Name Surname :_ _ _ , _ _ _ _ Grupa Nº _____2023_

J.C. Kendrew and Max Perutz awarded by Nobel Prize in chemistry 1962 for **myoglobin**, **Hemoglobin** **Chromo** proteins hem $Fe^{2+}$ myoglobin, Haemoglobin, $Fe^{3+}$in Catalases, Peroxidases, CytochromesP450
Task for student practical use of Interactive Molecules:

Chem Scape Raswin MAGE ISIS Draw FireFox Do the Notes on Practical:
Download: http://aris.gusc.lv/ChemFiles/htdocsTGF.zip lunch KineMAGE: 1MBODeOxyLopez.kin
Āris Kaksis RSU 2023. M.A. Lopez; and P.A. Kollman, 1975, Protein Sci., 2 (1993) :

**1.** What **helixes** constitute Myoglobine? ..., ... ..., ..., ..., ..., ..., ....What structure fold? ...
Find, call the N-terminal and **C**-terminal amino acids with position number on chain! Val...., Gly....

**2.** What are amino acid and peptide bonds on polypeptide chain?.....amino acids.....peptide bonds.

**3.** Where is adsorbed **oxygen** molecule on Myoglobin? by donor acceptor-bondto heme.....

**4.** Describe **triplet oxygen** molecule **•:O≡:::≡O:•**, on heme iron $Fe^{2+}$ by donor-acceptor bond? **Triplet** has **•:O≡:::≡O:•** ....., however one electron pair dismissed as degenerate orbital
anti-bonding radical on degenerate orbital, therefore sum in **triplet** gives .....

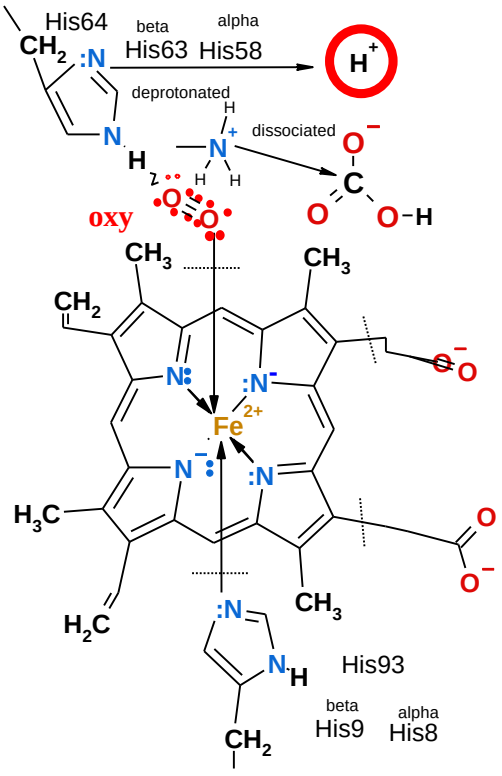
**5.** Enzymes **singlet oxygen** **•::O-:-O::•** one covalent bond create: on heme with atom .....
....., .....as AIR **oxygen** heated over >.....º C higher temperature.

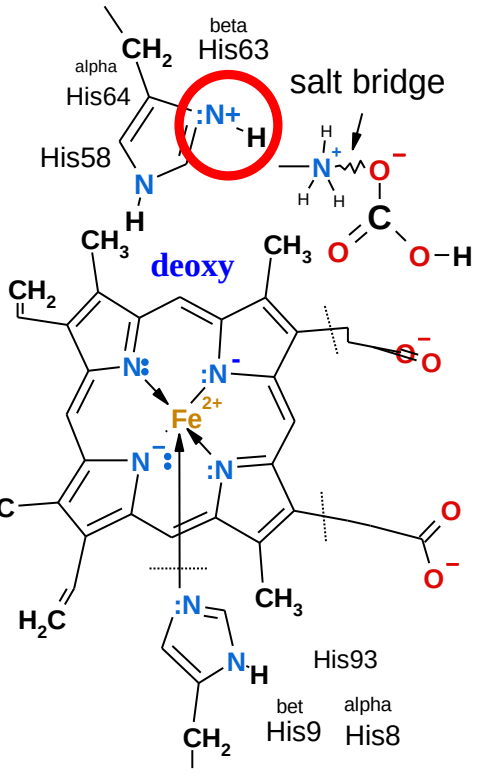
**6. Proximal** histidine number His..... **N** atom touch to heme iron $Fe^{2+}$ by donor acceptor-bond and call it?
**Distal** histidine numberHis..... is **N** atom is protonated $H^+$ **deoxy**, deprotonated **okxy**? Put Romen **number coordination =VI**..... of iron $Fe^{2+}$ bound to number of atoms **N**.....and **O** .....?

**7.** Haw many free delocalized electrons $e^-$ are present into Heme structure **n= 15*2=** .....?

| 29 sphere | Amino acids |
|---|---|
| 1-..... | 16-..... |
| 2-..... | 17-..... |
| 3-..... | 18-..... |
| 4-..... | 19-..... |
| 5-..... | 20-..... |
| 6-..... | 21-..... |
| 7-..... | 22-..... |
| 8-..... | 23-..... |
| 9-..... | 24-..... |
| 10-..... | 25-..... |
| 11-..... | 26-..... |
| 12-..... | 27-..... |
| 13-..... | 28-..... |
| 14-..... | 29- ..... |
| 15-..... | G 29.invisebl |

**8.** What 29 amino acids compose hydrophobic Heme pocket tertiary 3° structure of Myoglobin molecule?
Complete 29 amino acids with sequence number on chain!




$O_{2aqua}$ + **4** $\mathbb{H}_3O^+$ + **4** $e^-$<=>**6** $\mathbb{H}_2O$ , **E=E°**+0,0591/**4•lg([**$O_{2aqua}$**]*[**$H_3O^+$**]⁴/[**$\mathbb{H}_2O$**]⁶); E°**=1.0868 V;
..... ..... **Absolute** standard potential

Ox-Red system has high power of **oxidation** with **absolute** standard potential **E°**=1.0868.

**9.** What component **oxidation** prevent properties of heme pocket amino acids? oxidation.....

**10.** Determine Myoglobin.htm **E** helix turns count:
If 3.6 amino acids Cα alpha carbons backbone in one ring turns conection times to calculate 20/3,6=.....
Ser58,Glu59,Asp60,Leu61,Lys62.Lys63,His64,Gly65,Val66,Thr67,Val68,L69,T70,A71,Leu72,Gly73,Ala74,Ile75,Leu76Lys77

**10a** Cradle deoxy <=> oxy: http://aris.gusc.lv/ChemFiles/ChromoHem/MyoGlobOxDeoxCoBiliverdin/oxydeoxy.avi
lungs $O_{2aqua}$+(H⁺His64)Val1($NH_4^+$salt bridge**$HCO_3^-$**)**$Mb_T$**<=>(His64)Val1($NH_4^+$)**$Mb_R$**($O_2$)+**$H^+$**+**$HCO_3^-$** tissue

**11.1 – 11.5** Perform isoelectric point IEP=pH=$pK_{a\text{-}vid}$ analysis at physiologic pH=7,36 of medium .
calculate water solution pH at **myoglobin** concentration $C=10^{-7,3559}$ M ($^{mol}/_{Litrā}$)!
Sperm vale myoglobin (**1MBO.pdb**) $O_2 \Leftrightarrow H^+ . HCO_3^-$ shuttle in myocites C=0.6 mM myoglobine

| AA | $pKa_{COO-}$ | $pKa_{NH3+}$ | $pK_{RR}$ | Nr | |
|---|---|---|---|---|---|
| V | 0 | 9.62 | 0 | 1 | 1 |
| E | 0 | 0 | 4.25 | 4 | 2 |
| E | 0 | 0 | 4.25 | 6 | 3 |
| H | 0 | 0 | 6 | 12 | 4 |
| K | 0 | 0 | 10.53 | 16 | 5 |
| E | 0 | 0 | 4.25 | 18 | 6 |
| D | 0 | 0 | 3.65 | 20 | 7 |
| H | 0 | 0 | 6 | 24 | 8 |
| D | 0 | 0 | 3.65 | 27 | 9 |
| R | 0 | 0 | 12.48 | 31 | 10 |
| K | 0 | 0 | 10.53 | 34 | 11 |
| H | 0 | 0 | 6 | 36 | 12 |
| E | 0 | 0 | 4.25 | 38 | 13 |
| E | 0 | 0 | 4.25 | 41 | 14 |
| K | 0 | 0 | 10.53 | 42 | 15 |
| D | 0 | 0 | 3.65 | 44 | 16 |
| R | 0 | 0 | 12.48 | 45 | 17 |
| K | 0 | 0 | 10.53 | 47 | 18 |
| H | 0 | 0 | 6 | 48 | 19 |
| K | 0 | 0 | 10.53 | 50 | 20 |
| E | 0 | 0 | 4.25 | 52 | 21 |
| E | 0 | 0 | 4.25 | 54 | 22 |
| K | 0 | 0 | 10.53 | 56 | 23 |
| E | 0 | 0 | 4.25 | 59 | 24 |
| D | 0 | 0 | 3.65 | 60 | 25 |
| K | 0 | 0 | 10.53 | 62 | 26 |
| K | 0 | 0 | 10.53 | 63 | 27 |
| H | 0 | 0 | 6 | 64 | 28 |
| K | 0 | 0 | 10.53 | 77 | 29 |
| K | 0 | 0 | 10.53 | 78 | 30 |
| K | 0 | 0 | 10.53 | 79 | 31 |
| H | 0 | 0 | 6 | 81 | 32 |
| H | 0 | 0 | 6 | 82 | 33 |
| E | 0 | 0 | 4.25 | 83 | 34 |
| E | 0 | 0 | 4.25 | 85 | 35 |
| K | 0 | 0 | 10.53 | 87 | 36 |
| H | 0 | 0 | 6 | 93 | 37 |
| K | 0 | 0 | 10.53 | 96 | 38 |
| H | 0 | 0 | 6 | 97 | 39 |
| K | 0 | 0 | 10.53 | 98 | 40 |
| K | 0 | 0 | 10.53 | 102 | 41 |
| Y | 0 | 0 | 10.07 | 103 | 42 |
| E | 0 | 0 | 4.25 | 105 | 43 |
| E | 0 | 0 | 4.25 | 109 | 44 |
| H | 0 | 0 | 6 | 113 | 45 |
| H | 0 | 0 | 6 | 116 | 46 |
| R | 0 | 0 | 12.48 | 118 | 47 |
| H | 0 | 0 | 6 | 119 | 48 |
| D | 0 | 0 | 3.65 | 122 | 49 |
| D | 0 | 0 | 3.65 | 126 | 50 |
| K | 0 | 0 | 10.53 | 133 | 51 |
| E | 0 | 0 | 4.25 | 136 | 52 |
| R | 0 | 0 | 12.48 | 139 | 53 |
| K | 0 | 0 | 10.53 | 140 | 54 |
| D | 0 | 0 | 3.65 | 141 | 55 |
| K | 0 | 0 | 10.53 | 145 | 56 |
| Y | 0 | 0 | 10.07 | 146 | 57 |
| K | 0 | 0 | 10.53 | 147 | 58 |
| E | 0 | 0 | 4.25 | 148 | 59 |
| Y | 0 | 0 | 10.07 | 151 | 60 |
| G | 2.34 | 0 | 0 | 153 | 61 |

http://aris.gusc.lv/ChemFiles/ChromoHem/MyoGlobOxDeoxCoBiliverdin/1MBOpI.xls
http://aris.gusc.lv/ChemFiles/ChromoHem/MyoGlobOxDeoxCoBiliverdin/1MBOaaLin153.doc

```
     SQ SEQUENCE 153 >1MBO:A|PDBID|CHAIN|SEQUENCE
1    VLSEGEWQLV LHVWAKVEAD VAGHGQDILI RLFKSHPETL EKFDRFKHLK
51   TEAEMKASED LKKHGVTVLT ALGAILKKKG HHEAELKPLA QSHATKHKIP
101  IKYLEFISEA IIHVLHSRHP GDFGADAQGA MNKALELFRK DIAAKYKELG
151  YQG
```

61 pKa values in table make the sum 449,21.....

Calculate the sum of 61 pKa values from table .....

## Myoglobin shuttle charges on in **lungs** $O_2$ in **tissue** $H^+.HCO_3^-$

Protolytic constant $pK_a = pK_{mean}$ 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 $NpK_a$:

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

**11.1** Summary acid groups on protein molecule number NpKa=59....+2.....=.... 153 amino acids on molecule chain 59+2 of them protolytic constants $pK_a$ for side groups. N-terminal valine V $pK_{aNterminal}$=9.62 and C-terminal glycine G $pKa_{Cterminal}$=2.34
Sum calculate as

$\Sigma pKa_{Rside\ group}+pK_{aNterminal}+pKa_{Cterminal}=$ .....

**11.2** Average acid group constant $pK_{mean}= pK_a$ = IEP **ISOELEKTRIC POINT** NpKa=59.....+2.....=..... IEP=449,21 / 61 =.....
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** & **-$NH_3^+$** pozitiv **-$COO^-$** & **-$NH_3^+$** negativ **-$COO^-$** & **-$NH_2$**
Underline existing: positive (+) or zero charge or negative (-)!

**11.3** Myoglobin molecule charge Signe + zero „**0**” or – at physiologic pH=7.36

Underline existing:

**COOH**,**$NH_3^+$** positive+ pH=7.36<IEP=**7,3641** negative **-$COO^-$**,**$NH_2$**.

**11.4** Myoglobin molecule charge + zero „**0**” or – at e**lectrophoresis** pH **8.8**

Underline existing:

**COOH**,**$NH_3^+$** positive+ IEP=**7,3641**<pH=**8,8** negative **-$COO^-$**,**$NH_2$**.

**11.5** Calculate $C=10^{-7,3559}$ M sperm vale myoglobin solution

by *Ostwald dilution law* concentration C in logarithm: $pH=\frac{pK_a - \log C}{2}=$

$$=\frac{7,3641-\log 10^{-7,3559}}{2}=\frac{7,3641+7,3559}{2}=$$ **14,720** / 2 =.....

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

David Richardson, Celia Bonaventura, and Jane Richardson, Protein Science vol. 3. Oct.1994

Download: http://aris.gusc.lv/ChemFiles/ChromoHem/HbOxDeoxCO/2HCOProTour8.kin MAGE aplication
Text 1994 2023: Āris Kaksis RSU 2023; O2Solutions.pdf Āris Kaksis RSU 2023 [6]

**B.** Open the folder "HbOxDeoxCO" and click on "2HCOProTour8.kin" will be lunched KineMAGE application of representation for Human Haemoglobin investigation for Hb structure conformation change:
THE PROTEIN TOURIST #8 - THE **T- R**, DEOXY-OXY TRANSITION IN HUMAN HAEMOGLOBIN

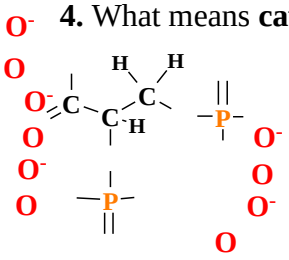
Read, sea and let to understand of given problem from description on 4 pages
that You have, try to investigate on given interactive structure presentations 3HHB and 2HCO.

To answer to following questions! ..... α...., α...., β...., β...

**1.** How menu quaternary 4° structure subunits has **Haemoglobin** molecule? To name it's!

**2.** What means **Tense state** of Haemoglobin? 3. What means **Relax state**?

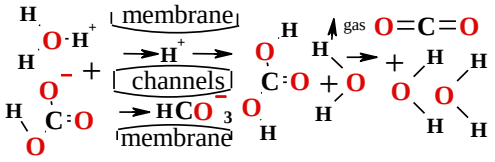
**4.** What means **cavity** of Haemoglobin molecule? [$BPG^{5-}$] shift equilibrium to .....



**5.** Put in $BPG^{5-}$ structure atoms **O**, $O^-$ ! $BPG^{5-}$ switch equilibrium as response to **blood plasma** [$O_{2aqua}$] concentration decrease and change Haemoglobin (**Hb**) from **oxy R** to **deoxy T** conformation. Reaching venous [$O_{2aqua}$]=**0,426•10$^{-5}$M** after 4 $O_2$ desorbtion. Write 4$O_2$ oxygen molecules adsorbtion equilibrium in **lungs** releasing 4$H^+$ and 4$HCO_3^-$:
in **lungs** $4O_{2aqua}+(H^+(HCO_3^-))_4$**deoxyT-**$BPG^{5-}$<=>**oxyR**$(O_2)_4+4H^++BPG^{5-}+4HCO_3^-$.....

**6** By ..... drive $H_3O^++HCO_3$ transport down gradients of membranes channels exhaling $CO_{2gas}+H_2O$ and $O_{2aqua}+H_2O$ opposite gradients osmosis in aquaporins inhaling oxygen $O_2$. [14] CA

Tissues consume oxygene 4 $O_{2aqua}$ in exoergic oxidation reactions. Homeostasis in **lungs** arterial concentration is [$O_{2aqua}$]=**6•10$^{-5}$M** and erytrocite [$BPG^{5-}$]=**5mM**. Inhale oxygen $O_2$ from air promotes $CO_2\uparrow$ gas release through increase concentration of hydrogen 4$H^+$ and bicarbonate $HCO_3^-$ ions. Blood buffer system dominate pH=7,36 bicarbonate $HCO_3^-$ ions and with protons $H^+$ cross membrane in parallel channels on surface of alveoli epithelia cells have joined! Reactants supply elevate exhaled $CO_2$.




citosol => surface

**Hb** adsorbs $O_2$ and departures $H^++HCO_3^-$. **Endothermic** reaction consume heat so cooling lungs $H^+ + HCO_3^- + Q <=> H_2O + CO_2\uparrow$.
Surface of epthelia pH 5.5 anti-bacterial and anti-septic prevent infection in organism.

1) hydrogen $H^+$ ion acidity increase shifts equilibrium to ....
**Hb** adsorbs $O_2$ yield $H^+$.Cell surface pH=5.5 has anti-bacterial as well as anti-septic properties to prevent infection in organism.; 2) bicarbonate $HCO_3^-$ concentration increase shifts equilibrium to .....
3) heating + **Q** shifts equilibrium as Air breathing human as well as animals have the lungs located inside body and equipped with heat producing cells in alveolar area as heating shifts equilibrium to .....

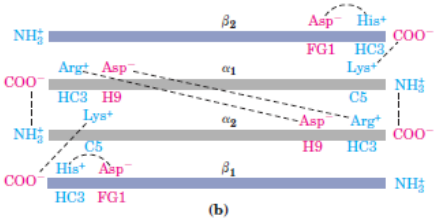
**7.** Where **hems** lay and how many **hems** has Haemoglobin molecule? β..., β....,.α....,.α..... in sub units.

**8.** What is the maximum adsorbed oxygen molecules $O_2$ into Haemoglobin molecule and what the main sense where occur the Haemoglobin molecules in animal bodies? on 4 heme $Fe^{2+}$ .....with 4$O_2$ molecules.

**9**. Which **proximal** α, β. His....., ..... N atom touch to heme iron $Fe^{2+}$ by donor acceptor-bond?

**10**. Which **distal** α, β. histidine His....., .... N atom is protonated $H^+$ **deoxy**, deprotonated **okxy**?

**11.** View2 PO4 site" un „View3 dimmer rot" identify ten = 2*5 five salt bridges pairs of amino acids!
See 8th page 21.fig. and 14th page in lecture :




| -COO$^-$..$^+$H$_3$N- | -COO$^-$..$^+$H$_3$N- |
|---|---|
| 1 α1Arg.....α2Val..... | 6 α2Asp.....α1Arg..... |
| 2 α2Arg.....α1Val..... | 7 β2Asp.....β2His..... |
| 3 α1Arg.....α2Lys..... | 8 β1Asp.....β1His..... |
| 4 α2Arg.....α1Lys..... | 9 β2His.....α1Lys..... |
| 5 α1Asp.....α2Arg..... | 10 β1His.....α2Lys..... |

Close the MAGE Observe and describe: http://aris.gusc.lv/ChemFiles/ChromoHem/HbOxDeoxCO/tetramer.avi

**12.** **Tense state** and **Relax state** change of conformation in **Haemoglobine** molecule!

Shuttle reaction homeostasis equilibrium **Deoxy**<=>**oxy** $O_{2aqua}$<=>$H^++HCO_3^-$ stabilizes concentrations:
lungs $4O_{2aqua}+(H^+$His63,58$)_4$Val1$(NH_4^+$salt bridge $HCO_3^-)_4Hb_T$<=>$(Val1(NH_4^+))_4Hb_R(O_2)_4+4H^++4HCO_3^-$ tissue
Hypoxia deficiency stress concentration less [$O_{2aqua}$]= .....**M**. 2023: Āris Kaksis
and homeostasis arterial blood concentration [$O_{2aqua}$] = .....**M**.
homeostasis concentration [$HCO_3^-$]+[$CO_{2aqua}$]=.....**M** and [$CO_{2aqua}$]=.....**M**.
mitochondrial produced amount of one blood circuit [$HCO_3^-$]+[$CO_{2aqua}$]=..... M.
mitochondrial produced amount of one blood circuit [$O_{2aqua}$]=..... M.
daily human organism uses 500 g $O_2$ oxygen amount $n_{O_2}=^{500\ g}/_{32\ g/mol}$=..... mol.
What is daily carbon dioxide amount respiration out of human organism ? $n_{CO_2}$=..... mol

**C.** FireFox professor **Eric Marz** tutorial **Haemoglobin** at RSU Sickle Cell anaemia.

**1.** What kind of intermolecular bonds don't have Haemoglobin molecule? Underline it!

Are known 5 units of intermolecular bonds in Biochemistry – 1.**Hydrogen**, 2.**Hydrophobic,** 3.**Salt bridges,**

**4.Sulfur -S-S- disulfide bridges** and **5.Coordinative donor-acceptor bonds**

**2.** 8 **helixes** make up Haemoglobin molecule β (beta) subunit?.Call its and call into which one structure kind 8 helixes are lying !. ..., ..., ..., ..., ..., ..., ..., ..., .....structure

**3.** What kind of intermolecular bonds make up the **helix** secondary 2° structure of proteins? .....

**4.** Whichever seven amino acid residues make up the hydrophobic property of E helix protein chain?

Check and call them! ALA....., PRO....., PHE....., LEU.....,VAL....., VAL....., ALA.....

**5.** Whichever seven amino acid residues make up the **hydrophilic** property of E helix protein chain?

Check and call them! ! ASP....., SER....., LYS....., LYS....., HIS....., LYS....., LYS....

**6.** Into which one kind of pocket is placed **heme hydrophilic** or hydrophobic? Are there present or absent ?

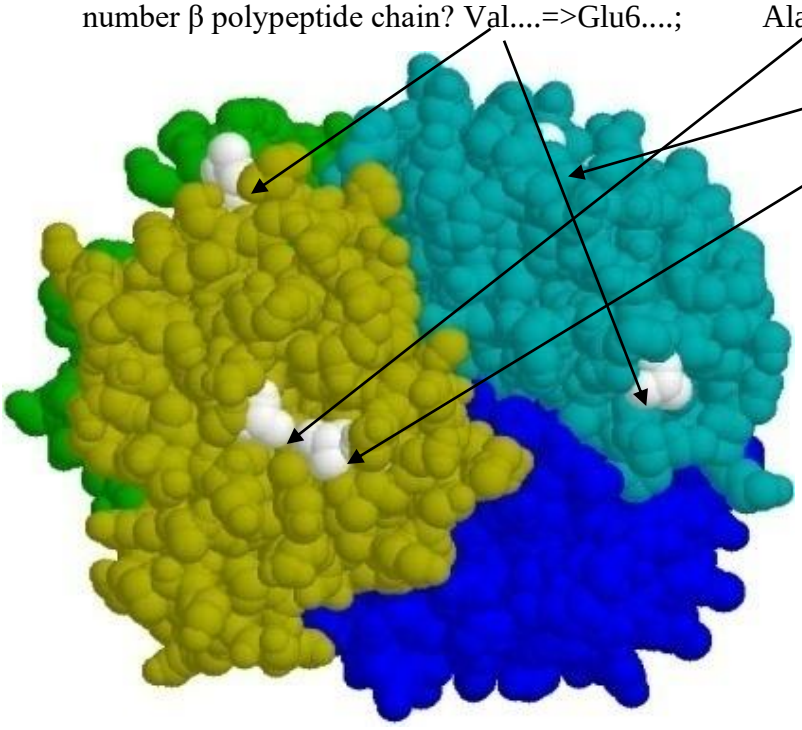
**water $\mathbb{H}_2O$, Hydroxonium ions $\mathbb{H}O^+$, oxygen $O_2$, free** (delocalized) **electrons 30e$^-$**?

..... , ..... ..... .....

**7.** What intermolecular bonds bind Haemoglobin molecule subunits α1, β1, β2, α2 and which type in biochemistry known bond perform conformation change between **Relax** to **Tense state** after entrance in to cavity

2,3-bisphosphoglycerate anion? Underline which of typed five bonds are its and which one type conformation!- 1.**Hydrogen** 2.**Hydrophobic** 3.**Salt bridge 4.sulfur -S-S-disulfide bridge** 5.**coordinative** donor-acceptor **bond** Slides on 19.page:

**8.** Which three amino acids replace and cause the sickle cell anemia, to call them and show its position number β polypeptide chain? Val....=>Glu6....; Ala.....un Leu.....



4th class work Experimental Research in Medical Chemistry
Open solutions CHromo Proteins: HromoProteinsAS.pdf
Open answers on work papers HromoProteinsAnswere.doc
HromoProteinsAnswere.doc and
Send for evaluation 4_Sername.doc with e-mail to me.
Download and open six experimental research applications :
http://aris.gusc.lv/ChemFiles/ChromoHem/MyoGlobOxDeoxCoBiliverdin/1MBODeOxyLopez.kin
Open FireFox link of Santa Barbara University for 11 questions:
http://aris.gusc.lv/ChemFiles/ChromoHem/MyoGlobOxDeoxCoBiliverdin/Myoglobin.htm
Continue 11 answerers open video move oxy- deoxy Myoglobin:
http://aris.gusc.lv/ChemFiles/ChromoHem/MyoGlobOxDeoxCoBiliverdin/oxydeoxy.avi
Open MAGE publication link and perform research-amswers next 12 questions:
http://aris.gusc.lv/ChemFiles/ChromoHem/HbOxDeoxCO/2HCOProTour8.kin
Continue 12 jautajumus atbildēt ar video filmu par oksī - deoksī Hb:
http://aris.gusc.lv/ChemFiles/ChromoHem/HbOxDeoxCO/tetramer.avi
Lunch after the download: http://aris.gusc.lv/ChemFiles/htdocsTGF.zip and unzipped on
FireFox professor **Eric Marz** tutorial **Haemoglobin** adapted to Riga Stradin's University:
htdocsTGF/hemoglobEricMarzUMas/INDEX.htm Sickle Cell anaemia.
Se you in onpractical class work .

sciencerly,
Aris Kaksis

**References.**


[1] David R. Lide. CRC Handbook of Chemistry and Physics .90th ed. Taylor and Francis Group LLC; 2010 .
[2] Prigogine I., Defey R. Chemical Thermodynamics. Longmans Green & co ©; 1954.
[3] Prigogine I., Nicolis G. Self-Organization in Non-Equilibrium Systems. Wiley, 1977.
[4] Prigogine I. Time, Structure and Fluctuations. Lecture, The Nobel Praise in Chemistry; 1977.
[5] Kuman M. New light on the attractors creating order out of the chaos. Int J Complement Alt Med.; 2018; 11(6); 337.;
[6] Nelson DL, Cox MM. Lehninger Principles of Biochemistry. 5th ed. New York: W.H. Freman and company; 2008.
[7] Xing W, Yin G, Zhang J. Rotating Electrode Method and Oxygen Reduction Electrocatalysts. *Elsevier*; 6 (2014) .
[8] Alberty RA. Biochemical Thermodynamic's : Applications of Mathematics. John Wiley & Sons, Inc. 1-463, (2006).
[9] Pinard MA, Mahon B, McKenna R. Probing the Surface of Human Carbonic Anhydrase for Clues towards the Design of Isoform Specific Inhibitors. *BioMed Research International*; **2015**, 3 (2015).
[10] Kotz JC, Purcell KF. Chemistry and chemical reactivity. Saunders College Publishing; 1991.
[11] White VM. THE CARBON CYCLE, ISOTOPES, AND CLIMATE I and II. Lectures 37, 38; 2003 .
[12] Hanania J, Pomerantz C, Stenhouse K, Toor J, Donev J. Carbon cycle. University of Calgary's 2020 .
[13] Der wohltemperierte Planet. *Der Spiegel*. 2007 Nr.19:148-154. German .
[14] Kaksis A. The Biosphere Self-Organization Attractors drive perfect order homeostasis reactions to link bioenergetic with functionally activate oxygen and carbon dioxide molecules. 7th International Conference on New Trends in Chemistry September 25-26, 2023.27-32.