

Glycoproteins, Chromoproteins, Nucleoproteins,
 lipids transport (extra cellular): Lipoproteins, Lipocalins, Albumin
 lipids transport (intra cellular) START and otherlipids binding proteins,

Theoretical concepts and key terms.

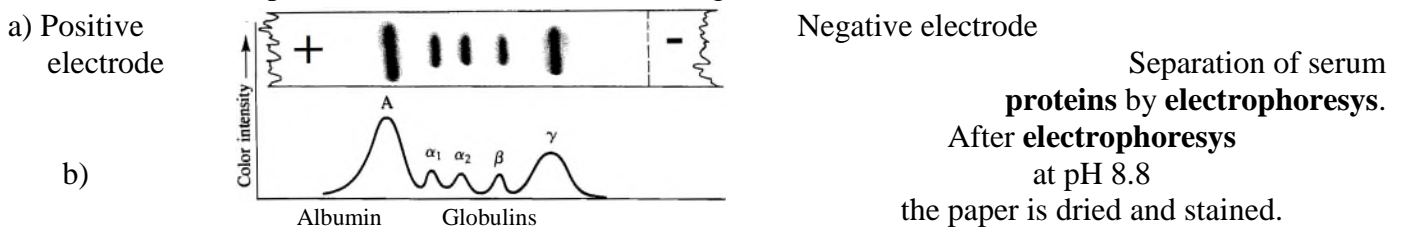
1. Immunoglobulin, extra cellular space to blood plasma faced proteins.
2. Myoglobin, hemoglobin, peroxidases, cytochroP450 oxidoreductases: Heme containing
3. Nucleosomes, ribosomes.
4. Lipoproteins. 5. Lipocalins, 6. START and otherwater soluble proteins transporters of:
 Phospholipids, Ssphingolipids, cholesterol, steroids, A, D, K and E vitamins.
7. Human serum albumin transporters of fatty acids, aspirin, warfarin, paracetamol.

Plasma Proteins: Electrophoresys

Human blood consists of a fluid water (90-92%) solution portion (plasma- various inorganic ions and a heterogeneous mixture of organic molecules) and cellular components. The cellular components, which make up 40-50% of volume of whole blood, consist of red blood cells (erythrocytes), white blood cells (leukocytes) and blood platelets (thrombocytes). **Electrophoresys** is the method of separating **proteins** of biological fluids into fractions in human plasma, urine and cerebrospinal fluid.

A sample of plasma is applied as a narrow line to a cellulose acetate strip. The ends of the strip are then immersed in a buffer of pH 8.8 and a voltage is applied to the strip. At pH 8.8, **plasma proteins** have net negative charges and migrate toward the positive electrode. The **protein** in spot is determined as peak.

Proteins move to the positive electrode direction <= Origin



Protein spots on stripe a) refer to peaks on the graph b).

Table

human serum	Fraction	(g/L)	%	
	albumin	35-50	52-67	0,6 mM albumin regulates the osmotic pressure in blood. Albumin is seven 7 fatty acids, aspirin, warfarin, ibuprofen transporter through blood circulation in organism.
globulin	α_1	1-4	2.5-4.5	The α_1 and α_2 fractions are transporter lipoprotein vesicles of lipids fats, cholesterol, phospholipids but lipocalins load and unload cholesterol,steroids as well vitamins K, E, D, A along movement to target cells in tissues.
globulin	α_2	5-11	6.6-13.6	
globulin	β	6-12	9.1-14.7	
globulin	γ	05-15	9.0-21.6	

The four **globulin** fractions are arbitrarily designated α_1 , α_2 , β and γ according to their **electrophoretic** mobility. Serum **albumin** isoelectric point **IEP** in range from **7.32** to **7.40** and migrates farthest toward the positive electrode. **Gamma-globulin** , **immunoglobulin**, which isoelectric point **IEP 7.9** with 2.18 times grater molar mass as for **albumin** 66473,4 g/mol for γ -**globulin** mass $2 \cdot 49750,3 + 2 \cdot 22801,5 - 34,3 = 145067,3$ g/mol and migrates the shortest distance.

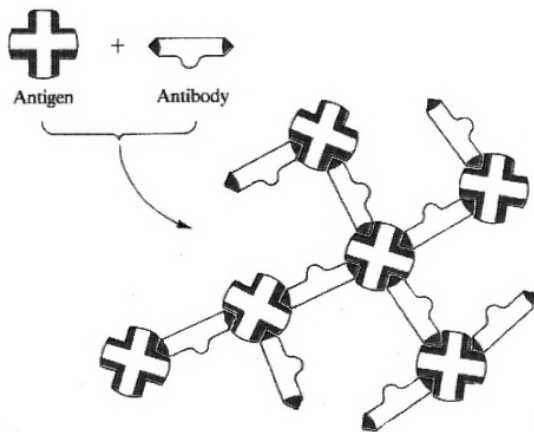
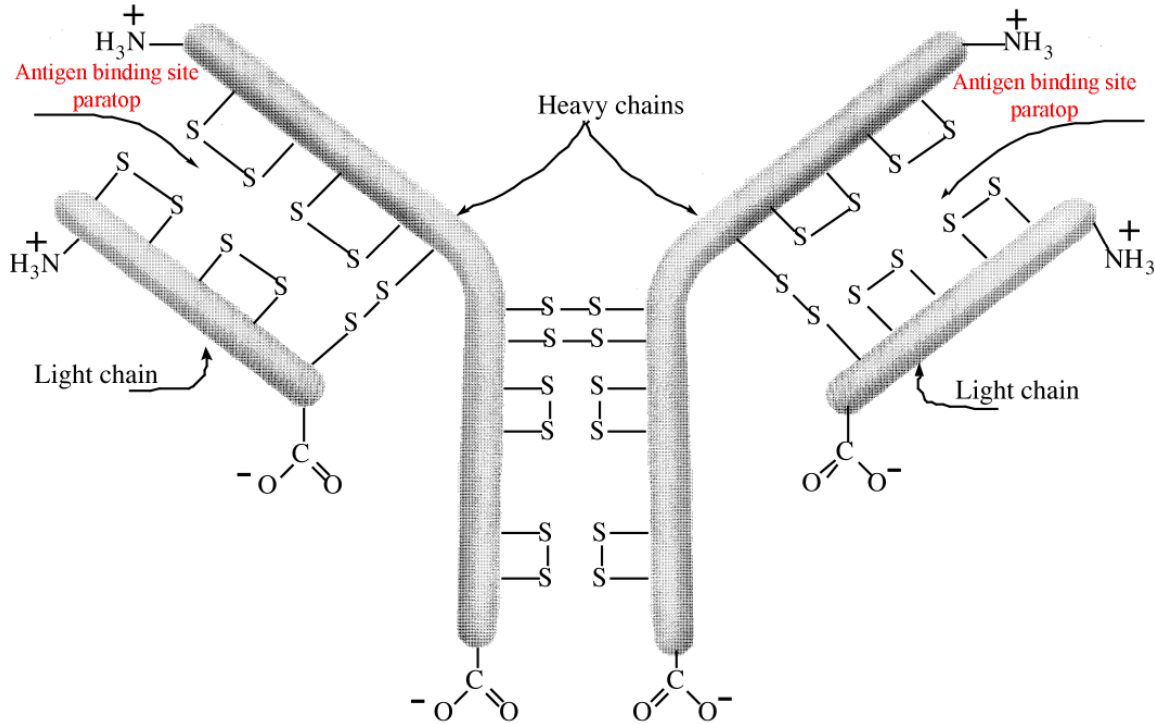
How many times immunoglobulin mass is grater than albumin?

To calculate it! $145067,3 / 66473,4 = 2,18$ times

The α_1 fraction contains antitrypsin, a protein that inhibits the protein-digesting enzyme trypsin. Alpha α_2 fraction contains **haptoglobin**, which binds any **hemoglobin** released from destroyed red blood cells and

ceruloplasmin, the principal copper-containing **protein** of the body. Alpha α_2 fraction contains **prothrombin**, an inactive form of the blood-clotting enzyme thrombin. Beta β fraction contains a variety of transport **proteins**, as well as substances involved in blood clotting.

Gamma γ -globulin fraction **antibodies - immunoglobulins**, whose function is to combat **antigens** (non host **proteins**) introduced into the host body. The response is the basis for immunization against infectious diseases (poliomyelitis, tetanus and diphtheria etc.). **Antibody** is quaternary structure of two **heavy** ($2 \times 49750,3 = 99500,6$ g/mol mas) and two **light** ($2 \times 22801,5 = 45603$ g/mol mas) polypeptide chains held together with four **disulfide bonds Cys—S—S—Cys**. Each **antibody** has two identical binding sites **paratops** that react with specific **antigen** to form an insoluble complex called **precipitin** and binding it remove following breakdown by white blood cells (leucocytes-macrophages).



Four protein subunit chains projection of the quaternary structure **antibody**. The interaction between **antibody** and its specific **antigen** to form an inactive **precipitin** complex. The precipitated **antigen-antibody** complex is then ingested and broken down with blood cells. **Precipitin** complex (insoluble). Antibody immunoglobulin IgG1 with antigenic bodies binding in plasma:

What the name of insoluble antigen antibody complex precipitates the **Precipitin**.....

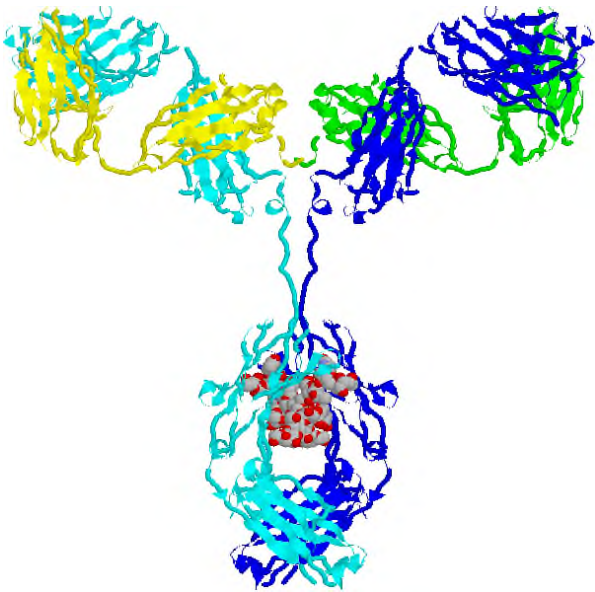
<http://aris.gusc.lv/ChemFiles/ChimAntibodyMarz/2frmcont.htm>

Lysozyme – Fab₂ (antigen binding dimer fragment) complementary bound lysozyme.

1FDL.pdb protein data bank structure file of Fab₂-Lysozyme fragment structure.

IgG1-all.pdb:

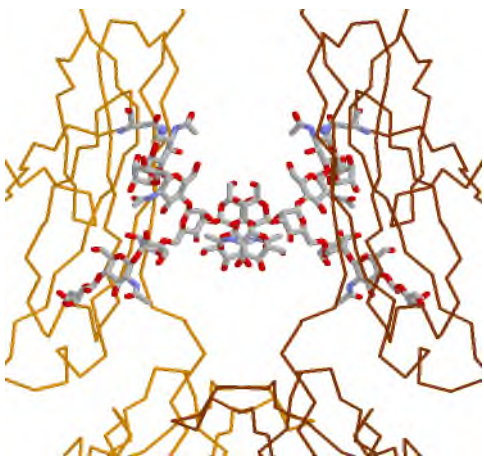
<http://aris.gusc.lv/ChemFiles/ChimAntibodyMarz/2frmcont.htm>



Carbohydrate chains have one immunological marker
fucose FUC2 ($\beta 1 \rightarrow 6 \uparrow$) with

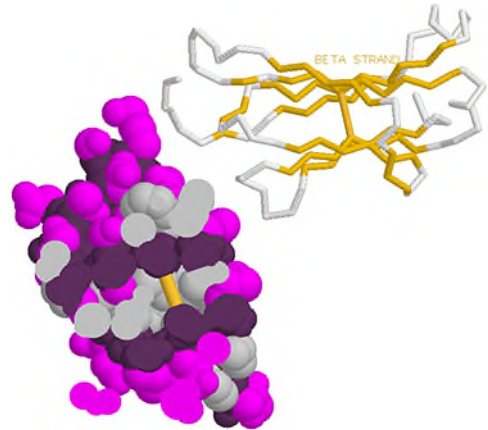
glycoside bond -O- NAG1
at Nacetyl-glucoseamine :

ASN306-NAG1-NAG3-MAN4-MAN5-NAG6
FUC2($\beta 1 \rightarrow 6 \uparrow$) -GAL7-MAN8-NAG9



12 tertiary 3° structure domains build two beta
sheet secondary 2° structures
folded in to domain of tertiary 3° structure with
inter molecular forces:

1. Hydrogen bonds; $>\text{N-H}\dots\text{O}=\text{C}<$
2. Hydrophobic bonds
 $(\text{H}_2\text{O})_4 \rightarrow \diamond \diamond \leftarrow (\text{H}_2\text{O})_4$;
3. Disulfide bonds Cys-S-S-Cys



Four disulfide bonds S-S connect
4 protein polypeptide chains.



Calculate mas for two heavy chain polypeptides in **IgG1-all.pdb** molecule!

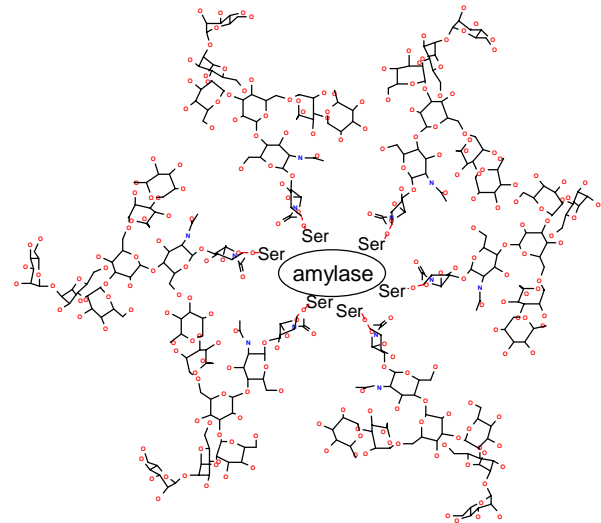
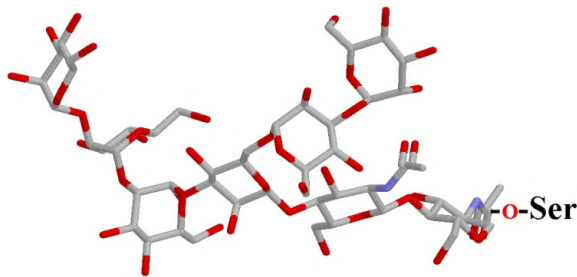
two **heavy** ($2 \cdot 49750,3 = 99500,6$ g/mol mass) and

Calculate mas for two light chain polypeptides in **IgG1-all.pdb** molecule!

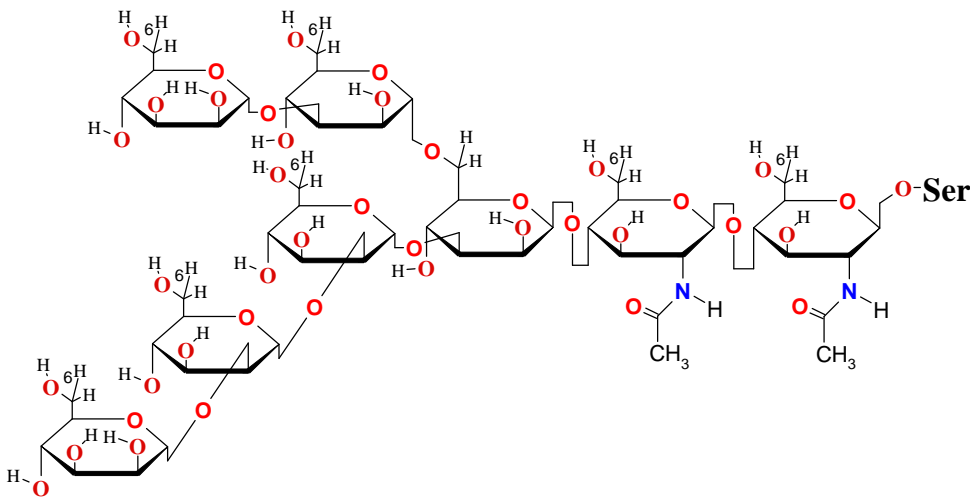
two **light** ($2 \cdot 22801,5 = 45603$ g/mol mass) polypeptide chains.

Glycoproteins Carbohydrates polysaccharides + protein

[http://aris.gusc.lv/ChemFiles/Saccharides/PolySaccharides/HyalurChondroitHeparKeratMucHTM/Muc3Man6\(2Man\)2NAcGal.html](http://aris.gusc.lv/ChemFiles/Saccharides/PolySaccharides/HyalurChondroitHeparKeratMucHTM/Muc3Man6(2Man)2NAcGal.html)



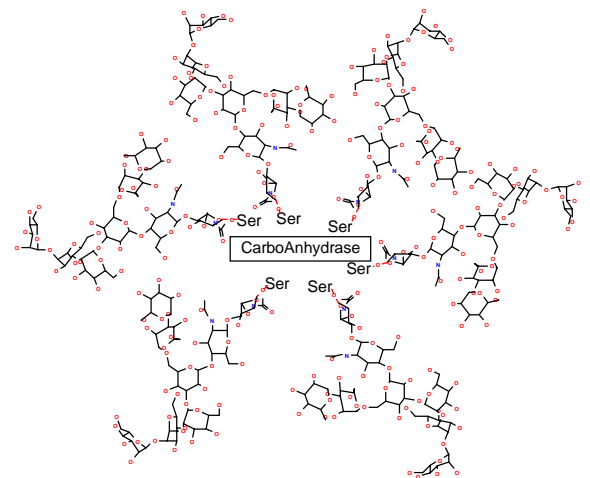
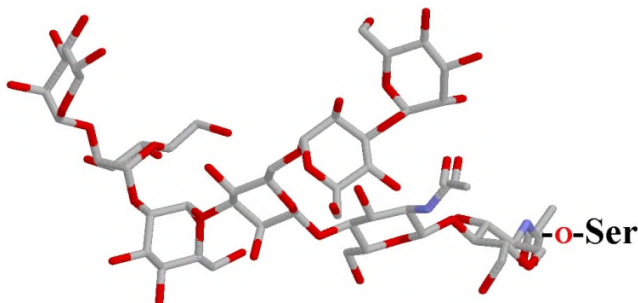
Mucin shield **Amylase enzyme** protect from degradation by peptidases in digestive tract



(forked) branched $\text{Man}(\alpha 1 \rightarrow 3)\text{Man}(\alpha 1 \rightarrow 6)\downarrow$

$\text{Man}(\alpha 1 \rightarrow 2)\text{Man}(\alpha 1 \rightarrow 2)\text{Man}(\alpha 1 \rightarrow 3)\text{Man}(\beta 1 \rightarrow 4)\text{GlcNAc}(\beta 1 \rightarrow 4)\text{GlcNAc}-\beta$

Stick molecular picture glycoside bond **O**-linked to serine hydroxyl group **H-O-Ser**



Mucin shield **CarboAnhydrase enzyme** protect from degradation by peptidases in digestive tract

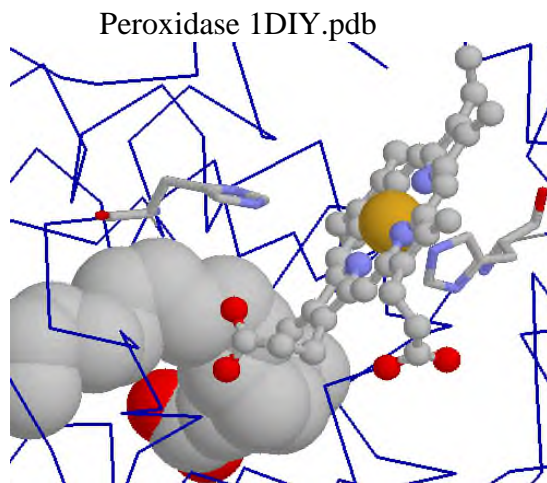
How mucin shield protect against degradation with peptidases in intestinal tract?

Chromoproteins: Cytochrome, Hemoglobin, Catalase, Peroxidase etc.

<http://aris.gusc.lv/ChemFiles/hemoglobEricMarzUMas/2frmcont.htm>;

<http://aris.gusc.lv/ChemFiles/CycloOxygenase/cycox.html>

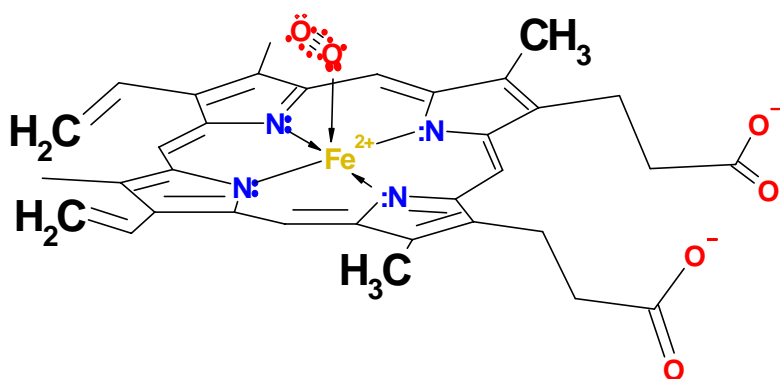
Hemoglobin 2hhd.pdb, Myoglobin 1MBO.pdb



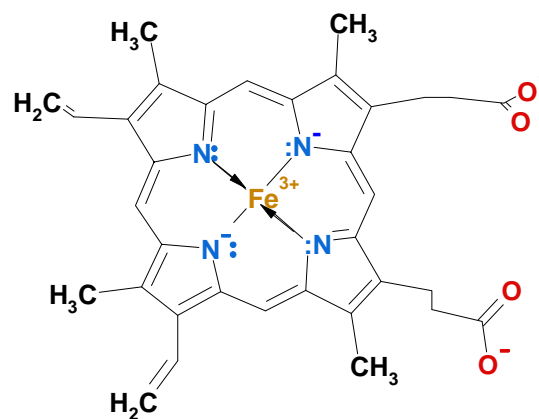
Heme prosthetic group surrounded by globular proteins :

Hemoglobin, Myoglobin,

Peroxidase, CytochromP450, Catalase etc.



Triplet oxygen in human organism!



Singlet oxygen in human organism!

Triplet oxygen molecule is inactive on heme iron(II) Fe^{2+} locked by donor acceptor-bond $••O≡:::≡O••$ because has three covalent bonds.

That is biochemical reaction less **oxygen** at absence of water in heme pocket and therefore safe storage form for organism.

Singlet oxygen $••:O-:-O:••$ is active molecule having one covalent bond and is found on heme iron(III) Fe^{3+} atom in certain oxidising enzymes: Peroxidase, CytochromeP450, Catalase.

Biochemical reactive form of **oxygen** is located in isolated pocket of enzyme active site.

In water dissolute oxygen $O_{2\text{aqua}}$ is inactive **triplet** state up to temperature $100^{\circ}C$, but

AIR **triplet** oxygen $••O≡:::≡O••$ turns to **singlet**-active state oxygen $••:O-:-O:••$, when heated AIR atmosphere up to and over $>80^{\circ}C$ temperature, than start combustion processes of organics.

Singlet oxygen risk increases five times in pure atmosphere 100% O_2 as concentration increases from 20% to 100%. Reaction velocity is proportional to concentration. Pure oxygen concentration $[O_2]$ is times $100\%/20\%=5$ five..... increased. Singlet oxygen risk increases five..... times:

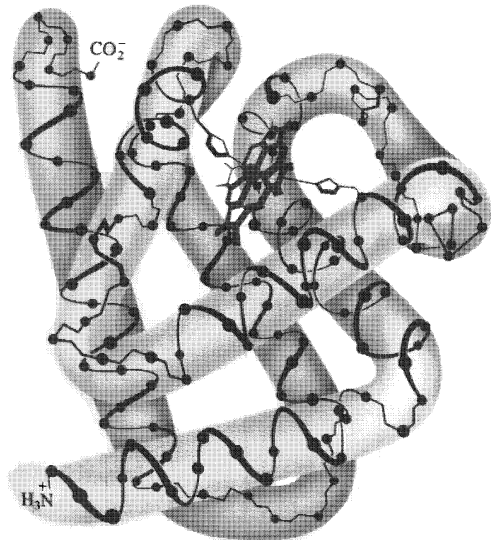
$$\rightarrow$$

$$v \sim [O_2]$$

<http://aris.gusc.lv/ChemFiles/ChromoHem/MyoGlobOxDeoxCoBiliverdin/1MBODEOxyLopez.kin>

Myoglobin $O_2 \rightleftharpoons H^+, HCO_3^-$ shuttle exchange stored oxygen molecule with H^+, HCO_3^- in concentration sensitive oxy \leftrightarrow deoxy equilibrium maintaining $[O_{2,aqua}] = 1.5 \cdot 10^{-5}$ M and pH=7.36 values. **Myoglobin** consists of 153 amino acids starting from Val1 to Gly153 coordinated around the single iron(II) atom heme complex center. Isoelectric point **IEP** 7.36.

J.C. Kendrew awarded by Nobel Prize in chemistry in 1963 for **myoglobin**. The secondary and tertiary structure of **myoglobin** shown in **Figure** of the three-dimensional structure. **Heme** group on one frame plane disposed adjacent symmetrical joined polygons.



The **N**-terminal amino acid Val1 indicated by protonated group — NH_3^+ is at the lower left.

C-terminal amino acid Gly153 indicated by deprotonated carboxyl — COO^- is at the upper left.

Alpha carbon atoms between the peptide bonds are located on **backbone trace**.

1. The **backbone** consists of eight sections of α -helixes **A, B, C, D, E, F, G, H**, each separated by a β -bend with **hydrogen bonds** between peptide bonds atom of hydrogen donor $>N-H \dots O=C<$ hydrogen acceptor atom **O**.

2. Non polar side chains of 29 amino acids as Phe, Ala, Val, Leu, Ile, Gly and Met are clustered around heme pocket, which shield oxygen O_2 from water and hydronium ions H_3O^+ contacts. **Hydrophobic interactions** between non polar side chains fold eight α -helixes into tertiary 3° structure of **myoglobin protein**.

3. The **myoglobin** surface is coated with hydrophilic amino acids Lys, Arg, Ser, Glu, His and Gln, which interact with the aqueous environment create water soluble **hydrate coat**.

4. Tertiary 3° structure support electrostatic attractions called **salt bridges**. Positive Lys— NH_3^+ attract with negative charged Glu carboxylic COO^- group.

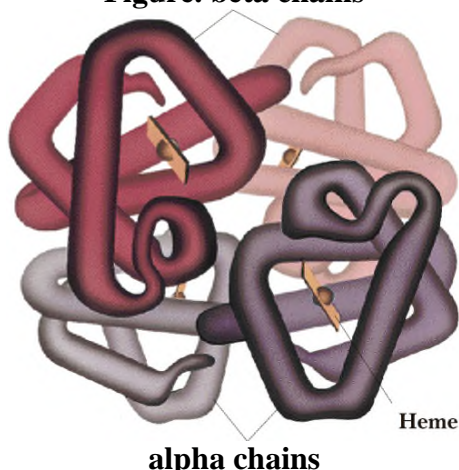
Tertiary 3° structures of globular proteins fold α -helix and β -pleated sheet secondary 2° structures.

Max Perutz awarded by Nobel Prize in chemistry on 1963 for **Hemoglobin**.

<http://aris.gusc.lv/ChemFiles/ChromoHem/HbOxDeoxCO/2HCOProTour8.kin>

Quaternary 4° Structure. Hemoglobin consists of 4 synthesised protein monomers

Figure. beta chains



- subunits: $\alpha 1, \alpha 2$ 141 amino acids on **chains** and $\beta 1, \beta 2$ 146 amino acids on **chains** with

five **5 intermolecular forces** bounded together. The flat disks represent four **hemes** in its pockets.

The chief factors stabilizing the binding of protein subunits is **hydrophobic interaction** and ten **10 salt bridges** support oxy-deoxy venous oxygen concentration $[O_{2,aqua}] = 1.85 \cdot 10^{-5}$ M sensitive equilibrium as conformation changes to deoxy state.

1 -- $\alpha 1$ Arg141— $COO^- \dots H_3^+ N$ — $\alpha 2$ Lys127,

2 $\alpha 1$ Arg141— $COO^- \dots H_3^+ N$ — $\alpha 2$ Val1,

3- $\beta 2$ Asp94— $COO^- \dots H_3^+ N$ — $\beta 2$ His146,

4 - $\beta 2$ His146— $COO^- \dots H_3^+ N$ — $\alpha 1$ Lys40,

5- $\alpha 2$ Arg141— $NH_3^+ \dots COO^-$ — $\alpha 1$ Asp126,

4th page on **Proteins** <http://aris.gusc.lv/NutritionBioChem/38Olbalt10311Eng.doc>

Sickle cell hemoglobin SC **hydrophobic** spots stick excluding water causing aggregation. Adjacent β chain Val6 bound to neighbor molecule Ala70 and Leu88.

<http://aris.gusc.lv/ChemFiles/hemoglobEricMarzUMas/INDEX.htm>

To call five intermolecular bonds of proteins!

Hydrogen bond, Hydrophobic bond, Salt bridge, Disulfide bond, Coordinative donor-acceptor bond...

Transport proteins for insoluble lipids are two type extra cellular and intra cellular.

Extra cellular lipids transport proteins: albumin, lipoprotein vesicles, lipocalins,

Intra cellular lipids transport proteins STARD1-15 and other transport:

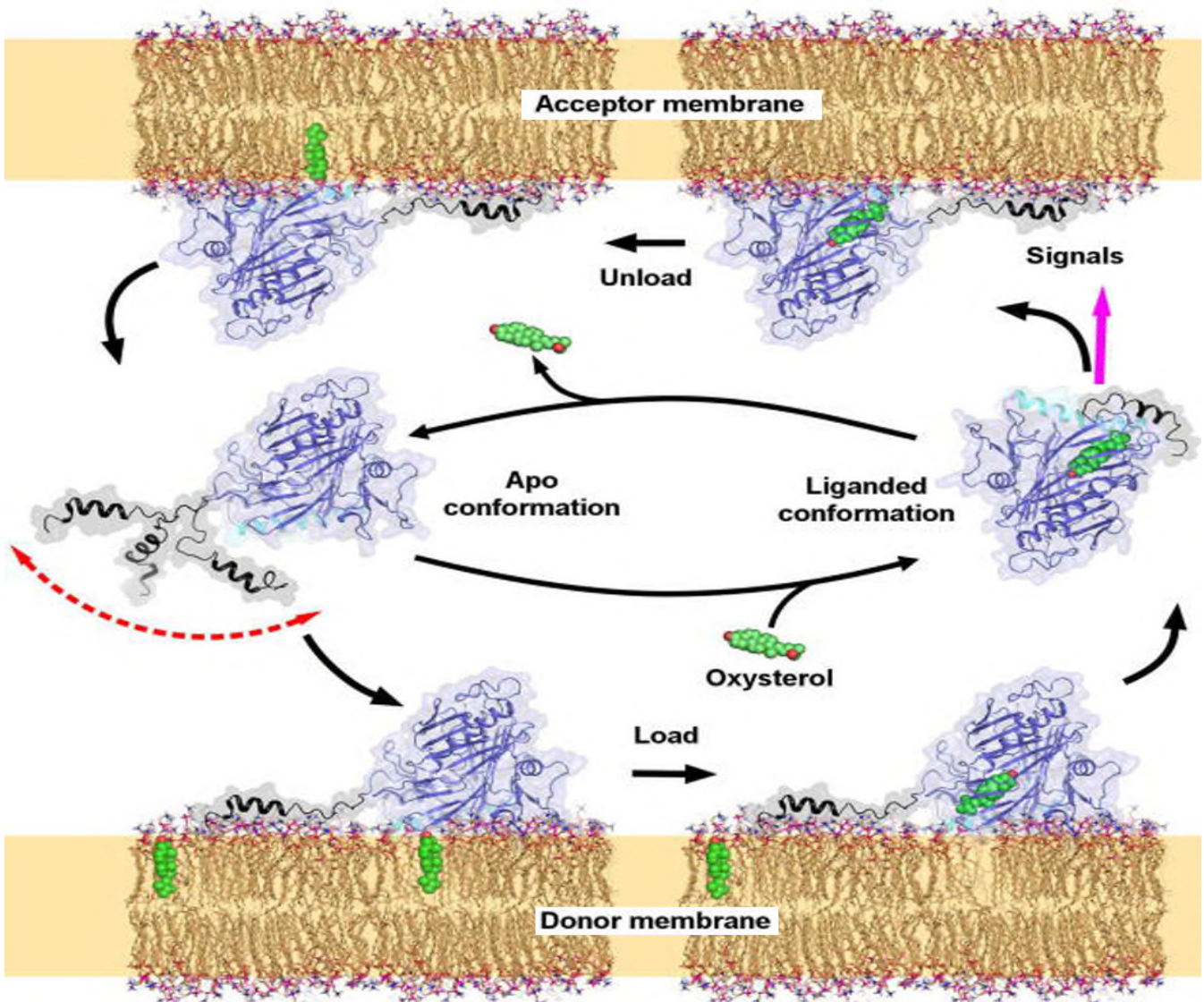
cholesterol, steroids, phospholipids ceramide, diglyceride DAG, fatty acids, vitamins K, E, D, A

Lipocalins extra cellular water transport of cholesterol, steroids, vitamins K, E, D, A.

OSBP (oxy-sterol binding protein) oxi-sterol transport protein involved in cholesterol metabolic transport across membranes surface load from and unload to membranes, that keep homeostasis 33.3% mass fraction 1/3 of 100% membrane mass.

<http://aris.gusc.lv/ChemFiles/BilipidCholine/Membrane/Cholest5ene3-20diol/Cholesterol5.htm>

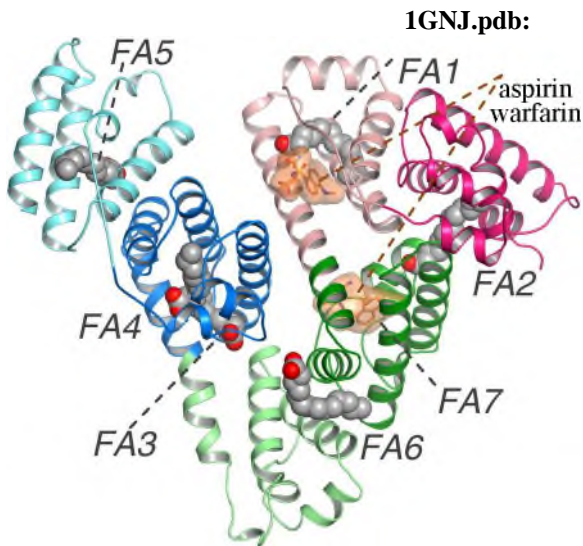
Lipocalins mechanism like **OSBP**, retinol **ORPs** and other **Lipocalins** for A,K,E,D vitamin transport proteins. Human organism has 12 **OSBP** iso forms. **Osh4** human protein isoform **OSBP4** cholesterol exchange. **OSB4 lipocalin** molecule exterior surface around the lid of the tunnel contains seven highly conserved basic positive charged residues Lys15, Lys173, Lys334, Arg344, Arg347, Lys348, Lys353, Lys407, Arg410, Lys411, $-\text{NH}_3^+$ attract to negative charged $>\text{PO}_4^-$ phosphate on surface as three tentacle helices. After attraction load into **lipocalin** from donor membrane and unload cholesterol on empty membrane. Structure **1ZHY**.pdb with cholesterol:



Steroids and vitamins are water insoluble like cholesterol. **Lipocalins** transfer these hydrophobic molecules to target sites for physiological functions like cholesterol unloaded in membranes. Nature. 2005 September 1; 437(7055): 154–158

What is normal mole ratio in red blood cell for cholesterol / phospholipid composite membrane 1978 publication: C/PL= 1 one cholesterol per one phospholipid molecule.....

Human serum albumin **HSA** is the most abundant protein in blood plasma



<http://aris.gusc.lv/ChemFiles/Albumin/cycox.html>

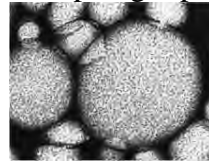
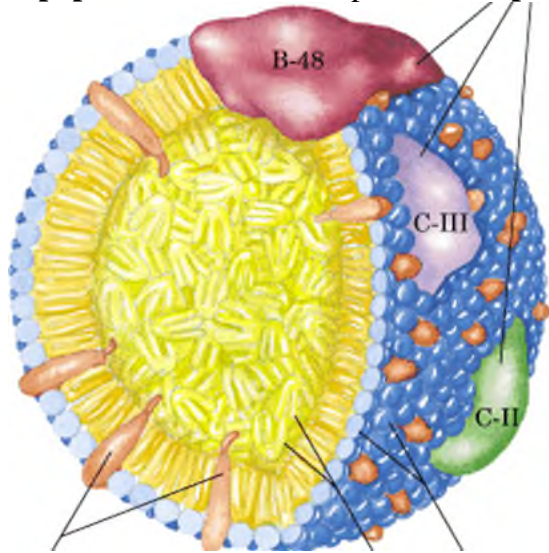
Human serum albumin HSA

in blood plasma has typical circulating concentration 0.6 mM.

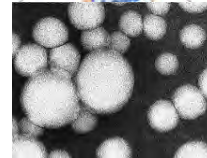
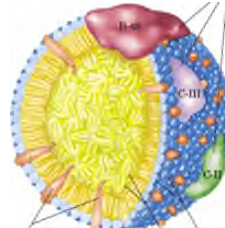
Albumin blood plasma

transport lipoprotein for 7 Fatty acids FA and **water** insoluble drugs : warfarin, ibuprofen, aspirin etc.

Lipoprotein vesicles transport of **fats, phospholipids, sphingolipids, cholesterol**



80...200 nm

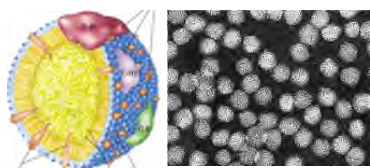


28...70 nm

Chylomicrons diameter range from about 100 nm to about 500 nm. Vesicle comprise up to million (10^6) molecules of **Fats** and **Cholesterin**.

VLDLs, LDL very low density lipoprotein,

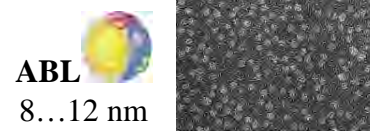
low density lipoprotein vesicles. When the diet contains more fatty acids in excess, the liver converts them to triacylglycerols, which are packaged with specific apolipo- proteins into **VLDLs, LDL**.



20...25 nm

20...25 nm

The **VLDLs, LDL** are transported in the blood to adipose tissues, where the triacylglycerols are removed and stored in lipid droplets within adipocytes.



ABL

8...12 nm

8...12 nm

HDL high density lipoproteins vesicles.

Cholesteryl esters and Cholesterol metabolizing within **HDL** vesicles by esterification and have been up taken in liver and in extra hepatic cells

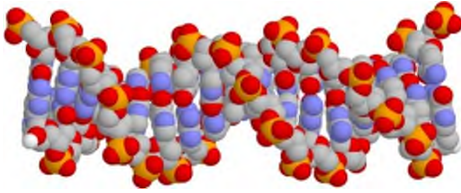
Myoglobin molecule Mb oxygen adsorbtion bind long chain fatty acids 6C,8C,10C,12C,14C,**16C**,18C,20C acylkarnitin. Oxygen desorption $O_2 \rightleftharpoons H^+, HCO_3^-$ of shuttle molecules Mb instantly release acylkarnitin but binde oxidation products of Krebs cycle H^+, HCO_3^- . So maintain concentration $[O_{2aqua}]$, pH=7,36 stable. Mb shuttle serves as fuel suppliers to muscle and cardio myocyte cells physiologic sustain homeostasis $[O_{2aqua}]$, pH=7,36.

© 2016 J.Biol.Chem. 291:25133-25143. Binding **energy** from -15,8 to -30,7 kJ/mol .

Nucleoproteins

1d66-pwz.pdb:

DNA atoms CPK color scheme



http://aris.gusc.lv/ChemFiles/DnaMarzHTM/fs_pairs.htm

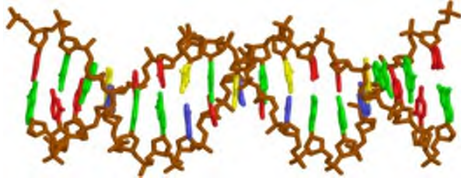
1. What base pairs constitute DNA fragment?
17 base pairs.....
2. What net charge of phosphates $>PO_4^-$ bears fragment?
 $(-17 >PO_4^-) + (-17 >PO_4^-) = -34$ net charge.....
3. What names bases paired by two hydrogen bonds?
adenin **A=T** thymine.....

4. What names bases paired by three hydrogen bonds? guanine **G=C** cytosine.....

1d66-pwz.pdb:

http://aris.gusc.lv/ChemFiles/DnaMarzHTM/fs_code.htm

DNA color scheme for bases **A** adenin **T** thymine **G** guanine **C** cytosine



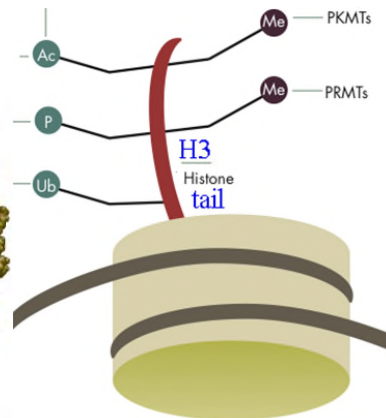
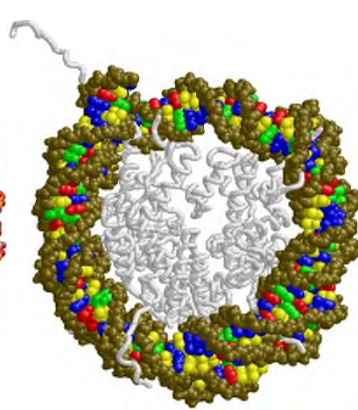
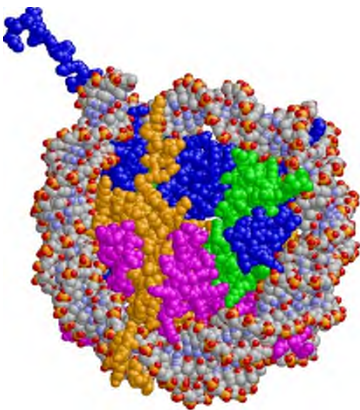
5. What type DNA strands parallel or anti parallel?
Anti parallel.....
6. Draw the 5' end functional group and 3' end group!
 $5' - O_3P - O -$ phosphate..... $3' - OH$ hydroxyl.....

Nucleosome quaternary 4° structure histones disk of 8 subunits with **H3** N-terminus tail

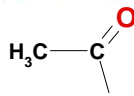
1A0I.pdb:

<http://aris.gusc.lv/ChemFiles/CLUnucleosome/nucleosome.htm>

Nucleic acid 146 DNA base pairs binding proteins on disk of 8 histones
two 2***H2A**, two 2***H2B**, two 2***H3** and two 2***H4**.

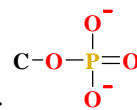


Ac, acetylation deacetylation enzymes marker



acyl group;

P, Phosphorylation enzymes=kinases, phosphate ester marker



formation or remove;

Ub, ubiquitination enzymes Ligases polypeptide chain degradation and remove;

PKMT: Lys (K) and PRMT: Arg (R) methyl transferases; methylation, demethylation

EPIGENETIC FACTORS

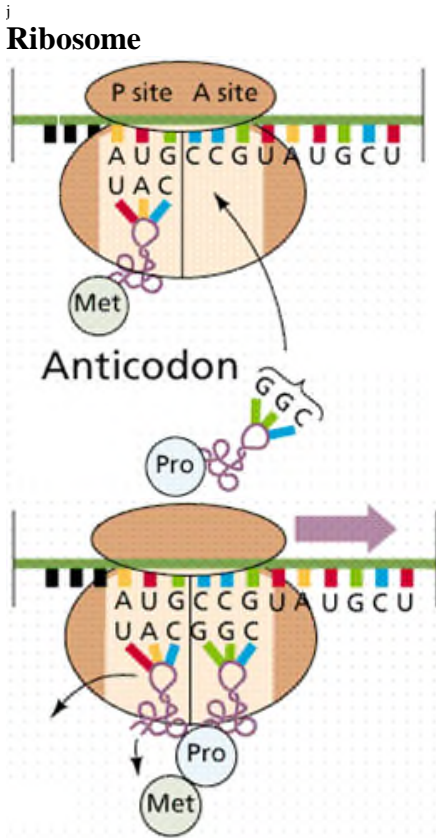
The binding of epigenetic factors to histone H3 "tails" alters the extent to which DNA is wrapped around histone disks and the availability of genes in the DNA to be activated for expression.

HEALTH ENDPOINTS:

Cancer; Autoimmune disease; Mental disorders; Diabetes

Nucleic acids **RNA** binding proteins **Table 1. The genetic code.**

Messenger RNA **mRNA** three base sequence **Genetic Code**



1st position (5'end)↓	2nd position				3rd position (3'end)↓
	U	C	A	G	
U	Phe Phe Leu Leu	Ser Ser Ser Ser	Tyr Tyr STOP STOP	Cys Cys S-SelCys Trp	U C A G
C	Leu Leu Leu Leu	Pro Pro Pro Pro	His His Gln Gln	Arg Arg Arg Arg	U C A G
A	Ile Ile Ile Met init	Thr Thr Thr Thr	Asn Asn Lys Lys	Ser Ser Arg Arg	U C A G
G	Val Val Val Val	Ala Ala Ala Ala	Asp Asp Glu Glu	Gly Gly Gly Gly	U C A G

Translation in ribosome start with methionine:

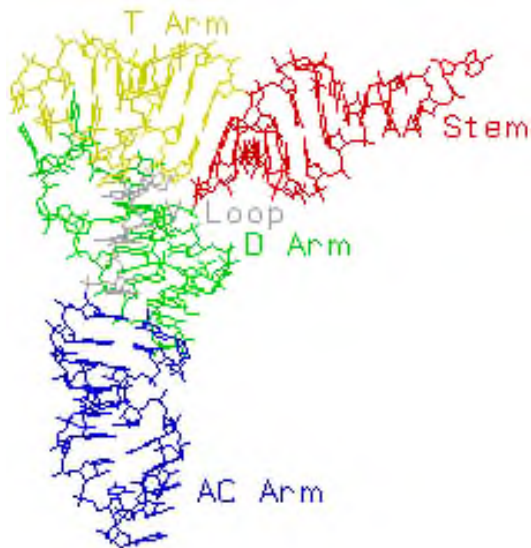
Met init, Pro, Tyr, Ala

1, 2, 3, 4

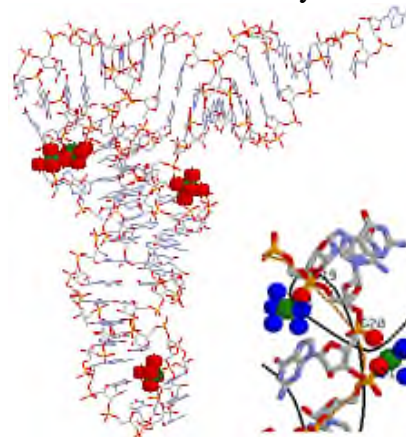
4 amino acids encoded on mRNA messenger RNA

http://aris.gusc.lv/ChemFiles/CarnegieMellonUChem/Programs/Courses/BiochemMols/tRNA_Tour/tRNAMain.htm

tRNA.pdb: Transfer tRNA for Phe phenylalanine amino acid translation in ribosomes.



T Arm T 5-Methyluridine, Ψ pseudo uridine
V loop; variable loop
AA Stem amino acid Stem for Phe
D Arm Dihydro uridine Loop



AC Arm anti codon loop

Three Mg^{2+} clusters in the D Arm loop and one Mg^{2+} in the AC Arm loop.

The Mg^{2+} ion-oxygen distances are about $2\text{\AA} = 0,2\text{ nm}$ ($1\text{\AA} = 0,1\text{ nm}$).

Five waters oxygens (blue) and a phosphate oxygen (red) from G19.

Four waters and phosphate oxygens from G20 and A21.

Phosphate ribose diester backbone is shown as thin string.

To call five intermolecular bonds!

Hydrogen bond, Hydrophobic bond, Salt bridge, Disulfide bond, Coordinative donor-acceptor bond...