

Supplementary Materials

Structural analysis of human serum albumin in complex with the fibrate drug gemfibrozil

Stefano Liberi ^{1,†,‡}, Sara Linciano ^{2,†}, Giulia Moro ², Luca De Toni ³, Laura Cendron ^{1,*}
and Alessandro Angelini ^{2,4,*}

1 Department of Biology, University of Padua, Viale G. Colombo 3, 35131 Padua, Italy;
stefano.liberi@iusspavia.it

2 Department of Molecular Sciences and Nanosystems, Ca' Foscari University of Venice, Via Torino 155,
30172 Mestre, Italy; giulia.moro@unive.it (S.L.); sara.linciano@unive.it (G.M.)

3 Department of Medicine, Unit of Andrology and Reproductive Medicine, University of Padova, Via
Giustiniani 2, 35128 Padova, Italy; luca.detoni@unipd.it

4 European Centre for Living Technology (ECLT), Ca' Bottacin, Dorsoduro 3911, Calle Crosera,
30123 Venice, Italy

* Correspondence: laura.cendron@unipd.it (L.C.); alessandro.angelini@unive.it (A.A.)

† The authors contributed equally to this work.

‡ Present address: The Armenise-Harvard Laboratory of Structural Biology, Department of Biology and
Biotechnology "L. Spallanzani", University of Pavia, Via Ferrata 9, 27100 Pavia, Italy.

Supplementary tables

Data collection *	HSA–GEM–Myr
Beamline	ID30B
Wavelength (Å)	0.9686
Space group	C2
Cell parameters	
a, b, c (Å); α , β , γ (°)	184.90, 38.64, 96.21; 90, 104.58, 90
Resolution (Å)	46.56 – 2.20 (2.27 – 2.20)
Unique observations	33831 (2911)
Multiplicity	3.2 (3.2)
R_{merge}	0.069 (0.561)
R_{pim}	0.058 (0.533)
$\langle I / \sigma(I) \rangle$	8.8 (1.7)
CC1/2	0.997 (0.753)
Completeness (%)	99.2 (98.6)
Refinement	
No. reflections (used for R_{free} calculation)	33826 (3419)
$R_{\text{work}}/R_{\text{free}}$	0.219/0.255
Number non-hydrogen atoms	4838
protein (chain A)	4626
ligands (GEM, Myr)	68
solvent	68
Others (MPD, PO4, PG4, PGE)	76

Geometry

RMSD values	
bond lengths (Å)	0.008
bond angles (°)	1.398
Ramachandran plot (%)	
most favoured	94.82
additionally allowed	4.98
outliers	0.2
Rotamers outliers (%)	3.73
Average B-factor	56.0

Supplementary Table S1. Statistics on X-ray diffraction data collection and refinement. Frames were measured in 0.1° oscillation steps at 100 K. A single crystal was used to collect all diffraction data. The highest-resolution shell statistics are shown within brackets.

HSA—Sudlow's site II (FA3–FA4)	GEM1 ligand
HSA atom/residue	atom, type of interaction, distance (Å)
CB/Tyr411	C02 (NP, 3.77)
CG/Tyr411	C07 (NP, 3.65)
CD1/Tyr411	C06 (NP, 3.76)
CD1/Tyr411	C07 (NP, 3.85)
CD1/Tyr411	O08 (NP, 3.88)
CE1/Tyr411	O08 (NP, 3.81)
CE1/Tyr411	C09 (NP, 3.52)
CE1/Tyr411	C10 (NP, 3.87)
CE1/Tyr411	O17 (NP, 3.40)
CZ/Tyr411	C09 (NP, 3.72)
CZ/Tyr411	O17 (NP, 3.33)
OH/Tyr411	O17 (HB, 2.45)
OH/Tyr411	C10 (NP, 3.89)
OH/Tyr411	C11 (NP, 3.46)
OH/Tyr411	C12 (NP, 3.83)
OH/Tyr411	C15 (NP, 3.47)
CD2/Leu423	C01 (NP, 3.90)
CD2/Leu423	C03 (NP, 3.77)
CB/Val426	C01 (NP, 3.49)
CG1/Val426	C01 (NP, 3.46)
OG/Ser427	C01 (NP, 3.87)
CD2/Leu460	C01 (NP, 3.89)
CD/Arg485	C13 (NP, 3.68)
CD1/Phe488	C18 (NP, 3.82)
CA/Ser489	O16 (NP, 3.25)
CB/Ser489	O16 (NP, 3.26)
OG/Ser489	O16 (HB, 2.60)
OG/Ser489	C13 (NP, 3.46)

OG/Ser489	C15 (NP, 3.70)
CD1/Leu491	C18 (NP, 3.45)

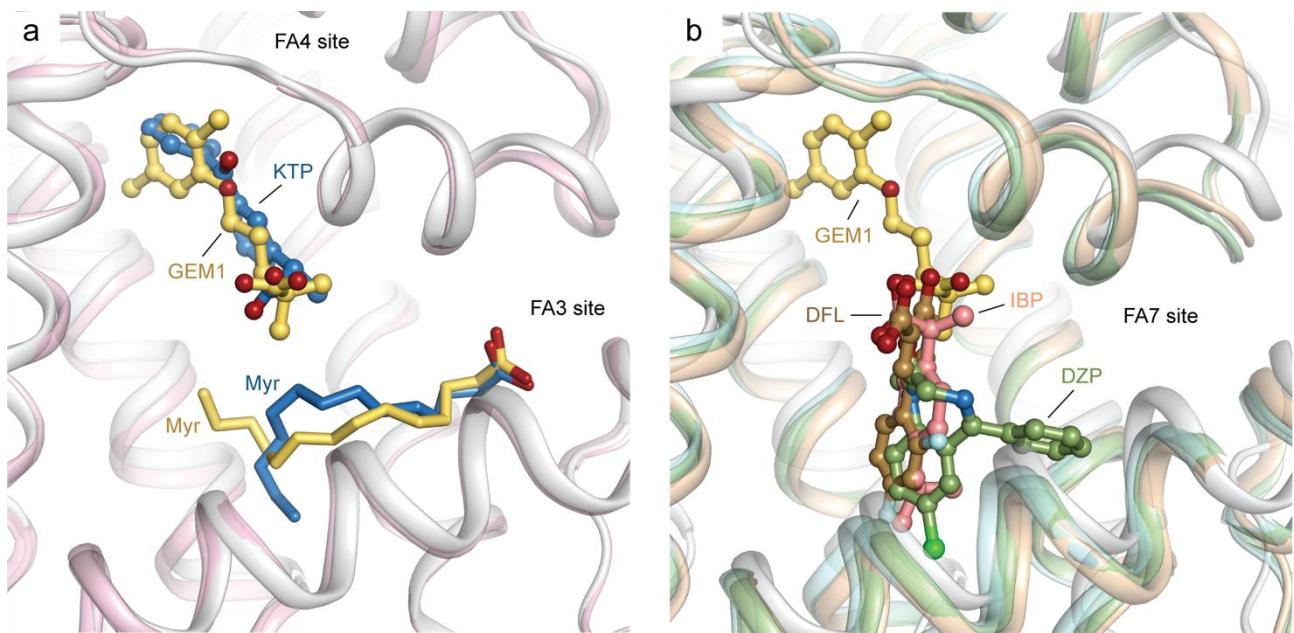
Supplementary Table S2. Inter-molecular interactions in the complexes between [HSA](#) and GEM1. Optimal inter-molecular hydrogen bonds (HB), polar interactions (PI), and non-polar interactions (NP) were defined using the web server PROFUNC [1] and LigPlot + [2].

HSA—Sudlow's site I (FA7)	GEM2 ligand
HSA atom/residue	atom, type of interaction, distance (Å)
CA/Lys199	C18 (NP, 3.89)
O/Lys199	C04 (NP, 3.55)
O/Lys199	C05 (NP, 3.86)
O/Lys199	C18 (NP, 3.56)
CB/Lys199	C18 (NP, 3.48)
CG/Lys199	C18 (NP, 3.68)
C/Ser202	C03 (NP, 3.76)
O/Ser202	C03 (NP, 3.79)
CB/Ser202	C03 (NP, 3.76)
N/Leu203	C03 (NP, 3.84)
N/Leu203	C04 (NP, 3.55)
CA/Leu203	C04 (NP, 3.79)
CD2/Phe211	C03 (NP, 3.69)
CD2/Phe211	C04 (NP, 3.14)
CD2/Phe211	C05 (NP, 3.02)
CD2/Phe211	C06 (NP, 3.44)
CD2/Phe211	C09 (NP, 3.63)
CD2/Phe211	C18 (NP, 3.36)
CE2/Phe211	C05 (NP, 3.35)
CE2/Phe211	C06 (NP, 3.72)
CE2/Phe211	C09 (NP, 3.23)
CE2/Phe211	C10 (NP, 3.38)
CE2/Phe211	C18 (NP, 3.12)
CD1/Trp214	C09 (NP, 3.62)
CG/Arg218	O17 (NP, 3.55)
NH1/Arg222	O16 (PI, 3.88)
NH2/Arg222	O17 (PI, 4.2)

CD2/Leu238	C13 (NP, 3.23)
CE1/His242	C14 (NP, 3.63)
NE2/His242	C14 (NP, 3.14)
CD2/Leu481	C01 (NP, 3.50)

Supplementary Table S3. Inter-molecular interactions in the complexes between HSA and GEM2. Optimal inter-molecular hydrogen bonds (HB), polar interactions (PI), and non-polar interactions (NP) were defined using the web server PROFUNC [1] and LigPlot+ [2].

Supplementary figure



Supplementary Figure S1. Comparison of the ligand occupancy at Sudlow's binding sites II (FA3–FA4) and I (FA7) in HSA–GEM and other ligands. (a) Detailed view of the superimposed GEM1 (yellow orange) and ketoprofen (KTP, sky blue) bound to Sudlow's site II (FA3–FA4) crystallised in the presence of myristic acid (My). The α -helices of HSA in complex with GEM1 (PDB identification code: 7QFE) and KTP (PDB identification code: 7JWN) are represented by cartoon loops and coloured in white and light pink, respectively. Bound ligands are shown in a ball-and-stick representation and coloured by atom type (GEM1: carbon = yellow orange and oxygen = firebrick; KTP: carbon = sky blue, oxygen = firebrick, and nitrogen = sky blue; Myr co-crystallised in complex with GEM1: carbon = sky blue and oxygen = firebrick; Myr co-crystallised in complex with KTP: carbon = sky blue and oxygen = firebrick); (b) detailed view of the superimposed GEM1 (yellow orange), ibuprofen (IBP, salmon), diazepam (DZP, smudge), and diflunisal (DFL, brown) bound to Sudlow's site I (FA7) crystallised in the absence of myristic acid (My). The α -helices of HSA in complex with GEM1 (PDB identification code: 7QFE), IBP (PDB identification code: 2BXG), DZP (PDB identification code: 2BXF), and DFL (PDB identification code: 2BXE) are represented by cartoon loops and coloured in white, pale green, wheat, and pale cyan,

respectively. Bound ligands are shown in a ball-and-stick representation and coloured by atom type (GEM1: carbon = yellow orange and oxygen = firebrick; IBP: carbon = salmon and oxygen = firebrick; DZP: carbon = smudge, oxygen = firebrick, nitrogen = sky blue, and chlorine = light green; and DFL: carbon = brown, oxygen = firebrick, and fluorine = pale cyan). The three-dimensional structure models were generated and rendered using Pymol [3].

References

1. Laskowski, R.A.; Watson, J.D.; Thornton, J.M. ProFunc: a server for predicting protein function from 3D structure. *Nucleic Acids Res.* **2005**, *33*, W89–W93, doi:10.1093/nar/gki414.
2. Laskowski, R.A.; Swindells, M.B. LigPlot+: Multiple ligand-protein interaction diagrams for drug discovery. *J. Chem. Inf. Model.* **2011**, *51*, 2778–2786, doi:10.1021/ci200227u.
3. Janson, G.; Zhang, C.; Prado, M.G.; Paiardini, A. PyMod 2.0: improvements in protein sequence-structure analysis and homology modeling within PyMOL. *Bioinformatics* **2017**, *33*, 444–446, doi:10.1093/bioinformatics/btw638.