Fatty acid binding to serum albumin: Molecular simulation approaches

Shin-ichi Fujiwara, Takashi Amisaki

PII: S0304-4165(13)00126-8
DOI: doi: 10.1016/j.bbagen.2013.03.032
Reference: BBAGEN 27504

To appear in: *BBA - General Subjects*

Received date: 4 February 2013
Revised date: 26 March 2013
Accepted date: 28 March 2013

Please cite this article as: Shin-ichi Fujiwara, Takashi Amisaki, Fatty acid binding to serum albumin: Molecular simulation approaches, *BBA - General Subjects* (2013), doi: 10.1016/j.bbagen.2013.03.032

Biochim. Biophys. Acta General subjects

Review Article

Fatty acid binding to serum albumin: Molecular simulation approaches

Shin-ichi Fujiwara* and Takashi Amisaki

Department of Biological Regulation, Faculty of Medicine, Tottori University, 86 Nishi-cho, Yonago 683-8503, Japan

*To whom correspondence should be addressed:
Shin-ichi Fujiwara, Ph.D.
Department of Biological Regulation
Faculty of Medicine
Tottori University, Yonago, 683-8503, Japan
Tel: +81-859-38-6356
Fax: +81-859-38-6350
E-mail: fujiwara@med.tottori-u.ac.jp

Abstract

Background: Binding affinity for human serum albumin (HSA) is one of the most important factors affecting the distribution and free blood concentration of many ligands. The effect of fatty acids (FAs) on HSA-ligand binding has long been studied. Since the elucidation of the 3-dimensional structure of HSA, molecular simulation approaches have been applied to studies of the structure-function relationship of HSA-FA binding.

Scope of review: We review current insights into the effects of FA binding on HSA, focusing on the biophysical insights obtained using molecular simulation approaches such as docking, molecular dynamics (MD), and binding free energy calculations.

Major conclusions: Possible conformational changes on binding of FA molecules to HSA have been observed through MD simulations. High- and low-affinity FA-binding sites on HSA have been identified based on binding free energy calculations. The relationship between the warfarin binding affinity of HSA and FA molecules has been clarified based on the results of simulations of multi-site FA binding that cannot be experimentally observed.

General significance: Molecular simulation approaches have great potentials to provide detailed biophysical insights into HSA as well as the effects of the binding of FAs or other ligands to HSA.

Key words

human serum albumin; fatty acid; simulation; molecular dynamics; free energy; docking

1. Introduction

Human serum albumin (HSA) is the most abundant protein in blood plasma and has ligand-binding and enzymatic properties. HSA is a transporter and depot protein for numerous endogenous compounds (e.g., fatty acids [FAs]) and exogenous compounds, and is also capable of binding to many commonly used drugs. Binding to HSA is one of the factors influencing drug disposition [1-3]. Recently, interactions between HSA and environmentally hazardous substances such as carbon nanoparticles [4] and PCB153 [5] have been also reported.

Since the first report of the 3-dimensional structure of HSA in 1992 [6], more than 70 such structures of HSA have been deposited in the Protein Data Bank (PDB). X-ray crystallography [6-30] and nuclear magnetic resonance (NMR) spectroscopy [31, 32] have revealed not only the structure of HSA but also those of its ligand-binding modes. Based on these structures, additional significant insights, such as the dynamic properties of HSA, have been revealed using molecular simulation approaches. Molecular dynamics (MD) simulation is a well-established method for the analysis of macromolecular conformations, especially focusing on the dynamic nature of macromolecules. Currently, MD simulations are playing a larger role in the study of macromolecules as a result of continuous improvements in algorithms, software and hardware [33-35]. In this review, we describe current insights into the conformation and function of HSA obtained with molecular simulation approaches, such as MD, molecular docking, and binding free energy calculations, focusing on the effects of FA binding.

2. FA binding to HSA: experimental approaches

2.1 Interaction between HSA and FA

FAs play critical roles in energy metabolism and the synthesis of membrane phospholipids. In the body, FAs are transported via the lymphatic and vascular systems. Owing to their low solubility in water, FAs require a transporter to increase their concentration in vascular and interstitial compartments. HSA is the main FA-binding protein in extracellular fluid [36]. Under normal physiological conditions, HSA binds with approximately 0.1–2 mol FA per mole protein [37]. The FA/HSA molar ratio increases to 6 during fasting or maximum exercise [38, 39] or under pathological conditions such as diabetes [40, 41] and cardiovascular disease [42].

Interaction between HSA and FAs has long been studied. Early affinity constants of HSA reported for FAs indicated that multiple FA-binding sites exist on HSA [43-47]. Later, the presence of 7 FA-binding sites on the protein was elucidated through X-ray crystallographic studies (Figure 1). These FA-binding sites are common for medium-/long-chain or monosaturated/polysaturated FAs [7, 10, 11, 48]. The FA-binding affinity (high/low) of each site has been also identified with ^{13}C NMR spectroscopy [49, 50] and site-directed mutagenesis of HSA [51] (Figure 1). Comparison of the 3-dimensional structures of defatted HSA and HSA-FA complexes has revealed that the binding of FA molecules to HSA causes a relative rearrangement at the I-II and II-III domain interfaces [7, 10, 52] and conformational changes of the side chains of subdomain IIA [17].

2.2 Effect of FA on HSA-ligand binding

Ligand-binding affinity for HSA is among the most important factors affecting the distribution and free concentration of many ligands, and the binding affinity is likely to be influenced by the binding of FAs to HSA, because some of the FA-binding sites overlap with ligand-binding sites [17]. Table 1 shows the ligands that bind to FA-binding sites, which have been revealed by X-ray crystallography. FA-binding site 7 (subdomain IIA), and sites 3 and 4 (subdomain IIIA) are known as major drug-binding sites I and II, respectively [17, 53]. In

general, bulky heterocyclic anions bind preferentially to FA-binding site 7, while sites 3 and 4 are preferred by aromatic carboxylates with an extended conformation [52, 54]. FA-binding site 1 is also a major ligand-binding site, especially for endogenous compounds such as heme [13, 15], bilirubin [21], and prostaglandins [23]. The binding of heme to this site has been reported to reduce the affinity of ligands for drug-binding site I [52]. Few ligands have been reported that bind to FA-binding sites 2, 5, and 6. Sites 5 and 6 have been identified as the sites with highest and low FA affinities, respectively [50]. The binding of FA to site 2, as well as the binding of heme to site 1, has been reported to stabilize the rotated conformation of domain I relative to domain II [7, 10, 52, 55]. Details on an expected correlation between preferred binding sites and classes of bound ligands are well summarized in recent reviews [3, 52, 54]. Numerous experimental studies have also indicated that ligand-binding affinity to HSA can be modulated through simultaneous binding of FAs [55-68]. The modulation is caused by competitive binding between a ligand and an FA at the same binding site [17] or allosteric effects from the binding of FAs [62, 66-68].

3. Molecular simulation of HSA and its application to HSA-FA binding studies

3.1 Molecular simulation studies of HSA to date

X-ray crystallography and NMR spectroscopy have made significant contributions to the structural analyses of HSA. In addition to these experimental techniques, molecular simulation approaches have become feasible for further structural-functional analyses of HSA. In this section, we review docking, MD, and binding free energy calculation studies of HSA.

3.1.1 Molecular modeling studies using electrostatic potential calculation or molecular docking simulations

The electrostatic potential around HSA has been analyzed using the determined HSA structure to find ligand-binding sites on HSA and bound conformations of ligands. Grymonpré et al. [69] have predicted the binding site of hyaluronic acid from the calculated electrostatic potential around HSA. Song and Gunner [70] have analyzed the binding of chloride ions using multi-conformation continuum electrostatics.

Molecular docking approaches have been widely used for the molecular modeling of HSA-ligand binding. Although more than 70 HSA structures on PDB database have given insights into ligand-binding sites on HSA and bound conformations of many ligands [3, 52, 54], it is still difficult to predict the exact binding modes (ligand-binding site and bound conformation) of unknown HSA-ligand complexes, because of the existence of multiple ligand-binding sites on HSA and flexible amino acid side chains at those sites. Docking simulations have been performed to estimate HSA-ligand binding modes computationally. Currently, more than 60 HSA-ligand docking studies have been reported (Table 2). The binding modes obtained in these studies can help further structural-functional analyses of HSA.

The results of docking simulation are generally reported along with experimental studies such as equilibrium dialysis, circular dichroism, fluorometry, calorimetry, and spectroscopy [5, 63, 67, 71-124]. In spite of this, choosing the correct target for docking can sometimes be difficult owing to the existence of multiple ligand-binding sites on HSA [3, 17, 54]. Hence, the results of such studies should be interpreted with caution. In the case of bilirubin, for example, docking simulation has been carried out for subdomain IIA (drug-binding site I) of HSA [125]. Although the study reported that bilirubin was docked to subdomain IIA in a robust manner, X-ray crystallography identified that bilirubin binds to subdomain IB instead [21]. Many of the experimental studies have examined only subdomains IIA and IIIA (drug-binding site II) as target sites for docking, because these sites are primary drug-binding

sites [53]. Experiments that examine the binding of a ligand at sites other than drug-binding sites I and II may be useful to select the correct site for docking simulations.

3.1.2 MD simulations for analyzing conformations of HSA or HSA-ligand complexes

The first MD simulation of HSA was reported in 2001, and it analyzed the influence of the protonation states of Lys195 and Lys199 on HSA conformation [126]. Simulations of the binding of divalent cations (Co^{2+}, Cu^{2+}, Ni^{2+} and Cd^{2+}) to the N-terminus of HSA were reported in 2004 [127]. These simulations used part of the HSA molecule. The MD simulation of the whole HSA molecule was first published in 2005 and reported that inter-domain motion of the unliganded HSA molecule was observed in a 2-ns MD simulation [128]. Fujiwara and Amisaki [129] later carried out 10-ns MD simulations of defatted and FA-bound HSA to analyze the conformational changes of HSA brought about by the binding of FA molecules, as described in section 3.2.1. The interaction of HSA with macromolecules has also been analyzed using MD simulations with a chrysotile surface [130], a carbon nanotube surface [131], self-assembling monolayers [132], poly(amidoamine) dendrimers [133], and HSA-HSA adsorption [134].

Three-dimensional structures of HSA obtained with X-ray crystallography are used in common for starting structures for MD simulations. HSA-ligand structures obtained through docking simulations have been also used for MD to confirm the stability of docked complexes [80, 84, 87, 90, 100, 104, 119, 120, 135-138]. Some of the HSA-ligand structures obtained using docking and MD simulations have been applied to quantum mechanics calculations of the excitation energies [138] and quantitative structure-activity relationship analyses of HSA-ligand binding affinity [139]. In addition, longer time scale (≥100 ns) MD simulations have been recently performed for analyses of the HSA-aspirin complex [140], the HSA-heme complex [141], the structural role of disulfide bridges in HSA [142], and the conformational flexibility of the unliganded HSA [119]. Thus, MD simulations are playing an increasingly important role in structural-functional studies of HSA.

3.1.3 HSA-ligand binding free energy calculations based on MD trajectory data

An MD trajectory of ligand-bound structures is a collection of estimated equilibrium conformations. Trajectory data have been used for the calculation of HSA-ligand binding free energy as well as conformational analyses. The HSA-ligand binding free energy (binding affinity) correlates with percentage plasma protein binding [143]. To date, calculated binding free energies have been reported for Gd-AAZTA complex (AAZTA = 6-amino-6-methylperhydro-1,4-diazepine tetraacetic acid) [144], zidovudine and its derivatives [63], FAs (see section 3.2.2) [145], levamlodipine [76], flavones [78], perfluorooctanoic acid and perfluorooctane sulfonate [146], mexiletine [110], hydroxyquinoline molecules [119], PCB153 [5], and warfarin [147]. Fujiwara and Amisaki [147] have calculated binding free energies under various FA/HSA molar ratios, as described in section 3.2.3. In these reports, calculated binding affinities were consistent with those from experimental approaches, indicating the appropriateness of the calculations.

3.2 Applications of molecular simulations to HSA-FA binding

As of Febrary 2013, eight studies have been published that report the application of molecular simulations to HSA-FA binding. In this section, we review these published studies concerning (1) conformational changes of HSA caused by FA binding [129], (2) conformation and binding affinity of an FA molecule at each FA binding site [145, 148], and (3) effect of FA binding on HSA-ligand interaction [63, 72, 144, 147, 149].

3.2.1 MD studies of conformational changes in HSA caused by binding of FA molecules

Fujiwara and Amisaki [129] carried out conformational analyses of the unliganded HSA and the HSA-FA complex with 10-ns MD simulations. The radius of gyration of MD simulations of the unliganded HSA was almost the same that of the experimental value, indicating that the equilibrium state of HSA molecules in aqueous solution was reproduced well in the MD simulations. The main differences between the unliganded HSA and the HSA-FA complex were observed in the primary internal motions characterized by the first 3 principal components at domains I and III (Figure 2). The directional motion projected on the first principal component of the unliganded HSA was conserved in the HSA-FA complex as the third principal directional motion with higher frequency. Thus, their MD study provides insights into the possible conformational changes of HSA caused by the binding of FA molecules on a scale of 10-ns. A method to obtain a full impression of the conformational freedom of HSA is to perform simulations over longer time periods. Continuing improvements in MD algorithms and software, and enhanced hardware performance will enable longer MD simulations, which may provide further insights into the effect of FA binding on the conformation of HSA.

3.2.2. Identification of high-affinity FA binding sites on HSA by molecular simulations

When considering the interaction between FA molecules and other ligands, the identification of high-affinity FA-binding sites on HSA is very important (see section 2.2). Seven possible FA-binding sites have been revealed with X-ray crystallography [10, 11]. High- and low-affinity FA binding sites have also been experimentally determined with ^{13}C NMR spectroscopy [49, 50].

Rizzuti et al. [148] have analyzed the structural basis of high-affinity FA-binding site 5 using MD simulations. They observed that Lys525 was important because the residue anchored FA head-groups. Fujiwara and Amisaki [145] have quantitatively examined the HSA-FA affinity at each FA-binding site using MD simulations and binding free energy calculations. The calculated value of each absolute binding free energy deviated greatly from the experimental binding free energies as estimated using the HSA-FA affinity constants [45] (Figure 3). However, the spectrum of the affinity (high/low) over FA binding sites was successfully identified. They identified FA-binding sites 5, 4, and 2 as high-affinity sites, and 1, 3, 6, and 7 as low-affinity sites, identical to those of the experimental approaches [50] (see Figure 1). Binding free energy calculation may be useful for comparison of the relative stabilities of HSA-ligand complexes, although the accurate calculation of absolute binding free energy is one of the challenges to be tackled in theoretical studies.

3.2.3. Effect of FA molecules on HSA-ligand binding

The published X-ray structures of HSA-ligand-FA complexes have given insights into the effect of FA on HSA-ligand binding [17, 54]. However, the number of the structures is not nearly large enough to cover the binding modes of the complexes, because of the existence of multiple ligand-binding sites on HSA and flexible amino acid chains at those sites. As the second best approach, molecular simulations have been performed to analyze the effect of FA molecules on HSA-ligand binding. One of the advantages of molecular simulation approaches is that conditions that are not observed experimentally can be simulated computationally. The effects of FA molecules on the interaction between HSA and ligands have been analyzed through docking simulations. Paal and Shkarupin [149] have reported reduced binding affinities of paclitaxel, in comparison to the defatted HSA, at paclitaxel-binding sites on the HSA-FA complex. Gianolio et al. [144] have observed that Gd-AAZTA binds with different affinities to defatted (low affinity) and FA-bound HSA (high affinity) as a consequence of the conformational changes upon FA binding. Fanali et al. [72] have performed docking analyses of 3 anti-HIV drugs in 4 of the 7 FA-binding sites to compare intermolecular energies of the

drugs at each site. Quevedo et al. [63] have observed that reduced affinities of zidovudine derivatives in the presence of FAs were caused by an intense electrostatic repulsion between FA and ligands with negative charges.

An approach focusing on the number of FA molecules bound to HSA has been also reported. Fujiwara and Amisaki [147] have analyzed the relationship between HSA-warfarin binding affinity and the positions of bound FA molecules. Based on the affinity at each FA-binding site (section 3.2.2), they constructed 11 "virtual" HSA-warfarin-FA complexes, each with different FA molecule positions. These virtual complexes were used for MD simulations and binding free energy calculations for HSA-warfarin binding (Figure 4). The results indicate that unfavorable steric effects on HSA-warfarin binding affinity (in terms of the van der Waals energy contribution) were caused by the binding of an FA molecule to FA-binding site 2, which is closest to the warfarin-binding site (see Figure 1). Conversely, the magnitude of HSA-warfarin binding free energy was discovered to be largest (i.e., the HSA-warfarin binding affinity was strongest) when 3 FA molecules were bound to the high-affinity sites. The relationship between HSA-warfarin binding affinity and the number of bound FA molecules (Figure 4) coincided with the previous observations [59]. This study clarified the structural and energetic properties of these steric/allosteric effects of FAs on HSA-warfarin binding affinity. The molecular simulation approach described above may be applicable to binding studies of interactions between other ligands and HSA.

4. Conclusions

We reviewed recent molecular simulation studies to analyze the structure-function relationship of HSA, focusing on the HSA-FA binding. Differences in the directional motions of domains I and III between the unliganded HSA and the HSA-FA complex have been analyzed with MD simulations (see section 3.2.1). High- and low-affinity FA-binding sites on HSA have been identified quantitatively with MD simulations and binding free energy calculations (see section 3.2.2). In addition, HSA-ligand binding free energies were calculated with respect to the positions of FA molecules bound to HSA (see section 3.2.3). Such approaches will continue to evolve in themselves in terms of simulation theory and computer technology. For example, MD studies on millisecond time scales are now available [150]. To date, MD simulations on nanosecond time scales ($\geq$100 ns) have been reported for HSA. Longer-time-scale MD calculations may elucidate unknown conformational characteristics of HSA.

One of the characteristics of HSA-FA binding is the binding of multiple FA molecules. We expect that HSA is useful as a model of multiple-ligand-binding proteins, and that additional advances in molecular simulation approaches may lead to the elucidation of the relationship between conformation and function of HSA.

Acknowledgement

This study was supported in part by Grants-in-Aid for Scientific Research from the Ministry of Education, Culture, Sports, Science and Technology of Japan.

References

[1] U. Kragh-Hansen, V.T.G. Chuang, M. Otagiri, Practical aspects of the ligand-binding and enzymatic properties of human serum albumin, Biol. Pharm. Bull. 25 (2002) 695-704.
[2] M. Otagiri, A molecular functional study on the interactions of drugs with plasma proteins, Drug Metab. Pharmacokinet. 20 (2005) 309-323.
[3] G. Fanali, A. di Masi, V. Trezza, M. Marino, M. Fasano, P. Ascenzi, Human serum albumin: from bench to bedside, Mol. Aspects Med. 33 (2012) 209-290.
[4] S. Mandal, M. Hossain, P.S. Devi, G.S. Kumar, K. Chaudhuri, Interaction of carbon nanoparticles to serum albumin: elucidation of the extent of perturbation of serum albumin conformations and thermodynamical parameters, J. Hazard. Mater. 248-249C (2013) 238-245.
[5] C. Han, S. Fang, H. Cao, Y. Lu, Y. Ma, D. Wei, X. Xie, X. Liu, X. Li, D. Fei, C. Zhao, Molecular interaction of PCB153 to human serum albumin: Insights from spectroscopic and molecular modeling studies, J. Hazard. Mater. 248-249C (2013) 313-321.
[6] X.M. He, D.C. Carter, Atomic structure and chemistry of human serum albumin, Nature 358 (1992) 209-215.
[7] S. Curry, H. Mandelkow, P. Brick, N. Franks, Crystal structure of human serum albumin complexed with fatty acid reveals an asymmetric distribution of binding sites, Nat. Struct. Biol. 5 (1998) 827-835.
[8] S. Sugio, A. Kashima, S. Mochizuki, M. Noda, K. Kobayashi, Crystal structure of human serum albumin at 2.5 angstrom resolution, Protein Eng. 12 (1999) 439-446.
[9] A.A. Bhattacharya, S. Curry, N.P. Franks, Binding of the general anesthetics propofol and halothane to human serum albumin. High resolution crystal structures, J. Biol. Chem. 275 (2000) 38731-38738.
[10] A.A. Bhattacharya, T. Grüne, S. Curry, Crystallographic analysis reveals common modes of binding of medium and long-chain fatty acids to human serum albumin, J. Mol. Biol. 303 (2000) 721-732.
[11] I. Petitpas, T. Grüne, A.A. Bhattacharya, S. Curry, Crystal structures of human serum albumin complexed with monounsaturated and polyunsaturated fatty acids, J. Mol. Biol. 314 (2001) 955-960.
[12] I. Petitpas, A.A. Bhattacharya, S. Twine, M. East, S. Curry, Crystal structure analysis of warfarin binding to human serum albumin: anatomy of drug site I, J. Biol. Chem. 276 (2001) 22804-22809.
[13] M. Wardell, Z. Wang, J.X. Ho, J. Robert, F. Ruker, J. Ruble, D.C. Carter, The atomic structure of human methemalbumin at 1.9 A, Biochem. Biophys. Res. Commun. 291 (2002) 813-819.
[14] I. Petitpas, C.E. Petersen, C.E. Ha, A.A. Bhattacharya, P.A. Zunszain, J. Ghuman, N.V. Bhagavan, S. Curry, Structural basis of albumin-thyroxine interactions and familial dysalbuminemic hyperthyroxinemia, Proc. Natl. Acad. Sci. USA 100 (2003) 6440-6445.
[15] P.A. Zunszain, J. Ghuman, T. Komatsu, E. Tsuchida, S. Curry, Crystal structural analysis of human serum albumin complexed with hemin and fatty acid, BMC Struct. Biol. 3 (2003) 6.
[16] S. Lejon, I.M. Frick, L. Bjorck, M. Wikstrom, S. Svensson, Crystal structure and biological implications of a bacterial albumin binding module in complex with human serum albumin, J. Biol. Chem. 279 (2004) 42924-42928.
[17] J. Ghuman, P.A. Zunszain, I. Petitpas, A.A. Bhattacharya, M. Otagiri, S. Curry, Structural basis of the drug-binding specificity of human serum albumin, J. Mol. Biol. 353 (2005) 38-52.
[18] F. Yang, C. Bian, L. Zhu, G. Zhao, Z. Huang, M. Huang, Effect of human serum albumin on drug metabolism: structural evidence of esterase activity of human serum albumin, J. Struct. Biol. 157 (2007) 348-355.

[19] S. Lejon, J.F. Cramer, P. Nordberg, Structural basis for the binding of naproxen to human serum albumin in the presence of fatty acids and the GA module, Acta Crystallogr. F 64 (2008) 64-69.
[20] L. Zhu, F. Yang, L. Chen, E.J. Meehan, M. Huang, A new drug binding subsite on human serum albumin and drug-drug interaction studied by X-ray crystallography, J. Struct. Biol. 162 (2008) 40-49.
[21] P.A. Zunszain, J. Ghuman, A.F. McDonagh, S. Curry, Crystallographic analysis of human serum albumin complexed with 4Z,15E-bilirubin-IXα, J. Mol. Biol. 381 (2008) 394-406.
[22] S. Guo, X. Shi, F. Yang, L. Chen, E.J. Meehan, C. Bian, M. Huang, Structural basis of transport of lysophospholipids by human serum albumin, Biochem. J. 423 (2009) 23-30.
[23] S. Yamaguchi, G. Aldini, S. Ito, N. Morishita, T. Shibata, G. Vistoli, M. Carini, K. Uchida, Δ^{12}-prostaglandin J_2 as a product and ligand of human serum albumin: formation of an unusual covalent adduct at His146, J. Am. Chem. Soc. 132 (2010) 824-832.
[24] K.L. Hein, U. Kragh-Hansen, J.P. Morth, M.D. Jeppesen, D. Otzen, J.V. Moller, P. Nissen, Crystallographic analysis reveals a unique lidocaine binding site on human serum albumin, J. Struct. Biol. 171 (2010) 353-360.
[25] D. Buttar, N. Colclough, S. Gerhardt, P.A. MacFaul, S.D. Phillips, A. Plowright, P. Whittamore, K. Tam, K. Maskos, S. Steinbacher, H. Steuber, A combined spectroscopic and crystallographic approach to probing drug-human serum albumin interactions, Bioorg. Med. Chem. 18 (2010) 7486-7496.
[26] A.J. Ryan, J. Ghuman, P.A. Zunszain, C.W. Chung, S. Curry, Structural basis of binding of fluorescent, site-specific dansylated amino acids to human serum albumin, J. Struct. Biol. 174 (2011) 84-91.
[27] A.J. Ryan, C.W. Chung, S. Curry, Crystallographic analysis reveals the structural basis of the high-affinity binding of iophenoxic acid to human serum albumin, BMC Struct. Biol. 11 (2011) 18.
[28] Y. He, T. Ning, T. Xie, Q. Qiu, L. Zhang, Y. Sun, D. Jiang, K. Fu, F. Yin, W. Zhang, L. Shen, H. Wang, J. Li, Q. Lin, H. Li, Y. Zhu, D. Yang, Large-scale production of functional human serum albumin from transgenic rice seeds, Proc. Natl. Acad. Sci. USA 108 (2011) 19078-19083.
[29] Y. Wang, Z. Luo, X. Shi, H. Wang, L. Nie, M. Huang, A fluorescent fatty acid probe, DAUDA, selectively displaces two myristates bound in human serum albumin, Protein Sci. 20 (2011) 2095-2101.
[30] Z. Luo, X. Shi, Q. Hu, B. Zhao, M. Huang, Structural evidence of perfluorooctane sulfonate transport by human serum albumin, Chem. Res. Toxicol. 25 (2012) 990-992.
[31] T. Oltersdorf, S.W. Elmore, A.R. Shoemaker, R.C. Armstrong, D.J. Augeri, B.A. Belli, M. Bruncko, T.L. Deckwerth, J. Dinges, P.J. Hajduk, M.K. Joseph, S. Kitada, S.J. Korsmeyer, A.R. Kunzer, A. Letai, C. Li, M.J. Mitten, D.G. Nettesheim, S. Ng, P.M. Nimmer, J.M. O'Connor, A. Oleksijew, A.M. Petros, J.C. Reed, W. Shen, S.K. Tahir, C.B. Thompson, K.J. Tomaselli, B. Wang, M.D. Wendt, H. Zhang, S.W. Fesik, S.H. Rosenberg, An inhibitor of Bcl-2 family proteins induces regression of solid tumours, Nature 435 (2005) 677-681.
[32] A. Almogren, P.B. Furtado, Z. Sun, S.J. Perkins, M.A. Kerr, Purification, properties and extended solution structure of the complex formed between human immunoglobulin A1 and human serum albumin by scattering and ultracentrifugation, J. Mol. Biol. 356 (2006) 413-431.
[33] T. Hansson, C. Oostenbrink, W. van Gunsteren, Molecular dynamics simulations, Curr. Opin. Struct. Biol. 12 (2002) 190-196.
[34] J.L. Klepeis, K. Lindorff-Larsen, R.O. Dror, D.E. Shaw, Long-timescale molecular dynamics simulations of protein structure and function, Curr. Opin. Struct. Biol. 19 (2009) 120-127.

[35] J.D. Durrant, J.A. McCammon, Molecular dynamics simulations and drug discovery, BMC Biol. 9 (2011) 71.
[36] G.J. van der Vusse, Albumin as fatty acid transporter, Drug Metab. Pharmacokinet. 24 (2009) 300-307.
[37] D.S. Fredrickson, R.S. Gordon, Jr., The metabolism of albumin-bound C^{14}-labeled unesterified fatty acids in normal human subjects, J. Clin. Invest. 37 (1958) 1504-1515.
[38] R. Brodersen, S. Andersen, H. Vorum, S.U. Nielsen, A.O. Pedersen, Multiple fatty acid binding to albumin in human blood plasma, Eur. J. Biochem. 189 (1990) 343-349.
[39] R. Bahr, A.T. Hostmark, E.A. Newsholme, O. Gronnerod, O.M. Sejersted, Effect of exercise on recovery changes in plasma levels of FFA, glycerol, glucose and catecholamines, Acta Physiol. Scand. 143 (1991) 105-115.
[40] D.P. Cistola, D.M. Small, Fatty acid distribution in systems modeling the normal and diabetic human circulation. A ^{13}C nuclear magnetic resonance study, J. Clin. Invest. 87 (1991) 1431-1441.
[41] G. Paolisso, P.A. Tataranni, J.E. Foley, C. Bogardus, B.V. Howard, E. Ravussin, A high concentration of fasting plasma non-esterified fatty acids is a risk factor for the development of NIDDM, Diabetologia 38 (1995) 1213-1217.
[42] V.A. Kurien, M.F. Oliver, Free fatty acids during acute myocardial infarction, Prog. Cardiovasc. Dis. 13 (1971) 361-373.
[43] D.S. Goodman, The interaction of human serum albumin with long-chain fatty acid anions, J. Am. Chem. Soc. 80 (1958) 3892-3898.
[44] A.A. Spector, K. John, J.E. Fletcher, Binding of long-chain fatty acids to bovine serum albumin, J. Lipid Res. 10 (1969) 56-67.
[45] J.D. Ashbrook, A.A. Spector, E.C. Santos, J.E. Fletcher, Long chain fatty acid binding to human plasma albumin, J. Biol. Chem. 250 (1975) 2333-2338.
[46] A.O. Pedersen, R. Brodersen, Myristic acid binding to human serum albumin investigated by dialytic exchange rate, J. Biol. Chem. 263 (1988) 10236-10239.
[47] G.V. Richieri, A. Anel, A.M. Kleinfeld, Interactions of long-chain fatty acids and albumin: determination of free fatty acid levels using the fluorescent probe ADIFAB, Biochemistry 32 (1993) 7574-7580.
[48] S. Curry, P. Brick, N.P. Franks, Fatty acid binding to human serum albumin: new insights from crystallographic studies, Biochim. Biophys. Acta 1441 (1999) 131-140.
[49] J.R. Simard, P.A. Zunszain, C.E. Ha, J.S. Yang, N.V. Bhagavan, I. Petitpas, S. Curry, J.A. Hamilton, Locating high-affinity fatty acid-binding sites on albumin by x-ray crystallography and NMR spectroscopy, Proc. Natl. Acad. Sci. USA 102 (2005) 17958-17963.
[50] J.R. Simard, P.A. Zunszain, J.A. Hamilton, S. Curry, Location of high and low affinity fatty acid binding sites on human serum albumin revealed by NMR drug-competition analysis, J. Mol. Biol. 361 (2006) 336-351.
[51] U. Kragh-Hansen, H. Watanabe, K. Nakajou, Y. Iwao, M. Otagiri, Chain length-dependent binding of fatty acid anions to human serum albumin studied by site-directed mutagenesis, J. Mol. Biol. 363 (2006) 702-712.
[52] P. Ascenzi, M. Fasano, Allostery in a monomeric protein: the case of human serum albumin, Biophys. Chem. 148 (2010) 16-22.
[53] G. Sudlow, D.J. Birkett, D.N. Wade, The characterization of two specific drug binding sites on human serum albumin, Mol. Pharmacol. 11 (1975) 824-832.
[54] S. Curry, Lessons from the crystallographic analysis of small molecule binding to human serum albumin, Drug Metab. Pharmacokinet. 24 (2009) 342-357.
[55] G. Fanali, R. Fesce, C. Agrati, P. Ascenzi, M. Fasano, Allosteric modulation of myristate and Mn(III)heme binding to human serum albumin. Optical and NMR spectroscopy

characterization, FEBS J 272 (2005) 4672-4683.
[56] K. Maruyama, S. Awazu, H. Nishigori, M. Iwatsuru, Effects of fatty acid on the specific drug-binding sites of human serum albumin, Chem. Pharm. Bull. 34 (1986) 3394-3402.
[57] P. Claudepierre, S. Urien, O. Chassany, J.P. Tillement, Analysis of free fatty acid effect on methotrexate binding to albumin, Biochem. Pharmacol. 47 (1994) 415-417.
[58] A.M.L. Zaton, J.M. Ferrer, J.C.R. Degordoa, M.A. Marquinez, Binding of coumarins to site I of human serum albumin. Effect of the fatty acids, Chem. Biol. Interact. 97 (1995) 169-174.
[59] H. Vorum, B. Honore, Influence of fatty acids on the binding of warfarin and phenprocoumon to human serum albumin with relation to anticoagulant therapy, J. Pharm. Pharmacol. 48 (1996) 870-875.
[60] N. Takamura, S. Shinozawa, T. Maruyama, A. Suenaga, M. Otagiri, Effects of fatty acids on serum binding between furosemide and valproic acid, Biol. Pharm. Bull. 21 (1998) 174-176.
[61] V.T.G. Chuang, M. Otagiri, How do fatty acids cause allosteric binding of drugs to human serum albumin? Pharm. Res. 19 (2002) 1458-1464.
[62] P. Ascenzi, A. Bocedi, S. Notari, G. Fanali, R. Fesce, M. Fasano, Allosteric modulation of drug binding to human serum albumin, Mini Rev. Med. Chem. 6 (2006) 483-489.
[63] M.A. Quevedo, S.R. Ribone, G.N. Moroni, M.C. Briñón, Binding to human serum albumin of zidovudine (AZT) and novel AZT derivatives. Experimental and theoretical analyses, Bioorg. Med. Chem. 16 (2008) 2779-2790.
[64] B. Bojko, A. Sulkowska, M. Maciazek, J. Rownicka, F. Njau, W.W. Sulkowski, Changes of serum albumin affinity for aspirin induced by fatty acid, Int. J. Biol. Macromol. 42 (2008) 314-323.
[65] G.J. Amirtharaj, S.K. Natarajan, A. Mukhopadhya, U.G. Zachariah, S.K. Hegde, G. Kurian, K.A. Balasubramanian, A. Ramachandran, Fatty acids influence binding of cobalt to serum albumin in patients with fatty liver, Biochim. Biophys. Acta 1782 (2008) 349-354.
[66] G. Fanali, G. De Sanctis, M. Gioia, M. Coletta, P. Ascenzi, M. Fasano, Reversible two-step unfolding of heme-human serum albumin: a ^{1}H-NMR relaxometric and circular dichroism study, J. Biol. Inorg. Chem. 14 (2009) 209-217.
[67] A. Bolli, M. Marino, G. Rimbach, G. Fanali, M. Fasano, P. Ascenzi, Flavonoid binding to human serum albumin, Biochem. Biophys. Res. Commun. 398 (2010) 444-449.
[68] G. Fanali, Y. Cao, P. Ascenzi, M. Fasano, Mn(II) binding to human serum albumin: a ^{1}H-NMR relaxometric study, J. Inorg. Biochem. 117 (2012) 198-203.
[69] K.R. Grymonpré, B.A. Staggemeier, P.L. Dubin, K.W. Mattison, Identification by integrated computer modeling and light scattering studies of an electrostatic serum albumin-hyaluronic acid binding site, Biomacromolecules 2 (2001) 422-429.
[70] Y.F. Song, M.R. Gunner, Using multiconformation continuum electrostatics to compare chloride binding motifs in α-amylase, human serum albumin, and Omp32, J. Mol. Biol. 387 (2009) 840-856.
[71] Y. Li, W. He, Y. Dong, F. Sheng, Z. Hu, Human serum albumin interaction with formononetin studied using fluorescence anisotropy, FT-IR spectroscopy, and molecular modeling methods, Bioorg. Med. Chem. 14 (2006) 1431-1436.
[72] G. Fanali, A. Bocedi, P. Ascenzi, M. Fasano, Modulation of heme and myristate binding to human serum albumin by anti-HIV drugs. An optical and NMR spectroscopic study, FEBS J 274 (2007) 4491-4502.
[73] J. Tang, N. Lian, C. Bi, W. Li, Analysis of eupatilin-human serum albumin interactions by means of spectroscopic and computational modelling, J. Pharm. Pharmacol. 59 (2007) 637-643.
[74] F. Cui, X. Kong, L. Qin, G. Zhang, Q. Liu, B. Lei, X. Yao, Specific interaction of

4'-O-(a-l-Cladinosyl) daunorubicin with human serum albumin: The binding site II on HSA molecular using spectroscopy and modeling, J. Photochem. Photobiol. B 95 (2009) 162-169.
[75] F. Cui, Y. Yan, Q. Zhang, J. Du, X. Yao, G. Qu, Y. Lu, Characterization of the interaction between 2'-deoxyuridine and human serum albumin, Carbohydr. Res. 344 (2009) 642-647.
[76] Z. Liu, X. Zheng, X. Yang, E. Wang, J. Wang, Affinity and specificity of levamlodipine-human serum albumin interactions: insights into its carrier function, Biophys. J. 96 (2009) 3917-3925.
[77] G. Fanali, V. Rampoldi, A. di Masi, A. Bolli, L. Lopiano, P. Ascenzi, M. Fasano, Binding of anti-Parkinson's disease drugs to human serum albumin is allosterically modulated, IUBMB Life 62 (2010) 371-376.
[78] H. Liu, W. Bao, H. Ding, J. Jang, G. Zou, Binding modes of flavones to human serum albumin: insights from experimental and computational studies, J. Phys. Chem. B 114 (2010) 12938-12947.
[79] F. Ding, W. Liu, X. Zhang, L. Zhang, Y. Sun, Fluorescence and circular dichroism studies of conjugates between metsulfuron-methyl and human serum albumin, Colloids Surf. B 76 (2010) 441-448.
[80] B. Sudhamalla, M. Gokara, N. Ahalawat, D.G. Amooru, R. Subramanyam, Molecular dynamics simulation and binding studies of β-sitosterol with human serum albumin and its biological relevance, J. Phys. Chem. B 114 (2010) 9054-9062.
[81] F. Ding, L. Wei, J.X. Diao, Y. Sun, Characterization of Alizarin Red S binding sites and structural changes on human serum albumin: A biophysical study, J. Hazard. Mater. 186 (2011) 352-359.
[82] Z. Omidvar, K. Parivar, H. Sanee, Z. Amiri-Tehranizadeh, A. Baratian, M.R. Saberi, A. Asoodeh, J. Chamani, Investigations with spectroscopy, zeta potential and molecular modeling of the non-cooperative behaviour between cyclophosphamide hydrochloride and aspirin upon interaction with human serum albumin: binary and ternary systems from multi-drug therapy, J. Biomol. Struct. Dyn. 29 (2011) 181-206.
[83] G. Fanali, Y. Cao, P. Ascenzi, V. Trezza, T. Rubino, D. Parolaro, M. Fasano, Binding of ∆9-tetrahydrocannabinol and diazepam to human serum albumin, IUBMB Life 63 (2011) 446-451.
[84] C. Calderon, E. Abuin, E. Lissi, R. Montecinos, Effect of human serum albumin on the kinetics of 4-methylumbelliferyl-beta-D-N-N'-N" triacetylchitotrioside hydrolysis catalyzed by hen egg white lysozyme, Protein J. 30 (2011) 367-373.
[85] P. Ascenzi, A. Bolli, A. di Masi, G.R. Tundo, G. Fanali, M. Coletta, M. Fasano, Isoniazid and rifampicin inhibit allosterically heme binding to albumin and peroxynitrite isomerization by heme-albumin, J. Biol. Inorg. Chem. 16 (2011) 97-108.
[86] H. Vahedian-Movahed, M.R. Saberi, J. Chamani, Comparison of binding interactions of lomefloxacin to serum albumin and serum transferrin by resonance light scattering and fluorescence quenching methods, J. Biomol. Struct. Dyn. 28 (2011) 483-502.
[87] M.J. Kimzey, H.N. Yassine, B.M. Riepel, G. Tsaprailis, T.J. Monks, S.S. Lau, New site(s) of methylglyoxal-modified human serum albumin, identified by multiple reaction monitoring, alter warfarin binding and prostaglandin metabolism, Chem. Biol. Interact. 192 (2011) 122-128.
[88] Y. Sun, B. Su, Q. Xu, R. Liu, Insights into the binding of 2-aminobenzothiazole with human serum albumin (HSA): spectroscopic investigation and molecular modeling studies, Appl. Spectrosc. 66 (2012) 791-797.
[89] M.R. Housaindokht, Z. Rouhbakhsh Zaeri, M. Bahrololoom, J. Chamani, M.R. Bozorgmehr, Investigation of the behavior of HSA upon binding to amlodipine and propranolol: Spectroscopic and molecular modeling approaches, Spectrochim. Acta A 85

(2012) 79-84.
[90] F. Samari, M. Shamsipur, B. Hemmateenejad, T. Khayamian, S. Gharaghani, Investigation of the interaction between amodiaquine and human serum albumin by fluorescence spectroscopy and molecular modeling, Eur. J. Med. Chem. 54 (2012) 255-263.
[91] F. Ding, L. Zhang, J.X. Diao, X.N. Li, L. Ma, Y. Sun, Human serum albumin stability and toxicity of anthraquinone dye alizarin complexone: an albumin-dye model, Ecotoxicol. Environ. Saf. 79 (2012) 238-246.
[92] T. Zohoorian-Abootorabi, H. Sanee, H. Iranfar, M.R. Saberi, J. Chamani, Separate and simultaneous binding effects through a non-cooperative behavior between cyclophosphamide hydrochloride and fluoxymesterone upon interaction with human serum albumin: multi-spectroscopic and molecular modeling approaches, Spectrochim. Acta A 88 (2012) 177-191.
[93] O.K. Abou-Zied, Revealing the ionization ability of binding site I of human serum albumin using 2-(2'-hydroxyphenyl)benzoxazole as a pH sensitive probe, Phys. Chem. Chem. Phys. 14 (2012) 2832-2839.
[94] A. Hosainzadeh, M. Gharanfoli, M. Saberi, J. Chamani, Probing the interaction of human serum albumin with bilirubin in the presence of aspirin by multi-spectroscopic, molecular modeling and zeta potential techniques: insight on binary and ternary systems, J. Biomol. Struct. Dyn. 29 (2012) 1013-1050.
[95] M.I. Sabela, N.J. Gumede, L. Escuder-Gilabert, Y. Martin-Biosca, K. Bisetty, M.J. Medina-Hernandez, S. Sagrado, Connecting simulated, bioanalytical, and molecular docking data on the stereoselective binding of (+/-)-catechin to human serum albumin, Anal. Bioanal. Chem. 402 (2012) 1899-1909.
[96] X.L. Han, F.F. Tian, Y.S. Ge, F.L. Jiang, L. Lai, D.W. Li, Q.L. Yu, J. Wang, C. Lin, Y. Liu, Spectroscopic, structural and thermodynamic properties of chlorpyrifos bound to serum albumin: A comparative study between BSA and HSA, J. Photochem. Photobiol. B 109 (2012) 1-11.
[97] J. Zhu, L. Wu, Q. Zhang, X. Chen, X. Liu, Investigation the interaction of daphnin with human serum albumin using optical spectroscopy and molecular modeling methods, Spectrochim. Acta A 95 (2012) 252-257.
[98] R. Huo, C. Li, F. Cui, G. Zhang, Q. Liu, X. Yao, Spectroscopic and molecular modeling studies of the interaction between 4'-O-(α-L-oleandrosyl)daunorubicin and human serum albumin and its analytical application, J. Fluoresc. 22 (2012) 111-119.
[99] Y. Yue, J. Liu, M. Yao, X. Yao, J. Fan, H. Ji, The investigation of the binding behavior between ethyl maltol and human serum albumin by multi-spectroscopic methods and molecular docking, Spectrochim. Acta A 96 (2012) 316-323.
[100] S. Jana, S. Dalapati, S. Ghosh, N. Guchhait, Study of microheterogeneous environment of protein human serum albumin by an extrinsic fluorescent reporter: a spectroscopic study in combination with molecular docking and molecular dynamics simulation, J. Photochem. Photobiol. B 112 (2012) 48-58.
[101] A. Sengupta, W.D. Sasikala, A. Mukherjee, P. Hazra, Comparative study of flavins binding with human serum albumin: a fluorometric, thermodynamic, and molecular dynamics approach, Chemphyschem. 13 (2012) 2142-2153.
[102] S.R. Feroz, S.B. Mohamad, N. Bujang, S.N. Malek, S. Tayyab, Multispectroscopic and molecular modeling approach to Investigate the interaction of flavokawain B with human serum albumin, J. Agric. Food Chem. (2012).
[103] I.P. Caruso, W. Vilegas, M.A. Fossey, M.L. Cornelio, Exploring the binding mechanism of Guaijaverin to human serum albumin: Fluorescence spectroscopy and computational approach, Spectrochim. Acta A 97C (2012) 449-455.
[104] B. Hemmateenejad, M. Shamsipur, F. Samari, T. Khayamian, M. Ebrahimi, Z. Rezaei,

Combined fluorescence spectroscopy and molecular modeling studies on the interaction between harmalol and human serum albumin, J. Pharm. Biomed. Anal. 67-68 (2012) 201-208.
[105] F. Ding, J.X. Diao, Y. Sun, Bioevaluation of human serum albumin-hesperidin bioconjugate: insight into protein vector function and conformation, J. Agric. Food Chem. 60 (2012) 7218-7228.
[106] H.H. Sun, J. Zhang, Y.Z. Zhang, L.Y. Yang, L.L. Yuan, Y. Liu, Interaction of human serum albumin with 10-hydroxycamptothecin: spectroscopic and molecular modeling studies, Mol. Biol. Rep. 39 (2012) 5115-5123.
[107] M. Banerjee, U. Pal, A. Subudhhi, A. Chakrabarti, S. Basu, Interaction of Merocyanine 540 with serum albumins: photophysical and binding studies, J. Photochem. Photobiol. B 108 (2012) 23-33.
[108] J. Sochacka, W. Baran, The investigation of the binding of 6-mercaptopurine to site I on human serum albumin, Protein J. 31 (2012) 689-702.
[109] F. Ding, X.N. Li, J.X. Diao, Y. Sun, L. Zhang, Chiral recognition of metalaxyl enantiomers by human serum albumin: evidence from molecular modeling and photophysical approach, Chirality 24 (2012) 471-480.
[110] L.S. Yu, Y.J. Hong, L. Li, Y.X. Jin, M.Y. Zheng, H.L. Jiang, S. Zeng, Enantioselective drug-protein interaction between mexiletine and plasma protein, J. Pharm. Pharmacol. 64 (2012) 792-801.
[111] F. Deng, C. Dong, Y. Liu, Characterization of the interaction between nitrofurazone and human serum albumin by spectroscopic and molecular modeling methods, Mol. Biosyst. 8 (2012) 1446-1451.
[112] X.M. Zhou, W.J. Lu, L. Su, Z.J. Shan, X.G. Chen, Binding of phthalate plasticizers to human serum albumin in vitro: a multispectroscopic approach and molecular modeling, J. Agric. Food Chem. 60 (2012) 1135-1145.
[113] Y. Sun, Z. Ji, X. Liang, G. Li, S. Yang, S. Wei, Y. Zhao, X. Hu, J. Fan, Studies on the binding of rhaponticin with human serum albumin by molecular spectroscopy, modeling and equilibrium dialysis, Spectrochim. Acta A 87 (2012) 171-178.
[114] T. Chatterjee, A. Pal, S. Dey, B.K. Chatterjee, P. Chakrabarti, Interaction of virstatin with human serum albumin: spectroscopic analysis and molecular modeling, PLoS One 7 (2012) e37468.
[115] S. Tabassum, W.M. Al-Asbahy, M. Afzal, F. Arjmand, Synthesis, characterization and interaction studies of copper based drug with human serum albumin (HSA): spectroscopic and molecular docking investigations, J. Photochem. Photobiol. B 114 (2012) 132-139.
[116] D. De, H. Kaur, A. Datta, Unusual binding of a potential biomarker with human serum albumin, Chem. Asian J. 8 (2013) 728-735.
[117] C. Dong, S. Ma, Y. Liu, Studies of the interaction between demeclocycline and human serum albumin by multi-spectroscopic and molecular docking methods, Spectrochim. Acta A 103 (2013) 179-186.
[118] N. Zaidi, E. Ahmad, M. Rehan, G. Rabbani, M.R. Ajmal, Y. Zaidi, N. Subbarao, R.H. Khan, Biophysical insight into furosemide binding to human serum albumin: a study to unveil its impaired albumin binding in uremia, J. Phys. Chem. B 117 (2013) 2595-2604.
[119] O.K. Abou-Zied, N. Al-Lawatia, M. Elstner, T.B. Steinbrecher, Binding of hydroxyquinoline probes to human serum albumin: combining molecular modeling and forster's resonance energy transfer spectroscopy to understand flexible ligand binding, J. Phys. Chem. B 117 (2013) 1062-1074.
[120] N. Fani, A.K. Bordbar, Y. Ghayeb, A combined spectroscopic, docking and molecular dynamics simulation approach to probing binding of a Schiff base complex to human serum albumin, Spectrochim. Acta A 103 (2013) 11-17.
[121] H. Pu, H. Jiang, R. Chen, Combined multispectroscopic and molecular docking

investigation on the interaction between strictosamide and human serum albumin, Luminescence (2013) in press, doi: 10.1002/bio.2480.
[122] S.K. Ghorai, S.K. Samanta, M. Mukherjee, P. Saha Sardar, S. Ghosh, Tuning of "antenna effect" of Eu(III) in ternary systems in aqueous medium through binding with protein, Inorg. Chem. 52 (2013) 1476-1487.
[123] S. Aggarwal, A.K. Tiwari, P. Srivastava, N. Chadha, V. Kumar, G. Singh, A.K. Mishra, Investigation for the interaction of tyramine-based anthraquinone analogue with human serum albumin by optical spectroscopic technique, Chem. Biol. Drug Des. 81 (2013) 343-348.
[124] M. Domínguez-García, C. Ortega-Zúñiga, E. Meléndez, New tungstenocenes containing 3-hydroxy-4-pyrone ligands: antiproliferative activity on HT-29 and MCF-7 cell lines and binding to human serum albumin studied by fluorescence spectroscopy and molecular modeling methods, J. Biol. Inorg. Chem. 18 (2013) 195-209.
[125] Z. Moosavi-Movahedi, H. Bahrami, M. Zahedi, K. Mahnam, J. Chamani, S. Safarian, A.A. Saboury, A.A. Moosavi-Movahedi, A theoretical elucidation of bilirubin interaction with HSA's lysines: first electrostatic binding site in IIA subdomain, Biophys. Chem. 125 (2007) 375-387.
[126] N. Díaz, D. Suárez, T.L. Sordo, K.M. Merz, Jr., Molecular dynamics study of the IIA binding site in human serum albumin: influence of the protonation state of Lys195 and Lys199, J. Med. Chem. 44 (2001) 250-260.
[127] S. de Silva, R.M. de Silva, K.M. Nalin de Silva, Molecular mechanics (MM), molecular dynamics (MD) and semi-empirical study of Co^{2+}, Cu^{2+}, Ni^{2+} and Cd^{2+} binding to N-terminal of human serum albumin (HSA), J. Mol. Struct. 711 (2004) 73-81.
[128] R. Artali, G. Bombieri, L. Calabi, A. Del Pra, A molecular dynamics study of human serum albumin binding sites, Farmaco 60 (2005) 485-495.
[129] S. Fujiwara, T. Amisaki, Molecular dynamics study of conformational changes in human serum albumin by binding of fatty acids, Proteins 64 (2006) 730-739.
[130] R. Artali, A. Del Pra, E. Foresti, I.G. Lesci, N. Roveri, P. Sabatino, Adsorption of human serum albumin on the chrysotile surface: a molecular dynamics and spectroscopic investigation, J. R. Soc. Interface 5 (2008) 273-283.
[131] J.W. Shen, T. Wu, Q. Wang, Y. Kang, Induced stepwise conformational change of human serum albumin on carbon nanotube surfaces, Biomaterials 29 (2008) 3847-3855.
[132] H.J. Hsu, S.Y. Sheu, R.Y. Tsay, Preferred orientation of albumin adsorption on a hydrophilic surface from molecular simulation, Colloids Surf. B 67 (2008) 183-191.
[133] J. Giri, M.S. Diallo, A.J. Simpson, Y. Liu, W.A. Goddard, R. Kumar, G.C. Woods, Interactions of poly(amidoamine) dendrimers with human serum albumin: binding constants and mechanisms, ACS Nano 5 (2011) 3456-3468.
[134] H.W. Fang, M.C. Hsieh, H.T. Huang, C.Y. Tsai, M.H. Chang, Conformational and adsorptive characteristics of albumin affect interfacial protein boundary lubrication: From experimental to molecular dynamics simulation approaches, Colloids Surf. B 68 (2009) 171-177.
[135] R. Nasiri, H. Bahrami, M. Zahedi, A.A. Moosavi-Movahedi, N. Sattarahmady, A theoretical elucidation of glucose interaction with HSA's domains, J. Biomol. Struct. Dyn. 28 (2010) 211-226.
[136] J. Li, X. Zhu, C. Yang, R. Shi, Characterization of the binding of angiotensin II receptor blockers to human serum albumin using docking and molecular dynamics simulation, J. Mol. Model. 16 (2010) 789-798.
[137] C. Malleda, N. Ahalawat, M. Gokara, R. Subramanyam, Molecular dynamics simulation studies of betulinic acid with human serum albumin, J. Mol. Model. 18 (2012) 2589-2597.
[138] K. Aidas, J.M. Olsen, J. Kongsted, H. Ågren, Photoabsorption of acridine yellow and

proflavin bound to human serum albumin studied by means of quantum mechanics/molecular dynamics, J. Phys. Chem. B 117 (2013) 2069-2080.
[139] O. Deeb, M.C. Rosales-Hernandez, C. Gomez-Castro, R. Garduno-Juarez, J. Correa-Basurto, Exploration of human serum albumin binding sites by docking and molecular dynamics flexible ligand-protein interactions, Biopolymers 93 (2010) 161-170.
[140] H.A. Alvarez, A.N. McCarthy, J.R. Grigera, A molecular dynamics approach to ligand-receptor interaction in the aspirin-human serum albumin complex, J. Biophys. 2012 (2012) 642745.
[141] T.R. Guizado, S.R. Louro, C. Anteneodo, Dynamics of heme complexed with human serum albumin: a theoretical approach, Eur. Biophys. J. 41 (2012) 1033-1042.
[142] G. Paris, S. Kraszewski, C. Ramseyer, M. Enescu, About the structural role of disulfide bridges in serum albumins: evidence from protein simulated unfolding, Biopolymers 97 (2012) 889-898.
[143] N.A. Kratochwil, W. Huber, F. Muller, M. Kansy, P.R. Gerber, Predicting plasma protein binding of drugs: a new approach, Biochem. Pharmacol. 64 (2002) 1355-1374.
[144] E. Gianolio, G.B. Giovenzana, D. Longo, I. Longo, I. Menegotto, S. Aime, Relaxometric and modelling studies of the binding of a lipophilic Gd-AAZTA complex to fatted and defatted human serum albumin, Chemistry 13 (2007) 5785-5797.
[145] S. Fujiwara, T. Amisaki, Identification of high affinity fatty acid binding sites on human serum albumin by MM-PBSA method, Biophys. J. 94 (2008) 95-103.
[146] M. Salvalaglio, I. Muscionico, C. Cavallotti, Determination of energies and sites of binding of PFOA and PFOS to human serum albumin, J. Phys. Chem. B 114 (2010) 14860-14874.
[147] S. Fujiwara, T. Amisaki, Steric and allosteric effects of fatty acids on the binding of warfarin to human serum albumin revealed by molecular dynamics and free energy calculations, Chem. Pharm. Bull. 59 (2011) 860-867.
[148] B. Rizzuti, M. Pantusa, R. Guzzi, The role of Lys525 on the head-group anchoring of fatty acids in the highest affinity binding site of albumin, Spectroscopy 24 (2010) 159-163.
[149] K. Paal, A. Shkarupin, Paclitaxel binding to the fatty acid-induced conformation of human serum albumin--automated docking studies, Bioorg. Med. Chem. 15 (2007) 7568-7575.
[150] D.E. Shaw, M.M. Deneroff, R.O. Dror, J.S. Kuskin, R.H. Larson, J.K. Salmon, C. Young, B. Batson, K.J. Bowers, J.C. Chao, M.P. Eastwood, J. Gagliardo, J.P. Grossman, C.R. Ho, D.J. Ierardi, I. Kolossvary, J.L. Klepeis, T. Layman, C. McLeavey, M.A. Moraes, R. Mueller, E.C. Priest, Y.B. Shan, J. Spengler, M. Theobald, B. Towles, S.C. Wang, Anton, a special-purpose machine for molecular dynamics simulation, Commun. ACM 51 (2008) 91-97.
[151] T. Seto, H. Isogai, M. Ozaki, S. Nosaka, Noble gas binding to human serum albumin using docking simulation: nonimmobilizers and anesthetics bind to different sites, Anesth. Analg. 107 (2008) 1223-1228.
[152] F. Ding, W. Liu, L. Zhang, B. Yin, Y. Sun, Sulfometuron-methyl binding to human serum albumin: Evidence that sulfometuron-methyl binds at the Sudlow's site I, J. Mol. Struct. 968 (2010) 59-66.
[153] S.W. Sarsam, D.R. Nutt, K. Strohfeldt, K.A. Watson, Titanocene anticancer complexes and their binding mode of action to human serum albumin: a computational study, Metallomics 3 (2011) 152-161.
[154] W. Humphrey, A. Dalke, K. Schulten, VMD: Visual molecular dynamics, J. Mol. Graphics 14 (1996) 33-38.

Table 1. Ligands bound to FA-binding sites on human serum albumin (HSA).

Site [a]	Bound ligand identified by X-ray crystallography
1	Azapropazone [17], AZT[b] [20], bilirubin-IXα [21], dansyl-L-asparagine [26], dansyl-L-arginine [26], dansyl-L-glutamate [26], fusidic acid [21], heme [13, 15], indomethacin [17], iophenoxic acid [27], naproxen [19], Δ^{12}-prostaglandin J_2 [23], salicylic acid [18, 20], triiodobenzoic acid [7]
2	Halothane [9]
3, 4	CMPF[c] [17], dansyl-L-asparagine [26], dansyl-L-norvaline [26], dansyl-L-phenylalanine [26], dansyl-L-sarcosine [26], diazepam [17], diflunisal [17], halothane [9], ibuprofen [17], indoxyl sulfate [17], iophenoxic acid [27], propofol [9]
5	Fusidic acid [21], oxyphenbutazone [17], propofol [9]
6	DAUDA[d] [29], diflunisal [17], halothane [9], ibuprofen [17]
7	Aspirin [18], azapropazone [17], AZT[b] [20], citric acid [16], CMPF[c] [17], dansyl-L-arginine [26], dansyl-L-asparagine [26], dansyl-L-glutamate [26], dansyl-L-phenylalanine [26], DAUDA[d] [29], diflunisal [17], halothane [9], indomethacin [17], indoxyl sulfate [17], iodipamide [17], iophenoxic acid [27], lysophosphatidylethanolamine [22], oxyphenbutazone [17], phenylbutazone [17], salicylic acid [18, 20], triiodobenzoic acid [7], warfarin [12, 17]

[a] The numbering of the FA binding sites was sourced from Bhattacharya et al. [10].
[b] 3'-Azido-3'-deoxythymidine.
[c] 3-carboxy-4-methyl-5-propyl-2-furanpropanoic acid.
[d] 11-(Dansylamino) undecanoic acid.

Table 2. HSA-ligand docking simulations reported as of Febrary 2013.

Year	Ligand docked to HSA
2006	Formononetin [71]
2007	Anti-HIV drugs (abacavir, nevirapine, atazanavir) [72], bilirubin [125], eupatilin [73], paclitaxel [149],
2008	Noble gas [151], zidovudine and its derivatives [63]
2009	Daunorubicin [74], deoxyuridine [75], levamlodipine [76],
2010	Angiotensin II receptor blockers [136], anti-Parkinson's disease drugs (apomorphine and benserazide) [77], flavones [78], flavonoids [67], glucose [135], metsulfuron-methyl [79], perfluorooctanoic acid and perfluorooctane sulfonate [146], β-sitosterol [80], sulfometuron-methyl [152]
2011	Anthraquinone dye [81], cyclophosphamide hydrochloride and aspirin [82], diazepam and ∆9-tetrahydrocannabinol [83], hen egg white lysozyme and triacetylchitotrioside [84], isoniazid and rifampicin [85], lomefloxacin [86], titanocene [153], warfarin (docked to methylglyoxal-modified HSA) [87]
2012	2-aminobenzothiazole [88], amlodipine and propranolol [89], amodiaquine [90], anthraquinone dye [91], anti-breast cancer drugs (fluoxymesterone, cyclophosphamide) [92], benzoxazole [93], betulinic acid [137], bilirubin [94], catechin [95], chlorpyrifos [96], daphnin [97], daunorubicin analog [98], ethyl maltol [99], extrinsic fluorescent probe [100], flavins [101], flavokawain B [102], guaijaverin [103], harmalol [104], hesperidin [105], 10-hydroxycamptothecin [106], merocyanine 540 [107], 6-mercaptopurine [108], metalaxyl [109], mexiletine [110], nitrofurazone [111], phthalate plasticizers [112], rhaponticin [113], virstatin [114], water soluble copper(II) complex [115]
2013	Acridine yellow and proflavin [138], [2,2'-bipyridyl]-3,3'-diol [116], demeclocycline [117], furosemide [118], hydroxyquinoline derivatives [119], PCB153 [5], Schiff base complex [120], strictosamide [121], tetracycline hydrochloride [122], tyramine-based anthraquinone analogue [123], water-soluble tungstenocene derivatives [124]

Figure legends

Figure 1. Ribbon model of the human serum albumin (HSA)-palmitate complex derived from X-ray crystallography (PDB ID: 1E7H). HSA is composed of 3 homologous domains, I-III, each is divided into subdomains A and B. The 7 palmitate molecules are shown in blue (identified as high-affinity fatty acid [FA] binding sites) or yellow (identified as low-affinity FA-binding sites) in a space-filling representation [50]. The numbering of the FA binding sites was sourced from Bhattacharya et al. [10]. Molecular graphics images were prepared with VMD (version 1.9.1) [154].

Figure 2. Directional motions of the unliganded HSA and the HSA-FA complex projected on the first, second, and third principal components (PCs 1-3). The arrows in the figure indicate the approximate directions of cooperative motions of C_α atoms. The directional motion projected on PC1 of the unliganded HSA is similar to that projected on PC3 of the HSA-FA complex. Molecular graphics images were prepared with VMD (version 1.9.1) [154]. This figure was reproduced and adapted from Fig. 8 of PROTEINS: Structure, Function, and Bioinformatics 64 (2006) 730-739 [129].

Figure 3. Relationship between experimental and calculated HSA-FA (myristate, palmitate) binding free energy for 3 high-affinity FA binding sites (sites 5, 4, 2). Affinity constants (K_1, K_2, K_3) were taken from Ashbrook et al. [45]. The experimental binding free energy ($\Delta G_{bind,expt}$) was calculated using the equation $\Delta G_{bind,expt} = -RT \ln K$, where R and T are the gas constant and the absolute temperature, respectively. The calculated values of absolute binding free energies deviated considerably from the experimental binding free energies (red lines). This figure was reproduced and adapted from Fig. 4 of Biophysical Journal 64 (2008) 95-103 [145], with permission from Elsevier.

Figure 4. Four of the 11 "virtual" HSA-warfarin-myristate complexes and the relationship between calculated HSA-warfarin binding free energies (ΔG_{bind}) and the number of bound FA (myristate) molecules. Based on molecular dynamics simulations of the 11 virtual HSA-warfarin-myristate complexes, ΔG_{bind} was calculated for each complex. In the graph, the position of bound FA molecules is also indicated. This figure was reproduced in part with permission from Chemical and Pharmaceutical Bulletin Vol. 59 No.7 [147]. Copyright 2011 The Pharmaceutical Society of Japan.

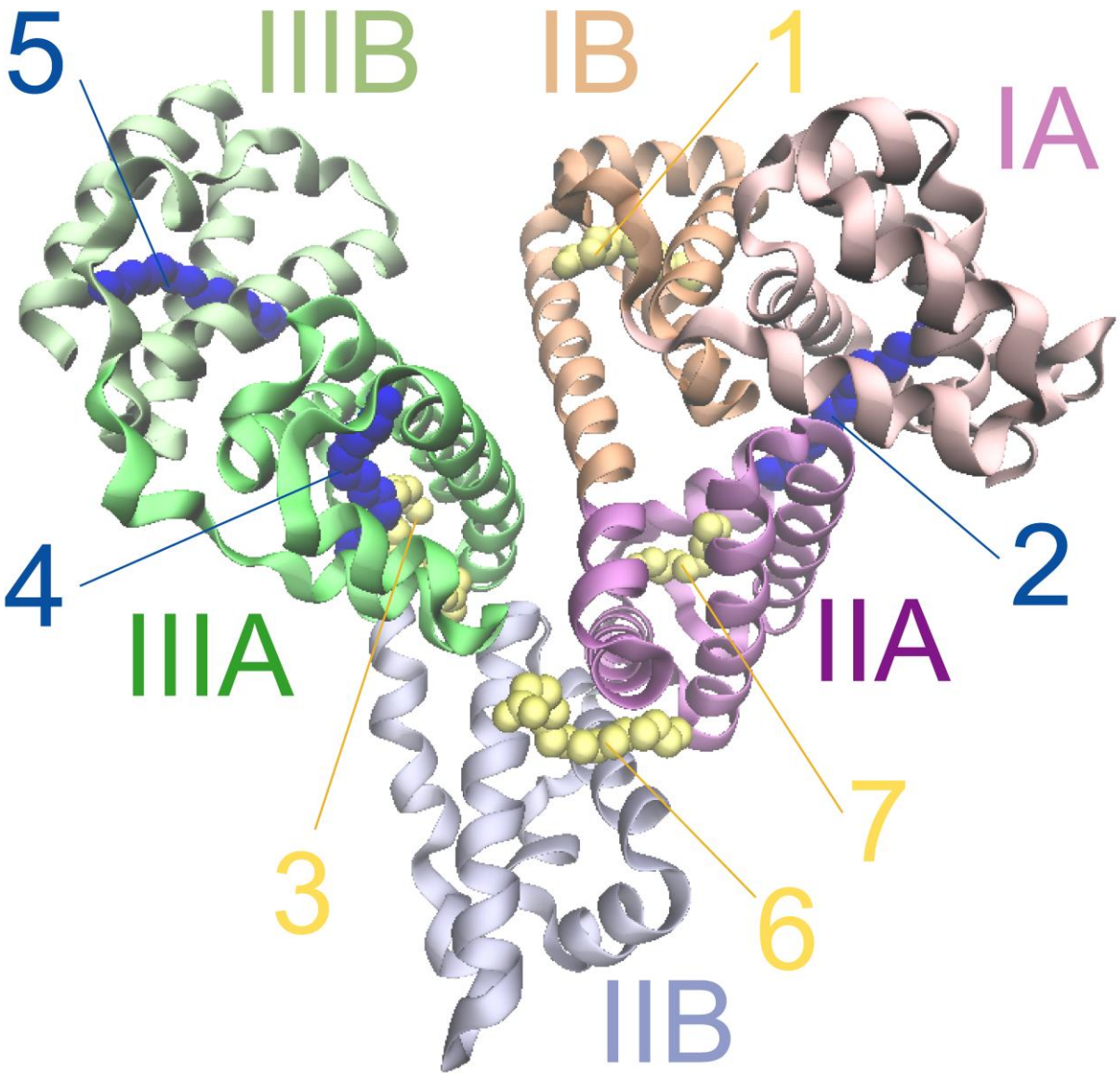

Figure 1

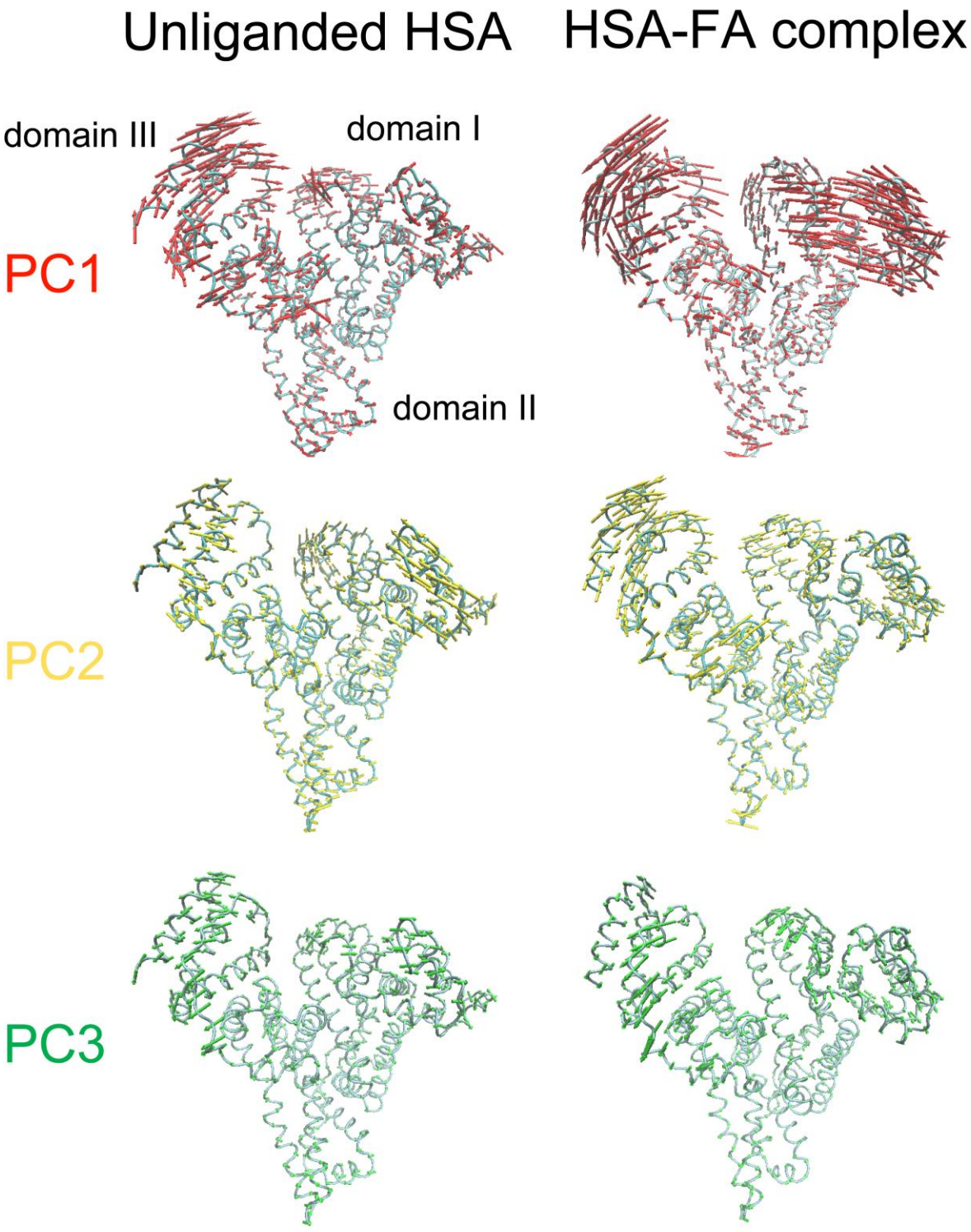

Figure 2

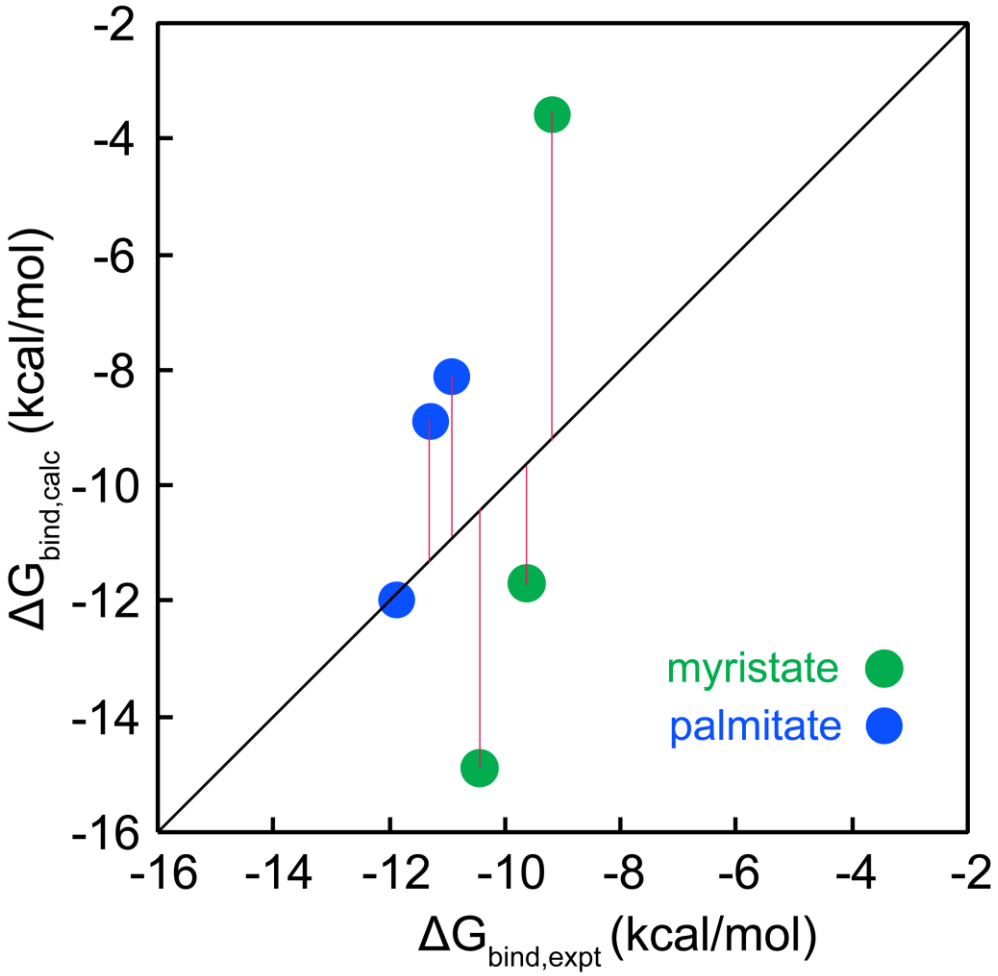

Figure 3

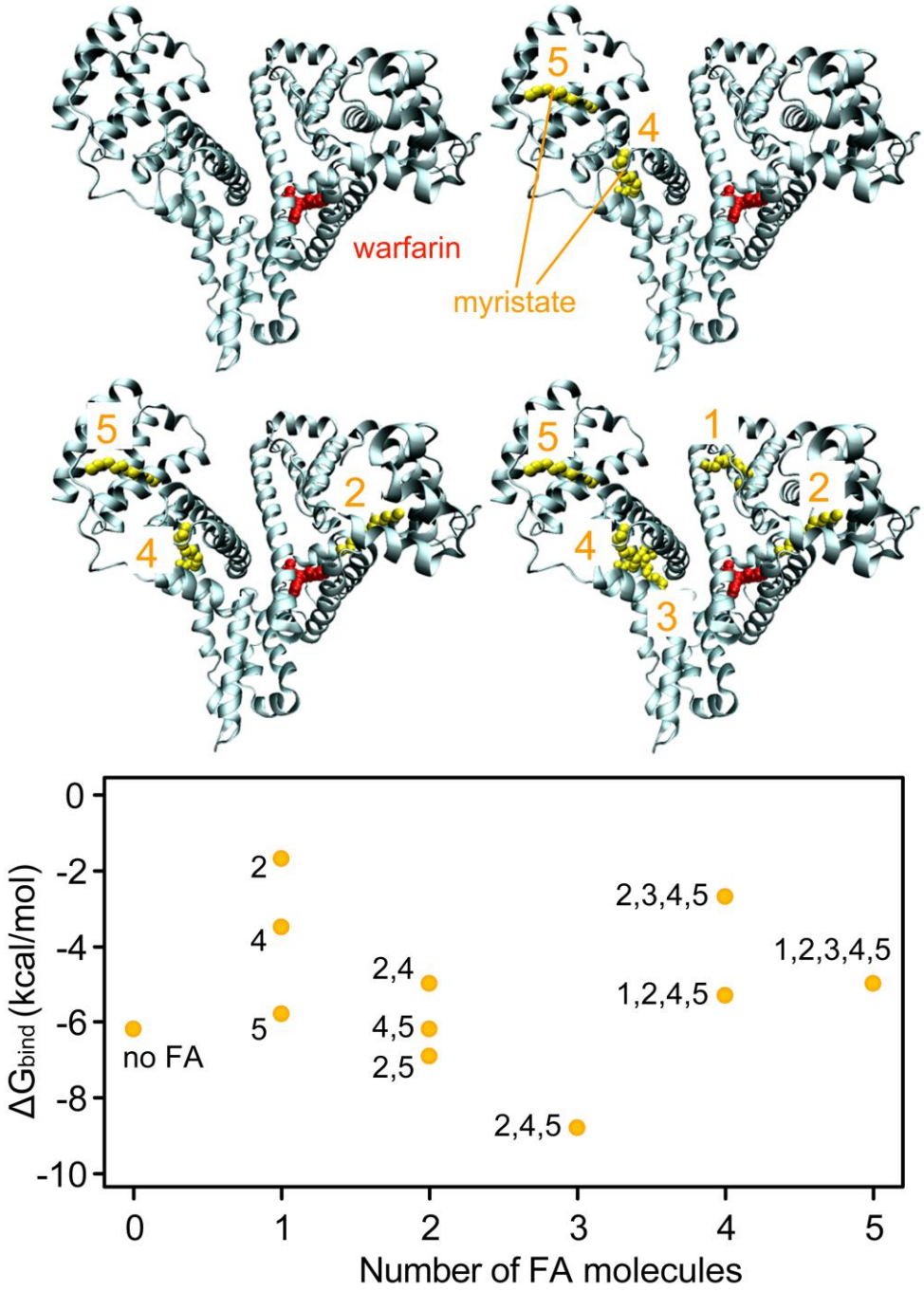
warfarin
myristate
5
4
5
2
4
5
1
2
4
3
0
-2
-4
-6
-8
-10
ΔGbind (kcal/mol)
no FA
2
4
5
2,4
4,5
2,5
2,4,5
2,3,4,5
1,2,4,5
1,2,3,4,5
0
1
2
3
4
5
Number of FA molecules

Figure 4

Highlights

- Binding of FA molecules to HSA can modulate ligand binding affinity to HSA.
- Molecular simulation approaches have been applied to structural analyses of HSA.
- Possible conformational changes of HSA-FA binding were analyzed by MD simulations.
- Binding free energy calculations identified high/low affinity FA binding sites.
- Molecular simulation analyzes conditions that cannot be experimentally observed.