






Water Channel Proteins WCPS: [Aquaporin 1 AQP1](#) cross erythrocyte [membranes](#) A. [Task](#)

ChemScape MDL  **RasMol**  (RasMac ); MAGE   Firefox application.  
htdocsLocal <http://aris.gusc.lv/ChemFiles/Aquaporins/AquaPorin1.htm>: [Sui H](#), [Han BG](#), [Lee JK](#),  
[Walian P](#), [Jap BK](#) Life Sciences Division University of California, Berkeley [Nature](#). 2001

[1J4N.pdb](#) and [1j4nbio1.pdb](#) tetramer RSU Aris Kaksis 2025 RSU tutorial [solutions](#):

1. What N-terminus and C-terminus amino acids have primary structure of Aquaporin 1? Using **Backbone** Display option **N-terminus** domain starting amino acid is Met1..... and finishing amino acid is Ser249..... ? What is total number of amino acids on chain 249.....?
2. Call and explain building the molecules of E7 class transport enzymes AQP 1 channels and generally for Aquaporin substrates? ....**H<sub>2</sub>O**, ....**O<sub>2</sub>**, ....**NO**, ....**CO** are small neutral..... molecules and membrane integrated protein formed channels..... .
3. How many **alpha helices** constitute Aquaporin 1 polypeptide molecule? ...**9 Alpha-helices**.  
**H1, H2, H3, H4, H5, H6, H7, HB, HE**.
4. Are constituting Aquaporin 1 **beta structure** and **sheets** with **beta strands**? .....**no**.....
5. How many quaternary 4° structure components 3° subunits and what tertiary 3° structure domain units with its secondary 2° structure components are found in given biological unit of Aquaporin 0? What type of secondary 2° structure segments constitutes AQP 0 [eye-lens cells](#)?  
Each of four 4 tetramer unit have; **8** .....**Alpha-helices**; **no**.....**parallel beta strands**
6. Select in menu Distance **tetramer** size tall and wide in angstroms are: ....49.Å..... 68-60.Å....
7. What **Channel** distance long and at constriction region wide in angstroms?...39.Å.....35-23.Å
8. Define the protein primary, secondary, tertiary and quaternary structures!  
Primary (1°) Structure polymer chain polypeptide linear linked amino acids.....  
Secondary (2°) are folded primary chains in alpha.....and beta..... secondary structures.  
Tertiary (3°) are folded secondary structure units globular.....and fibrillar.....  
Quaternary (4°) are by five intermolecular forces in globular and fibrillar linked :  
tertiary structure units.....
5. How many quaternary 4° structure components 3° subunits and what tertiary 3° structure domain units with its secondary 2° structure components are found in given biological unit of Aquaporin1? What type of secondary 2° structure segments constitutes Aquaporin 1?  
...4 tetramer units; .....**9 Alpha-helices ;no-parallel beta strands**.....
6. In **menu** shoos Distance and measure tall and wide in angstroms? .....48.Å.....77-58.Å.
7. What **Channel** length and at constriction region wide in angstroms distance?...28.Å.....23.Å.
8. What number of **water** molecules, which are present in AQP1 [1J4N.pdb](#)! .....10...
9. Which 15 amino acid residues **Hydrophobic** channel walls direct water flow into single file as **hydrophobic** lining of the **H<sub>2</sub>O** pathway and near **H4** and **H5** as well?.....  
Phe24.....,Ile25.....,Ile29....(**H1**),Phe58.....,Ile62....(**H2**),Ala75.....,Leu77....(**LB**)  
Val81.....,Leu85....(**HB**),Val155.....,Leu151.....(**H4**),  
Ile172....., Ile174.....,Val178.....(**H5**), Ile193.....(**LE**);
10. What two **hydrophobic** amino acids roughly pointing to the Two aqueous pathways 2-fold symmetry in that **NPA** (**asparagine, proline, alanine**;) region, respectively, on the **NPA** motifs are completely symmetric on **HB/HE** contact? **Pro79**..... and **Pro195**.....  
**NPA** (**Asn, Pro, Ala**) Asn78,**Pro79**,Ala80 and Asn194,**Pro195**,Ala196
11. What **NPA** (**Asn, Pro, Ala**) motif amino acids and Leu77(**LB**), Ile193(**LE**) constriction site with hydrogen bonds turn over water molecule: Leu77...,**LB**, Asn194..., Asn78..., Ile193...**LE**

- 12.** What four amino acid residues the Ar/R constriction site line one side of the pore hydrophilic line, whereas two other opposite side channel locates hydrophobic?  
Arg197 .....,His182..... hydrophilic, Phe58 .....,Cys191..... hydrophobic .....
- 13.** Amino acid which involved in reversible blocking water transport by mercury  $\text{Hg}^{2+}$  cation from extracellular side of channel? Cys191.....
- 13a.** Amino acid analog on cytosolic site to Cys191 mercury  $\text{Hg}^{2+}$  cation bound  
On extracellular site of channel? Ala75.....
- 14.** Two water molecules are stopped by mercury cation? HOH301....., HOH346.....
- 15.** Ten amino acids of **NPA** four at Selectivity Filter (**SF**) region and six at **Ar/R constriction** site have formed Water molecules in lane a single file through the pore ?  
Ala196.....,Pro195....., Asn194.....,Ala80.....,  
Ala75.....- Asn78.....-Arg197.....,His182.....,Phe58.....,His76.....-Cys191.....
- 16.** What is the size of the narrowest region of the pore that physical limitation on the size of substrates allowed to permeate the AQP1 pore, which is only slightly larger than the 2.8 Å diameter of the Water molecule, as pore constriction prevents permeation of all molecules bigger than Water, including hydrates and ions-protons  $\text{H}^+$ ?.....3 Å diameter.
- 17.** What is the rate of extreme Water molecules AQP1 monomer and tetramer permeability?  
..... $3 \times 10^9$  Water/sec in tetramer four times faster  $4 \times 3 \times 10^9 = 12 \times 10^9$ .....  $\text{sec}^{-1}$ ..
- 17a.**How many molecules of  $\text{O}_2$  in second cross **AQP1** membrane, if  
arterial concentration is  $[\text{O}_2] = 6 \cdot 10^{-5} \text{ M}$  and  $[\text{H}_2\text{O}] = 55,3 \text{ M}$  and  
if AQP1 direct interface to water is  $[\text{O}_2] = 9,768 \cdot 10^{-5} \text{ M}$  due to water solubility from air 20,95% ?  
 $[\text{O}_2] / [\text{H}_2\text{O}] \cdot 3 \cdot 10^9 = 6 \cdot 10^{-5} / 55,3 \cdot 3 \cdot 10^9 = 3255$ .....  $\text{O}_2$  molecules per second.  
 $[\text{O}_2] / [\text{H}_2\text{O}] \cdot 3 \cdot 10^9 = 9,768 \cdot 10^{-5} / 55,3 \cdot 3 \cdot 10^9 = 5299$ .....  $\text{O}_2$  molecules per second.
- 18.** What compound conduction strictly prevents AQP1, that is physiologically very important, as the passage of this compound through the pore would annihilate the proton gradient across the cell membrane, that serves as a major energy transfer mechanism from stored energy molecules of fatty acids and glucose ?  $\text{H}^+$  proton.....
- 19.** What two amino acid residues the positive charges of  $-\text{NH}_2^+$  of extracellular vestibule as well as six amino acid residues in the cytoplasmic vestibule would also help to repel protons from entering the pore?  
Arg197.....,His182.....His71.....His76.....Arg162.....Arg163.....Arg164.....Thr159.....

20. What **AQP1** isoelectric point  $IEP=pH=pK_{a-vid}$  at physiologic  $pH=7,36$  ? To determine water solution pH with **AQP1** concentration  $C=0.000000082=10^{-7,082}$  M (mol/Litre)!

**Erythrocyte aquaporin-1 (AQP1)** specific water pore of molecules **H<sub>2</sub>O**, **O<sub>2</sub>** rate per second  $3 \cdot 10^9$   
<http://aris.gusc.lv/ChemFiles/Aquaporins/1J4NpIStud.doc> ; <http://aris.gusc.lv/ChemFiles/Aquaporins/1J4NpI.xls>

Sequence of 271 AA Amino Acids in AQP1 molecule 1J4N.pdb :

10 20 30 40 50 60 70 80  
 MASEFKKKLFWRAVVAEFLAMILFIFISIGSALGFHYPIKSNQTTGAVQDNVVKVSLAFGLSIATLAQSVGHISGAHLNPA  
 VTLGLLLSCQISVLRIMYIIAQCVGAIVATAILSGITSSLPDNLGLNALAPGVNSGQGLGIEIIGTLQLVLCVLTATD  
 RRRRDLGSGPLAIGFSVALGHLLAIDYTGCGINPARSFGSSVITHNFQDHWIFWVGPFIGAALAVLIYDFILAPRSSDL  
 TDRVKVWTSQVVEYDLDDADDINSRVEMKPK; Sum of 46 pKa values in table 412,45;  $pK_{a-vid}=7.64$

AA	pKa <sub>COO</sub>	pKa <sub>NH3+</sub>	pK <sub>RR</sub>	Nr
M	9,21			1
E	4,25			4
K	10,53			6
K	10,53			7
K	10,53			8
R	12,48			12
E	4,25			17
H	6			36
Y	10,07			37
K	10,53			40
D	3,65			50
K	10,53			53
H	6			71
H	6			76
C	8,18			89
R	12,48			95
Y	10,07			99
C	8,18			104
D	3,65			123
E	4,25			144
C	8,18			154
D	3,65			160
R	12,48			161
R	12,48			162
R	12,48			163
R	12,48			164
D	3,65			165
H	6			182
D	3,65			187
Y	10,07			188
C	8,18			191
R	12,48			197
H	6			206
D	3,65			210
H	6			211
Y	10,07			229
D	3,65			230
R	12,48			236
D	3,65			239
D	3,65			242
R	12,48			243
K	10,53			245
E	4,25			253
E	4,25			254
Y	10,07			255
D	3,65			256
D	3,65			258
D	3,65			260
D	3,65			261
R	12,48			265
E	4,25			267
K	10,53			269
K	2,18			271

In account are present 4 Cysteine residues  $Cys = pK_{RR} = 8.18$ ;

Sum of 46 pKa values in table 412,45.....

### Calculation tasks for Aquaporine 1 molecule AQP1

Protolytic constant  $pK_a$  isoelectric point  $IEP=pK_a$  calculate  
 of side chains  $\Sigma pK_{a\text{side group}}$ ,  $pK_{a\text{NterminalNH}_3}$  and  $pK_{a\text{CterminalCOO}}$ -constants  
 sum divide with number of acid groups  $NpK_a$ :

$$IEP=pK_a=(\Sigma pK_{a\text{side group}}+ pK_{a\text{Nterminal}}+ pK_{a\text{Cterminal}})/NpK_a$$

**20.1** Acid groups number in sum  $NpK_a=52.....+2.....= 54.....$

271 amino acids of them protolytic constants  $pK_a$  for side groups  $52+2$ .

N-terminal Methionine M  $pK_{a\text{Nterminal}}=9.21$  and

C-terminal Leucine K  $pK_{a\text{Cterminal}}=2.18$

Sum are calculate as

$$\Sigma pK_{a\text{side group}}+pK_{a\text{Nterminal}}+pK_{a\text{Cterminal}}=412,45.....$$

**20.2** Average acid group constant  $pK_{\text{mean}}=pK_a=IEP$  **ISOELEKTRIC POINT**

$$NpK_a=52.....+2.....=54.....$$

$$IEP=412,45 / 54 = 7,637963.....$$

At pH value of amino acid and protein on isoelectric point  $pH=IEP$

**total charge is zero „0”**

plus (+)—zero charge „0”  $IEP=pH$ —minus (-) —→ 14 pH scale

**-COOH & -NH<sub>3</sub><sup>+</sup> positive -COO<sup>-</sup> & -NH<sub>2</sub> charge negative -COO<sup>-</sup> & -NH<sub>2</sub>**

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

**20.3** AQP1 molecule charge sign (+). zero „0” or (-) at physiologic  $pH=7.36$

Underline existing:

**-COOH, -NH<sub>3</sub><sup>+</sup> positive (+)  $pH=7.36 < IEP=7,64$  negative(-) -COO<sup>-</sup>, -NH<sub>2</sub>.**

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

Underline existing:

**-COOH, -NH<sub>3</sub><sup>+</sup> positive (+)  $IEP=7,64 < pH=8,8$  negative(-) -COO<sup>-</sup>, -NH<sub>2</sub>.**

**20.5** Calculate  $C=0.000000082=10^{-7,082}$  M solution pH

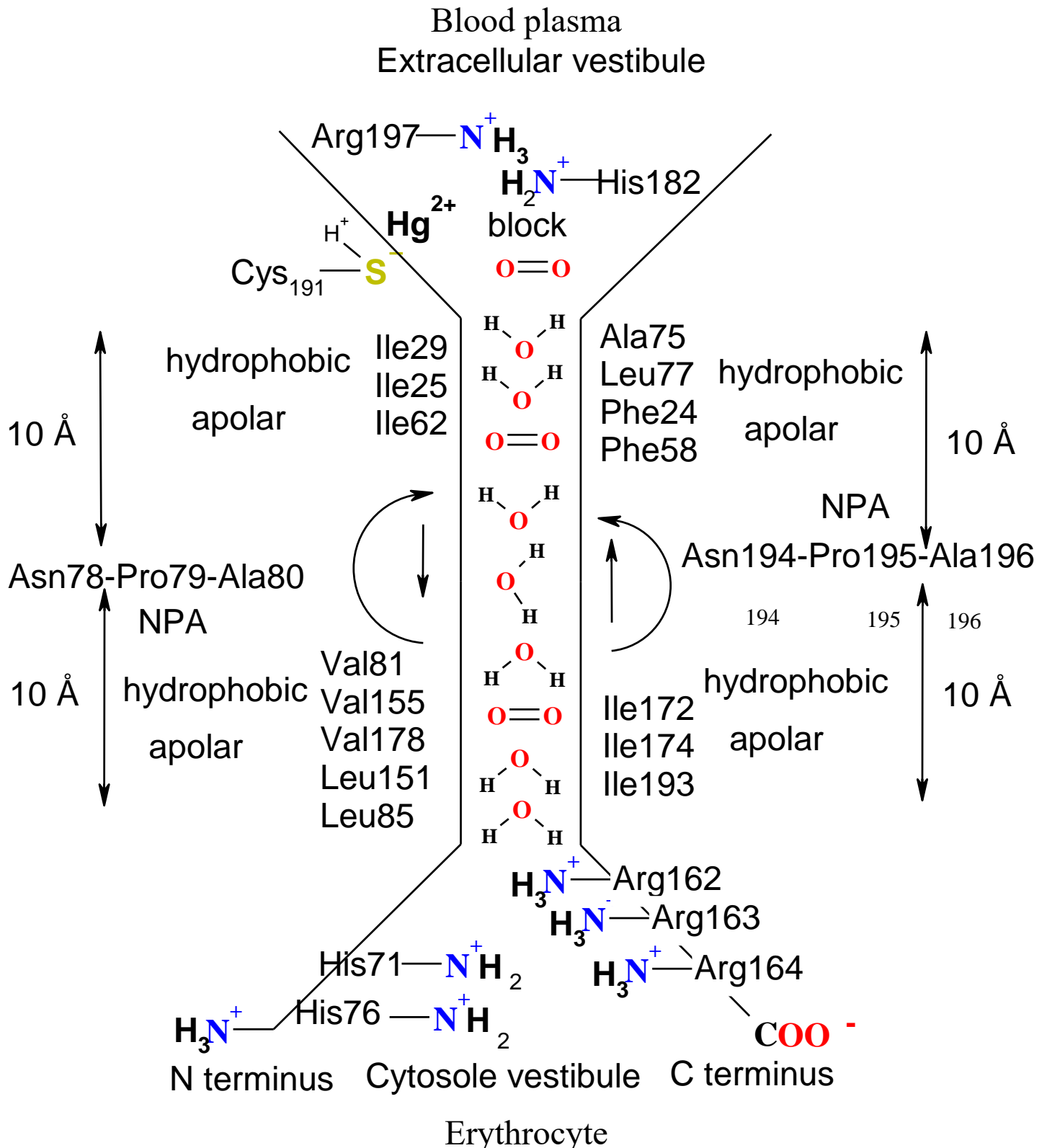
by *Ostwald dilution law* concentration C in logarithm:

$$pH = \frac{pK_a - \log C}{2} = \frac{7,637963 - \log 0.000000082}{2} =$$

$$pH = \frac{pK_a - \log C}{2} = \frac{7,637963 + 7,082}{2} = 14,719963 / 2 = 7,36.....$$

Attractor 7,36 AQP1 concentration is  $10^{-7,082}.....M$ .

20. Put amino acids numbers 28-Å-long, **cylindrical** bAQP1 **channel** is flanked by shallow vestibules on each end! Channel volume show in the background, with responsible channel forming amino acids (put numbers)! The central region show with diameter of <2.5 Å, the regions both side shown with diameter from >2.5 Å to <10 Å. The distance from center 0 is 10 Å! Starting from the extracellular side, the vestibule show funnel like diameter of 10 Å with 4 water in center **HOH** 301,302,303,304 **oxygen** atoms **H<sub>2</sub>O** between residues show oriented into the center line of the channel which are responsible for most of the narrowing of the vestibules up to channel narrowest diameter of 1.99 Å ! Set both side in funnels charges of groups **-NH<sub>3</sub><sup>+</sup>** and **-COO<sup>-</sup>**!



<http://aris.gusc.lv/06Daugavpils/Research/tgf/AQPskemS.tgf>