Name Surname :__ _Teacher_ , ___Teacher_ _____ Group Nº _____2025

A. human serum albumin HSA studies by molecule viewers:

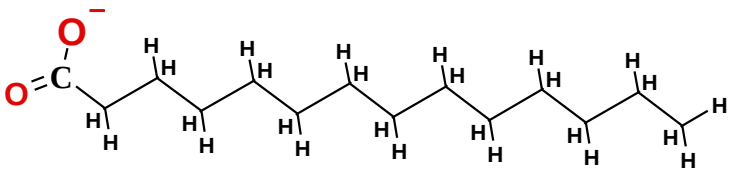
ChemScape MDLi **RasMol**; MAGE FireFox application.

B. Task RSU Aris Kaksis 2025 **HSA** on Home Page**:** http://aris.gusc.lv/ChemFiles/Albumin/cycox.html

1. Backbone Display option **N-terminus** domain starting amino acid is .....His3 & **C-terminus** amino acid is .....Gly584? What number of amino acids on **HSA** chain 585.....! (See page 2nd) and what number on **1E7Gmyrist.pdb** primary structures 584-3+1=582...?

2. What are **lipid-like** molecules transported as cargo with **HSA** in blood plasma? 1. Fatty acids....., 2. warfarin....., 3 aspirin....., are **diphilic** with **hydrophilic** and **hydrophobic** (non-polar) groups.

3. Put in deprotonate structure formula of myristic acid **C14** oxygen atoms in anion!



3a. What is the maximum of fatty acids bound to **HSA** Human Serum Albumin **1E7G.pdb**? ...7.....

4. What number of **alpha helices** constitute human serum albumin **HSA**? **33 Alpha-helices**.....

5. How many domains constitute **HSA** molecule and what its names? Three **domains I**, **II**, **III** ...

6. What & how many amino acids line on polypeptide sequence of each **domain**?.....

I G207-H3+1=205... **II** K372-E208+1=165..... **III** G584-V373+1=212.....

7. What circulating concentration of Human serum albumin **HSA** in blood plasma? 0.6 mM ....

8. How many disulfide bonds **–S-S-** connect 33 individual helices! .....17 **–S-S-**.....

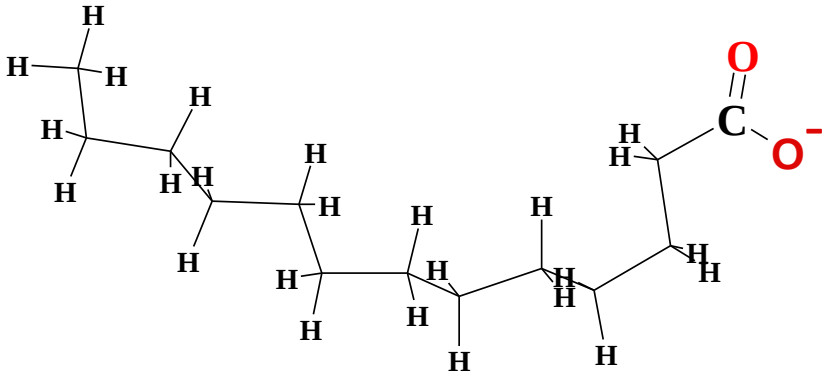
9. What seven(6 **IIA**)- helices are in homologous domains **IA-IIA-IIIA**? **IA**.....

H1,H2,H3,H4,H5,H6,H7IIA.....H12,H13,H14,H15,H16,H17

IIIA.....H23,H24,H25,H26,H27,H28,H29

10. What four(5)-helices are in homologous domains **IB-IIB-IIIB**? **IB** H8,H9,H10,H11

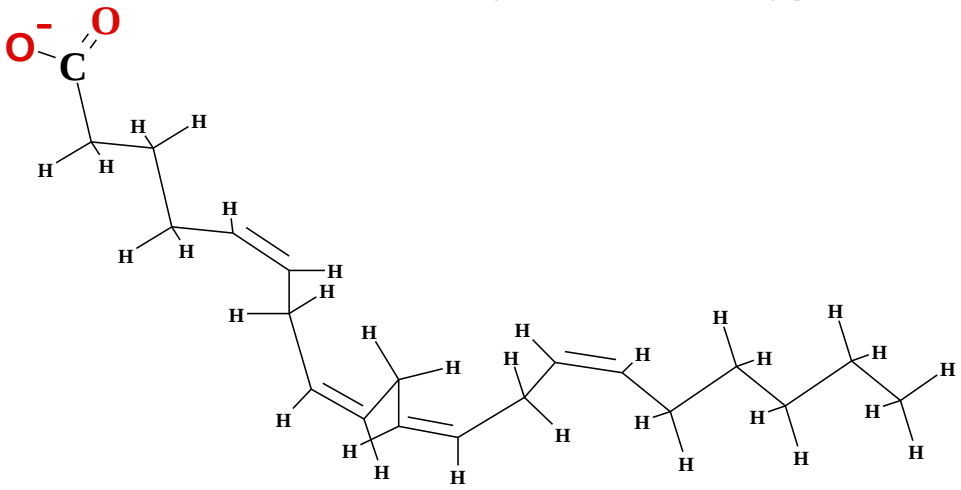
IIB .....H19,H20,H21,H22 **IIIB** H30,H31,H32,H3



11. Put in stearate **C18** carboxylate oxygen atoms **C=O**, **C-O⁻**!

12. What is the maximum of fatty acids bound to **HSA** Human Serum Albumin **1E7I.pdb**? ...7...

11. Put in arachidonate **C20:4** carboxylate anion two oxygen atoms and four double bonds!



14. What is the maximum of fatty acids bound to **HSA** Human Serum Albumin **1GNJ.pdb**? ...7...

Human serum albumin **HSA** circulating concentration 0.6 mM in blood plasma

http://aris.gusc.lv/ChemFiles/Albumin/1E7GpI.pdf ; http://aris.gusc.lv/ChemFiles/Albumin/1E7GpI.xls

Sequrnce of 585 AA Amino Acids in Albumin molecule 1E7G.pdb:

DAHKSEVAHRFKDLGEENFKALVLIAFAQYLQQCPFEDHVKLVNEVTEFAKTCVADESAENCDKSLHTLFGDKLCTVATL
RETYGEMADCCAKQEPERNECFLQHKDDNPNLPRLVRPEVDVMCTAFHDNEETFLKKYLYEIARRHPYFYAPELLFFAKR
YKAAFTECCQAADKAACLLPKLDELRDEGKASSAKQRLKCASLQKFGERAFKAWAVARLSQRFPKAEFAEVSKLVTDLTK
VHTECCHGDLLECADDRADLAKYICENQDSISSKLKECCEKPLLEKSHCIAEVENDEMPADLPSLAADFVESKDVCKNYA
EAKDVFLGMFLYEYARRHPDYSVVLLLRLAKTYETTLEKCCAAADPHECYAKVFDEFKPLVEEPQNLIKQNCELFEQLGE
YKFQNALLVRYTKKVPQVSTPTLVEVSRNLGKVGSKCCKHPEAKRMPCAEDYLSVVLNQLCVLHEKTPVSDRVTKCCTES
LVNRRPCFSALEVDETYVPKEFNAETFTFHADICTLSEKERQIKKQTALVELVKHKPKATKEQLKAVMDDFAAFVEKCCK
ADDKETCFAEEGKKLVAASQAALGL

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
D	0	9.6	3.65	1	1	R	0	0	12.48	144	55	K	0	0	10.53	286	109	K	0	0	10.53	439	163
H	0	0	6	3	2	R	0	0	12.48	145	56	H	0	0	6	288	110	H	0	0	6	440	164
K	0	0	10.53	4	3	H	0	0	6	146	57	E	0	0	4.25	292	111	E	0	0	4.25	442	165
E	0	0	4.25	6	4	Y	0	0	10.07	148	58	E	0	0	4.25	294	112	K	0	0	10.53	444	166
H	0	0	6	9	5	Y	0	0	10.07	150	59	D	0	0	3.65	296	113	R	0	0	12.48	445	167
R	0	0	12.48	10	6	E	0	0	4.25	153	60	E	0	0	4.25	297	114	E	0	0	4.25	450	168
K	0	0	10.53	12	7	K	0	0	10.53	159	61	D	0	0	3.65	301	115	D	0	0	3.65	451	169
D	0	0	3.65	13	8	R	0	0	12.48	160	62	D	0	0	3.65	308	116	Y	0	0	10.07	452	170
E	0	0	4.25	16	9	Y	0	0	10.07	161	63	E	0	0	4.25	311	117	H	0	0	6	464	171
E	0	0	4.25	17	10	K	0	0	10.53	162	64	K	0	0	10.53	313	118	E	0	0	4.25	465	172
K	0	0	10.53	20	11	E	0	0	4.25	167	65	D	0	0	3.65	314	119	K	0	0	10.53	466	173
Y	0	0	10.07	30	12	D	0	0	3.65	173	66	K	0	0	10.53	317	120	D	0	0	3.65	471	174
E	0	0	4.25	37	13	K	0	0	10.53	174	67	Y	0	0	10.07	319	121	R	0	0	12.48	472	175
D	0	0	3.65	38	14	K	0	0	10.53	181	68	E	0	0	4.25	321	122	K	0	0	10.53	475	176
H	0	0	6	39	15	D	0	0	3.65	183	69	K	0	0	10.53	323	123	E	0	0	4.25	479	177
K	0	0	10.53	41	16	E	0	0	4.25	184	70	D	0	0	3.65	324	124	R	0	0	12.48	484	178
E	0	0	4.25	45	17	R	0	0	12.48	186	71	Y	0	0	10.07	332	125	R	0	0	12.48	485	179
E	0	0	4.25	48	18	D	0	0	3.65	187	72	E	0	0	4.25	333	126	E	0	0	4.25	492	180
K	0	0	10.53	51	19	E	0	0	4.25	188	73	Y	0	0	10.07	334	127	D	0	0	3.65	494	181
D	0	0	3.65	56	20	K	0	0	10.53	190	74	R	0	0	12.48	336	128	E	0	0	4.25	495	182
E	0	0	4.25	57	21	K	0	0	10.53	195	75	R	0	0	12.48	337	129	Y	0	0	10.07	497	183
E	0	0	4.25	60	22	R	0	0	12.48	197	76	H	0	0	6	338	130	K	0	0	10.53	500	184
D	0	0	3.65	63	23	K	0	0	10.53	199	77	D	0	0	3.65	340	131	E	0	0	4.25	501	185
K	0	0	10.53	64	24	K	0	0	10.53	205	78	Y	0	0	10.07	341	132	E	0	0	4.25	505	186
H	0	0	6	67	25	E	0	0	4.25	208	79	R	0	0	12.48	348	133	H	0	0	6	510	187
D	0	0	3.65	72	26	R	0	0	12.48	209	80	K	0	0	10.53	351	134	D	0	0	3.65	512	188
K	0	0	10.53	73	27	K	0	0	10.53	212	81	Y	0	0	10.07	353	135	E	0	0	4.25	518	189
R	0	0	12.48	81	28	R	0	0	12.48	218	82	E	0	0	4.25	354	136	K	0	0	10.53	519	190
E	0	0	4.25	82	29	R	0	0	12.48	222	83	E	0	0	4.25	358	137	E	0	0	4.25	520	191
Y	0	0	10.07	84	30	K	0	0	10.53	225	84	K	0	0	10.53	359	138	R	0	0	12.48	521	192
E	0	0	4.25	86	31	E	0	0	4.25	227	85	D	0	0	3.65	365	139	K	0	0	10.53	524	193
D	0	0	3.65	89	32	E	0	0	4.25	230	86	H	0	0	6	367	140	K	0	0	10.53	525	194
K	0	0	10.53	93	33	K	0	0	10.53	233	87	E	0	0	4.25	368	141	E	0	0	4.25	531	195
E	0	0	4.25	95	34	D	0	0	3.65	237	88	Y	0	0	10.07	370	142	K	0	0	10.53	534	196
E	0	0	4.25	97	35	K	0	0	10.53	240	89	K	0	0	10.53	372	143	H	0	0	6	535	197
R	0	0	12.48	98	36	H	0	0	6	242	90	D	0	0	3.65	375	144	K	0	0	10.53	536	198
E	0	0	4.25	100	37	E	0	0	4.25	244	91	E	0	0	4.25	376	145	K	0	0	10.53	538	199
H	0	0	6	105	38	H	0	0	6	247	92	K	0	0	10.53	378	146	K	0	0	10.53	541	200
K	0	0	10.53	106	39	D	0	0	3.65	249	93	E	0	0	4.25	382	147	E	0	0	4.25	542	201
D	0	0	3.65	107	40	E	0	0	4.25	252	94	E	0	0	4.25	383	148	K	0	0	10.53	545	202
D	0	0	3.65	108	41	D	0	0	3.65	255	95	K	0	0	10.53	389	149	D	0	0	3.65	549	203
R	0	0	12.48	114	42	D	0	0	3.65	256	96	E	0	0	4.25	393	150	D	0	0	3.65	550	204
R	0	0	12.48	117	43	R	0	0	12.48	257	97	E	0	0	4.25	396	151	E	0	0	4.25	556	205
E	0	0	4.25	119	44	D	0	0	3.65	259	98	E	0	0	4.25	400	152	K	0	0	10.53	557	206
D	0	0	3.65	121	45	K	0	0	10.53	262	99	Y	0	0	10.07	401	153	K	0	0	10.53	560	207
H	0	0	6	128	46	Y	0	0	10.07	263	100	K	0	0	10.53	402	154	D	0	0	3.65	562	208
D	0	0	3.65	129	47	E	0	0	4.25	266	101	R	0	0	12.48	410	155	D	0	0	3.65	563	209
E	0	0	4.25	131	48	D	0	0	3.65	269	102	Y	0	0	10.07	411	156	K	0	0	10.53	564	210
E	0	0	4.25	132	49	K	0	0	10.53	274	103	K	0	0	10.53	413	157	E	0	0	4.25	565	211
K	0	0	10.53	136	50	K	0	0	10.53	276	104	K	0	0	10.53	414	158	E	0	0	4.25	570	212
K	0	0	10.53	137	51	E	0	0	4.25	277	105	E	0	0	4.25	425	159	E	0	0	4.25	571	213
Y	0	0	10.07	138	52	E	0	0	4.25	280	106	R	0	0	12.48	428	160	K	0	0	10.53	573	214
Y	0	0	10.07	140	53	K	0	0	10.53	281	107	K	0	0	10.53	432	161	K	0	0	10.53	574	215
E	0	0	4.25	141	54	E	0	0	4.25	285	108	K	0	0	10.53	436	162	L	2.36	0	0	585	216

In account not present 66 Cysteine residues Cys = pK$_{RR}$ =8.18. which are busy in 17 disulfide bonds;
Seven fatty acids considered as7 Nonanoic acids pKa=4.96; 7*4.96=34.72.....
Sum of 217 pKa values in table 1604.91..... to add plus 7 fatty acids 7*4.96=34.72.....

Sum of 217 pKa values in table and to add plus 7 fatty acids pKa : 1604.91+ 34.72=1639.63.....

Calculation tasks for Human Serum Albumin molecule

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 NpK_a:

$$IEP=pK_a=(\Sigma pKa_{Rside\ group}+pKa_{Nterminal}+pKa_{Cterminal})/NpKa$$

14.1 Protolytic equilibrium number in sum NpKa=215.....+2.....+7.....=217.....+7.....=224.....

585 amino acids of them protolytic constants pK_a for side groups 215+2 ,
N-terminal aspartate D $pK_{aNterminal}$=9.6 and C-terminal leucine L $pKa_{Cterminal}$=2.36 and 7 fatty acids pK_a=4.96.

Sum on 2nd page are calculate as $\Sigma pKa_{Rside\ group}+pK_{aNterminal}+pKa_{Cterminal}+7*pK_a$= 1639.63.....

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

no 7 fatty acids NpKa=215.....+2.....=217..... IEP=1604.91 / 217 =**7.3958986**.....

with 7 fatty acids IEP=1639.63 / 224 =**7.3197768**.....

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 existing and to cut incorrect given answerecharge:

14.3 Determine albumin without 7 fatty acids molecule charge sign (+). zero „**0**” or (-) at physiologic pH=7.36

Underline existing and to cut incorrect given answerecharge:

(+).....small positive.charge almost zero “0”.....underline

-COOH & **-NH_3^+** positive (+) charge ……. pH=7.36 < IEP=**7.4** ..... charge negative(-) **-COO^-** & **-NH_2**.

14.4 Determine albumin +7 fatty acids molecule charge sign (+). zero „**0**” or (-) at physiologic pH=7.36

Underline existing and to cut incorrect given answerecharge:

(-).....small negative.charge almost zero “0”.....underline

-COOH & **-NH_3^+** positive (+) charge ……. IEP=**7.32**< pH=7.36 ..... charge negative(-) **-COO^-** & **-NH_2**.

14.5 Determine albumin molecule charge sign (+). zero „**0**” or (-) at **electrophoresis** pH **8.8**

Underline existing and to cut incorrect given answerecharge:

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

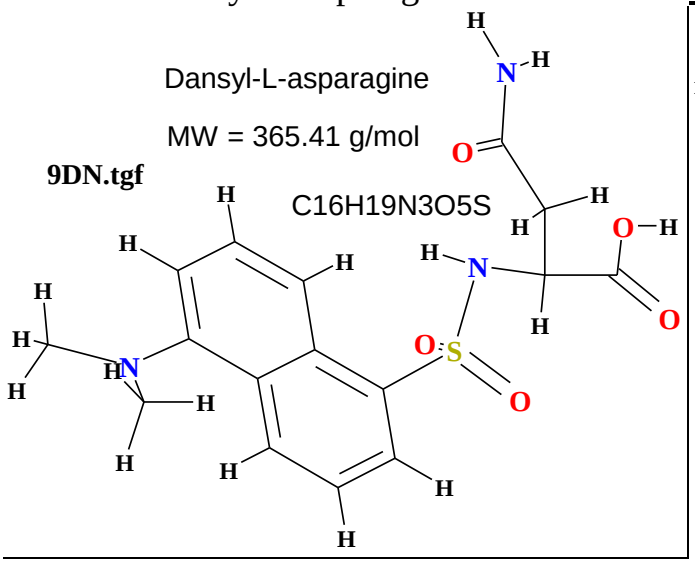
-COOH & **-NH_3^+** positive (+) charge …….IEP=**7.32**< pH=8.8 ..... charge negative(-) **-COO^-** & **-NH_2**.

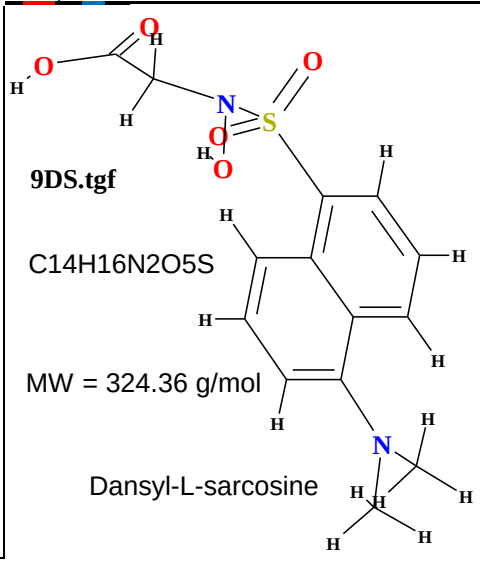
14.6 Calculate $10^{-7,4}$ M albumin +7 fatty acids solution pH by *Ostwald dilution law* concentration C in

logarithm: : $pH=\frac{pK_a-\log C}{2}=\frac{7,3198-\log 10^{-7,4002}}{2}=\frac{7,3198+7,4002}{2}$ = **14,720** / 2 = 7,36.....

Attractor 7,36 Albumin concentration is $10^{-7,4}$ M.

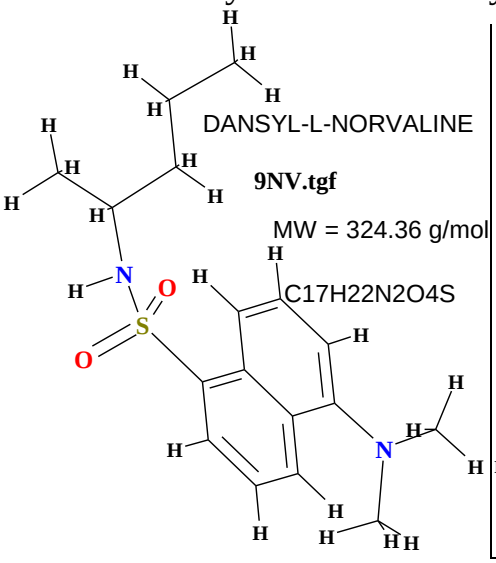
15. Draw Dansyl-L-asparagine Asn amide >C=O, -NH2 atoms of **HSA** Human Serum Albumin **2XVV.pdb** of Protein Data Bank structure!

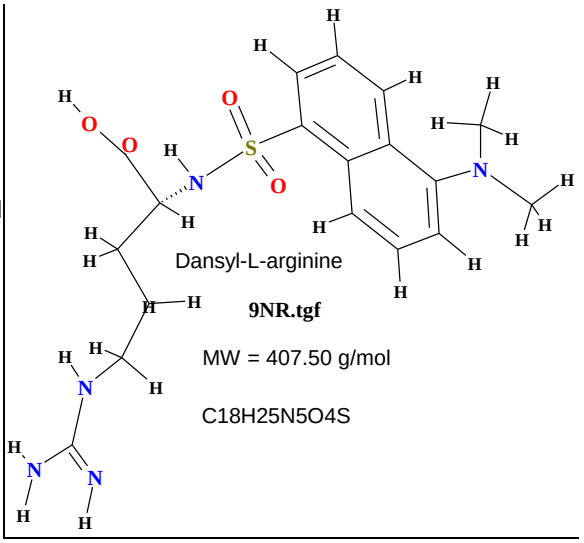




Protein Data Bank structure **2XVQ.pdb**

16. Draw Dansyl-L-sarcosine Gly carboxyl group >C=O un-O-H atoms **9DS**.pdb!



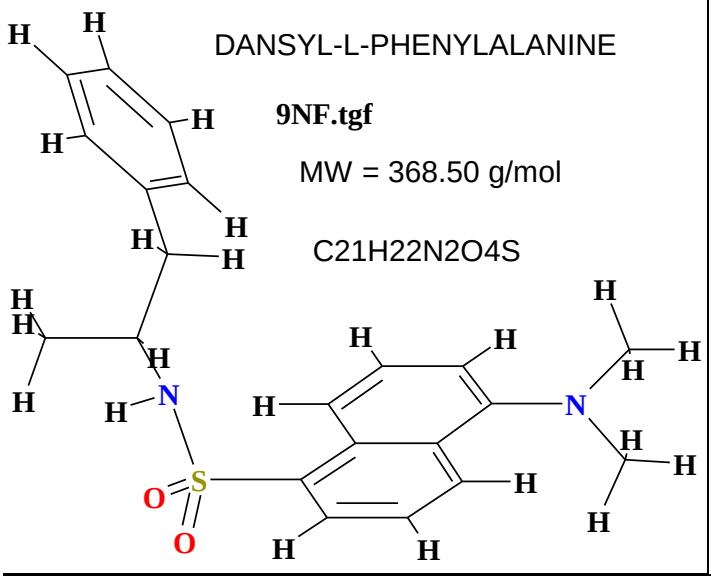


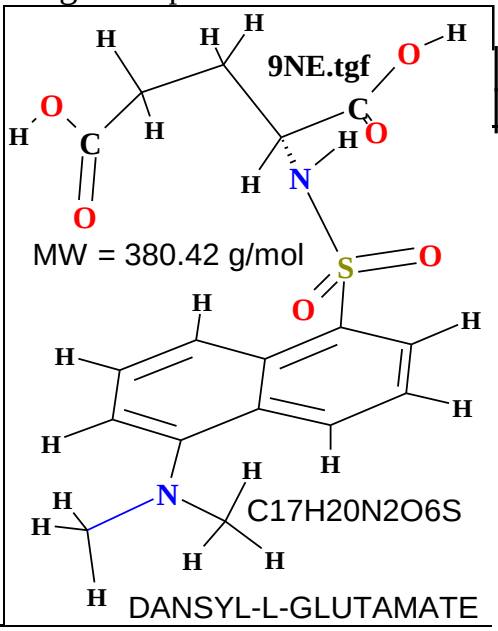
17. Draw Dansyl-L-arginine Arg guanidin three nitrogen –N< atoms **9NR**.pdb **HSA** Human Serum Albumin! **2XVW.pdb** Protein Data Bank

Protein Data Bank structure **2XW1.pdb**

18. Draw Dansyl-L-norvaline two –CH_3 methil groups **9NV**.pdb

19. Draw Dansyl-L-phenylalanine Phe benzene ring **9NF**.pdb from **HSA** Human Serum Albumin

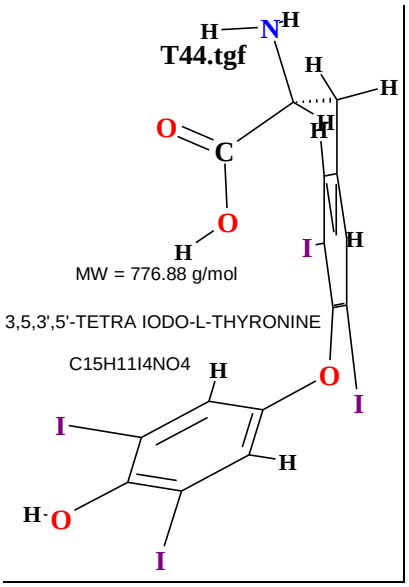


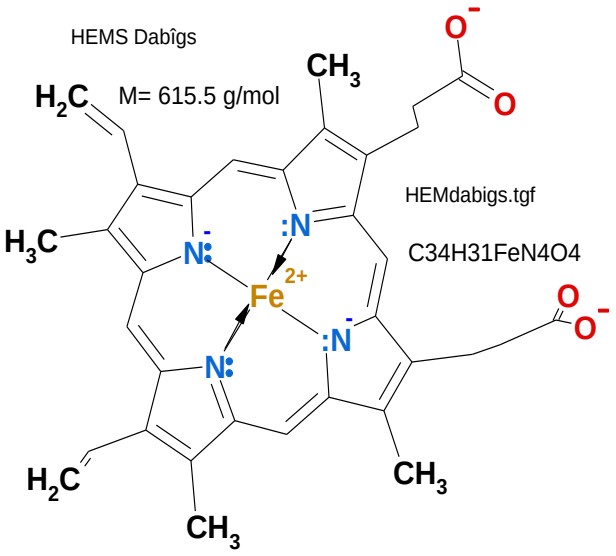


2XW0.pdb Protein Data Bank structure

20. Put in Dansyl-L-glutamate Glu carboxyl group >C=O and -O-H atoms **9NE**.pdb from **HSA** Human Serum Albumin Protein Data Bank structure PDB **2XSI.pdb**

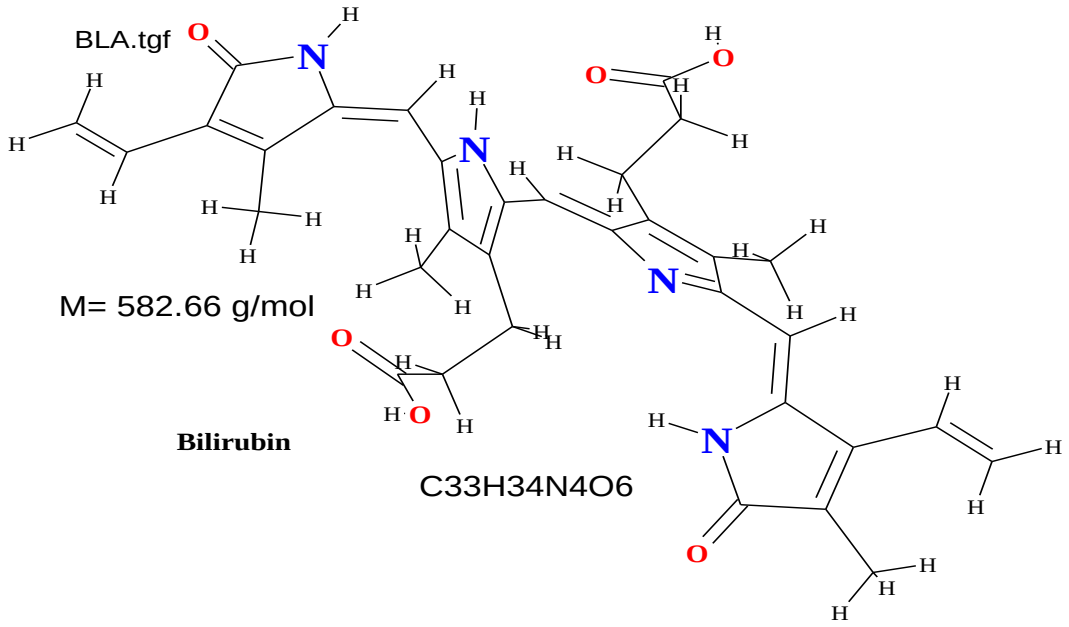
21. Draw **L-Thyroxin** Tyr carboxyl >C=O$^-$,-O-H & -NH$_2$ atoms of thyroid hormone secreted by the follicular cells of thyroid gland bound on **HSA** Human Serum Albumin **1HK1.pdb** Protein Data Bank structure



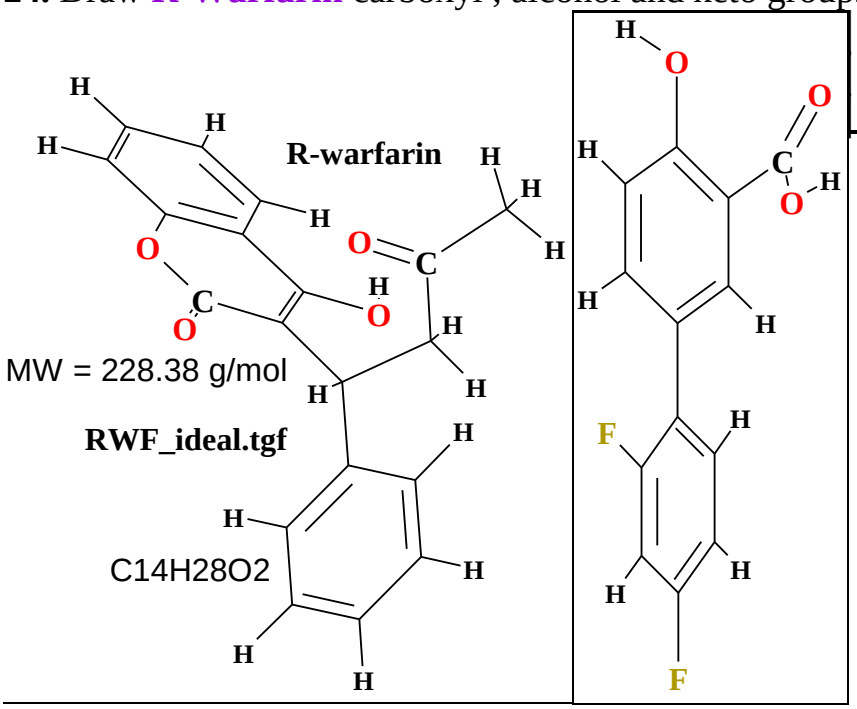


22. Draw 4 pyrol N atoms on Heme coordinated by iron(II) **HEMdabigs**.pdb from **HSA** Human Serum Albumin Protein Data Bank structure **1O9X.pdb**

23. Draw 4 pyrol N atoms on Prosthetic group metabolite **Bilirubin: BLA**.pdb bound on **HSA** Human Serum Albumin Protein Data Bank structure **2VUE.pdb**

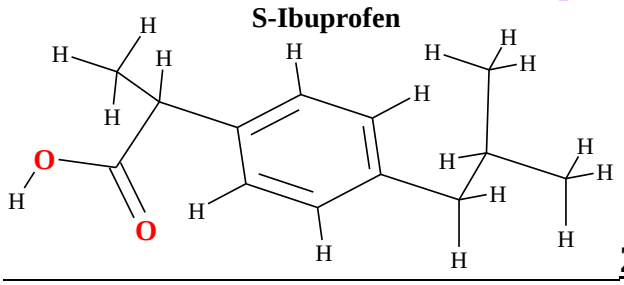


24. Draw **R-Warfarin** carboxyl , alcohol and keto groups >C=O$^-$, - O-H atoms anti-clotting human blood medicine bound Human Serum Albumin **2BXD.pdb** Protein Data Bank structure .

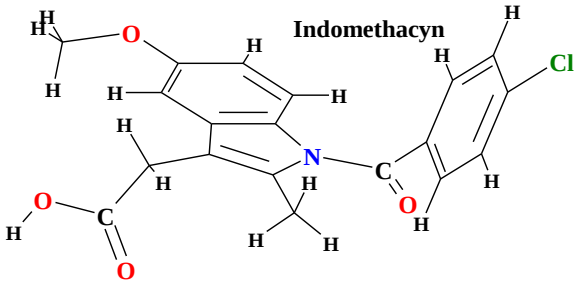


25. Draw **Diflunisal** salicylate carboxyl group >C=O$^-$, - O-H atoms non-steroidal anti-inflammatory agent **NSAIA** structural formula **1FL**.pdb from **HSA** Human Serum Albumin Protein Data Bank structure **2BXE.pdb**

26. Draw **Ibuprofen** carboxyl group >**C=O**$^{-}$, - **O-H** atoms non-steroidal anti-inflammatory agent **NSAIA** human blood medicine bound on **HSA** Human Serum Albumin

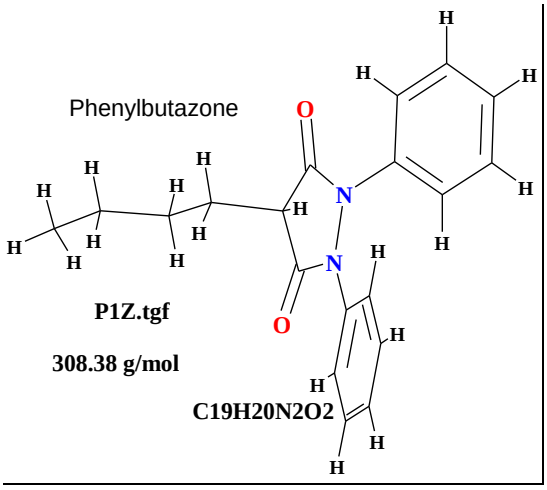


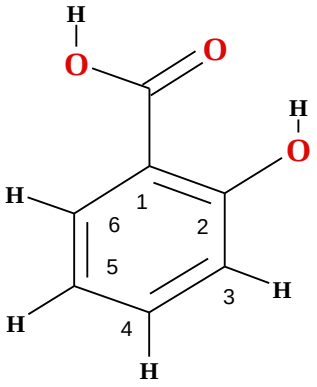
2BXG.pdb Protein Data Bank structure **IBP**.pdb.



27. Draw **IndoMethacin** carboxyl , ether and keto groups >**C=O**$^{-}$, - **O-H** atoms non-steroidal anti-inflammatory agent **NSAIA** structural formula **IMN**.pdb from **HSA** Human Serum Albumin Protein Data Bank structure **2BXM.pdb**

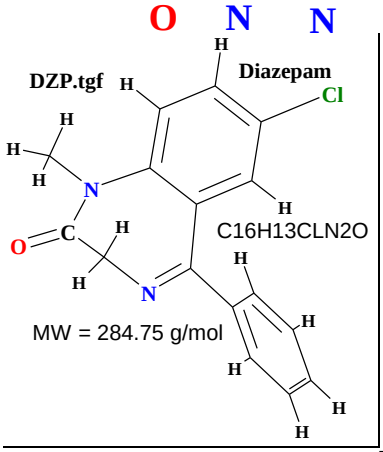
28. Draw **phenyl Butazon** keto group >**C=O** un –**N**< 2 atoms non-steroidal , antipyretic, anti-inflammatory, analgesic structural formula **P1Z**.pdb from **HSA** Human Serum Albumin Protein Data Bank structure **2BXM.pdb** .



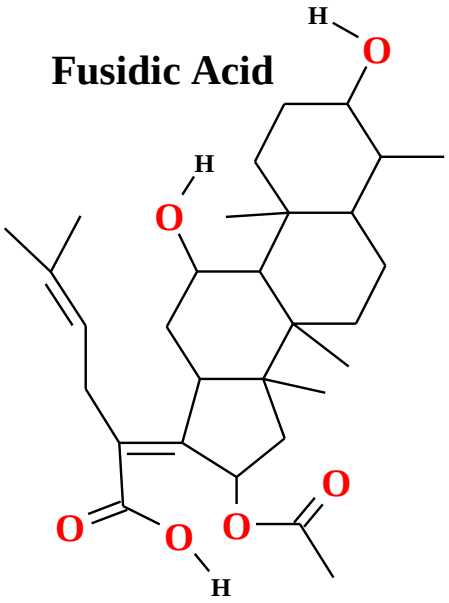


29. Draw **Salicylic Acid 2-hydroxy-benzoic Acid** carboxyl and alcohol groups >**C=O**$^{-}$, -**O-H** atoms Anti-inflammation drug Aspirin Acylating hydroxyl group at C2 on benzene ring **SAL**.pdb from **HSA** Human Serum Albumin **2BXM.pdb** Protein Data Bank

30. Put in **Diazepam** given three atoms anticonvulsant, anxiolytic, sedative, relaxant, amnesic human body medicine formula **DZP**.pdb from Human Serum Albumin Protein Data Bank structure **2BXF.pdb**



31. Put in **Fusidic Acid** given six atoms antibiotic, Anti-Bacterial Agent, Protein Synthesis Inhibitor structural formula **FUA**.pdb from **HSA** Human Serum Albumin Protein Data Bank structure **2VUF.pdb.**



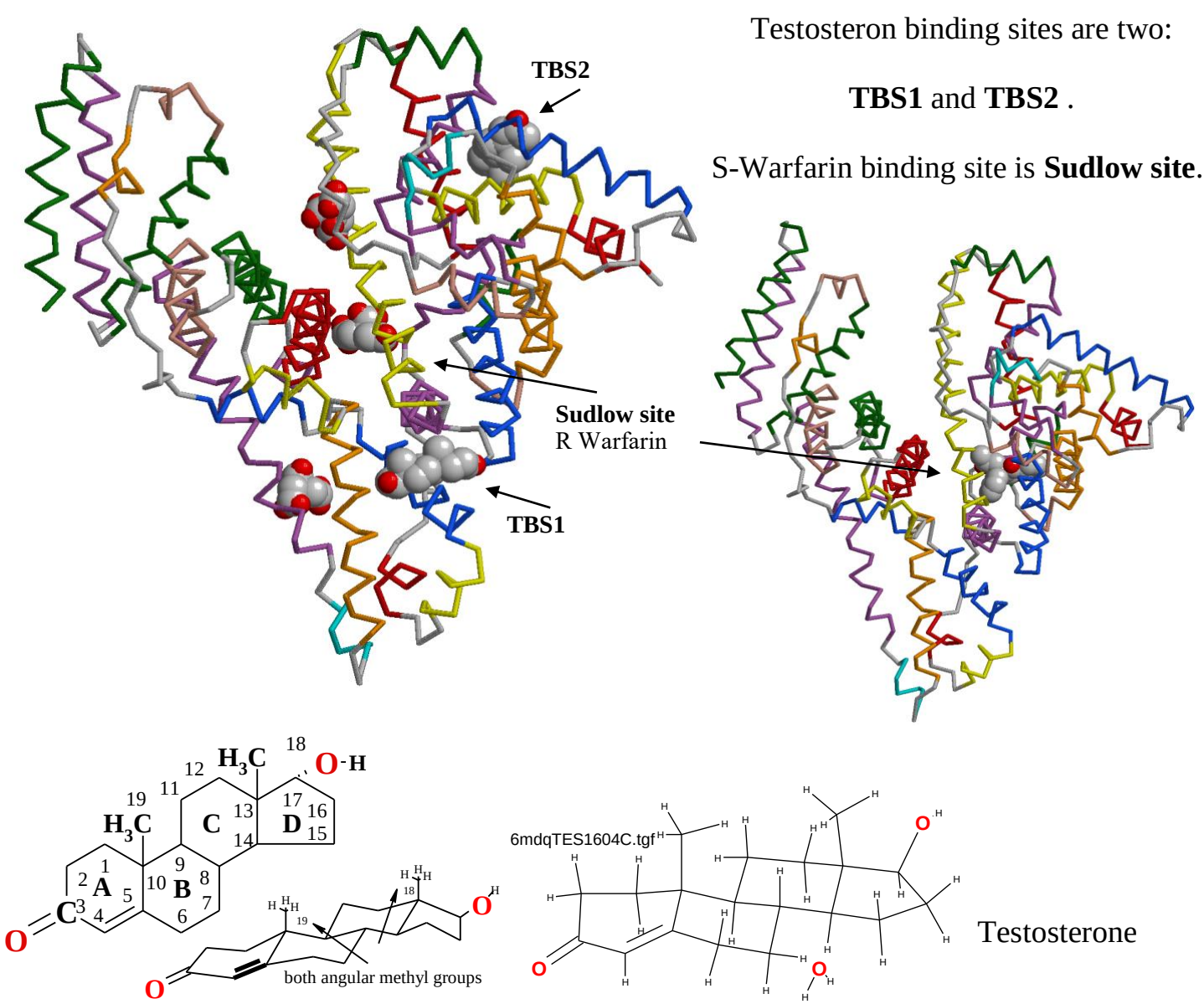


Fig. 5 ESA domains and testosterone binding sites. The testosterone (yellow) and citrate molecules (magenta) are shown with atoms as spheres. Warfarin (from structure of HSA complexed with warfarin, PDB ID: 2BXD), which is bound at Sudlow site I,6 is shown with atoms as blue spheres. Testosterone was predicted to bind in Sudlow site I by Peters.1 The interactive collection of superpositions of the ESA–testosterone complex and other SA complexes with selected compounds that bind in TBS1 or TBS2 is available at ; https://molstack.bioreproducibility.org/c/hYYh/. and in: Chem Sci. 2019 Feb 14; 10(6): 1607-1618.