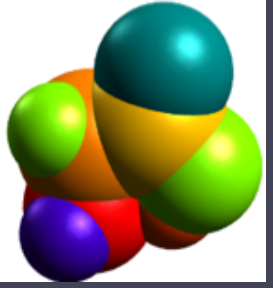

Computational Chemistry Day

Formation of a ternary human serum albumin-indomethacin-quercetin complex and energy transfer

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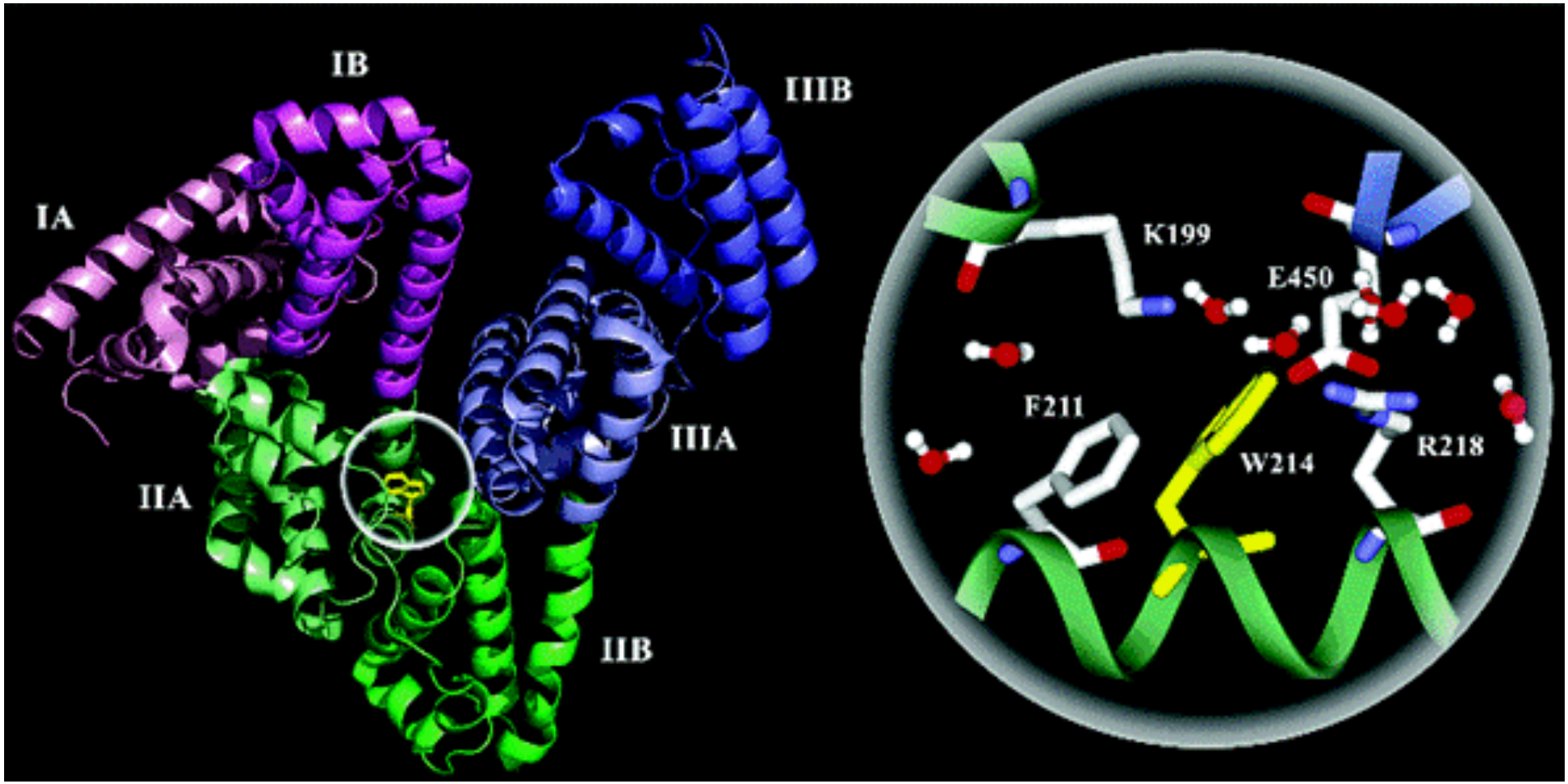
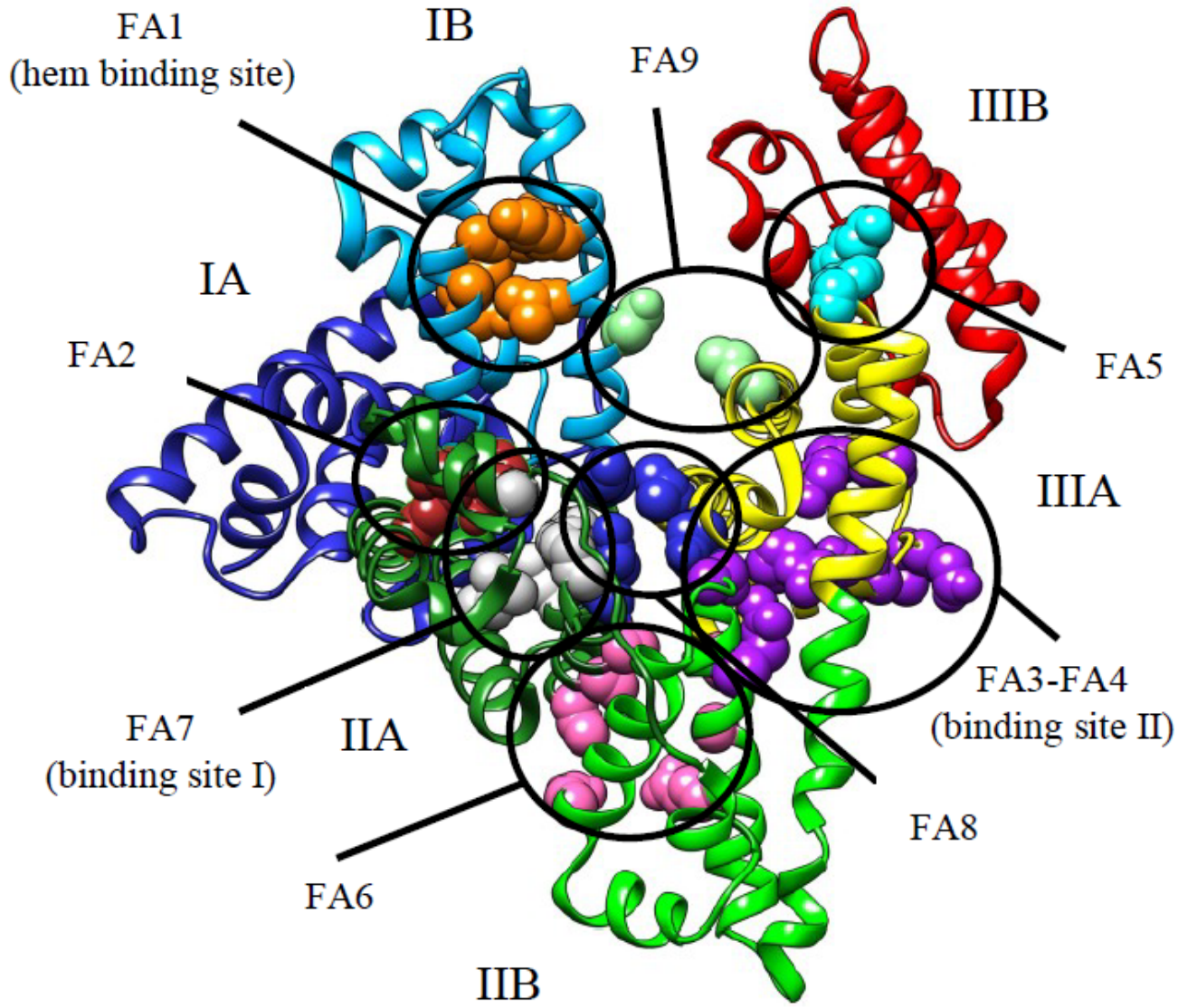
Human serum albumin (HSA)

- M_r = 66500
- ~60% of all plasma proteins
- Functions:
  - Oncotic pressure regulation
  - Plasma pH regulation
  - Transfer and storage of hydrophobic molecules (bilirubin, fatty acids, steroid and thyroid hormones, hem…)
- Multiple binding sites:
  - IIA, IIIA, and IB
  - 9 fatty acid binding sites
  - Binding sites for metal ions

Human serum albumin (HSA)



Binding of physiological ligands

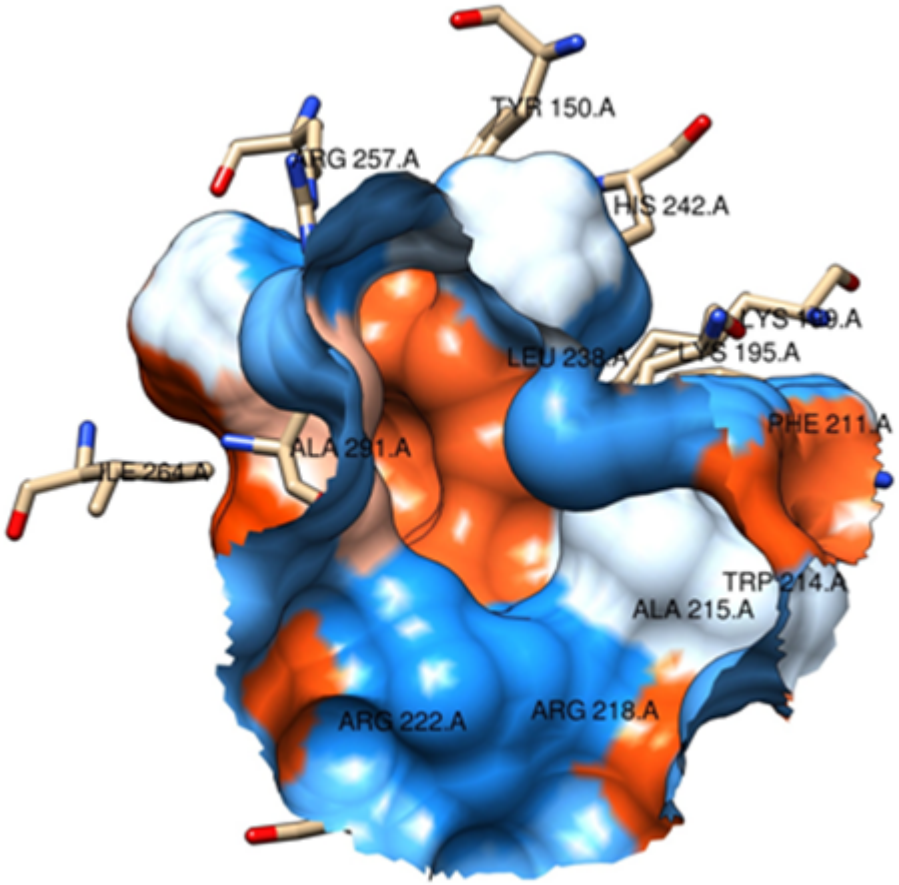


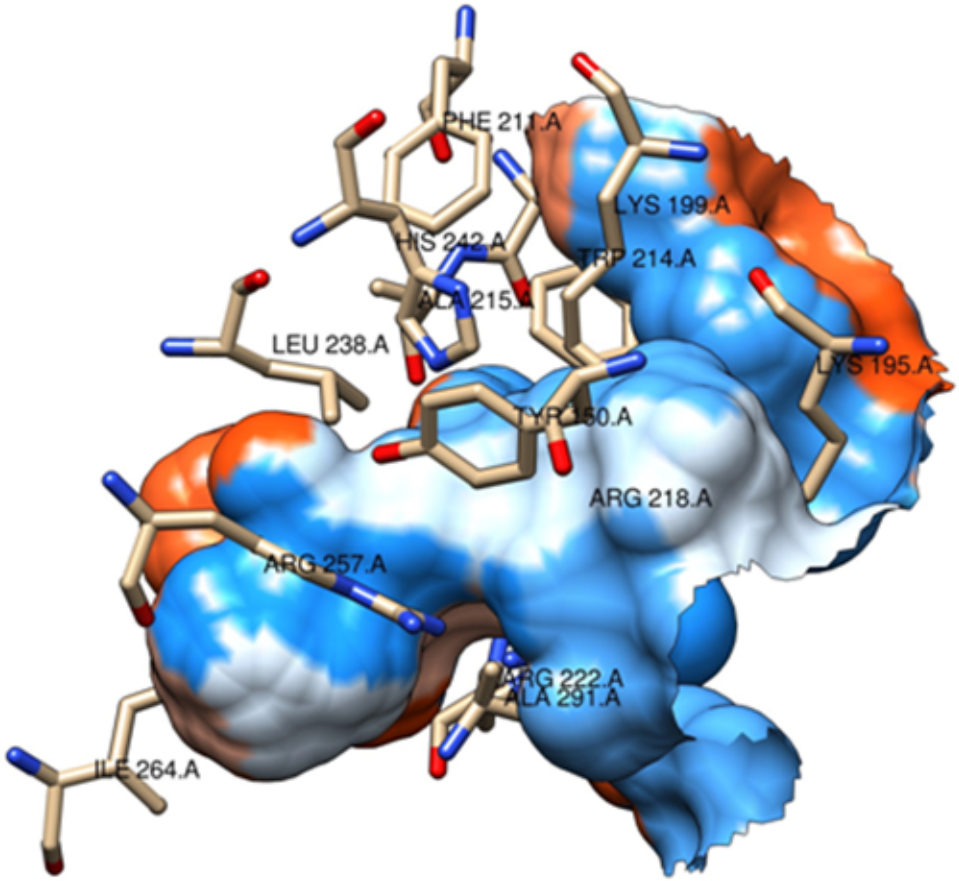
Sudlow binding site I

- Warfarin-azapropazone binding site
- In the IIA subdomain
- Binds the largest number of drugs
- Dicarboxylic acids or medium size heterocyclic molecules
- Negative charge in the center of the ligand
- Consists of two non-polar binding pockets
- Several centrally located polar amino acids

Sudlow binding site I

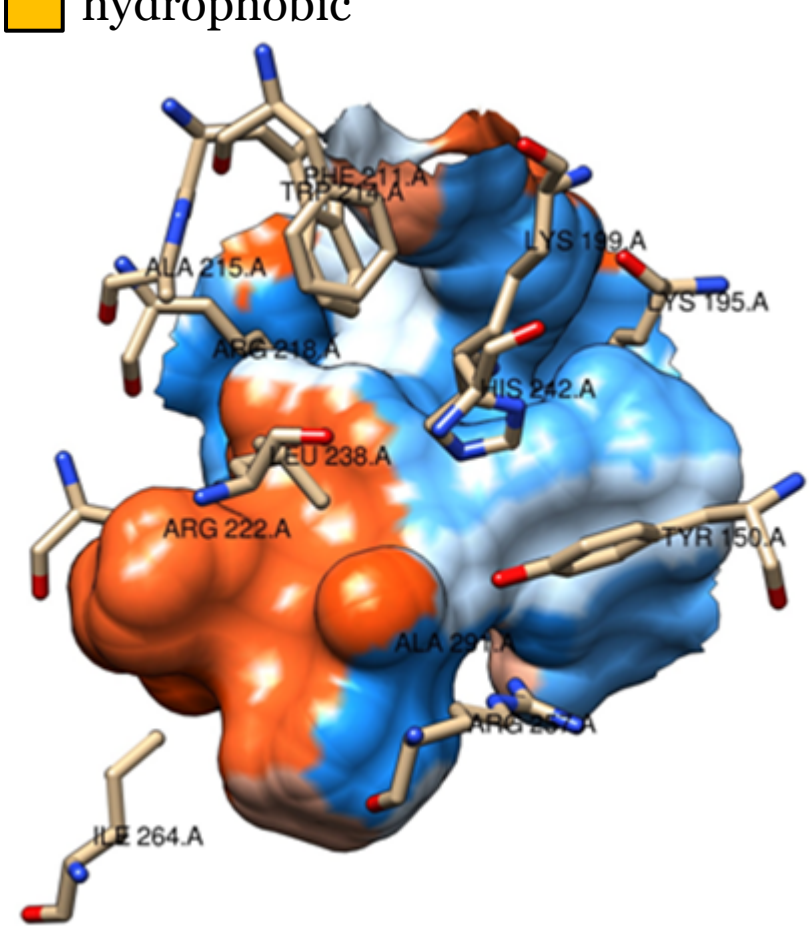
- hydrophilic
- neutral
- hydrophobic

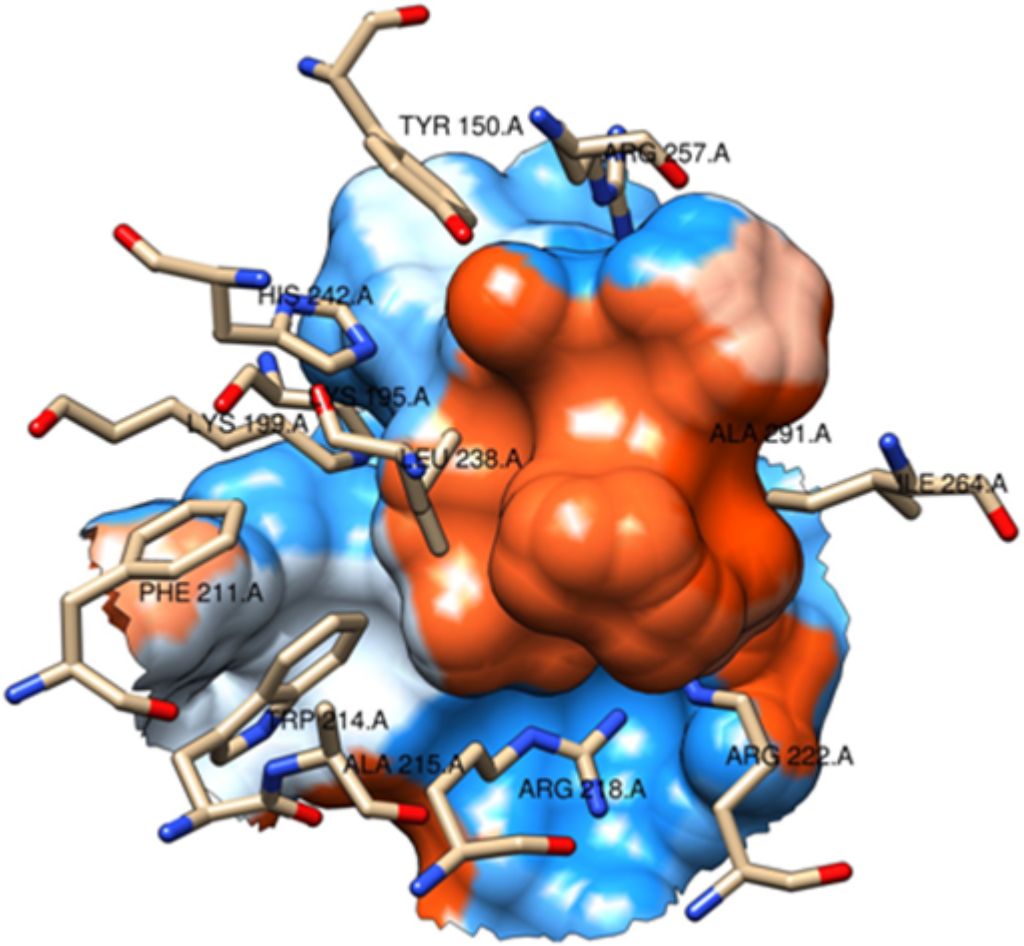




Sudlow binding site I

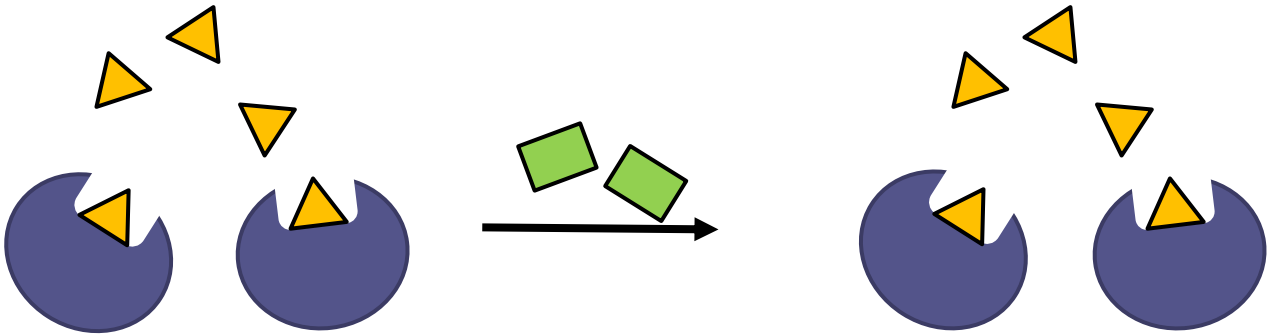
- hydrophilic
- neutral
- hydrophobic





Significance of drug-plasma protein binding

- Active inactive drug fraction
- Pharmacokinetic interactions



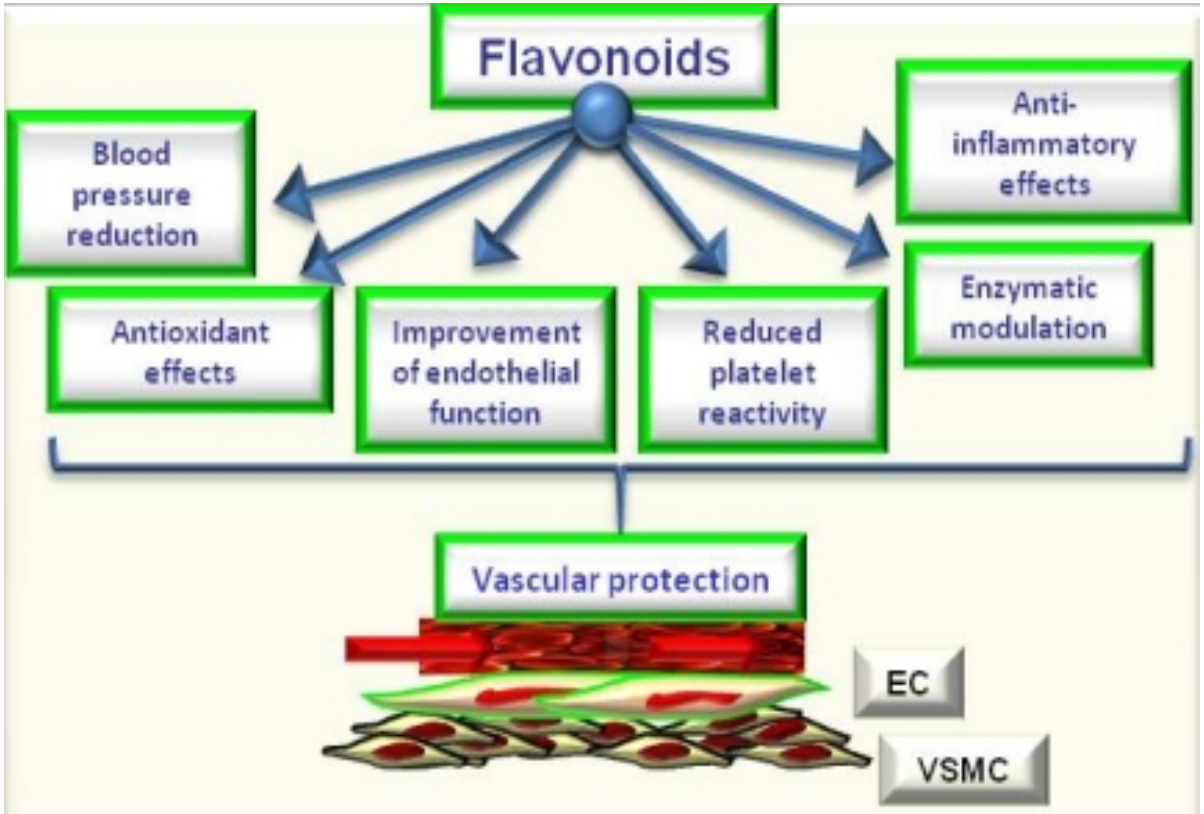
Significance of drug-plasma protein binding

- Active inactive drug fraction
- Pharmacokinetic interactions

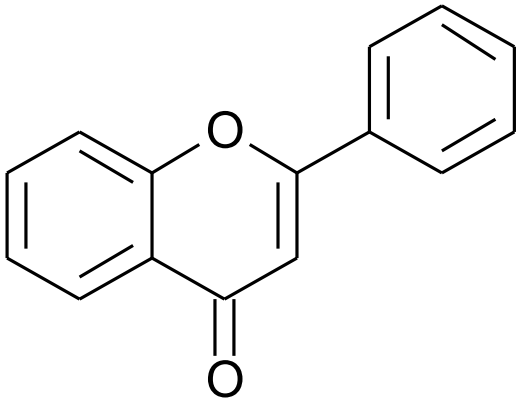
Drugs with high HSA binding percentage				
Phenytoin (90%)	Midazolam (95%)	Mycophenolic acid (97%)	Tiagabine (96%)	Tamoxifen (>90%)
Lamotrigine (>90%)	Piroxicam (>99%)	Protease inhibitors (>90%)	Furosemide (>99%)	Valproate (90–95%)
Oxcarbazepine (>90%)	Warfarin (>99%)	Efavirenz (>99%)	Ezetimibe (>99%)	Vancomycin (>90%)
Stiripentol (99%)	Sertraline (98%)	Spironolactone (>90%)	Alfentanil (92%)	Tacrolimus (98%)
Clopidogrel (98%)	Carbamazepine (>90%)	Canrenone (>90%)	Tolbutamide (96%)	Indomethacin (>99%)

Flavonoids

- Natural products
- Abundant in fruits and vegetables
- Primarily bound to the binding site I

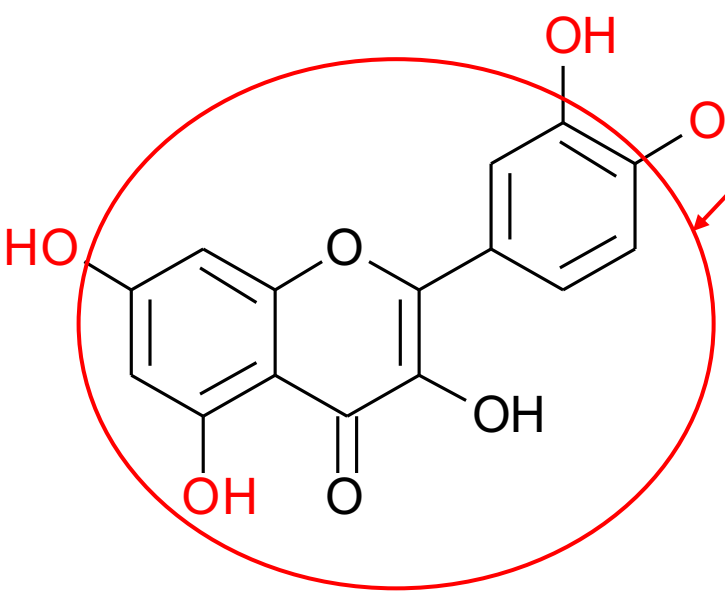


Flavonoids

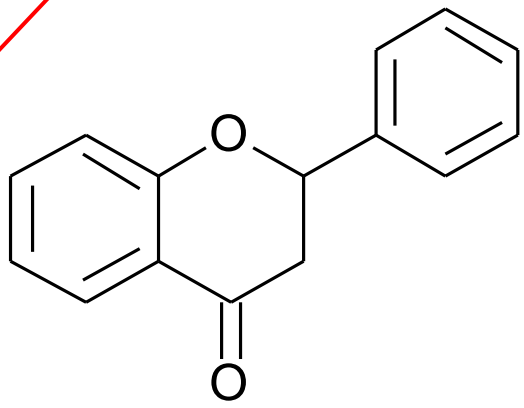


flavons

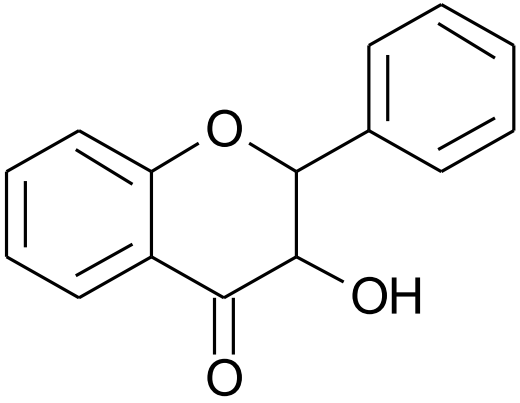
Quercetin



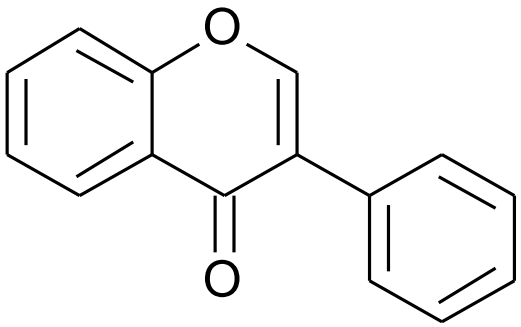
flavonols



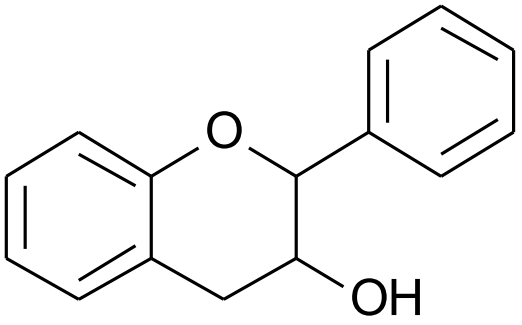
flavanons



flavanonols



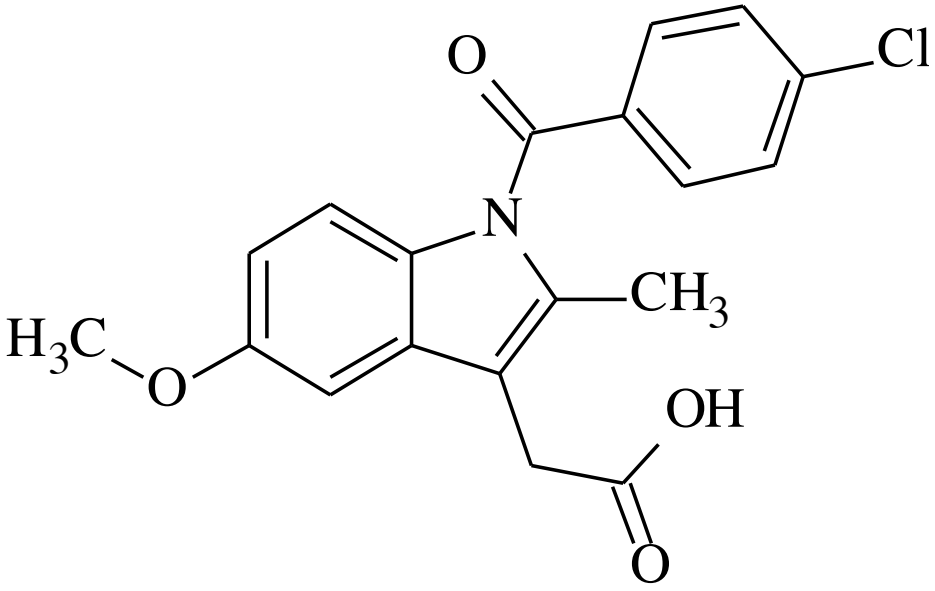
isoflavons



flavanols

Indomethacin

- Nonsteroidal anti-inflammatory drug (NSAID)
- Arylalcane acid (derivative of methylated indole)
- Inhibits both COX-1 and COX-2 enyzmes (greater affinity for COX-1)
- Inhibits synthesis of inflammatory mediators

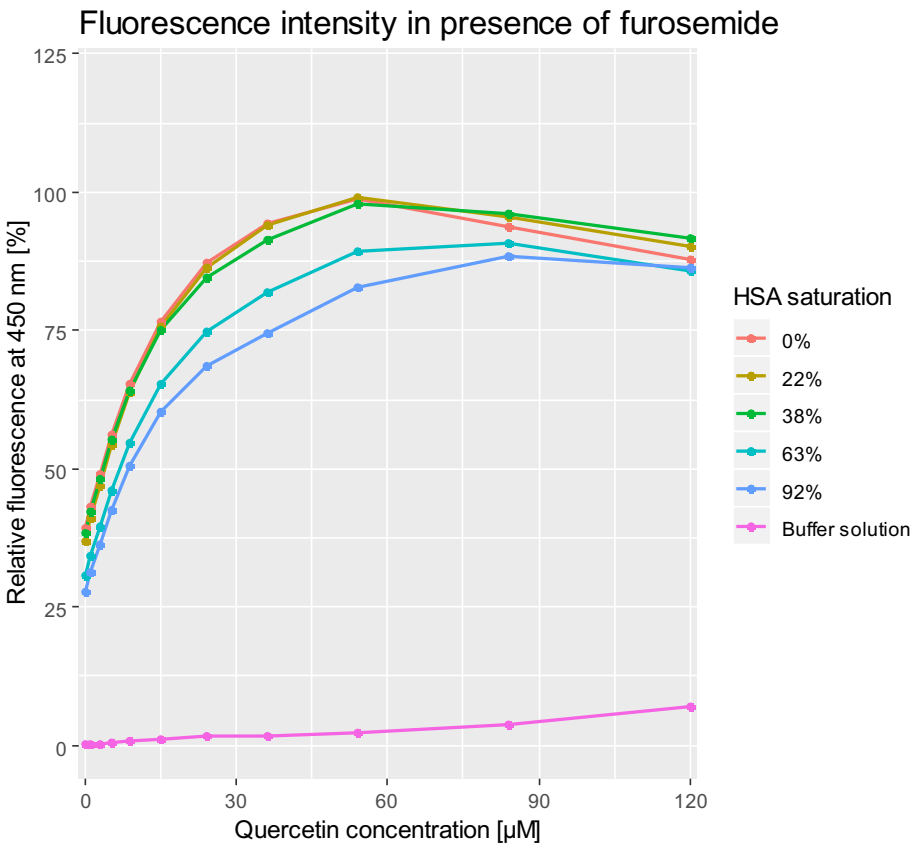


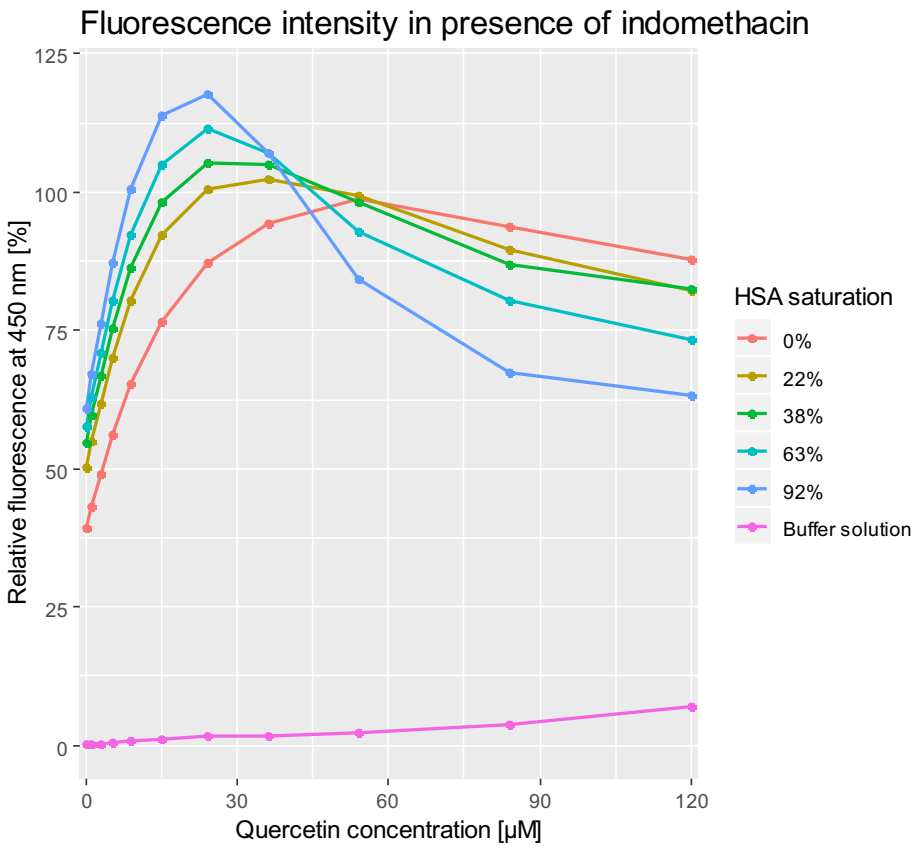
Methods

- Fluorescence spectroscopy:
  - ↓ fluorescence of HSA (Trp): λ_{abs} = 295 nm, λ_{em} = 340 nm
  - ↑ fluorescence of flavonoids: λ_{abs} = 450 nm, λ_{em} = 526 nm

- Molecular modeling
  - Binary and ternary HSA-ligand(s) complex(es)
    - Docking (AutoDock 4.2.6.)
      - PDB entry 2BXM (HSA + indomethacin, 2.5 Å, monomer A), added missing side chains and hydrogen atoms, water molecules removed, total charge -14 at pH 7.4
      - Docking grid 80×80×80 Å centered at Trp214, resolution 0.375 Å
      - 100 docking attempts using Lamarckian Genetic Algorithm with population size of 150, maximum number of energy evaluations of 25 000 000, 27 000 generations, mutation rate 0.02, crossover rate 0.08, and RMSD of 2.0 Å as a criterion for cluster separation
    - Molecular dynamics (Amber 16)
      - 300 ns simulation, 300K, protein force field – FF14SB, octaedar of 20 Å TIP3P water, force field parameters created using Antechamber and GAFF force field
      - Charge of both ligands and HSA was neutralized by adding equivalent amount of Na^+ ions

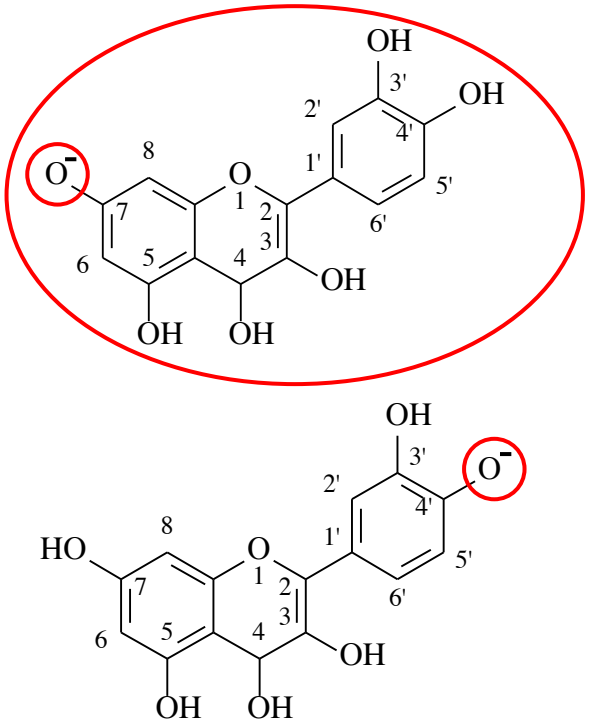
Fluorescence spectroscopy



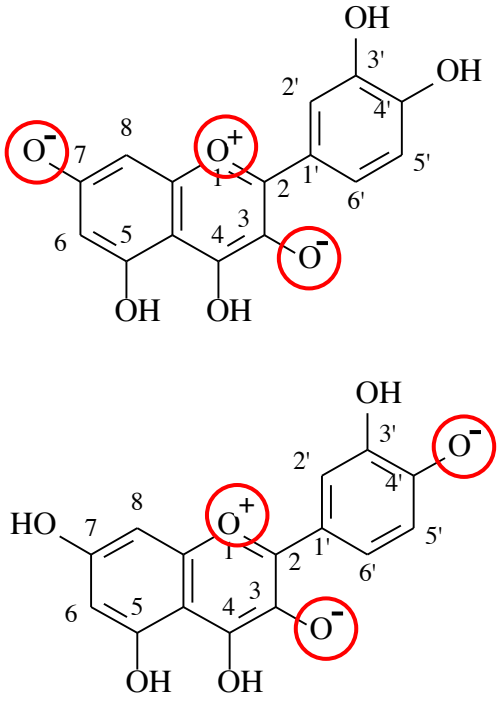


Docking studies

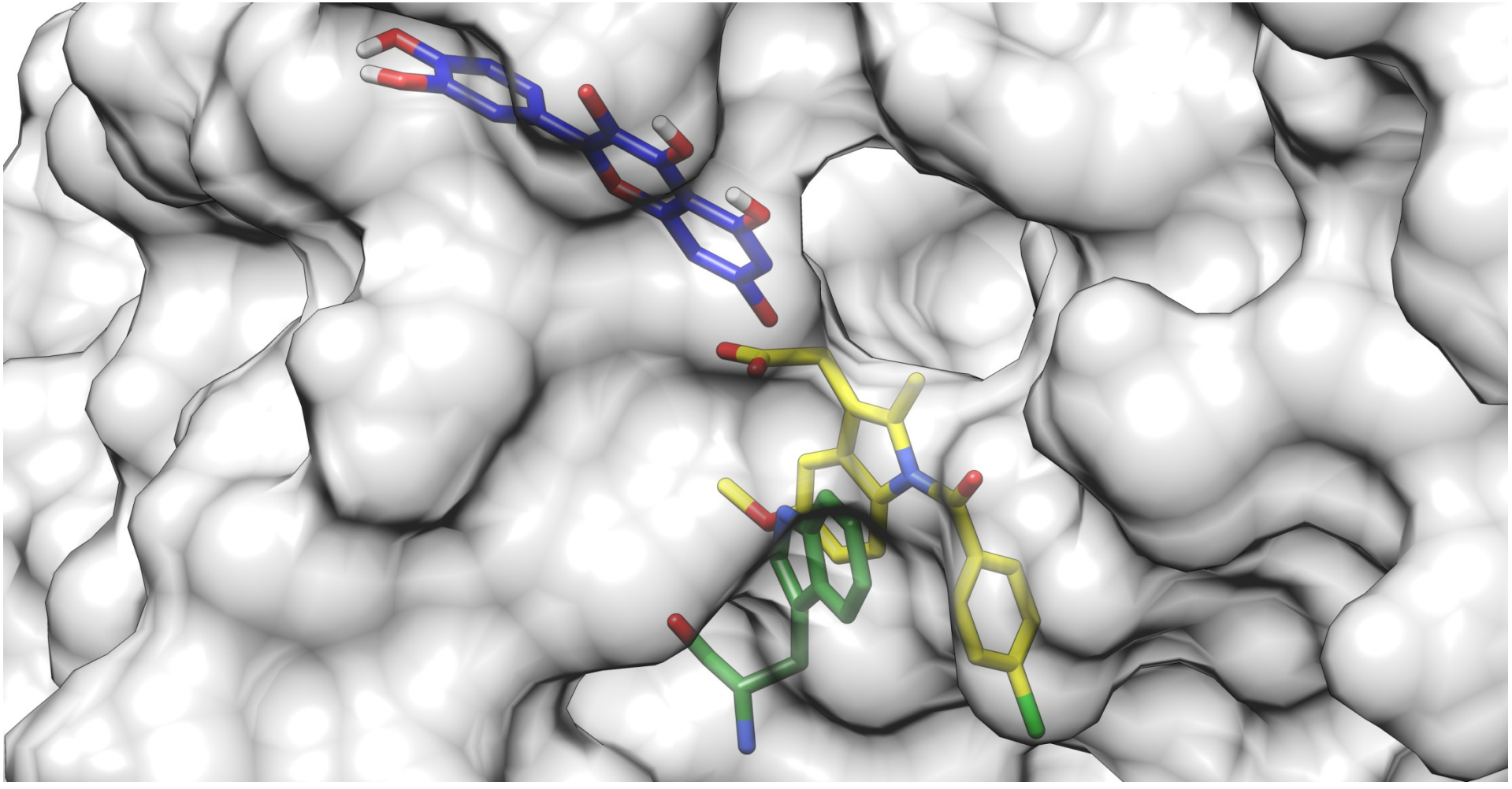
Quercetin anions



Quercetin fluorescent anions

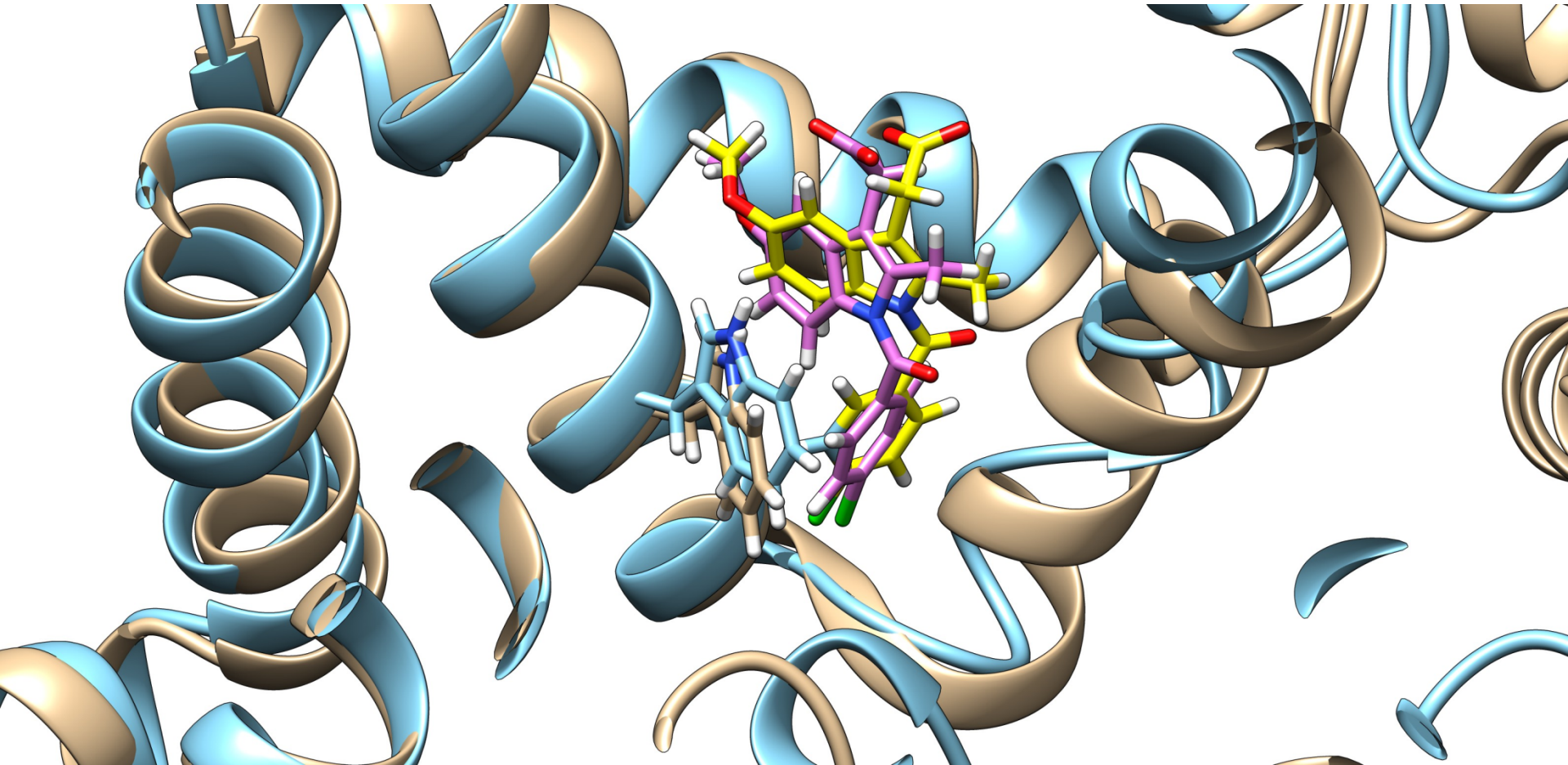


Docking studies



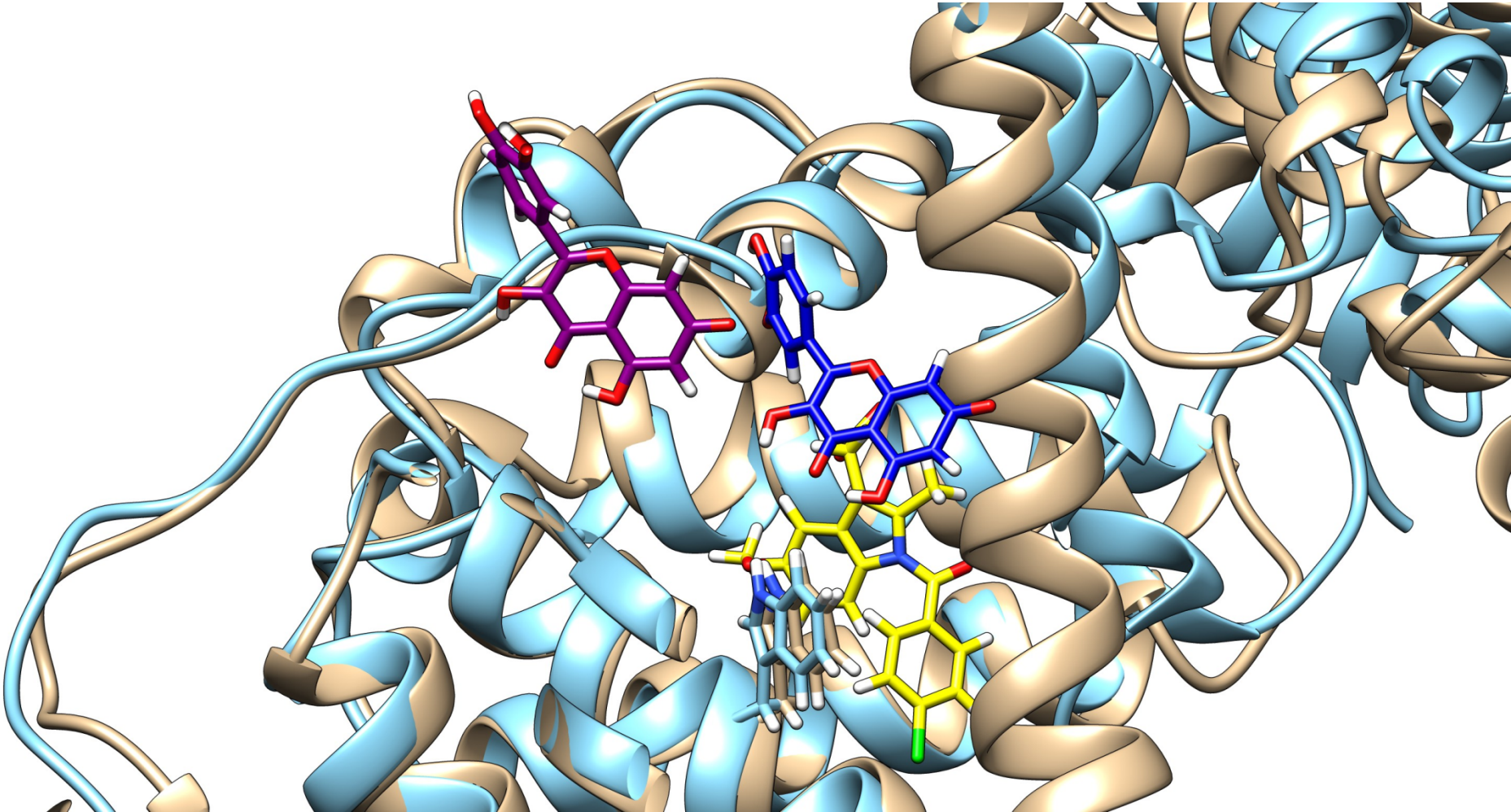
Molecular dynamics

- Indomethacin



Molecular dynamics

- Quercetin

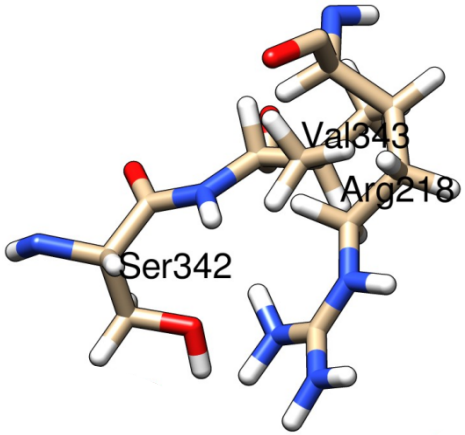


Decomposition of binding energy

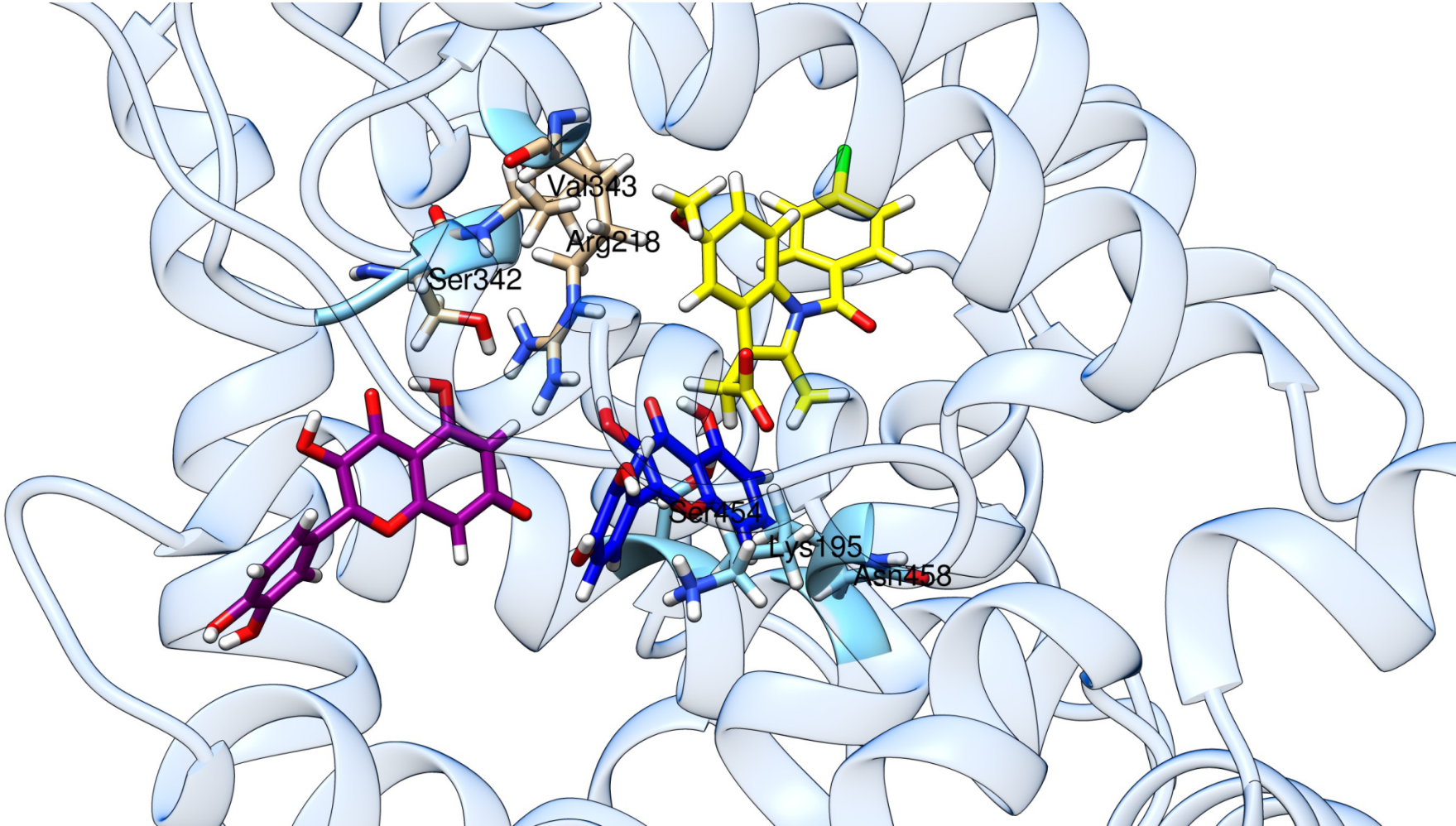
ΔG_{bind} (MMGBSA)

HSA-quercetin complex			HSA-indomethacin-quercetin complex		
Residue name	Residue #	kcal/mol	Residue name	Residue #	kcal/mol
Que	/	**-9.205**	**Que**	/	**-12.119**
Ser	342	-2.703	Asn	458	-4.170
Arg	218	-2.691	**Lys**	195	**-1.875**
Val	343	-2.500	Ser	454	-1.846
Pro	447	-1.090	Val	455	-1.675
Trp	214	-0.627	Tyr	452	-1.593
Lys	195	**-0.602**	**Ind**	/	**-1.405**
Ser	192	-0.533	Ala	194	-1.245
Gln	196	-0.325	Leu	198	-0.803
Glu	450	-0.321	Asp	451	-0.769
Met	446	-0.229	Ala	191	-0.480
…	…	…	…	…	…
Total		**-20.034**	**Total**		**-30.378**

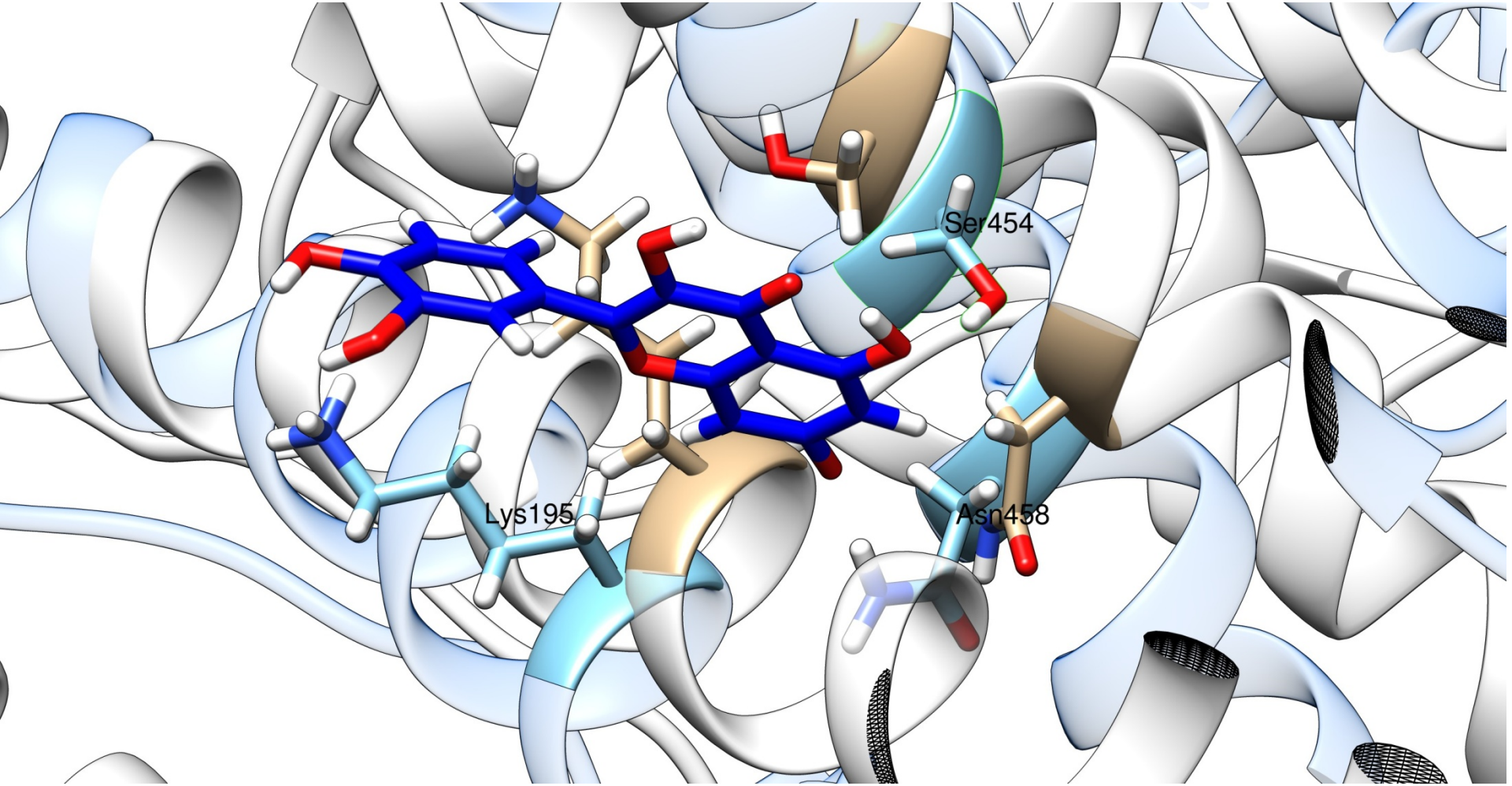
Decomposition by residues



Decomposition by residues



Decomposition by residues



Conclusion

- No displacement interaction between indomethacin and quercetin
  - Indomethacin increases quercetin binding constant ($\Delta\Delta G$ = -10.343 kcal/mol) and fluorescence intensity
  - Quercetin decreases indomethacin binding constant ($\Delta\Delta G$ = 4.232 kcal/mol)
- Translocation of quercetin deeper into the hydrophobic cleft with conformation change of HSA
- HSA is highly adaptive and displacement interactions are more complicated than previously thought

Acknowledgements

- Tana Tandarić (RBI)
- dr. sc. Robert Vianello (RBI)
- dr. sc. Mirza Bojić (FBF)

Thank you for your time!

Questions?