis* in Latvian), because water structure press together nonpolar amino acids under influence of hydrophobic force. Amino acids lies in adjacent chains of neighboring mutual contacting proteins (polypeptide). Hydrophobic bond forming amino acids are involved in tertiary 3° and quaternary 4° protein structure to folding secondary structure units of alpha **α** helix or/and beta **β** sheet.

Hydrophobic <u>bond</u> $(H_2O)_4 \rightarrow \Diamond\Diamond \leftarrow (H_2O)_4$ <u>water structure</u>

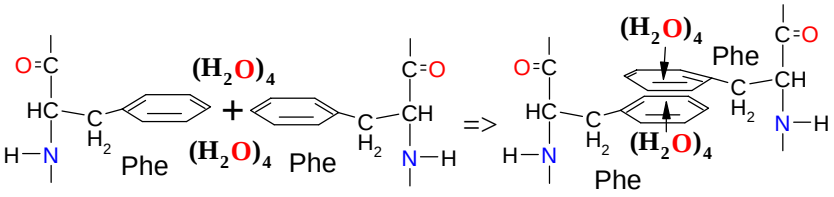
<u>press together</u>

<u>nonpolar</u> ◊ benzene residues of phenylalanine:

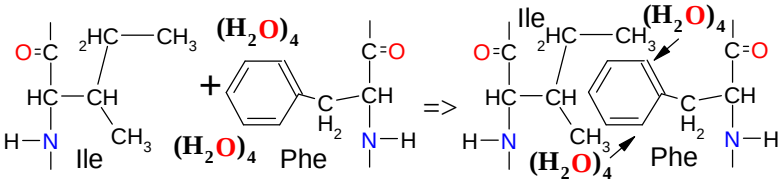


Task 3.

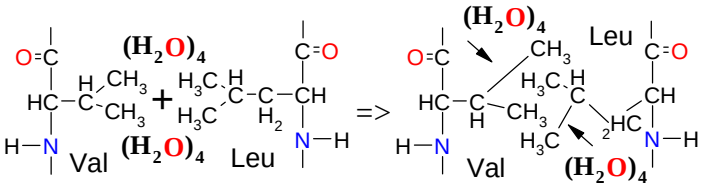
1) Write hydrophobic bond in protein chains with two phenylalanine benzene rings:



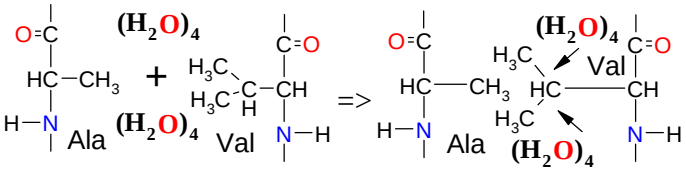
2) Hydrophobic bond in protein chains with isoleucine and phenylalanine residue:



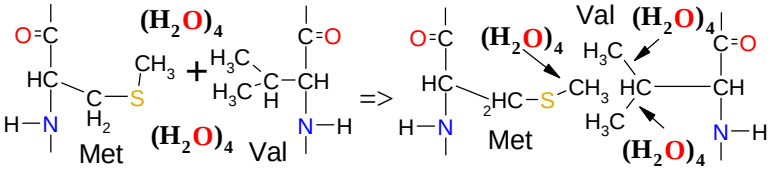
3) Hydrophobic bond in protein chains with valine and leucine residue:



4) Hydrophobic bond in protein chains with alanine and valine residue:



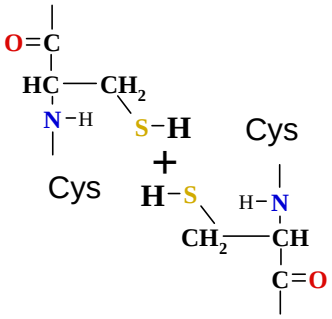
5) Hydrophobic bond in protein chains with methionine and valine residue:



4th **Disulfide bond** forms under mild oxidation conditions between two protein chains joint adjacent strands cysteines (Cys[C]) amino acids oxidizing sulf-hydryl groups removing two hydrogen atoms. Disulfide bond forming cysteine residues are found in tertiary 3° and quaternary 4° protein structure to folding secondary 2° structure units of alpha **α** helix or / and beta **β** sheet.

Cys—SH +HS—Cys => Cys—S—S—Cys +2H **Task**

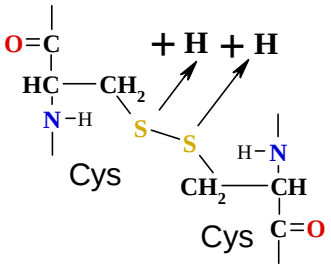
1) Write oxidation product disulfide bond between two cysteine residues :



Oxidation is removing hydrogen atoms

- 2 **H**

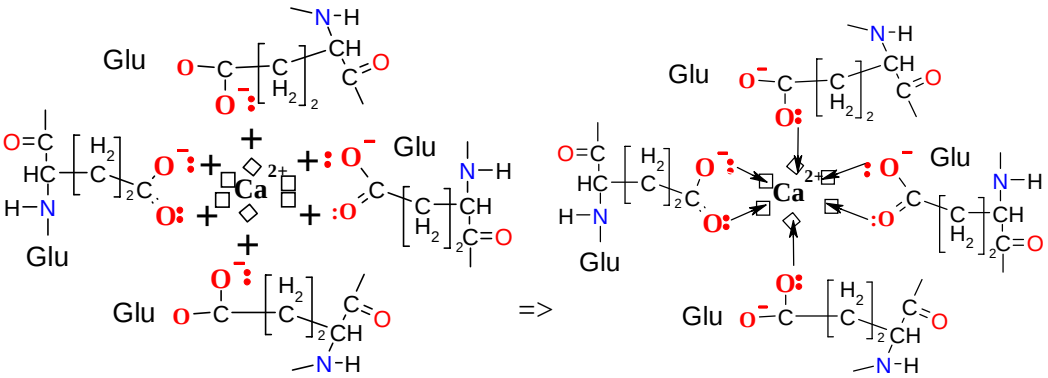
=========>



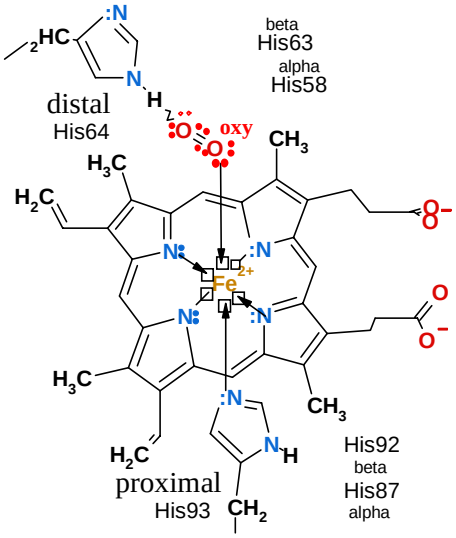
5th **Coordinative bond** form complex makers (see http://aris.gusc.lv/BioThermodynamics/CrystalloGraphy.pdf and http://aris.gusc.lv/BioThermodynamics/4KompleksiA.pdf) which are metallic ions: iron(II) ions Fe^{2+}, iron(III) ions Fe^{3+}, calcium ions Ca^{2+}, magnesium ions Mg^{2+} also zinc ions Zn^{2+} or cooper ions Cu^{2+} and others, which are acceptors of donor oxygen and nitrogen unshared electron pairs, and, which (Fe^{2+}, Fe^{3+}, Ca^{2+}, Mg^{2+}) with coordination number 6 or (Zn^{2+}, Cu^{2+}) with coordination number 4 coordinates around metallic ion 6 or 4 oxygen **O** and nitrogen **N** atoms from enveloping proteins, stabilizing tertiary 3° and quaternary 4° structure of proteins. **Coordinative** donor acceptor **bond** calcium ion with carboxyl groups of Glu —COO^-:→□Ca^{2+}□←:^-OOC— or **iron(II)** ion on center of hem O=O:→□Fe^{2+}□←:N,

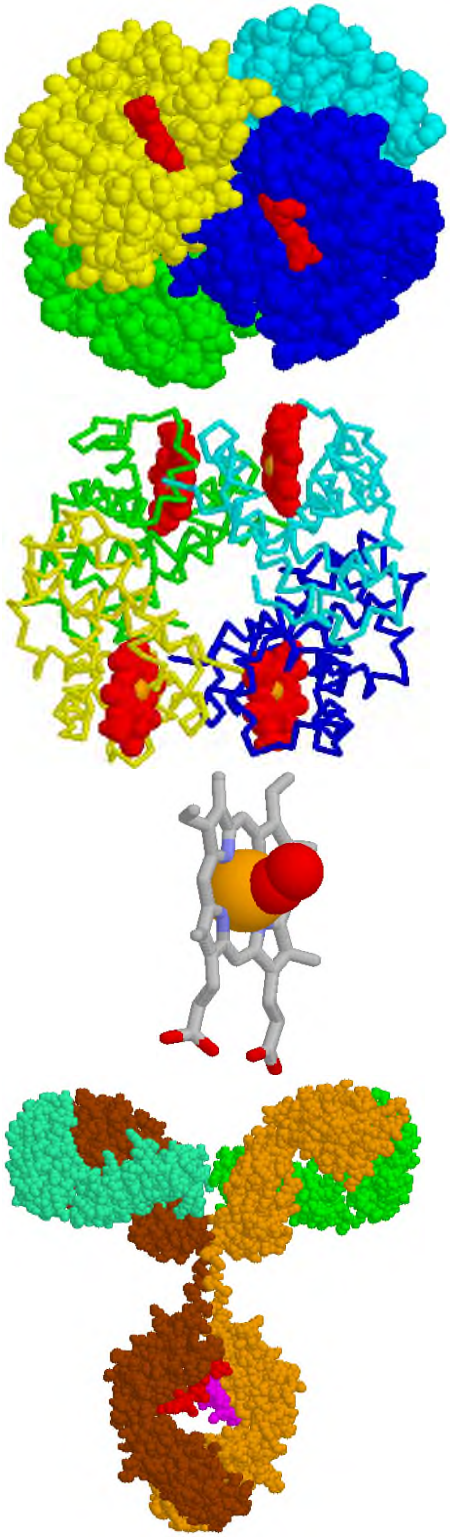
Task Ligand donor :→□ acceptor central metallic ion with empty □ electron orbital. Symbols :→□ shows donor acceptor coordinative bond features.

1)Ca^{2+} ion coordinative bonds in protein myosine with four glutamate residues:



2) Depicted 6 coordinative bonds in protein myoglobin and hemoglobin of Fe^{2+} iron(II) central ion, complex makers in heme structure with four nitrogen **N** atoms, with oxygen molecule O_2 to Fe^{2+} iron(II), with proximal histidine His93, βHis92 or αHis87 to Fe^{2+} iron(II). Distal histidine His64, βHis63 or αHis58 hydrogen bonded to oxygen O_2 molecule.



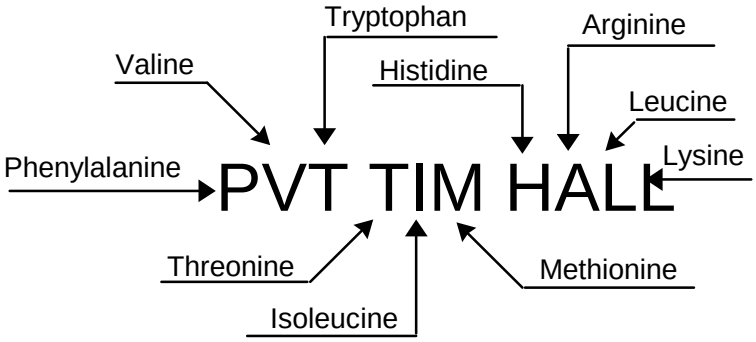


16 Fig. In human hemoglobin are build in four hems on which iron(II) atoms adsorbs four oxygen molecules, immunoglobulin body guard defense protein against infections and foreign bodies.

8 Proteins

Proteins are living nature multiform building materials, construction elements and machine tools of chemical reactions, which works like on conveyer gradually for maintenance of organism living functions. Amino acids are building blocks linking into protein chains.

Adult human body contains mass fractions 19% of proteins, which as polypeptide chains in polycondensation reactions forms 20 proteinogenic amino acids. Ten of 20 amino acids are essential amino acids, which human organism self can not synthesize, therefore essential amino acids have to uptake with nutrition. That easier remember names to recall of essential amino acids, is suggested PVT TIM HALL, what may find helpful for ten essential amino acids according its Latin name first letter:



The others ten amino acids are alanin, aspargin, aspartic acid, cystein, glutamine, glutamic acid, glycin, prolin and serine, what human organism can synthesize self.

Amino acid account on protein chains are very widely bounds from some tenth amino acids to 34000 amino acids on titin molecule. Scientists have calculated average statistic amino acids count on chain of human proteins 184 amino acids in molecule. Calculated from 20 amino acids combinations and variations number on polypeptide chain with account length 184 amino acids obtains number $1.9 \cdot 10^{240}$ which is $3 \cdot 10^{215}$ times greater as Avogadro $6 \cdot 10^{23}$ one molar number;
3. page: http://aris.gusc.lv/NutritionBioChem/32ProteinsC.pdf

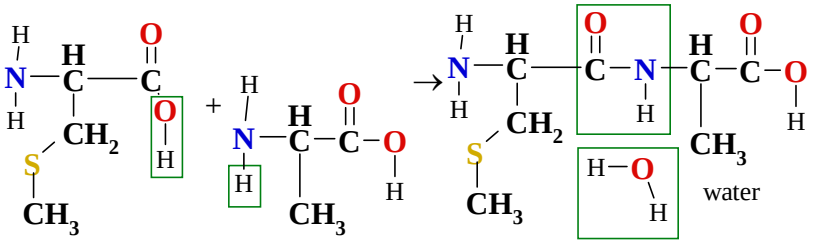
Mapping the human genome DNA (deoxyribonucleic acid) in year 2001. shows pool encoded proteins number 31078, from what ascertain in action of human body 23371 and unknown 7707 proteins.

According structure proteins classify as fibrous (threadlike) and (globe-shaped) globular proteins. Fibrous proteins are water insoluble and from such proteins are made muscles, connective tissues, hares and those fiber proteins hold bones, assign framework mechanical persistence of bone material. Globulins, for example, lipoprotein, hemoglobin, immunoglobulin are globular water soluble proteins, which carry out water insoluble lipids emulgation and transport, maintains constant oxygen concentration $C_{O_2} = 6 \cdot 10^{-5}$ mol/liter arterial blood plasma, its pH=7,36 – in this water solution, defends the blood medium from undesirable proteins or foreign bodies and infections.

9.1 Peptide bond – primary structure

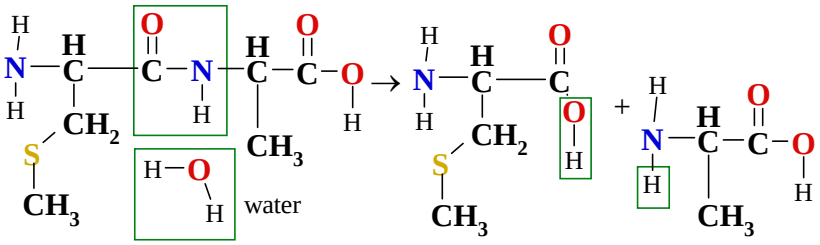
Proteins in cells synthesize ribosomes. Ribosomes are biocatalysts – combined enzymes complexes – biological sewing-machine of amino acids, which in polycondensation reaction with correct sequence of gene encoded one chain by peptide bonds bind in sequence each following of 20+1 different amino acids, forming dipeptides, tripeptides and polypeptides.

All 31078 encoded proteins synthesizes in ribosomes and first amino acid from messenger RNA molecule read methionine (Met[M]), with which start polycondensation reaction of polypeptide chain synthesis according on messenger RNA molecule encoded amino acid sequence. For example, linking to first amino acid methionine alanin produces dipeptide methioninalanin or in three letter abbreviation Met-Ala or in one letter symbol MA dipeptide and water:



methionine (Met[M]) alanin (Ala[A]) methioninalanin

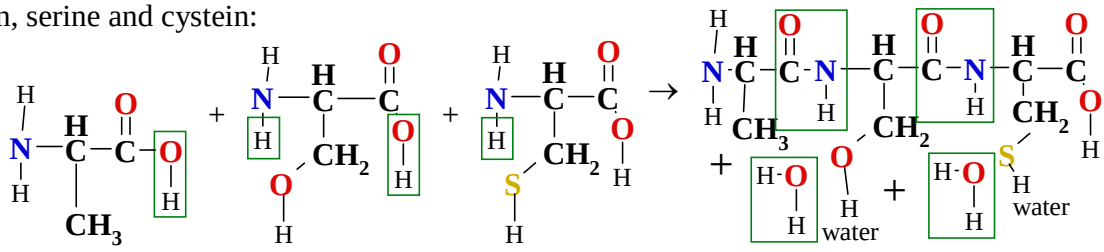
In polycondensation reaction arises water molecule, therefore the hydrolyze is reverse reaction. Hydrolyze reaction is reaction with water,



methioninalanin methionine (Met[M]) alanin (Ala[A])

in which the hydrolyze products of polypeptide-protein are free amino acids solution in water. Therefore cooking meet in soup hydrolyzes free amino acid solution in water, what calls about bouillon.

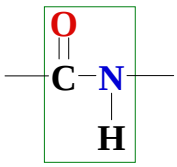
Tripeptide alaninserincystein forms in polycondensation of three amino acids alanin, serine and cystein:



alanin (Ala[A]) serine (Ser[S]) Cysteine (Cys[C]) alaninserincysteine

Three letters are Ala-Ser-Cys and one letter symbol abbreviation is ASC. Amino acid number one alanin on tripeptide chain has free amino group **H_2N**— and its call about N terminal. C terminal is last amino acid cystein number three on chain with free carboxyl group —**COOH**.

amino acid **amino acid**



*17 Fig. Peptide-bond –**HN-CO**- . Covalent bond binds two amino acids on polypeptide chain.*

9.2 Secondary, tertiary, quaternary structure of Proteins and five intermolecular forces

Five intermolecular forces strengthen folding of proteins in three different structure shapes, which calls one about secondary structure, tertiary structure and quaternary structure. In former chapter we consider primary structure of proteins, which forms polypeptide chains. Five intermolecular forces are:

1. and b) Hydrogen bonds,
2. and a) Salt bridge,
3. and c) Hydrophobic bonds,
4. and e) Coordinative bonds and
5. and d) Disulphide bonds.

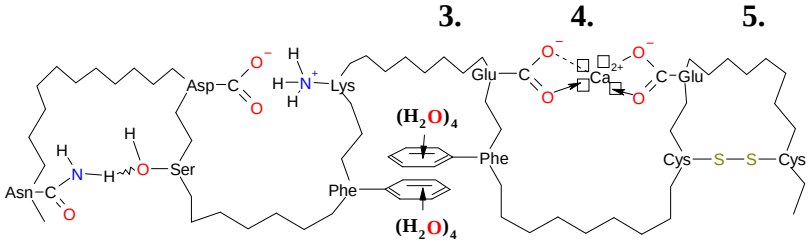
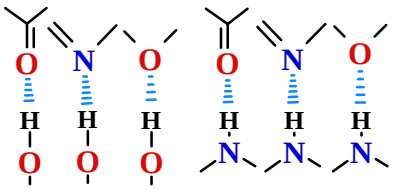


Fig.18 Stylistic picture of **disulfide** **bond** Cys—**S—S**—Cys, **coordinative** donor acceptor **bond** calcium ion with carboxyl groups —**COO:** →□Ca^{2+}□←**:OOC**— or **iron(II)** ion on center of hem →□Fe^{2+}□←, **salt bridge** Asp—**COO-**…$^{+}$**H$_3$N**—Lys, **hydrophobic bond** **$(H_2O)_4$**→◊◊← **$(H_2O)_4$** water press together nonpolar ◊ residues of amino acids, **hydrogen bond** Asn=**O**…**H**—**O**—Ser.

1. Hydrogen bond forms if between electronegative chemical elements oxygen atoms =**O**…**H**—**O**— or nitrogen atoms =**N**—**H**…**N**≡ stand hydrogen atom, which covalently bind with one of atoms.

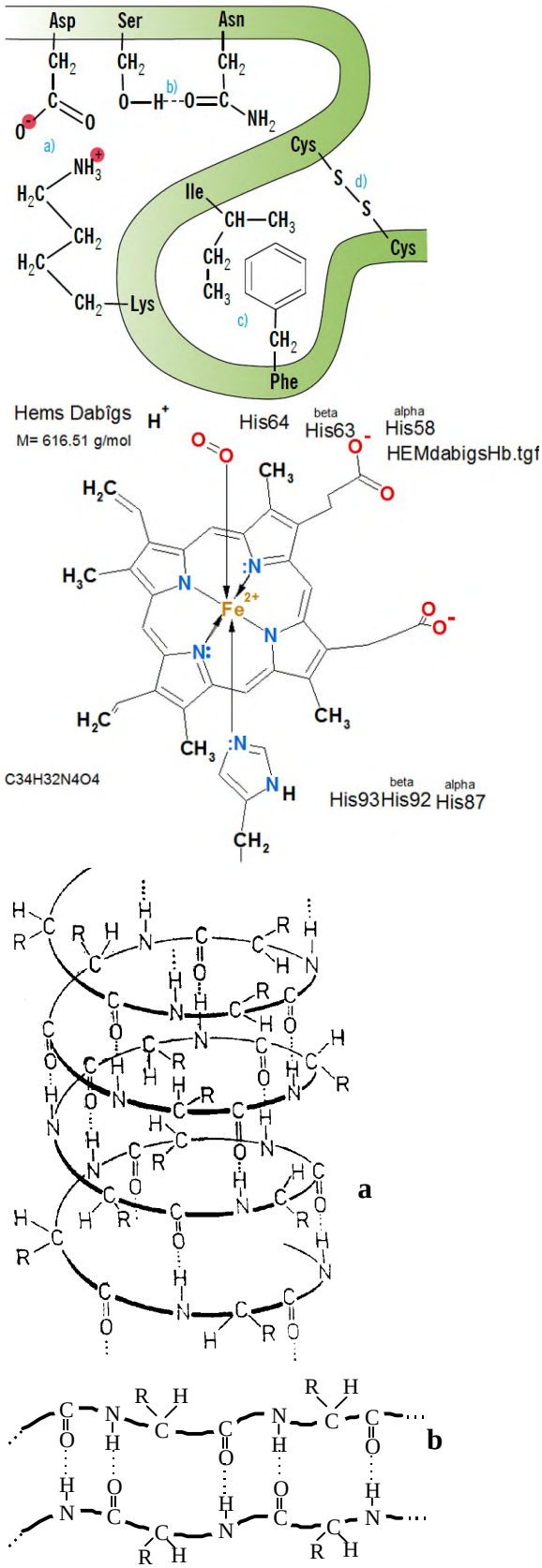
Oxygen or nitrogen atoms are hydrogen atom acceptors



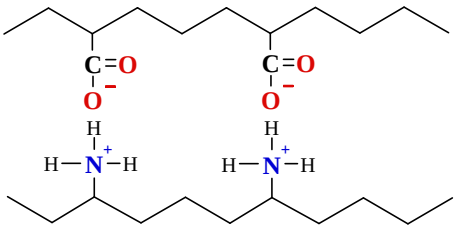
Oxygen or nitrogen atoms are hydrogen atom donors.

Secondary structure 2°

Hydrogen bond fastens secondary structure of alpha helixes, beta sheets and beta loops for proteins.



*19 Fig. Polypeptide chain secondary structure of proteins folds together with hydrogen bonds into the alpha helix (**a**) and into the beta sheet (**b**).*

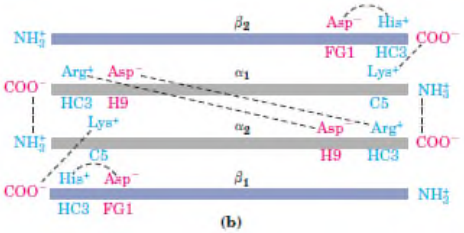


20 Fig. Salt bridge joint two chains of proteins with opposite charged negative carbonic acid **—COO$^-$** *and positive charged ammonium* **H_3^+N—** *functional groups.*

2,3 DiPhosphoGlicerate^{5-}

2,3DPG$^-$**OOC**— **$PO4^{2-}H_3^+N$**—beta1Val1,
$PO4^{2-}H_3^+N$—beta2Val1,

1 - α1Arg141—**COO$^-$**...**H_3^+N**—α2Val1,
2 - α2Arg141—**COO$^-$**...**H_3^+N**—α1Val1,
3 - α1Arg141...α2Lys127,
4 - α2Arg141...α1Lys127,
5 - α2Arg141...α1Asp126,
6 - α1Arg141...α2Asp126,
7 - β2Asp94...β2His146,
8 - β1Asp94...β1His146,
9 - β2His146...α1Lys40,
10- β1His146...α2Lys40,

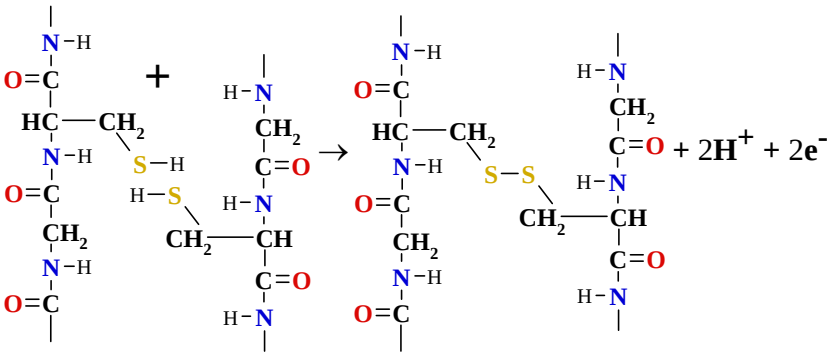


21 Fig. Venous blood hemoglobin has ten salt bridges, which joint four alpha1, alpha2, beta1 and beta2 protein chains with opposite charged negative carbonic acid **—COO$^-$** *and positive charged ammonium* **H_3^+N—** *functional groups.* 2,3DPG *phosphate ions* **$PO4^{2-}$** *with ionic bond are bound to free end* **N** *terminal amino acid number one valine* (Val1) *ammonium ions* **H_3^+N—** of *beta1 and beta2 protein chains***,** which lie in cavity of entrance with amino phosphate net charge **-2** for allosteric regulation of hemoglobin if oxygen concentration is below blood plasma concentration **$[O_2] = 6•10^{-5}$ M.**

2. Salt bridge-ionic bond forms between negative charged carbonic acid and positive charged neighbor on protein chains ammonium functional groups **—COO$^-$…H_3^+N—**

3. Disulfide bond forms under mild oxidation conditions between two protein chains joint opposite strand cystein (Cys[C]) amino acid oxidizing sulfhydryl groups

Cys—SH +HS—Cys → Cys—S—S—Cys +2H$^+$+2e$^-$



4. Hydrophobic bond forms in water medium. Meeting two protein chains and touching residues of nonpolar amino acids, for example, phenylalanine and leucine or isoleucine, water molecules press together with force, which is ten times stronger as Van der Walls forces. Hydrophobic force influences cooling of heated gelatin water solution, which forms jelly, similar as cooked legs or hade of pig in soup, which after cooling turns into jelly or (zilc in Latvian), because water press together nonpolar amino acids under influence of hydrophobic forces, which lies in adjacent chains of neighboring mutual contacting proteins (polypeptide).

5. Coordinative bond form complex makers (look A.Rauhvarger, General Chemistry, vol.III, Complex compounds, part 12, p. 236) which are metallic ions: iron(II) ions **Fe^{2+},** iron(III) ions **Fe^{3+},** calcium ions **Ca^{2+},** magnesium ions **Mg^{2+}** also zinc ions **Zn^{2+}** or cooper ions **Cu^{2+}** and others, which are acceptors of donor oxygen and nitrogen unshared electron pairs, and, which (**Fe^{2+}**, **Fe^{3+}**, **Ca^{2+}**, **Mg^{2+}**) with coordination number 6 or (**Zn^{2+}**, **Cu^{2+}**) with coordination number 4 coordinates around metallic ion 6 or 4 oxygen **O** and nitrogen **N** atoms from enveloping proteins, stabilizing tertiary and quaternary structure of proteins.

Tertiary structure 3° In tertiary structure folds secondary structure elements: alpha helixes, which resembles to tube of coiled protein chain, as well as beta sheets and beta loops, which provides parallel location tightly binding with hydrogen bonds of protein chains into beta sheets. In formation of tertiary structure take a place intermolecular interaction forces and some times all five: 1. Hydrogen bonds, 2. Salt bridges, 3. Disulfide bonds, 4. Hydrophobic bonds and 5.Coordinative bonds.

Quaternary structure 4° Quaternary structure is several protein separated chains aggregates, which bind together five intermolecular forces 1. Hydrogen bonds, 2. Salt bridges, 3. Disulfide bonds, 4. Hydrophobic bonds and 5.Coordinative bonds. For example:

In **hemoglobin** molecule four protein chains of tertiary structure alpha1, alpha2, beta1 and beta2 binds 1. Hydrogen bonds, 2. Salt bridges, 4. Hydrophobic bonds and 5.Coordinative bonds Fe^{2+}.

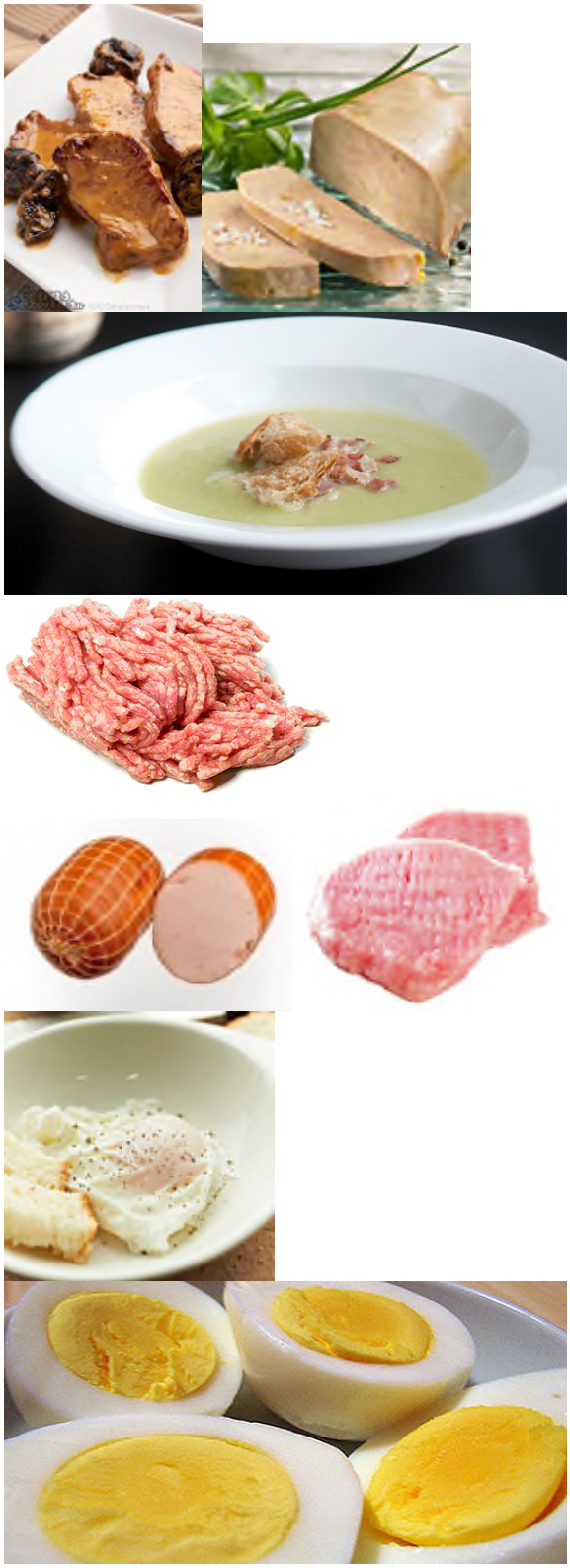
In **immunoglobulin** molecule two heavy and two light protein chains of tertiary structure bind :1. Hydrogen bonds, 2. Salt bridges, 3. Disulfide bonds, 4. Hydrophobic bonds.

Denaturation of proteins

Destruction of protein quaternary, tertiary and secondary structure is called as well **denaturation**. For example, egg white is transparent fluid viscous liquid, which natural appearance determines included proteins primary, secondary, tertiary and quaternary structure. Boiled eggs white are white congealed mass, because high temperature boiling breaks four intermolecular forces: 1. Hydrogen bonds, 2. Salt bridges, 4. Hydrophobic bonds and 5.Coordinative bonds.
3. Disulfide bonds demolish only at presence of reducing agents and present disulfide bonds in curdle can not break with heating only. Therefore heating curdle can obtain next cooking product cheese.

To prepare meal humans have learned denaturate proteins for nutrition, which perfect would be used in food containing amino acids, because organism absorbs just free amino acids. Therefore peoples in cooking meal apply the same methods as in chemistry labor methods: separation or grind into smaller peaces, heating and boiling, adding of acids, for example, acetic acid, citric acid or vine addition, in which always are present acids.

Mentioned denaturation actions with food applied proteins demolish quaternary, tertiary and secondary protein structure, but reaction of hydrolyze break the primary structure and release free amino acids, which absorb human organism from food prepared meals, that inside cells in ribosomes as new would synthesize for organism necessary proteins.



22 Fig. Protein denaturation from food. Cookery photographs: soup of beef tea, prepared meat , fishes, eggs and milk meals. On preparation of meals proteins are denaturate.