Name Surname :_____ , _____ **Group Nº** _____**2023**

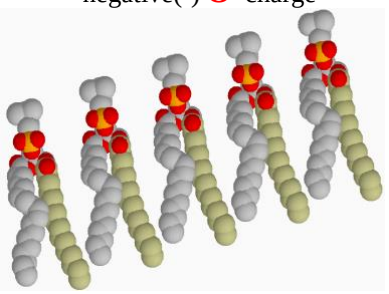
Phospholipids Bilayer Membrane composite materials for compartmentation of organelles: Task for practical research works: for Interactive Molecule:

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** **Spacefill** 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)

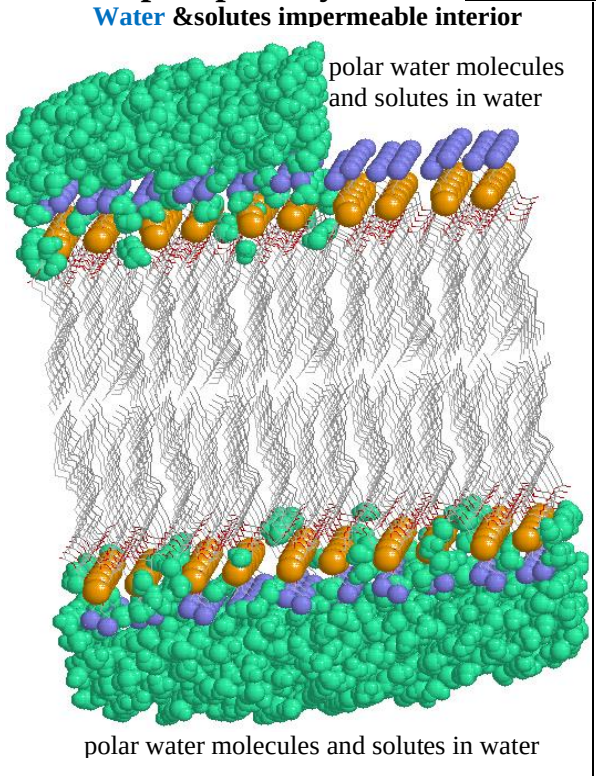


Hydrophobic non polar molecule part

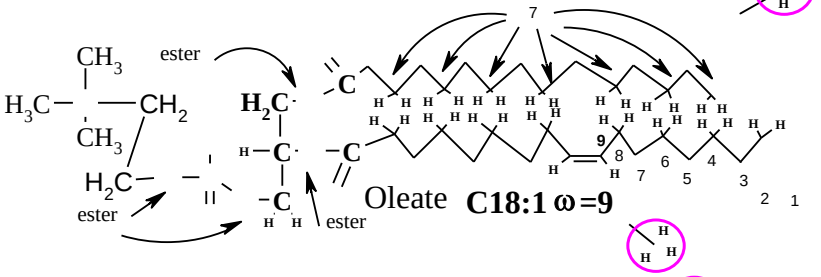
1. Write the main Physiological function of **Phospholipid Bilayer Membranes** in Life?
Membrane Interior is impermeable for .....and **water** .....
so work as **isolating** cellular wall for compartment .....

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 can not cross membrane Interior .....

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



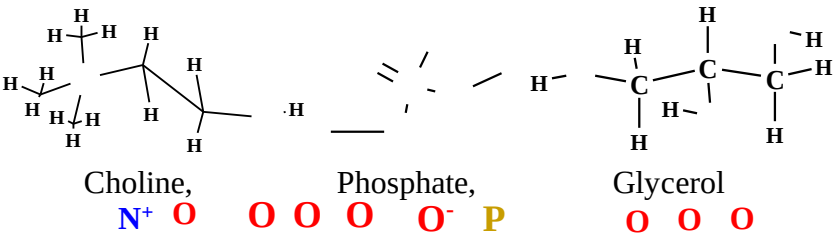
N^+ O O O O O O O O^- P



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

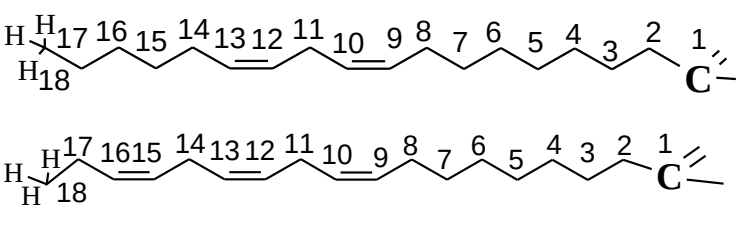
1.4 size Å **104.5** bonding angle ° **+0.8** charge **H H** **O** **-0,8** charge



N^+ O O O O O^- P O O O

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=.....** and **C18:3 ω=.....**

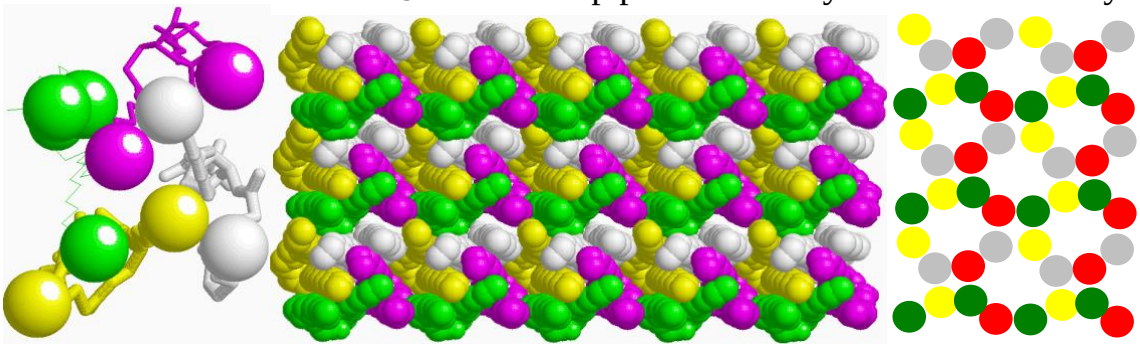


linoleate

α-linolenate

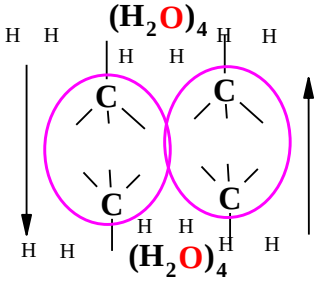
6. Calculate accumulate London Forces -2 $^{kJ}/_{mol}$ Bonding **E**nergy between two hydrogen contacts at each of 7 carbon methylene **–CH_2-** on Palmitate & Oleate chains contact **n**=7•2=... points!
E= -2•7•2= -2• **n**= -2•**14**=..... $^{kJ}/_{mol}$

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
E_C=3•**E**=;
E_C=3*-28=..... $^{kJ}/_{mol}$
E_C= ..... $^{kJ}/_{mol}$

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



9. Calculate hydrophobic contact -10 $^{kJ}/_{mol}$ **e**nergy E_H for **water** tetramer structure (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 n_H=2•3=6 for one phosphatidyl choline molecule E_H=-10•3/2=-60/2 =..... $^{kJ}/_{mol}$?

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. E_{Bond} = -168 +(-30) = ... $^{kJ}/_{mol}$

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 .....**Å**. Get the average size of Membrane thickness as mean $Dist_{mean}$ =.....Å as nanometers ..... nm !

12. What number of 1.4 Å size water molecules cover the distance 56 Å? 56/1.4= ..... 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=.....m meters long .

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

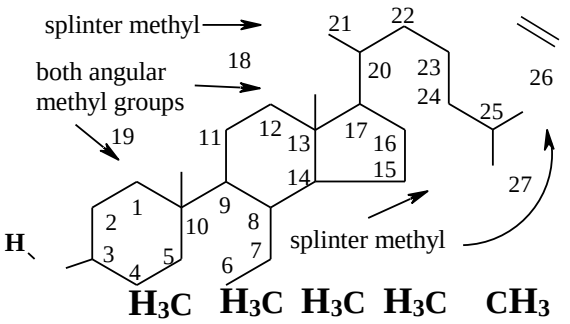
15. What mass fraction the Phosphatidyl Choline molecules in cellular membranes total mass 100%? Phosphatidyl Choline in **Membrane** constitute 1/3 part .....of **Membranes** mass 100%.

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

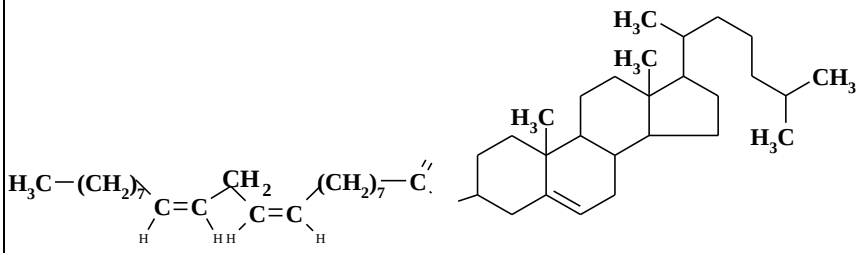
The **Cholesterol/PhosphoLipid** C/PL mole ratio of human red cell membranes ranges from a normal value of 0.9–1.0 (Journal of Cellular Biochemistry 2004 V8, 4, p 413-430). 1 mol cholesterol against 1 mol Phospholipid.

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



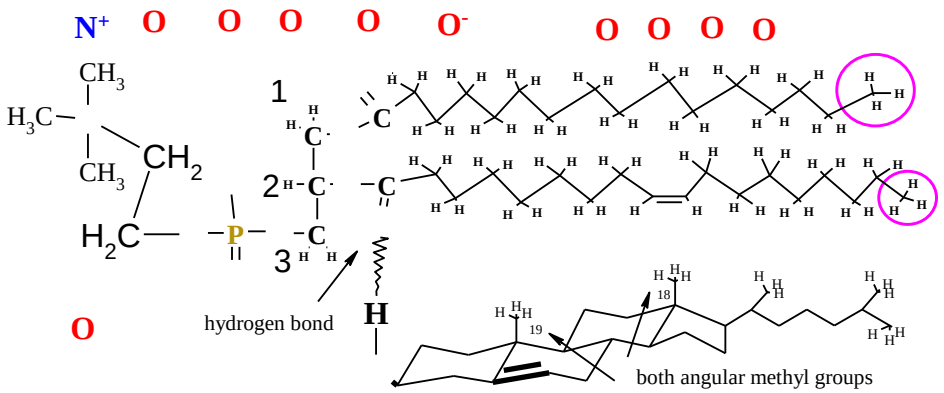
hydrocarbon four circle symbols in structure!

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? membranes mechanically .....
.....production and Steroid .....

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

5. Cholesterol/Phospholipid rate in erythrocyte membranes published =.....! 1978. Year
$\frac{\text{Cholesterol}}{\text{Phospho\_Lipid}}$ mole ratio $\frac{C}{PL}$ of human red blood cell membranes a normal value $\frac{C}{PL}$ =.....
Cholesterol and **Phospho Lipid** complex C/PL=1/1 in cell membranes

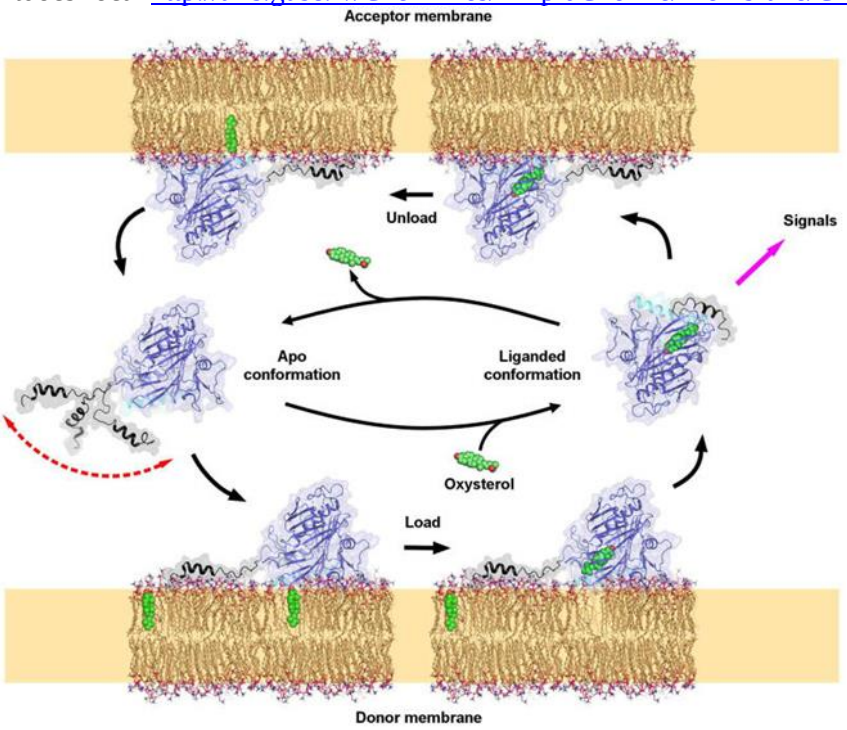


5. a Put given atoms in Phosphatidyl choline (lecithin) Cholesterol composite complex structure

5.b. Mass fraction of phospholipids 1/3 in eukaryotes membranes of total 100%? .....%
5.c. 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 .....
and as hydrogen donor hydroxyl group **HO**- of ..... molecule,
2. Van der Walls dispersion forces among no polar hydrocarbons structured atoms in lipid chains of..... and of .....

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



OSBP oxi-sterol transport protein involved in cholesterol metabolic transport through membranes surface, that keep 33.3% mass fraction 1/3 100% membrane mass. Cholesterol upload 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...MET... and **C-terminus** amino acid LEU.....!

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

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

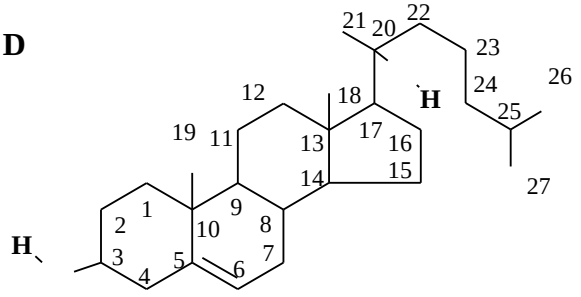
A B C D

two oxygen atoms **O** **O⁻**

angular **–CH₃** groups C18, C19 : **H₃C** **H₃C**

and 3 metil groups **–CH₃**: **H₃C** **H₃C**

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



9. Count hydroxyl groups **-OH** in cholesterol and 20-hydroxicholesterol? 1.....un 2.....

10. Put in 25-hydroxicholesterol

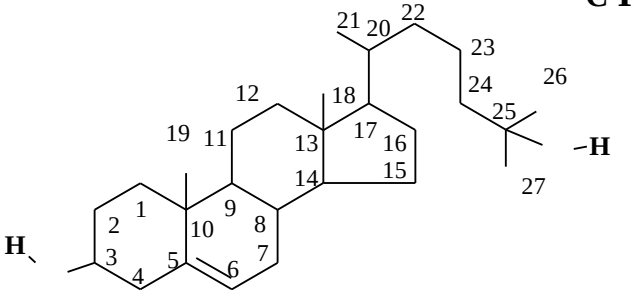
four circle symbols: **A B C D**

two oxygen atoms **O** **O⁻**

angular **–CH₃** groups C18, C19 : **H₃C** **H₃C**

and 3 metil groups **–CH₃**: **H₃C** **H₃C**

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



11. Put in 7-hidroksiholesterolā

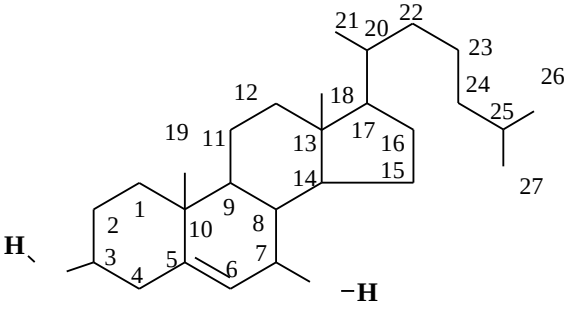
four circle symbols: **A B C D**

two oxygen atoms **O** **O⁻**

angular **–CH₃** groups C18, C19 : **H₃C** **H₃C**

and 3 metil groups **–CH₃**: **H₃C** **H₃C**

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? .....;..... transport proteins

.....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.pdb 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 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:

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

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

12.1. Acid groups sum NpKa=Sum of 147 + 2 = ... pKa values in table: .....

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 .

12.2 Sum are calculate as $\Sigma pKa_{Rside\ group}+pK_{aNterminal}+pKa_{Cterminal}$ = ...

Average acid group constant **ISOELEKTRIC POINT** pK_{mean}=IEP=1107,65/149=.....

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 KES1 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:

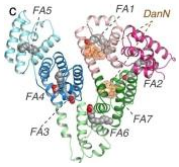
-COOH & **-NH_3^+** positive (+) charge IEP=**7.43**< pH=8.8 charge negative(-) **-COO^-** & **-NH_2**.

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

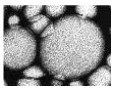
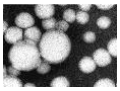
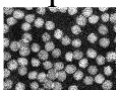
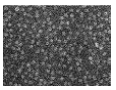
$$pH=\frac{pK_a-\log C}{2}=\frac{7,4338926-\log 10^{-7,2861074}}{2}=\frac{7,4338926+7,2861074}{2}$$ = **14,72** / 2 = ...

7,36 Attractor YEAST_KES1 concentration is C=.....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.

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

.....

…….....

14. What compounds transport lipoproteins enclosed interior!

.....;.....;.....;.....;

.....; Fat soluble .....

..... and .....;..... other .....

15. Write two transport forms of lipocalins! ..... and .....

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

.....

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

beta half-barrel of ..... and

..... **beta sheets** from ..... **strands** in 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?

Trp.....,Phe.....,Leu.....,Ile.....,Leu.....,Ala.....

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

HOH....., HOH....., HOH.....

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

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

Gln.....,Trp.....,Tyr.....,Asn.....,Gln.....

24. 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**? => ....., ..., ..., ..., ..., ..., ...

10: Pro...,Ala...,Leu...,Leu...,Ala...,Pro...,Ile...,Leu...,Phe...,Leu...,

6: Gly...,Pro....,Leu....., Pro.....,Pro.....,Val.....,

8: Ala.....,Ile.....,Ala.....,Phe.....,Leu.....,Leu.....,Val.....,Phe.....,

8: Pro.....,Pro.....,Pro.....,Val....,Pro.....,Pro.....,Ile.....,Ile.....,

6: Leu....,Val.....,Ala.....,Pro.....,Phe.....,Val.....

5: Leu.....,Leu.....,Pro.....,Leu.....,Ala**25.** Where disposed **C-terminus** polypeptide chain residues from 308 to 434 amino acids in

protein? .....,where enclosed buried lipid

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

Lys.....,Lys.....,Lys.....,Arg.....,Arg.....,

Lys.....,Lys.....,Lys.....,Arg.....,Lys.....