Name Surname :___ Teacher _ , ___ Teacher _____ Group Nº ___ Teacher _____2021

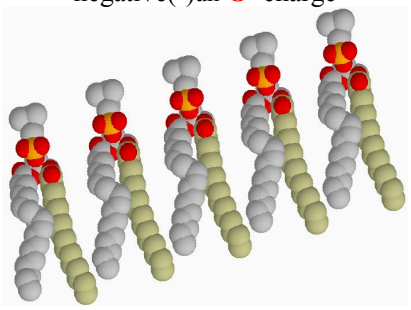
Steroidogenic Acute Regulatory protein-related lipid Transfer START domain STARTD1-13: http://aris.gusc.lv/06Daugavpils/Research/StartS.pdf **A.** Task for practical research works: http://aris.gusc.lv/06Daugavpils/Research/PhosphLipidBilayerMembran.pdf for Interactive Molecule research: Chemscape MDLi ISIS Draw RasMol FireFox 3.5.5v **B** tested molecule structure lunch the Riga Stradin's University assistant professor **Aris Kaksis 2021.** prepared **StART domain START1-13** molecules experimental research practical study: http://aris.gusc.lv/ChemFiles/START/START.htm

Lipids Hydrophobic & Hydrophilic Biphilic molecules in the **CPK** color scheme 1965 :

at Display conditions: **Stick** (on Menu Stripe) **Ball & Stick** **Spacefill**

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)

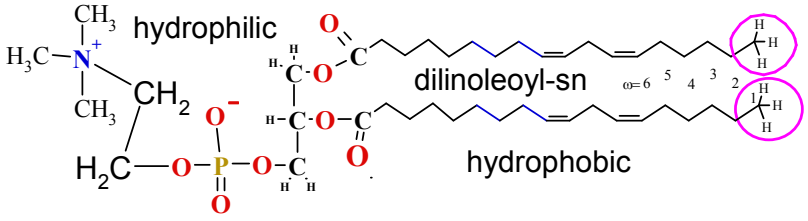
Published in **Nature Journal** by **Corey, Pauling, Koltun** positive(+)N^+ and negative(-)un O^- charge



Hydrophobic non polar molecule part

START domain of PCTP Phosphatidyl Choline Transfer Protein 1LN1 molecule complexed with DLPC, dilinoleoyl-*sn*-glycerol-3-phosphoryl choline

1. DLPC, dilinoleoyl-*sn*-glycerol-3-phosphoryl choline draw, to identify fatty acids essential omega number from methyl–CH_3 tail ω =6..... and amphiphatic property!
To show at physiologic pH=7.36!



2.Secondary structure units for 1LN1! are:.....
Alpha **helixes** 1,2,3,4,5,6 .....
.....
Bets **sheet** strands 1,2,3,4,5,6,7,8,9,10....

3. Hydrophobic pocket amino acids for DLPC: Val34,56,85,99,103,152,160,171,196, ...
Leu33,51,60,68,88,159,187,200,Ile41,71,133,150,161,183,188,Ala69,135,136,191,192,204,206,..
Phe199,Pro65,106,108,179,184.....

4. Check and to indicate water molecules in hydrophobic cavity HOH 506,513,521,532 .....

5. Check and to indicate salt bridge and hydrogen bonding pocket amino acids with DLPC head groups! .....Tyr72,Gln157,Arg78,Asp82.....

6. Check and to indicate the lipid quaternary amine methyl groups in cation–$N^+(CH_3)_3$ contacting amino acids as three-walled aromatic cage formed by the ring faces .....
Val103,Tyr116,Tyr175,Trp101,Tyr114,Tyr155.....

7.1-7.5 Analyses of PCTP isoelectric point IEP=pH=$pK_{a\text{-}mean}$ at physiologic pH=7,36 .

Determine at solution pH with PCTP concentration C=$10^{-6,7418}$ M ($^{mol}/_{Liter}$)!

Human phosphatidylcholine transfer protein H_PCTP concentration $10^{-6,7418}$ M

http://aris.gusc.lv/ChemFiles/Albumin/START/1LN1pIStudS.doc;http://aris.gusc.lv/ChemFiles/START/1LN1pI.xls

SQ SEQUENCE 214 >1LN1:A|PDBID|CHAIN|SEQUENCE **1LN1** PhosphCholin8-210(1-214)

MELAAGSFSEEQFWEACAELQQPALAGADWQLLVETSGISIYRLLDKKTGLYEYKVFGVLEDCSPTLLADIYMDSDYRKQ
WDQYVKELYEQECNGETVVYWEVKYPFPMSNRDYVYLRQRRDLDMEGRKIHVILARSTSMPQLGERSGVIRVKQYKQSLA
IESDGKKGSKVFMYYFDNPGGQIPSWLINWAAKNGVPNFLKDMARACQNYLKKT

AA	Nr	pKa_{COOH}	pKa_{NH3+}	pK_{RR}	Nr	AA	pKa_{COOH}	pKa_{NH3+}	pK_{RR}	Nr
M	1	9,21		1	K	40		10,53	104	M
E	2		4,25	2	Y	41		10,07	105	E
E	3		4,25	10	R	42		12,48	112	E
E	4		4,25	11	D	43		3,65	113	E
E	5		4,25	15	Y	44		10,07	114	E
C	6		8,18	17	Y	45		10,07	116	C
E	7		4,25	19	R	46		12,48	118	E
D	8		3,65	29	R	47		12,48	120	D
E	9		4,25	35	R	48		12,48	121	E
Y	10		10,07	42	D	49		3,65	122	Y
R	11		12,48	43	D	50		3,65	124	R
D	12		3,65	46	E	51		4,25	126	D
K	13		10,53	47	R	52		12,48	128	K
K	14		10,53	48	K	53		10,53	129	K
Y	15		10,07	52	H	54		6	131	Y
E	16		4,25	53	R	55		12,48	136	E
Y	17		10,07	54	E	56		4,25	145	Y
K	18		10,53	55	R	57		12,48	146	K
E	19		4,25	61	R	58		12,48	151	E
D	20		3,65	62	K	59		10,53	153	D
C	21		8,18	63	Y	60		10,07	155	C
D	22		3,65	70	K	61		10,53	156	D
Y	23		10,07	72	E	62		4,25	162	Y
D	24		3,65	74	D	63		3,65	164	D
D	25		3,65	76	K	64		10,53	166	D
Y	26		10,07	77	K	65		10,53	167	Y
R	27		12,48	78	K	66		10,53	170	R
K	28		10,53	79	Y	67		10,07	174	K
D	29		3,65	82	Y	68		10,0	175	D

1LN1 622,3 ;76+2=78 ;622,3/78=7,97820513

ISOELEKTRIC POINT zero charge "0"

Sum are calculate as 90 protolytic equilibria

pK_a values in table 622.3.....

$\Sigma pK_{aRside\ group} + pK_{aNterminus} + pK_{aCterminus}$ = 622.3.....

Protolysis mean constant pK_a isoelectric point IEP=pK_a calculate as sum of constants: side chains $\Sigma pK_{aRside\ group}$,,

$pK_{aNterminus}NH_{3+}$ and $pK_{aCterminus}COO–$

divided with number of protolytic acid groups NpK_a:

Protolytic constant pK_a isoelectric point IEP calculate as sum of constant side chains $\Sigma pK_{aRside\ group}$,,

$pK_{aNterminus}NH_3$ and $pK_{aCterminus}COO–$

divided with number of protolytic acid groups NpK_a:

$IEP = pK_{mean} = (\Sigma pK_{aRside\ group} + pK_{aNterminus} + pK_{aCterminus})/NpK_a$

pK_{mean} = IEP = 622.3 / 78=7.9782.....,

7.1 Acid groups number sum NpK_a=76.....+2.....= 78.....

of 214 amino acids 76+2 of them

protolytic constants pK_a for side groups,

N-terminus methionine M $pK_{aNterminus}$=9.21 and

C- terminus threonine T $pKa_{Cterminus}$=2.11

7.2 Average acid group constant pK_{mean}= IEP

ISOELEKTRIC POINT IEP= 622.3 / 78=**7.9782**.....

							7		
Y	30	10,07	84	D	69	3,65	177	Y	
K	31	10,53	86	K	70	10,53	193	K	
E	32	4,25	87	K	71	10,53	201	E	
Y	33	10,07	89	D	72	3,65	202	Y	
E	34	4,25	90	R	73	12,48	205	E	
E	35	4,25	92	C	74	8,18	207	E	
C	36	8,18	93	Y	75	10,07	210	C	
E	37	4,25	96	K	76	10,53	212	E	
Y	38	10,07	100	K	77	10,53	213	Y	
E	39	4,25	102	T	2,11	78	214	E	

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 (+)cor zero or negative (-)!

7.3 Determine molecule charge sign (+), zero „**0**” or (-) at physiologic pH=7.36 of blood plasma

Underline existing:

-COOH & **-NH_3^+** positive (+) charge ……. pH=7.36 < IEP=**8**..... charge negative(-) **-COO^-** & **-NH_2**.

7.4 Determine molecule charge sign (+), zero „**0**” or (-) at **electrophoresis** pH **8.8** on stripe

Underline existing:

-COOH & **-NH_3^+** positive (+) charge ……. IEP=**8**< pH=8.8 electrophoresis charge negative(-) **-COO^-** & **-NH_2**.

7.5 Calculate C = $10^{-6,7418}$ M PCTP solution pH by *Ostwald dilution law* concentration C in logarithm:

$$pH=\frac{pK_a - \log C}{2}=\frac{7,9782-\log 10^{-6,7418}}{2}=\frac{7,9782+6,7418}{2}$$ = **14,72** / 2 = 7,36.....

7,36 Attractor H_PCTP concentration is C=$10^{-6,7418}$.....M .

8. Check and to indicate the helix 1 residues rests numbers against the back of this beta-sheet! ... from residue 9.....-to 22.....

9. Check and to indicate the helices 2 and 3 residues rests numbers which are inserted between beta strands 3 and 4! 64-82.....

10. Check and to indicate the long amphiphatic C-terminal helix 4a-4b, is fold at Pro 197 and ... lays over the top of a tunnel! 1from residue 84.....-to 209.....

11a. Check and to indicate the helix identifiers and residue ranges for human PC-TP are .....

....**H1**9-22.....,**H2**64-74....., **H3**75-82.....,**H4**184-209.....

11b. Check and to indicate the beta strand identifiers and residue ranges are.....

.....**B1**(31-36),**B2**(39-46),**B3**(51-61),**B4**(84-93),**B5**(96-104),**B6**(111-123),**B7**(130-138),.....

..... **B8**(150-162),**B9**(168-178).....

12. Check and to indicate the loop identifiers and residue ranges which are .....

.....**L1**(105-110) and **L2**(139-149).....

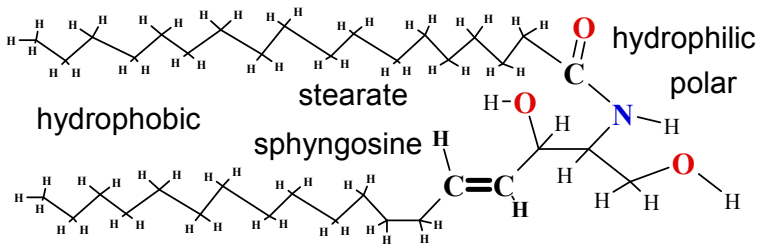
13. Check and to indicate the presence of a disulfide bond joining two conserved Cysteine .....

.....residues! Cys6—Cys207.....

14. What is the soluble concentration limit of phosphatidyl choline (PC) molecules?10^{-10} M.....

Ceramide transfer CERT cytosolic 68-kDa protein 2E3R is responsible for the trafficking of ceramide from the endoplasmic reticulum ER to the *trans* Golgi network where it is converted to sphingomyelin SM.

1. CERT, DLPC D-erythro-C18-ceramide draw, to indicate fatty acids and amphiphatic property! To show at physiologic pH=7.36!



2.Which membrane is ceramide donor membrane and which membrane is ceramide acceptor membrane!
The endoplasmic reticulum (ER) is....
donor membrane.....
The *trans* Golgi network membrane is...
ceramide acceptor membrane.....

3. What ranges have ceramide molecular species amide-acyl chains? from C14.....- to C20.....

4. What cavity property has ceramide transfer CERT cytosolic 68-kDa protein? amphiphatic.....

5. What specific tertiary structure folding name? helix.....-grip.....fold

6. Check and to indicate the helix 1 residues rests numbers against the back of this beta-sheet!

from residue PHE367.....- to SER382.....

7. Check and to indicate the helices 2 and 3 residues numbers inserted between beta strands 3, 4!

from THR428.....- to GLU446.....and from SER543.....- to ASN546.....

8. Check and to indicate the long amphiphatic C-terminal helix 5-5a, is kinked at Pro 577 and ....

lays over the top of a tunnel! from PRO564.....number – to ALA592.....number

9a. Check and to indicate the helix identifiers and residue ranges for human PC-TP are .....

..**H1**367-382,.....**H2**428-438,.....**H3**441-446,.....**H4**543-546,.....**H5**564-592.....

9b. Check and to indicate the beta strand identifiers and residue ranges are .....

..**B1**393-398,.....**B2**401-405,.....**B3**418-425,.....**B4**548-558,.....**B5**522-532,.....

..**B6**499-506,.....**B7**478-489,.....**B8**462-469,.....**B9**449-459.....

10. Analyses of Human CERT isoelectric point IEP=pH=$pK_{a\text{-}vid}$ at physiologic pH=7,36 .
2E3R.pdb 347-624 determine pH with CERT cytosolic 70,829-kDa concentration $C=10^{-7,3684}$ M ($^{mol}/_{Liter}$)!
http://aris.gusc.lv/ChemFiles/Albumin/START/2E3RpIStudS.doc;http://aris.gusc.lv/ChemFiles/START/2E3RpI.xls

```
SQ SEQUENCE 624 >2E3R,2RSG,4N:A|PDBID|CHAIN|SEQUENCE 348-624 2E3R.pdb
MSDNQSWNSSGSEEDPETESGPPVERCGVLSKWTNYIHGWQDRWVVLKNNALSYYKSEDETEYGCRGSICLSKAVITPHD
FDECRFDISVNDSVWYLRAQDPDHRQQWIDAIEQHKTESGYGSESSLRRHGSMVSLVSGASGYSATSTSSFKKGHSLREK
LAEMETFRDILCRQVDTLQKYFDACADAVSKDELQRDKVVEDDEDDFPTTRSDGDFLHSTNGNKEKLFPHVTPKGINGID
FKGEAITFKATTAGILATLSHCIELMVKREDSWQKRLDKETEKKRRTEEAYKNAMTELKKKSHFGGPDYEEGPNSLINEE
EFFDAVEAALDRQDKIEEQSQSEKVRLHWPTSLPSGDAFSSVGTHRFVQKPYSRSSSMSSIDLVSASDDVHRFSSQVEEM
VQNHMTYSLQDVGGDANWQLVVEEGEMKVYRREVEENGIVLDPLKATHAVKGVTGHEVCNYFWNVDVRNDWETTIENFHV
VETLADNAIIIYQTHKRVWPASQRDVLYLSVIRKIPALTENDPETWIVCNFSVDHDSAPLNNRCVRAKINVAMICQTLVS
PPEGNQEISRDNILCKITYVANVNPGGWAPASVLRAVAKREYPKFLKRFTSYVQEKTAGKPILF plekstrin PH domane
```

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	K	46	10,53	116	H	91	6		230	E	136		4,25	337	E	181		4,25	476
D	2		3,65	3	E	47	4,25	118	K	92	10,53		234	E	137		4,25	338	H	182		6	479
E	3		4,25	13	Y	48	10,07	121	D	93	3,65		240	E	138		4,25	343	E	183		4,25	482
E	4		4,25	14	E	49	4,25	124	K	94	10,53		242	K	139		10,53	344	D	184		3,65	486
D	5		3,65	15	R	50	12,48	128	E	95	4,25		244	R	140		12,48	346	Y	185		10,07	492
E	6		4,25	17	R	51	12,48	129	K	96	10,53		249	H	141		6	348	H	186		6	495
E	7		4,25	19	H	52	6	130	H	97	6		261	D	142		3,65	357	K	187		10,53	496
E	8		4,25	25	Y	53	10,07	143	C	98	8,18		262	H	143		6	365	R	188		12,48	497
R	9		12,48	26	K	54	10,53	152	E	99	4,25		264	R	144		12,48	366	R	189		12,48	504
C	10		8,18	27	K	55	10,53	153	K	100	10,53		268	K	145		10,53	370	D	190		3,65	505
K	11		10,53	32	H	56	6	155	R	101	12,48		269	Y	146		10,07	372	Y	191		10,07	508
Y	12		10,07	36	R	57	12,48	158	E	102	4,25		270	R	147		12,48	374	R	192		12,48	513
H	13		6	38	E	58	4,25	159	D	103	3,65		271	D	148		3,65	382	K	193		10,53	514
D	14		3,65	42	K	59	10,53	160	K	104	10,53		275	D	149		3,65	388	E	194		4,25	520

R	15	12,48	43	E	60	4,25	163	R	10512,48	276	D	150	3,65	389	D	195	3,65	522	
K	16	10,53	48	E	61	4,25	165	D	1063,65	278	H	151	6	391	E	196	4,25	524	
Y	17	10,07	54	R	62	12,48	168	K	10710,53	279	R	152	12,48	392	C	197	8,18	529	
Y	18	10,07	55	D	63	3,65	169	E	1084,25	280	E	153	4,25	398	D	198	3,65	534	
K	19	10,53	56	C	64	8,18	172	E	1094,25	282	E	154	4,25	399	H	199	6	535	
E	20	4,25	58	R	65	12,48	173	K	11010,53	283	H	155	6	404	D	200	3,65	536	
D	21	3,65	59	D	66	3,65	176	K	11110,53	284	Y	156	10,07	407	R	201	12,48	543	
E	22	4,25	60	K	67	10,53	180	R	11212,48	285	D	157	3,65	411	C	202	8,18	544	
E	23	4,25	62	Y	68	10,07	181	R	11312,48	286	D	158	3,65	415	R	203	12,48	546	
Y	24	10,07	63	D	69	3,65	183	E	1144,25	288	E	159	4,25	423	K	204	10,53	548	
C	25	8,18	65	C	70	8,18	185	E	1154,25	289	E	160	4,25	424	C	205	8,18	555	
R	26	12,48	66	D	71	3,65	187	Y	11610,07	291	E	161	4,25	426	E	206	4,25	563	
C	27	8,18	70	K	72	10,53	191	K	11710,53	292	K	162	10,53	428	E	207	4,25	567	
K	28	10,53	73	D	73	3,65	192	E	1184,25	297	Y	163	10,07	430	R	208	12,48	570	
H	29	6	79	E	74	4,25	193	K	11910,53	299	R	164	12,48	431	D	209	3,65	571	
D	30	3,65	80	R	75	12,48	196	K	12010,53	300	R	165	12,48	432	C	210	8,18	575	
D	31	3,65	82	D	76	3,65	197	K	12110,53	301	E	166	4,25	433	K	211	10,53	576	
E	32	4,25	83	K	77	10,53	198	H	1226	303	E	167	4,25	435	Y	212	10,07	579	
C	33	8,18	84	E	78	4,25	201	D	1233,65	308	E	168	4,25	436	R	213	12,48	595	
R	34	12,48	85	D	79	3,65	202	Y	12410,07	309	D	169	3,65	442	K	214	10,53	599	
D	35	3,65	87	D	80	3,65	203	E	1254,25	310	K	170	10,53	445	R	215	12,48	600	
D	36	3,65	92	E	81	4,25	204	E	1264,25	311	H	171	6	448	E	216	4,25	601	
Y	37	10,07	96	D	82	3,65	205	E	1274,25	319	K	172	10,53	451	Y	217	10,07	602	
R	38	12,48	98	D	83	3,65	206	E	1284,25	320	H	173	6	456	K	218	10,53	604	
D	39	3,65	101	R	84	12,48	211	E	1294,25	321	E	174	4,25	457	K	219	10,53	607	
D	40	3,65	103	D	85	3,65	213	D	1303,65	324	C	175	8,18	459	R	220	12,48	608	
H	41	6	104	D	86	3,65	215	E	1314,25	327	Y	176	10,07	461	Y	221	10,07	612	
R	42	12,48	105	H	87	6	218	D	1323,65	331	D	177	3,65	466	E	222	4,25	615	
D	43	3,65	110	K	88	10,53	224	R	13312,48	332	R	178	12,48	468	K	223	10,53	616	
E	44	4,25	113	E	89	4,25	225	D	1343,65	334	D	179	3,65	470	K	224	10,53	620	
H	45	6	115	K	90	10,53	226	K	13510,53	335	E	180	4,25	472	F	2,13	225		624

Sum of 225 pKa values in table 1654.1; on 624 amino acids for chain IEP=7,351556

Protolytic constant pK_a isoelectric point IEP=pK_a 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:

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

Calculation tasks for Human CERT cytosolic 70,829-kDa concentration $10^{-7,3684}$ M

Protolytic constant pK_a isoelectric point IEP=pK_a 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:

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

10.1 Acid groups number in sum NpKa=223.....+2.....=225.....

624 amino acids for side groups 223+2 of them protolytic constants pK_a,
N-terminal methionin M $pK_{aNterminal}$=9.21 and C-termināl phenylalanine F $pKa_{Cterminal}$=2.36
Sum on 2nd page are calculate as $\Sigma pKa_{Rside\ group}+pK_{aNterminal}+pKa_{Cterminal}$= 1654.1.....

10.2 Average acid group constant **ISOELEKTRIC POINT** pK_{mean}= IEP =1654.1 / 225=**7.351556**.....

NpKa=223.....+2.....=225.....

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 (-)!

10.3 Determine CERT molecule charge sign (+). zero „**0**” or (-) at physiologic pH=7.36

Underline existing:
-COOH & **-NH_3^+** positive (+) charge ……. IEP=**7.35** < pH=7.36..... charge negative(-) **-COO^-** & **-NH_2**.

10.4 Determine CERT molecule charge sign (+). zero „**0**" or (-) at **electrophoresis** pH **8.8**

Underline existing:

-COOH & **-NH_3^+** positive (+) charge ……. IEP=**7.35**< pH=8.8 ..... charge negative(-) **-COO^-** & **-NH_2**.

10.5 Calculate $10^{-7,3684}$ M CERT solution pH by *Ostwald dilution law* concentration C in logarithm:

$$pH=\frac{pK_a-\log C}{2}=\frac{7,3515556-\log 10^{-7,3684444}}{2}=\frac{7,3515556+7,3684444}{2}=\mathbf{14,72}\ /\ 2=7,36.....$$

7,36 Attractor CERT concentration is $C=10^{-7,3684}$.....M .

11. Check and to indicate the loop identifiers and residue ranges for .2E3R.pdb!

....**L1** from 444..... to 449..... number and from **L2**469..... to 478.....

12. Check and to indicate the disulfide bond Cys—**S**—**S**—Cys joining conserved Cysteines!

between Cys433.....—**S**—**S**—Cys529.....

13. What is **S**—**S** bond distance in Ångströms Cys433—Cys529? d=3.666 Å..... Ångströms

14. Indicate salt bridge and hydrogen bonding pocket 5 amino acids with DLPC head groups!

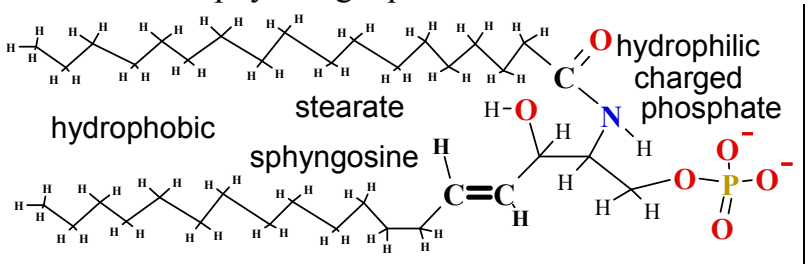
...Arg442.....,Glu446.....,Gln467.....,Asn504.....,Tyr553.....

15. Check and to indicate water molecules in hydrophobic cavity HOH1105,1112,1294.....

...H**O**H number 1105....., H**O**H number 1112....., H**O**H number 1294.....

Phosphorylated sphingolipids ceramide-1-phosphate C1P and sphingosine-1-phosphate S1P have emerged as key regulators of cell growth, survival, migration and inflammation 4K8N.pdb. C1P produced by ceramide kinase is the rate-limiting releaser of arachidonic acid used for pro-inflammatory eicosanoid production, which contributes to disease pathogenesis in: asthma or airway hyper-responsiveness, cancer, atherosclerosis and thrombosis. Provoke efficient targeting, trafficking and presentation of C1P to specific cellular receptors

1. C1P, ceramide-1-phosphate draw, to indicate fatty acids and amphiphatic property!
To show at physiologic pH=7.36!



2.Which disease pathogenesis limiting rate depends on C1P:
asthma or airway.....
hyper-responsiveness, ...
cancer, atherosclerosis and.....
thrombosis.....

3. Check and to indicate the helix residue numbers for human 4K8N.pdb!... **H1**367-382,.....

...**H2**428-438.....,**H3**441-446.....,**H4**543-546.....,**H5**564-592.....

4. Check and to indicate the β-strands residue numbers for human 4K8N.pdb!...**B1**393-398,.....
...**B2**401-405.....,**B3**418-425.....,**B4**548-558.....,**B5**522-532.....,
...**B6**499-506.....,**B7**478-489.....,**B8**462-469.....,**B9**449-459.....

5. Hydrophobic pocket of 25 aliphatic amino acids for C1P in 4K8N.pdb molecule?...Ala114....,
..Phe42....,50....,52....,162.....,171.....,121.....Val40.....,57.....,158.....,163.....Ile63,.....
..Leu10....,14....,39....,43....,46....,111.....,118.....,122.....,146.....,16.....Trp36....,117.....Met175.....

6. Iindicate salt bridge and hydrogen bonding pocket 6 amino acids which plugs at phosphate the binding tunnel! Arg66.....,Arg 97.....,Arg106.....,Arg110.....,Arg113.....,Lys60.....

7.1-7.5 Analyses CPTP molecule isoelectric point IEP=pH=$pK_{a\text{-}vid}$ at physiologic pH=7,36 .

4K80.pdb 1-214 determine pH with H_CPTP cytosolic 70,829-kDa concentration C=$10^{-6,9075}$ M ($^{mol}/_{Liter}$)!

Human ceramide-1-phosphate C1P and sphingosine -1-phosphate S1P

SQ SEQUENCE 214 >4K80:A|PDBID|CHAIN|SEQUENCE Human_CPTP
MDDSETGFNLKVVLVSFKQCLDEKEEVLLDPYIASWKGLVRFLNSLGTIFSFISKDVVSKLRIMERLRGGPQSEHYRSLQ
AMVAHELSNRLVDLERRSHHPESGCRTVLRLHRALHWLQLFLEGLRTSPEDARTSALCADSYNASLAAYHPWVVRRAVTV
AFCTLPTREVFLEAMNVGPPEQAVQMLGEALPFIQRVYNVSQKLYAEHSLLDLP

http://aris.gusc.lv/ChemFiles/START/4K80pI.xls;http://aris.gusc.lv/ChemFiles/START/4K80pILat.doc

aa	Nr	pKa$_{COOH}$	pKa$_{NH3+}$	pK$_{RR}$	aa	Nr	pKa$_{COOH}$	pKa$_{NH3+}$	pK$_{RR}$
M	1	9,21		1	H		36	6	100
D	2		3,65	2	E		37	4,25	102
D	3		3,65	3	C		38	8,18	105
E	4		4,25	5	R		39	12,48	106
K	5		10,53	11	R		40	12,48	110
K	6		10,53	18	H		41	6	112
C	7		8,18	20	R		42	12,48	113
D	8		3,65	22	H		43	6	116
E	9		4,25	23	E		44	4,25	123
K	10		10,53	24	R		45	12,48	126
E	11		4,25	25	E		46	4,25	130
E	12		4,25	26	D		47	3,65	131
D	13		3,65	30	R		48	12,48	133
Y	14		10,07	32	C		49	8,18	138
K	15		10,53	37	D		50	3,65	140
R	16		12,48	41	Y		51	10,07	142
K	17		10,53	55	Y		52	10,07	149
D	18		3,65	56	H		53	6	150
K	19		10,53	60	R		54	12,48	155
R	20		12,48	62	R		55	12,48	156
E	21		4,25	65	C		56	8,18	163
R	22		12,48	66	R		57	12,48	168
R	23		12,48	68	E		58	4,25	169
E	24		4,25	74	E		59	4,25	173
H	25		6	75	E		60	4,25	181
Y	26		10,07	76	E		61	4,25	189
R	27		12,48	77	R		62	12,48	196
H	28		6	85	Y		63	10,07	198
E	29		4,25	86	K		64	10,53	203
R	30		12,48	90	Y		65	10,07	205
D	31		3,65	93	E		66	4,25	207
E	32		4,25	95	H		67	6	208
R	33		12,48	96	D		68	3,65	212
R	34		12,48	97	P	1,99	69		214
H	35		6	99					

sum=539,06 ;67+2=69 ; $^{539,06}/_{69}$=7,8124638=IEP

ISOELEKTRIC POINT charge "0" Sum are calculate as

69 protolytic equilibria pK$_a$ values in table is 539,06.....

$\Sigma pK_{aRside\ group}$+ $pK_{aNterminus}$+ $pK_{aCterminus}$ = 539,06.....

Protolysis mean constant pK$_a$ isoelectric point IEP=pK$_a$ calculate as sum of constants: side chains $\Sigma pK_{aRside\ group}$, $pK_{aNterminus}NH_3^+$ and $pK_{aCterminus}COO^-$ divided with number of protolytic acid groups NpK$_a$: 69.....

$IEP=pK_a=(\Sigma pK_{aRside\ group}+pK_{aNterminus}+pK_{aCterminus})/NpK_a$

pK_{mean}= pK$_a$ = IEP = 539,06 / 69 = 7,8124638.....,

7.1 Acid groups number sum NpK$_a$=67.....+2.....=69..... of 214 amino acids 67+2 of them;protolytic constants pK$_a$ for side groups, N-terminus methionine M $pK_{aNterminus}$=9.21 and C-terminus proline P $pKa_{Cterminus}$=1.99

7.2 Average acid group constant pK_{mean} calculates as IEP

ISOELEKTRIC POINTpK_{mean}= IEP= 539,06/69 = **7,8124638**.....

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 (-)!

7.3 Determine H_CPTP molecule charge sign (+), zero „**0**" or (-) at physiologic pH=7.36 of blood plasma

Underline existing:

-COOH & **-NH_3^+** positive (+) charge ……. pH=7.36< IEP=**7,81**..... charge negative(-) **-COO^-** & **-NH_2**.

7.4 Determine A thioesterase molecule charge sign (+), zero „**0**" or (-) at **electrophoresis** pH 8.8 on stripe

Underline existing:

-COOH & **-NH_3^+** positive (+) charge IEP=**7,81** < pH=8.8 electrophoresis charge negative(-) **-COO^-** & **-NH_2**.

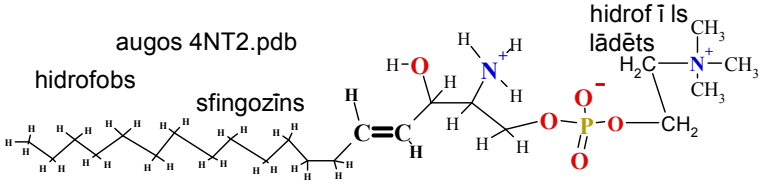
Electrophoresis pH=8.8 show the movement towards positive electrode.

7.5 Calculate $10^{-6,9075}$ M ($^{mol}/_{Liter}$) protein solution pH by *Ostwald dilution law* concentration C in logarithm:

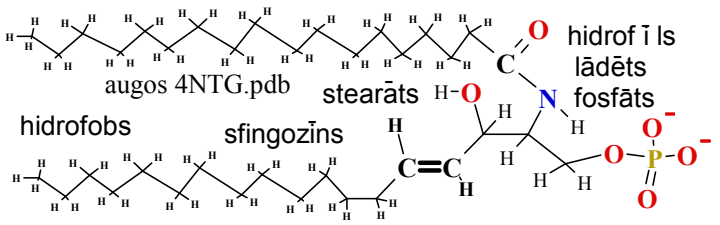
$$pH=\frac{pK_a-\log C}{2}=\frac{7,8124638-\log 10^{-6,9075362}}{2}=\frac{7,8124638+6,9075362}{2}$$ = **14,72** / 2 = 7,36.....

7,36 Attractor H_CPTP concentration is C=$10^{-6,9075}$.....M .

C-terminal STARD 230 amino acids domane Sphingosine-1-phosphatidylCholin 4NT2.pdb and **Ceramide-1-phosphate inter membrane transfer protein** 4NTG.pdb ACD11



The accelerated-cell-death11 (ACD11) protein molecule 4NT2 provides a genetic model for studying immune response activation and localized cellular suicide that halts



pathogen spread during infection in plants.

ACD11 structure/function 4NTG.pdb and show that ACD11 disruption dramatically alters the in vivo balance of sphingolipid mediators that regulate eukaryotic programmed cell death. Normally low ceramide-1-phosphate C1P levels elevate and cell death inducer phytoceramide, rises acutely . ACD11 exhibits selective inter membrane transfer of C1P and phyto-C1P.

1. Check and to indicate salt bridge and hydrogen bonding pocket amino acids with ACD11 phosphate head groups 4NT2, 4NTG!.....Lys64,Arg99,Arg103.....

2. What is salt bridge distance 4NT2,4NTG? Asp60,67 and His143,97?

..d=2.725..... Å Ångströms....., d=2.514..... Å Ångströms.....

Ceramide-1-phosphate C1P 4NT2.pdb and sphingosine-1-phosphate 24,322 kDa transport protein H_ACD11.
http://aris.gusc.lv/ChemFiles/START/4NT2pI.xls; http://aris.gusc.lv/ChemFiles/START/4NT2pIStudS.doc

```
SQ SEQUENCE 206 >4NT2:A|PDBID|CHAIN|SEQUENCE ACD11_ARATH Accelerated cell death 11
MADSEADKPLRKISAAFKKLAIIVNSPNPEVPVTQFSHACSLVSPLFGCLGIAFKFAEMDYVAKVDDLVRASSSISTLVV
MMDKDIEADCVRKAGSHTRNLLRVKRGLDMVKVLFEQIIASEGDNSLKDPATKSYAQVFAPHHGWAIRKAVSLGMYALPT
RAHLLNMLKEDEAAAKIHMQSYVNSSAPLITYLDNLFLSKQLGIDW
```

aa	Nr	pKa_{COOH}	pKa_{NH3+}	pK_{RR}	aa	Nr	pKa_{COOH}	pKa_{NH3+}	pK_{RR}
M	1	9,21		1	R	32		12,48	103
D	2		3,65	3	K	33		10,53	105
E	3		4,25	5	R	34		12,48	106
D	4		3,65	7	D	35		3,65	109
K	5		10,53	8	K	36		10,53	112
R	6		12,48	11	E	37		4,25	116
K	7		10,53	12	E	38		4,25	122
K	8		10,53	18	D	39		3,65	124
K	9		10,53	19	K	40		10,53	128
E	10		4,25	30	D	41		3,65	129
H	11		6	38	K	42		10,53	133
C	12		8,18	40	Y	43		10,07	135
C	13		8,18	49	H	44		6	142
K	14		10,53	55	H	45		6	143
E	15		4,25	58	R	46		12,48	148
D	16		3,65	60	K	47		10,53	149
Y	17		10,07	61	Y	48		10,07	156
K	18		10,53	64	R	49		12,48	161
D	19		3,65	66	H	50		6	163
D	20		3,65	67	K	51		10,53	169
R	21		12,48	70	E	52		4,25	170
D	22		3,65	83	D	53		3,65	171
K	23		10,53	84	E	54		4,25	172
D	24		3,65	85	K	55		10,53	176
E	25		4,25	87	H	56		6	178
D	26		3,65	89	Y	57		10,07	182
C	27		8,18	90	Y	58		10,07	192
R	28		12,48	92	D	59		3,65	194
K	29		10,53	93	K	60		10,53	200
H	30		6	97	D	61		3,65	205
R	31		12,48	99	W	2,38	62		206

sum = 475,9; 60+2=62 ; pKavid=475,9 / 62 =7,6758065

ISOELEKTRIC POINT charge "0" Sum are calculate as

62 protolytic equilibria pK_a values in table is 475,9.....

$\Sigma pK_{aRside\ group} + pK_{aNterminus} + pK_{aCterminus}$ = 475,9.....

Protolysis mean constant pK_a isoelectric point IEP=pK_a calculate as sum of constants: side chains $\Sigma pK_{aRside\ group}$, $pK_{aNterminus}NH_3^+$ and $pK_{aCterminus}COO^-$ divided with number of protolytic acid groups NpK_a: 62.....

$IEP=pK_a=(\Sigma pK_{aRside\ group}+pK_{aNterminus}+pK_{aCterminus})/NpK_a$

$pK_{mean}= pK_a = IEP = 475,9 / 62 = 7,6758065$....., where acid groups number sum NpK_a=60.....+2.....=62..... of 206 amino acids 60+2 of them;protolytic constants pK_a for side groups, N-terminus methionine M $pK_{aNterminus}$=9.21 and C-terminus Triptophane W $pKa_{Cterminus}$=2.38

a. Average acid group constant pK_{mean} calculates as IEP

ISOELEKTRIC POINT pK_{mean}= IEP= 475,9 / 62 = **7,6758065**.....

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 (-)!

b. Determine C1P_ACD11 molecule charge sign (+), zero „**0**" or (-) at physiologic pH=7.36 of blood plasma Underline existing:

-COOH & **-NH_3^+** positive (+) charge ……. pH=7.36< IEP=**7,68**..... charge negative(-) **-COO^-** & **-NH_2**.

c. Determine C1P_ACD11 molecule charge sign (+), zero „**0**" or (-) at **electrophoresis** pH 8.8 on stripe

Underline existing:

-COOH & **-NH_3^+** positive (+) charge IEP=**7,68** < pH=8.8 electrophoresis charge negative(-) **-COO^-** & **-NH_2**.

Electrophoresis pH=8.8 show the movement towards positive electrode.

d. Calculate $10^{-7,0442}$ M ($^{mol}/_{Liter}$) protein solution pH by *Ostwald dilution law* concentration C in logarithm:

$$pH=\frac{pK_a-\log C}{2}=\frac{7,6758065-\log 10^{-7,0441935}}{2}=\frac{7,6758065+7,0441935}{2}$$ = **14,72** / 2 = 7,36.....

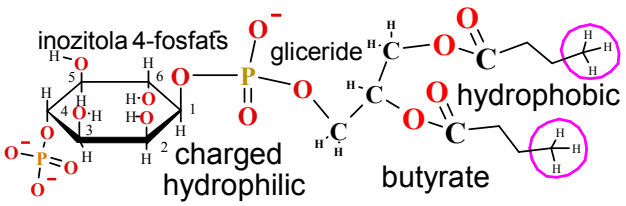
7,36 Attractor C1P_ACD11 concentration is C=$10^{-7,0442}$.....M .

ACD11 structures establish C1P binding via a surface-localized, phosphate head group recognition center connected to an interior hydrophobic pocket that adaptively ensheaths lipid chains via a cleft-like gating mechanism. A pi-helix (pi-bulge) near the lipid-binding cleft distinguishes apo-ACD11 from other GLTP-folds. The global two-layer 'sandwich' topology, alpha-helically-dominated, displaying C1P-selective binding identifies ACD11 as the plant prototype of a new GLTP-fold subfamily. Ceramide (Cer), ceramide-1-phosphate (C1P), and the long chain bases (LCB), sphingosine and sphingosine-1-phosphate (S1P), are bioactive lipids that function as messenger signals and mediators of eukaryotic processes such as cell growth, development, embryogenesis, senescence, inflammation, and programmed cell death (PCD) (Fyrst and Saba, 2010; Hannun and Obeid, 2008; Michaelson, 2010).

The dynamic balance between Cer (sphingoid base amide-linked to a fatty acyl chain) and its phosphorylated derivative, C1P, critically regulates PCD in plants and animals (Berkey et al., 2012; Chen, 2009; Pata et al., 2010; Reape and McCabe, 2008). PCD occurs during development, during disease symptoms associated with virulent infections, and during the hypersensitive response (HR) induced by a virulent stress effectors (Lam, 2004). Hallmarks of HR are local accumulation of reactive oxygen species, nitric oxide, and the phytohormone, salicylic acid. By inducing localized cell death triggered when resistance proteins recognize specific pathogen-derived molecules, HR potentiates defensive resistance.

(DiNitto and Lambright,2006; Lemmon,2008; Lemmon et al.,1996; Moravcevic et al.,2012).

Pleckstrin Homology PH Domain 2RSG.pdb **from the Ceramide Trafficking Protein CERT** is regulatory components of hundreds of human proteins involved in signaling, membrane traffic, and actin cytoskeleton remodeling



3. Draw CERT, PH domane Golgi membrane bound phosphatidyl inositol-4-phosphat componentes and identify amphiphatic properties! at physiologic pH conditions 7.36!

4. What organel membranes are VAMP (Vesicle Associated Membrane Protein)

For CERT protein binding? ER endoplasmatic reticulum.....,

Golgi aparatus.....

N-teminal 100 amino acids domain chain 2RSG.pdb central cave of CERT protein is defined structurally as a sandwich of seven beta strands capped at one end by an alpha helix. A subset of PH domains binds to phosphor inositides and proteins. Several mechanisms by which ligand binding to PH domains regulates protein activity. Cooperative phospholipid binding to the C and A sites of the PH domain of ASAP1 is the mechanism underlies rapid switching between active and inactive ASAP1. Major function of PH domains is to localize proteins to specific membrane regions through binding to specific phospho inositides (Moravcevic et al.,2012). Proteins containing phosphatidylinositol 3,4,5-triphosphate (PtdIns(3,4,5)P3)-binding PH

domains are recruited to membranes in which PtdIns(3,4,5)P3 is produced. Membrane localization may be further specified by the coincidence of two signals, which was first described for two independent domains within a single protein, each domain having distinct ligand specificities (Moravcevic et al.,2012). Coincidence detection may also be mediated by a single PH domain binding two distinct ligands (Balla, 2005), as recently described for FAPP1, which simultaneously binds phospho inositides and Arf1-GTP (Godi et al.,2004; He et al.,2011; Liu et al., 2014) and Grp1, which simultaneously binds PtdIns(3,4,5)P3 and Arf6-GTP (DiNitto et al.,2007; Malaby et al.,2013).

The two different PtdIns(4,5)P2 binding sites are illustrated as A site formed at the dimer interface and C site formed between ß1/ß2 and ß3/ß4 loops.

5. Aprakstīt vienīgo C-termināla α-spirāles 11 aminoskābju secības kārtas numurus 2RSG.pdb!

Gln106.....,107.....,108.....,109.....,110.....,111.....,112.....,113.....,114.....,Thr115...

6. Human CERT PH domain beta sheets and anti parallel strands numbers in 2RSG.pdb !
.....seven anti parallel β-strands and two β-sheets.....

7. Indicate amino acid sequence numbers on PH domain 2RSG.pdb ß-sandwich strandss!
First sandwich layer four anti parallel beta strands:
B1(26.....-33.....), **B2**(40.....-48.....), **B3**(51.....-55.....), **B4**(67.....-70.....),
Second sandwich layer three anti parallel beta strands:
B5(75.....-78.....), **B6**(85.....-90.....), **B7**(93.....-98.....)

Polipeptide chane trace bent orthogonal (perpendicular) direction in CERT PH domain ß- sandwich ß1-ß4 and ß5-ß7. C-terminal α-helix close ß-sandwich side-top, but two loops ß1/ß2 conect Thr34-39 and ß3/ß4 Lys56-66.

8. Indicate amino acid sequence numbers on PH domain 2RSG.pdb for two loops!
L1(34.....39.....), **L2**(56.....-66.....),

9. Exponate ß-sandwich end ß1/ß2 anti-parallel strands are two times larger as ß3/ß4.
Amino acid sequence numbers in ß1/ß2 anti-parallel strands are:
B1(Arg26.....-Trp33.....), **B2**(Trp40.....-Lys48.....),
ß3/ß4 anti-parallel strands are:
B3(Ala51.....-Tyr55.....), **B4**(Gly67.....-Cys70.....),

ß1/ß2 loop Trp33-Trp40 protroodes ß-sandwich.
ß1/ß2, ß3/ß4Tyr55-67, and ß7/αCArg98-106 CERT PH domain loop is larger as others.

10. Indicate amino acid sequence numbers on PH 2RSG.pdb four cysteins!

..Cys27..... Cys65..... Cys70..... Cys84.....

11.1-11.5 Analyses of CERT isoelectric point IEP=pH=$pK_{a\text{-}vid}$ at physiologic pH=7,36 .
Determine at solution pH with CERT concentration C=$10^{-7,3684}$ M ($^{mol}/_{Litrā}$)!
2E3R.pdb 347-624 Human Ceramide transfer CERT cytosolic 70,829-kDa koncentrācijā $10^{-7,2952}$ M
http://aris.gusc.lv/ChemFiles/Albumin/START/2E3RpIStudS.doc;http://aris.gusc.lv/ChemFiles/START/2E3RpI.xls

```
SQ SEQUENCE 624 >2E3R,2RSG,4N:A|PDBID|CHAIN|SEQUENCE 348-624 2E3R.pdb
MSDNQSWNSSGSEEDPETESGPPVERCGVLSKWTNYIHGWQDRWVVLKNNALSYYKSEDETEYGCRGSICLSKAVITPHD
FDECRFDISVNDSVWYLRAQDPDHRQQWIDAIEQHKTESGYGSESSLRRHGSMVSLVSGASGYSATSTSSFKKGHSLREK
LAEMETFRDILCRQVDTLQKYFDACADAVSKDELQRDKVVEDDEDDFPTTRSDGDFLHSTNGNKEKLFPHVTPKGINGID
FKGEAITFKATTAGILATLSHCIELMVKREDSWQKRLDKETEKKRRTEEAYKNAMTELKKKSHFGGPDYEEGPNSLINEE
EFFDAVEAALDRQDKIEEQSQSEKVRLHWPTSLPSGDAFSSVGTHRFVQKPYSRSSSMSSIDLVSASDDVHRFSSQVEEM
VQNHMTYSLQDVGGDANWQLVVEEGEMKVYRREVEENGIVLDPLKATHAVKGVTGHEVCNYFWNVDVRNDWETTIENFHV
VETLADNAIIIYQTHKRVWPASQRDVLYLSVIRKIPALTENDPETWIVCNFSVDHDSAPLNNRCVRAKINVAMICQTLVS
PPEGNQEISRDNILCKITYVANVNPGGWAPASVLRAVAKREYPKFLKRFTSYVQEKTAGKPILF plekstrin PH domane
```

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	K	46	10,53	116	H	91	6	230	E	136	4,25	337	E	181	4,25	476
D	2	3,65	3		E	47	4,25	118	K	92	10,53	234	E	137	4,25	338	H	182	6	479
E	3	4,25	13		Y	48	10,07	121	D	93	3,65	240	E	138	4,25	343	E	183	4,25	482
E	4	4,25	14		E	49	4,25	124	K	94	10,53	242	K	139	10,53	344	D	184	3,65	486
D	5	3,65	15		R	50	12,48	128	E	95	4,25	244	R	140	12,48	346	Y	185	10,07	492
E	6	4,25	17		R	51	12,48	129	K	96	10,53	249	H	141	6	348	H	186	6	495
E	7	4,25	19		H	52	6	130	H	97	6	261	D	142	3,65	357	K	187	10,53	496
E	8	4,25	25		Y	53	10,07	143	C	98	8,18	262	H	143	6	365	R	188	12,48	497
R	9	12,48	26		K	54	10,53	152	E	99	4,25	264	R	144	12,48	366	R	189	12,48	504
C	10	8,18	27		K	55	10,53	153	K	100	10,53	268	K	145	10,53	370	D	190	3,65	505
K	11	10,53	32		H	56	6	155	R	101	12,48	269	Y	146	10,07	372	Y	191	10,07	508
Y	12	10,07	36		R	57	12,48	158	E	102	4,25	270	R	147	12,48	374	R	192	12,48	513
H	13	6	38		E	58	4,25	159	D	103	3,65	271	D	148	3,65	382	K	193	10,53	514
D	14	3,65	42		K	59	10,53	160	K	104	10,53	275	D	149	3,65	388	E	194	4,25	520
R	15	12,48	43		E	60	4,25	163	R	105	12,48	276	D	150	3,65	389	D	195	3,65	522
K	16	10,53	48		E	61	4,25	165	D	106	3,65	278	H	151	6	391	E	196	4,25	524
Y	17	10,07	54		R	62	12,48	168	K	107	10,53	279	R	152	12,48	392	C	197	8,18	529
Y	18	10,07	55		D	63	3,65	169	E	108	4,25	280	E	153	4,25	398	D	198	3,65	534
K	19	10,53	56		C	64	8,18	172	E	109	4,25	282	E	154	4,25	399	H	199	6	535
E	20	4,25	58		R	65	12,48	173	K	110	10,53	283	H	155	6	404	D	200	3,65	536
D	21	3,65	59		D	66	3,65	176	K	111	10,53	284	Y	156	10,07	407	R	201	12,48	543
E	22	4,25	60		K	67	10,53	180	R	112	12,48	285	D	157	3,65	411	C	202	8,18	544
E	23	4,25	62		Y	68	10,07	181	R	113	12,48	286	D	158	3,65	415	R	203	12,48	546
Y	24	10,07	63		D	69	3,65	183	E	114	4,25	288	E	159	4,25	423	K	204	10,53	548
C	25	8,18	65		C	70	8,18	185	E	115	4,25	289	E	160	4,25	424	C	205	8,18	555
R	26	12,48	66		D	71	3,65	187	Y	116	10,07	291	E	161	4,25	426	E	206	4,25	563
C	27	8,18	70		K	72	10,53	191	K	117	10,53	292	K	162	10,53	428	E	207	4,25	567
K	28	10,53	73		D	73	3,65	192	E	118	4,25	297	Y	163	10,07	430	R	208	12,48	570
H	29	6	79		E	74	4,25	193	K	119	10,53	299	R	164	12,48	431	D	209	3,65	571
D	30	3,65	80		R	75	12,48	196	K	120	10,53	300	R	165	12,48	432	C	210	8,18	575
D	31	3,65	82		D	76	3,65	197	K	121	10,53	301	E	166	4,25	433	K	211	10,53	576
E	32	4,25	83		K	77	10,53	198	H	122	6	303	E	167	4,25	435	Y	212	10,07	579
C	33	8,18	84		E	78	4,25	201	D	123	3,65	308	E	168	4,25	436	R	213	12,48	595
R	34	12,48	85		D	79	3,65	202	Y	124	10,07	309	D	169	3,65	442	K	214	10,53	599
D	35	3,65	87		D	80	3,65	203	E	125	4,25	310	K	170	10,53	445	R	215	12,48	600
D	36	3,65	92		E	81	4,25	204	E	126	4,25	311	H	171	6	448	E	216	4,25	601
Y	37	10,07	96		D	82	3,65	205	E	127	4,25	319	K	172	10,53	451	Y	217	10,07	602
R	38	12,48	98		D	83	3,65	206	E	128	4,25	320	H	173	6	456	K	218	10,53	604
D	39	3,65	101		R	84	12,48	211	E	129	4,25	321	E	174	4,25	457	K	219	10,53	607
D	40	3,65	103		D	85	3,65	213	D	130	3,65	324	C	175	8,18	459	R	220	12,48	608
H	41	6	104		D	86	3,65	215	E	131	4,25	327	Y	176	10,07	461	Y	221	10,07	612
R	42	12,48	105		H	87	6	218	D	132	3,65	331	D	177	3,65	466	E	222	4,25	615
D	43	3,65	110		K	88	10,53	224	R	133	12,48	332	R	178	12,48	468	K	223	10,53	616
E	44	4,25	113		E	89	4,25	225	D	134	3,65	334	D	179	3,65	470	K	224	10,53	620
H	45	6	115		K	90	10,53	226	K	135	10,53	335	E	180	4,25	472	F	2,13	225	624

Sum of 225 pKa values in table 1654.1; on 624 amino acids for chain IEP=7,351556

Protolytic constant pK_a isoelectric point IEP=pK_a 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:

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

Calculation tasks for Human CERT cytosolic 70,829-kDa concentration $10^{-7,3684}$ M

11.1 Acid groups number in sum NpKa=223.....+2.....=225.....

624 amino acids of them protolytic constants pK_a for side groups 223+2 ,

N-terminal methionin M $pK_{aNterminal}$=9.21 and C-termināl phenylalanine F $pKa_{Cterminal}$=2.36

Sum on 2nd page are calculate as $\Sigma pKa_{Rside\ group}+pK_{aNterminal}+pKa_{Cterminal}$= 1654.1.....

11.2 Average acid constant **ISOELEKTRIC POINT** $pK_{mean}=(\Sigma pKa_{Rside\ group}+pKa_{Nterminal}+pKa_{Cterminal})/NpKa$

IEP =1654.1 / 225=**7.351556**.....

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 (-)!

11.3 Determine CERT molecule charge sign (+). zero „**0**” or (-) at physiologic pH=7.36

Underline existing:

-COOH & **-NH_3^+** positive (+) charge ……. IEP=**7.35** < pH=7.36..... charge negative(-) **-COO^-** & **-NH_2**.

11.4 Determine CERT molecule charge sign (+). zero „**0**" or (-) at **electrophoresis** pH **8.8**

Underline existing:

-COOH & **-NH_3^+** positive (+) charge ……. IEP=**7.35**< pH=8.8 ..... charge negative(-) **-COO^-** & **-NH_2**.

11.5 Calculate $10^{-7,3684}$ M CERT solution pH by *Ostwald dilution law* concentration C in logarithm:

$$pH=\frac{pK_a-\log C}{2}=\frac{7,3515556-\log 10^{-7,3684444}}{2}=\frac{7,3515556+7,3684444}{2}=\mathbf{14,72}\ /\ 2=7,36.....$$

7,36 Attractor H_ CERT concentration is $C=10^{-7,3684}$.....M .

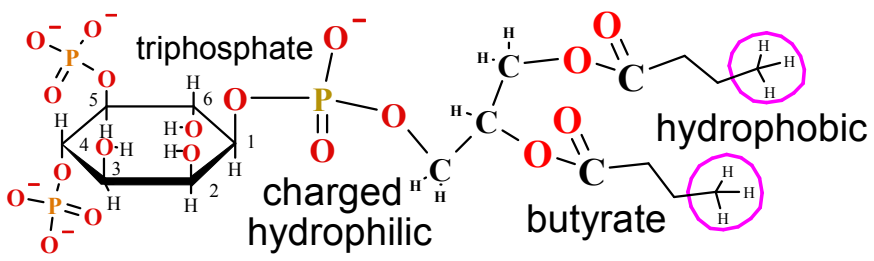
Membrane binding PH Domain to Anionic Phospholipids of the Arf GAP ASAP1 5C79AB.pdb

PH domains are regulatory components of hundreds of human proteins involved in signaling, membrane traffic, and actin cytoskeleton remodeling (DiNitto and Lambright,2006; Lemmon,2008; Lemmon et al.,1996; Moravcevic et al.,2012).

Domain is defined structurally as a sandwich of seven beta strands capped at one end by an alpha helix. A subset of PH domains binds to phosphor inositides and proteins. Several mechanisms by which ligand binding to PH domains regulates protein activity. Cooperative phospholipid binding to the C and A sites of the PH domain of ASAP1 is the mechanism underlies rapid switching between active and inactive ASAP1. Major function of PH domains is to localize proteins to specific membrane regions through binding to specific phospho inositides (Moravcevic et al.,2012). Proteins containing phosphatidylinositol 3,4,5-triphosphate (PtdIns(3,4,5)P3)-binding PH domains are recruited to membranes in which PtdIns(3,4,5)P3 is produced. Membrane localization may be further specified by the coincidence of two signals, which was first described for two independent domains within a single protein, each domain having distinct ligand specificities (Moravcevic et al.,2012). Coincidence detection may also be mediated by a single PH domain binding two distinct ligands (Balla, 2005), as recently described for FAPP1, which simultaneously binds phospho inositides and Arf1-GTP (Godi et al.,2004; He et al.,2011; Liu et al., 2014) and Grp1, which simultaneously binds PtdIns(3,4,5)P3 and Arf6-GTP (DiNitto et al.,2007; Malaby et al.,2013).

The two different PtdIns(4,5)P2 binding sites are illustrated as A site formed at the dimer interface and C site formed between ß1/ß2 and ß3/ß4 loops.

1. Dibutyryl PtdIns(4,5)P2, dibutyryl phosphatidyl inositol-4,5-diphosphate put in given atoms and amphiphatic properties!To show at physiologic pH=7.36!



2.Which four side chains residues interact with the bound PtdIns(4,5)P2 in A site: Lys349.....,Lys355.....,Trp357.....,Gln412..... 5C79AB.pdb

3. What two distances from Lys349, Lys355 >N –H…..O= to phosphate groups at positions 1, 5 of the inositol ring in salt bridges?..d=3.339.....Å Ångströms,..d=2.338.....Å Ångström.

4. Which 7 amino acids at the C site, to bind PtdIns(4,5)P2 contacts more than eight residues..... .... phosphate-4 with Arg360.....,His373.....,Arg378.....,Ala374..... .... 5-phosphate interacts with Lys348.....,Arg407.....,Asp351.....

5. What total amino acids account at the A and C site to bind contacting PtdIns(4,5)P2.....

phosphates with Arg360,His373,Arg378,Ala374-four 4.....Lys348,Arg407,Asp351-three 3..... Lys349,Lys355, Trp357,Gln412-four 4.....totally 4+3+4=11 eleven amino acids.....

6.1-6.5 Analyses of ASAP1 isoelectric point IEP=pH=$pK_{a\text{-}vid}$ at physiologic pH=7,36 .
Determine at solution pH with ASAP1 concentration $C=10^{-7,0794}$ M ($^{mol}/_{Liter}$)!

5C97.pdb Mouse PH domain Arf GAP ASAP1 + phospholipids membrane 127,5808 kDa concentration $10^{-7,0794}$ M

http://aris.gusc.lv/ChemFiles/START/5C79pIStudS.doc ; http://aris.gusc.lv/ChemFiles/START/5C79pI.xls

```
    SQ SEQUENCE 1147 >5C79:A|PDBID|CHAIN|SEQUENCE                    ASAP1_MOUSE
MRSSASRLSSFSSRDSLWNRMPDQISVSEFIAETTEDYNSPTTSSFTTRLHNCRNTVTLLEEALDQDRTALQKVKKSVKA
IYNSGQDHVQNEENYAQVLDKFGSNFLSRDNPDLGTAFVKFSTLTKELSTLLKNLLQGLSHNVIFTLDSLLKGDLKGVKG
DLKKPFDKAWKDYETKFTKIEKEKREHAKQHGMIRTEITGAEIAEEMEKERRLFQLQMCEYLIKVNEIKTKKGVDLLQNL
IKYYHAQCNFFQDGLKTADKLKQYIEKLAADLYNIKQTQDEEKKQLTALRDLIKSSLQLDPKEVGGLYVASRANSSRRDS
QSRQGGYSMHQLQGNKEYGSEKKGFLLKKSDGIRKVWQRRKCAVKNGILTISHATSNRQPAKLNLLTCQVKPNAEDKKSF
DLISHNRTYHFQAEDEQDYIAWISVLTNSKEEALTMAFRGEQSTGENSLEDLTKAIIEDVQRLPGNDICCDCGSSEPTWL
STNLGILTCIECSGIHREMGVHISRIQSLELDKLGTSELLLAKNVGNNSFNDIMEANLPSPSPKPTPSSDMTVRKEYITA
KYVDHRFSRKTCASSSAKLNELLEAIKSRDLLALIQVYAEGVELMEPLLEPGQELGETALHLAVRTADQTSLHLVDFLVQ
NCGNLDKQTSVGNTVLHYCSMYGKPECLKLLLRSKPTVDIVNQNGETALDIAKRLKATQCEDLLSQAKSGKFNPHVHVEY
EWNLRQDEMDESDDDLDDKPSPIKKERSPRPQSFCHSSSISPQDKLALPGFSTPRDKQRLSYGAFTNQIFASTSTDLPTS
PTSEAPPLPPRNAGKGPTGPPSTLPLGTQTSSGSSTLSKKRPPPPPPGHKRTLSDPPSPLPHGPPNKGAIPWGNDVGPLS
SSKTANKFEGLSQQASTSSAKTALGPRVLPKLPQKVALRKTETSHHLSLDRTNIPPETFQKSSQLTELPQKPPLGELPPK
PVELAPKPQVGELPPKPGELPPKPQLGDLPPKPQLSDLPPKPQMKDLPPKPQLGDLLAKSQAGDVSAKVQPPSEVTQRSH
TGDLSPNVQSRDAIQKQASEDSNDLTPTLPETPVPLPRKINTGKNKVRRVKTIYDCQADNDDELTFIEGEVIIVTGEEDQ
EWWIGHIEGQPERKGVFPVSFVHILSD                                      5C79
```

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	AA	pKa$_{COO-}$	pKa$_{NH3+}$	pK$_{RR}$	Nr
M	1	9,21		1	R	62	12,48		185	Y	123	10,07		338	K	184	10,53		513	K	244	10,53		711	K	304	10,53		941
R	2		12,48	2	E	63	4,25		186	E	124	4,25		341	E	185	4,25		518	H	245	6		715	E	305	4,25		947
R	3		12,48	7	H	64	6		187	K	125	10,53		342	K	186	10,53		523	H	246	6		717	K	306	10,53		951
R	4		12,48	14	K	65	10,53		189	K	126	10,53		343	D	187	3,65		532	E	247	4,25		719	E	307	4,25		956
D	5		3,65	15	H	66	6		191	K	127	10,53		348	E	188	4,25		535	Y	248	10,07		720	K	308	10,53		960
R	6		12,48	20	R	67	12,48		195	K	128	10,53		349	K	189	10,53		544	E	249	4,25		721	E	309	4,25		963
D	7		3,65	23	E	68	4,25		197	D	129	3,65		351	D	190	3,65		550	R	250	12,48		725	K	310	10,53		967
E	8		4,25	29	E	69	4,25		202	R	130	12,48		354	R	191	12,48		554	D	251	3,65		727	E	311	4,25		972
E	9		4,25	33	E	70	4,25		205	K	131	10,53		355	K	192	10,53		555	E	252	4,25		728	K	312	10,53		976
E	10		4,25	36	E	71	4,25		206	R	132	12,48		359	E	193	4,25		556	D	253	3,65		730	E	313	4,25		979
D	11		3,65	37	E	72	4,25		208	R	133	12,48		360	Y	194	10,07		557	E	254	4,25		731	K	314	10,53		983
Y	12		10,07	38	K	73	10,53		209	K	134	10,53		361	K	195	10,53		561	D	255	3,65		733	D	315	3,65		988
R	13		12,48	49	E	74	4,25		210	C	135	8,18		362	Y	196	10,07		562	D	256	3,65		734	K	316	10,53		992
H	14		6	51	R	75	12,48		211	K	136	10,53		365	D	197	3,65		564	D	257	3,65		735	D	317	3,65		997
C	15		8,18	53	R	76	12,48		212	H	137	6		373	H	198	6		565	D	258	3,65		737	K	318	10,53		1001
R	16		12,48	54	C	77	8,18		219	R	138	12,48		378	R	199	12,48		566	D	259	3,65		738	K	319	10,53		1005
E	17		4,25	61	E	78	4,25		220	K	139	10,53		382	R	200	12,48		569	K	260	10,53		739	D	320	3,65		1006
E	18		4,25	62	Y	79	10,07		221	C	140	8,18		388	K	201	10,53		570	K	261	10,53		744	K	321	10,53		1010
D	19		3,65	65	K	80	10,53		224	K	141	10,53		391	C	202	8,18		572	K	262	10,53		745	D	322	3,65		1015
D	20		3,65	67	E	81	4,25		227	E	142	4,25		395	K	203	10,53		578	E	263	4,25		746	K	323	10,53		1019
R	21		12,48	68	K	82	10,53		229	D	143	3,65		396	E	204	4,25		581	R	264	12,48		747	D	324	3,65		1024
K	22		10,53	73	K	83	10,53		231	K	144	10,53		397	E	205	4,25		584	R	265	12,48		750	K	325	10,53		1028
K	23		10,53	75	K	84	10,53		232	K	145	10,53		398	K	206	10,53		587	C	266	8,18		755	E	326	4,25		1034
K	24		10,53	76	D	85	3,65		235	D	146	3,65		401	R	207	12,48		589	H	267	6		756	R	327	12,48		1038
K	25		10,53	79	K	86	10,53		242	H	147	6		405	D	208	3,65		590	D	268	3,65		764	H	328	6		1040
Y	26		10,07	82	Y	87	10,07		243	R	148	12,48		407	Y	209	10,07		598	K	269	10,53		765	D	329	3,65		1043
D	27		3,65	87	Y	88	10,07		244	Y	149	10,07		409	E	210	4,25		600	R	270	12,48		775	R	330	12,48		1051
H	28		6	88	H	89	6		245	H	150	6		410	E	211	4,25		603	D	271	3,65		776	D	331	3,65		1052
E	29		4,25	92	C	90	8,18		248	E	151	4,25		414	E	212	4,25		606	K	272	10,53		777	K	332	10,53		1056
E	30		4,25	93	D	91	3,65		253	D	152	3,65		415	E	213	4,25		610	R	273	12,48		779	E	333	4,25		1060
Y	31		10,07	95	K	92	10,53		256	E	153	4,25		416	E	214	4,25		614	Y	274	10,07		782	D	334	3,65		1061
D	32		3,65	100	D	93	3,65		259	D	154	3,65		418	E	215	4,25		617	D	275	3,65		796	D	335	3,65		1064
K	33		10,53	101	K	94	10,53		260	Y	155	10,07		419	H	216	6		621	E	276	4,25		804	E	336	4,25		1071
R	34		12,48	109	K	95	10,53		262	K	156	10,53		430	R	217	12,48		625	R	277	12,48		811	R	337	12,48		1078
D	35		3,65	110	Y	96	10,07		264	E	157	4,25		431	D	218	3,65		628	K	278	10,53		815	K	338	10,53		1079
D	36		3,65	113	E	97	4,25		266	E	158	4,25		432	H	219	6		633	K	279	10,53		839	K	339	10,53		1084
K	37		10,53	120	K	98	10,53		267	R	159	12,48		439	D	220	3,65		636	K	280	10,53		840	K	340	10,53		1086
K	38		10,53	126	D	99	3,65		271	E	160	4,25		441	C	221	8,18		642	R	281	12,48		841	R	341	12,48		1088
E	39		4,25	127	Y	100	10,07		273	E	161	4,25		446	D	222	3,65		646	H	282	6		849	R	342	12,48		1089
K	40		10,53	133	K	101	10,53		276	E	162	4,25		450	K	223	10,53		647	K	283	10,53		850	K	343	10,53		1091
H	41		6	141	D	102	3,65		280	D	163	3,65		451	H	224	6		657	R	284	12,48		851	Y	344	10,07		1094
D	42		3,65	148	E	103	4,25		281	K	164	10,53		454	Y	225	10,07		658	D	285	3,65		855	D	345	3,65		1095
K	43		10,53	152	E	104	4,25		282	E	165	4,25		458	C	226	8,18		659	H	286	6		862	C	346	8,18		1096
D	44		3,65	154	K	105	10,53		283	D	166	3,65		459	Y	227	10,07		662	K	287	10,53		867	D	347	3,65		1099
K	45		10,53	156	K	106	10,53		284	R	167	12,48		462	K	228	10,53		664	D	288	3,65		875	D	348	3,65		1101
K	46		10,53	159	R	107	12,48		290	D	168	3,65		467	E	229	4,25		666	K	289	10,53		883	D	349	3,65		1102
D	47		3,65	161	D	108	3,65		291	C	169	8,18		469	C	230	8,18		667	K	290	10,53		887	E	350	4,25		1103
K	48		10,53	163	K	109	10,53		294	C	170	8,18		470	K	231	10,53		669	E	291	4,25		889	E	351	4,25		1108
K	49		10,53	164	D	110	3,65		300	D	171	3,65		471	R	232	12,48		673	K	292	10,53		901	E	352	4,25		1110
D	50		3,65	167	K	111	10,53		302	C	172	8,18		472	K	233	10,53		675	R	293	12,48		907	E	353	4,25		1117
K	51		10,53	168	E	112	4,25		303	E	173	4,25		476	D	234	3,65		679	K	294	10,53		911	E	354	4,25		1118
K	52		10,53	171	Y	113	10,07		308	C	174	8,18		489	E	235	4,25		686	K	295	10,53		915	D	355	3,65		1119

D	53		3,65	172	R	114	12,48	312	E	175		4,25	491	D	236		3,65	690	R	296		12,48	919	E		356	4,25	1121
Y	54		10,07	173	R	115	12,48	317	C	176		8,18	492	K	237		10,53	693	K	297		10,53	920	H		357	6	1126
E	55		4,25	174	R	116	12,48	318	H	177		6	496	R	238		12,48	694	E	298		4,25	922	E		358	4,25	1128
K	56		10,53	176	D	117	3,65	319	R	178		12,48	497	K	239		10,53	696	H	299		6	925	E		359	4,25	1132
K	57		10,53	179	R	118	12,48	323	E	179		4,25	498	C	240		8,18	700	H	300		6	926	R		360	12,48	1133
E	58		4,25	181	Y	119	10,07	327	H	180		6	502	E	241		4,25	701	D	301		3,65	930	K		361	10,53	1134
K	59		10,53	182	H	120	6	330	R	181		12,48	505	D	242		3,65	702	R	302		12,48	931	H		362	6	1143
E	60		4,25	183	K	121	10,53	336	E	182		4,25	510	K	243		10,53	708	E	303		4,25	937	D	1,88	363	3,65	1147
K	61		10,53	184	E	122	4,25	337	D	183		3,65	512	IEP=7,6405495 ; sum=2782,93 ; 364 protolytic constants																		

Protolytic average constant isoelectric point IEP=pK_{amean} 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 Mouse PH domain binde Arf GAP ASAP1 to phospholipid membrane 127,5808-kDa molecule

6.1 Acid groups number in sum NpKa=Sum of 362 + 2 = 364 pKa values in table: 2782,93.....

1147 amino acids of them protolytic constants pK_a for side groups 362+2 ,

N-terminal methionine M $pK_{aNterminal}$=9.21 and C-terminal aspartate D $pKa_{Cterminal}$=1.88 .

Sum are calculate as $\Sigma pKa_{Rside\ group}+pK_{aNterminal}+pKa_{Cterminal}$ = 2782,93.....

6.2 Average acid group constant **ISOELEKTRIC POINT** pK_{mean}= IEP = 2782,93 / 364 =**7.6405495**.....

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 (-)!

6.3 Determine ASAP1 molecule charge sign (+). zero „**0**" or (-) at physiologic pH=7.36

Underline existing:

-COOH & **-NH_3^+** positive (+) charge ……. pH=7.36 < IEP=**7.64** ..... charge negative(-) **-COO^-** & **-NH_2**.

6.4 Determine ASAP1 molecule charge sign (+). zero „**0**" or (-) at **electrophoresis** pH **8.8**

Underline existing:

-COOH & **-NH_3^+** positive (+) charge ……. IEP=**7.64**< pH=8.8 ..... charge negative(-) **-COO^-** & **-NH_2**.

6.5 Calculate C = $10^{-7,0794}$ moli / Liter M ASAP1 solution pH by *Ostwald dilution law* in logarithm of C:

$$pH=\frac{pK_a-\log C}{2}=\frac{7,6405495-\log 10^{-7,0794505}}{2}=\frac{7,6405495+7,0794505}{2}=\mathbf{14,72}\ /\ 2=7,36.....$$

7,36 Attractor mouse Arf GAP ASAP1 concentration is C=$10^{-7,0794}$.....M .

2D1X.pdb Cilvēka PH domēns Arf GAP ASAP1 + fosfolipīdu membrānā 132,787 kDa koncentrācijā $10^{-7,1294}$ M

http://aris.gusc.lv/ChemFiles/START/2D1XpIStudS.doc ; http://aris.gusc.lv/ChemFiles/START/2D1XpI.xls

Protolytic average constant isoelectric point IEP=pK_{amean} 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 Human PH domain binde Arf GAP ASAP1 to phospholipid membrane 132,787 kDa molecule

Acid groups number in sum NpKa=Sum of 358 + 2 = 360 pKa values in table: 2732,62.....

1129 amino acids of them protolytic constants pK_a for side groups 358+2 ,

N-terminal methionine M $pK_{aNterminal}$=9.21 and C-terminal aspartate D $pKa_{Cterminal}$=1.88 .

Sum are calculate as $\Sigma pKa_{Rside\ group}+pK_{aNterminal}+pKa_{Cterminal}$ = 2732,62.....

a. Average acid group constant **ISOELEKTRIC POINT** pK_{mean}= IEP = 2732,62 / 360 =**7.5906111**.....

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 (-)!

b. Determine ASAP1 molecule charge sign (+). zero „**0**" or (-) at physiologic pH=7.36

Underline existing:

-COOH & **-NH_3^+** positive (+) charge ……. pH=7.36 < IEP=**7.59** ..... charge negative(-) **-COO^-** & **-NH_2**.

c. Determine ASAP1 molecule charge sign (+). zero „**0**" or (-) at **electrophoresis** pH **8.8**

Underline existing:

-COOH & **-NH_3^+** positive (+) charge ……. IEP=**7.59**< pH=8.8 ..... charge negative(-) **-COO^-** & **-NH_2**.

d. Calculate C = $10^{-7,0794}$ moli / Liter M ASAP1 solution pH by *Ostwald dilution law* in logarithm of C:

$$pH=\frac{pK_a-\log C}{2}=\frac{7,5906111-\log 10^{-7,1293889}}{2}=\frac{7,5906111+7,1293889}{2}=\mathbf{14,72}\ /\ 2=7,36.....$$

7,36 Attractor human Arf GAP ASAP1 concentration is C=$10^{-7,1294}$.....M .

SQ SEQUENCE 1129 >1D1X:A|PDBID|CHAIN|SEQUENCE ASAP1_Human

MRSSASRLSSFSSRDSLWNRMPDQISVSEFIAETTEDYNSPTTSSFTTRLHNCRNTVTLLEEALDQDRTALQKVKKSVKA
IYNSGQDHVQNEENYAQVLDKFGSNFLSRDNPDLGTAFVKFSTLTKELSTLLKNLLQGLSHNVIFTLDSLLKGDLKGVKG
DLKKPFDKAWKDYETKFTKIEKEKREHAKQHGMIRTEITGAEIAEEMEKERRLFQLQMCEYLIKVNEIKTKKGVDLLQNL
IKYYHAQCNFFQDGLKTADKLKQYIEKLAADLYNIKQTQDEEKKQLTALRDLIKSSLQLDQKEDSQSRQGGYSMHQLQGN
KEYGSEKKGYLLKKSDGIRKVWQRRKCSVKNGILTISHATSNRQPAKLNLLTCQVKPNAEDKKSFDLISHNRTYHFQAED
EQDYVAWISVLTNSKEEALTMAFRGEQSAGENSLEDLTKAIIEDVQRLPGNDICCDCGSSEPTWLSTNLGILTCIECSGI
HREMGVHISRIQSLELDKLGTSELLLAKNVGNNSFNDIMEANLPSPSPKPTPSSDMTVRKEYITAKYVDHRFSRKTCSTS
SAKLNELLEAIKSRDLLALIQVYAEGVELMEPLLEPGQELGETALHLAVRTADQTSLHLVDFLVQNCGNLDKQTALGNTV
LHYCSMYSKPECLKLLLRSKPTVDIVNQAGETALDIAKRLKATQCEDLLSQAKSGKFNPHVHVEYEWNLRQEEIDESDDD
LDDKPSPIKKERSPRPQSFCHSSSISPQDKLALPGFSTPRDKQRLSYGAFTNQIFVSTSTDSPTSPTTEAPPLPPRNAGK
GPTGPPSTLPLSTQTSSGSSTLSKKRPPPPPPGHKRTLSDPPSPLPHGPPNKGAVPWGNDGGPSSSSKTTNKFEGLSQQS
STSSAKTALGPRVLPKLPQKVALRKTDHLSLDKATIPPEIFQKSSQLAELPQKPPPGDLPPKPTELAPKPQIGDLPPKPG
ELPPKPQLGDLPPKPQLSDLPPKPQMKDLPPKPQLGDLLAKSQTGDVSPKAQQPSEVTLKSHPLDLSPNVQSRDAIQKQA
SEDSNDLTPTLPETPVPLPRKINTGKNKVRRVKTIYDCQADNDDELTFIEGEVIIVTGEEDQEWWIGHIEGQPERKGVFP
VSFVHILSD

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	AA	pKa$_{COO-}$	pKa$_{NH3+}$	pK$_{RR}$	Nr
M	1	9,21		1	K	61	10,53		184	K	121	10,53		327	K	181	10,53		498	K	241	10,53		696	E	301	4,25		929
R	2	12,48		2	R	62	12,48		185	K	122	10,53		328	E	182	4,25		503	H	242	6		700	K	302	10,53		933
R	3	12,48		7	E	63	4,25		186	Y	123	10,07		330	K	183	10,53		508	H	243	6		702	D	303	3,65		938
R	4	12,48		14	H	64	6		187	K	124	10,53		333	D	184	3,65		517	E	244	4,25		704	K	304	10,53		942
D	5	3,65		15	K	65	10,53		189	K	125	10,53		334	E	185	4,25		520	Y	245	10,07		705	E	305	4,25		945
R	6	12,48		20	H	66	6		191	D	126	3,65		336	K	186	10,53		529	E	246	4,25		706	K	306	10,53		949
D	7	3,65		23	R	67	12,48		195	R	127	12,48		339	D	187	3,65		535	R	247	12,48		710	D	307	3,65		954
E	8	4,25		29	E	68	4,25		197	K	128	10,53		340	R	188	12,48		539	E	248	4,25		712	K	308	10,53		958
E	9	4,25		33	E	69	4,25		202	R	129	12,48		344	K	189	10,53		540	E	249	4,25		713	E	309	4,25		961
E	10	4,25		36	E	70	4,25		205	R	130	12,48		345	E	190	4,25		541	D	250	3,65		715	K	310	10,53		965
D	11	3,65		37	E	71	4,25		206	K	131	10,53		346	Y	191	10,07		542	E	251	4,25		716	D	311	3,65		970
Y	12	10,07		38	E	72	4,25		208	C	132	8,18		347	K	192	10,53		546	D	252	3,65		718	K	312	10,53		974
R	13	12,48		49	K	73	10,53		209	K	133	10,53		350	Y	193	10,07		547	D	253	3,65		719	D	313	3,65		979
H	14	6		51	E	74	4,25		210	H	134	6		358	D	194	3,65		549	D	254	3,65		720	K	314	10,53		983
C	15	8,18		53	R	75	12,48		211	R	135	12,48		363	H	195	6		550	D	255	3,65		722	K	315	10,53		987
R	16	12,48		54	R	76	12,48		212	K	136	10,53		367	R	196	12,48		551	D	256	3,65		723	D	316	3,65		988
E	17	4,25		61	C	77	8,18		219	C	137	8,18		373	R	197	12,48		554	K	257	10,53		724	K	317	10,53		992
E	18	4,25		62	E	78	4,25		220	K	138	10,53		376	K	198	10,53		555	K	258	10,53		729	D	318	3,65		997
D	19	3,65		65	Y	79	10,07		221	E	139	4,25		380	C	199	8,18		557	K	259	10,53		730	K	319	10,53		1001
D	20	3,65		67	K	80	10,53		224	D	140	3,65		381	K	200	10,53		563	E	260	4,25		731	D	320	3,65		1006
R	21	12,48		68	E	81	4,25		227	K	141	10,53		382	E	201	4,25		566	R	261	12,48		732	K	321	10,53		1010
K	22	10,53		73	K	82	10,53		229	K	142	10,53		383	E	202	4,25		569	R	262	12,48		735	E	322	4,25		1016
K	23	10,53		75	K	83	10,53		231	D	143	3,65		386	K	203	10,53		572	C	263	8,18		740	K	323	10,53		1020
K	24	10,53		76	K	84	10,53		232	H	144	6		390	R	204	12,48		574	H	264	6		741	H	324	6		1022
K	25	10,53		79	D	85	3,65		235	R	145	12,48		392	D	205	3,65		575	D	265	3,65		749	D	325	3,65		1025
Y	26	10,07		82	K	86	10,53		242	Y	146	10,07		394	Y	206	10,07		583	K	266	10,53		750	R	326	12,48		1033
D	27	3,65		87	Y	87	10,07		243	H	147	6		395	E	207	4,25		585	R	267	12,48		760	D	327	3,65		1034
H	28	6		88	Y	88	10,07		244	E	148	4,25		399	E	208	4,25		588	D	268	3,65		761	K	328	10,53		1038
E	29	4,25		92	H	89	6		245	D	149	3,65		400	E	209	4,25		591	K	269	10,53		762	E	329	4,25		1042
E	30	4,25		93	C	90	8,18		248	E	150	4,25		401	E	210	4,25		595	R	270	12,48		764	D	330	3,65		1043
Y	31	10,07		95	D	91	3,65		253	D	151	3,65		403	E	211	4,25		599	Y	271	10,07		767	D	331	3,65		1046
D	32	3,65		100	K	92	10,53		256	Y	152	10,07		404	E	212	4,25		602	D	272	3,65		781	E	332	4,25		1053
K	33	10,53		101	D	93	3,65		259	K	153	10,53		415	H	213	6		606	E	273	4,25		789	R	333	12,48		1060
R	34	12,48		109	K	94	10,53		260	E	154	4,25		416	R	214	12,48		610	R	274	12,48		796	K	334	10,53		1061
D	35	3,65		110	K	95	10,53		262	E	155	4,25		417	D	215	3,65		613	K	275	10,53		800	K	335	10,53		1066
D	36	3,65		113	Y	96	10,07		264	R	156	12,48		424	H	216	6		618	K	276	10,53		824	K	336	10,53		1068
K	37	10,53		120	E	97	4,25		266	E	157	4,25		426	D	217	3,65		621	K	277	10,53		825	R	337	12,48		1070
K	38	10,53		126	K	98	10,53		267	E	158	4,25		431	C	218	8,18		627	R	278	12,48		826	R	338	12,48		1071
E	39	4,25		127	D	99	3,65		271	E	159	4,25		435	D	219	3,65		631	H	279	6		834	K	339	10,53		1073
K	40	10,53		133	Y	100	10,07		273	D	160	3,65		436	K	220	10,53		632	K	280	10,53		835	Y	340	10,07		1076
H	41	6		141	K	101	10,53		276	K	161	10,53		439	H	221	6		642	R	281	12,48		836	D	341	3,65		1077
D	42	3,65		148	D	102	3,65		280	E	162	4,25		443	Y	222	10,07		643	D	282	3,65		840	C	342	8,18		1078
K	43	10,53		152	E	103	4,25		281	D	163	3,65		444	C	223	8,18		644	H	283	6		847	D	343	3,65		1081
D	44	3,65		154	E	104	4,25		282	R	164	12,48		447	Y	224	10,07		647	K	284	10,53		852	D	344	3,65		1083
K	45	10,53		156	K	105	10,53		283	D	165	3,65		452	K	225	10,53		649	D	285	3,65		860	D	345	3,65		1084
K	46	10,53		159	K	106	10,53		284	C	166	8,18		454	E	226	4,25		651	K	286	10,53		868	E	346	4,25		1085
D	47	3,65		161	R	107	12,48		290	C	167	8,18		455	C	227	8,18		652	K	287	10,53		872	E	347	4,25		1090
K	48	10,53		163	D	108	3,65		291	D	168	3,65		456	K	228	10,53		654	E	288	4,25		874	E	348	4,25		1092
K	49	10,53		164	K	109	10,53		294	C	169	8,18		457	R	229	12,48		658	K	289	10,53		886	E	349	4,25		1099
D	50	3,65		167	D	110	3,65		300	E	170	4,25		461	K	230	10,53		660	R	290	12,48		892	E	350	4,25		1100
K	51	10,53		168	K	111	10,53		302	C	171	8,18		474	D	231	3,65		664	K	291	10,53		896	D	351	3,65		1101
K	52	10,53		171	E	112	4,25		303	E	172	4,25		476	E	232	4,25		671	K	292	10,53		900	E	352	4,25		1103
D	53	3,65		172	D	113	3,65		304	C	173	8,18		477	D	233	3,65		675	R	293	12,48		904	H	353	6		1108
Y	54	10,07		173	R	114	12,48		308	H	174	6		481	K	234	10,53		678	K	294	10,53		905	E	354	4,25		1110
E	55	4,25		174	Y	115	10,07		312	R	175	12,48		482	R	235	12,48		679	D	295	3,65		907	E	355	4,25		1114

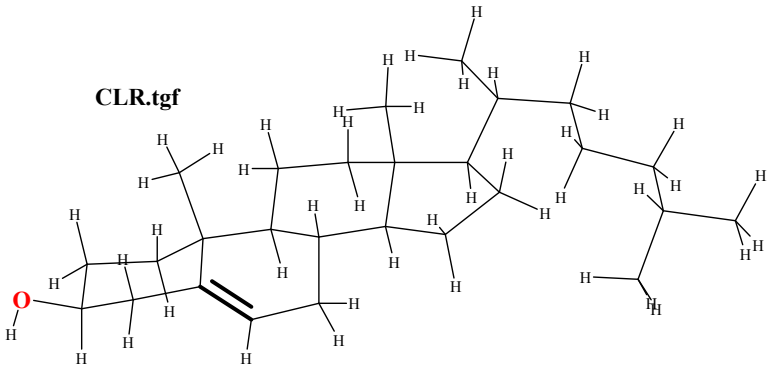
K	56		10,53	176	H	116	6	315	E	176		4,25	483	K	236		10,53	681	H	296		6	908	R		356		12,48	1115							
K	57		10,53	179	K	117	10,53	321	H	177		6	487	C	237		8,18	685	D	297		3,65	912	K		357		10,53	1116							
E	58		4,25	181	E	118	4,25	322	R	178		12,48	490	E	238		4,25	686	K	298		10,53	913	H		358		6	1125							
K	59		10,53	182	Y	119	10,07	323	E	179		4,25	495	D	239		3,65	687	E	299		4,25	919	D	1,88	359		3,65	1129							
E	60		4,25	183	E	120	4,25	326	D	180		3,65	497	K	240		10,53	693	K	300		10,53	923													

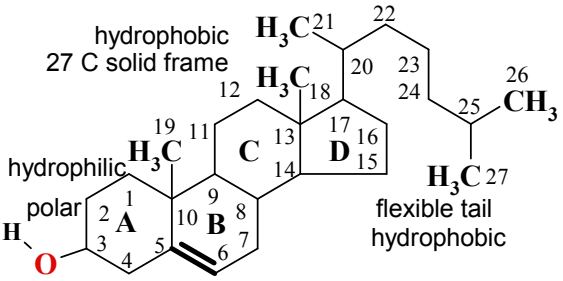
IEP=7,5906111= pK_{amean} ; sum=2732,62 ; 360 protolytic constants

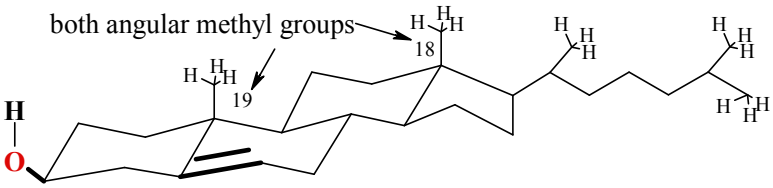
STARD4 5BRL, 1JSS.pdb**; STARD1,3 ,** 2I93,pdb, **membrane** interactions and **sterol** binding

The steroidogenic acute regulatory transfer (START) protein-related lipid binding domain family is defined by a conserved 210-amino acid sequence that folds into an α/ß helix-grip structure. Members of this protein family bind a variety of ligands, including cholesterol, phospholipids, sphingolipids, and bile acids, with putative roles in non vesicular lipid transport, metabolism, and cell signaling. STARD4 is expressed in most tissues as well has previously been shown to transfer sterol and membrane interaction sterol binding-uploader.

1. CLR.tgf -cholesterol put in given atoms, double bond, cycle symbols & amphiphatic property! Cholesterol steroids source!







Cholesterol four rings of the steroid are labeled **A**, **B**, **C** and **D**. Double bond between C5 and C6 atoms and alcohol **HO**- at C_3.**Cholesterol** is roughly planar with both angular methyl groups–$\mathbf{CH_3}$ labeled atoms C18 and C19 above the molecule plane.

2.Which 41 aliphatic and aromatic side chains residues interact within the cholesterol bound in pocket of STARD1 protein 3P0L.pdb?

Ala171.....,190.....,200.....,203.....,218.....,Gly145.....,201.....,221.....,
Leu122.....,124.....,137.....,138.....,199.....,227.....,243.....,247.....,251.....,260.....,271.....,275.....
Val126.....,151.....,156.....,178.....,179.....,256.....,Ile154.....,216.....,245.....,255.....,256.....,
Phe120.....,165.....,184.....,267.....,Trp147.....,241.....,250.....,His220.....,270.....,Tyr134.....

3. 44 aliphatic and aromatic side chains residues interact within the cholesterol bound in pocket of protein 2I93.pdb? Ala86.....,171.....,172.....,174.....,175.....,218.....,Gly145.....,201.....,221...
Leu122...,133...,137...,170...,178...,199....,227....,239....,243....,247....,251....,260....,271....,275....,
Val126.....,138.....,151.....,156.....,179.....,198.....,256.....,259.....,Ile154.....,245.....,256.....,
Phe120.....,165.....,184.....,267.....,Trp147.....,241.....,250.....,His220.....,270.....,Tyr134.....

4. Which 50 aliphatic and aromatic side chains residues interact within the cholesterol bound in pocket of STARD4 protein 1JSS.pdb? Val54.....,56.....,79.....,82.....,162.....,202.....,
Leu93.....,98.....,102.....,123.....,124.....,145.....,185.....,193.....,210.....,217.....,221....
Ile83.....,86.....,126.....,127.....,189.....,197....., Ala48....51....,71....,120.....,205.....,207.....,211.....,
Gly73.....,89.....,121.....,149.....,151.....,164.....,170.....,187.....,195.....,220.....,
Phe64.....,132.....,213.....,Trp95.....,171.....,His107.....,Tyr67.....,69.....,117.....,214.....,

5. Which 55 aliphatic and aromatic side chains residues interact within the cholesterol bound in pocket of STARD5 protein 2R55.pdb? Leu61....,110.....,163.....,172.....,180....,184....,189...,
Val36...,38...,57....,64....,68....,77....,83....,98....,117.....,120.....,122.....,149.....,188.....,189.....,
Ala134....., Gly35.....,48.....,53.....,59.....,73.....,74.....,85.....,147.....,151.....,157.....,184.....,

Ile56...,88...,89...,111.....,130.....,Phe46...,86...,116.....,176.....,192.....,193.....,200....,211....,214....,
Trp40.....,65.....,79.....,His136.....,212.....,Tyr51.....,58.....,201.....

6. What is defined conserved 210-amino acid sequence type folding structure? .....
.....alpha/beta helix-gripe

7. Indicate sequence numbers on alpha/beta helix-grip of STARD, 2I93,pdb? **H1**(69.....-91.....),
H2(129.....-139.....),**H3**(142.....-147.....),**H4**(231.....-233.....),**H5**(252.....-278.....).

8. Which beta strands constitute the U-shaped beta-barrel of STARD, 2I93.pdb molecule?
B1(97-101....),**B2**(107-113.....),**B3**(117-126.....),**B9**(237-243.....),
B8(223-229.....),**B7**(197-203.....),**B6**(182-192.....),**B5**(164-171.....),**B4**(151-160.....).

9. What three helixes near the N-and C-terminus are closed U-shaped beta-barrel?.....
H2(129.....-139.....), **H3**(142.....-147.....), **H5**(252.....-278.....).

10. Which alpha helix and which two loops enable the access of cholesterol to hydrophobic cavity by conformational changes in these molecule of STARD, 2I93.pdb?
H5(252.....-278.....), **L3**(174.....-180.....), **L2**(145.....-151.....).

11. Indicate beta-strands 7-6-5-4 including the 3,2-loops **L3** connecting beta5,6 , alpha3-helix in STARD, 2I93.pdb molecule **B7**197-203.....,**B6**182-192.....,
... **B5**164-171.....,**B4**151-160.....,**L3**174-180.....,**L2**145-151.....

12. What is distance in hydrogen bond cholesterol binding with the 3beta-hydroxyl group of cholesterol to Leu199 peptide bond carbonyl group Leu199>C=**O**...H-**O**-CLR in these molecule of STARD, 2I93.pdb?...distance 2,498 Å Angstroms (Ångströms).....

13. What five residues of STARD, 2I93.pdb are possible gate keepers in lipid ligand loading?
..piecas aminoskābes Trp96.....,Trp147.....,Arg217.....,Asp183.....,Asn148.....

14. What are five key residues in cholesterol binding. These side chains will likely change conformation upon ligand binding. Hydrogen bond between the cholesterol hydroxyl and either the Arg188 side chain or the backbone carbonyl of Leu199 as distance 2.498 Å Angstroms (Ångströms) measured in these molecule of STARD, 2I93.pdb? Ser186.....
...Glu169.....,Arg188.....,Leu199.....,His220.....

13. What mass fraction relates to phospholipids; cholesterol; inegral membrane proteins , ...if total sell membranes mass is 100%?

33.3....%.Phosfolipids in **membrane** constitute 1/3 part 33.3....% from membrane composite mass 100%

33.3....%.Cholesterol in **membrane** constitute 1/3 part 33.3....% from membrane composite mass 100%

33.3....%..Proteins in **membrane** constitute 1/3 part 33.3....% from membrane composite mass 100%

14. What fraction of membrane aquaporin proteins and for which purpose?

...60% mass fraction secure water $\mathbf{H_2O}$ and oxygen $\mathbf{O_{2}}_{aqua}$ osmosis in living organisms.....

15.1-15.5 STARD4 25.578 kDa isoelectric point IEP=pH=$pK_{a\text{-}vid}$ at physiologic pH=7,36 . Determine at solution pH with STARD4 concentration C=$10^{-7,0704}$ M ($^{mol}/_{Liter}$)!

http://aris.gusc.lv/ChemFiles/START/5BRLpIStudS.doc ; http://aris.gusc.lv/ChemFiles/START/5BRLpI.xls

```
     SQ SEQUENCE   224 >5BRL:A|PDBID|CHAIN|SEQUENCE                    STAR4_MOUSE
MADPESPWSQIGRKIKLEGLSDVASISTKLQNTLIQYHSIKEDEWRVAKKVKDVTVWRKPSEEFNGYLYKAQGVMDDVVN
NVIDHIRPGPWRLDWDRLMTSLDVLEHFEENCCVMRYTTAGQLLNIISPREFVDFSYTVGYEEGLLSCGVSVEWSETRPE
FVRGYNHPCGWFCVPLKDSPSQSLLTGYIQTDLRGMIPQSAVDTAMASTLANFYSDLRKGLRKA
```

AA	pKa_{COO^-}	pKa_{NH3^+}	pK_{RR}	Nr	AA	pKa_{COO^-}	pKa_{NH3^+}	pK_{RR}	Nr
M	1	9,21		1	H	39	6		107
D	2		3,65	3	E	40	4,25		109
E	3		4,25	5	E	41	4,25		110
R	4		12,48	13	C	42	8,18		112
K	5		10,53	14	C	43	8,18		113
								12,4	
K	6		10,53	16	R	44	8		116
								10,0	
E	7		4,25	18	Y	45	7		117

sum=574,18;73+2=75 ; 574,18 / 75 = 7,6496=IEP

ISOELEKTRIC POINT charge "0" Sum are calculate as

75 protolytic equilibria pK_a values in table is 574,18.....

$\Sigma pK_{aRside\ group} + pK_{aNterminus} + pK_{aCterminus}$ = 574,18.....

						12,4		
D	8	3,65	22	R	46	8		130
K	9	10,53	29	E	47	4,25		131
Y	10	10,07	37	D	48	3,65		134
						10,0		
H	11	6	38	Y	49	7		137
						10,0		
K	12	10,53	41	Y	50	7		141
E	13	4,25	42	E	51	4,25		142
D	14	3,65	43	E	52	4,25		143
E	15	4,25	44	C	53	8,18		148
R	16	12,48	46	E	54	4,25		153
K	17	10,53	49	E	55	4,25		156
						12,4		
K	18	10,53	50	R	56	8		158
K	19	10,53	52	E	57	4,25		160
						12,4		
D	20	3,65	53	R	58	8		163
						10,0		
R	21	12,48	58	Y	59	7		165
K	22	10,53	59	H	60	6		167
E	23	4,25	62	C	61	8,18		169
E	24	4,25	63	C	62	8,18		173
						10,5		
Y	25	10,07	67	K	63	3		177
Y	26	10,07	69	D	64	3,65		178
						10,0		
K	27	10,53	70	Y	65	7		188
D	28	3,65	76	D	66	3,65		192
						12,4		
D	29	3,65	77	R	67	8		194
D	30	3,65	84	D	68	3,65		203
						10,0		
H	31	6	85	Y	69	7		214
R	32	12,48	87	D	70	3,65		216
						12,4		
R	33	12,48	92	R	71	8		218
						10,5		
D	34	3,65	94	K	72	3		219
						12,4		
D	35	3,65	96	R	73	8		222
						10,5		
R	36	12,48	97	K	74	3		223
					2,3			
D	37	3,65	103	A	4	75		224
E	38	4,25	106					

Protolysis mean constant isoelectric point IEP=pKa_{mean} calculate as sum of constants: side chains $\Sigma pK_{aRside\ group}$, $pK_{aNterminus}NH_3^+$ and $pK_{aCterminus}COO^-$ divided with number of protolytic acid groups NpK_a: 75.....

$IEP=pK_a=(\Sigma pK_{aRside\ group}+pK_{aNterminus}+pK_{aCterminus})/NpK_a$

$pK_{mean}= pK_a = IEP = 574,18 / 75 = 7,6496$.....,

15.1 Acid groups number sum NpK_a=73.....+2.....=75..... of 2224 amino acids 73+2 of them;protolytic constants pK_a for side groups, N-terminus methionine M $pK_{aNterminus}$=9.21 and C-terminus alanine A $pKa_{Cterminus}$=2.34

15.2 Average acid group constant pK_{mean} calculates as IEP

ISOELEKTRIC POINTpK_{mean}= IEP = 574,18 / 75=**7,6496**.....

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 (-)!

15.3 Determine STARD4 molecule charge sign (+), zero „**0**” or (-) at physiologic pH=7.36 of blood plasma

Underline existing:

-COOH & **-NH_3^+** positive (+) charge ……. pH=7.36< IEP=**7,65**..... charge negative(-) **-COO^-** & **-NH_2**.

15.4 Determine STARD4 molecule charge sign (+), zero „**0**” or (-) at **electrophoresis** pH 8.8 on stripe

Underline existing:

-COOH & **-NH_3^+** positive (+) charge IEP=**7,65** < pH=8.8 electrophoresis charge negative(-) **-COO^-** & **-NH_2**.

Electrophoresis pH=8.8 show the movement towards positive electrode.

15.5 Calculate $10^{-7,0704}$ M ($^{mol}/_{Liter}$) STARD4 solution pH by *Ostwald dilution law* concentration C in logarithm:

$$pH=\frac{pK_a-\log C}{2}=\frac{7,6496-\log 10^{-7,0704}}{2}=\frac{7,6496+7,0704}{2}=\mathbf{14,72}\ /\ 2=7,36.....$$

7,36 Attractor mouse STARD4 concentration is $C=10^{-7,0794}$.....M .

16.1-16.5 Analyses of STARD1 isoelectric point IEP=pH=$pK_{a\text{-}vid}$ at physiologic pH=7,36 .
Determine at solution pH with STARD1 concentration C=$10^{-6,6743}$ M ($^{mol}/_{Liter}$)!

2I93.pdb Cilvēka STARD1 mitohondrijas 31,927 kDa koncentrācijā $10^{-6,6743}$ M
http://aris.gusc.lv/ChemFiles/START/2I93pIStudS.doc ; http://aris.gusc.lv/ChemFiles/START/2I93pI.xls

```
SQ SEQUENCE 285 >2I93:A|PDBID|CHAIN|SEQUENCE: STAR1+Cholesterol  67-280 (1-285)
MLLATFKLCAGSSYRHMRNMKGLRQQAVMAISQELNRRALGGPTPSTWINQVRRRSSLLGSRLEETLYSDQELAYLQQGE
EAMQKALGILSNQEGWKKESQQDNGDKVMSKVVPDVGKVFRLEVVVDQPMERLYEELVERMEAMGEWNPNVKEIKVLQKI
GKDTFITHELAAEAAGNLVGPRDFVSVRCAKRRGSTCVLAGMATDFGNMPEQKGVIRAEHGPTCMVLHPLAGSPSKTKLT
WLLSIDLKGWLPKSIINQVLSQTQVDFANHLRKRLESHPASEARC
```

AA		pKa_{COO-}	pKa_{NH3+}	pK_{RR}	Nr	AA		pKa_{COO-}	pKa_{NH3+}	pK_{RR}	Nr
M	1	9,21			1	E		45	4,25		139
K	2		10,53		7	R		46	12,48		140
C	3		8,18		9	E		47	4,25		142
Y	4		10,07		14	E		48	4,25		146
R	5		12,48		15	K		49	10,53		152
H	6		6		16	E		50	4,25		153
R	7		12,48		18	K		51	10,53		155
K	8		10,53		21	K		52	10,53		159
R	9		12,48		24	K		53	10,53		162
E	10		4,25		34	D		54	3,65		163
R	11		12,48		37	H		55	6		168
R	12		12,48		38	E		56	4,25		169
R	13		12,48		53	E		57	4,25		173
R	14		12,48		54	R		58	12,48		182
R	15		12,48		55	D		59	3,65		183
R	16		12,48		62	R		60	12,48		188
E	17		4,25		64	C		61	8,18		189
E	18		4,25		65	K		62	10,53		191
Y	19		10,07		68	R		63	12,48		192
S	20				69	R		64	12,48		193
D	21		3,65		70	C		65	8,18		197
E	22		4,25		72	D		66	3,65		205
Y	23		10,07		75	E		67	4,25		211
E	24		4,25		80	K		68	10,53		213
E	25		4,25		81	R		69	12,48		217
K	26		10,53		85	E		70	4,25		219
E	27		4,25		94	H		71	6		220
K	28		10,53		97	C		72	8,18		224
K	29		10,53		98	H		73	6		228
E	30		4,25		99	K		74	10,53		236
D	31		3,65		103	K		75	10,53		238
D	32		3,65		106	D		76	3,65		246
K	33		10,53		107	K		77	10,53		248
K	34		10,53		111	K		78	10,53		253
D	35		3,65		115	D		79	3,65		266
K	36		10,53		118	H		80	6		270
R	37		12,48		121	R		81	12,48		272
E	38		4,25		123	K		82	10,53		273
D	39		3,65		127	R		83	12,48		274
E	40		4,25		131	E		84	4,25		276
R	41		12,48		132	H		85	6		278
Y	42		10,07		134	E		86	4,25		282
E	43		4,25		135	R		87	12,48		284
							1,9				
E	44		4,25		136	C	6	88	8,18		285

IEP = pKa_{mean} = 8,0456818 ; summa = 708.02; 88 protolytic constants

ISOELEKTRIC POINT zero charge "0"

Sum are calculate as 88 protolytic equilibria pK_a values in table is

$\Sigma pK_{aRside\ group} + pK_{aNterminus} + pK_{aCterminus}$ = 708.02.....

Protolysis mean constant pK_a isoelectric point IEP=pK_{a_vid}
calculate as sum of constants: side chains $\Sigma pK_{aRside\ group}$,,
$pK_{aNterminus}NH_{3+}$ and $pK_{aCterminus}COO-$
divided with number of protolytic acid groups NpK_a:

Protolytic mean constant pKa_{mean} isoelectric point IEP calculate
as sum of constant side chains $\Sigma pK_{aRside\ group}$,,
$pK_{aNterminus}NH_3$ and $pK_{aCterminus}COO-$
divided with number of protolytic acid groups NpK_a:

$IEP=pK_a=(\Sigma pK_{aRside\ group}+pK_{aNterminus}+pK_{aCterminus})/NpK_a$

$pK_{mean}= pK_a$ = IEP = 708.02 / 88=8.0456818.....,

16.1 Acid groups number sum NpK_a=86.....+2.....=88.....
of 445 amino acids 42+2 of them
protolytic constants pK_a for side groups,
N-terminus methionine M $pK_{aNterminus}$=9.21 and
C-termināl cysteine C $pKa_{Cterminus}$=1.96

16.2 Average acid group constant **ISOELEKTRIC POINT**
pKa_{mean}=IEP = = 708.02 / 88=**8.0456818**.....
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^+** is negative **-COO^-**&**-NH_2**
Underline and determine existing: positive (+) or zero or negative (-)!

16.3 Determine STARTD1 molecule charge sign (+), zero „**0**" or (-) at physiologic pH=7.36
Underline existing:
-COOH & **-NH_3^+** positive (+) charge ……. pH=7.36 < IEP=**8.05** ..... charge negative(-) **-COO^-** & **-NH_2**.

16.4 Determine START-HUMAN molecule charge sign (+), zero „**0**" or (-) at **electrophoresis** pH **8.8** on stripe
Underline existing:
-COOH & **-NH_3^+** positive (+) charge …IEP=**8.05**< pH=8.8 electrophoresis charge negative(-) **-COO^-** & **-NH_2**.

16.5 Calculate C=$10^{-6,6743}$ M(mol/Liter) START solution pH by *Ostwald dilution law* concentration C logarithm:

$$pH=\frac{pK_a-\log C}{2}=\frac{8,0456818-\log 10^{-6,6743182}}{2}=\frac{8,0456818+6,6743182}{2}$$ = **14,72** / 2 = 7,36.....

7,36 Attractor human STARD1 concentration is C=$10^{-6,6743}$.....M .
Trp147 is located in a helical loop region as Trp147 interacts with the C-terminal helix

H5 (253-280) Leu271,275,Arg272,274. Binding mechanism via local unfolding or a significant conformational change in the C-terminal helix **H5** could be STARD family wide phenomenon. Five amino acids unfolding C-terminal helix binds Trp96,Trp147,Arg217,Asp183,Asn148.... Trp96,Asp183,Arg217 are all on the 'back' face of the beta-sheet. Trp147 is absolutely conserved across the family, and since the Trp147 side chain interacts with hydrophobic residues of the C-terminal helix in all the structures, this region is likely important for lipid access to the cavity due to flexibility and hydrophobic nature.
Mutation in the adjacent to Trp147, highly conserved residue Asn148 has been observed in congenital lipoid adrenal hyperplasia (lipoid CAH) [15].

Cholesterol components in eukaryote cells (human cells) plasma membranes 7-fold greater ~35% as total lipid amount and strongly increases in ERC endocytic recycling compartments. Comparing with endoplasmic reticulum (ER) just ~5% of total lipid amount, however in ER organelles cholesterol is synthesized.

Protein families classified as lipid transporters transfer lipids from membranes to other membrane. StAR proteins (START steroidogenic (or steroid-metabolizing enzymes) acute regulatory transfer protein) domains familie STARD.

Cellular 11-cis-retinaldehyde-binding protein domain CRALBP

3HY5.pdb mutant R234W molecule, 3HX3.pdb within R234 molecule, 1XGH.pdb molecule **rhodopsin** 1U19 **11-cis-retinylidene**, **bathorhodopsin** 2G87all **trans-retinylidene**.....
Cellular retinaldehyde-binding protein (**CRALBP**) is essential for mammalian vision by routing 11-cis-retinoids for the conversion of photobleached opsin molecules into photosensitive visual pigments. The arginine-to-tryptophan missense mutation in position 234 (R234W) in the human gene RLBP1 encoding **CRALBP** compromises visual pigment regeneration and is associated with Bothnia dystrophy - Night blindness occurs.

1. Call cellular retinaldehyde-binding protein 3HY5.pdb conserved 317 sequence folding type structure at C-terminus core domain? alpha/beta/alpha..... helix-grip domain in ten 10 helices **H7**...,**H8**...,**H9**...,**H10**....,**H11**....,**H12**....,**H13**....,**H14**....,**H15**....,**H16**...., comprises β-sheet..... β1-β5 with 1 anti parallel and 4 parallel.beta strands

2. Which are two helices packed against the concave face (ielekta virsma, struktūra) over core of the C-terminal alpha/beta/alpha domain?....**H6** alfa6..... un **H7** alfa7.....

3. Which are four helices packed against the convex (izliekta) face of the sheet in core of the .....C-terminal alpha/beta/alpha domain? **H9**.....,**H10**.....,**H11**.....,**H12** alpha9–alpha12.....

4. Which three helices and loop are third structural motif, formed between the aperiodic segment N-terminus residues and C terminus residues in 3HY5.pdb? helix **H1**..., loop. **L**(23.....- 42.....) with helices **H13** alpha13....., **H14** alpha14.....

5. Which secondary structure units delimited by the convex side of the alpha/beta/alpha ....C-terminal core αβα domain of 3HY5.pdb? beta-sheet..... and the 6 adjacent helices **H7**.....,**H8**.....,**H9**.....,**H10**.....,**H11**.....,**H12**.....

6. What four helixes govern C-terminal domain core and N-terminal α domain inter domain contacts by hydrophobic interactions between two helixes of the N-terminal α domain and two of the C-terminal αβα domain of 3HY5? N-terminal α domain **H4**.....,**H6**,α4,6 helix..... and of the C-terminal αβα domain **H7**.....,**H8** α7,8 helices.....

7. Which four amino acid side chains with two hydrogen bonds of helixes **H4**,**H6**,**H7**,**H8** control inter domain contact between helix alpha6-helix alpha8 and between helix α6, α10 3HY5.pdb?

... between helix alpha6 and helix alpha8 Tyr117.....,Glu185..... and

.....between helix alpha6, alpha10 Tyr124.....,Asp225.....

8. Which three amino acids in side chain flips consequent the side-chain of Ile238 C delta methyl group rotation into the retinal-binding cavity. This rotation is the only significant structural alteration in the R234W mutant retinal-binding pocket 3HY5.pdb not in R234 3HX3.pdb?.....
..Phe198.....,Phe235.....,Ile238.....Cdelta methyl group is rotated in mutant R234W.

9. Which 26 amino acid residues are forming hydrophobic retinal-binding pocket? Trp166.....,

..Leu177,215,220,227,258,262,263.....,....., ..., ..., ..., ..., ..., Ala212.....,

..Phe161,173,204,207,240,247, ..., ..., ..., ..., ..., ..., Met223.....,

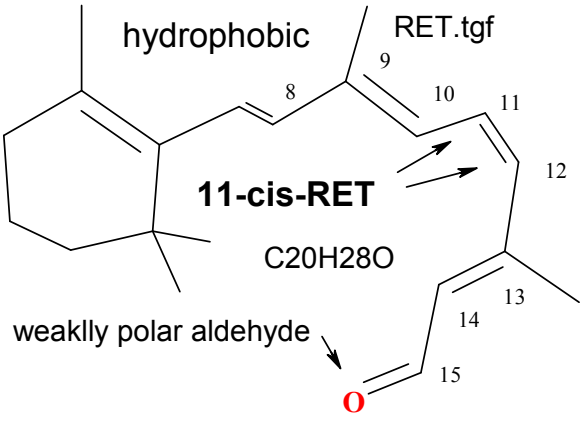
..Ile163,176,238,241, ..., ..., ..., ..., Val224,254,266,268....., ..., ..., ...,

..Pro145,244....., ...,C delta methyl group is rotated in mutant R234W 3HY5.pdb.....

10. Which three helices of the N-terminal domain, three last C-terminal helices and the three helical gate helices with beta-strands in yellow are indicated in molecule 3HY5.pdb? .....
The position of R234 is indicated cpk sphere, the 11-cis-retinal ligand is shown as cpk dots, and the cavity surface in the Retinoid-Binding Pocket is11-cis-retinal sequestered completely from bulk water solvent. The pocket volumes were calculated and rolling probe with radius of 1.0 Å. volume is 6.45×10^{2}Å^{3}.?..... N-terminal **H1**,**H2**,**H3**, ...

.... C-terminal **H14**,**H15**,**H16**; helical gate **H11**,**H12**,**H13**;.....

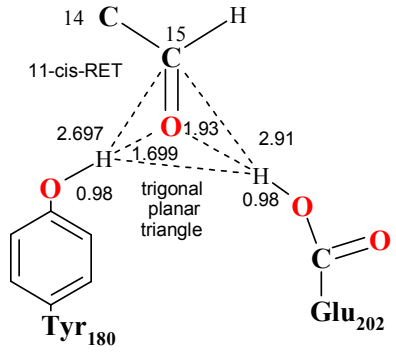
11. Cellular 11-cis-retinaldehyde put in given atom and amphipathic properties!



12. The pocket volumes were calculated by rolling probe with radius of 1.0 Å.....
....What is the appreciated cavity surface volume in the 11-cis-retinal Retinoid-Binding Pocket is sequestered completely from bulk water solvent in hydrophobic cavity of molecule 3HY5.pdb?
.....volume is 6.45×10^{2}Å^{3}.....

13. What two amino acid residues stacked the alpha, beta unsaturated retinal aldehyde between the phenyl ring and the sulfur atom in molecule 3HY5.pdb? .
sulfur atom of Met223..... phenyl ring of Phe161.....

14. What 2 amino acids serves as hydrogen bond donors to the aldehyde carbonyl oxygen and measure the distance between oxygen atoms >C=**O**...H-**O**- in 3HY5.pdb? d=2.697.....Å for Tyr180,.....
d=2.91..... Å for Glu202.....



14a. Put in two hydrogen bonds trigonal planar geometry for retinal carbonyl group >C=**O** as acceptor >C=**O**...H-**O**- with 2 hydrogen donor side chain hydroxyl: H-**O**-Tyr180 and H-**O**-(C=**O**)-Glu202 in molecule 3HY5.pdb?

15. 13 amino acid residues are fixing the beta-ionone ring and the polyene chain by van der Waals interactions with the apolar amino acids of helixes **H7**,**H8**,**H9**,**H11**,**H12** in 3HY5.pdb?
Ile163....., Trp166....., Phe173....., Leu215....., Leu220....., Val224....., Leu227.....,

Ile238....., Phe240....., Phe247....., Tyr251....., Val254....., Leu258.....,

16. Which 3 amino acid residues are connected by network of hydrogen bonds 2.984 Å, 2.959 Å, and 3.367 Å to alpha8 and to the carbonyl oxygen on beta 2 strand in molecule 3HY5.pdb?...

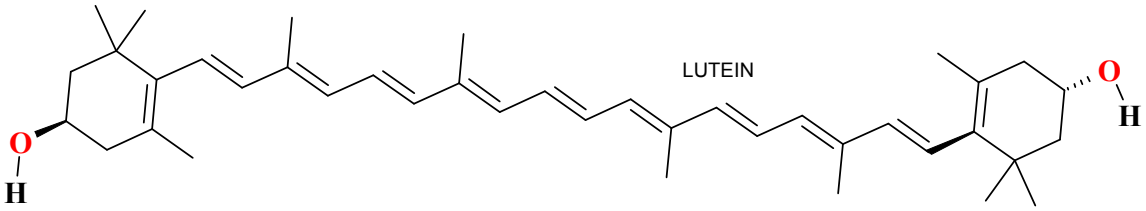
2.984 Å, 2.959 Å, and 3.367 Å to Gly155....., Thr193..... un Asn190.....

17. What 6 adjacent helixes represent central building block of the CRAL-TRIO fold as defined one wall of the 11-cis-retinal-binding cavity. in molecule 3HY5.pdb?.....

...**H6**.....,**H7**.....,**H8**.....,**H9**.....,**H10**.....,**H11**.....

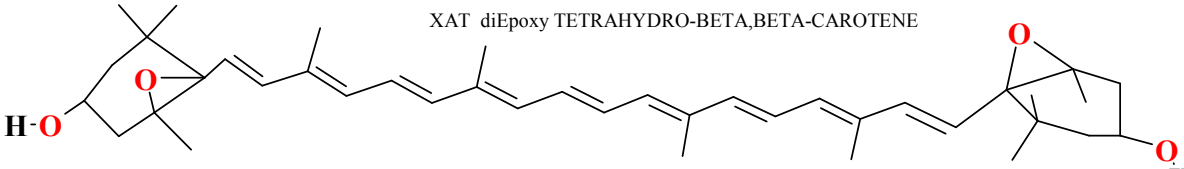
Carotenoid-binding proteins Structure of human StARD3 with lutein-binding domain 5I9J.pdb

1. Put in luteine LUT.pdb and di epoxy beta carotine XAT.pdb oxygen atoms?.



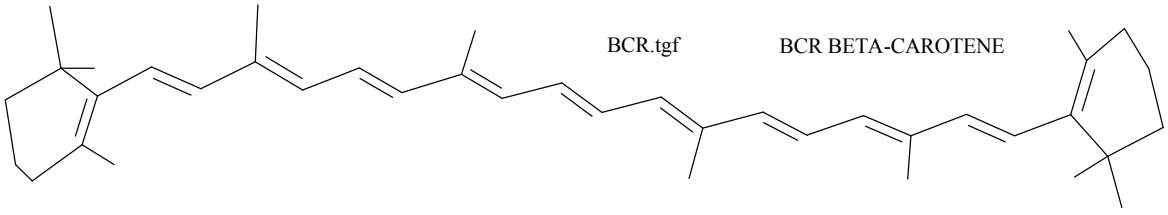
LUT.pdb.....

XAT diEpoxy tetra hydro-beta-carotene



XAT.pdb....

2. Draw the beta carotine BCR.pdb? .....BCR beta carotine.....



BCR.pdb.....

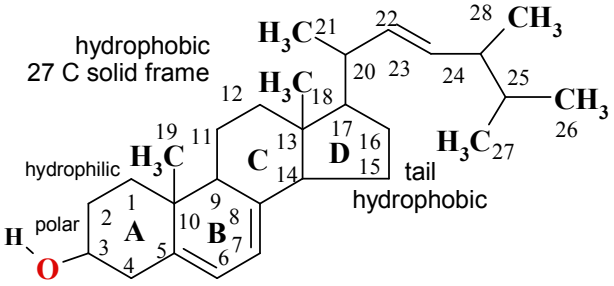
. Carotenoid-binding proteins. These proteins have sequence homology to the N-terminal domain (NTD) of the Orange Carotenoid Protein (OCP), and are referred to as Helical Carotenoid Proteins (HCPs). These proteins comprise at least nine distinct clades and are found in diverse organisms, frequently as multiple paralogs representing the distinct clades. These seem to be out-paralogs maintained from ancient duplications associated with subfunctionalization. All of the HCPs share conservation of the residues for carotenoid binding, and we confirm that carotenoid binding is a fundamental property of HCPs. We solved two crystal structures of the Nostoc sp. PCC 7120 HCP1 protein, each binding a different carotenoid, suggesting that the proteins flexibly bind a range of carotenoids.

Pigment-protein and pigment-pigment interactions are of fundamental importance to the light-harvesting and photoprotective functions essential to oxygenic photosynthesis. The orange carotenoid protein (OCP) functions as both a sensor of light and effector of photoprotective energy dissipation in cyanobacteria. We report the atomic-resolution structure of an active form of the OCP consisting of the N-terminal domain and a single noncovalently bound carotenoid pigment.

Ergosterol-cholesterol oxysterols / phosphatidylinositol 4-phosphate PI(4)P
inter membrane exchanger **Osh/Orp proteins** 2AIB.pdb,1ZHZ.pdb,4FES.pdb,3SPW.pdb,1ZHW.pdb

Oxysterols are oxidized derivatives of cholesterol that by enzymatic pathways involve cytochrome P450 enzymes (e.g. CYP27A1, CYP7A) and non-enzymatic involve action of reactive oxygen and nitrogen species. Oxysterols regulate cholesterol biosynthesis and are intermediates in the synthetic pathway of the bile acids as well steroid hormones, in cell development and differentiation, and cytotoxic and pro-apoptoic processes as well pro-inflammatory signaling pathway. Pathogenic effects of some oxysterols (e.g. cholesterol-5,6-epoxide, 7-ß-hydroxycholesterol) have been described in various diseases such as cardiovascular diseases, osteoporosis, Alzheimer's disease, and cancer.

1. Ergosterol draw, to indicate amphiphatic property in Elicitins 2AIB.pdb, 1ZHZ.pdb,4FES.pdb!



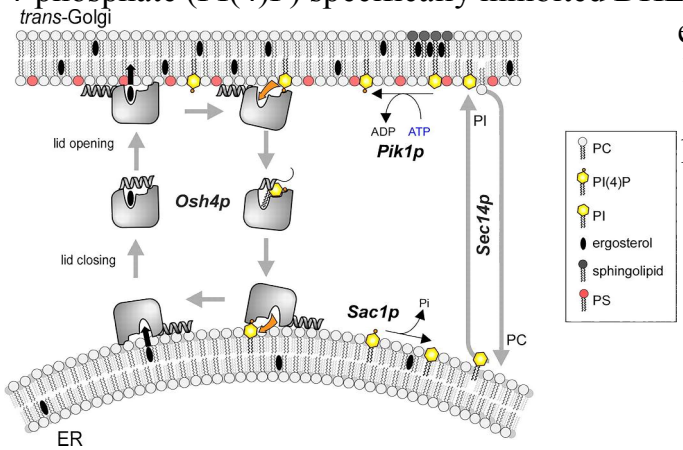
2. Check and to indicate the helix number by identifier and residue ranges for Elicitin 2AIB.pdb!.....
4-16,17-20,21-32,43-53,53-66,84-97...
H1...,H2...,H3...,H4...,H5...,H6.....

3. Check and to indicate the helix number by identifier ranges for Elicitin 1ZHZ.pdb!.....
0-4,7-19,23-27,30-32,40-47,49-55,56-59,63-68,76-105,255-263,293-295,304-308,312-327,328-354,382-392,406-411,422-428.....
H1..,H2..,H3..,H4..,H5..,H6..,H7..,H8..,H9..,H10..,H11..,H12..,H13..,H14..,H15..H16..,H17..

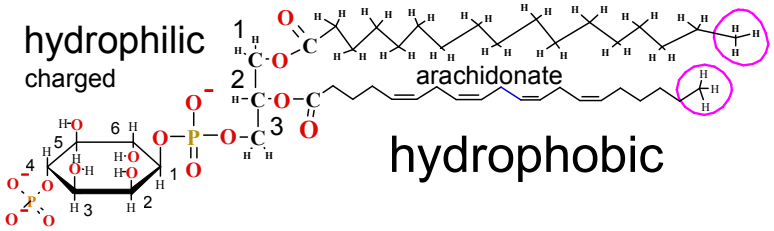
4. Check and to indicate the helix number by identifier ranges for Elicitin 1ZHy.pdb!.....
0-4,7-18,23-27,30-32,40-47,49-59,63-68,76-105,255-263,304-308,312-317,317-327,328-354,382-392,406-411,422-428.....
H1..,H2..,H3..,H4..,H5..,H6..,H7..,H8..,H9..,H10..,H11..,H12..,H13..,H14..,H15..H16...

5. Check and to indicate the helix number by identifier ranges for Elicitin 4FES.pdb!.....
0-5,7-19,23-27,30-32,40-47,49-55,56-59,63-67,76-105,255-263,293-295,304-308,312-326,328-353,382-392,406-411,422-428.....
H1..,H2..,H3..,H4..,H5..,H6..,H7..,H8..,H9..,H10..,H11..,H12..,H13..,H14..,H15..H16..,H17..

Osh/Orp link proteins transport sterols between organelles and are involved in phosphoinositide metabolism. The influence of membrane composition on the ability of Osh4p/Kes1p to extract, deliver, or transport dehydroergosterol (DHE). Phosphatidylinositol 4-phosphate (PI(4)P) specifically inhibited DHE extraction because PI(4)P was itself efficiently extracted by Osh4p. Osh4p selectively substitutes PI(4)P for sterol. Osh4p quickly exchanges DHE for PI(4)P and, thereby, can transport these two lipids between membranes along opposite routes. Osh4p transports sterol from the ER to late compartments pinpointed by PI(4)P and, in turn, transports PI(4)P backward. Coupled to PI(4)P metabolism, this transport cycle would create sterol gradients. Because the residues that recognize PI(4)P are conserved in Osh4p homologues, other Osh/Orp are potential sterol/phosphoinositol phosphate exchangers. In eukaryotic cells, sterols reside in all compartments but are unevenly distributed. Cholesterol (ergosterol in yeast) is synthesized in the ER, but is rare in this compartment and more concentrated at the trans Golgi, endosomes, and the plasma membrane (PM). Suppression of the entire Osh family is lethal, depriving the PM in ergosterol and stopping endocytosis. Conversely, expressing any of the seven Osh restores cell viability.



6. Phosphatidylinositol 4-phosphate (PI(4)P) draw, to indicate amphiphatic property of Elicitins .....3SPW.pdb! …….. heptadecanoate C17 and C20



7. Check and to indicate the 16 helix number identifier ranges for Elicitin 3SPW.pdb!...

0-4,7-18,23-27,30-32,40-47,49-59,63-68,76-105,255-263,304-308,312-317,317-327,328-354,382-392,406-411,422-428.....

H1..,**H2**..,**H3**..,**H4**..,**H5**..,**H6**..,**H7**..,**H8**..,**H9**..,**H10**..,**H11**..,**H12**..,**H13**..,**H14**..,**H15**..**H16**...

8.1-8.5 Analyses of 1ZHZ.pdb KES1 isoelectric point IEP=pH=$pK_{a\text{-}vid}$ at physiologic pH=7,36 . Determine at solution pH with KES1 concentration C=$10^{-7,286}$ M ($^{mol}/_{Liter}$)!

1ZHZ,3SPW.pdb KES1_YEAST oxysterol-binding protein 4 homolog 49,493 kDa koncentrācijā $10^{-7,286}$ 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_{amean} 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

8.1 Acid groups number in 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.....

8.2 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 (-)!

8.3 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**.

8.4 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**.

8.5 Calculate C = $10^{-7,0794}$ moli / Litrā 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 .

9. Check and to indicate the 17 helix number by identifier ranges for Elicitin 1ZHW.pdb!.....
0-4,7-18,23-27,30-32,40-47,49-55,56-59,63-67,76-105,255-263,282-286,304-308,312-327,328-354,382-392,406-411,422-428.....
H1..,**H2**..,**H3**..,**H4**..,**H5**..,**H6**..,**H7**..,**H8**..,**H9**..,**H10**..,**H11**..,**H12**..,**H13**..,**H14**..,**H15**..**H16**..,**H17**..

Osh/Orp structure consists of a beta-barrel forming a sterol-binding pocket. When Osh4p is empty, the N-terminal segment 29 amino-acids is unfolded and leaves the pocket open. Upon sterol binding, this segment forms a lid that blocks the sterol molecule in the pocket. Osh4p adapted to transport sterols and move sterol between membranes. All Osh proteins of the human oxysterol-binding protein-related protein contain a sterol-binding domain akin to Osh4p. The sterol transport activity of Osh4p should have general implications. To properly allocate sterol in the cell, Osh/Orp must ensure a one-way transport of sterol between defined compartments. This way to enrich one membrane with sterol at the expense of another. Such transport must involve transient and sequential interactions between the protein, the sterol molecule, and two lipid bilayers, one acting as a donor membrane and one as an acceptor. Osh4p adopts two different structures depending on its empty or sterol-bound state, and this is likely critical for proper protein targeting to donor and acceptor membranes during a cycle of transport. Osh4p has to transport sterol from the PM to the ER. Sterol uptake is facilitated by phosphatidylinositol (PI) 4,5 bisphosphate PI(4,5)P2, PM cytosole side lipid. The level of ergosterol at the PM increases upon Osh4p expression. Osh4p has a specific function at the Golgi, and that this function depends on another phosphoinositide, PI 4-phosphate PI(4)P, present at the trans side. Osh4p is the only Osh whose expression is lethal in Sec14p-deficient yeast. Sec14p is a key protein, which maintains a lipid composition permissive for vesicular transport. In the absence of Sec14p, the amount of PI(4)P is limiting. Osh4p, whose endogenous expression is high, monopolizes all remaining PI(4)P molecules at the expense of essential proteins of vesicular transport. Osh4p could distinguish a PI(4)P- from a PI(4,5)P2-containing membrane. No link has been found between PI(4)P and sterol transport by Osh4p. Osh4p acts as a sterol/PI(4)P exchanger. Osh4p quickly extracts and solubilizes the fluorescent ergosterol dehydroergosterol (DHE) from neutral liposomes.

Lipid transfer proteins selectively convey lipids between membranes is crucial for understanding how organelles maintain their composition (Lev, 2010). Osh4p to transport sterol from the PM, marked by PI(4,5)P2, to the ER (Raychaudhuri et al., 2006). Osh4p physically exchanges sterols for PI(4)P between membranes. The distribution of sterol may be controlled by phosphoinositide metabolism.

Osh4p efficiently solubilizes PI(4)P from membranes and uses roughly the same binding site as for sterol extraction. The structure of Osh4p is bound to PI(4)P. First, the sterol-binding pocket accommodates the PI(4)P acyl chains. Second, a shallow pocket at the entrance of the tunnel contains critical residues such as K336 and the H143/H144 pair, which selects the polar head of PI(4)P with high specificity. This interaction is essential for compensating loose binding of the PI(4)P acyl chains. Third, the N-terminal lid secures the bound PI(4)P molecule by wrapping its glycerol moiety. Osh4p displays a similar affinity for PI(4)P and sterols.

Our kinetic analysis suggests that Osh4p is designed to promote rapid exchange of sterols for PI(4)P between membranes according to the cycle shown in Figure. In this scheme, sterol release in the acceptor membrane is followed by PI(4)P extraction; conversely, PI(4)P release in the donor membrane precedes sterol extraction.

Osh4p overexpression reduces both the cellular level of PI(4)P and the Golgi targeting of a PI(4)P reporter (Fairn et al., 2007). Explained by retrograde transport of PI(4)P from the Golgi to the ER by Osh4p, where PI(4)P is metabolized by Sac1p, an ER-resident PI(4)P-phosphatase, as well alternative explanation for the interplay between Osh4p and Sac1p. Osh proteins to facilitate the hydrolysis of PI(4)P in "trans" by Sac1p by forming membrane contact sites between organelles (Stefan et al., 2011). This mechanism is appealing in the case of Osh proteins with additional domains that can effectively bridge two membranes. However, Osh4p lacks such domains and efficiently exchanges sterol for PI(4)P between liposomes without tethering them. Osh4p supplies the ER with PI(4)P, which is then hydrolyzed by Sac1p.

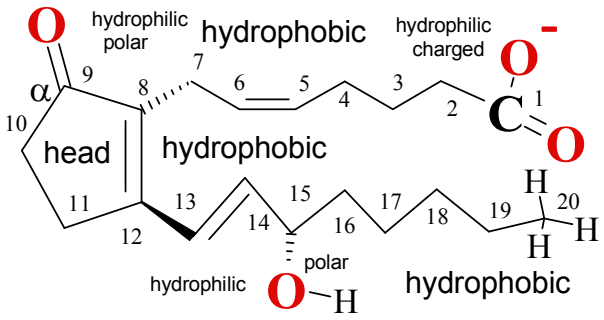
Phosphoinositide cycle along opposite routes in which Sec14p and Osh4p transport PI and PI(4)P. PI(4)P being converted into PI at the ER by Sac1p and PI being phosphorylated into PI(4)P at the trans Golgi by Pik1p (Fig.). When considered from the sole "point of view" of phosphoinositides, this cycle seems futile: ATP is used to continually regenerate PI(4)P. However, from a sterol-centric point of view, the consumption of ATP by Pik1p ensures net transfer of sterol in the forward direction and PC in the backward direction. As such, Osh4p could drive sterols up a concentration gradient and promote sterol enrichment of membranes of the late secretory pathway (TGN, PM) at the expense of the ER, where sterol is synthesized.

Notably, the mechanism by which Osh4p targets the ER remains to be addressed. Osh4p quickly (t1/2 of ~7 s) and totally solubilizes DHE from neutral (DOPC) liposomes under conditions where DHE was present at only 0.5 mol%. Thus, Osh4p should be able to efficiently pick up sterol from the ER, wherein this lipid is rare. Interestingly, we found previously that the lid of Osh4p corresponds to an ALPS motif (Drin et al., 2007). ALPS are unstructured sequences that fold into amphipathic helices upon binding to neutral membranes whose composition and high curvature result in loose lipid packing. The ER, which is characterized by a low content of anionic lipids, a high content of unsaturated lipids, and a highly tubulated structure, seems well adapted to the transient adsorption of the Osh4p ALPS/lid region. The membrane curvature. Extreme curvature and high electrostatic favors Osh4p retention on the liposomes, impairs lipid transport, and causes membrane aggregation through the multiple potential membrane-binding sites of Osh4p (ALPS motif, basic patches).

Most of residues that contact the PI(4)P headgroup are conserved in Osh/Orp proteins, which suggests that extraction of PI(4)P is a general hallmark of this family. Determining how this biochemical feature translates to a function will require further investigation for each Osh/Orp.

Wheat **nonspecific lipid transfer protein** with **prostaglandin B2** 1CZ3.pdb

1. Prostaglandin B2 draw, to indicate amphiphatic property in 1CZ2!



2. Check and to indicate the helix number by identifier and residue ranges for 1CZ3.pdb!..... 4-20,24-38,40-54,62-68,69-74... **H1**...,**H2**...,**H3**...,**H4**...,**H5**.....

3. What 4 aliphatic amino acids are on helix **H1** hydrophobic pocket-cavity for PGB2 in 1CZ3.pdb? ...Val6.....,Leu9.....,Val10.....,Leu14.....,Val17..... in helix **H1**.....

4. What 3 aliphatic amino acids are on helix **H2** hydrophobic pocket-cavity for PGB2 in 1CZ3.pdb? ....Val31.....,Leu34.....,Ala38..... in **H2**

5. What 4 aliphatic amino acids are on helix **H3** hydrophobic pocket-cavity for PGB2 in 1CZ3.pdb? ....Ala47.....,Leu51.....,Ile54.....,Ala55..... in **H3**

6. What 3 amino acids are on L3 and helix **H4** hydrophobic pocket-cavity for PGB2 in molecule 1CZ3.pdb? .....cilpā L3 Leu61....., un Ala66.....,Ile69..... in **H4**

7. What 3 amino acids are in the C-terminal region hydrophobic pocket-cavity for PGB2 in 1CZ3.pdb? .....Leu77.....,Ile81.....,Leu83..... C-terminal region .....

8. What 6 amino acids stick from parallel **H2** and **H5**-loop helixes in the C-terminal region hydrophobic pocket-cavity for PGB2 in1CZ3.pdb?..... parallel to **H2** helix of the **H5**-loop..... in the C-terminus Cys73.....,Gly74.....,Val75.....,Asn76.....,Leu77.....,Pro78.....

9. What 6 amino acids are fixing the PGB2 ring close to the centre of **H1** and **H3** helixes hydrophobic pocket-cavity for PGB2 in1CZ3.pdb?..... The PGB2 ring is close to the centre..... ... of **H1** and **H3** helixes Val6,Asp7,Val10,Cys50,Leu51,Leu54 .....

10. What is distance in hydrogen bonding of PGB2 with the hydrogen bond Tyr79-**O** –H and ... ˉ**O**-**O**=**C**<PGB2 carboxy group in hydrophobic pocket-cavity for PGB2 in1CZ3.pdb? ....Tyr79-O-H.....ˉ**O**-**O**=C<PGB2 distance in Angstroms (Ångströms) d=2.502 Å.....

11. What 8 amino acids four disulfide bonds in molecule 1CZ3.pdb?..... Cys3...,,Cys13...,,Cys27...,,Cys28...,,Cys48...,,Cys50...,,Cys73...,,Cys87...

12.1-12.5 Analyses of NLTP1 isoelectric point IEP=pH=$pK_{a\text{-}vid}$ at physiologic pH=7,36 .
Determine at solution pH with NLTP1_WHEAT concentration C=$10^{-6,58}$ M ($^{mol}/_{Liter}$)!

1CZ2.pdb NLTP1_WHEAT Non-specific lipid-transfer protein 11,9049 kDa koncentrācijā $10^{-6,58}$ M

http://aris.gusc.lv/ChemFiles/START/1CZ2pIStudS.doc ; http://aris.gusc.lv/ChemFiles/START/1CZ2pI.xls

```
SQ SEQUENCE  113 >1CZ2:A|PDBID|CHAIN|SEQUENCE                    NLTP1_WHEAT
 AQVMLMAVALVLMLAAVPRAAVAIDCGHVDSLVRPCLSYVQGGPGPSGQCCDGVKNLHNQARSQSDRQSACNCLKGIARG
 IHNLNEDNARSIPPKCGVNLPYTISLNIDCSRV
```

AA	pKa_{COO^-}	pKa_{NH3^+}	pK_{RR}	Nr	AA	pKa_{COO^-}	pKa_{NH3^+}	pK_{RR}	Nr
A 1	9,6	9		1	A 1	9,6	9		1
R2	12,4	8	19	**1**	R 2	12,4	8	19	**1**
D3	3,65	8	25	**2**	D 3	3,65	8	25	**2**
C4	**8,18**	**8,18**	**26**	**1**	H 4	6	6	28	**2**
H5	6	8	28	**2**	D 5	3,65	12,4	30	**2**
D6	3,65	12,4	30	**2**	R 6	8	10,0	34	**2**
R7	8	4,25	34	**2**	Y 7	7		39	**2**
C8	**8,18**	**8,18**	**36**	**2**	D 8	3,65	10,5	52	**2**
Y9	7		39	**2**	K 9	3		55	**2**
C0	**8,18**	**8,18**	**50**	**2**	H 10	6	12,4	58	**2**
C1	**8,18**	**8,18**	**51**	**2**	R 11	8		62	**2**
D2	3,65	10,5	52	**2**	D 12	3,65		66	**2**
K3	3		55	**3**					
H4	6	12,4	58	**3**					
R5	8		62	**3**					
D6	3,65		66	**3**					

AA	pKa_{COO^-}	pKa_{NH3^+}	pK_{RR}	Nr
R	12,4	8	13	67
K	10,5	3	14	75
R	12,4	8	15	79
H	6	6	16	82
E	4,25	17		86
D	3,65	12,4	18	87
R	8	10,5	19	90
K	3	10,0	20	95
Y	7		21	10
D	3,65	12,4	22	9
R	8		23	11
V	2,3	2	24	2
				3

IEP=8,1465625 ;
sum=260,69 ; 32 protolytic constants

8 cysteine forms 4 disulfide bonds
Cys 26,36,50,51,71,73,96,110

IEP=8,1354167 ;
Sum =195,25 ; 24 protolytic constants
AA 1-113 NLTP1_WHEAT
Cys26,Cys36,Cys50,Cys51,Cys71,Cys73,Cys96,Cys110

1CZ2.pdb -23=> 24-113 => 1-90

Cys3,Cys13,Cys27,Cys28,Cys48,Cys50,Cys73,Cys87

IEP = pKa_{mean} = 8,13542 ; sum = 195,25; 24 protolytic constants

Protolytic mean constant pKa_{mean} isoelectric point IEP calculate as sum of constant side chains $\Sigma pK_{aRside\ group}$,, $pK_{aNterminus}NH_3$ and $pK_{aCterminus}COO^-$ divided with number of protolytic acid groups NpK_a:

$$IEP=pK_a=(\Sigma pK_{aRside\ group}+pK_{aNterminus}+pK_{aCterminus})/NpK_a$$

Calculate NLTP1_WHEAT Non-specific lipid-transfer protein+PGB2 11,9049 kDa molecule

12.1 Acid groups number NpK_a==22.....+2.....= 24.....of 113 amino acids 22+2 of them protolytic constants pK_a for side groups, N-terminus alanine A $pK_{aNterminus}$=9.69 and C-terminal valine V $pKa_{Cterminus}$=2.32

Sum calculates one as $\Sigma pK_{aRside\ group}+pK_{aNterminus}+pK_{aCterminus}$ = 195,25.....

Average acid groups constant **ISOELEKTRIC POINT** for 1CZ2.pdb NLTP1_WHEAT

12.2 Average acid constant pKa_{mean}=IEP=($\Sigma pK_{aRside\ group}+pK_{aNterminus}+pK_{aCterminus}$)/$NpK_a$=195,25/24=**8,1354167**.....

0---------- plus (+) acidic-zero charge **0 IEP**=pH-minus (-) basic----------→ 14 pH scale

-COOH&-NH_3^+ positive charge **-COO^-** & **-NH_3^+** is negative **-COO^-&-NH_2**

Underline and determine existing: positive (+) or zero or negative (-)!0——— skābā vidē plus (+)———nulles

12.3 Determine NLTP1 molecule charge sign (+), zero „**0**" or (-) at physiologic pH=7.36

Underline existing:
-COOH & **-NH_3^+** positive (+) charge ……. pH=7.36 < IEP=**8.14**..... charge negative(-) **-COO^-** & **-NH_2**.

12.4 Determine NLTP1 molecule charge sign (+), zero „**0**" or (-) at **electrophoresis** pH **8.8** on stripe
Underline existing:
-COOH & **-NH_3^+** positive (+) charge …IEP=**8.14**< pH=8.8 electrophoresis charge negative(-) **-COO^-** & **-NH_2**.

12.5 Calculate $C=10^{-6,58}$ M (mol / Liter) NLTP1 solution pH by *Ostwald dilution law* concentration C logarithm:

$$pH=\frac{pK_a-\log C}{2}=\frac{8{,}1354167-\log 10^{-6{,}5845833}}{2}=\frac{8{,}1354167+6{,}5845833}{2}$$ = **14,72** / 2 = 7,36.....

7,36 Attractor NLTP1_WHEAT concentration is $C=10^{-6,58}$..... M .

Untill next Medical Chemistry experimental research!