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

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

Molecule viewers ChemScape MDLi 〔MDL〕 **RasMol** ⚛; MAGE ▢ 🦊 FireFox application.
Task 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, 2023
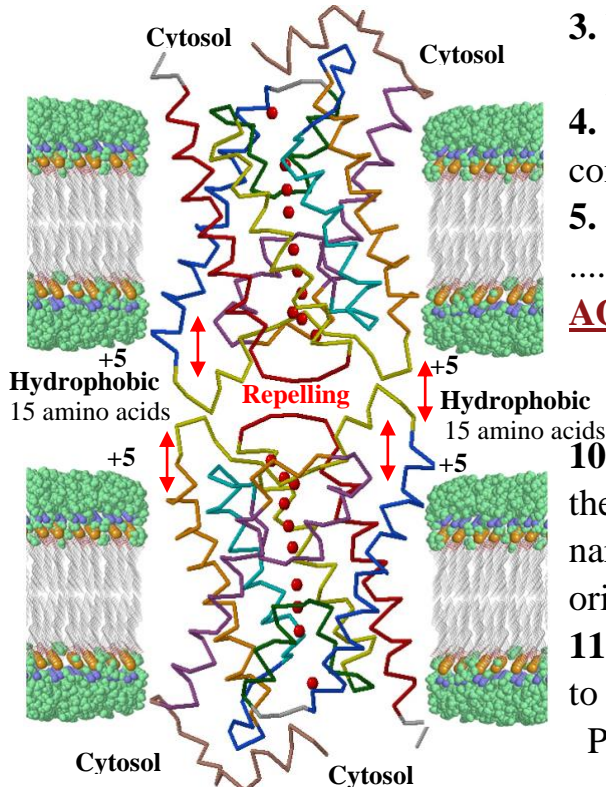by Aris Kaksis preparation at Riga Stradin's University. **Corey, Pauling, Koltun** the **CPK**

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	**Mg²⁺**	**Green**	+**2** (coordination up to 6)
Calcium ion	**Ca²⁺**	**Gray** Dark	+**2** (coordination up to 6)
Iron ion	**Fe²⁺**	**Yellow** Gray	+**2** (coordination up to 6)
Iron ion	**Fe³⁺**	**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-C**OO⁻** **negative** *charge*
**Basic–NH₃⁺** **positive** *charge*
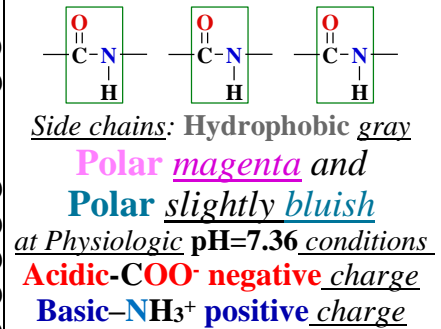
1. N-terminus amino acid is Ser.... and **C-terminus** amino acid is Gly...... What is total
number of amino acids on primary structure of Aquaporin 0 see 6. page ... ?
2. What type secondary structures dose contains the Aquaporin 0(**1YMG**?...., no...
3. What number of **alpha helices** constitute Aquaporin 0 polypeptide molecule? ....**Alpha-helices**
4. Are **beta structure** as **sheets** with **beta strands** constituting Aquaporin 0 **eye**-lens cells? ....

Cytosol   **Cytosol**
+5
Hydrophobic
15 amino acids   **Repelling**   **+5**
Hydrophobic
15 amino acids
+5   **+5**
Cytosol   **Cytosol**

3. What number of **alpha helices** constitute Aquaporin 0
polypeptide molecule? ....**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 ..., ..., …,
.... substrates small neutral molecules transport channels
AQP0.
9. What number oxygen atoms **O** of **water** molecules
are present in AQP0**1YMG.pdb**! ....
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? Asn...., Thr...., His.... 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**?
Phe...., His...., Met...., Ala...., Arg.....

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?
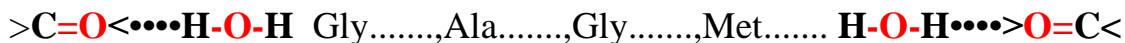
>**C**=**O**<••••**H**-**O**-**H** Gly.......,Ala.......,Gly.......,Met....... **H**-**O**-**H**••••>**O**=**C**<
13. Three amino acids **water** molecules ordered in line with three donor Hydrogen bonds

with four acceptor bonds?   4 **H₂O**<••••**H**-**N**=**C**< Asn.......,Arg.......,His...

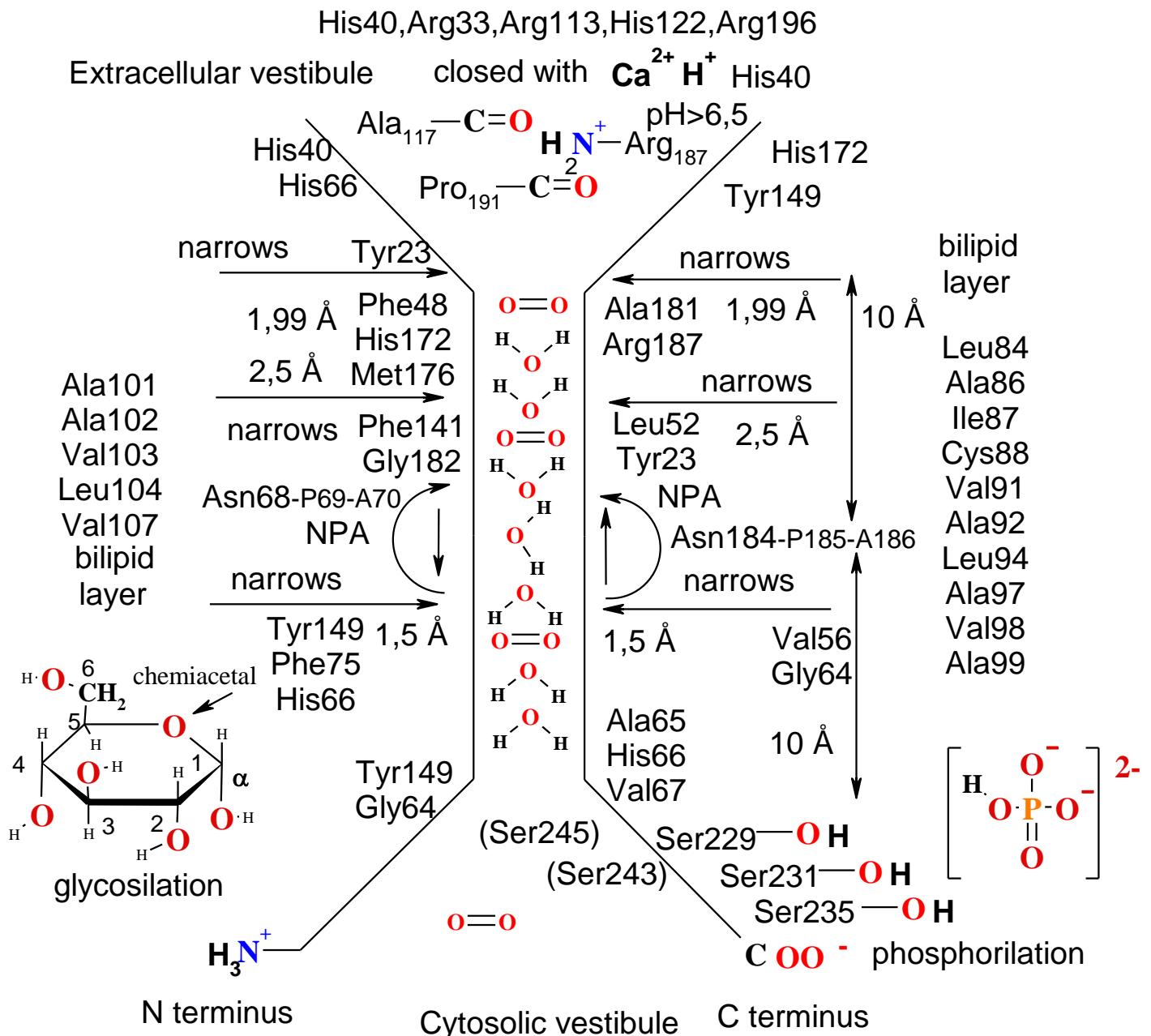**14.** Four amino acids <u>constricts the channel</u> diameter to 2.5 Å with side chain directly

toward the central axis ?   Phe....,Leu...,Leu....,Tyr2....to central axis of the channel

15. Two amino acid side chains just after the Tyr23 constriction are **NPA–NPA** <u>signature</u>

<u>motifs</u> that orient the key central **water** molecule what are responsible for the reorientation

which so are necessary for prevent and restrict any proton conduction? Asn.........,Asn.......

16. Four <u>cytoplasmic side</u> amino acids of the **NPAs** <u>signature</u> <u>motifs</u> 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?   Gly...........,Ala...........,His...........,Val........

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** <u>longitudinal</u> **1.4** Å

   and **0.55 Å** <u>bent size of dipole</u> with a maximum diameter of 1.5 Å as **water** specific bent-

   angular structure?   Tyr........,Val........,Gly......,His......,Phe.......

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? ...**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 <u>alanine40</u>, <u>aspartate40</u>, or <u>lysine40</u>, and they

showed that treatment of oocytes expressing the mutants no longer displayed the **pH**-<u>dependent</u>

closing as **pH** was raised from **>6.5**. Reaction of histidines40 in AQP0 with diethylpyrocarbonate

(DEPC) removed **pH** <u>dependence</u> and actually increased **water** conductance (restored by

histidine40-specific reversal with hydroxylamine), adding support to the case that titration of

histidines40 alters conductance! His-.................... increases conductance at pH > ....

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

1....   2...   3...   **4.**-**S-S**-...   **5.**...

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

1....   2...   3...

22. Put amino acids numbers in 28-Å-long, **cylindrical** AQP0 **channel** is <u>flanked by shallow</u> **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 <u>extracellular</u> 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 **-NH₃⁺** and **–COO⁻** both side of membrane prevent conductance of ions!

hydrophobic   trans cellular junction

Gly124,Pro123,Leu121,Phe198,Leu194

Leu39,Pro38,Gly37,Ala35,Gly114   Val112,Ala111,Pro110,Pro109;Val125

positive charged network AQP0 repulses junction AQP0

His40,Arg33,Arg113,His122,Arg196

Extracellular vestibule   closed with $Ca^{2+} H^+$ His40

Ala_{117}—C=O H_2N^+—Arg_{187} pH>6,5

His40
His66 Pro_{191}—C=O   His172
Tyr149

narrows   Tyr23   narrows   bilipid
                                                layer
1,99 Å Phe48   O=O   Ala181   1,99 Å   10 Å
        His172   H H   Arg187   Leu84
2,5 Å   Met176   O   narrows   Ala86
Ala101   H H   Leu52 2,5 Å   Ile87
Ala102 narrows Phe141 O   Tyr23   Cys88
Val103   Gly182 O=O   Val91
Leu104 Asn68-P69-A70 H H   NPA   Ala92
Val107   NPA   O   Asn184-P185-A186   Leu94
bilipid   H   Ala97
layer   narrows   O   narrows   Val98
                        H   Ala99
        Tyr149 1,5 Å   O   1,5 Å Val56
        Phe75   H H   Gly64   10 Å
        His66   O=O
                        O
Tyr149   H H   Ala65
Gly64   O   His66
                       H H   Val67 10 Å

chemiacetal   (Ser245)   Ser229—O H
                       (Ser243)   Ser231—O H
glycosilation   Ser235—O H

                       O=O   C OO⁻ phosphorilation

H_3N^+

N terminus   Cytosolic vestibule   C terminus

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

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

.... <u>cells as </u>inter <u>cellular</u> .... <u>or as</u> ......

between <u>thin</u> **fiber**.. <u>cells.</u>

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!

Tyr.....,Tyr.....,His.....,His.....,His....

25. What three C-terminal **2B6P**.pdb amino acids are Posttranslational modificate and have also

been suggested in the regulation of AQP0 activity, as C-terminal chain have been implicated as

regulatory **phosphorylation** sites in human AQP0? What C-terminal mobile helix name and

what three of 24 amino acids 239–263 suit for phosphorilation in structure

of **bAQP0**? **H**.......Ser.......(Ser245),Ser.......(Ser243,Ser240),Ser.......

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 $^{kJ}/_{mol}$.

Leu...., Pro...., Gly...., Ala...., Gly...., Val...., Ala...., Pro....,

Pro...., Val...., Gly...., Pro...., Leu....; Phe...., Leu.....

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 <u>from</u> Arg>NH...O=C<Pro......

and from Asn>NH...O=C<Ala.......

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

on **Eye-lens** extracellular side. His..., Arg...., Arg....., His....., Arg.......

29. Arg-187, Asn68 3. Conformation in the central "selectivity filter **SF**" make channel closure

with two <u>Hydrogen</u> bonds Arg-187>NH...O=C< un Asn68>NH...O=C< :

>NH...O=C<Pro...., O=C<Phe...., O=C<Asn, O=C<Arg...., O=C<Ser....;

>NH to O=C<Asn...., O=C<Asn...., O=C<Ala...., O=C<Asn.... and

What two amino acids which are conserved throughout all AQPs Val...., Tyr..... ?

30. What **AQP0** isoelectric point IEP=pH=pK$_{a-vid}$ 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$_{aR-COO}$⁻=4.25<7.36, pK$_{aCOO}$⁻=2.19<7.36 and for amine is greater as physiologic pH: 9.67=pK$_{a-NH3+}$>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$_{a-NH3+}$+ pK$_{a-COOH}$)/NpK$_a$

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

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⁻** & **-**

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

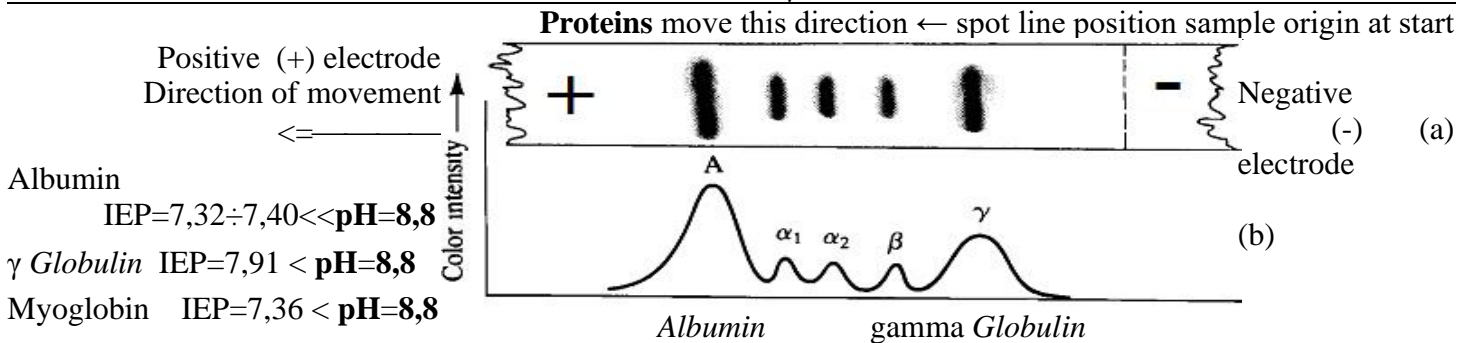gamma *Globulin* IgG1.pdb molecule has positive (+) charge, as at physiologic pH=7.36 is greater IEP=7.91.

Iso electric point IEP=pK$_a$ as well protolytic constant pK$_a$ calculates one of side residues R constants sum ΣpK$_{aRside\,residue}$ plus pK$_{aNterminusNH3+}$ and plus pK$_{aCterminusCOO–}$ sum dividing with number NpKa of acidic groups in molecule

IEP=pK$_a$=(ΣpK$_{aR\,side\,residue}$+ 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**

Negative (-) (a) electrode

(b)

Albumin   gamma *Globulin*

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 pK$_3$ 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).

```
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

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 ....

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$_a$=(ΣpK$_{aRside group}$+ pK$_{aNterminal}$+ pK$_{aCterminal}$)/NpK$_a$

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

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 pK$_{aCterminal}$=2.36

Sum are calculate as

ΣpK$_{aRside group}$+pK$_{aNterminal}$+pK$_{aCterminala}$=...........

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

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

IEP=389,69 / 46 =.............

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

<u>Underline</u> and determine existing: positive (+) or <u>zero charge</u> or negative (**-**)!

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

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

<u>Underline</u> existing:
-COOH,-NH₃⁺ positive (+) pH=7.36<IEP=**8,52** negative(-) **-COO⁻,-NH₂**.

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

<u>Underline</u> existing:

-COOH,-NH₃⁺ positive (+) IEP = **8,52** < pH = **8,8** negative (-) **-COO⁻,-NH₂**.

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

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

Attractor 7,36 AQP0 concentration is ....M.

Furthered follows aquaporin one **AQP1** from erythrocytes