Name Surname :___ Teacher _ , ___ Teacher _____ Group Nº ___ Teacher _____2023

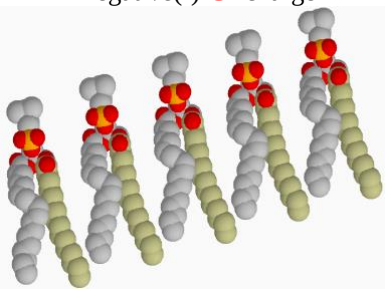
Phospholipids Bilayer Membrane composite materials for compartmentation of organelles: Task for practical research works: for Interactive Molecule:

Chemscape MDLi ISIS Draw RasMol FireFox 3.5.5v **B** task to lunch the Riga Stradin's University **Aris Kaksis** 2023rd prepared **Phospholipids Bilayer Membrane** experimental research practical work :

htdocsLocal http://aris.gusc.lv/ChemFiles/BilipidCholine/Membrane/Membrane/membrane/Membrane.html

Phosphatidyl Choline, Lecithin: molecule Biphilic Hydrophobic & Hydrophilic **CPK** color scheme 1965: at Display conditions: **Stick** (on Menu Stripe) **Ball & Stick** **Space fill** In **Nature Journal Corey, Pauling, Koltun** publication for atomic modeling Positive(+)N^+ and negative(-) O^- charge

Atom Name	Symbol	Color	Valence Number
Carbon	**C**	**Gray** lightly or **Black**	**4**
Hydrogen	H	White	**1**
Oxygen	O^-	**Red**	**2** (donor acceptor ligand up to 4)
Nitrogen	**N**	**Bluish**	**3** +1(donor acceptor ligand up to 4)
Sulfur	**S**	**Yellow**	**-2 , +6**
Phosphor	**P**	**Yellow** Intensive dark	**5** (& 3)
Sodium ion	Na^+	**Blue**	**+1** (coordination up to 6)
Magnesium ion	Mg^{2+}	**Green**	**+2** (coordination up to 6)
Calcium ion	Ca^{2+}	**Gray** Dark	**+2** (coordination up to 6)
Iron ion	Fe^{2+}	**Yellow** Gray	**+2** (coordination up to 6)
Iron ion	Fe^{3+}	**Yellow** Gray	**+3** (coordination up to 6)



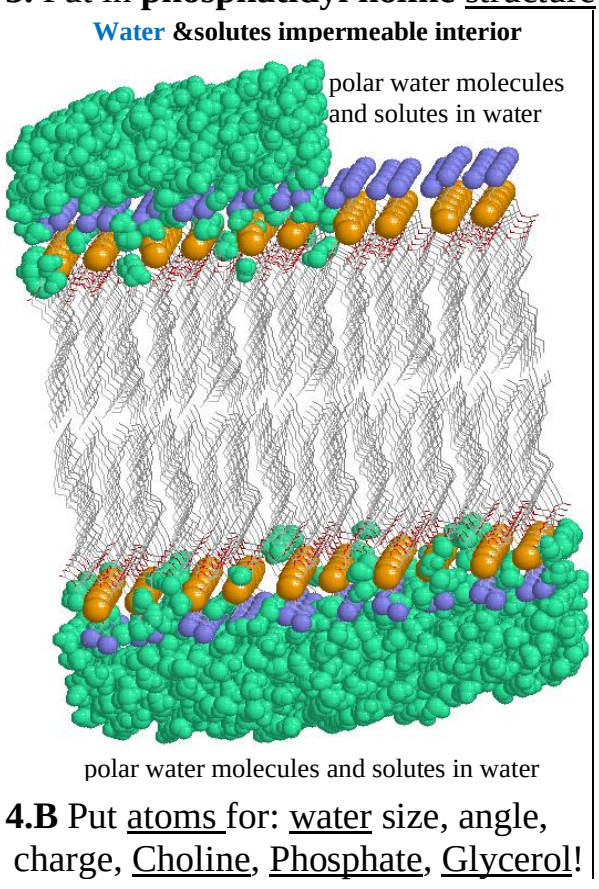
Hydrophobic non polar molecule part

1. Write the main Physiological function of **Phospholipid Bilayer Membranes** in Life?

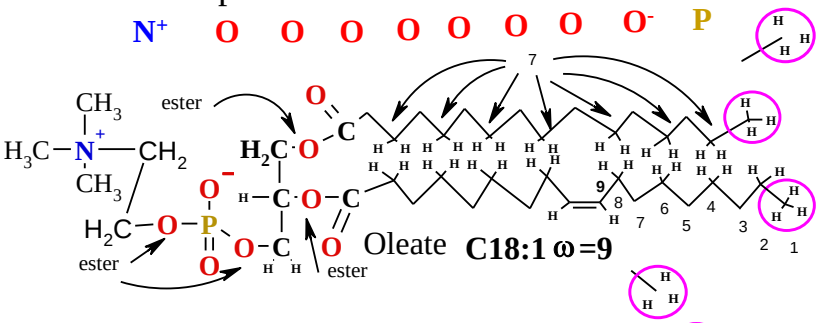
Membrane waterless Interior is impermeable for **water** ..... and **water solutes**..... so work as **isolating** cellular wall for compartment solutions components.....

2. What the Structure Properties has Interior, Exterior. What the biological destiny has cells wall building in liposomes, organelles and **Monolayer** in lipoprotein vesicles. Water medium **green**. Components exterior cannot cross membrane Interior impermeable.....

3. Put in **phosphatidyl holine** structure!



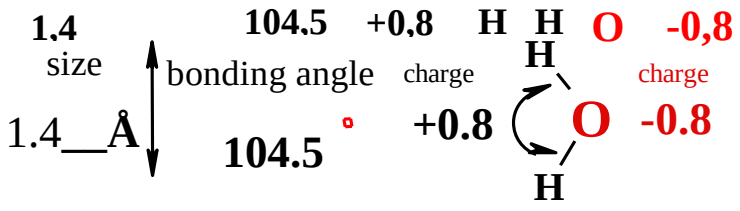
polar water molecules and solutes in water

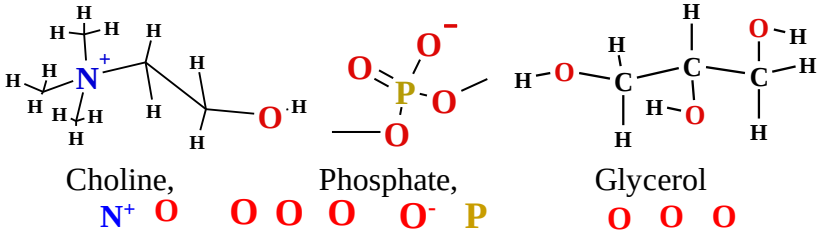


ω number are double bond from methyl group –**CH_3**

Essential omega Fatty Acids **: ω=6** and **ω =3**

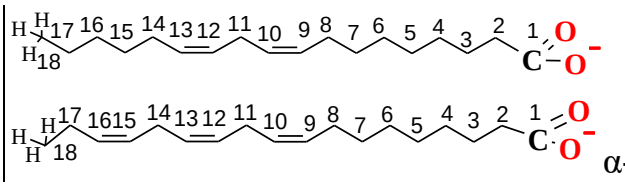
4.A Put in water molecule proper parameters





4.B Put atoms for: water size, angle, charge, Choline, Phosphate, Glycerol!

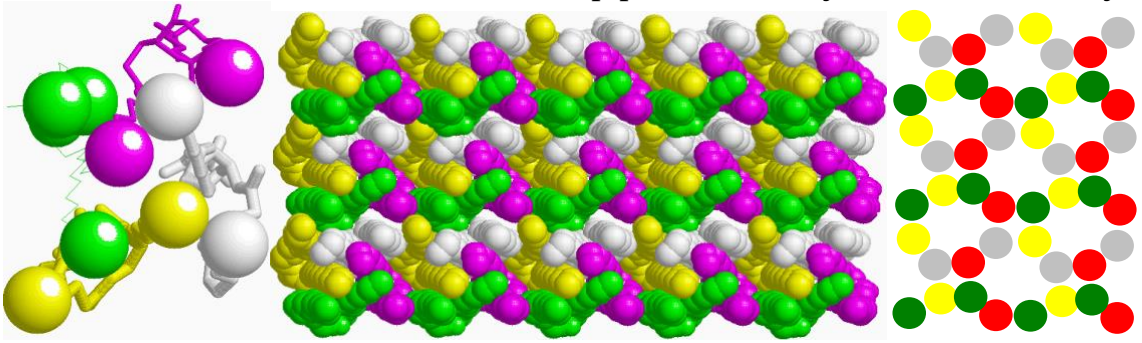
5. Put in <u>essential</u> Fatty Acid Salts oxygen atoms at pH=7.36 : **C18:2 ω=6**..... and **C18:3 ω=3**.....


linoleate

α-linolenate

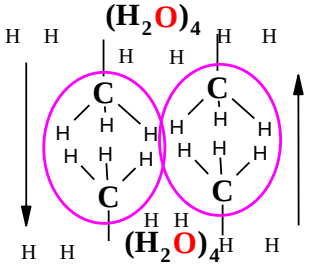
6. Calculate accumulate London Forces -2 $^{kJ}/_{mol}$ Bonding **E**nergy between two hydrogen contacts at each of 7 carbon methylene **–CH_2-** on Palmitate & Oleate chains contact **n**=7•2=14 points!
E= -2•7•2= -2• $\mathbf{n_C}$= -2•14=...-28... $^{kJ}/_{mol}$

7. On drawn tetramer **-CH_3** structure top pattern of fatty acids chains Bilayer detect Number 3 of chains each other surrounded contact!



London Forces per one chain is
$\mathbf{E_C}$=3•**E**=;
$\mathbf{E_C}$=3*-28=..... $^{kJ}/_{mol}$
$\mathbf{E_C}$= -84..... $^{kJ}/_{mol}$

8. What is London Forces made Energy $\mathbf{E_L}$ of Phosphatidyl Choline molecule having two Fatty Acid hydrocarbon chains? $\mathbf{n_L}$=2*$\mathbf{n_C}$=2***42**= 84..... contacts $\mathbf{E_L}$= -2*$\mathbf{n_L}$= -168..... $^{kJ}/_{mol}$



9. Calculate hydrophobic contact -10 $^{kJ}/_{mol}$ **e**nergy $\mathbf{E_H}$ for **water** tetramer structure $(\mathbf{H_2O})_4$ with six contact points in two methyl groups–CH_3 palmitate **C16** and oleate **C18:**1 between two phosphatidyl choline molecules on interface membrane interior of bilayer contacts $\mathbf{n_H}$=2•3=6 for one phosphatidyl choline molecule $\mathbf{E_H}$=-10•3/2=-60/2 =-30..... $^{kJ}/_{mol}$?
9.a Put in structure 12 H given atoms for six hydrophobic bonds!

10. What total Bonding Force Energy per one Bilayer **Phosphatidyl Choline** molecule?
Each single phospholipid in membrane distributed London Forces -2 $^{kJ}/_{mol}$ for 84 contact points -168 $^{kJ}/_{mol}$ adds **hydrophobic** interactions **E**nergy -30 kJ/mol forming total sum on Phosphatidyl Choline. $\mathbf{E}_{Bond}$ = -168 +(-30) = -198..... $^{kJ}/_{mol}$

11. Measure the thickness of **Phospholipid Bilayer Membrane** using <u>right button</u> click on interactive picture and in menu chose "**select**", "**Mouse Click Action**" , "**Distance**". Experimentally measure the thickness of **Membrane** performing by mouse two clicks: 1) on **blue nitrogen** atom one side and following click on **blue nitrogen** atom opposite side of **Membrane**. On status bar is shown distance value in angstroms 56.....**Å**. Get the average size of Membrane thickness as mean $\mathbf{Dist_{mean}}$ =56.....Å as nanometers 5.6..... nm!

12. What number of 1.4 Å size water molecules cover the distance 56 Å? 56/1.4= 40..... times

13. What would be thickness in meters of home buildings walls if human tall size is 1.75 meters?
So wall (membrane) cross channel would be 1.75*40=70.....m meters long .

14. What kind of pure Phosphatidyl Choline bilayer membrane physical state is liquid or solid? liquid....

15. What mass fraction constitute the Phospho Lipid molecules in cellular membranes total mass 100%? 33.3%..... ...

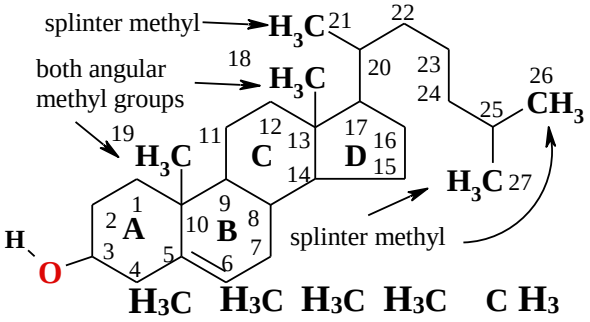
Phosphatidyl Choline in **Membrane** constitute 1/3 part 33.3% of **Membranes** mass 100%.

I) 1/3 part constitute **Phospholipids** which mass fraction of **Membranes** to make **33.3%** of total mass 100%;
II) second **1/3** part **Cholesterols** which mass fraction of **Membranes** to make **33.3%** of total mass 100%;
III) third **1/3** part **Membranes integral Proteins** which mass fraction to make 33.3% of total mass 100%
Bulk mass fraction **20%** goes to **Aquaporins \nd** for other **Proteins** remains **13.3**.....**%** :

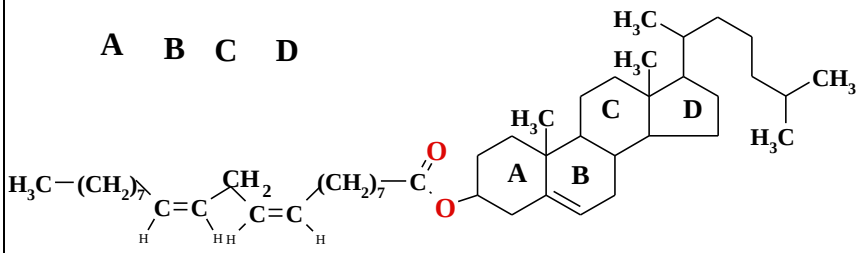
The **Cholesterol/Phospho Lipid** C/PL mole ratio of human red cell membranes ranges from a normal value of 0.9–1.0 (Journal of Cellular Biochemistry 2004 V8, 4, p 413-430). 1 mol cholesterol against 1 mol Phospholipid.

C. Task **Cholesterol Steroid Lipid** All atoms **C27 ℍ46 O** colored **CPK** labels in work2:
htdocsLocal http://aris.gusc.lv/ChemFiles/BilipidCholine/Membrane/Cholesterol/CholesterolMembran.html;

1. Put in **cholesterol** hydrocarbon circle symbols **A B C D**
double bond >**C=C**<
oxygen in alcohol **HO**- at C3, **O**



2. Put in **cholesterol and linoleate C18:2** ester **O O** oxygen atoms and



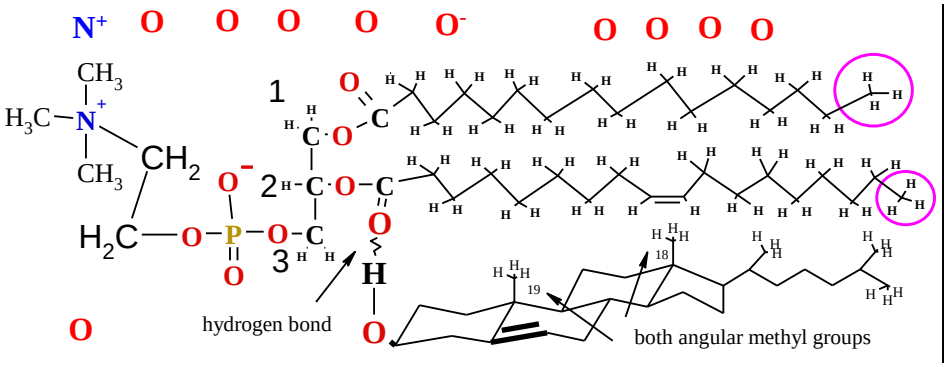
hydrocarbon four circle symbols in structure!

angular methyl **–CH₃** groups C18, C19, 3 metil groups **–CH₃** : splinter, fork, rod are good clutch fixing chains of hydrocarbon mechanically in phosphatidyl choline bilayer **membrane**.

3. What three Cholesterol functions of Human body? Mechanically unbroken membranes.....
.....Bile salt production, Steroid hormones.....

4. What is Cholesterol 1/3 mass fraction eukaryote membranes of total 100%?33,3%.....

5. Cholesterol/Phospholipid rate in erythrocyte membranes 0,9-1/1.....!published in 1978. Year **Cholesterol** and **Phospho Lipid** complex C/PL=1/1 in cell membranes



5. a Put given atoms in Phosphatidyl choline (lecithin) Cholesterol composite complex structure

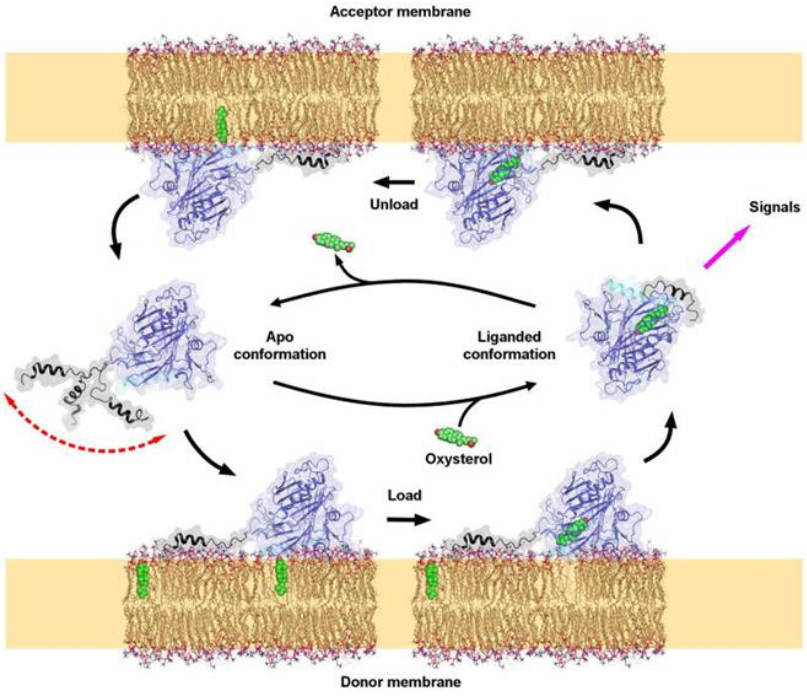
$\frac{\text{Cholesterol}}{\text{Phospho\_Lipid}}$ mole ratio $\frac{C}{PL}$ of human red blood cell membranes a normal value $\frac{C}{PL}$ =0,9 ÷ 1.....

5.a. Mass fraction of phospholipids 1/3 in eukaryotes membranes of total 100%? 33,3%.....

5.b. What two 1. and 2. inter molecular forces bind membranes phospholipids and cholesterol molecules assigned mechanic strength and elastic flexibility?

1. Hydrogen bond between hydrogen acceptor phospholipid carboxylic group >**C=O**.....
And as hydrogen donor hydroxyl group **HO**- of cholesterol..... molecule,
2. Van der Walls dispersion forces among no polar hydrocarbons structured atoms in lipid chains of .....cholesterol and of fatty acids chains.....

htdocsLocal http://aris.gusc.lv/ChemFiles/BilipidCholine/Membrane/Cholest5ene3-20diol/Cholesterol5Membran.html;



OSBP oxi-sterol transport protein involved in cholesterol metabolic transport across membranes surface, that keep 33.3% mass fraction 1/3 of 100% membrane mass. Cholesterol load and unload from to membranes. **Lipocalins** mechanism like **OSBP**, retinol **ORPs** and other **Lipocalins** A,K,E,D vitamin transport proteins. Human organism has 12 **OSBP** iso forms. To investigate **Osh4** human protein iso form **OSBP4** for steroids:

Cholesterol CRL.pdb,

20-hidroksiholesterols HC2_ideal.pdb, 25-hidroksiholesterols HC3_ideal.pdb, 7-hidroksiholesterols HCR_ideal.pdb

6. In **Osh4** protein **1ZHY**Marz.**pdb** at Display **Backbone**, Termini to determine domain **N-terminus** amino acid...MET1... and **C-terminus** amino acid LEU434.....!

7. Count amino acids in **OSBP** sterol membrane-membrane transport polypeptide chains primary structure of **1ZHY.pdb**, **1ZHW.pdb**, **1ZHX.pdb**, **1ZHT.pdb**...434-1=433+1.....?

8. Put in **oxy-sterol** four circle symbols

two oxygen atoms

angular **–CH₃** groups C18, C19 :

and 3 metil groups **–CH₃**:

as splinter, fork, rod on flexible tail

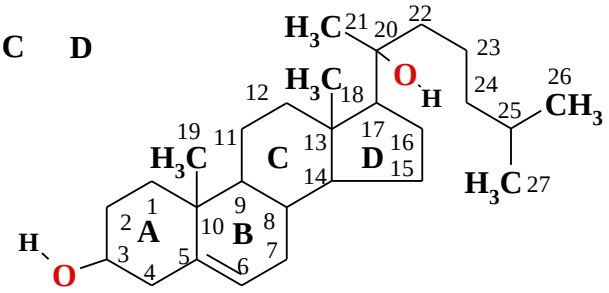
A B C D

O O⁻

H_3C H_3C

H_3C H_3C

C H_3



9. What number hydroxyls **-OH** found in cholesterol and 20-hidroxy cholesterol? 1.....un 2.....

10. Put in 25-hydroxicholesterol

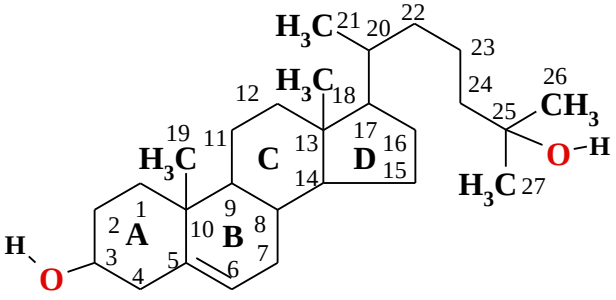
four circle symbols: A B C D

two oxygen atoms O O⁻

angular **–CH₃** groups C18, C19 : H_3C H_3C

and 3 metil groups **–CH₃**: H_3C H_3C

as splinter, fork, rod on flexible tail C H_3



11. Put in 7-hidroksiholesterolā

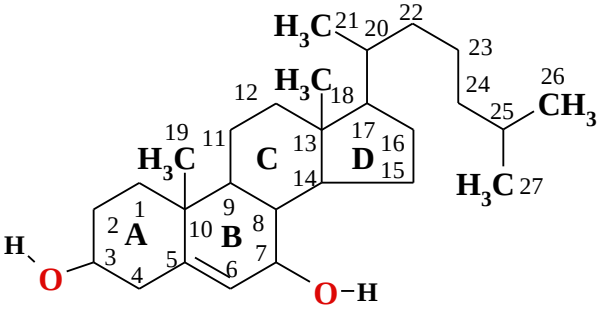
four circle symbols: A B C D

two oxygen atoms O O⁻

angular **–CH₃** groups C18, C19 : H_3C H_3C

and 3 metil groups **–CH₃**: H_3C H_3C

as splinter, fork, rod on flexible tail C H_3



12. What molecules are in human body transporting cholesterol, A vitamins, hormones fats un fatty acids? Lipocalins.....**OSBP** oxy-sterol transport proteins.....

...**ORPs** retinol (retinal) transport proteins.....

12.1-12.5. 1ZHY.pdb KES1 isoelectric point IEP=pH=$pK_{a\text{-mean}}$ at physiologic pH=7,36 .
Determine at solution pH with KES1 concentration C=$10^{-7,286}$ M ($^{mol}/_{Liter}$)!

1ZHY, 3SPW.pdb KES1_YEAST oxysterol-binding protein 4 homolog 49,493 kDa concentration $10^{-7,286}$ M
http://aris.gusc.lv/ChemFiles/START/1ZHZpIStudS.doc ; http://aris.gusc.lv/ChemFiles/START/1ZHZpI.xls

SQ SEQUENCE 434 >1ZHZ:A|PDBID|CHAIN|SEQUENCE: RLBP1+Retinal -1-434 (1-434)
MSQYASSSSWTSFLKSIASFNGDLSSLSAPPFILSPISLTEFSQYWAEHPELFLEPSFINDDNYKEHCLIDPEVESPELA
RMLAVTKWFISTLKSQYCSRNESLGSEKKPLNPFLGELFVGKWENKEHPEFGETVLLSEQVSHHPPVTAFSIFNDKNKVK
LQGYNQIKASFTKSLMLTVKQFGHTMLDIKDESYLVTPPPLHIEGILVASPFVELEGKSYIQSSTGLLCVIEFSGRGYFS
GKKNSFKARIYKDSKDSKDKEKALYTISGQWSGSSKIIKANKKEESRLFYDAARIPAEHLNVKPLEEQHPLESRKAWYDV
AGAIKLGDFNLIAKTKTELEETQRELRKEEEAKGISWQRRWFKDFDYSVTPEEGALVPEKDDTFLKLASALNLSTKNAPS
GTLVGDKEDRKEDLSSIHWRFQRELWDEEKEIVL

AA	pKa$_{COO^-}$	pKa$_{NH3^+}$	pK$_{RR}$	Nr	AA	pKa$_{COO^-}$	pKa$_{NH3^+}$	pK$_{RR}$	Nr	AA	pKa$_{COO^-}$	pKa$_{NH3^+}$	pK$_{RR}$	Nr	AA	pKa$_{COO^-}$	pKa$_{NH3^+}$	pK$_{RR}$	Nr
M	1	9,21		1	C	26	8,18		98	H	51	6		184	K	76	10,53		258	Y	101	10,07		318	E	126	4,25		379
Y	2	10,07		4	R	27	12,48		100	D	52	3,65		188	D	77	3,65		259	D	102	3,65		319	K	127	10,53		380
K	3	10,53		15	E	28	4,25		102	K	53	10,53		190	K	78	10,53		260	K	103	10,53		325	D	128	3,65		381
D	4	3,65		23	E	29	4,25		107	D	54	3,65		191	E	79	4,25		261	D	104	3,65		328	D	129	3,65		382
E	5	4,25		41	K	30	10,53		108	E	55	4,25		192	K	80	10,53		262	K	105	10,53		334	K	130	10,53		386
Y	6	10,07		45	K	31	10,53		109	Y	56	10,07		194	Y	81	10,07		265	K	106	10,53		336	K	131	10,53		396
E	7	4,25		48	E	32	4,25		117	H	57	6		202	K	82	10,53		276	E	107	4,25		338	D	132	3,65		406
H	8	6		49	K	33	10,53		122	E	58	4,25		204	K	83	10,53		279	E	108	4,25		340	K	133	10,53		407
E	9	4,25		51	E	34	4,25		124	E	59	4,25		214	K	84	10,53		282	E	109	4,25		341	E	134	4,25		408
E	10	4,25		55	K	35	10,53		126	E	60	4,25		216	K	85	10,53		283	R	110	12,48		344	D	135	3,65		409
D	11	3,65		61	E	36	4,25		127	K	61	10,53		218	E	86	4,25		284	E	111	4,25		345	R	136	12,48		410
D	12	3,65		62	H	37	6		128	Y	62	10,07		220	E	87	4,25		285	R	112	12,48		347	K	137	10,53		411
Y	13	10,07		64	E	38	4,25		130	C	63	8,18		229	R	88	12,48		287	K	113	10,53		348	E	138	4,25		412
K	14	10,53		65	E	39	4,25		133	E	64	4,25		232	Y	89	10,07		290	E	114	4,25		349	D	139	3,65		413
E	15	4,25		66	E	40	4,25		139	R	65	12,48		236	D	90	3,65		291	E	115	4,25		350	H	140	6		418
H	16	6		67	H	41	6		143	Y	66	10,07		238	R	91	12,48		294	E	116	4,25		351	R	141	12,48		420
C	17	8,18		68	H	42	6		144	K	67	10,53		242	E	92	4,25		298	K	117	10,53		353	R	142	12,48		423
D	18	3,65		71	D	43	3,65		155	K	68	10,53		243	H	93	6		299	R	118	12,48		359	E	143	4,25		424
E	19	4,25		73	K	44	10,53		156	K	69	10,53		247	K	94	10,53		303	R	119	12,48		360	D	144	3,65		427
E	20	4,25		75	K	45	10,53		158	R	70	12,48		249	E	95	4,25		306	K	120	10,53		363	E	145	4,25		428
E	21	4,25		78	K	46	10,53		160	Y	71	10,07		251	E	96	4,25		307	D	121	3,65		364	E	146	4,25		429
R	22	12,48		81	Y	47	10,07		164	K	72	10,53		252	H	97	6		309	D	122	3,65		366	K	147	10,53		430
K	23	10,53		87	K	48	10,53		168	D	73	3,65		253	E	98	4,25		312	Y	123	10,07		367	E	148	4,25		431
K	24	10,53		94	K	49	10,53		173	K	74	10,53		255	R	99	12,48		314	E	124	4,25		372	L	2,36	149		434
Y	25	10,07		97	K	50	10,53		180	D	75	3,65		256	K	100	10,53		315	E	125	4,25		373					

IEP=7,4338926 ; sum=1107,65 ; 149 protolytic constants

protolytic average constant isoelectric point IEP=pK_{a_mean} calculate of side chains $\Sigma pK_{aRside\ group}$.. $pK_{aNterminal}NH_3$ and $pK_{aCterminal}COO^-$ constants sum divide with number of acid groups NpK_a:

$$IEP=pK_{amean}=(\Sigma pKa_{Rside\ group}+pKa_{Nterminal}+pKa_{Cterminal})/NpKa$$

Calculate Yeast KES1 49,493-kDa molecule concentration $10^{-7,286}$ M

12.1 Acid groups sum NpKa=Sum of 147 + 2 = 149 pKa values in table: 1107,65.....
434 amino acids of them protolytic constants pK_a for side groups 147+2 ,
N-terminal methionine M $pK_{aNterminal}$=9.21 and C-terminal aspartate D $pKa_{Cterminal}$=1.88 .
Sum are calculate as $\Sigma pKa_{Rside\ group}+pK_{aNterminal}+pKa_{Cterminal}$ = 1107,65.....

12.2 Average acid group constant **ISOELEKTRIC POINT** pK_{mean}= IEP = 1107,65 / 149 =**7.4338926**.....
At pH value of amino acid and protein on isoelectric point pH=**IEP total charge** is zero „**0**”
0——— plus (+) acidic————zero charge „**0**” **IEP**=pH———— minus (-) basic———→ 14 pH scale
-COOH & **-NH_3^+** positive charge .....**-COO^-** & **-NH_3^+**.....charge is negative **-COO^-** & **-NH_2**
Underline and determine existing: positive (+) or zero or negative (-)!

12.3 Determine ASAP1 molecule charge sign (+). zero „**0**” or (-) at physiologic pH=7.36
Underline existing:

-COOH & **-NH_3^+** positive (+) charge ……. pH=7.36 < IEP=**7.43** ..... charge negative(-) **-COO^-** & **-NH_2**.

12.4 Determine KES1 molecule charge sign (+). zero „**0**” or (-) at **electrophoresis** pH **8.8**
Underline existing:

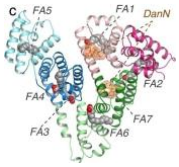
-COOH & **-NH_3^+** positive (+) charge ……. IEP=**7.43**< pH=8.8 ..... charge negative(-) **-COO^-** & **-NH_2**.

12.5 Calculate C = $10^{-7,0794}$ mol / Liter M KES1 solution pH by *Ostwald dilution law* in logarithm of C:

$$pH=\frac{pK_a-\log C}{2}=\frac{7,4338926-\log 10^{-7,2861074}}{2}=\frac{7,4338926+7,2861074}{2}$$ = **14,72** / 2 = 7,36.....

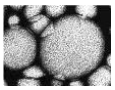
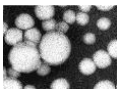
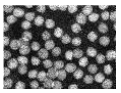
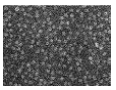
7,36 Attractor YEAST_KES1 concentration is C=$10^{-7,0794}$.....M .

13. Write five lipids transport forms for lipoprotein aggregates under photography!



Albumin.....

HDL join esterify outstanding cam Cholesterol molecule which protrude on membrane surface as insoluble. So avoid atherosclerosis and keep healthy cardiovascular state.

80…200 nm  28…70 nm  20…25 nm  8…12 nm 

……..... chylomicron..... VLDL.....LDL.....HDL....

14. What compounds transport lipoproteins enclosed interior! .....

Fatty acids, aspirin, warfarin, Ibuprofen, cholesterin.....

Fat soluble vitamins: K-E-D-A, fats and oils Triglycerides, other medicaments.....

15. Write two transport forms of lipocalins! **OSBP** and **ORPs**.....

16. What three mass fractions 1/3,1/3,1/3 form cell **membranes** 100% mass**?**

...cholesterol.....phospholipids.....membrane proteins.....

17. What secondary structures comprise **OSBP4 1ZHY.pdb**?

17 alpha helixes.....

..**beta half-barrel** of **12 strands** and **two beta sheets** from **two strands** each.....

18. What 6 nonpolar amino acids **OSBP4** protein closing lid on **N-terminus** helix **H2**

... serve as support floor for cholesterol tail methyl groups **–CH_3** of carbons C26 and C27? .....

...Trp10,Phe13,Leu14,Ile17,Leu27,Ala29.....

19. Which 3 water molecules HOH bound buried C3 hydroxyl group -**OH**?

... HOH2003, HOH2004, HOH2018.....

20. What inter molecular bonds bound hydroxyl group -**OH** with water molecules on bottom

..... of tunnel?.....hydrogen bonds.....

21. What 5 amino acids bind with hydrogen bonds buried C3 hydroxyl group –**OH** and three water molecules HOH2003, HOH2004, HOH2018?Gln96,Trp46,Tyr97,Asn165, Gln181.....

22. What 7 amino acids of 20 proteino genic form hydrophobic pocket tunnel bottom interior envelope together 38 non polar amino acids buried in tunnel lipid molecule closing with lid helix **H2**?... 7=Pro, Gly, Ala, Val, Leu, Ile, Phe.....

...Pro1,Ala5,Leu24,Leu27,Ala29,Pro31,Ile33,Leu39,Phe42,Leu93,Gly105,Pro110,Leu111,...13

...Pro145,Pro146,Val147,Ala149,Ile167,Ala169,Phe171,Leu175,Leu177,Val179,Phe182,.....11

...Pro198,Pro199,Pro200,Ile203,Ile206,Leu207,Val208,Ala209,Pro211,Phe212,Val213,.....11

...Leu215,Leu290,Pro304,Leu305,Ala321.....5

23. Where disposed **C-terminus** polypeptide chain residues from 308 to 434 amino acids in protein? behind tunnel of beta barrel, where enclosed buried lipid.....

24. What 10 basic amino acids outside on **OSBP4 H2** lid surface are positively charged?.....

...Lys15,Lys173,Lys334,Arg344,Arg347,Lys348,Lys353,Lys407,Arg410,Lys411.....