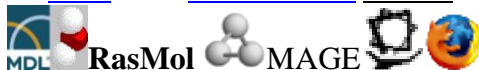


Enzyme Proteins A.Task liver CATALASE studies for student practical introduction helping material:



ChemScape MDL, RasMol, MAGE, Firefox application. B.Lunch Kenyon University: <http://aris.gusc.lv/ChemFiles/catalaseKenyon/cat1.htm> professor Elizabeth M. Boon '97, Aaron Downs '00, David Marcey and Aris Kaksis 2023 Riga Stradin's University prepared molecular tutorial

1. Call the cell organelles where first enzyme CATALASE found and classification number!

EC1.11.1.6.....organelles peroxisomes.....

2 High rate protolysis activate $\text{GH}_2\text{O} + \text{GHOOH} = 0 + 365 = 365 \text{ kJ/mol}$ to $\text{GH}_3\text{O}^+ + \text{GHOO}^- = 22.44 + 418.32 = 440.76 \text{ kJ/mol}$;

1) Protolysis $\text{HOOH} + \text{H}_2\text{O} \rightleftharpoons \text{H}_3\text{O}^+ + \text{HOO}^-$ $pK_a = 11,75$ make possible collisions of anions $\text{HOO}^- > < \text{OOH}$.

2) High activation energy $E_a = 79000 \text{ J/mol}$ colliding $\text{HOO}^- > < \text{OOH}$ with slow velocity $\vec{k} = 1.416 \cdot 10^{-16} \text{ M}^{-2}\text{s}^{-1}$ constant disproportionate OOH oxidize atoms to $\text{O}_{2\text{aqua}}$ and second atoms OOH reduce about 2 OH^- ions,

3) which neutralizing to water: $2\text{OH}^- + 2\text{H}_3\text{O}^+ \Rightarrow 2\text{H}_2\text{O} + 2\text{H}_2\text{O}$. In summary produce $\text{O}_{2\text{aqua}} + 2\text{H}_2\text{O} + 2\text{H}_2\text{O} + \text{Q}$:

$2\text{H}_2\text{O}_2 + 2\text{H}_2\text{O} \rightarrow \text{H}_3\text{O}^+ + \text{HOO}^- > < \text{OOH} + \text{H}_3\text{O}^+ \rightarrow \text{O}_{2\text{aqua}} + 2\text{H}_2\text{O} + 2\text{H}_2\text{O} + \text{Q}$ the Life resources.....

2.a Collision negative to positive $\text{HOO}^- > \text{Fe}^{3+}$ have $E_a = 29 \text{ J/mol}$ indispensable law activation energy with protonation the His74 and Asn 147 velocity constant increases over absent CATALASE 30 million times as geometric factor A improve to

$A = 0.131$: $\text{H}_2\text{O}_2 + \text{H}_2\text{O}_2 + \text{Fe}^{3+} \rightarrow \text{His74-H}^+ + \text{HOO}^- > \text{Fe}^{3+} < \text{OOH} + \text{Asn147-H}^+ \rightarrow \text{O}_{2\text{aqua}} + \text{H}_2\text{O} + \text{H}_2\text{O} + \text{Q}_{\text{exothermic}} + \text{Fe}^{3+}$.

Protolysis activate transition state complex oxygen+ water+ heat+ CATALASE,

Negative anions collisions with positive iron Fe^{3+} ion produce life resources 30 million times faster.

Write disproportionation reaction first and second peroxide molecule:

1. $\text{H-O-O-H}_{\text{protolysis}} + \text{Fe(III)} + \text{His74} \Rightarrow \text{Fe(IV)-O} + (\text{HO}^-_{\text{Red}} + \text{H}^+_{\text{protonation}} - \text{His74}) \Rightarrow \text{H}_2\text{O}$

2. $\text{H-O-O-H}_{\text{protolysis}} + \text{Fe(IV)-O}_{\text{Ox}} + \text{Asn147} \Rightarrow \text{Fe(III)} + \text{O}=\text{O}_{\text{Ox}} + (\text{HO}^-_{\text{Red}} + \text{H}^+_{\text{protonation}} - \text{Asn147}) \Rightarrow \text{H}_2\text{O}$

3. Two non enzymatic and Biochemical peroxide sources in oxygen dissolute water !

1) Heavy metal compounds iron Fe^{3+} , cooper Cu^{2+} , manganese Mn^{4+} , lead Pb^{4+}

2) Ionization radiation: ultraviolet UV radiation....., roentgen x-ray radiation....., gamma γ radiation....., beta (β^- and β^+) radiation....., alpha particles α^{2+} radiation.....

3) Peroxisomal ethylene $-\text{CH}_2-\text{CH}_2-$ dehydrogenation to cis double bond $\text{H} > \text{C} = \text{C} < \text{H}$

4. What N-terminus Asn3... and C-terminus Asn500.... amino acids have 8CAT.pdb?

What number of amino acid has CATALASE polypeptide chain? 527.....

What is number of amino acids on chain of 8CAT.pdb $500 - 3 = 497$; $497 + 1 = 498$?

4a 1-4. What 8CAT.pdb isoelectric point $\text{IEP} = \text{pH} = \text{pK}_{a\text{-vid}}$, total charge at $\text{pH} = 7,36$?

To determine water solution pH with CATALASE concentration $C = 10^{-7,021667} \text{ M}$ (mol/Litre)!

Liver Catalase (EC 1.11.1.6), present in the peroxisomes

SQ SEQUENCE 527 >8CAT:A|PDBID|CHAIN|SEQUENCE 67527,6 g/mol

MADNRDPASD	QMKHWKEQRA	AQKPDVLT	TGGGNPVGDKLNSLTVGPRGPLLVQDVVFTDEMAHFRERIPERVVHAKGAG
AFGYFEVTHD	ITRYSKAKVF	EHIGKRTPIA	VRVSTVAGESGSADTVRDRPGFAVKFYTEDGNWDLVGNNTPIFFIRDALL
FPSFIHSQKR	NPQTHLKD	PDVWDFWLSLRPESLHQVSLFSDRGI	PDGHRHMNGYGSHTFKLVNANGEAVYCKFHYKTDQ
GIKNLSVEDA	ARLAHEDPDYGLRDLFN	AIATGNYP	SWTLYIQVMTFSEAEIFPFNPFDLTKVWPHGDYPLIPVGKLVLNR
NPVNYFAEVE	QLAFDPSNMP	PGIEPSPDKMLQGR	LFAYPDTHRRLGPNYLQIPVNC
APNYYPNFS	SAPEHQPSALEHR	THFGSDVQRFNSANDDNVTQVRTFY	LKVLNEEQRKRLCENIAGHLKDAQLFIQKKAVK
NFSDVHPEY	GSRIQALLDKYNEEK	PKNAVHTYVQHGSHLSAREKANL	
10	20	30	40
MADNRDPASD	QMKHWKEQRA	AQKPDVLT	TGGGNPVGDKLNSLTVGPRGPLLVQDVVFTDEMAHFRERIPERVVHAKGAG
90	100	110	120
AFGYFEVTHD	ITRYSKAKVF	EHIGKRTPIA	VRVSTVAGESGSADTVRDRPGFAVKFYTEDGNWDLVGNNTPIFFIRDALL
170	180	190	200
FPSFIHSQKR	NPQTHLKD	PDVWDFWLSLRPESLHQVSLFSDRGI	PDGHRHMNGYGSHTFKLVNANGEAVYCKFHYKTDQ
250	260	270	280
GIKNLSVEDA	ARLAHEDPDYGLRDLFN	AIATGNYP	SWTLYIQVMTFSEAEIFPFNPFDLTKVWPHGDYPLIPVGKLVLNR
330	340	350	360
NPVNYFAEVE	QLAFDPSNMP	PGIEPSPDKMLQGR	LFAYPDTHRRLGPNYLQIPVNC
410	420	430	440
APNYYPNFS	SAPEHQPSALEHR	THFGSDVQRFNSANDDNVTQVRTFY	LKVLNEEQRKRLCENIAGHLKDAQLFIQKKAVK
490	500	510	520
NFSDVHPEY	GSRIQALLDKYNEEK	PKNAVHTYVQHGSHLSAREKANL	CATA_BOVIN

$$pK_{\text{mean}} = pK_{\text{sum}} / NpK = 1339,51 / 174 = \dots pK_a \text{ count } 172 + 2 = 174 = NpK$$

Val73, Val74, Ala76, Val116, Ala117, Pro129, Gly131, Val146, Gly147, Phe153, Phe154, Ile155, Ala158, Leu159, Leu160, Phe161, Pro162, Phe164, Ile165, Phe198, Leu199, Phe200, Leu299, Ala333, Phe334, Pro336, Met350, Leu351, Gly353, Phe356, Ala357, Pro359, Ala435 Val72, Val73, Ala75, Val115, Ala116, Pro128, Gly130, Val145, Gly146, Phe152, Phe153, Ile154, shift -1 Ala157, Leu158, Leu159, Phe160, Pro161, Phe163, Ile164, Phe197, Leu198, Phe199, Leu298, Ala332, Phe333, Pro335, Met349, Leu350, Gly352, Phe355, Ala356, Pro358, Ala434

In account are present four Cysteine residues $pK_{RR} = 8.18$; Sum of 174 pKa in table sum

AA	pK _{acoo-}	pK _{NH3+}	pK _{RR}	Nr	AA	pK _{acoo-}	pK _{NH3+}	pK _{RR}	Nr	AA	pK _{acoo-}	pK _{NH3+}	pK _{RR}	Nr	AA	pK _{acoo-}	pK _{NH3+}	pK _{RR}	Nr	
A	--	9,69		1	R		12,48		129	45	D		3,65	256	89	E		4,25	419	133
D	--	3,65		2	K		10,53		134	46	D		3,65	258	90	H		6	420	134
R	--	12,48		4	Y		10,07		136	47	Y		10,07	259	91	R		12,48	421	135
D	--	3,65		5	E		4,25		138	48	R		12,48	262	92	H		6	423	136
D	--	3,65		9	D		3,65		139	49	D		3,65	263	93	D		3,65	427	137
K	--	10,53		12	D		3,65		143	50	Y		10,07	273	94	R		12,48	430	138
H	--	6		13	R		12,48		155	51	Y		10,07	279	95	D		3,65	436	139
K	--	10,53		15	D		3,65		156	52	E		4,25	287	96	D		3,65	437	140
E	--	4,25		16	H		6		165	53	E		4,25	289	97	R		12,48	443	141
R	--	12,48		18	K		10,53		168	54	D		3,65	297	98	Y		10,07	446	142
K	--	10,53		22	R		12,48		169	55	K		10,53	300	99	K		10,53	448	143
D	--	3,65		24	H		6		174	56	H		6	304	100	E		4,25	452	144
D	--	3,65		36	K		10,53		176	57	D		3,65	306	101	E		4,25	453	145
K	--	10,53		37	D		3,65		177	58	Y		10,07	307	102	R		12,48	455	146
R	--	12,48		46	D		3,65		179	59	K		10,53	314	103	K		10,53	456	147
D	--	3,65		53	D		3,65		183	60	R		12,48	319	104	R		12,48	457	148
D	--	3,65		58	R		12,48		188	61	Y		10,07	324	105	C		8,18	459	149
E	--	4,25		59	E		4,25		190	62	E		4,25	327	106	E		4,25	460	150
H	--	6		62	H		6		193	63	E		4,25	329	107	H		6	465	151
D	--	3,65		64	D		3,65		201	64	D		3,65	334	108	K		10,53	467	152
R	--	12,48		65	R		12,48		202	65	E		4,25	343	109	D		3,65	468	153
E	--	4,25		66	D		3,65		206	66	D		3,65	347	110	K		10,53	475	154
R	--	12,48		67	H		6		208	67	K		10,53	348	111	K		10,53	476	155
E	--	4,25		70	R		12,48		209	68	R		12,48	353	112	K		10,53	479	156
R	--	12,48		71	H		6		210	69	Y		10,07	357	113	D		3,65	484	157
H	--	6		74	D		3,65		212	70	D		3,65	359	114	H		6	486	158
K	--	10,53		76	Y		10,07		214	71	H		6	361	115	E		4,25	488	159
Y	--	10,07		83	H		6		217	72	R		12,48	362	116	Y		10,07	489	160
E	--	4,25		85	K		10,53		220	73	H		6	363	117	R		12,48	492	161
H	--	6		88	D		3,65		225	74	R		12,48	364	118	D		3,65	498	162
D	--	3,65		89	E		4,25		227	75	Y		10,07	369	119	K		10,53	499	163
R	--	12,48		92	Y		10,07		230	76	C		8,18	376	120	Y		10,07	500	164
Y	--	10,07		93	C		8,18		231	77	Y		10,07	378	121	E		4,25	502	165
K	--	10,53		95	K		10,53		232	78	R		12,48	379	122	E		4,25	503	166
K	--	10,53		97	H		6		234	79	R		12,48	381	123	K		10,53	504	167
E	--	4,25		100	Y		10,07		235	80	Y		10,07	385	124	K		10,53	506	168
H	--	6		101	K		10,53		236	81	R		12,48	387	125	H		6	510	169
K	--	10,53		104	D		3,65		238	82	D		3,65	388	126	Y		10,07	512	170
R	--	12,48		105	K		10,53		242	83	C		8,18	392	127	H		6	515	171
R	--	12,48		111	E		4,25		247	84	D		3,65	395	128	H		6	518	172
E	--	4,25		118	D		3,65		248	85	Y		10,07	403	129	R		12,48	522	173
D	--	3,65		123	R		12,48		251	86	Y		10,07	404	130	E		4,25	523	174
R	--	12,48		126	H		6		254	87	E		4,25	412	131	K		10,53	524	175
D	--	3,65		127	E		4,25		255	88	H		6	413	132	L	2,36		527	176

Tasks for catalase molecule 8CAT.pdb

Protolytic constant pK_a isoelectric point $IEP=pK_a$ calculate of side chains

$\Sigma pK_{aRside\ group}$; **N**terminal $pK_{aNterminal}NH_3^+$ and $pK_{aCterminal}COO^-$ constants

sum divide with number of acid groups NpK_a :

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

Acid groups number in sum $NpK_a=172.....+2.....= 174.....$

527 amino acids of them for pK_a side groups number $172+2$.

N-terminal Methionine M $pK_{aNterminal}=9.21$ and

C-terminal Lysine K $pK_{aCterminal}=2.34$

Sum are calculate as

$$\Sigma pK_a=\Sigma pK_{aRside\ group}+pK_{aNterminal}+pK_{aCterminal}=1339,51.....$$

1. Average constant $pK_{mean}=pK_a=IEP$ **ISOELEKTRIC POINT**

$$NpK_a=172.....+2.....=174..... ;$$

$$IEP=\Sigma pK_a/ NpK_a =1339,51/ 174 =\mathbf{7,698333}.....$$

At pH value on isoelectric point $pH=IEP$ **total charge** is zero „0”

zero charge „0” $IEP=pH$

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

Underline existing!

2. 8CAT molecule charge sign (+). zero „0” or (-) at $pH=7.36$

Underline existing:

COOH,**NH₃⁺** positive + $pH=7.36 < IEP=7,7$ negative **-COO⁻**,**NH₂**.

3. 8CAT molecule charge +. zero „0” or - at **electrophoresis pH 8.8**

Underline existing:

COOH,**NH₃⁺** positive + $IEP=7,7 < pH = 8,8$ negative - **COO⁻**,**NH₂**

4. Calculate solution pH $C=10^{-7,021667}$ M 8CAT by *Ostwald dilution law*
concentration C in logarithm:

$$pH=\frac{pK_a - \log C}{2} = \frac{7,698333 - \log 10^{-7,021667}}{2} = \frac{7,698333 + 7,021667}{2} = \mathbf{14,7201} / 2 = 7,36.....$$

Attractor 7,36 concentration of 8CAT is $10^{-7,021667}.....M$.

4b. What 1QQW.pdb isoelectric point IEP=pH=pK_{a-vid} at physiologic pH=7,36 ? To determine

water solution pH with CATALASE concentration C=10^{-7,021667} M (mol/Litre)!

Catalase (EC 1.11.1.6), present in Human erythrocytes

SQ SEQUENCE 527 >1QQW:A|PDBID|CHAIN|SEQUENCE 67384, 637 g/mol

MADSRDPASDQMHWKEQRAAQKADVLTGAGNPGVDKLNVTIVGPRGPLLVQDVVFTDEMAHFDRERI PERVVHAKGAG
 AFGYFEVTHDITKYSKAKVFEHIGKKTPIAVRFSTVAGESGSADTVRDRPGFAVKFYTEDGNWDLVGNNTPIFFIRDPIIL
 FPSFIHSQKRNPPQTHLKDPMVWDFWSLRPESLHQVSFLFSDRGIPDGRHMNGYGSHTFKLVNANGEAVYCKFHYKTDQ
 GIKNLSVEDAARLSQEDPDYGIRDLFNAIATGKYPSTWTFYIQVMTFNQAETFFPNPFDLTKVWPHKDYPLIPVGKLVNLR
 NPVNYFAEVEQIAFDPSNMPGIEASPKMLQGRFLFAYPDTHRRLGPNYLHIVNCPYRARVANYQRDGPMPQDNQGG
 APNYYPNSFGAPEQQPSALEHSIQYSGEVRRFNTANDDNVTQVRAFVYVNLNNEQRKRLCENIAGHLKDAQIFIQKKA
 VKNFTEVHPDYGSHIQALLDKYNAEKPKNAIHTFVQSGSHLAAREKANL

AA	pK _{acoo-}	pK _{anH3+}	pK _{RR}	Nr	AA	pK _{acoo-}	pK _{anH3+}	pK _{RR}	Nr	AA	pK _{acoo-}	pK _{anH3+}	pK _{RR}	Nr	AA	pK _{acoo-}	pK _{anH3+}	pK _{RR}	Nr
M	--	9,21		1	K	--	10,53	135	45	D	--	3,65	264	89	Y	--	10,07	425	132
D	--	3,65	3	2	Y	--	10,07	137	46	K	--	10,53	273	90	E	--	4,25	428	133
R	--	12,48	5	3	E	--	4,25	139	47	Y	--	10,07	274	91	R	--	12,48	430	134
D	--	3,65	6	4	D	--	3,65	140	48	Y	--	10,07	280	92	R	--	12,48	431	135
D	--	3,65	10	5	D	--	3,65	144	49	E	--	4,25	290	93	D	--	3,65	437	136
K	--	10,53	13	6	R	--	12,48	156	50	D	--	3,65	298	94	D	--	3,65	438	137
H	--	6	14	7	D	--	3,65	157	51	K	--	10,53	301	95	R	--	12,48	444	138
K	--	10,53	16	8	H	--	6	166	52	H	--	6	305	96	Y	--	10,07	447	139
E	--	4,25	17	9	K	--	10,53	169	53	K	--	10,53	306	97	E	--	4,25	453	140
R	--	12,48	19	10	R	--	12,48	170	54	D	--	3,65	307	98	E	--	4,25	454	141
K	--	10,53	23	11	H	--	6	175	55	Y	--	10,07	308	99	R	--	12,48	456	142
D	--	3,65	25	12	K	--	10,53	177	56	K	--	10,53	315	100	K	--	10,53	457	143
D	--	3,65	37	13	D	--	3,65	178	57	R	--	12,48	320	101	R	--	12,48	458	144
K	--	10,53	38	14	D	--	3,65	180	58	Y	--	10,07	325	102	C	--	8,18	460	145
R	--	12,48	47	15	D	--	3,65	184	59	E	--	4,25	328	103	E	--	4,25	461	146
D	--	3,65	54	16	R	--	12,48	189	60	E	--	4,25	330	104	H	--	6	466	147
D	--	3,65	59	17	E	--	4,25	191	61	D	--	3,65	335	105	K	--	10,53	468	148
E	--	4,25	60	18	H	--	6	194	62	E	--	4,25	344	106	D	--	3,65	469	149
H	--	6	63	19	D	--	3,65	202	63	D	--	3,65	348	107	K	--	10,53	476	150
D	--	3,65	65	20	R	--	12,48	203	64	K	--	10,53	349	108	K	--	10,53	477	151
R	--	12,48	66	21	D	--	3,65	207	65	R	--	12,48	354	109	K	--	10,53	480	152
E	--	4,25	67	22	H	--	6	209	66	Y	--	10,07	358	110	E	--	4,25	484	153
R	--	12,48	68	23	R	--	12,48	210	67	D	--	3,65	360	111	H	--	6	486	154
E	--	4,25	71	24	H	--	6	211	68	H	--	6	362	112	D	--	3,65	488	155
R	--	12,48	72	25	Y	--	10,07	215	69	R	--	12,48	363	113	Y	--	10,07	489	156
H	--	6	75	26	H	--	6	218	70	H	--	6	364	114	H	--	6	492	157
K	--	10,53	77	27	K	--	10,53	221	71	R	--	12,48	365	115	D	--	3,65	498	158
Y	--	10,07	84	28	E	--	4,25	228	72	Y	--	10,07	370	116	K	--	10,53	499	159
E	--	4,25	86	29	Y	--	10,07	231	73	H	--	6	372	117	Y	--	10,07	500	160
H	--	6	89	30	C	--	8,18	232	74	C	--	8,18	377	118	E	--	4,25	503	161
D	--	3,65	90	31	K	--	10,53	233	75	Y	--	10,07	379	119	K	--	10,53	504	162
R	--	12,48	93	32	H	--	6	235	76	R	--	12,48	380	120	K	--	10,53	506	163
Y	--	10,07	94	33	Y	--	10,07	236	77	R	--	12,48	382	121	H	--	6	510	164
K	--	10,53	96	34	K	--	10,53	237	78	Y	--	10,07	386	122	H	--	6	518	165
K	--	10,53	98	35	D	--	3,65	239	79	R	--	12,48	388	123	R	--	12,48	522	166
E	--	4,25	101	36	K	--	10,53	243	80	D	--	3,65	389	124	E	--	4,25	523	167
H	--	6	102	37	E	--	4,25	248	81	C	--	8,18	393	125	K	--	10,53	524	168
K	--	10,53	105	38	D	--	3,65	249	82	D	--	3,65	396	126	L	2,36	--	527	169
R	--	12,48	106	39	R	--	12,48	252	83	Y	--	10,07	404	127					
R	--	12,48	112	40	E	--	4,25	256	84	Y	--	10,07	405	128					
E	--	4,25	119	41	D	--	3,65	257	85	E	--	4,25	413	129					
D	--	3,65	124	42	D	--	3,65	259	86	E	--	4,25	420	130					
R	--	12,48	127	43	Y	--	10,07	260	87	H	--	6	421	131					
D	--	3,65	128	44	R	--	12,48	263	88										

1303.23...../169.....=**7,48983**..... Number of pKa 167.....+2.....=169.....=NpK 1QQW
 Are present four Cysteine residues pK_{RR} =8.18; Sum of 169 pKa values in table 1303.23.....

Tasks for catalase molecule 1QQW.pdb

Protolytic constant pK_a isoelectric point IEP=pK_a calculate of side chains

$\Sigma pK_{aR\text{side group}}$; Nterminal pK_{aNterminal}NH₃⁺ and pK_{aCterminal}COO⁻ constants

sum divide with number of acid groups NpK_a:

$$IEP=pK_a=(\Sigma pK_{aR\text{side group}}+ pK_{aN\text{terminal}}+ pK_{aC\text{terminal}})/NpK_a$$

Acid groups number in sum NpK_a=167.....+2.....= 169.....

527 amino acids of them for pK_a side groups number 167+2.

N-terminal Methionine M pK_{aNterminal}=9.21 and

C-terminal Lysine K pK_{aCterminal}=2.34

Sum are calculate as

$$\Sigma pK_a=\Sigma pK_{aR\text{side group}}+pK_{aN\text{terminal}}+pK_{aC\text{terminal}}=1303.23.....$$

1. Average constant pK_{mean}=pK_a=IEP **ISOELEKTRIC POINT**

$$NpK_a=167.....+2.....=169.....$$

$$IEP=\Sigma pK_a/ NpK_a =1303.23/ 169 =\mathbf{7,48983}.....$$

At pH value on isoelectric point pH=IEP **total charge** is zero „0”

zero charge „0” IEP=pH

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

Underline existing!

2. 1QQW molecule charge sign (+). zero „0” or (-) at pH=7.36

Underline existing:

COOH,**NH₃⁺** positive + pH=7.36<IEP=**7,49** negative-**COO⁻**,**NH₂**.

3. 1QQW molecule charge +. zero „0” or - at **electrophoresis** pH **8.8**

Underline existing:

COOH,**NH₃⁺** positive +IEP=**7,49**<pH = **8,8** negative - **COO⁻**,**NH₂**

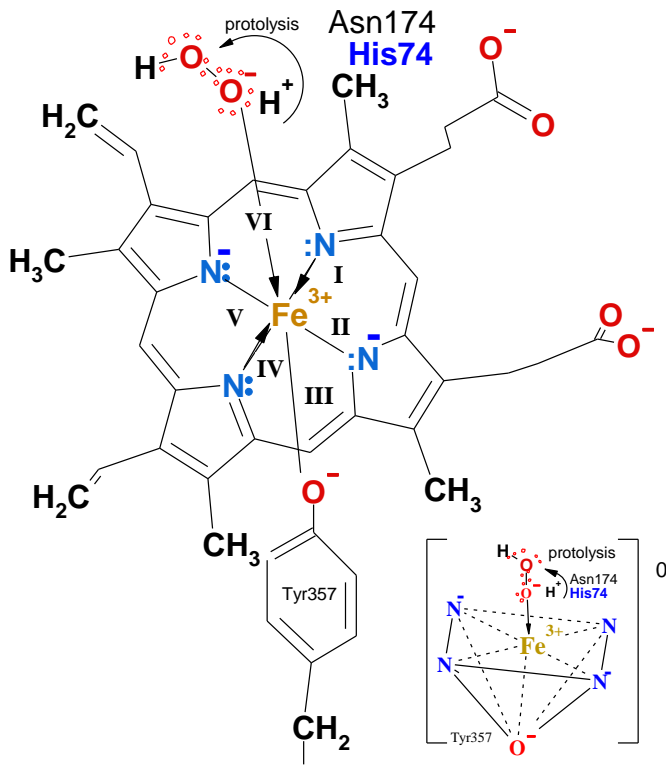
4. Calculate solution pH C=10^{-7,23017} M 1QQW by *Ostwald dilution law*

concentration C in logarithm:

$$pH=\frac{pK_a-\log C}{2}=\frac{7,48983-\log 10^{-7,23017}}{2}=\frac{7,48983+7,23017}{2}=\mathbf{14,7201} / 2 = 7,36.....$$

Attractor 7,36 concentration of 1QQW is 10^{-7,23017}M.

5. What secondary structures contains the CATALASE?**13 Alpha helices** and**beta barrel 8 strands**.
6. What **alpha helices** constitute CATALASE polypeptide molecule?**13 Alpha-helices**
7. What **beta structure** and **sheets** and how many **beta strands** constitute CATALASE?**beta-barrel structure** with**8 anti-parallel strands**.
8. What quaternary 4° structures components 3° subunits and what tertiary 3° structure domain units with its secondary 2° structures components are in given CATALASE unit?4 four tetramer units of.....4 fourprotein chains; How many secondary 2° structures?**13 Alpha-helices**;**beta-barrel structure****8 anti-parallel beta strands**.
9. **Funnel channel** long, wide, deep in angstroms distance?.....25.Å.....20-15 Å.....33-19Å
10. What are 11 **hydrophobic Funnel** AA numbers on polypeptide chain? **Val115**....., **Ala116**....., **Pro128**....., **Phe152**....., **Phe153**....., **Phe163**....., **Ile164**....., **Pro178**....., **Val181**....., **Leu198**....., **Phe199**.....
11. What are 14 **polar-charged Funnel** amino acids numbers on polypeptide chain ?!
.....**Ser113**....., **Glu118**....., **Ser121**....., **Arg126**....., **Asp127**....., **Gln167**....., **Lys168**.....,**Lys176**....., **Glu247**....., **His255**....., **Glu453**....., **Glu454**....., **Gln461**....., **His466**.....
12. What are 31 amino acids numbers on polypeptide chain of Heme pocket tertiary 3° structure? Val72....., Val73....., Ala75....., Val115....., Ala116....., Pro128....., Gly130....., Val145....., Gly146....., Phe152....., Phe153....., Ile154....., Ala157....., Leu158....., Leu159....., Phe160....., Pro161....., Phe163....., Ile164....., Phe197....., Leu198....., Phe199....., Leu298....., Ala332....., Phe333....., Pro335....., Met349....., Leu350....., Gly352....., Phe355....., Ala356....., Pro358....., Ala434.....
13. **Heme** pocket 8 **hydrophilic** amino acids numbers on polypeptide chain and 3 functions are?
...**Arg353**.....,**Tyr³⁵⁷**.....**Arg71**.....,**Arg111**.....,**Glu329**.....,**Arg364**.....
1) **His74, Asn147** catalysis**H⁺** deprotonation for peroxide dismutation on **heme** iron
2) **H3=156-168 and H9=347-366** bind the**heme** prosthetic group..
3) binding**heme** propionic acid residues.
14. First Tertiary domain starting amino acid =...Asn3. and finishing amino acid=...Ala75.?
15. What two first alpha helices names are depicted?**H1**....., **H2**.....
16. **Heme** moiety domain starting amino acid =...Lys76. and finishing amino acid=...Asn320.?
17. 5 Helix names in **heme** domain:.....**H3**.....,**H4**.....,**H5**.....,**H6**.....,**H7**.....,
18. Beta sheet name and strands account:**beta-barrel and****8 anti-parallel strands**.
19. Third domain starting amino acid =.....Pro321. and finishing amino acid=Asp436. ?
20. **Carboxy terminal** starting amino acid =..... Asp437. and finishing amino acid=Asn500.?
21. **Carboxy-terminal globulin** 4 Helix names:.. **H10**....., **H11**....., **H12**....., **H13**.....
22. How many polypeptide chains are linked to each **heme**?one polypeptide chain..
23. What number of **hemes** in biological unit of CATALASE **7cat.pdb**?.....four **hemes**.
24. What mass kD and number subunits make quaternary structure of CATALASE dumb-bell?..
.....four subunits, 60.....kD each subunit molar mass, tetramer mass 60*4=.....240 kD.
25. **Heme** location distance from center the tetramer in angstroms is 45,66/2=22.83.....Å
26. How much **salt bridges** are present in tetramer?...28 bridges, Show **Arg-Asp, Glu** Functional groups interaction! **Arg**≡C— **NH₃⁺**.....=>...**salt bridge**...<=.....**-OOC** —C≡**Asp, Glu**..
27. **Heme proximal** components 6 and of α helix **H9** 3 amino acids numbers on polypeptide chain? **Val¹⁴⁵**....., **Pro³³⁵**....., **His²¹⁷**....., **Arg³⁵³**....., **Ala³⁵⁶**....., **Tyr³⁵⁷**.....and **H9 Arg³⁵³**....., **Ala³⁵⁶**....., **Tyr³⁵⁷**.....
28. **Heme** location, distal side structural components? distal side facing the..... channel containsnon polar andpolar **residues**, some of which are contributed by the **beta-barrel**.....
29. What 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**
30. What four 4 intermolecular forces fold protein chains of CATALASE? Call identified!
1....**Hydrogen bond** 2....**Hydrophobic** 3... **Salt bridges** 5....**Coordinative bond**
31. Describe the CATALASE **heme** pocket vicinity components: pyrrole rings, propionic



acid residues, hydrophobic pocket complex maker
atom bonding properties are coordination number
N=VI.....

32. Put in line six atoms which form coordinative
bonds with ion **Fe³⁺**! **Iron!**

:N, :N-, :N-, :N-, -O-, H-::O-:-O-::-H.....
1 2 3 4 5 6.....

33. Do account coordination number N=VI... of
iron Fe on center of given CATALASE Heme!
Mark on left !

34. Which nonpolar, polar or ionic coordination
bond of **iron Fe** belongs to **-O-** group of Tyr357?
Ionic bond.....

35. Explain Asn147, His74 role in CATALASE!
Involved in proton **H⁺**... transfer

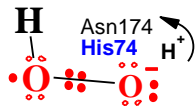
to connect with the hydroxyl group
HO- + H+ - Asn147, His74..... -> **H₂O**

36. Show collision semi hexagonal, reverse

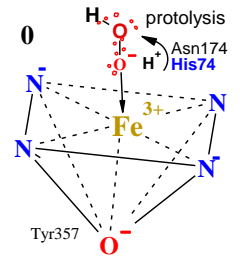
pyramidal coordination and give to coordinative complex neutral zero charge.....

37. Peroxide deprotonate **H-::O-:-O-:: + H⁺** and
explain anion free electron reactivity! Reactive
mean radical with free • electron anion with

.....negative charge **HOO-**.....



37.a Show peroxide anion
and **iron Fe(III)** collision
small activation energy
29 J/mol as negative attracts
positivecharge



38. What and how in complex reaction sequence favors and produce the **CATALASE**?

1) favors stable efficiency • 100% product essential unsaturated fatty acids $\omega=6, \omega=3$

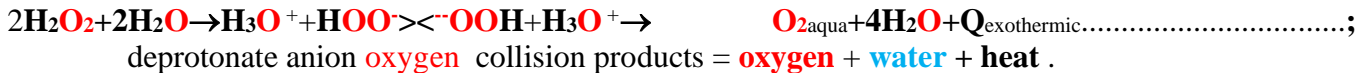
2) because erasing peroxide **H₂O₂** to zero $[\text{H}_2\text{O}_2]^2 = \dots \text{mol/liter}.$

3) **CATALASE** favors thirty million times $30 \cdot 10^6$ production of life resources:

and ethylene group **-CH₂-CH₂-** on dehydrogenation to cis

39. Reaction non catalytic spontaneous disproportionation. Protolysis create anions collision!

Protolysis $\text{pK}_a=11,75: 2\text{H}_2\text{O}_2 + 2\text{H}_2\text{O} \rightarrow \text{H}_3\text{O}^+ + \text{HOO}^- + \text{HO}_2^- + \text{H}_3\text{O}^+$create dismutation collision :



40. Write the dismutation reaction mechanism in steps with protonation **His74**, Asn174 of
peroxide deprotonation in collisions with active site **Fe(III)-O-Tyr357-E**:

1. **H-O-O-H** protolysis + **His74** + **Fe(III)-O-Tyr357-E** => **H⁺-His74** protonate + **HOO-** -> + **Fe(III)-O-Tyr357-E**

2. **H⁺His74** protonate + **HOO-** -> + **Fe(III)-O-Tyr357-E** => **HO-Red** + **H⁺His74** protonate + **OxO-Fe(IV)-O-Tyr357-E**.....

3. **HO-Red** + **H⁺His74** protonate + **OxO-Fe(IV)-O-Tyr357-E** => **RedH₂O** + **His74** + **OxO-Fe(IV)-O-Tyr357-E**

4. => **H-O-O-H** protolysis + **Asn174** + **O-Fe(IV)-O-Tyr357-E** => **H⁺Asn174** + **HOO-** -> + **O-Fe(IV)-O-Tyr357-E**

5. **H⁺Asn174** + **HOO-** -> + **O-Fe(IV)-O-Tyr357-E** => **HO-Red** + **H⁺Asn174** + **OxO-O-Fe(IV)-O-Tyr357-E**.....

6. => **HO-Red** + **H⁺Asn174** + **OxO-O-Fe(IV)-O-Tyr357-E** => **RedH₂O** + **Asn174** + **OxO-O-Fe(IV)-O-Tyr357-E**

7. **RedH₂O** + **OxO-O-Fe(IV)-O-Tyr357-E** => **RedH₂O** + **OxO=O** + **-Fe(III)-O-Tyr357-E**

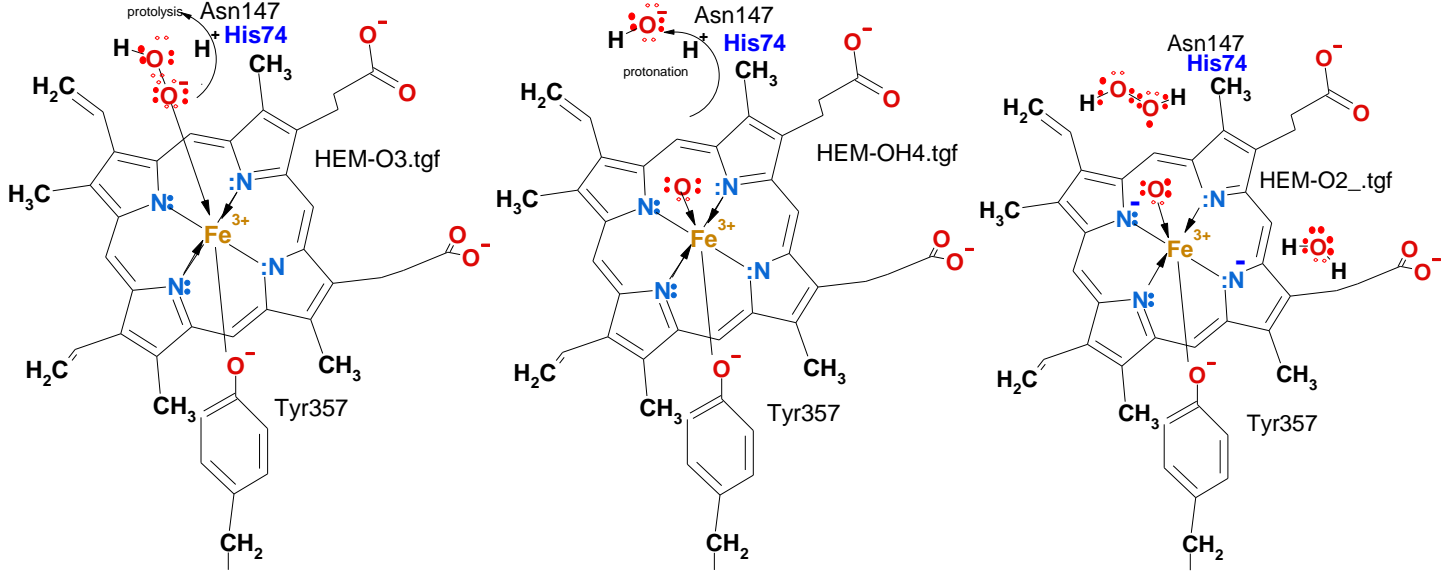
Summary: $2\text{H}_2\text{O}_2 + \text{CAT} \rightarrow \text{H}_3\text{O}^+ + \text{HOO}^- \leftarrow \text{Fe(III)} \leftarrow \text{OOH} + \text{H}_3\text{O}^+ \rightarrow \text{O}_{2\text{aqua}} + 2\text{H}_2\text{O} + \text{Q}_{\text{exothermic}} + \text{CAT}$

Protolysis activate transition state complex **oxygen** + **water** + **heat** + CATALASE,

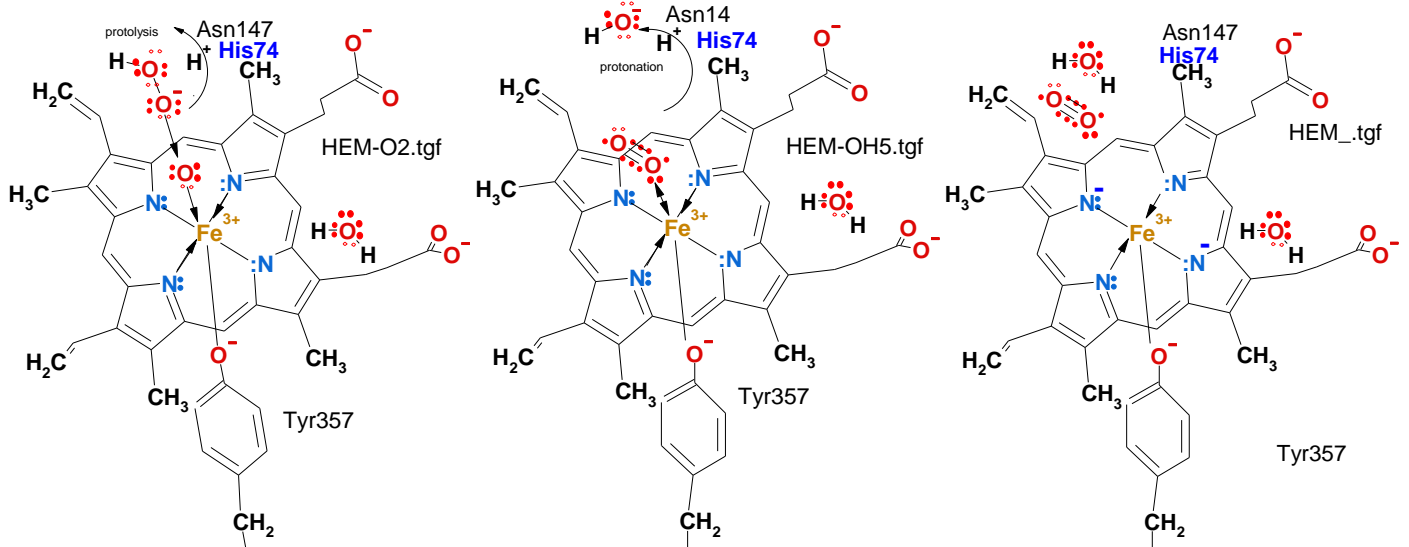
1. Colliding oxygen atom oxidized **Ox O-Fe(III)** and deprotonate on **H⁺ His74**, Asn174;.....

2. second oxygen atom reduced **HO-Red** and protonated neutral about **H₂O**.....

41. What is happen with CATALASE during life resources production $O_2+H_2O+Q?$



Hem pocket with 28 hydrophobic amino acids – in waterless medium oxidation-reduction reaction absent:



42. For what reason CATALASE is tightly bound to NADPH?

$Tyr357-OH+Fe^{2+}+NADP^++H_2O(H^+-His161)\rightleftharpoons Tyr357-O^-+Fe^{4+}+NADPH+H_3O^++(His161)$
 NADPH stabilizing $Tyr357-O^- + Fe^{3+}$ ionic bondshifts equilibrium to right
 with high rate protolysis attractors $pH=7,36.....$ and concentration $[H_2O]=55,3.....mol/L$

43. Two beta sheets Intermolecular hydrogen bonds connecting in tetramer subunits with 4 beta strands..... 1st [page](#):

44. Show Hydrogen bond between electronegative element atoms O and N peptide bond carbonyl $>C=O$ and imino group hydrogen $>C=O...H-N<.....$
 and imino group hydrogen $>N-H$ and carbonyl group $>N-H...O=C<.....$
 or between carbonyl $>C=O$ and hydroxyl Ser hydrogen, amino residues $>C=O...H-O-Ser....$
 or between carbonyl $>C=O$ and amino hydrogen His residue $>C=O...H-N<His.....$

45. Arrhenius velocity constant $k \rightarrow$ expression active collision number $e^{-E_a/(RT)} = \exp(-E_a/(RT)) = 0.988.....!$
 $\rightarrow k = A \dots \bullet e^{-E_a/(RT)} \dots$
 activation energy $E_a = 29..... J/mol$;
 geometric factor $A = 0,13.....$ and
 the temperature $T = 298,15..... K$.

46. How many times decreases activation energy E_a for CATALASE to compare absence catalyst?

$E_{a,CATALASE} = 29 \text{ J/mol}$; $E_{a(\text{absence catalyst})} = 79000 \text{ J/mol}$
 $79000/29 = 2724$ times smaller E_a for CATALASE.

47. How many times increases pre exponential - geometric factor for CATALASE?

$A_{\text{absence catalyst}} = 0.01$, $A_{CATALASE} = 0.13$ and
 $0.13/0.01 = 13$ times improve pre exponential - geometric factor $A_{CATALASE}$.

48. What physical meaning has Boltzmann exponential factor value $0.988 = \exp(-E_a/(RT))$?

$\rightarrow k = A \cdot e^{-\frac{E_a}{RT}} = A \cdot 0.988$ Correct performed collision number is 988 of total 1000.

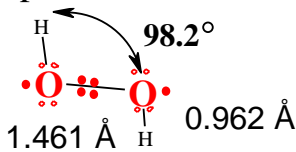
49. What physical meaning has pre exponential-geometric factor $1=A$?

Perfect geometry of peroxide molecules for collision is $A=1$...

as from 100 total collisions correct collision number is 100.....

50. What physical meaning has perfect pre exponential-geometric factor?

fraction perfect is 1 as mean 100% perfect collisions .



51. Measure the geometry of peroxide bond angle, distance between $O - H$ and $O - O$ put in text boxes of structure!

52. What many times increases CATALASE velocity constant for 1 mol in liter H_2O_2 conversion

to life resources $O_2 + H_2O + Q$?

oxygen+water+heat + CATALASE?

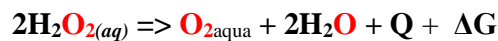
$\sqrt{\frac{k}{v}} = \frac{CAT}{k} \cdot [H_2O_2] = 0.36 \cdot [H_2O_2]$;

..... $\frac{CAT}{\sqrt{k}} / \sqrt{k} = 0,36/1,19 \cdot 10^{-8} = 30 \cdot 10^6$ times greater velocity constant.

53.0 Peroxide $H_2O_{2(aq)} + H_2O_{2(aq)}$ oxygen atoms dismutation to $\Rightarrow O_{2aqua} + H_2O + H_2O + Q$

Thermodynamics study ΔH_H , ΔS_H , ΔG_H dismutation. reaction as **exothermic**, **athermic**, **endothermic**?

Peroxide $2H_2O_2$ conversion to $O_{2aqua} + 2H_2O + Q$ at temperature 298.15 K, using the data table!



Substance	$\Delta H^\circ_H, \text{kJ/mol}$	$\Delta S^\circ_H, \text{J/mol/K}$	$\Delta G^\circ_H, \text{kJ/mol}$
H_3O^+	-285,81	-3,854	-213,275
O_{2aqua}	-11,715	110,876	16,4
O_{2aqua}	-11,70	-94,2	16,40
H_2O_{2l}	-237,129	69,91	-237,129
H_2O	-285,85	69,9565	-237,191
H_2O	-286,65	-453,188	-151,549
$H_2O_{2(aq)}$	-191,17	143,9	-134,03
$H_2O_{2(aq)}$	-191,99	-481,688	-48,39

CRC 2010 ;

Biothermodynamic 2006;

$\Delta H_{Hess} = \Delta H^\circ_{O_2} + 2\Delta H^\circ_{H_2O} - 2\Delta H^\circ_{H_2O_2} = -201,02 \dots = -201,06 \dots \text{kJ/mol}$
 $= -11,7 - 2 \cdot 286,65 - (2 \cdot -191,99) = -201,02 \dots \text{kJ/mol}$ **exothermic..**
 $= -11,7 - 2 \cdot 285,85 - (2 \cdot -191,17) = -201,06 \dots \text{kJ/mol}$

2. $\Delta S_{dispersed} = -\Delta H_H/T = -(-201,02)/298,15 = 674,2 \dots \text{J/mol/K}$; $\Delta S_{dispersed} = -\Delta H_H/T = -(-201,06)/298,15 = 674,36 \dots \text{J/mol/K}$;

$\Delta S_{Hess} = \Delta S^\circ_{O_2} + 2\Delta S^\circ_{H_2O} - 2\Delta S^\circ_{H_2O_2} = -94,2 + 2 \cdot 69,9565 - (2 \cdot -481,688) = -37,2 \dots \text{J/mol/K}$;

$\Delta S_{Hess} = \Delta S^\circ_{O_2} + 2\Delta S^\circ_{H_2O} - 2\Delta S^\circ_{H_2O_2} = 110,876 + 2 \cdot 69,9565 - (2 \cdot 143,9) = -37 \dots \text{J/mol/K}$;

2. $\Delta S_{total} = \Delta S_H + \Delta S_{dispersed} = -37,2 + 674,2 = 637 \dots \text{J/mol/K}$; $\Delta S_{total} = -37,011 + 674,36 = 637,35 \dots \text{J/mol/K}$;

4. $\Delta G_{Hess} = \Delta H_H - T \cdot \Delta S_H = -201,02 - 298,15 \cdot -0,0372 = -189,9 \dots \text{kJ/mol}$ **exoergic** **spontaneous**.....

$\Delta G_{Hess} = \Delta H_H - T \cdot \Delta S_H = -201,06 - 298,15 \cdot -0,037 = -190 \dots \text{kJ/mol}$;

$T \cdot \Delta S_{total} = 0,637 \cdot 298,15 = 189,9 \dots \text{kJ/mol}$; $T \cdot \Delta S_{total} = 0,63735 \cdot 298,15 = 190 \dots \text{kJ/mol}$;

High rate protolysis peroxide dismutation (oxidation – reduction).

$[H_2O_2]=1; 10^{(-10)}$ M Biochemistry concentrations $[O_{2(aq)}]=6 \cdot 10^{-5}$ M, $[H_3O^+]=10^{-7,36}$ M, $[H_2O]=55,3$ M.

Red $H_2O_2+2H_2O=O_2+2H_3O^++2e^-$; $E^\circ_{H_2O_2}=0,4495$ V **Absolute** standard potential;

$$E_{Red}=E^\circ_{H_2O_2}+0,0591/2 \cdot \lg([O_{2(aq)}] \cdot [H_3O^+]^2/[H_2O_2]/[H_2O]^2)=0,4495+0,0591/2 \cdot \lg(6 \cdot 10^{-5} \cdot 10^{(-7,36 \cdot 2)}/10^{(-10)}/55,3^2)=0,08227$$
 V

$$E_{Red}=E^\circ_{H_2O_2}+0,0591/2 \cdot \lg([O_{2(aq)}] \cdot [H_3O^+]^2/[H_2O_2]/[H_2O]^2)=0,4495+0,0591/2 \cdot \lg(6 \cdot 10^{(-5)} \cdot 10^{(-7,36 \cdot 2)}/1/55,3^2)=-0,2132$$
 V

Ox $H_2O_2 + 2 H_3O^+ + 2 e^- = 4 H_2O$; $-E^\circ_{OxH_2O_2}=-1,6855$ V **Absolute** **inverse** standard potential .

$$-E_{Ox}=-E^\circ_{H_2O_2Ox}+0,0591/2 \cdot \lg([H_2O]^4/[H_2O_2]/[H_3O^+]^2)=-1,6855+0,0591/2 \cdot \lg(55,3^4/10^{(-7,36 \cdot 2)}/10^{(-10)})=-0,749$$
 V;

$$-E_{Ox}=-E^\circ_{H_2O_2Ox}+0,0591/2 \cdot \lg([H_2O]^4/[H_2O_2]/[H_3O^+]^2)=-1,6855+0,0591/2 \cdot \lg(1 \cdot 10^{(-7,36 \cdot 2)}/55,3^4)=-2,3265$$
 V

$[H_2O_2]=1$ M; $[H_2O_2]=10^{(-10)}$ M; concentration $2H_2O_{2(aq)} \Rightarrow O_{2(aq)}+2H_2O$;

$$[H_2O_2]=10^{(-10)}$$
 M; $\Delta G_{eqBioChem}=(E^\circ_{Red}-E^\circ_{Ox}) \cdot F \cdot n=(0,08227-0,749) \cdot 96485 \cdot 2=(-1,236) \cdot 96485 \cdot 2=-128,7$ kJ/mol ;

$$[H_2O_2]=1$$
 M; $\Delta G_{eqBioChem}=(E^\circ_{Red}-E^\circ_{Ox}) \cdot F \cdot n=(-0,2132-2,3265) \cdot 96485 \cdot 2=(-2,5397) \cdot 96485 \cdot 2=-490,1$ kJ/mol ;

$$\Delta G_{Alberty}=G_{O_2Biochem_arteriaj}+2 \cdot G_{H_2OBioChemistry}-2 \cdot G_{H_2O_2}=78,08+2 \cdot 85,64-2 \cdot 364,79=-480,22$$
 kJ/mol;

Free energy change minimum mixture reaching at **favored absolute** potential standard equilibrium:

$$\Delta G_{eqStandard}=(E^\circ_{H_2O_2}-E^\circ_{H_2O_2Ox}) \cdot F \cdot n=(0,4495-1,6855) \cdot 96485 \cdot 2=(-1,1845) \cdot 96485 \cdot 2=-238,5$$
 kJ/mol ;

$$\Delta G_{HessAlberty}=G_{O_2}+2G_{H_2O}-2G_{H_2O_2}=330+2 \cdot (0)-(2 \cdot 284)=-238$$
 kJ/mol ; **Alberty**

Absolute potential scale is coinciding with **Alberty** absolute free energy scale.

$$\frac{[O_2]_{(aq)} \cdot [H_2O]^2}{[H_2O_2]_{(aq)}^2} = K_{eqStandard} = K_{H_2O_2} = \exp(-\Delta G_{eqStandard}/R/T) = \exp(238500/8,3144/298,15) = 10^{41,8}$$

Exothermic and exoergic H_2O_2 dismutation reaction Hess law

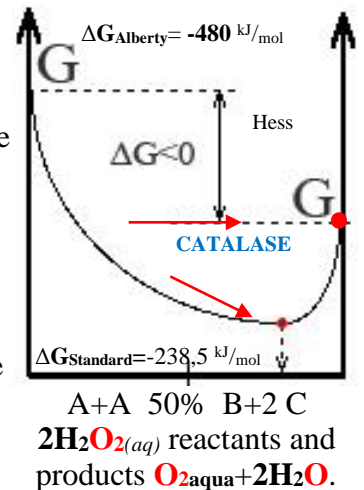
53.10 Homeostasis free energy change $\Delta G_{Alberty}=-480,22$ kJ/mol is negative,

53.10a Hess **Alberty** free energy change $\Delta G_{HessAlberty}=-238$ kJ/mol is negative,

53.11 **Absolute** potential scale $\Delta G_{eqStandard}=-238,5$ kJ/mol coinciding with Absolute free energy scaling. reaching equilibrium mixture constant

$$K_{eqBioChem}=10^{41,8}$$

Le Chatelier principle mean Prigogine attractor the free energy change minim ΔG_{min} reaching. High rate protolysis attractors pH=7,36, air oxygen 20,95% stay at equilibrium state, while homeostasis irreversibly continues, as is non equilibrium state. Prigogine attractors of Nobel Prize in Chemistry since 1977th. CATALASE erase peroxide molecules H_2O_2 in 100% efficiency for $\omega=6$, $\omega=3$ essential fatty acids elongation synthesis in peroxisomes. CATALASE reactivity is indispensable Brownian molecular engine for the irreversible homeostasis evolution and survival.



Conclusions



Protolysis activate transition state active complex **oxygen+water+heat** +CATALASE

CATALASE reactivity producing life resources $O_{2(aq)}+2H_2O+Q$

54.1. Catalyst CATALASE (CAT) is involved to reaction active transition state complex formation :

$H_2O_2 \dots CAT \dots H_2O_2$ and on finish released into products free unchanged CAT.

54.2. Catalyst CAT decrease activation energy E_a from 79000 J/mol to 29 J/mol times 2724 less.

54.3. Catalyst CAT improve geometric factor $A=0.01$ to $A=0.13$ times 13 better.

54.4. Catalyst CATALASE increase reaction velocity constant k from $1.9 \cdot 10^{-8} M^{-1}s^{-1}$ to $0.36 M^{-1}s^{-1}$ times $30 \cdot 10^6$ thirty million more.

54.5. Prigogine attractor CATALASE reactivity is indispensable life molecular engine converting processes to • 100% efficiency with erasing peroxide molecules.

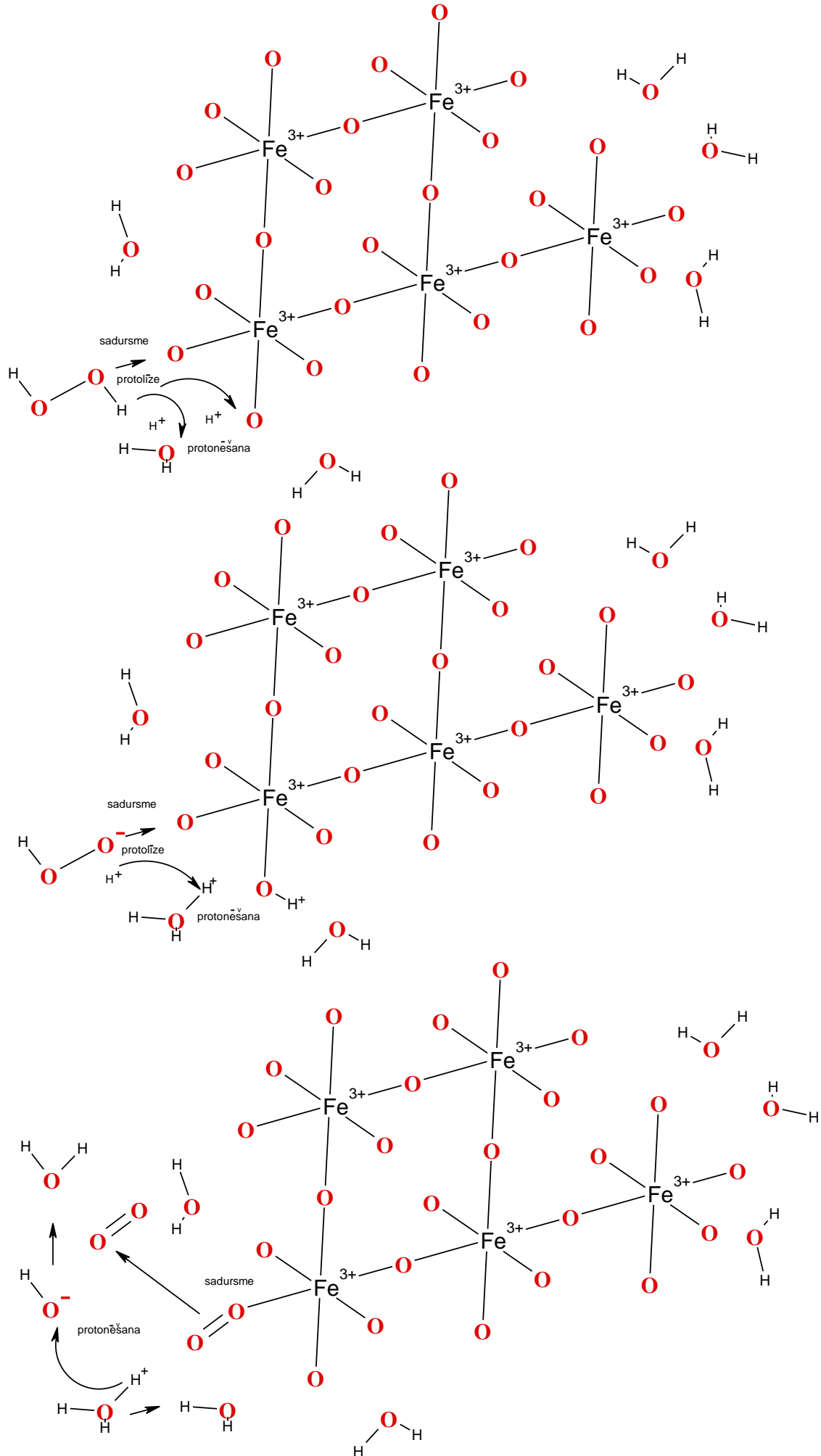
54.6. CATALASE reactivity produces the life resources:

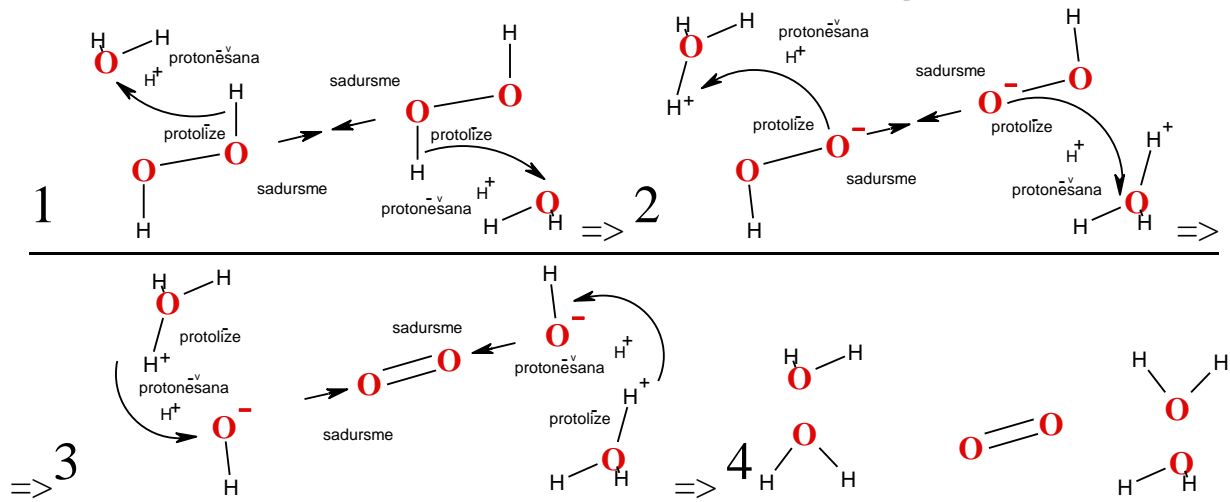
$\omega=6$, $\omega=3$ fatty acids **oxygen+water+heat**; $O_{2(aq)}+2H_2O+Q$

References.

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Catalyst by iron(III) oxide Fe_2O_3 .





Catalyst by manganese(II) oxide MnO.

