Āris Kaksis 2018.gadā, **Rīgas Stradiņa universitāte**:http://aris.gusc.lv/BioThermodynamics/ComposeProtein4.pdf

Composite proteīns with carbohydrates, **hemes**, **ltpids** , **cell membranes** and **nucleic acids**

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, **7.** FABP and **8.** Human serum Albumin HAS water soluble proteins!
.....Transporters of Phospholipids, Ssphingolipids, cholesterol, steroids, A, D, K and E vitamins.

Praktiskā nodarbība: **Asins plazmas olbaltumvielas: elektroforēze**

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. Sākums Parauga šaura līnija.

Amino Acid	pK_{aCOOH}	pK_{aNH3+}	$pK_{aRgroup}$
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.

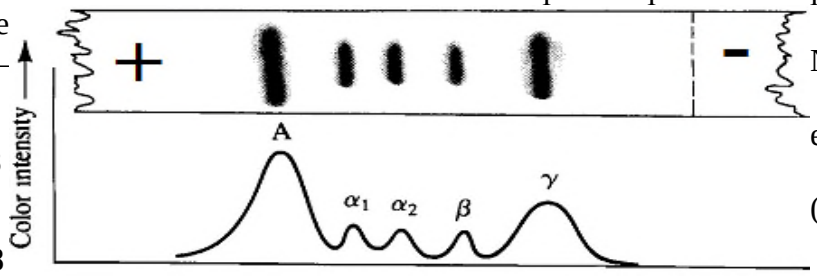
Iso electric point IEP=pK_a as well protolytic constant pK_a calculates one of side residues R constants sum $\Sigma pK_{aRside\ residue}$ plus $pK_{aNterminusNH3+}$ and plus $pK_{aCterminusCOO-}$ sum dividing with number NpKa of acidic groups in molecule
$IEP=pK_a=(\Sigma pK_{aR\ side\ residue}+ pK_{aNterminus}+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

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 each spot. γ *Globulin* moves slower as *Albumin*

Proteins move this direction ← spot line position sample origin at start

Positive (+) electrode
Direction of movement <=——

Albumin
IEP=7,32÷7,40<<**pH=8,8**
γ *Globulin* IEP=7,91 < **pH=8,8**
Myoglobin IEP=7,36 < **pH=8,8**



Albumin gamma *Globulin*

Table human **serum**	**Fraction**	(g/L)	**%**
albumin	albumin	35-50	52-67
globulin	**α_1**	1-4	2.5-4.5
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

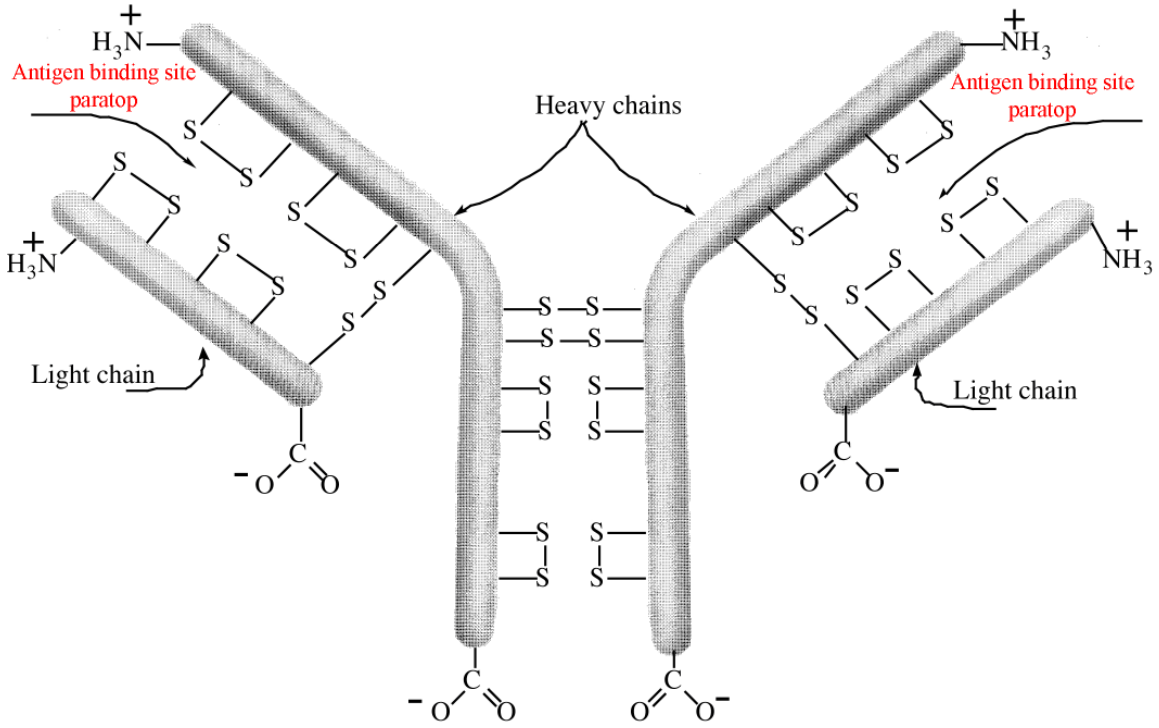
0,6 mM **albumin** regulates the osmotic pressure in blood. **Albumin** is seven 7 fatty acids, aspirin, warfarin, ibuprofen transporter through blood circulation in organism. The **α_1** and **α_2** fractions are transporter lipoprotein vesicles of lipids fats, cholesterol, phospholipids but lipocalins load and unload cholesterol,steroids as well vitamins K, E, D, A along movement to target cells in tissues. The four **globulin** fractions are arbitrarily designated **α_1**, **α_2**, **β** and **γ** according to their **electrophoretic** mobility.

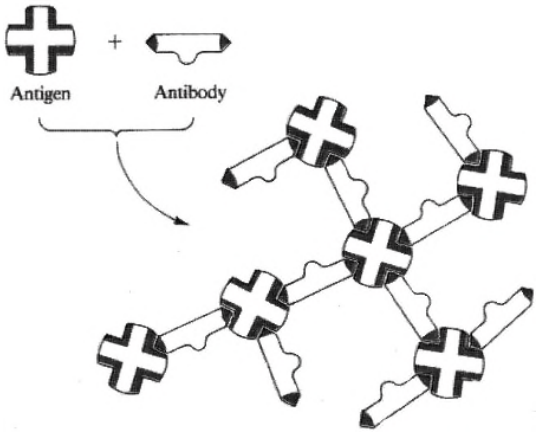
Serum **albumin** isoelectric point **IEP** in range from **7.32** to **7.40** and migrates toward the positive electrode. **Gamma-globulin** , **immunoglobulin**, which isoelectric point **IEP 7.9** with 2.18 times grater molar mass as **albumin** 66473,4 g/mol for **γ –globulin** mass 2*49750,3+2*22801,5-34,3=145067,3 g/mol and migrates shortest distance.

Calculate times 145067,3/66473,4=2,18.....

The α_1 fraction contains antitrypsin, a protein that inhibits the protein-digesting enzyme trypsin. Alpha α_2 fraction contains **haptoglobulin**, 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*49750,3=99500,6 g/mol mas) and two **light** (2*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:

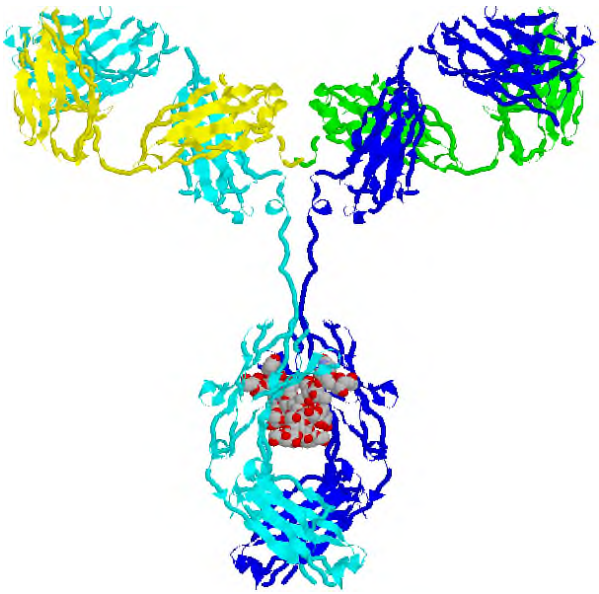
What is water insoluble antigen antibody complex precipitates the **Precipitin**.....

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

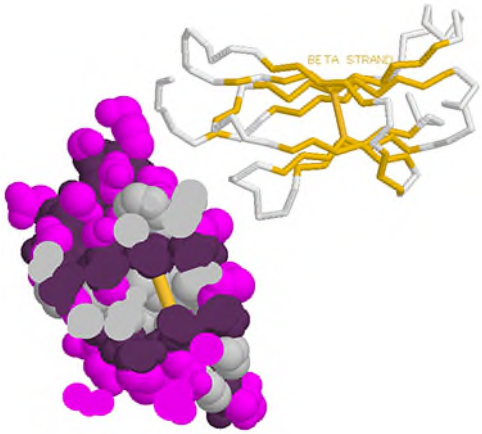
Lysozyme – **Fab$_2$** (antigen binding dimmer fragment) complementary bound lysozyme.

1FDL.pdb protein data bank structure file of Fab$_2$-Lysozyme fragment structure.

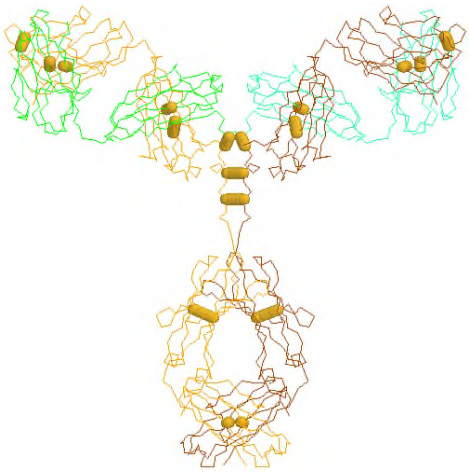
IgG1-all.pdb: http://aris.gusc.lv/ChemFiles/ChimAntibodyMarz/2frmcont.htm



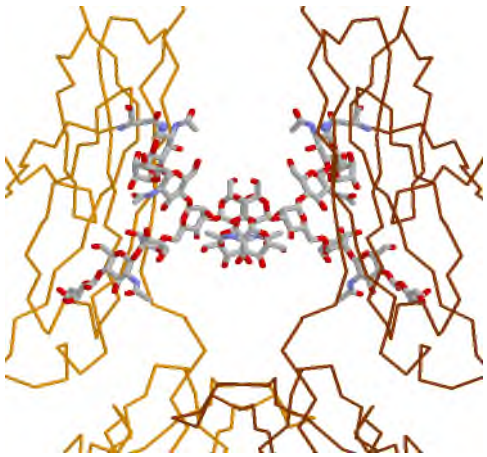
12 tertiary 3º structure domains build two beta sheet secondary 2º structures
folded in to domain of tertiary 3º structure with inter molecular forces:
1.Hydrogen bonds; >**N**-**H**... **O**=**C**<
2.Hydrophobic bonds
(H$_2$O)$_4$→◊◊←(H$_2$O)$_4$;
3. Disulfide bonds **Cys—S—S—Cys**



Four disulfide bonds **S—S** connect
4 protein polypeptide chains.



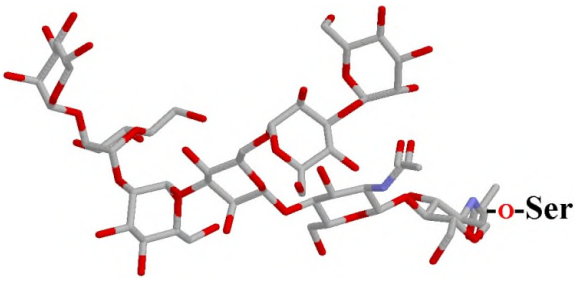
Carbohydrate chains have one immunological marker fucose
FUC2 (**β**1→6↑) with
glycoside bond **–O–** NAG1
at Nacetyl-glucoseamine :
ASN306-NAG1-NAG3-MAN4-MAN5-NAG6
FUC2(**β**1→6↑) -GAL7-MAN8-NAG9

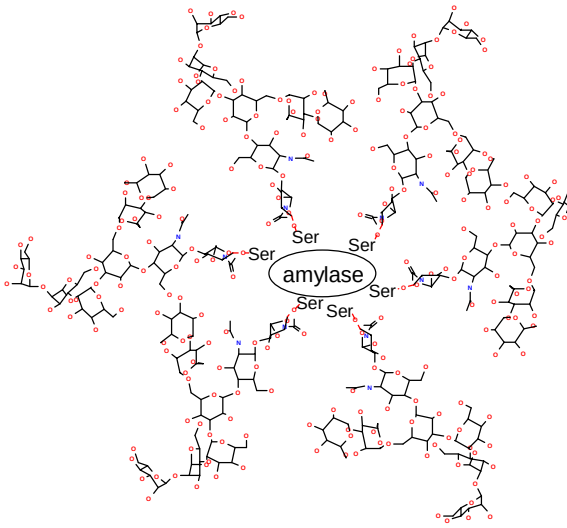


Calculate mas for two heavy chain polypeptides in **IgG1**-all.pdb molecule!
two **heavy** (2*49750,3=99500,6 ..... g/mol mass) and
Calculate mas for two light chain polypeptides in **IgG1**-all.pdb molecule!
two **light** (2*22801,5=45603 ..... g/mol mass) polypeptide chains.

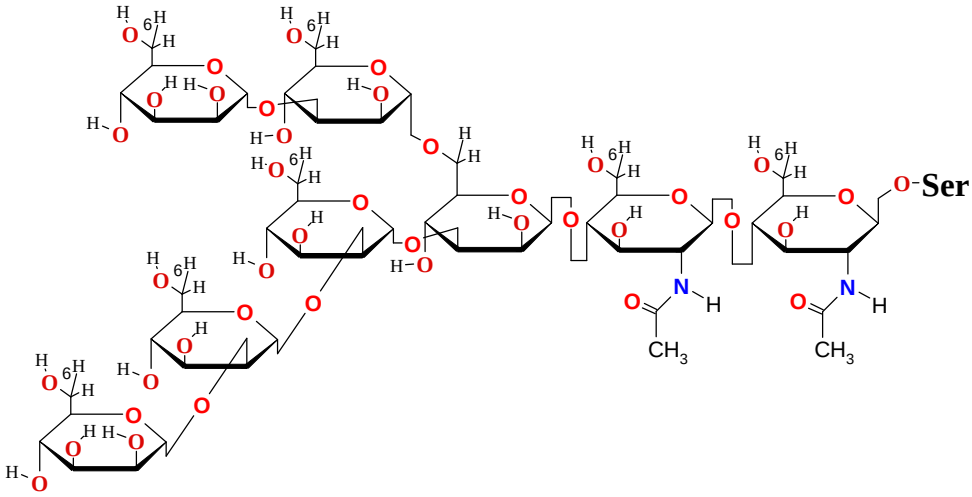
Glycoproteins Carbohydrates polysaccharides + protein

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





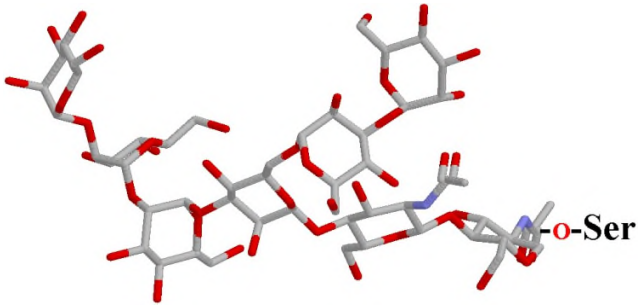
Mucin shield **Amylase enzyme** protect from degradation by peptidases in digestive tract

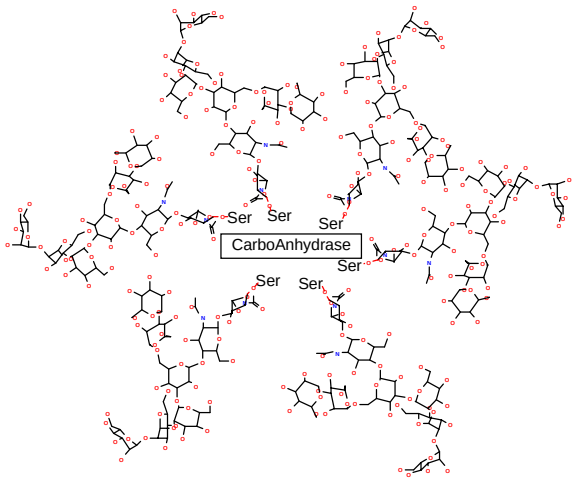


(forked) branched Man(**α**1→3)Man(**α**1→6)↓

Man(**α**1→2)Man(**α**1→2)Man(**α**1→3)Man(**β**1→4)GlcNAc(**β**1→4)GlcNAc-**β**

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





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

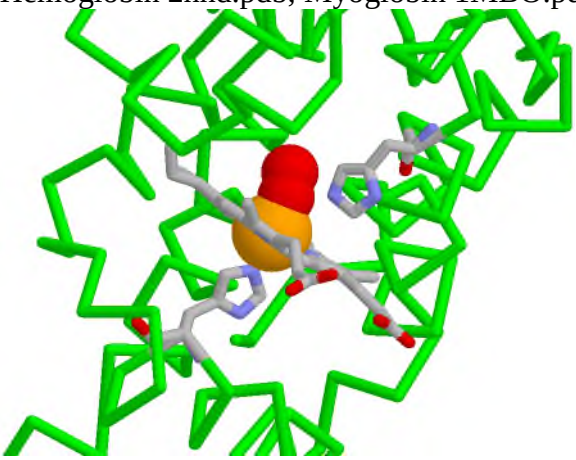
How mucin shield protect against degradation with peptidases in intestinal tract?

Chromoproteins: Cytochrome, **Hemoglobin**, **Catalase**, **Peroxidase** etc.

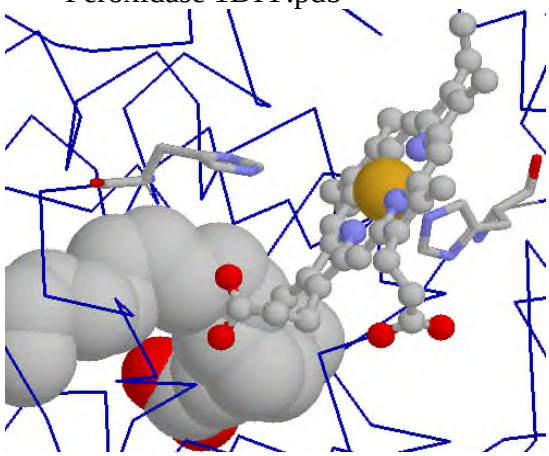
http://aris.gusc.lv/ChemFiles/hemoglobEricMarzUMas/2frmcont.htm

http://aris.gusc.lv/ChemFiles/CycloOxigenase/cycox.html

Hemoglobin 2hhd.pdb, Myoglobin 1MBO.pdb

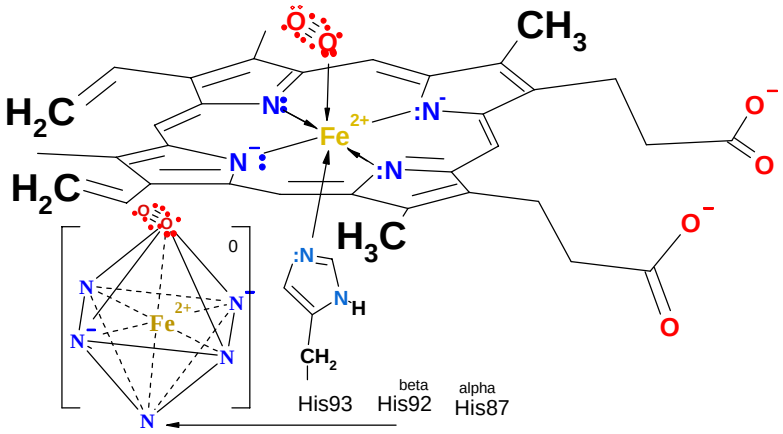


Peroxidase 1DIY.pdb



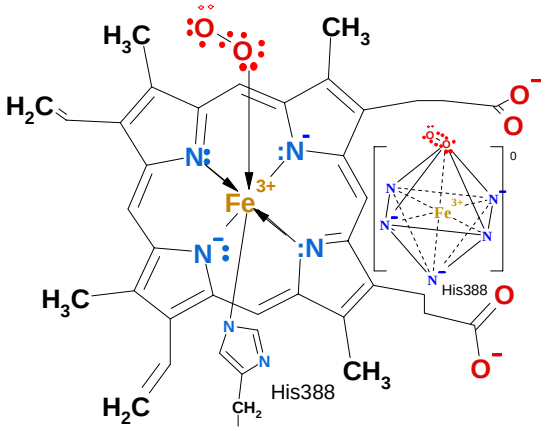
Heme prosthetic group surrounded by globular proteins :

Hemoglobin, Myoglobin,



Triplet oxygen in human organism!

Peroxidase, CytochromP450, Catalase etc.



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_{2aqua} is inactive **triplet** state up to temperature 100º C, but

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

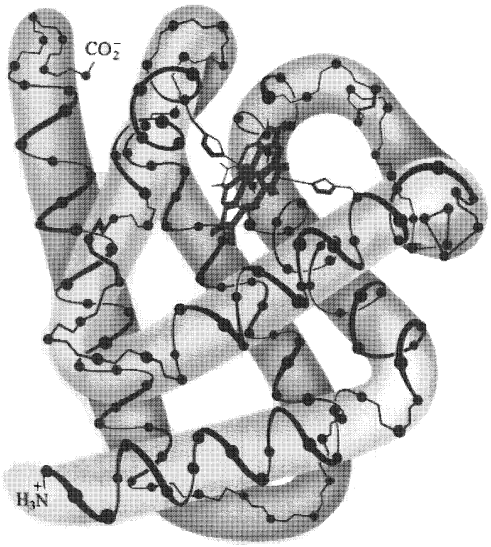
Singlet oxygen risk increases five times in pure atmosphere 100% O_2 as concentration increases from 20% to 100%. Reaction velocity is proportional to concentration. Pure oxygen concentration [O_2] is times 100%/20%=5 five..... increased. Singlet oxygen risk increases five..... times:

$$\vec{v} \sim [O_2]$$

http://aris.gusc.lv/ChemFiles/ChromoHem/MyoGlobOxDeoxCoBiliverdin/1MBODeOxyLopez.kin

Myoglobin $O_2 \Leftrightarrow H^+, HCO_3^-$ shuttle exchange stored oxygen molecule with H^+, HCO_3^- in concentration sensitive oxy⇔ deoxy equilibrium maintaining $[O_{2aqua}]=1.5\cdot10^{-5}$ 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**...**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 3º 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 3º structure support electrostatic attractions called **salt bridges**. Positive Lys—NH_3^+ attract with negative charged Glu carboxylic ^-OOC— group.

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

Max Perutz awarded by Nobel Prize in chemistry on 1963 for **Hemoglobin**.
http://aris.gusc.lv/ChemFiles/ChromoHem/HbOxDeoxCO/2HCOProTour8.kin

Quaternary 4º **Structure**. Hemoglobin consists of 4 synthesised protein monomers

Figure. beta chains

alpha chains

- subunits: **α1**, **α2** 141 amino acids on **chains** and **β1**, **β2** 146 amino acids on **chains** with five **5 intermolecular forces** bounded together. The flat disks represent four **heme**s 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 $[O_{2aqua}]=1.85\cdot10^{-5}$M sensitive equilibrium as conformation changes to deoxy state.

1 --α1Arg141—**COO**$^-$...**H**$_3^+$**N**—α2Lys127,
2 α1Arg141—**COO**$^-$...**H**$_3^+$**N**—α2Val1,
3- β2 Asp94—**COO**$^-$...**H**$_3^+$**N**—β2His146,
4 - β2 His146—**COO**$^-$...**H**$_3^+$**N**—α1Lys40,
5- α2 Arg141—**NH**$_3^+$...$^-$**OOC**—.α1Asp126,

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**....

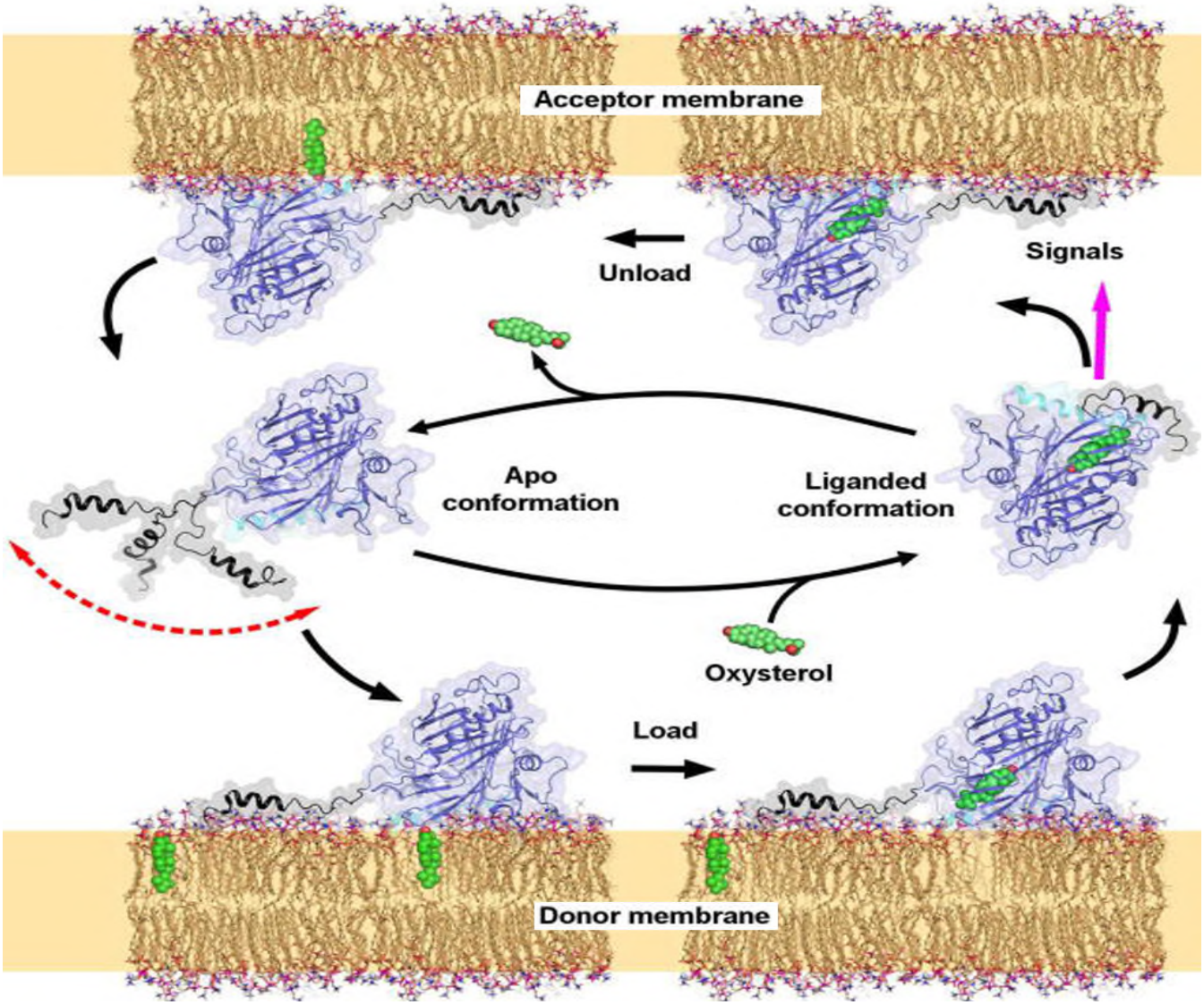
Transport proteins for insoluble lipids are two type extra cellular and intra cellular.

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

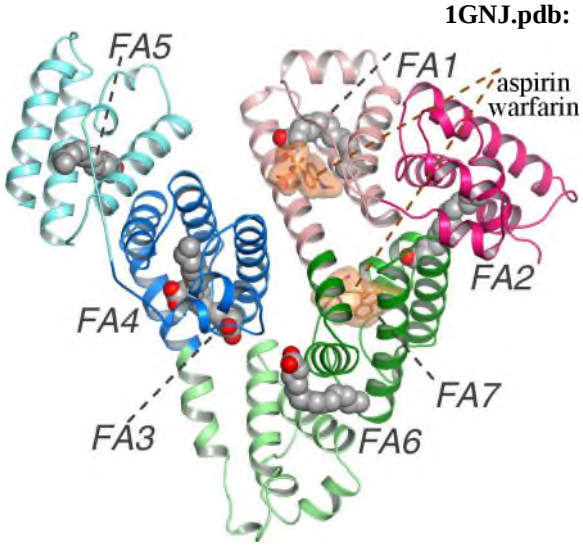
Lipocalins mechanism like **OSBP**, retinol **ORPs** and other **Lipocalins** for A,K,E,D vitamin transport proteins. Human organism has 12 **OSBP** iso forms. **Osh4** human protein isoform **OSBP4** cholesterol exchange. **OSB4 lipocalin** molecule exterior surface around the lid of the tunnel contains seven highly conserved basic positive charged residues Lys15, Lys173, Lys334, Arg344, Arg347, Lys348, Lys353, Lys407, Arg410, Lys411, $-NH_3^+$ attract to negative charged $>PO_4^-$ phosphate on surface as three tentacle helixes. 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

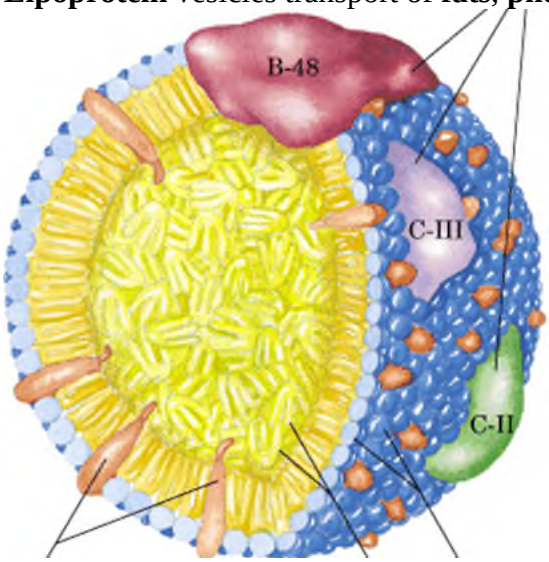
1GNJ.pdb: http://aris.gusc.lv/ChemFiles/Albumin/cycox.html

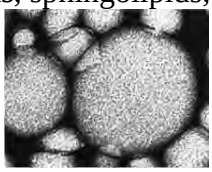


Human serum albumin **HSA** in blood plasma has typical circulating concentration 0.6 mM.

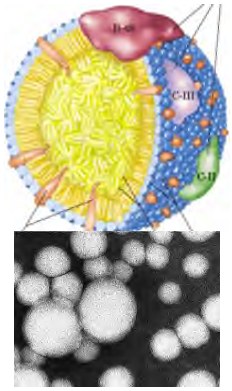
Albumin blood plasma transport lipoprotein for 7 Fatty acids FA1,FA2,FA3,FA4,FA5,FA6,FA7 and **water** insoluble drugs : warfarin, ibuprofen, aspirin etc.

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





80…200 nm



28…70 nm

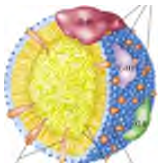
Chylomicrons diameter range from about 100 nm to about 500 nm.

Vesicle comprise up to million (10^6) molecules of **Fats** and **Cholesterin**.

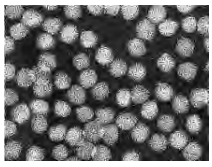
VLDLs, LDL very low density lipoprotein,

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

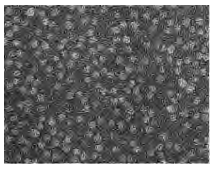


20…25 nm

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

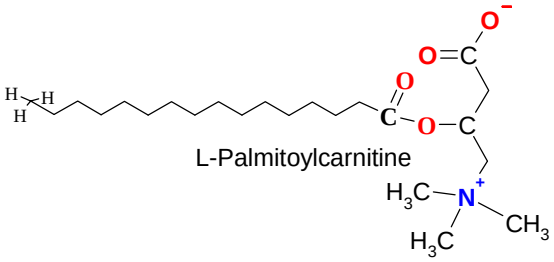


8…12 nm

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 with **energy** from -15,8 to -30,7 *kJ/mol* .. Oxygen desorption $O_2 \Leftrightarrow H^+, HCO_3^-$ of shuttle molecules Mb instantly release acylkarnitin but bind beta oxidation products H^+, HCO_3^-. So maintain concentration $[O_{2aqua}]$, pH=7,36 stable. Mb shuttle serves as fuel suppliers to muscle and cardio myocite cells physiologic sustain homeostasis $[O_{2aqua}]$, pH=7,36



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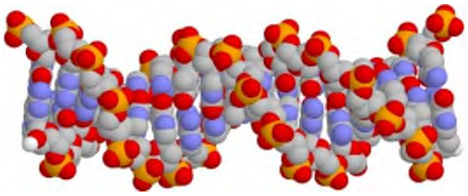
Nucleoproteins

1d66-pwz.pdb: http://aris.gusc.lv/ChemFiles/DnaMarzHTM/fs_pairs.htm

DNA atoms CPK color scheme



1. What base pares constitute DNA fragment?
17 base pairs.....

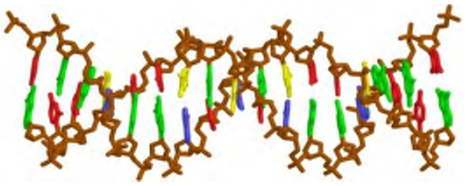
2. What net charge of phosphates >PO_4^- beers fragment?
(-17>PO_4^-) +(-17>PO_4^-) = - 34 net charge.....

3. What names bases paired by two hydrogen bonds?
adenin **A**=**T** thymin.....

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** thymin **T** **G** guanine **C** cytosine



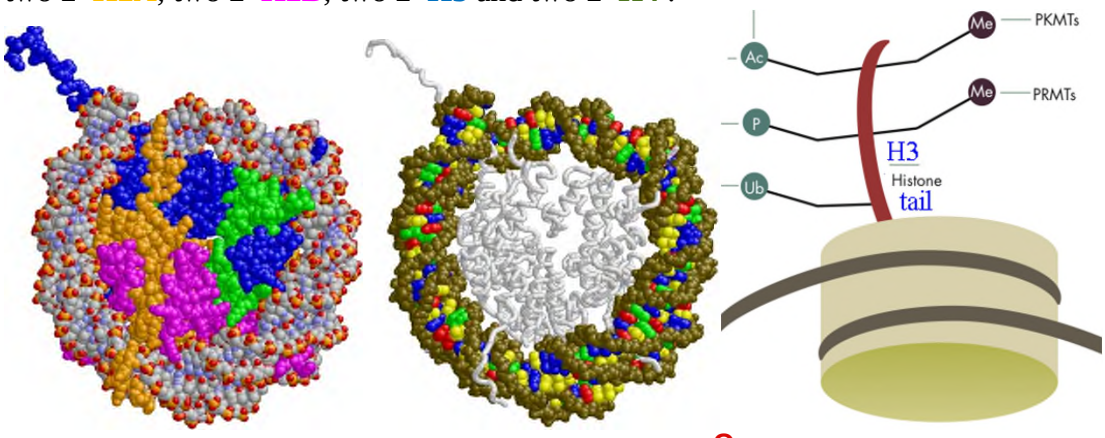
5. What type DNA strands parallel or anti parallel?
Anti parallel.....

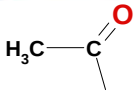
6. Draw the 5' end functional group and 3' end group !
5'$^-O_3P–O–$ phosphate.....3'$–OH^-$ hydroxyl.....

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

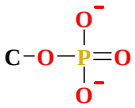
1AOI.pdb: http://aris.gusc.lv/ChemFiles/CLUnucleosome/nucleosome.htm

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 transferees; 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.

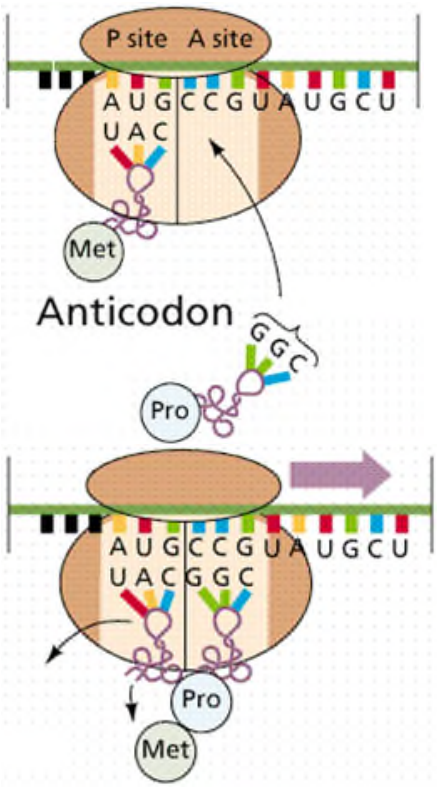
HEALTH ENDPOINTS: Cancer; Autoimmune disease; Mental disorders; Diabetes

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

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

j

Ribosome

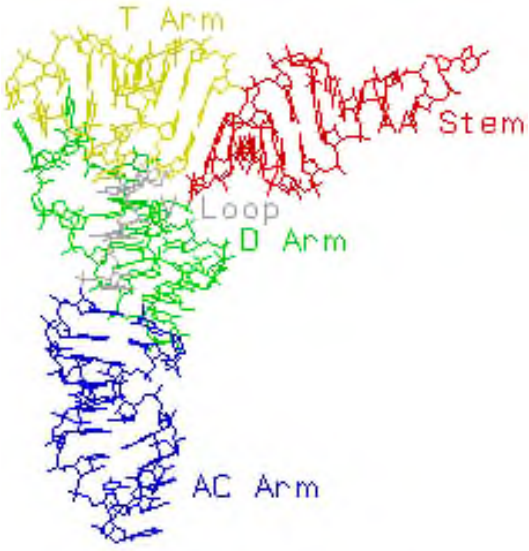


1st position (5'end)↓	**2**nd position U	C	A	G	**3**rd position (3'end)↓
U	Phe	Ser	Tyr	Cys	**U**
	Phe	Ser	Tyr	Cys	**C**
	Leu	Ser	**STOP**	**S-SelCys**	**A**
	Leu	Ser	**STOP**	Trp	**G**
C	Leu	Pro	His	Arg	**U**
	Leu	Pro	His	Arg	**C**
	Leu	Pro	Gln	Arg	**A**
	Leu	Pro	Gln	Arg	**G**
A	Ile	Thr	Asn	Ser	**U**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

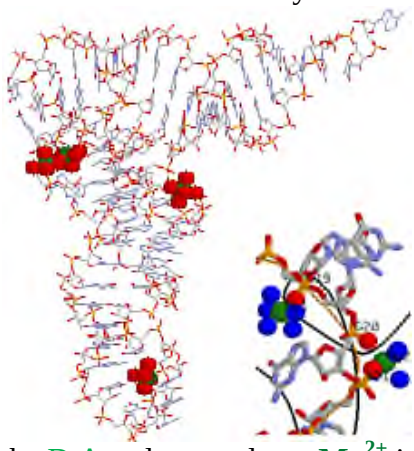
Translation in ribosome start with methionine:
Met init, Pro, Tyr, Ala
1, 2, 3, 4
4 amino acids encoded on mRNA messenger RNA

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

tRNA.pdb: Transfer t**RNA** 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 2Å = 0,2 nm (1 Å = 0,1 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**....