Name Surname :_____ Group Nº _____2023

Cyclo Oxygenase Enzyme Protein Task **COX** studies:

ChemScape MDLi **RasMol** (RasMac); MAGE FireFox application.

Aris Kaksis 2023 Riga Stradin's University prepared molecular tutorial based on **Eric Marz (1997)** ***Massachusetts University***: http://aris.gusc.lv/ChemFiles/CycloOxigenase/cycox.html

3. **N-terminus** domain starting amino acid is His33.... and **C-terminus** amino acid is Gln583.....

What total of amino acids are on Cyclooxigenase 2 **COX – 2** chain 604... (see 2nd page)

What number amino acids are on chain **3HS5.pdb**...550+1=551.....?

5. **N-terminus** amino acid is His33..... andfinishing **C-terminus** amino acid is Val582.....

What amino acids are on chain **3TZI.pdb**...549+1=550.....?

4. **N-terminus** amino acid is Pro32.....and **C-terminus** amino acid is ASP584.....

What number amino acids are on chain **1DIY.pdb**...552+1=553.....?

6. **N-terminus** amino acid is Val33.... and **C-terminus** amino acid is Pro583.....

and what are on **1PTH.pdb, 1EQG.pdb** chains 583-33=550; 550+1=551.....?

7. What secondary structures have Cyclo oxygenase COX? **31 Alpha-helices**, **beta sheets**.....

8. What number of **alpha helices** constitute COX polypeptide molecule?**31 Alpha-helices**.....

9. What type of **beta structure** and **sheets** and how many **beta strands** has COX molecule?

31PTH–43TZI-53N8Z beta structure three beta sheets with 2 anti-parallel strands in each sheet....

10. What units with its secondary 2º structure components are found in given tertiary 3º structure domain biological unit of COX enzyme Cyclo oxygenase? What type of secondary 2º structure segments constitutes COX enzyme Cyclo oxygenase?. **31 Alpha-helices**; .....

beta structure three beta sheets with 2 anti-parallel strands in each sheet.....

11. What amino acid binds non-steroidal anti-inflammatory drugs **NSAIDs** or Arachidonic Acid as substrate - ligand for prostaglandin H2 (PGH2) and G2 (PGG2) synthesis? .....**Ser530**....

36. What **COX-2** isoelectric point IEP=pH=$pK_{a\text{-}vid}$ at physiologic pH=7,36 ? To determine water solution pH with **COX-2** concentration $C=10^{-6,8473}$ M ($^{mol}/_{Litre}$)!

Prostaglandine G/H synthase – Cyclooxigenase 2 **COX** - 2

http://aris.gusc.lv/ChemFiles/CycloOxigenase/3TZIpIStudS.pdf; http://aris.gusc.lv/ChemFiles/CycloOxigenase/3TZIpI.xls

Sequrnce of 604 AA Amino Acids in COX-2 molecule 3HS5.pdb, 3TZI.pdb:

```
MLFRAVLLCAALGLSQAANPCCSNPCQNRGECMSTGFDQYKCDCTRTGFYGENCTTPEFLTRIKLLLKPTPNTVHYILTH
FKGVWNIVNNIPFLRSLIMKYVLTSRSYLIDSPPTYNVHYGYKSWEAFSNLSYYTRALPPVADDCPTPMGVKGNKELPDS
KEVLEKVLLRREFIPDPQGSNMMFAFFAQHFTHQFFKTDHKRGPGFTRGLGHGVDLNHIYGETLDRQHKLRLFKDGKLKY
QVIGGEVYPPTVKDTQVEMIYPPHIPENLQFAVGQEVFGLVPGLMMYATIWLREHNRVCDILKQEHPEWGDEQLFQTSRL
ILIGETIKIVIEDYVQHLSGYHFKLKFDPELLFNQQFQYQNRIASEFNTLYHWHPLLPDTFNIEDQEYSFKQFLYNNSIL
LEHGLTQFVESFTRQIAGRVAGGRNVPIAVQAVAKASIDQSREMKYQSLNEYRKRFSLKPYTSFEELTGEKEMAAELKAL
YSDIDVMELYPALLVEKPRPDAIFGETMVELGAPFSLKGLMGNPICSPQYWKPSTFGGEVGFKIINTASIQSLICNNVKG
CPFTSFNVQDPQPTKTATINASASHSRLDDINPTVLIKRRSTEL
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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	9,21		1	1	E		4,25	156	48	R		12,48	297	94	Y		10,07	446	140
R		12,48	4	2	D		3,65	159	49	C		8,18	299	95	E		4,25	451	141
C		8,18	9	3	K		10,53	161	50	D		3,65	300	96	Y		10,07	452	142
C		8,18	21	4	E		4,25	162	51	K		10,53	303	97	R		12,48	453	143
C		8,18	22	5	E		4,25	165	52	E		4,25	305	98	K		10,53	454	144
C		8,18	26	6	K		10,53	166	53	H		6	306	99	R		12,48	455	145
R		12,48	29	7	R		12,48	170	54	E		4,25	308	100	K		10,53	459	146
E		4,25	31	8	R		12,48	171	55	D		3,65	311	101	Y		10,07	461	147
C		8,18	32	9	E		4,25	172	56	E		4,25	312	102	E		4,25	465	148
D		3,65	38	10	D		3,65	176	57	R		12,48	319	103	E		4,25	466	149
Y		10,07	40	11	H		6	190	58	E		4,25	325	104	E		4,25	470	150
K		10,53	41	12	H		6	193	59	K		10,53	328	105	K		10,53	471	151
C		8,18	42	13	K		10,53	197	60	E		4,25	332	106	E		4,25	472	152
D		3,65	43	14	D		3,65	199	61	D		3,65	333	107	E		4,25	476	153
C		8,18	44	15	H		6	200	62	Y		10,07	334	108	K		10,53	478	154
R		12,48	46	16	K		10,53	201	63	H		6	337	109	Y		10,07	481	155
Y		10,07	50	17	R		12,48	202	64	Y		10,07	341	110	D		3,65	483	156
E		4,25	52	18	R		12,48	208	65	H		6	342	111	D		3,65	485	157
C		8,18	54	19	H		6	212	66	K		10,53	344	112	E		4,25	488	158
E		4,25	58	20	D		3,65	215	67	K		10,53	346	113	Y		10,07	490	159
R		12,48	62	21	H		6	218	68	D		3,65	348	114	E		4,25	496	160
K		10,53	64	22	Y		10,07	220	69	E		4,25	350	115	K		10,53	497	161
K		10,53	68	23	E		4,25	222	70	Y		10,07	359	116	R		12,48	499	162
H		6	75	24	D		3,65	225	71	R		12,48	362	117	D		3,65	501	163
Y		10,07	76	25	R		12,48	226	72	E		4,25	366	118	E		4,25	506	164
H		6	80	26	H		6	228	73	Y		10,07	371	119	E		4,25	510	165
K		10,53	82	27	K		10,53	229	74	H		6	372	120	K		10,53	518	166
R		12,48	95	28	R		12,48	231	75	H		6	374	121	C		8,18	526	167
K		10,53	100	29	K		10,53	234	76	D		3,65	379	122	Y		10,07	530	168
Y		10,07	101	30	D		3,65	235	77	E		4,25	384	123	K		10,53	532	169
R		12,48	106	31	K		10,53	237	78	D		3,65	385	124	E		4,25	539	170
Y		10,07	108	32	K		10,53	239	79	E		4,25	387	125	K		10,53	543	171
D		3,65	111	33	Y		10,07	240	80	Y		10,07	388	126	C		8,18	555	172
Y		10,07	116	34	E		4,25	246	81	K		10,53	391	127	K		10,53	559	173
H		6	119	35	Y		10,07	248	82	Y		10,07	395	128	C		8,18	561	174
Y		10,07	120	36	K		10,53	253	83	E		4,25	402	129	D		3,65	570	175
Y		10,07	122	37	D		3,65	254	84	H		6	403	130	K		10,53	575	176
K		10,53	123	38	E		4,25	258	85	E		4,25	410	131	H		6	585	177
E		4,25	126	39	Y		10,07	261	86	R		12,48	414	132	R		12,48	587	178
Y		10,07	133	40	H		6	264	87	R		12,48	419	133	D		3,65	589	179
Y		10,07	134	41	E		4,25	267	88	R		12,48	424	134	D		3,65	590	180
R		12,48	136	42	E		4,25	276	89	K		10,53	435	135	K		10,53	598	181
D		3,65	143	43	Y		10,07	287	90	D		3,65	439	136	R		12,48	599	182
D		3,65	144	44	R		12,48	293	91	R		12,48	442	137	R		12,48	600	183
C		8,18	145	45	E		4,25	294	92	E		4,25	443	138	E		4,25	603	184
K		10,53	152	46	H		6	295	93	K		10,53	445	139	L	2,36		604	185
K		10,53	155	47															

185 of 604 amino acids active pKa sum in tabele is 1456,06..... average constant value pKa=7,8727.....

Calculation tasks for COX-2 molecule 1DIY.pdb

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 NpKa:

$IEP=pKa_{mean}=(\Sigma pK_{aRside\ group}+pK_{aNterminal}+pK_{aCterminal})/NpKa$

1.Acid groups number in sum NpKa=183.....+2.....=185.....

604 amino acids of them protolytic constants pK_a for side groups 183+2 terminus N and C, N-terminal metionine M $pK_{aNterminal}$=9.21 and C-terminal leucine L $pKa_{Cterminal}$=2.36.

Sum are calculating as $\Sigma pK_{aRside\ group}+pK_{aNterminal}+pK_{aCterminal}$ = 1456,06.....

2. Average acid group constant pK_{mean}= pK_a = IEP **ISOELEKTRIC POINT**

NpKa=183.....+2.....=185..... IEP=1456,06 / 185 =**7.8727**.....

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 (-)!

3. Determine COX-2 molecule charge sign (+). zero „**0**" or (-) at physiologic pH=7.36

(+).....positive.charge "0".....underline

-COOH & **-NH_3^+** positive (+) charge ……. pH=7.36 < IEP=**7.87** ..... charge negative(-) **-COO^-** & **-NH_2**.

4. Determine COX-2 molecule charge sign (+). zero „**0**" or (-) at **electrophoresis** pH **8.8**

(-).....is negative charge .....underline

-COOH & **-NH_3^+** positive (+) charge ……. IEP=**7.87**< pH=8.8 ..... charge negative(-) **-COO^-** & **-NH_2**.

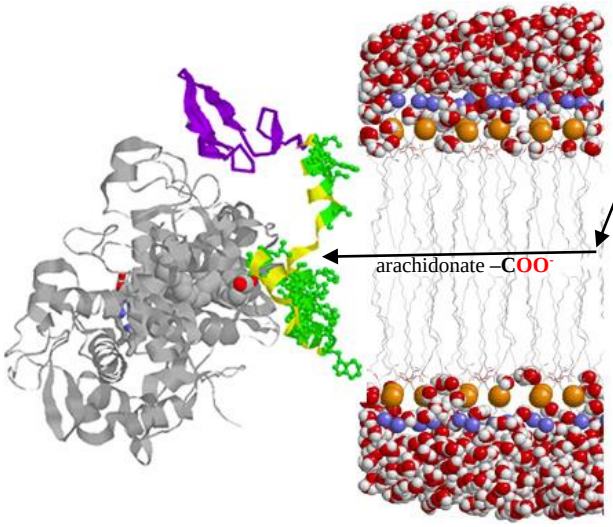
5. Calculate COX-2 solution pH at concentration $C=10^{-6,8473}$ M ($^{mol}/_{Litre}$)

by *Ostwald dilution law* concentration $C=10^{-6,8473}$ M in logarithm:

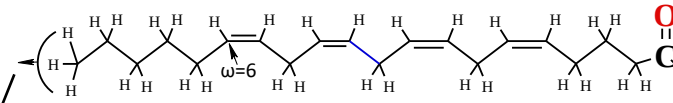
$$pH=\frac{pK_a-\log C}{2}=\frac{7.8727-\log 10^{-6,8473}}{2}=\frac{7.8727+6,8473}{2}=\mathbf{14,72}\ /\ 2=7,36.....$$

7,36 Attractor COX-2 concentration is $C=10^{-6,8473}$.....M .

6 COX-2 molecule have positive charged **^+H_3N-** fixation at negative **-PO_4^-** charged



phosphate groups **-PO_4^-**....><**^+H_3N-**..... bilateral of broken membrane, that could slip ω=6 metil group



-O^- in COX tunnel for prostaglandin synthesis. Cell membrane damage expose nonpolar hydrocarbon chains at which stick COX nonpolar (green) amino acids. Positive charged groups **N-NH_3^+**(+) faces molecule perpendicular to membrane hydrophobic binding to COX hydrocarbon chains.

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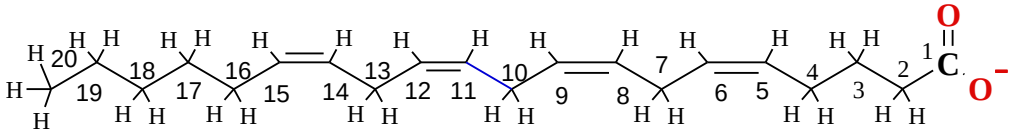
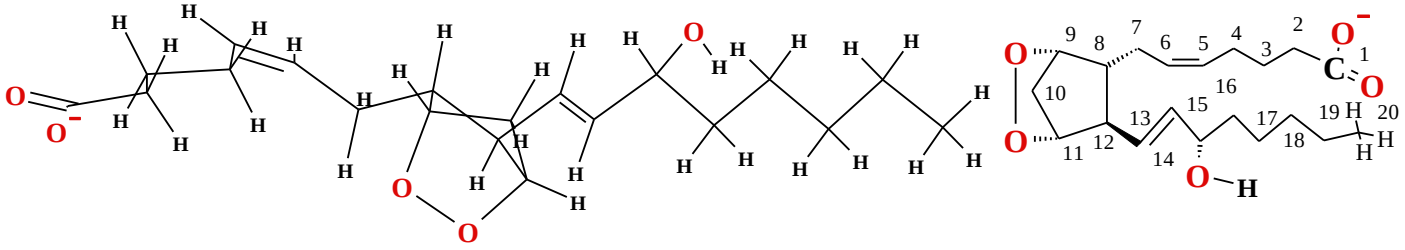
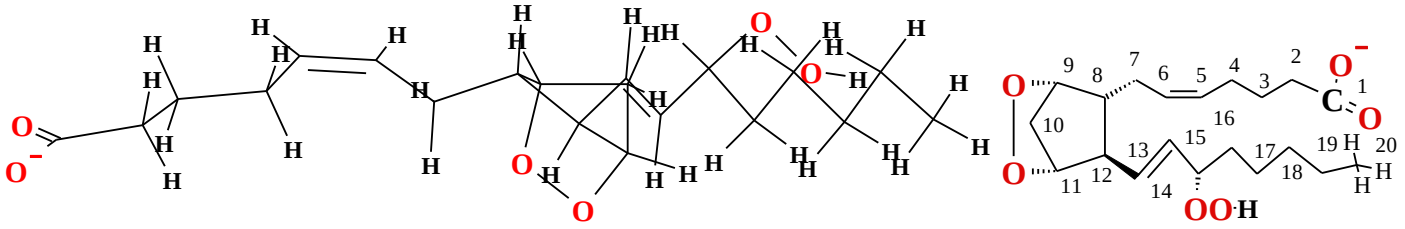
12. Appoint **O** atoms and account **C**..... **atoms** in structure pH=7.36!



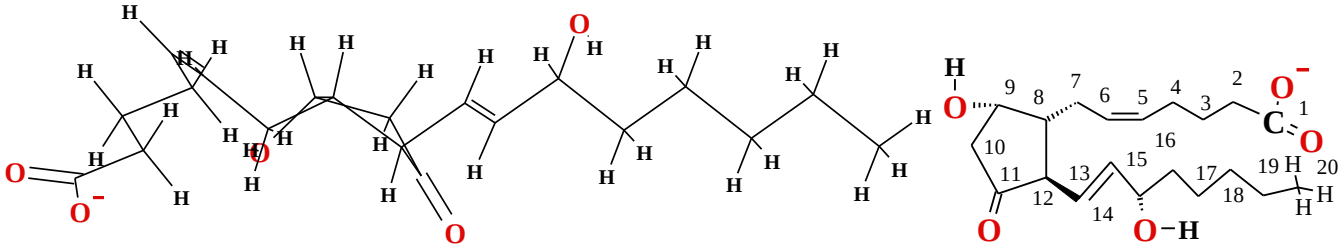
13. Appoint **O** atoms! and account **C**..... **atoms** in H2 (PGH_2) structure pH=7,36 !

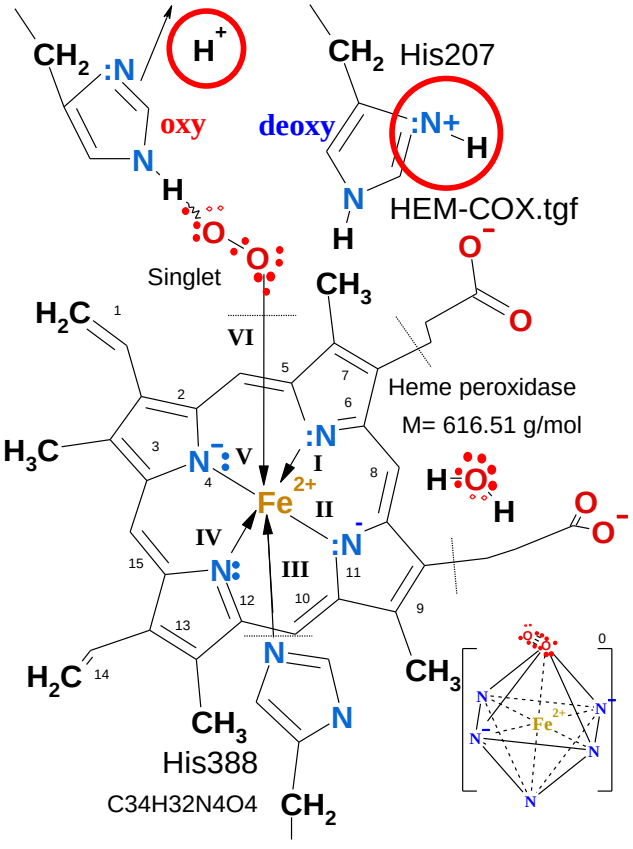


14. Appoint **O** atoms! and account **C**..... **atoms** in G2 (PGG_2) structure pH=7,36!



15. Appoint **O** atoms! and account **C**..... **atoms** in D2 (PGD_2) structure pH=7,36!





16. Which active sites contains the heme structure? ... active site heme is **peroxidase**.....

17. What is the central ion of coordinative sphere and what is oxidation number of central ion?
.....Fe^{2+} Iron(II).....

18. Triplet usual has three **•:O≡O:•** covalent bonds, as ever one electron pair degenerates is antybonding radical, therefore sum in **triplet** gives <u>double</u> bond **:O=O:**. Hem **peroxidase** iron Fe^{2+} adsorbs oxygen **singlet** molecule as **H:-••:O-:-O:••-:H** has one covalent bond **•::O-:-O::•**..... therefore is peroxidase enzyme COX.

19. Which atoms bind coordinative of iron Fe^{2+}?
:N, ...:N$^-$,.....:N, ...:N$^-$,.....:N Hist388.....
and singlet oxygen **•::O-:-O::•**.....
What is the coordination number?
coordination number is. **N=VI**.....

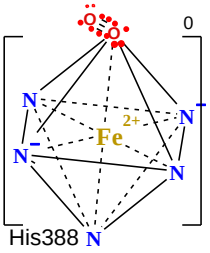
20. Haw many free delocalized electrons **e$^-$** are present into Heme structure including carbon to carbon double bond >C=C< and unshared electron pair at atoms **:N** in heme? **n**=2*15=**30**.....electrons

20a. Deep in the tunnel, at the far end, lies Tyr-385-**O-H**, a catalytically important residue with Heme-dependent peroxidase activity is required for radical reaction activity by cyclooxygenase. For the radical reaction is formed Tyrosine: Tyr-385-**O*** radical,…….

21. Which residues make module that is similar to epidermal growth factor? 33-72 .....

Which cell receptors signalin epidermal growth faktor in COX ? leucocytes.....

22. Appoint hexagonal symmetry iron(III) coordination geometry!



23. What residues are modules of membrane-binding motif!

.....

24. What residues of membrane-binding motif have the hydrophobic side chain amino acids?

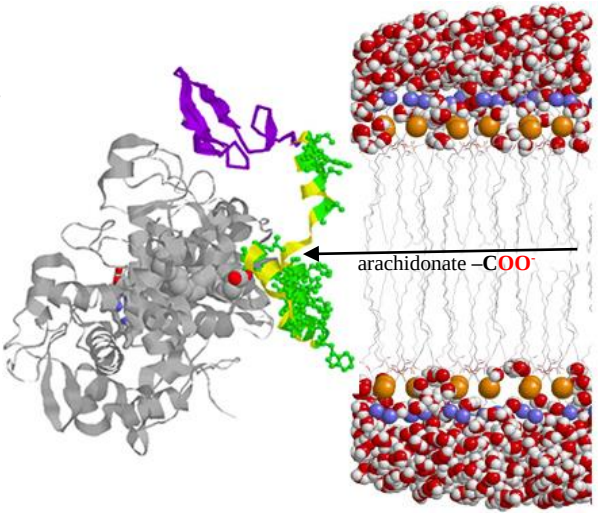
74,75,77-78,82,88-89,91-93,98-100,102-103,105,107-108,112,115,116.

25. What two kind active sites contain globular structure of COX enzyme? .....

.....contains both the **cyclo oxygenase** and **peroxidase** active sites .....

26. What hydrophobic side chains containing amino acids make the walls of the tunnel on ..... defined four **COX** alpha helices: **H5**:107-123, **H14**:325-353, **H17**:378-384, **H28**: 520-535?

H5:Phe107.....,Ile108.....,Leu112.....,Met113.....,
Leu115.....,Val116.....,Leu117.....,Val119.....,Leu123.....
H14:Leu328.....,Phe329.....,Ala333.....,Leu334.....,
Ile335.....,Leu336.....,Ile337.....,Gly338.....,Ile341.....,
Ile343.....,Val344.....,Ile345.....,Val349.....,Leu352.....
H17: Ala378.....,Met379.....,Phe381.....,Leu384.....
H28: Met522.....,Ile523.....,Met525.....,Gly526.....,
Ala527.....,Pro528.....,Phe529.....,Leu531.....,Gly533.....,
Leu534.....,Leu535.....



COX membrane damage site

28. What amino acid in the tunnel is covalently modified **–O–** Acylate by Warfarin, Aspirin and Bromoaspirin, thus irreversibly inactivating both COX-1 and COX-2? .....

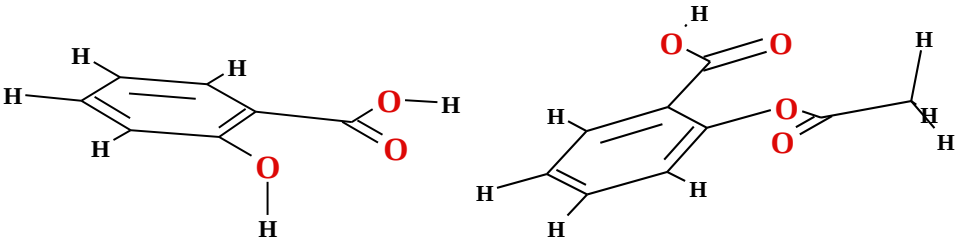
..... **Ser530** L-serine residue as **–O–**Acetyl-L-serine .....

29. What amino acid in the tunnel binds non-steroidal anti-inflammatory drugs **NSAIDs** Ibuprofen , Flourbiprofen and IndoMethacin form the salt bridge between drug carboxylate **–C=O-O-**....$^{+}$**H$_2$N-and** amino acid residue amonium**1PTH.pdb** , **1DIY.pdb**? **Arg-120** .....

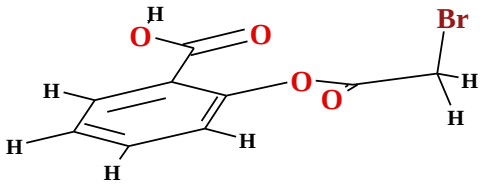
30. What is the name of transport protein in blood of aspirin, bromaspirin and warfarin and other water insoluble molecules like as fatty acids with carboxylate **–C=O-O-** groups?

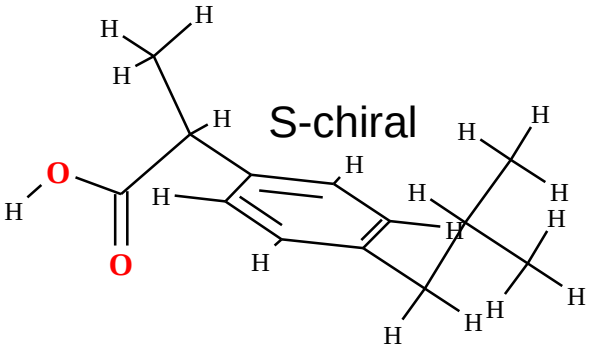
That are water insoluble bioorganic compounds called Lipids, which transporting in water **lipoproteins** Such protein is in blood plasma with 0.6 mM concentration: **albumin**.....

31. Appoint **O** atoms! in Salicilc acid, in Aspirin structures



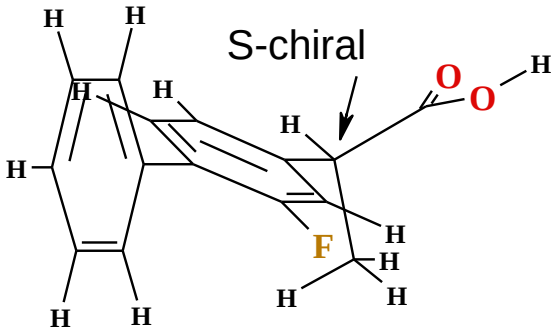
32. Appoint **O** atoms! in Bromaspirin in S-Ibuprofen structures

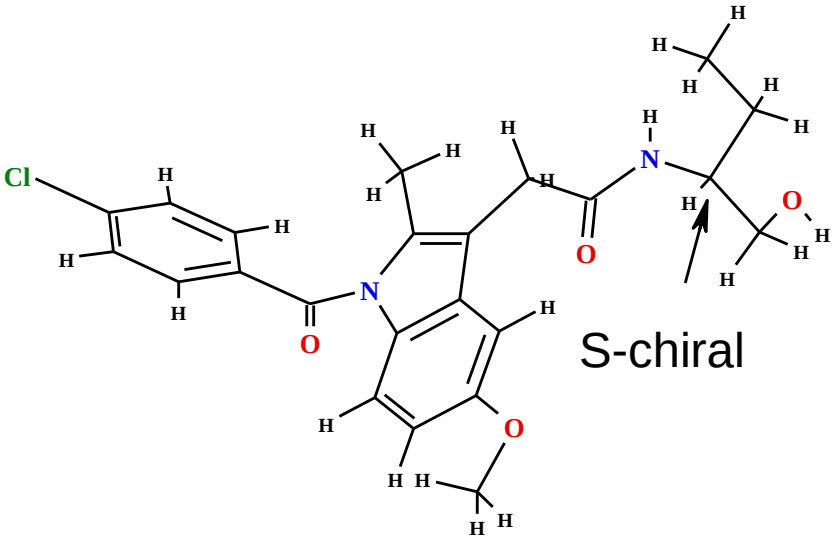




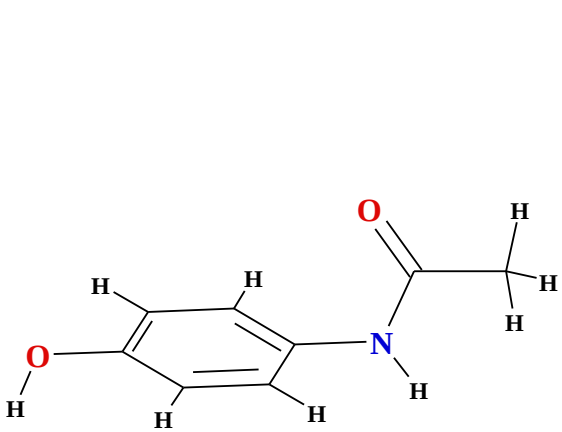
Bromaspirin **1PTH.pdb**

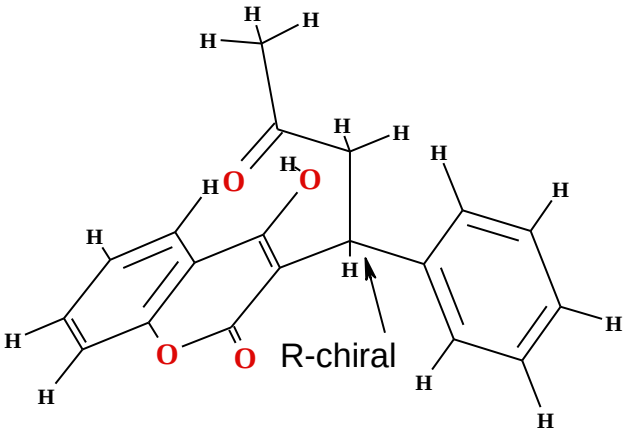
33. Appoint **O** atoms! in S-Fluribiprofen, in S-Indometacin structures!





33. Appoint **O** atoms! in R-Tailenol, in R Warfarin structures





R Warfarin **ACD 1DIY.pdb**

Cell signaling **Prostaglandins** PGG2, PGH2 controlled processes:

1) constriction of muscle cells around blood vessels,
2) aggregation of platelets during blood clotting, and
3) constriction of the uterus during labor. **Prostaglandins** also
4) deliver and signals to leucocytes, macrophages and
5) so strengthen pain too induce inflammation.

Almost all mammalian cells except erythrocytes produce one or more of eicosanoids

Prostaglandins are created from and have a common precursor molecule Arachidonic Acid.

20-carbon ω=6 compound (Greek eikosil , "twenty") with four double bonds **C20:4**:

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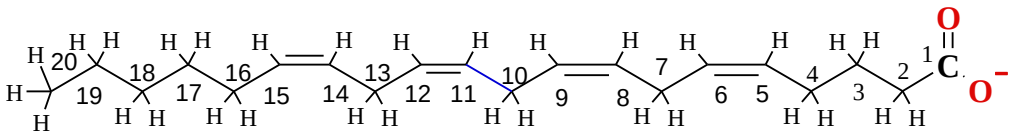
12. Appoint **O** atoms and account **C**..... **atoms** in structure pH=7.36!



prostaglandins(PGs), prostacyclins (PGIs), thromboxanes (TXs), leukotrienes (LTs).

For example: Prostaglandins: PGA2, PGE1, PGE2, PGE3, PGF2a, PGG2, PGH2

and Prostacyclin PGI2; Thromboxanes TXA2 and TXB2; Leukotriene LTE4 .

There are thought to be at least four different mechanisms of action for NSAIDs.

1.Warfarin**,** Aspirin (and also bromoaspirin) is the only one which covalently modifies - Acylate a Ser530 L-serine residue **as O-(bromoacetyl)-L-serine** in the tunnel, thus irreversibly inactivating both COX-1 and COX-2.

2. Ibuprofen acts instead by competing in a reversible fashion for the ibuprofene and Arg-120 binding site in the tunnel.

3. Members of the third class of inhibitors. Flourbiprofen and IndoMethacin cause a slow, time-dependent inhibition of COX-1 and COX-2, apparently via formation of a salt bridge between a carboxylate on the drug and Arg-120 (shown here in purple, blinking), which lies in the tunnel.

4. The drug SC-558 acts by a fourth mechanism, specifically inhibiting COX-2. It is a weak competitive inhibitor of COX-1 but inhibits COX-2 in a slow, time-dependent process. Specific COX-2 inhibitors will likely be the drugs of the future, since they will be able to selectively block the inflammation mediated by COX-2, without the potential for stomach lesions and renal toxicity **that arise from COX-1 inhibition**.