Name Surname: _____ Group Nº _____2023

**A. human serum albumin HSA** studies by molecule viewers:

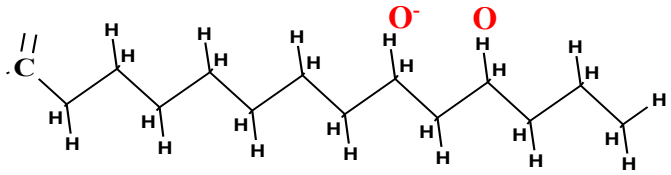
ChemScape MDLi **RasMol**; MAGE FireFox application.

**B.** Task RSU Aris Kaksis 2023 **HSA** on Home Page**:** http://aris.gusc.lv/ChemFiles/Albumin/cycox.html

**1. Backbone** Display option **N-terminus** domain starting amino acid is His..... & **C-terminus** amino acid is Gly..... and total number of amino acids on **HSA** chain is 584-3+1=.....?

**2. HSA** transported **lipid-like** molecules transported as cargo with **HSA**? 1. .....acids, 2…....., 3.....**diphilic hydrophilic** and **hydrophobic (non-polar)**

**3.** Put in myristate **C14** carboxylate anion given two oxygen atoms!



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

**4.** What number of **alpha helices** are in human serum albumin **HSA**? .....**Alpha-helices**

**5.** How many domains constitute **HSA** molecule and what its names? .....**domains** ...,...,...

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

**I** G207.....-H3+1=.....;**II** K372.....-E208.....+1=..... ;**III** G584.....-V373.....+1=.....

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

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

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

**IIA:** H.....,H.....,H.....,H.....,H.....,H...

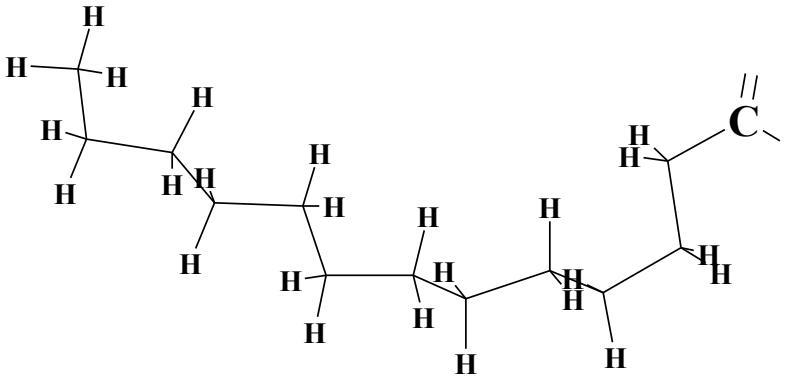
**IIIA**(6): H.....,H.....,H.....,H.....,H.....,H.....,H.....

**10.** What four(5)-helices are in homologous domains **IB-IIB-IIIB**? **IB** .....

**IB** H...,H...,H.....,H...,

**IIB** H....,H....,H....,H....,

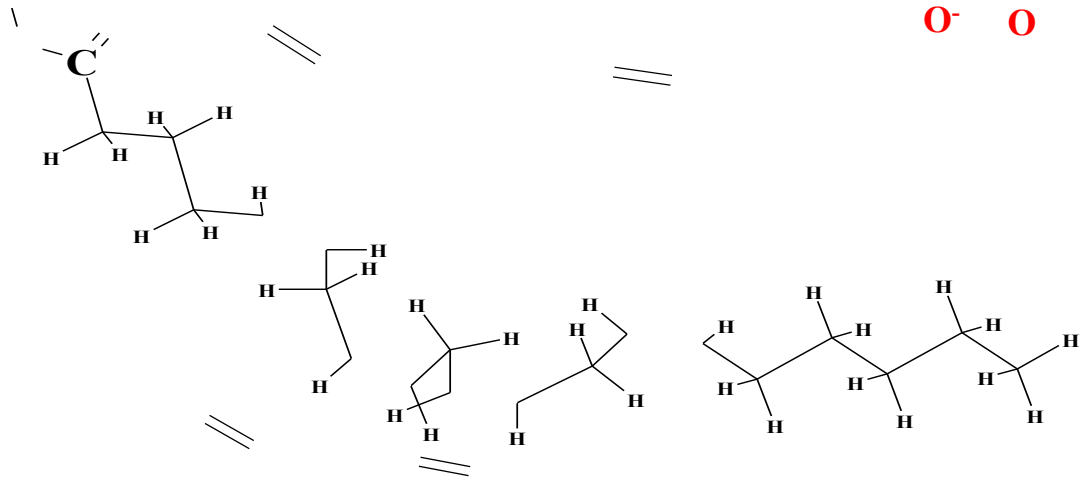
**IIIB** H....,H....,H....,H....,



**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**?
..... fatty acids

**13.** 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**?
..... fatty acids

# 14.1 – 14.6

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

http://aris.gusc.lv/ChemFiles/Albumin/1E7GpI.doc ; 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 Sum of 217 pKa values in molecule pKaSum : 1604.91

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=.....
Sum of 217 pKa values in table ..... to add plus 7 fatty acids 7*4.96=.....

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

## 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** Acid groups number in sum NpKa=215.....+2.....+7.....=.....+7.....=.....

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

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

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

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

**14.2b.** with 7 fatty acids IEP=1639.63 / 224 =.....

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

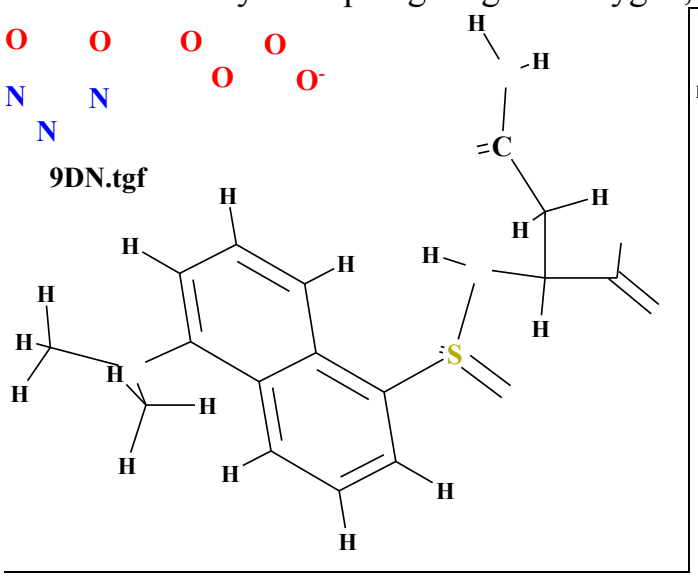
**14.5** Determine albumin molecule charge sign (+). zero „**0**” or (-) at **electrophoresis** pH **8.8**
Underline existing and to cut incorrect given answerecharge:
**-COOH** & **-$NH_3^+$** positive (+) charge ……. IEP=**7.32**< pH=8.8 ..... charge negative(-) **-$COO^-$** & **-$NH_2$**.

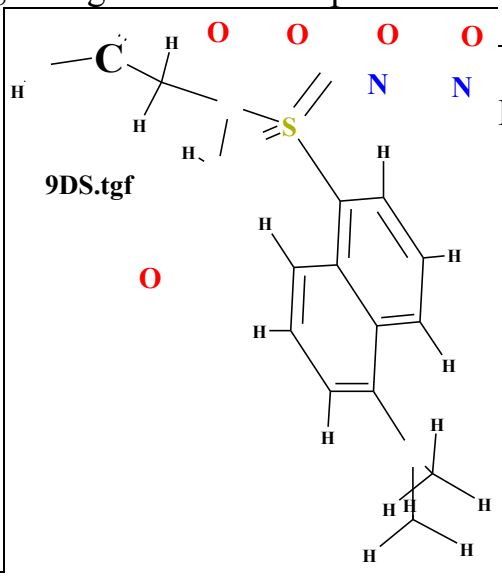
**14.6** Calculate $10^{-7,4}$ M albumin +7 fatty acids solution pH by *Ostwald dilution law* concentration C in logarithm:

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

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

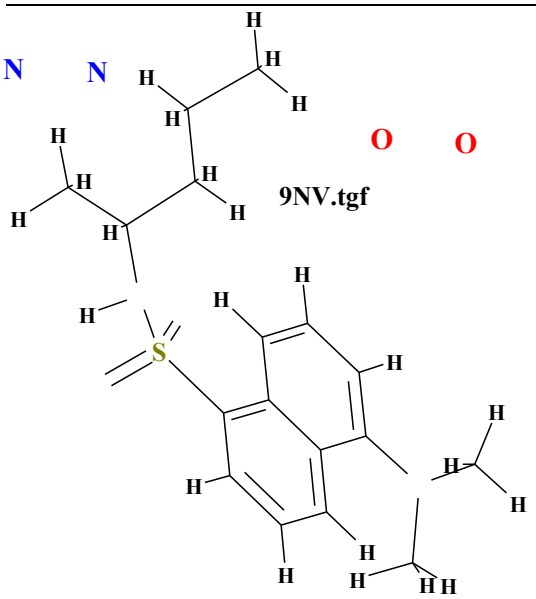
**15.** Put in Dansyl-L-asparagine given oxygen, nitrogen atoms **9DN**.pdb! **2XVV.pdb** of Protein Data Bank in HSA molecule

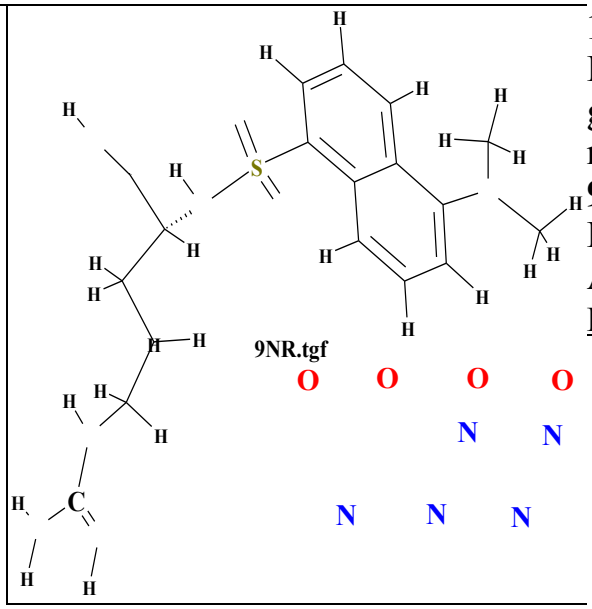



Protein Data Bank structure **2XVQ.pdb**

**16.** Put in Dansyl-L-sarcosine Gly given oxygen, nitrogen atoms **9DS**.pdb!



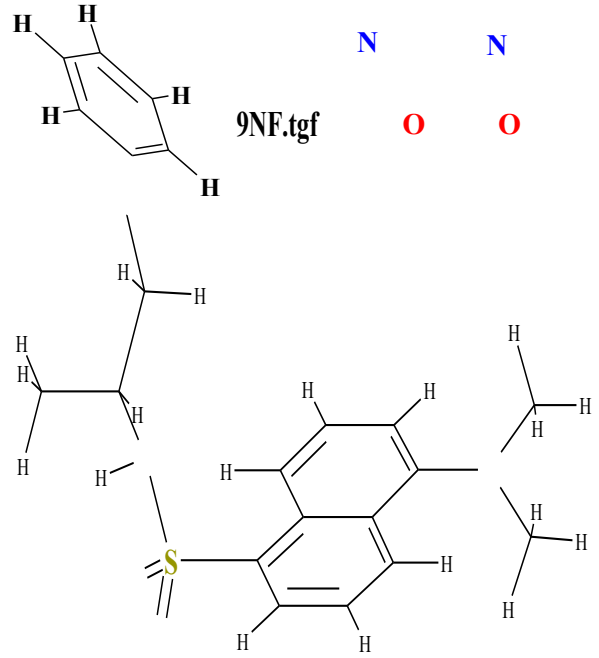



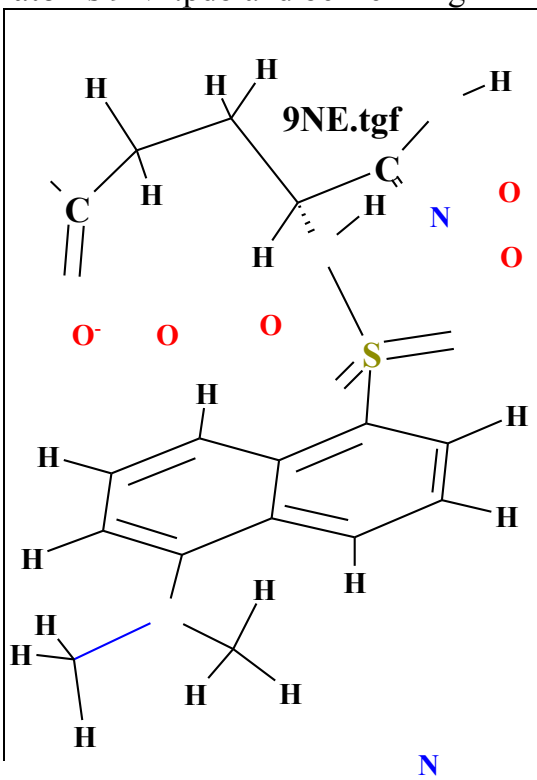

**17.** Put in Dansyl-L-arginine Arg given oxygen, nitrogen atoms **9NR**.pdb **HSA** Human Serum Albumin!**2XVW.pdb** Protein Data Bank

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

**18.** Put in Dansyl-L-norvaline given oxygen, nitrogen atoms **9NV**.pdb

**19.** Put in Dansyl-L-phenylalanine given atoms **9NF**.pdb and benzen ring **in** HSA

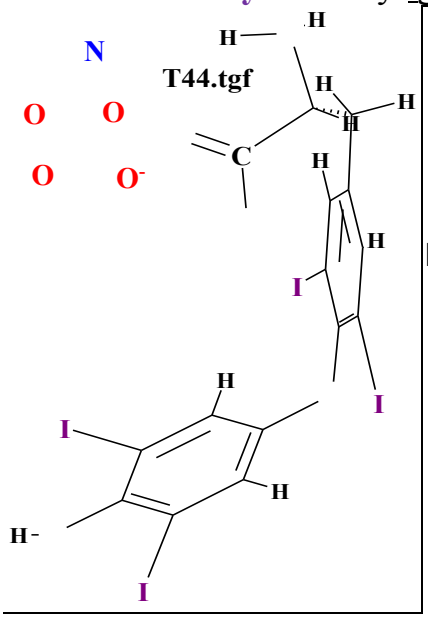


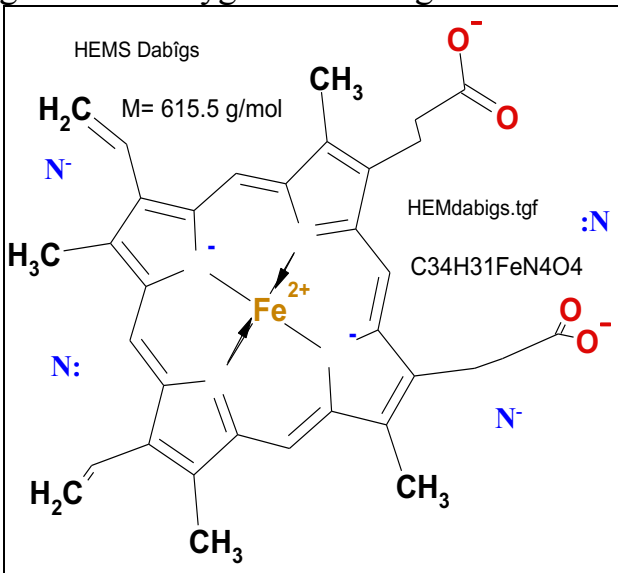




**2XW0.pdb** Protein Data Bank structure

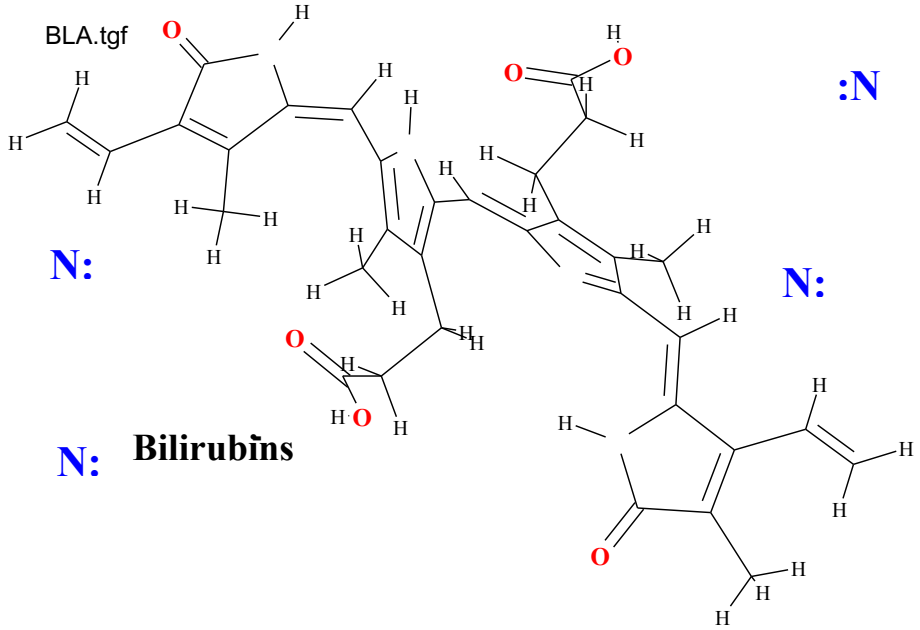
**20.** Put in Dansyl-L-glutamate Glu carboxyl group **>C=O** and **-O-H** atoms **9NE**.pdb from **HSA** Human Serum Albumin Protein Data Bank structure PDB **2XSI.pdb**

**21.** Put in **L-Thyroxin** Tyr given four oxygen and nitrogen atoms which secreted by the follicular cells of the thyroid gland bound on **HSA** Human Serum Albumin **1HK1.pdb** Protein Data Bank structure



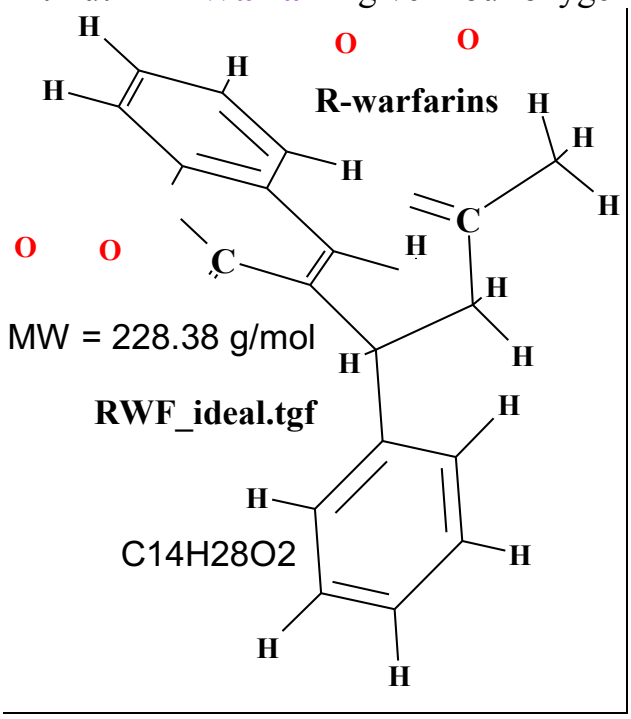




**22.** Put in 4 pyrol **N** atoms on Heme coordinated by iron(II) **HEMdabigs**.pdb from **HSA** Human Serum Albumin Protein Data Bank structure **1O9X.pdb**

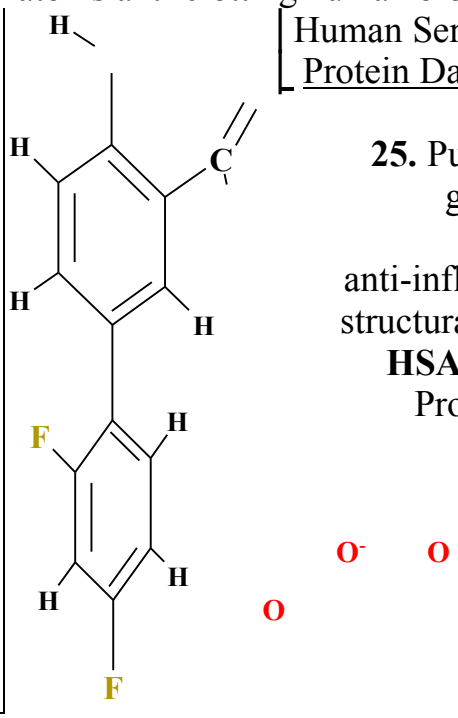


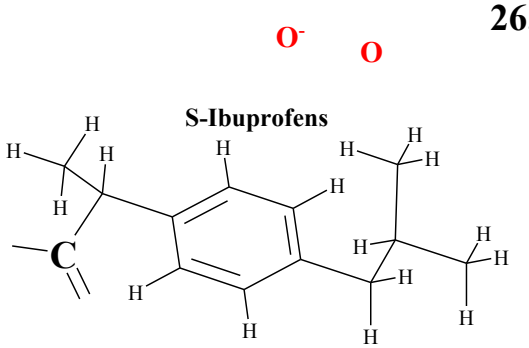

**23.** Put in 4 pyrol **N** atoms on Prosthetic group metabolite **Bilirubin: BLA**.pdb bound on **HSA** Human Serum Albumin Protein Data Bank structure **2VUE.pdb**

**24.** Put in **R-Warfarin** given four oxygen atoms anti-clotting human blood medicine bound to Human Serum Albumin **2BXD.pdb** Protein Data Bank structure.



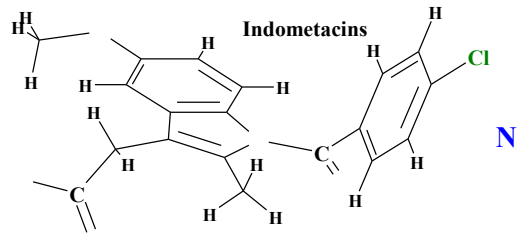

**25.** Put in **Diflunisal** salicylate given three oxygen atoms non-steroidal anti-inflammatory agent **NSAIA** structural formula **1FL**.pdb from **HSA** Human Serum Albumin Protein Data Bank structure **2BXE.pdb**





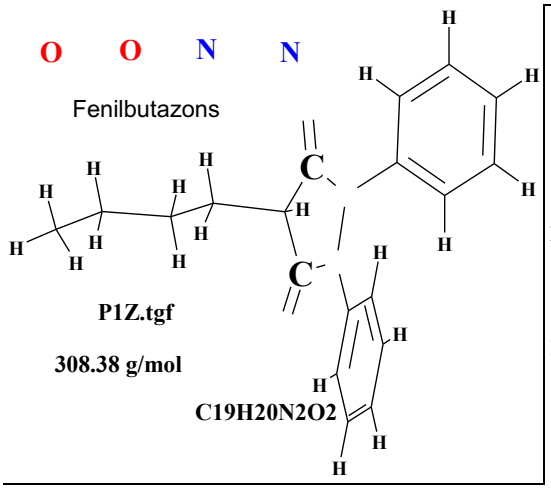

**26.** Put in **Ibuprofen** given two oxygen atoms non-steroidal anti-inflammatory agent **NSAIA** human blood medicine bound on **HSA** Human Serum Albumin

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

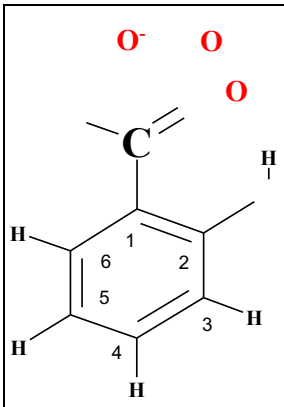



**27.** Put in **IndoMethacin** given four oxygen and nitrogen atoms non-steroidal anti-inflammatory agent **NSAIA** structural formula **IMN**.pdb from **HSA** Human Serum Albumin Protein Data Bank structure **2BXM.pdb**

**28.** Put in **phenyl Butazon** given two oxygen and two nitrogen atoms non-steroidal, antipyretic, anti-inflammatory, analgesic structural formula **P1Z**.pdb from **HAS**

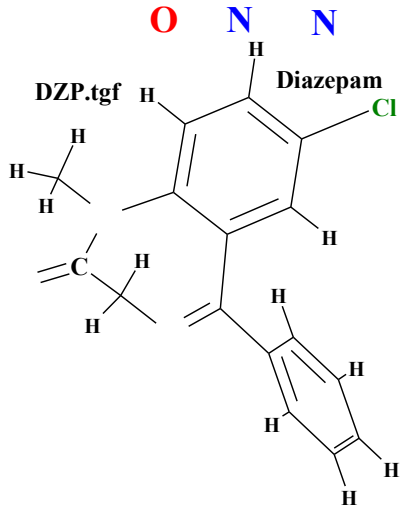


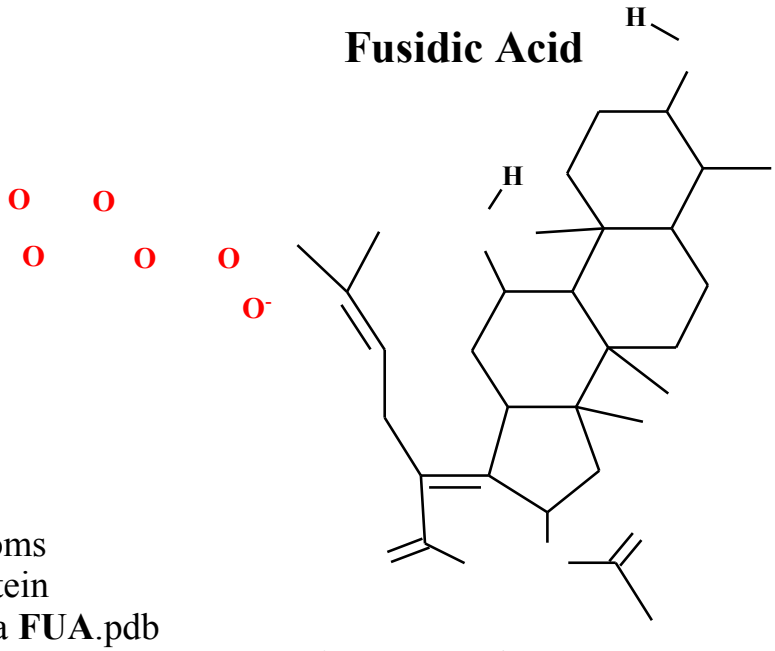

Human Serum Albumin Protein Data Bank structure **2BXM.pdb** .



**29.** Put in **Salicylic Acid** **2-hydroxy-benzoic Acid** given three oxygen atoms Anti-inflammation drug Aspirin Acylating hydroxyl group at C2 on benzene ring **SAL**.pdb **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




**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.**

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

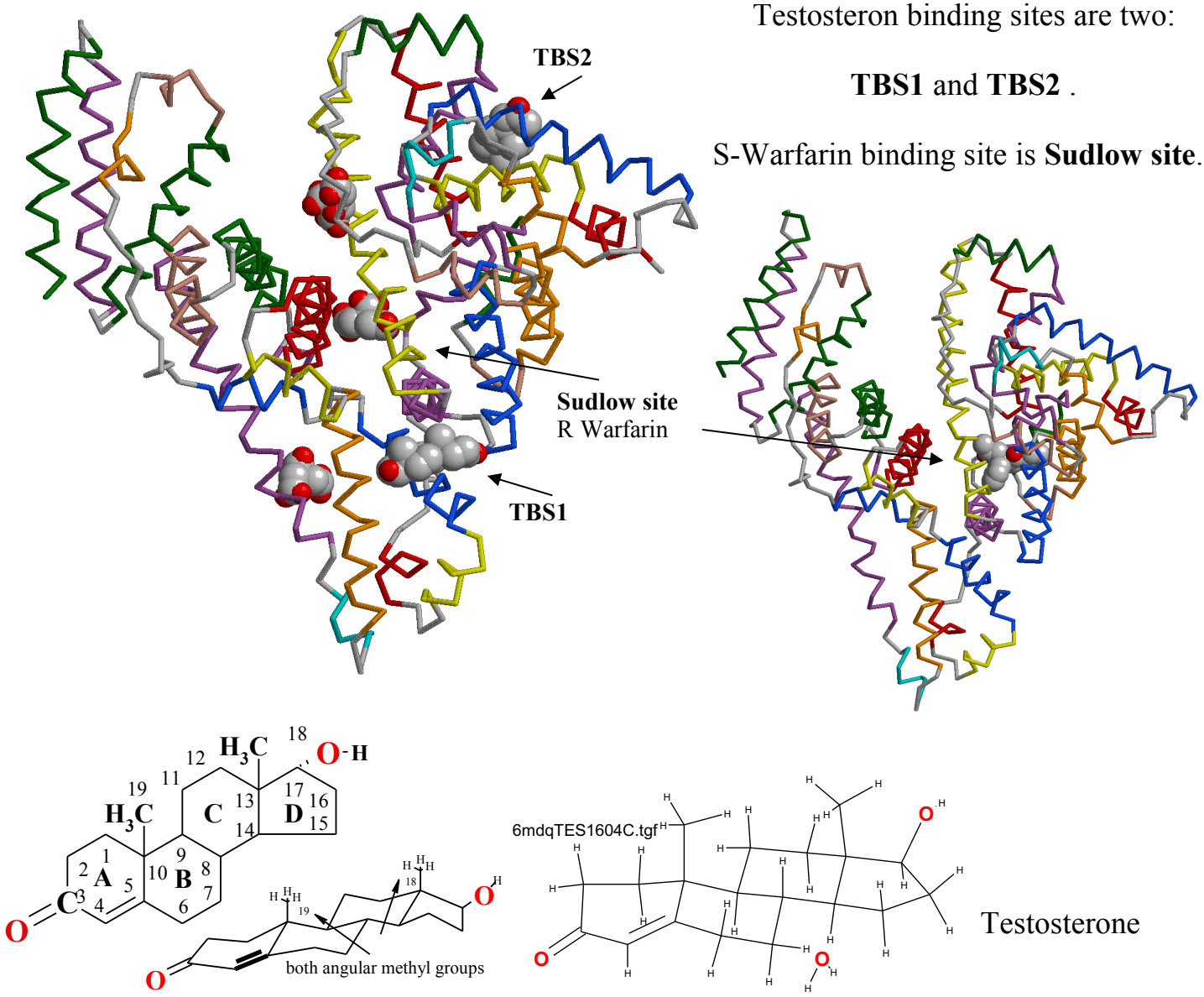



Fig. 5 ESA domains and testosterone binding sites. The testosterone (yellow) and citrate molecules (magenta) are shown with atoms as spheres. Warfarin (from structure of HSA complexed with warfarin, PDB ID: 2BXD), which is bound at Sudlow site I,6 is shown with atoms as blue spheres. Testosterone was 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.