Name Surname: __Teacher_____Teacher___ Group Nº _____2025

Aquaporin 0 AQP1-11.pdf: Water Channel Proteins WCPsAQPs09.pdf: Studies-research Aquaporin0S.pdf:

Molecule viewers ChemScape MDLi **RasMol**; MAGE FireFox application.

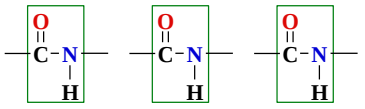
Research htdocsLocal: http://aris.gusc.lv/ChemFiles/Aquaporins/AquaPorin1-0.htm **Eye-lens** cells

William E. C. Harries,David Akhavan,Larry J. W. Miercke,Shahram Khademi,Robert M. Stroud Department of Biochemistry and Biophysics, University of California 2004 Proc.Natl.Acad.Sci. **1YMG.pdb** monomer 2025 by Aris Kaksis preparation at Riga Stradin's University. **Corey, Pauling, Koltun** the **CPK**

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)

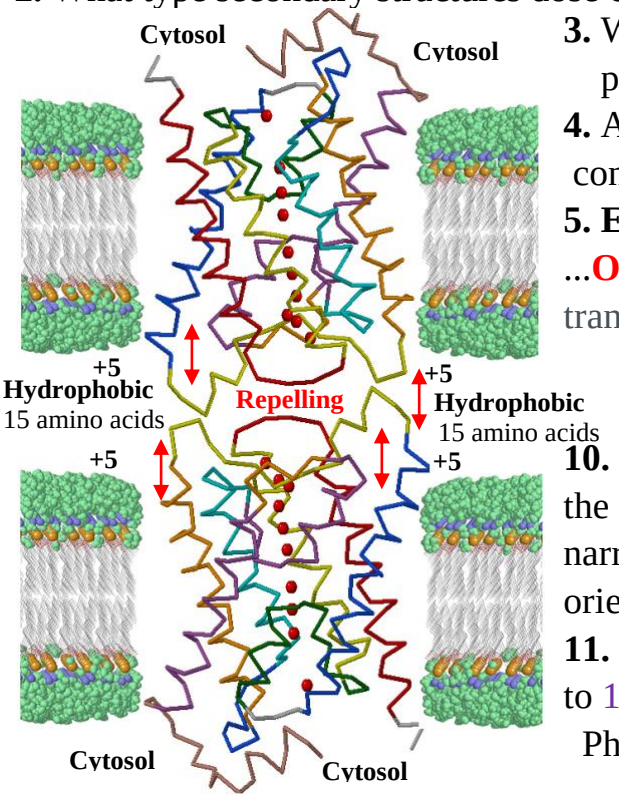
color scheme 1965 **USA** patent Protein **Backbone** is **Cα** carbon atoms of amino acids trace



Side chains: **Hydrophobic** *gray* **Polar** *magenta* and **Polar** *slightly bluish* *at Physiologic* **pH=7.36** *conditions* **Acidic-COO^- negative** *charge* **Basic–NH_3^+ positive** *charge*

1. **N-terminus** amino acid is Ser6.... and **C-terminus** amino acid is Gly239..... What is total number of amino acids on primary structure of Aquaporin 0 see 6. page 263... ?

2. What type secondary structures dose contains the Aquaporin 0 **1YMG**?….**alpha**, no **beta**...



3. What number of **alpha helices** constitute Aquaporin 0 polypeptide molecule? **8 Alpha-helices**

4. Are **beta structure** as **sheets** with **beta strands** constituting Aquaporin 0 **eye-lens** and fiber cells? **no**n....

5. **Eye-lens** and fiber cells junction proteins are ...H_2O, ...O_2, …NO, ...CO substrates small neutral molecules transport channels **AQP0**.

9. What number oxygen atoms **O** of **water** molecules are present in AQP0**1YMG.pdb**! 10....

10. Which three amino acid residues with one oriented into the center line of the channel are responsible for most of the narrowing of the vestibule? Asn115, Thr12 and His40 as oriented into the center line .

11. At which five amino acid residues narrow the channel to 1.99 Å on AQP0 polypeptide chain sequence **backbone**? Phe48, His172, Met176, Ala181, Arg187.

12. Four **backbone** carbonyls >**C=O<••••H-O-H** of successive amino acid residues the canonical **AQP0** channel Hydrogen bond acceptors that align **water**s through the channel?

>**C=O<••••H-O-H** Gly180.....,Ala181.....,Gly182.....,Met183..... **H-O-H••••>O=C<**

13. Three amino acids **water** molecules ordered in line with three donor Hydrogen bonds with four acceptor bonds? 4 **H_2O<••••H-N=C<** Asn119.....,Arg187.....,His172...

14. Four amino acids constricts the channel diameter to 2.5 Å with side chain directly toward the central axis ? Phe141....,Leu52...,Leu168....,Tyr23....to central axis of the channel

15. Three amino acid side chains just after the Tyr23 constriction are **NPA–NPA** signature motifs that orient the key central **water** molecule what are responsible for the reorientation which so are necessary for prevent and restrict any proton conduction? Asn68.....,Asn184.....

16. Four cytoplasmic side amino acids of the **NPAs** signature motifs line three of **backbone** carbonyl oxygens >**C=O<••••H-O-H** resumes along the wall point directly into the channel and it ends at Tyr149? Gly64.....,Ala65.....,His66.....,Val67.....

17. Five amino acids form another constriction that is the channel narrowest region? It accepts close average **radius** of **water pore** sphere **SF** ~**1.1 Å H–O–H** longitudinal **1.4** Å and **0.55 Å** bent size of dipole with a maximum diameter of 1.5 Å as **water** specific bent-angular structure? Tyr149.....,Val56.....,Gly64.....,His66.....,Phe75.....

18. What number of **water** molecules the line of eight **backbone** carbonyls >**C=O---H-O-H** and other channel-lining residues establishes a tight single-file pathway for **water** highlighted molecules in the Aquaporin 0 channel? ...Eight **water** molecules in a tight single-file pathway...

19. What amino acid side chain residue from **backbone** chain of Aquaporin 0 is responsible for the inhibitory effect of **pH** or **Ca^{2+}** on **water** transport rates? Therefore, Nemeth-Cahalan and Hall made mutations experiments that changed His-40 to alanine40, aspartate40, or lysine40, and they showed that treatment of oocytes expressing the mutants no longer displayed the **pH**-dependent **closing** as **pH** was raised from **>6.5**. Reaction of histidines40 in AQP0 with diethylpyrocarbonate (DEPC) removed **pH** dependence and actually increased **water** conductance (restored by histidine40-specific reversal with hydroxylamine), adding support to the case that titration of histidines40 alters conductance! His-40..... increases conductance at pH < 6.6.....

24. Two Tyrosines and three Histidines are present down the monomer channel z axis?
Show the positions of Tyrosine residues on **backbone trace** and what
the positions of the Histidines that are close to the channel and vestibules! .....
Tyr23.....,Tyr149.....,His40.....,His66.....,His172....

25. Which three C-terminal **2B6P**.pdb amino acids Post translation modification are involved in **AQP0** activity regulation by **phosphorylation** and glycosylation? Call position C-terminal 24 amino acids mobile helix of protein and secondary structural domain 239–263 string three amino acids subjects to phosphorylation!
mobile helix **H8**...,Ser229....., Ser231....., Ser235..... un
extra cellular vestibule are shoved six **OH** groups in phosphorylatable amino acids:
Ser31....., **Thr108**....., **Ser126**....., **Thr120**....., **Thr199**....., **Thr195**.....
Call C-terminal of 24 amino acids constitute mobile helix and three amino acids,
1. By right button hold down on molecule picture and choose indicate **Select>Residue>Ser** and second time hold down indicate **Display>Sticks** and again **Color>CPK**:
mobile helix **H8**. Ser245....., Ser243....., Ser240.....

26. In **Eye-lens** fiber cells 15 residues of amino acids non polar groups hydrophobic interaction forces between bound two aquaporins (AQP0's) create inter membranes cells 16 Å gap junction. Each contact point integrate energy -10 $^{kJ}/_{mol}$, if 15 contact points than are -150 $^{kJ}/_{mol}$.

Leu39...., Pro38...., Gly37...., Ala35...., Gly114...., Val112...., Ala111...., Pro110....,

Pro109...., Val125...., Gly124...., Pro123...., Leu121....; Phe198...., Leu194.....

27. Four amino acids as conserved throughout all **AQPs**, alternate conformation located in the central "selectivity filter **SF**" of the channel to contribute channel closure?

with two good hydrogen bonds from Arg187....>NH...O=C<Pro191.....

and from Asn68....>NH...O=C<Ala-117.....

28. Five amino acids on surface create positive +5 charge network repulsing **AQP0**

on **Eye-lens** extracellular side. His40..., Arg33...., Arg113....., His122....., Arg196.....

29. Arg-187, Asn68 3. Conformation in the central "selectivity filter **SF**" make channel closure with two Hydrogen bonds Arg-187>NH...O=C< un Asn68>NH...O=C< :

>NH...O=C<Pro191...., O=C<Phe189...., O=C<Asn184, O=C<Arg187...., O=C<Ser31....;

>NH to O=C<Asn115...., O=C<Asn115...., O=C<Ala-117...., O=C<Asn119.... and

What two amino acids which are conserved throughout all AQPs Val41...., Tyr177..... ?

23. Call the cell and organelles localization sites where transport enzymes Aquaporins 0 found!

..... **eye-lens** cells as inter cellular ..... **junction**s or as shunt.....

between thin **fiber**.....**junction**s cells.

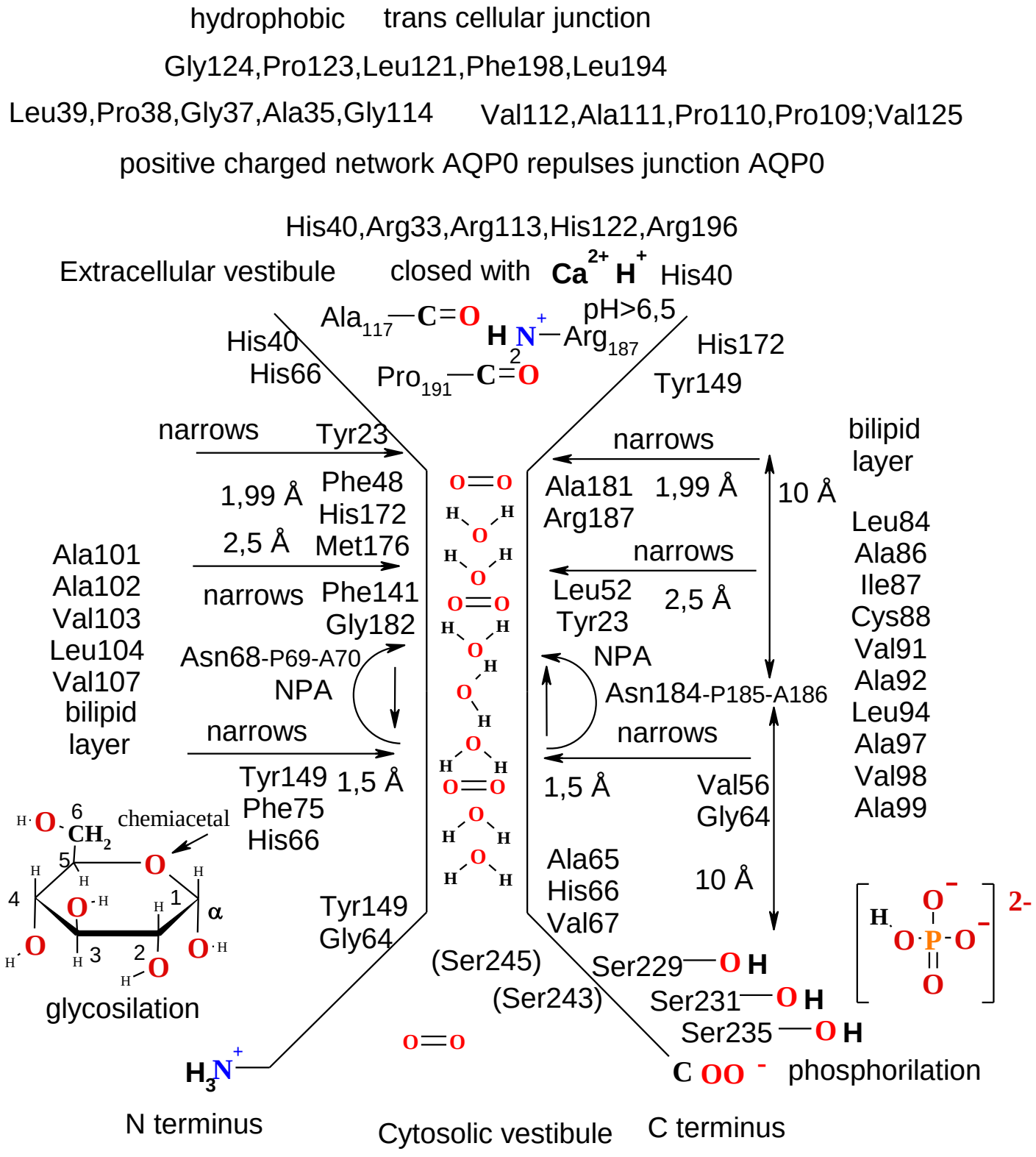
20. Five 5 intermolecular forces are known in biochemistry and physiology of proteins? ...

1....**Hydrogen bond** 2...**Hydrophobic** 3...**Salt bridges** **4.-S-S-…disulfide bonds 5.…Coordinative bond**

21. What **3** intermolecular forces fold protein chains of AQP0? Call three 3 identified present!

1....**Hydrogen bond** 2...**Hydrophobic** 3...**Salt bridges**

22. Put amino acids numbers in 28-Å-long, **cylindrical** AQP0 **channel** is flanked by shallow **vestibules** on each end! Channel volume show in the background, with major channel-forming residues amino acid names! The central region show with diameter of <2.5 Å, the regions both side shown with diameter of >2.5 Å and <10 Å long distance from central region center 0! Starting from the extracellular side, the vestibule funnel like diameter of <10 Å with 10 water molecules **HOH** 405, 415, 455, 411, 426, 407, 436, 439, 451, 435, 465 oxygen between residues show oriented into the center line. Drawn 7 **HOH**. Center amino acids are responsible for most of the narrowing of the vestibules up to channel narrowest diameter of 1.99 Å, 2,5 Å, 1.5 Å ! Seven charges -NH_3^+ and –COO^- both side of membrane prevent conductance of ions!



Download: http://aris.gusc.lv/06Daugavpils/Research/tgf/AQP0skemS.tgf

30. What **AQP0** isoelectric point IEP=pH=$pK_{a\text{-}vid}$ at physiologic pH=7,36 ? To determine water solution pH with **AQP0** concentration C=0.00000063=$10^{-6,2}$M ($^{mol}/_{Litre}$)!

21 L-α-Amino Acids proteins polypeptide protolysis pK_a value **isoelectric point IEP**

At physiologic pH=7, 36 ±0.01 carboxylic groups **R-COO⁻** negative charged and amino groups **R-NH₃⁺** positive charged. For example, glutamic acid pK_a reference to physiologic pH value smaller as $pK_{aR\text{-}COO^-}$=4.25<7.36, pK_{aCOO^-}=2.19<7.36 and for amine is greater as physiologic pH: 9.67=$pK_{a\text{-}NH3+}$>7.36 .

Table shown constants pK_a of four type parallel protolytic equilibria in each amino acid molecule:

acid	⇔ base	$+H^+$;
1. **R-COOH**	⇔**R-COO⁻**	$+H^+$;
2. **R-NH₃⁺**	⇔**R-NH₂**	$+H^+$;
3. **Tyr**-phenol-**OH**	⇔**Tyr**-phenol-**O⁻**	$+H^+$,
4. Cys-SH	⇔Cys-**S⁻**	$+H^+$

Parallel protolytic equilibria number NpKa average isoelectric point and constant pK_a value IEP= pK_a is calculated as

IEP= pK_a= (Σ $pK_{a\ R\ group}$+ $pK_{a\text{-}NH3+}$+ $pK_{a\text{-}COOH}$)/Npk$_a$

In *Ostwald's dilution law* calculate pH of solution at concentration C logarithm: $pH=\frac{pK_a - \log C}{2}$ =.....

Amino acid and protein at isoelectric point value pH=**IEP** sum of total overall **ion** charge is zero

0—— acidic charge (+)————zero „**0**" charge **IEP**———in basic medium charge minus (-)—→pH scale

-COOH & **-NH₃⁺** positive charge .....**-COO⁻** & **-NH₃⁺**..... charge is negative **-COO⁻** & **-NH₂**

Amino Acid		pK_{aCOOH}	pK_{aNH3+}	$pK_{aRgroup}$
Isoleucine	I	2.36	9.68	
Valine	V	2.32	9.62	
Leucine	L	2.36	9.60	
Phenylalanine	F	1.83	9.13	
Cysteine	C	1.96	10.28	8.18
Methionine	M	2.28	9.21	
Alanine	A	2.34	9.69	
Proline	F	1.99	10.96	
Glycine	G	2.34	9.60	
Threonine	T	2.11	9.62	
Serine	S	2.21	9.15	
Tryptophan	W	2.38	9.39	
Tyrosine	Y	2.20	9.11	10.07
Histidine	H	1.82	9.17	6.00
Aspartate	D	1.88	9.60	3.65
Glutamate	E	2.19	9.67	4.25
Asparagine	N	2.02	8.80	
Glutamine	Q	2.17	9.13	
Lysine	K	2.18	8.95	10.53
Arginine	R	2.17	9.04	12.48

Table5.3 Reginald H. Garrett, Charles M. Grishman, **Biochemistry**, University of Virginia 1995

Myoglobin IEP=7,36 is neutral zero „**0**" charged molecule, as IEP=7,36 is equal physiologic pH_{blood}=7,36 1MBO.pdb

Albumin E7G.pdb 7,32=IEP 7 fatty acids small - charge and 7,40=IEP absent 7 fatty acids + positive at pH=7.36, but

gamma *Globulin* IgG1.pdb molecule has positive (+) charge, as at physiologic pH=7.36 is greater IEP=7.91.

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

IEP=pK_a=(Σ$pK_{aR\ side\ residue}$+ $pK_{aNterminus}$+ $pK_{aCterminus}$)/NpKa

Figure Separation of serum proteins by **electrophoresis**. (a) A sample is applied as a narrow line at the origin. After **electrophoresis** at pH **8.8**, the paper is dried and stained. (b) A plot of color intensity of each spot. γ *Globulin* moves slower as *Albumin*

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

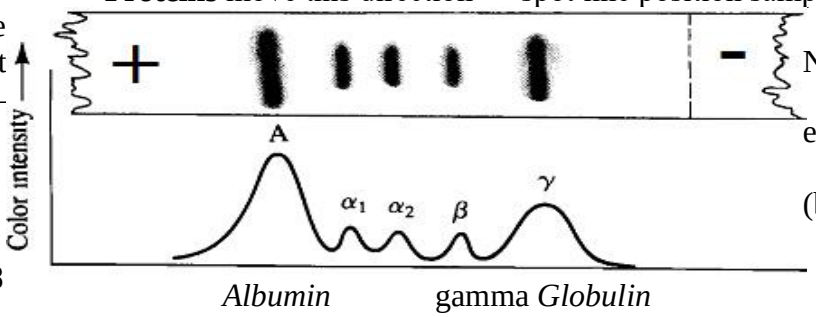
Positive (+) electrode

Direction of movement <=————

Albumin IEP=7,32÷7,40<<**pH=8,8**

γ *Globulin* IEP=7,91 < **pH=8,8**

Myoglobin IEP=7,36 < **pH=8,8**



Seleno cysteine, the 21st L-α-Amino Acid

Seleno cysteine is an L-α-amino acid found in a handful of proteins, including certain **peroxidases** and **reductases** where it participates in the catalysis of electron transfer reactions. As its name implies, a selenium **Se** atom replaces the sulfur **S** of its structural analog, cysteine. The p*K*$_3$ of seleno cysteine 5.2 is 3 units lower than that of cysteine 8.18. Since seleno cysteine is inserted into polypeptides during translation, it is commonly referred to as the "21st amino acid." However, like the other 20 genetically encoded amino acids, seleno cysteine is specified by a simple three-letter codon **UGA** (see class 16 week Nucleo proteins tRNA 62 codons).

Lens-specific **Aquaporin**-0 (**AQP**0) functions as a specific **water pore** and forms the thin **junctions** between **fibre cells**

http://aris.gusc.lv/ChemFiles/Aquaporins/1YMGpI.doc ; http://aris.gusc.lv/ChemFiles/Aquaporins/1YMGpI.xls

```
Sequence of 263 AA Amino Acids in AQP0 molecule 1YMGpI.pdb:      80*3+23=263
         10         20         30         40         50         60         70         80
MWELRSASFWRAICAEFFASLFYVFFGLGASLRWAPGPLHVLQVALAFGLALATLVQAVGHISGAHVNPAVTFAFLVGSQ
MSLLRAICYMVAQLLGAVAGAAVLYSVTPPAVRGNLALNTLHPGVSVGQATIVEIFLTLQFVLCIFATYDERRNGRLGSV
ALAVGFSLTLGHLFGMYYTGAGMNPARSFAPAILTRNFTNHWVYWVGPVIGAGLGSLLYDFLLFPRLKSVSERLSILKGS
RPSESNGQPEVTGEPVELKTQAL
```

AA	pKa$_{COO^-}$	pKa$_{NH3^+}$;	pK$_{RR}$	NchainAA;	Nr
M		9,21		1	1
E			4,25	3	2
R			12,48	5	3
R			12,48	11	4
C			8,18	14	5
E			4,25	16	6
Y			10,07	23	7
R			12,48	33	8
H			6	40	9
H			6	61	10
H			6	66	11
R			12,48	85	12
C			8,18	88	13
Y			10,07	89	14
Y			10,07	105	15
R			12,48	113	16
H			6	122	17
E			4,25	134	18
C			8,18	144	19
Y			10,07	149	20
D			3,65	150	21
E			4,25	151	22
R			12,48	152	23
R			12,48	153	24
R			12,48	156	25
H			6	172	26
Y			10,07	177	27
Y			10,07	178	28
R			12,48	187	29
R			12,48	196	30
H			6	201	31
Y			10,07	204	32
Y			10,07	219	33
D			3,65	220	34
R			12,48	226	35
K			10,53	228	36
E			4,25	232	37
R			12,48	233	38
K			10,53	238	39
R			12,48	241	40
E			4,25	244	41
E			4,25	250	42
E			4,25	254	43
E			4,25	257	44
K			10,53	259	45
L	2,63			263	46

In account are present 3 Cysteine residues Cys = pK$_{RR}$ =8.18;

Sum of 46 pKa values in table 389,69.....

Calculation tasks for Aquaporine 0 molecule AQP0

Protolytic constant pK$_a$ isoelectric point IEP=pK$_a$ calculate of side chains ΣpK$_{aRside\ group}$.. pK$_{aNterminal}$$NH_3$ and pK$_{aCterminal}$$COO^-$constants sum divide with number of acid groups NpK$_a$:

IEP=pK$_a$=(ΣpKa$_{Rside\ group}$+ pKa$_{Nterminal}$+ pKa$_{Cterminal}$)/NpKa

30.1 Acid groups number in sum NpKa=44.....+2.....= 46.....

263 amino acids of them protolytic constants pK$_a$ for side groups 44+2.
N-terminal Methionine M pK$_{aNterminal}$=9.21 and
C-terminal Leucine L pKa$_{Cterminal}$=2.36

Sum are calculate as

ΣpKa$_{Rside\ group}$+pK$_{aNterminal}$+pKa$_{Cterminala}$=389,69.....

30.2 Average acid group constant pK$_{mean}$=pK$_a$=IEP **ISOELEKTRIC POINT**

NpKa=44.....+2.....=46.....

IEP=389,69 / 46 =**8,5228261**.....

At pH value of amino acid and protein on isoelectric point pH=**IEP**
total charge is zero „**0**" **IEP**=pH

0————plus (+)—zero charge „**0**" —minus charge (-) ——→ 14 pH scale

Underline and determine existing: positive (+) or zero charge or negative (-)!

-COOH & **-NH$_3^+$** positive **-COO$^-$** & **-NH$_3^+$** charge negative **-COO$^-$** & **-NH$_2$**

30.3 AQP0 molecule charge sign (+). zero „**0**" or (-) at physiologic pH=7.36

Underline existing:
-COOH,**-NH$_3^+$** positive (+) pH=7.36<IEP=**8,52** negative(-) **-COO$^-$**,**-NH$_2$**.

30.4 AQP0 molecule charge (+). zero „**0**" or (-) at **electrophoresis** pH **8.8**

Underline existing:

-COOH,**-NH$_3^+$** positive (+) IEP = **8,52** < pH = **8,8** negative (-) **-COO$^-$**,**-NH$_2$**.

30.5 Calculate C=0.00000063=$10^{-6,2}$ M AQP0 solution pH
by *Ostwald dilution law* concentration C in logarithm:

$$pH=\frac{pK_a-\log C}{2}=\frac{8{,}5228261-\log 0.00000063\cdot}{2}=\frac{8{,}5228261+6{,}2}{2}=$$ **14,723** / 2 = 7,36.....

Attractor 7,36 AQP0 concentration is $10^{-6,2}$.....M.

Furthered follows aquaporin one **AQP1** from erythrocytes