

A. human serum albumin HSA studies by molecule viewers:

ChemScape MDL i  RasMol  ; MAGE  FireFox application.

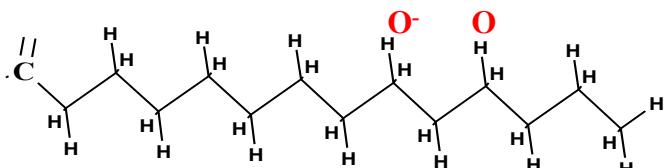
B. Task RSU Aris Kaksis 2025 HSA on Home Page: <http://aris.gusc.lv/ChemFiles/Albumin/cycox.html>

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

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

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

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

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

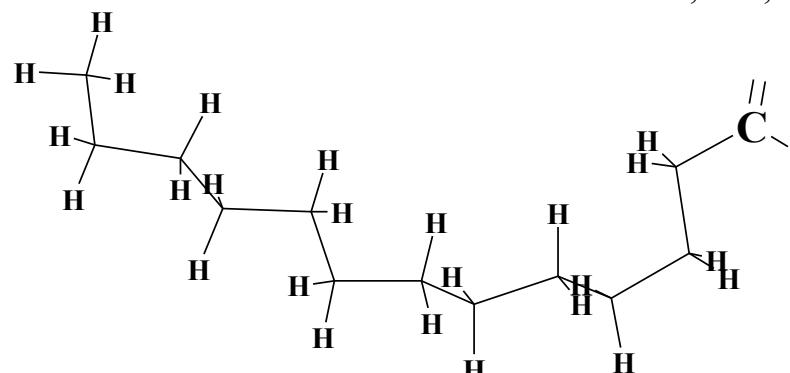
IIB H....,H....,H....,H....,

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

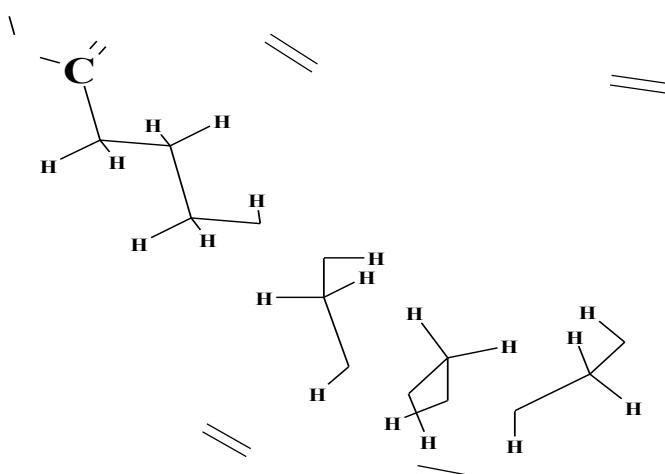


11. Put in stearate **C18** carboxylate oxygen atoms **C=O**, **C-O-**!

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

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

DAHKSEVAHFKDLGEENFKALVIAFAQYLQQCPFEDHVKLNEVTEFAKTCVADESAENCDKSLHTLFGDKLCTVATL
RETYGEMADCCAKQEPPERNECFLQHKDDNPNLPRLVPEVDVMCTAFHDNEETFLKKYLYEIARRHPFYAPELLFFAKR
YKAASFTECCQAADKAACLLPKLDELRDEGKASSAKQRLKCASLQKFGERAFAVARLSQRFPKAFAEVSKLVTDLTK
VHTECCHGDLLECADDRADLAKYICENQDSISSKLKECCEKPLLEKSHCIAEVENDEMPADLPSLAADFVESKDVCKNYA
EAKDVFGLGMFLYEYARRHPDYSVVLRLAKTYETTLEKCCAAADPHECYAKVFDEFKPLVEEPQNLIKQNCELFEQLGE
YKFQNALLVRYTKVPQVSTPTLVEVSRLGKVGSKCKHPEAKRMPCAEDYLSVVLNQLCVLHEKTPVSDRVTKCCTES
LVNRRPCFSALEVDETYVPKEFNAETFTFHADICLSEKERQIKKQTALVELVKHKPKATKEQLKAVMDDFAAFVEKCK
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 as 7 Nonanoic acids $pK_a = 4.96$; $7 \cdot 4.96 = \dots$

Sum of 217 pKa values in table to add plus 7 fatty acids $7 \cdot 4.96 = \dots$

Sum of 217 pKa values in table and to add plus 7 fatty acids $pK_a : 1604.91 + 34.72 = \dots$

Calculation tasks for Human Serum Albumin molecule

Prolytic constant pK_a isoelectric point $IEP = pK_a$ calculate of side chains $\Sigma pK_{aR\text{side group}}$, $pK_{a\text{NterminalNH}_3}$ and $pK_{a\text{CterminalCOO}}$ -constants sum divide with number of acid groups NpK_a :

$$IEP = pK_a = (\Sigma pK_{aR\text{side group}} + pK_{a\text{Nterminal}} + pK_{a\text{Cterminal}}) / NpK_a$$

14.1 Acid groups number in sum $NpK_a = 215 + 2 + 7 = \dots + 7 = \dots$

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

Sum on 2nd page are calculate as $\Sigma pK_{aR\text{side group}} + pK_{a\text{Nterminal}} + pK_{a\text{Cterminal}} + 7 \cdot pK_a = \dots$

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

no 7 fatty acids $NpK_a = 215 + 2 = \dots$ $IEP = 1604.91 / 217 = \dots$

14.2b. with 7 fatty acids $IEP = 1639.63 / 224 = \dots$

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 & -NH₃⁺ positive charge -COO⁻ & -NH₂ charge is negative -COO⁻ & -NH₂
Underline existing and to cut incorrect given answererecharge:

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 answererecharge:

-COOH & -NH₃⁺ positive (+) charge pH=7.36 < IEP=7.4 charge negative(-) -COO⁻ & -NH₂.

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 answererecharge:

-COOH & -NH₃⁺ positive (+) charge IEP=7.32 < pH=7.36 charge negative(-) -COO⁻ & -NH₂.

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

Underline existing and to cut incorrect given answererecharge:

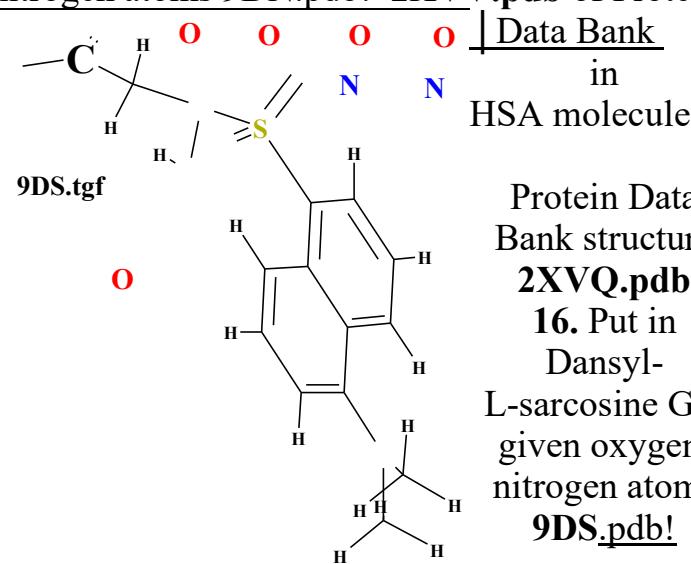
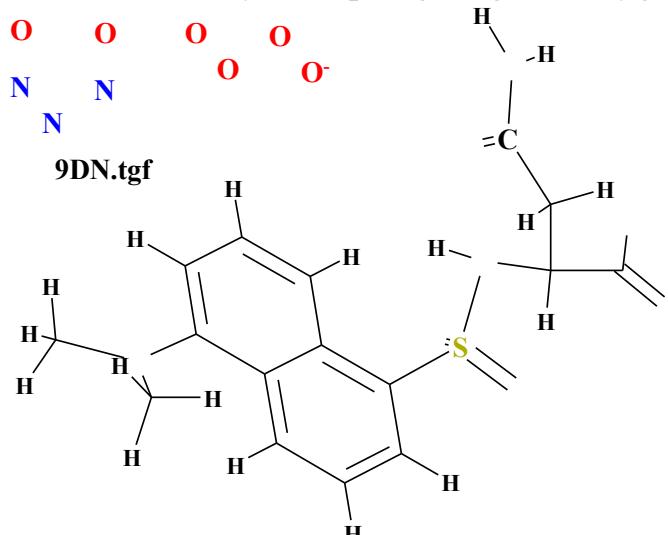
-COOH & -NH₃⁺ positive (+) charge IEP=7.32 < pH=8.8 charge negative(-) -COO⁻ & -NH₂.

14.6 Calculate $10^{-7.4}$ M albumin +7 fatty acids solution pH by *Ostwald dilution law* concentration C in logarithm:

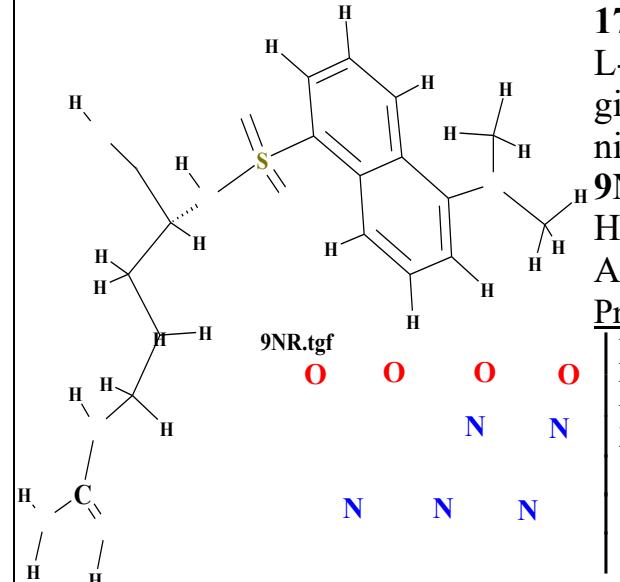
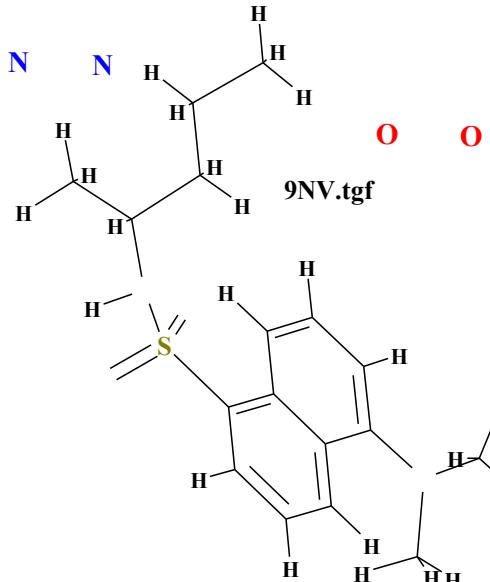
$$: pH = \frac{pK_a - \log C}{2} = \frac{7,3198 - \log 10^{-7,4002}}{2} = \frac{7,3198 + 7,4002}{2} = 14,720 / 2 = \dots$$

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



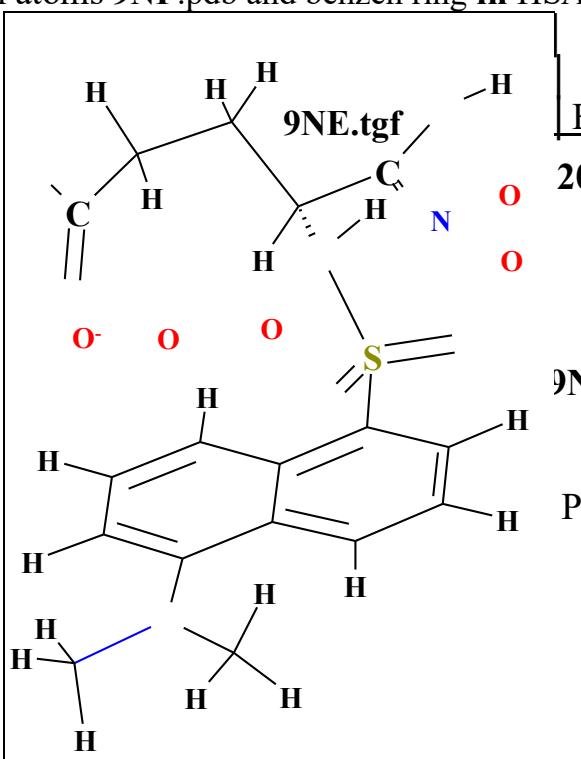
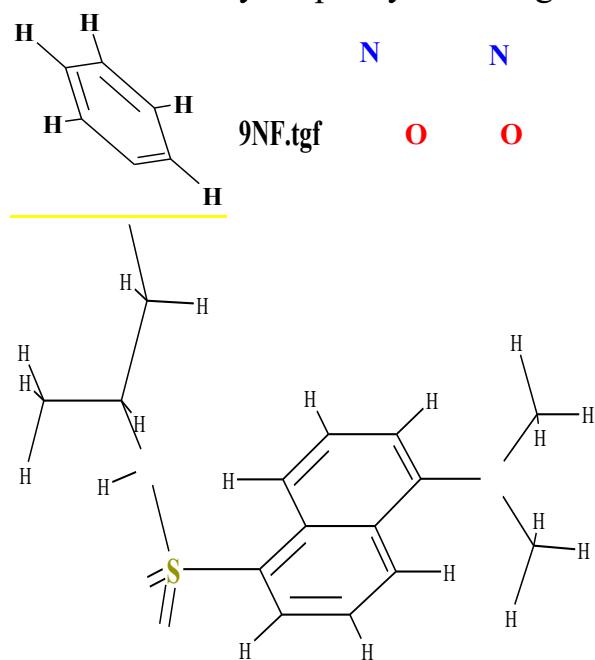
Protein Data Bank structure
2XVQ.pdb
16. Put in Dansyl-L-sarcosine Gly given oxygen, nitrogen atoms **9DS.pdb!**



17. Put in Dansyl-L-arginine Arg given oxygen, nitrogen atoms **9NR.pdb** **HSA Human Serum Albumin!2XVW.pdb** Protein Data Bank structure
2XW1.pdb

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

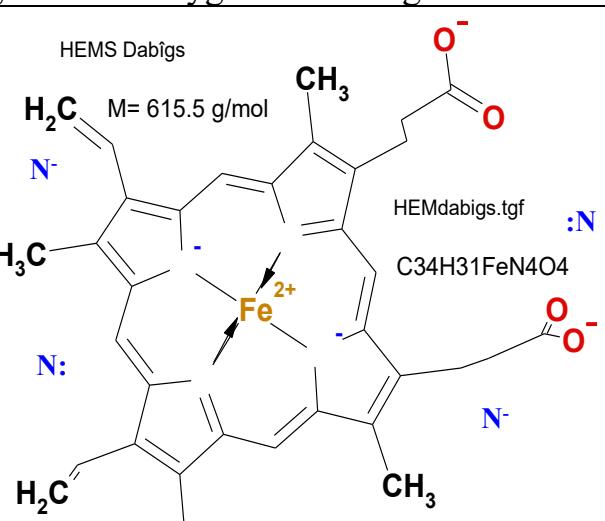
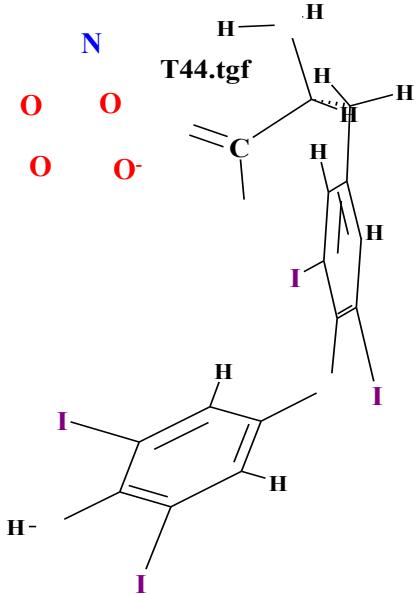
19. Put in Dansyl-L-phenylalanine given atoms **9NF.pdb** and benzen ring in HSA



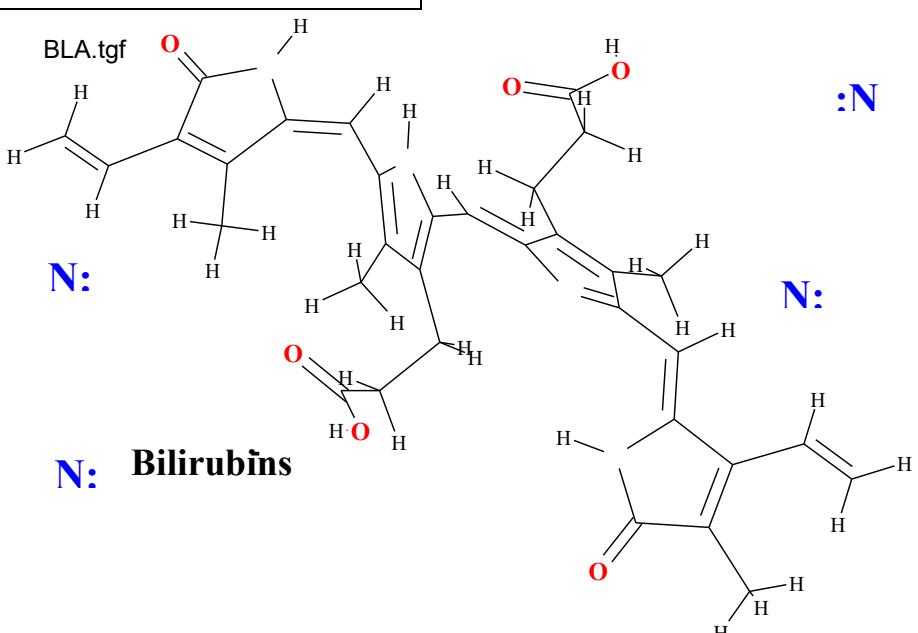
2XW0.pdb
Protein Data Bank structure

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



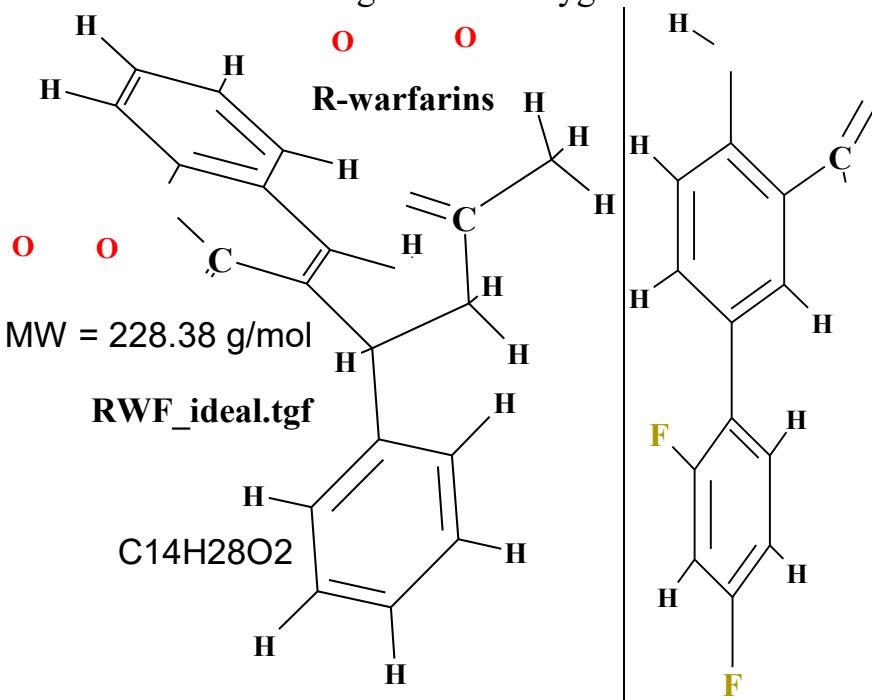
the follicular cells of the thyroid gland bound on
HSA Human Serum Albumin
1HK1.pdb Protein Data Bank structure



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**

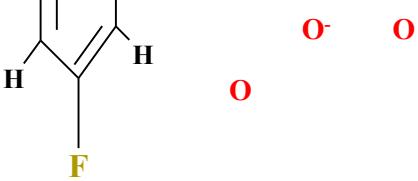
23. Put in 4 pyrol **N** atoms on Prosthetic group metabolite **Bilirubin**:
BLA.pdb bound on **HSA Human Serum Albumin** Protein Data Bank structure **2VUE.pdb**

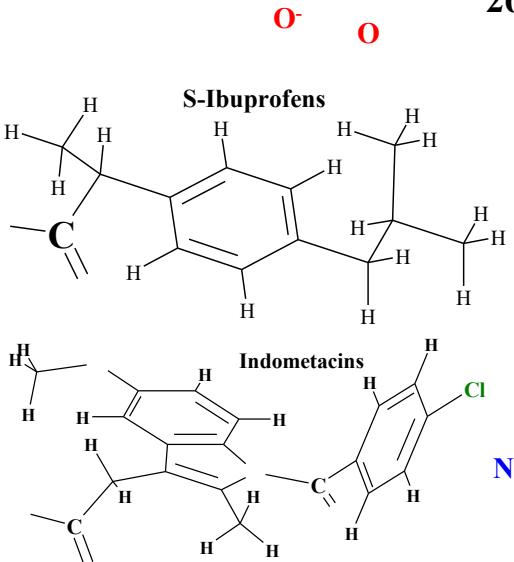
24. Put in **R-Warfarin** given four oxygen atoms anti-clotting human blood medicine bound to



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**



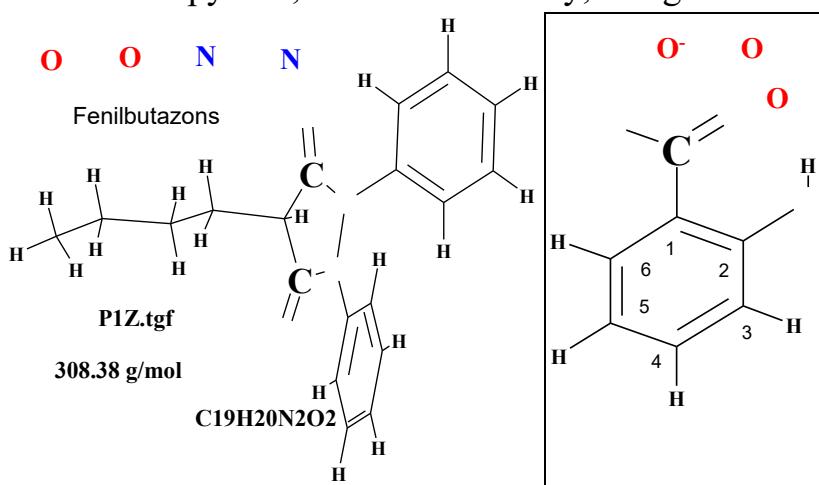


26. Put in **Ibuprofen** given two oxygen atoms non-steroidal anti-inflammatory agent **NSAIA** human blood medicine bound on **HSA** Human Serum Albumin

[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](#)

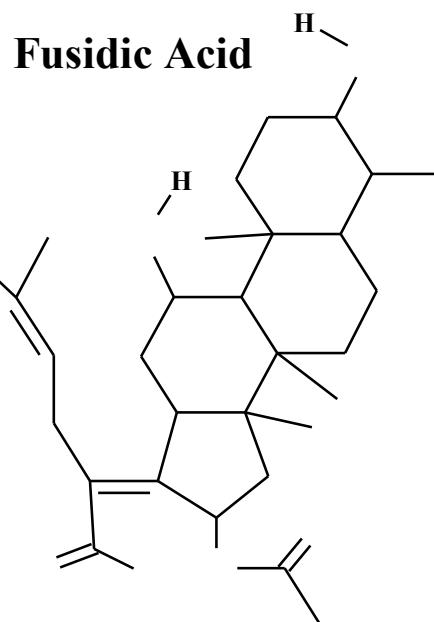
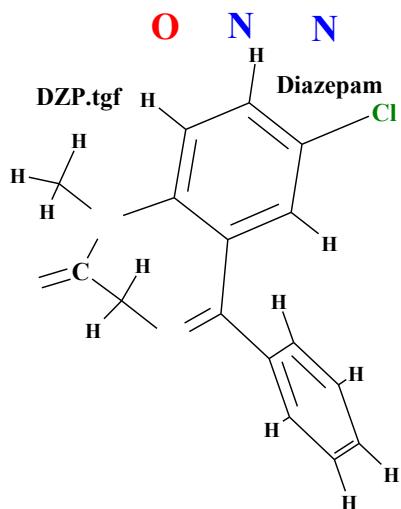
28. Put in **phenyl Butazon** given two oxygen and two nitrogen atoms non-steroidal, antipyretic, anti-inflammatory, analgesic structural formula **P1Z.pdb** from **HAS**



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



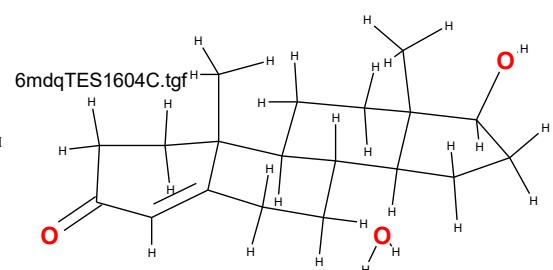
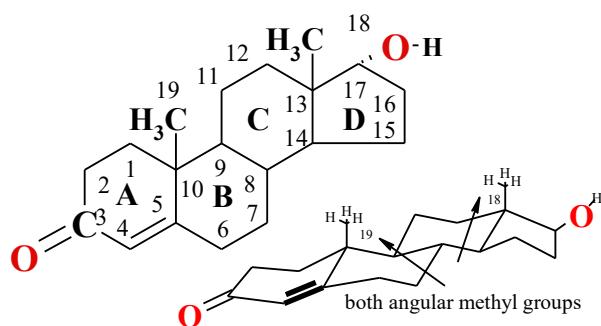
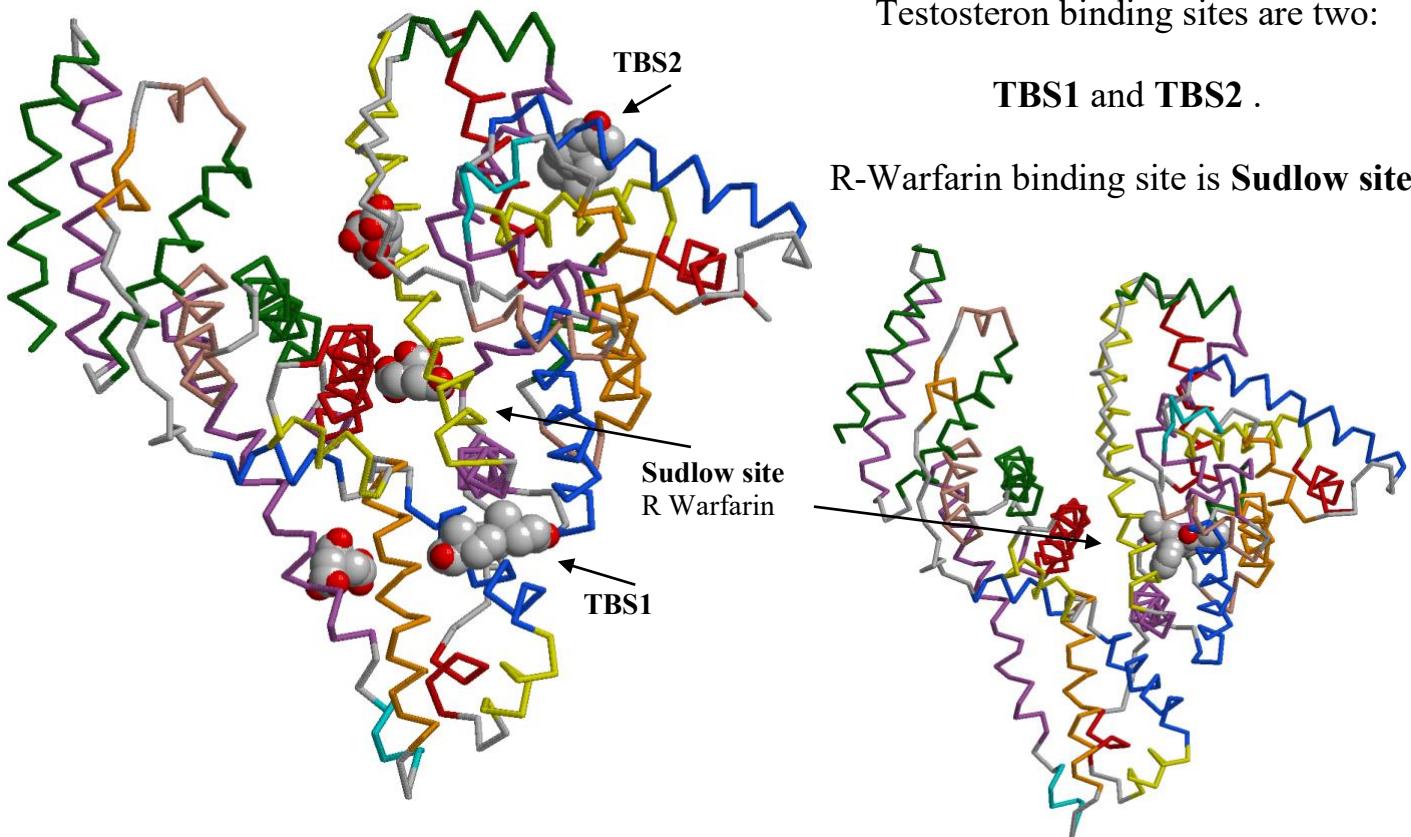
31. Put in **Fusidic Acid** given six atoms antibiotic, Anti-Bacterial Agent, Protein Synthesis Inhibitor structural formula **FUA.pdb** from **HSA** Human Serum Albumin Protein Data Bank structure **2VUF.pdb**.

[Protein Data Bank structure 2BXF.pdb](#)

Testosteron binding sites are two:

TBS1 and TBS2 .

R-Warfarin binding site is **Sudlow site.**



Testosterone

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.](#)