




Phospholipids Bilayer Membrane composite materials for compartmentation of organelles:

Task for practical research works: for Interactive Molecule:

Chemscape MDL  ISIS Draw  RasMol  FireFox 3.5.5v **B** task to lunch the Riga Stradin's University Aris Kaksis 2023rd prepared **Phospholipids Bilayer Membrane**

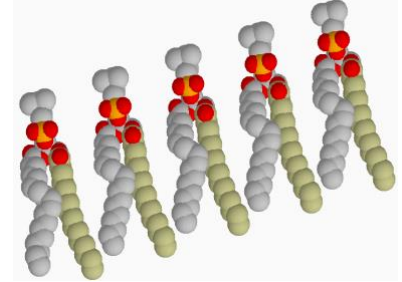
experimental research practical work :

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)



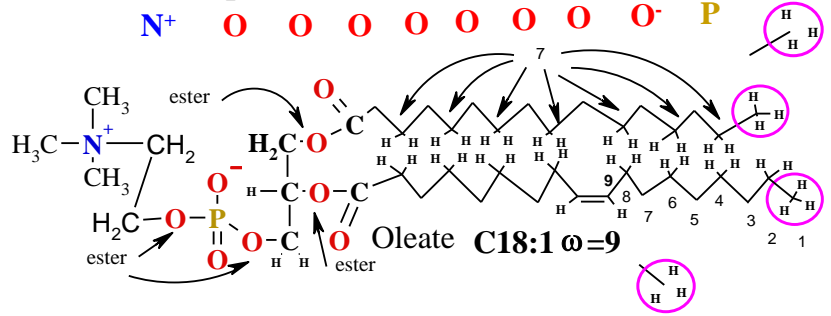
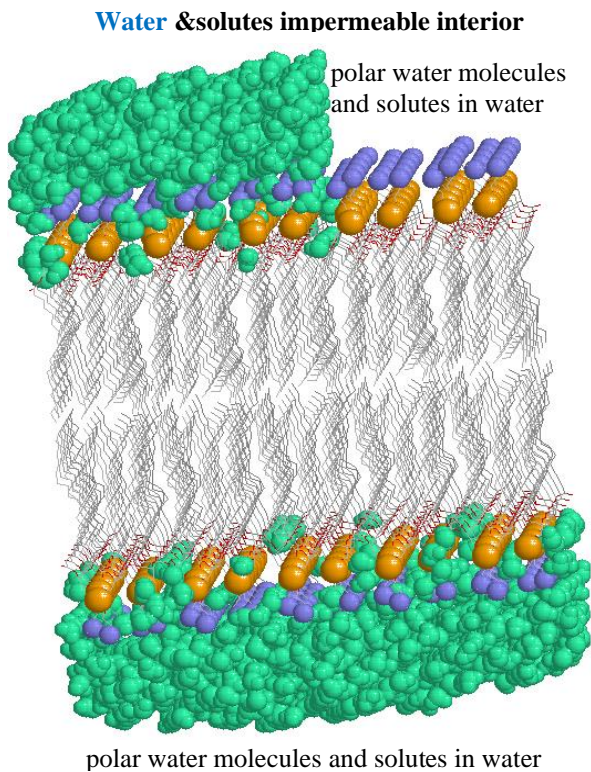
Hydrophobic non polar molecule part

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**.....

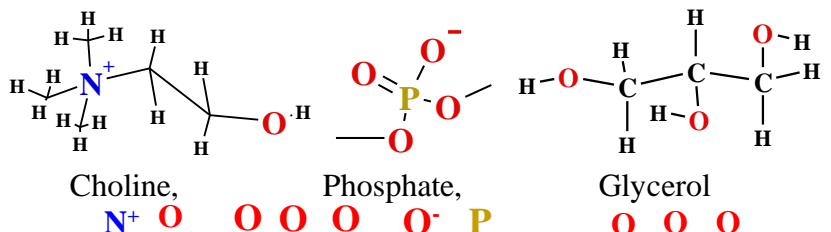
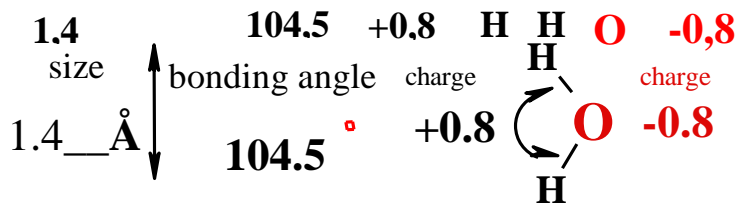
2. What the Structure Properties has Interior, Exterior. What the biological destiny has **cells** wall building in **liposomes, organelles** and **Monolayer** in lipoprotein vesicles. **Water medium green.** Components exterior cannot cross membrane Interior impermeable.....

3. Put in phosphatidyl holine structure!



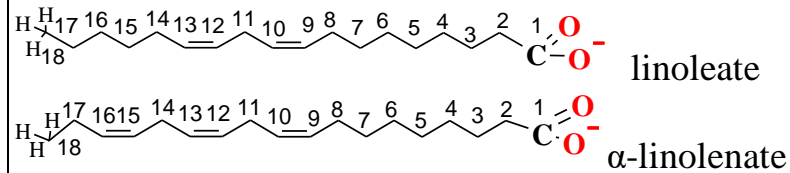
ω number are double bond from methyl group -CH₃
Essential omega Fatty Acids : ω=6 and ω=3

4.A Put in water molecule proper parameters



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

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

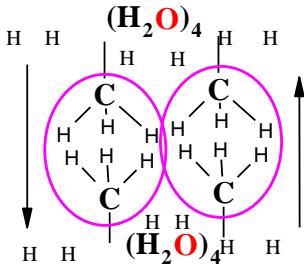


6. Calculate accumulate London Forces -2 kJ/mol Bonding Energy between two hydrogen contacts at each of 7 carbon methylene $-\text{CH}_2-$ on Palmitate & Oleate chains contact $n=7 \cdot 2=14$ points!
 $E = -2 \cdot 7 \cdot 2 = -2 \cdot n_C = -2 \cdot 14 = \dots -28 \dots \text{ kJ/mol}$

7. On drawn tetramer $-\text{CH}_3$ structure top pattern of fatty acids chains Bilayer detect Number 3 of chains each other surrounded contact! London Forces per one chain is

$E_C = 3 \cdot E = ;$
 $E_C = 3 \cdot -28 = \dots \text{ kJ/mol}$
 $E_C = -84 \dots \text{ kJ/mol}$

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



9. Calculate hydrophobic contact -10 kJ/mol energy E_H for **water** tetramer structure $(\text{H}_2\text{O})_4$ with six contact points in two methyl groups $-\text{CH}_3$ palmitate C16 and oleate C18:1 between two phosphatidyl choline molecules on interface membrane interior of bilayer contacts $n_H = 2 \cdot 3 = 6$ for one phosphatidyl choline molecule $E_H = -10 \cdot 3 / 2 = -60 / 2 = -30 \dots \dots \dots \text{ kJ/mol}$
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 Energy -30 kJ/mol forming total sum on Phosphatidyl Choline. $E_{\text{Bond}} = -168 + (-30) = -198 \dots \dots \dots \text{ kJ/mol}$

11. Measure the thickness of **Phospholipid Bilayer Membrane** using right button 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 $56 \dots \dots \dots \text{ \AA}$. Get the average size of Membrane thickness as mean $\text{Dist}_{\text{mean}} = 56 \dots \dots \dots \text{ \AA}$ as nanometers $5.6 \dots \dots \dots \text{ nm}$!

12. What number of 1.4 \AA size water molecules cover the distance 56 \AA ? $56 / 1.4 = 40 \dots \dots \dots$ 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 $1.75 \cdot 40 = 70 \dots \dots \dots \text{ m}$ 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.

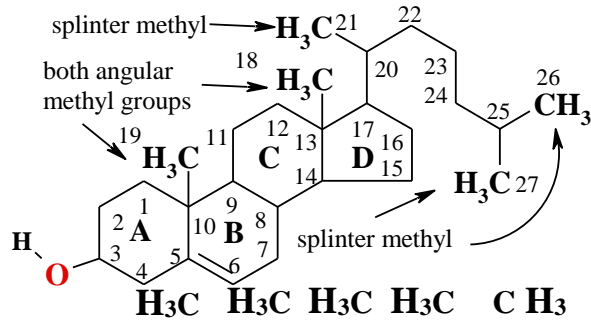
C. Task Cholesterol Steroid Lipid All atoms **C27** **H46** **O** colored **CPK** labels in work2:

htdocsLocal <http://aris.gusc.lv/ChemFiles/BilipidCholine/Membrane/Cholesterol/CholesterolMembran.html>;

1. Put in **cholesterol** hydrocarbon circle symbols

double bond $>C=C<$

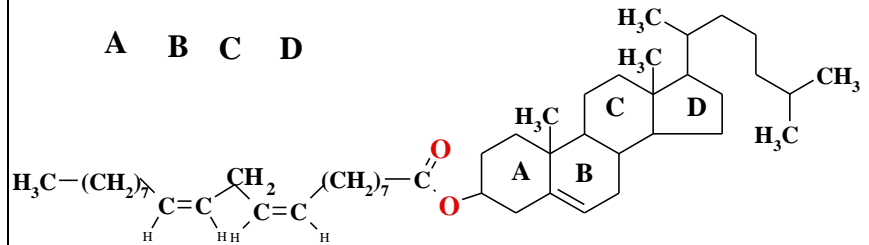
oxygen in alcohol **HO-** at C3, **O**



2. Put in **cholesterol and linoleate C18:2** ester

O O oxygen atoms and

A B C D



hydrocarbon four circle symbols in structure!

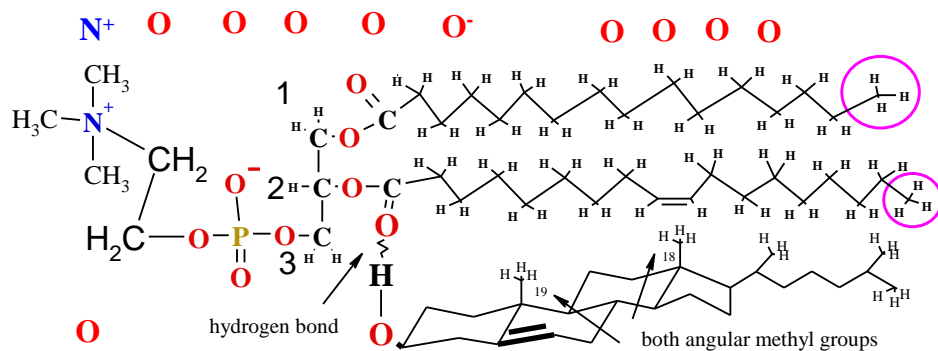
angular methyl $-CH_3$ groups C18, C19, 3 methyl groups $-CH_3$: splinter, fork, rod are good clutch fixing chains of hydrocarbon mechanically in phosphatidyl choline bilayer **membrane**.

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 $\frac{C}{PL} = 0,9 \div 1 \dots$

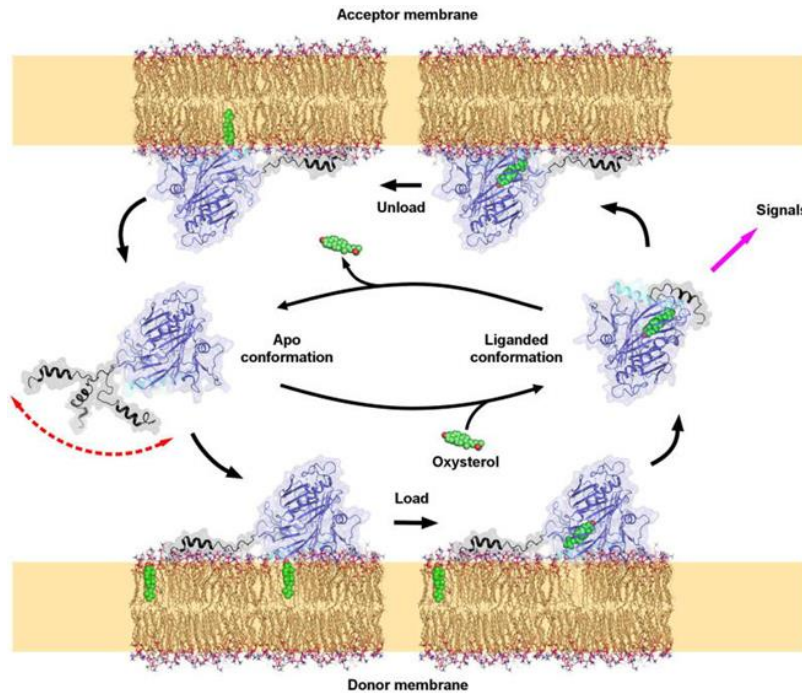
5.a. Mass fraction of phospholipids 1/3 in eukaryotes membranes of total 100%? 33,3%.....

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 Waals dispersion forces among non polar hydrocarbons structured atoms in lipid chains ofcholesterol and of fatty acids chains.....



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-hidroksiholessterols HC2_ideal.pdb,
- 25-hidroksiholessterols HC3_ideal.pdb,
- 7-hidroksiholessterols 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.....?

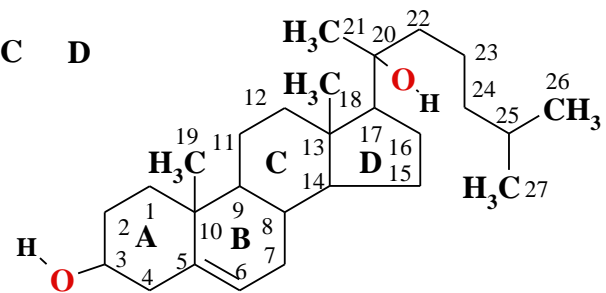
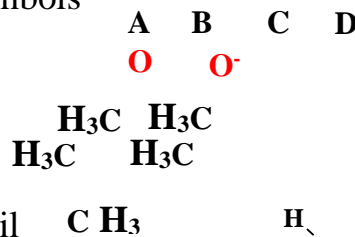
8. Put in **oxy-sterol** four circle symbols

two oxygen atoms

angular **-CH₃** groups C18, C19 :

and 3 metil groups **-CH₃**:

as splinter, fork, rod on flexible tail **C H₃**



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

10. Put in 25-hidroxiholessterol

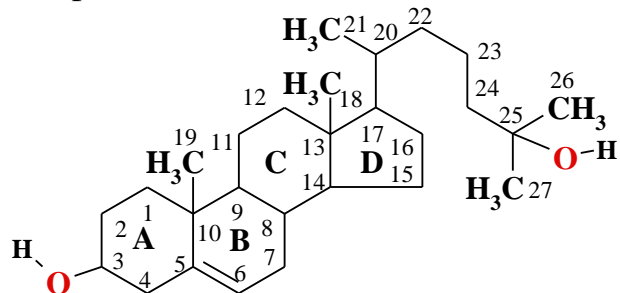
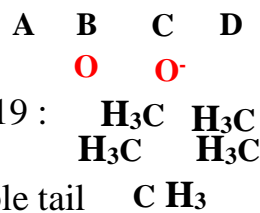
four circle symbols:

two oxygen atoms

angular **-CH₃** groups C18, C19 :

and 3 metil groups **-CH₃**:

as splinter, fork, rod on flexible tail **C H₃**



11. Put in 7-hidroksiholessterolā

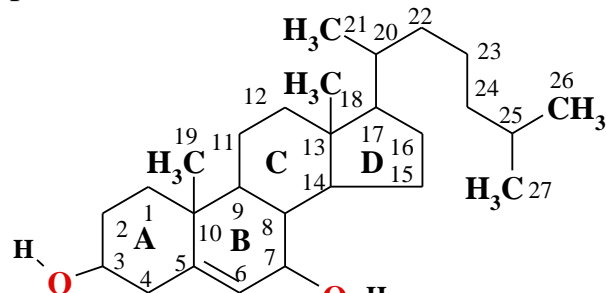
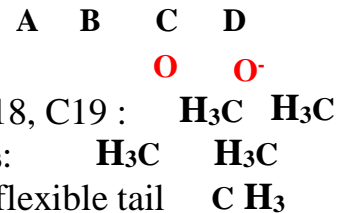
four circle symbols:

two oxygen atoms

angular **-CH₃** groups C18, C19 :

and 3 metil groups **-CH₃**:

as splinter, fork, rod on flexible tail **C H₃**



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-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 >1ZHZA: A|PDBID|CHAIN|SEQUENCE: RLBP1+Retinal -1-434 (1-434)

MSQYASSSSWTSFLKSIASFNGDLSSLSAPPFILSPISLTFESQYWAHEPELFLEPSFINDDNYKEHCLIDPEVESPELA
 RMLAVTKWFISTLKSQYCSRNESLGSSEKPLNPFLGELFVGVKQWENKEHPEFGETVLLSEQVSHHPPVTAFSIFNDKKNKVK
 LQGYNQIKASRYFTKSLMLTVKQFGHTMLDIKDESYLVTPLPHIEGILVASPFVELEGKSYIQSSSTGLLCVIEFSGRGRYFS
 GKKNFSFKARIYKDSKDKDEKALYTISGQWSSGSKIKANKKEESRLFYDAARIPAEHLNVKPLEEQHPLESRKAWYDV
 AGAIKLGDFNLIAKTKTELEETQRELKQEEAKGISWQRRWFKDFDYSVTPEEGALVPEKDDTFLKLASALNLSTKNAPS
 GTLVGDKEDRKEDLSSIHWRWFQRELWDEEKEIVL

AA	pK _{aCOO-}	pK _{aNH₃⁺}	pK _{aRR}	Nr	AA	pK _{aCOO-}	pK _{aNH₃⁺}	pK _{aRR}	Nr	AA	pK _{aCOO-}	pK _{aNH₃⁺}	pK _{aRR}	Nr	AA	pK _{aCOO-}	pK _{aNH₃⁺}	pK _{aRR}	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 ΣpK_{aRside group}..

pK_{aNterminal}NH₃ and pK_{aCterminal}COO-constants sum divide with number of acid groups NpK_a:

$$IEP=pK_{a-mean}=(\Sigma pK_{aRside\ group}+pK_{aNterminal}+pK_{aCterminal})/NpK_a$$

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

12.1 Acid groups sum NpK_a=Sum of 147 + 2 = 149 pK_a 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 pK_{aCterminal}=1.88 .

Sum are calculate as ΣpK_{aRside group}+pK_{aNterminal}+pK_{aCterminal} = 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 & -NH₃⁺ positive charge-COO⁻ & -NH₃⁺.....charge is negative -COO⁻ & -NH₂

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 & -NH₃⁺ positive (+) charge pH=7.36 < IEP=7.43 charge negative(-) -COO⁻ & -NH₂.

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

Underline existing:

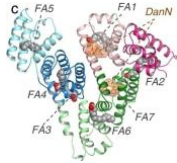
-COOH & -NH₃⁺ positive (+) charge IEP=7.43 < pH=8.8 charge negative(-) -COO⁻ & -NH₂.

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 .

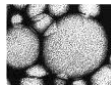
13. Write five lipids transport forms for lipoprotein aggregates under photography!



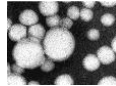
HDL join esterify outstanding cam Cholesterol molecule which protrude on membrane surface as insoluble. So avoid atherosclerosis and keep healthy cardiovascular state.

Albumin.....

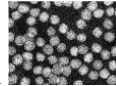
80...200 nm



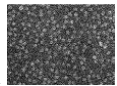
28...70 nm



20...25 nm



8...12 nm



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

14. What compounds transport lipoproteins enclosed interior!

Fatty acids, aspirin, warfarin, Ibuprofen, cholesterol.....

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 **-CH₃** of carbons C26 and C27?

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

19. Which 3 water molecules **H₂O** bound buried C3 hydroxyl group **-OH**?

... **H₂O**2003, **H₂O**2004, **H₂O**2018.....

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 **H₂O**2003, **H₂O**2004, **H₂O**2018?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.....