

# Computational Modeling of the Interactions of Drugs with Human Serum Albumin (HSA)

Sergio Mares-Sámamo<sup>1,2</sup>, Ramón Garduño-Juárez<sup>1</sup>

<sup>1</sup> Universidad Nacional Autónoma de México, Instituto de Ciencias Físicas, Mexico

<sup>2</sup> CONACYT, Mexico

{sergiom, ramón}@icf.unam.mx

**Abstract.** Human serum albumin (HSA) is the most abundant protein in the circulatory system that shows a remarkable capacity to bind a wide range of drugs impacting their therapeutic effect. Therefore, the binding to HSA represents a fundamental factor to consider when designing and developing new drugs. Although biophysical techniques (e.g. spectroscopy) are commonly employed to measure the extent to which drugs bind to HSA, these methods are time consuming and usually extremely expensive. Hence, there is an urgent need to incorporate more efficient methods in an attempt to streamline the development of new drugs. Here we present the implementation of a robust and cost-effective computational method to the prediction of the binding affinity of drugs towards HSA. Our method incorporates the program AutoDock Vina to perform in silico molecular docking of a highly diverse set of drugs against the 3D crystal structure of HSA. The 3D structure of HSA was retrieved from the Protein Data Bank and prepared to be used as receptor in our docking simulations. 3D structures of drugs were generated and optimized using Open Babel. Our protocol using AutoDock Vina as the docking engine was capable of reproducing the binding mode of indoxyl sulfate within the X-ray crystal structure of HSA (RMSD < 2.0 Å). In addition, our protocol correlated accurately predicted affinity values with experimentally determined association constants ( $r^2=0.61$ ). Our computational-based molecular docking approach incorporating AutoDock Vina may prove useful to the prediction of the binding affinities of drugs towards human serum albumin, and thus, could help alleviate a major bottleneck of the drug discovery process.

**Keywords.** Computer-aided drug design, modeling, docking.

## 1 Introduction

A recent benchmark study has shown that bringing a new pharmaceutical drug product to the market exceeds an investment of two thousand million (US) dollars and takes over ten years of development [35]. Consequently, there is an ever-growing effort to apply new technologies that could help streamline the discovery process. The publication of the article "Next Industrial Revolution:

Designing Drugs by Computer at Merck", in the early 1980s in the prestigious business magazine Fortune, marked the first public credit to the enormous potential of computers assisting the design of new drugs [1].

Over the past two decades, the explosive advancements in high-performance computing [32], together with the increasing availability of 3-dimensional structures of important pharmaceutical targets, have paved the way to the computer-aided design of drugs.

Particularly, the now wide spread access to supercomputers and the development of more efficient algorithms have positively impacted the discovery and development of new drugs. Computer-aided drug design (CADD) uses computational approaches to discover, develop, and analyze drugs [28].

Although CADD is an emerging discipline, its foundations date back to the seminal works of Emil Fischer (1894) [14] and Paul Ehrlich (1909) [12] when they proposed the lock and key model.

This model rationalizes the mechanism of drug action: a receptor structure (the lock) contains a region with a particular shaped pocket at which an appropriately shaped drug (the key) can interact [6]. Though a number of biomolecules within the human body can work as a receptor, protein molecules frequently play this role. In the same way in which a key should open only one lock – otherwise security may be compromised – drugs should be designed to act on only one receptor [1]. Deleterious side effects are due, at least in part, to the fact that the drug molecule interacts not only with its target receptor but also with other proteins not associated with the disease that it is proposed to treat.

Human serum albumin (HSA), the most abundant protein of the circulatory system, represents a very typical example of a promiscuous protein receptor that interacts with a broad range of drugs impacting their efficacy and safety [4, 31, 26]. This protein plays a crucial role in regulating the osmotic pressure and in transporting endogenous compounds such as fatty acids, hormones, toxic metabolites and metals. Due to its high capacity of binding a wide range of drugs, HSA determines, to a large extent, the safety and pharmacokinetics profile of most drugs. Regardless of the method of administration (e.g. oral, intravenous), drugs are transported through the blood, where they inevitably interact with the human serum albumin (HSA) before reaching their receptors.

Hence, drug molecules can circulate in the bloodstream either bound to HSA (or other plasma proteins) or in their unbound (free) form [26]. Interestingly, only unbound drug molecules can interact with the protein receptor to trigger their therapeutic effect [41]. Moreover, the distribution and elimination of a drug from the body depends on the strength with which it is bound to HSA [17].

Therefore, the study of the binding of drugs to HSA, at the molecular level, results of paramount importance to better outline the pharmacokinetics profile of drugs [23]. Molecular docking is central to the CADD strategies. The computational-based fitting of the 3D structure of a molecule (ligand) within the binding site of a protein receptor is called docking [8].

The docking process, in general, involves two independent steps: (1) the generation of the

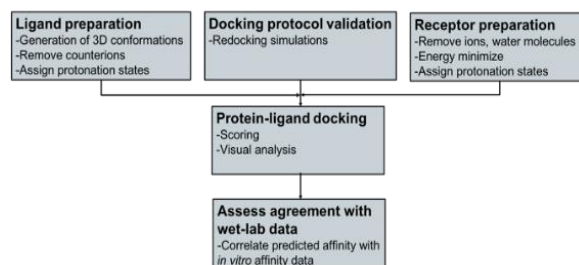


Fig. 1. Overall molecular docking workflow

correct pose (conformation and orientation) of ligands inside the receptor (protein) binding site and (2) the evaluation of the affinity of binding. [8].

Over the last few decades, docking techniques have successfully been incorporated to the standard laboratory experiments to the discovery of new drugs. This strategy has importantly contributed to reducing both the experimental work and the associated costs [1].

Although there are currently a wide range of docking software alternatives, AutoDock Vina [45] remains one of the top choices due to its high reliability [7]. AutoDock Vina is an open source program, free for academic use, that employs the 3D structures of the receptor and ligand to predict the best way of molecular interaction [45]. In the first stage, AutoDock Vina samples the conformational space to explore possible modes of protein–ligand interaction. Subsequently, in the second stage, the program uses a scoring function to determine the most suitable pose in terms of the energy of interaction [21].

The aim of the current study is to harness the significant increase, in recent years, of computing power, and the availability of 3D crystal structures of the HSA to develop a computational method that incorporates molecular docking, using AutoDock Vina, to predict the interactions of drugs with this plasma protein, which may potentially lead to a streamlined drug discovery process.

## 2 Methods

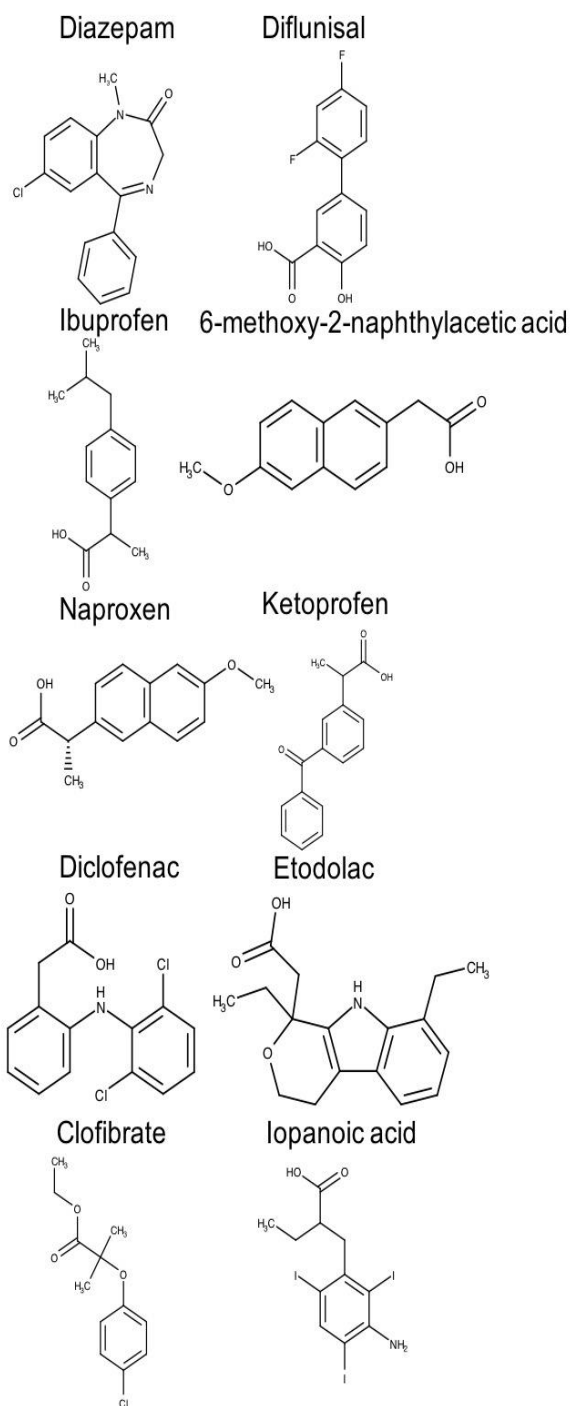
Figure 1 shows the overall methodology that was applied to conduct this study and some of the tools that were employed.

Protein-ligand docking requires as input a three-dimensional (3D) ligand database and the 3D structure of the protein receptor. Affinity of selected drug ligands against the human serum albumin (HSA) receptor were investigated using AutoDock Vina.

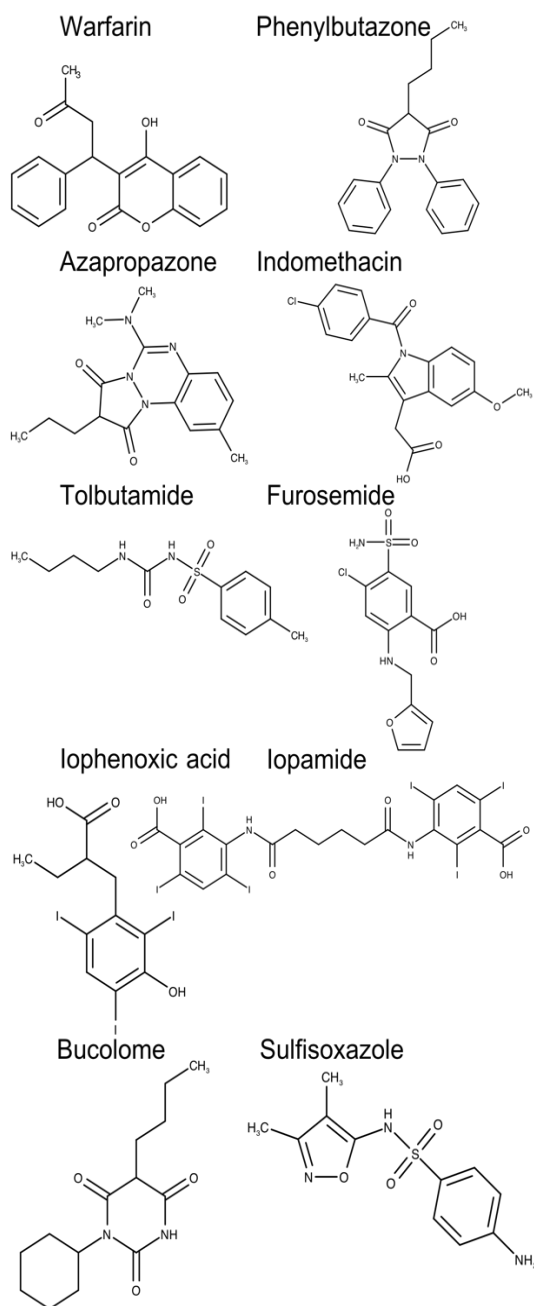
Before docking, ligand database and HSA receptor were prepared. Open Babel was used to the generation of 3D structures of ligands and to their optimization. HSA was prepared using, Scwrl4, Reduce and Gromacs. Finally, predicted affinity was compared with reported biological activity.

## 2.1 Receptor Preparation

The 3D crystal structure of human serum albumin (PDB ID 2BXH) [15] was retrieved from the Protein Data Bank [3] and subjected to an initial preparation phase where all water molecules and solutes contained in the structure were removed, including the ligand indoxyl sulfate, which was co-crystallized with the protein. Subsequently, the structure was inspected with Scwrl4 [27] to add missing atoms and side chains and to optimize them with respect to the entire hydrogen bond network of the protein. Scwrl4 uses an energy function based on a backbone-dependent rotamer library to estimate the positioning of residue side chains. When elucidating X-ray crystal structures, misorienting Asp and Glu side chains and His rings is quite common (except at extremely high resolutions) because the electron density is symmetric around these residues. The Reduce [49] program, included in the MolProbity server [48], was used to identify the preferred orientation for these side chains based on van der Waals contacts and H-bonding. In this process, flipping was accepted when the program indicated "clear evidence for flipping." Reduce software conducts surface protein analysis, employing all explicit hydrogen atoms and van der Waals contacts, to choose the most suitable side chain orientations of Asn, Gln, and His amino acids [49]. The protein was then energy minimized to obtain a stable, low energy conformation. The protein was minimized *in vacuo*, with no harmonic restraints, using the steepest descent minimizer of Gromacs 4.5.4 [18].



**Fig. 3** Two-dimensional chemical structures of drugs interacting at the drug-binding site 2 of human serum albumin (HAS)



**Fig. 2.** Two-dimensional chemical structures of drugs interacting at the drug-binding site 1 of human serum albumin (HAS)

Protonation states were assigned at pH=7.4 using the PDB2PQR [11] software, which

incorporates Propka [29] to the calculation of the pKa protein values. Then Gasteiger charges were added and the receptor file converted to the pdbqt format using the “prepare\_receptor4.py” script of AutoDockTools [33]. Finally, the quality of the protein receptor was assessed using a series of tests for structural consistency and reliability. The stereochemistry of the refined structure was evaluated through the analyses of Ramachandran plots [39] using the MolProbity server [48]. The statistics of non-bonded residue interactions, which determines the amino acid environment, was assessed with the ERRAT program [10]. This tool helps discriminate between correctly and incorrectly determined regions of protein structures based on characteristic atomic interactions [10].

## 2.2 Ligand Preparation

A total of 20 chemically and functionally highly-diverse drug molecules (Figures 2 and 3), for which association constants ( $k_a$ ) towards HSA had been determined, were identified from a literature search [51].

In order to utilize these molecules in the docking procedure, their two-dimensional (2D) structures, in smile format, were retrieved from the PubChem database (<https://pubchem.ncbi.nlm.nih.gov/>). Smiles strings were subsequently converted to 3-dimensional (3D) coordinates using the “gen3d” option of the Open Babel package [16], version 2.4.0 (<http://openbabel.org>).

Open Babel package was also used to remove salts and counterions, if present, to energy minimize and to assign protonation states (at pH=7.4) to ligand structures. Finally, the “prepare\_ligand4.py” script included in AutoDockTools [33] was employed to assign Gasteiger charges and to convert ligands to the pdbqt format.

## 2.3 Molecular Docking

Molecular docking is a computationally-based procedure to the prediction of the binding mode and binding affinity of a ligand (drug molecule) with a protein (receptor) of known 3D structure. AutoDock Vina [45] version 1.1.2 was used to dock drug molecules into the drug-binding sites of the HSA protein.

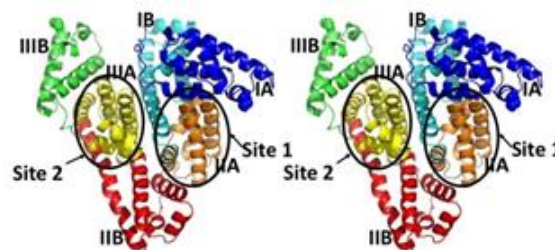
The input of the protocol includes the 3D structure of the protein receptor and those of the ligands, and a defined search conformational space. The output yields a set of poses (receptor-ligand) ranked by  $\Delta G$ , the predicted energy of binding in kcal/mol. AutoDock Vina was set to generate a maximum of 20 poses, through the *num\_modes* command. This program performs a user-defined number of individual sampling runs through the “exhaustiveness” parameter. In each run, the program perturbs the ligand structure to sample different three-dimensional arrays of the drug molecule within the active site of the receptor followed by a local optimization, using the Broyden-Fletcher-Goldfarb-Shanno algorithm [37].

The resulting ligand conformation is either accepted or rejected according to a Metropolis criterion [45]. Subsequently, the scoring function evaluates multiple times each local optimization. The number of scoring function evaluations is determined heuristically, and depends on the number of rotatable bonds of the ligand and whether the receptor contains flexible amino acids [45].

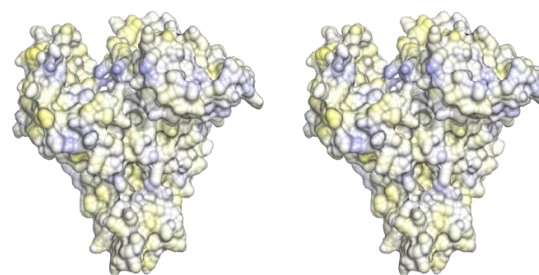
The protein receptor was maintained rigid throughout the docking process, while drug molecules were allowed flexibility. The search space was defined to cover two distinct HSA drug-binding sites. Binding site I was covered within a region with coordinates at the center along the X, Y and Z axis as -4.326, -4.756 and 9.449, and dimensions (Å) along the X, Y and Z axis were set to 10 Å. In the case of binding site II, the search space region was centered at the coordinates (X, Y, Z) 9.187, 3.021, -14.306. And the dimensions along these axes were also set to 10 Å. All other parameters were kept as the AutoDock Vina default values.

## 2.4 Validation of AutoDock Vina Performance

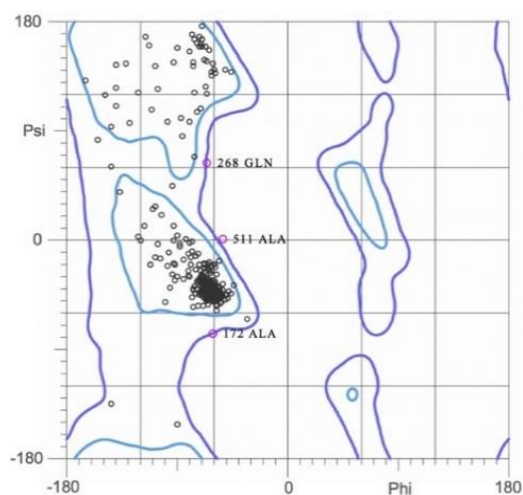
The performance of AutoDock Vina was evaluated assessing whether this program could reproduce accurately the experimental binding mode of ligand indoxyl sulfate within the X-ray crystal structure of Human Serum Albumin (PDB ID 2BXH) [15]. The root-mean-square deviation (RMSD) cut-off of 2 Å was used as a criterion of the correct bound prediction [38].



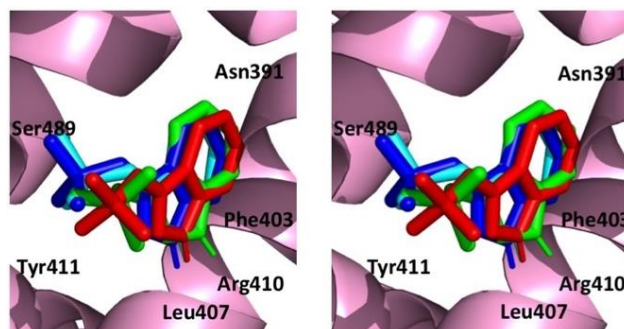
**Fig. 4.** Stereo view of the overall structure of the refined model of human serum albumin (HSA). Subdomain IA is shown in dark blue; subdomain IB, in light blue; subdomain IIA, in orange; subdomain IIB, in red; subdomain IIIA, in yellow; subdomain IIIB, in green



**Fig. 5.** Stereo view of the hydrophobic and hydrophilic potentials of the human serum albumin mapped onto a surface representation. Blue: area of predicted highest hydrophilic potential; yellow: area of predicted highest hydrophobic potential



**Fig. 6.** Ramachandran plot of the refined model of human serum albumin (HAS). 98.9% of the residues were located in the most favored regions



**Fig. 7.** Stereo view of the re-docking of indoxyl sulfate using AutoDock Vina. Top 1 pose (green), top 2 pose (light blue) and top 3 pose (dark blue) compared with the co-crystallised ligand indoxyl sulfate (red) viewed from the outside of the internal cavity of human serum albumin (HAS)

**Table 1.** Interactions of co-crystallized indoxyl sulfate with human serum albumin (Ids-HSA\*). For comparison, the interactions of the top 3 poses obtained from re-docking experiments are shown

	Ids-HSA* X-ray crystal structure	Pose 1	Pose 2	Pose 3
	Ile388	X	X	X
	Asn391	✓	✓	✓
	Phe403	✓	X	X
	Leu407	✓	✓	✓
Hydrophobic interactions	Arg410	✓	X	X
	Tyr411	✓	✓	✓
	Leu430	✓	✓	✓
	Gly434	✓	✓	✓
	Ser489	X	✓	X
Hydrophilic interactions	Arg410	✓	X	X
	Tyr411	✓	X	✓
	Leu430	✓	✓	✓

Also, in a comparative study, the correlation of predicted affinity of binding with experimentally

determined associations constants was evaluated as a measurement of the precision of the protocol.

**Table 2.** Predicted affinity (free energy of binding) of drugs docked, using AutoDock Vina, into the refined model of human serum albumin (HSA) and experimentally determined association constants ( $K_a$ ) for the interactions of these drugs with the protein. \*: 6-methoxy-2-naphthylacetic acid

Drug	$K_a$ ( $M^{-1}$ )	Log $K_a$	Predicted affinity (kcal/mol)
Azapropazone	280000	5.45	-8
Bucolome	1500000	6.17	-7.2
Furosemide	200000	5.30	-7.4
Indomethacin	1400000	6.15	-7.1
Iodipamide	9900000	6.99	-6.2
lophenoxic_acid	77000000	7.89	-5
Phenylbutazone	1500000	6.18	-7.3
Sulfisoxazole	180000	5.26	-7.3
Tolbutamide	40000	4.60	-7.6
Warfarin	340000	5.53	-7.9
6-MNA*	1200000	6.08	-7
Clofibrate	760000	5.88	-6.2
Diazepam	1300000	6.11	-6.9
Diclofenac	3800000	6.58	-6.6
Diflunisal	530000	5.72	-7.7
Etodolac	200000	5.30	-6.8
Ibuprofen	3500000	6.54	-6.6
Iopanoic_acid	6700000	6.83	-5.5
Ketoprofen	2500000	6.39	-7.1
Naproxen	1200000	6.08	-7.4

Structure analysis and visualization were performed using PyMOL (<https://pymol.org/2/>) and lipophilic potential was calculated using VASCO, version 1.02 [43].

### 3 Results

#### 3.1 Receptor Refinement and Preparation

Using state-of-the-art computational techniques, including side-chain protein optimization and energy minimization, we highly refined the structure of Human Serum Albumin (HSA) from the experimentally determined X-ray crystal. Figures 4

and 5 shows that our refined structure of HSA is composed of a single chain that contains three  $\alpha$ -helical homologous domains, namely, I (residues 1-195), II (residues 196-383), and III (residues 384-585). Each domain is divided into subdomains A and B. The protein features a heart-shaped structure with an approximate dimension of 80 x 80 x 30 Å. Studies conducted by Sudlow and co-workers [44] showed the presence of two distinct binding sites. Binding site I is located within the core of subdomain IIA including all its six helices and also a loop-helix fragment (residues 148-154) contributed by subdomain IB.

Although the pocket that defines this binding site contains predominantly apolar residues, two

groups of hydrophilic residues, Tyr150, His242, Arg257 and Lys195, Lys199, Arg218, Arg222 are also displayed in this pocket. Binding site 1 allocates a series of therapeutically important drugs such as warfarin, phenylbutazone, furosemide, among others (Figure 2).

Drug-binding site 2 comprises the six  $\alpha$ -helices of subdomain IIIA and predominantly includes a largely hydrophobic cleft measuring about 16 Å deep and 8 Å wide with a positively charged residue positioned near the surface (Figures 4 and 5).

Although topologically binding site 2 is similar to binding site 1, the former shows some key differences. Pocket of binding site 2 presents a smaller size, and unlike site 1, only contains a patch of hydrophilic residues, including Tyr411, Arg410, Lys414 and Ser489 (Figures 4 and 5).

Binding sites 1 and 2 are distinguishable not only in terms of structural architectures but also in terms of hydrophobicity profile, which ultimately determines the different binding specificities for both pockets. Figure 3 shows the structures of some drugs that bind site 2 (e.g. diazepam, ibuprofen, naproxen, ketoprofen).

### 3.2 Refined Structure Validation

The quality of the refined model was assessed using MolProbity, a server that includes the generation of Ramachandran plot analysis for structure verification. Figure 6 shows that the structure possesses 98.9% residues located in the most favoured regions of the plot and therefore they are stereochemically valid.

The non-bonded residue interactions were further evaluated by means of the ERRAT analysis. Our refined structure showed a 94.2% ERRAT score. High quality structures generally show >85% score [10].

The consistency of the folding pattern, the geometric quality of the backbone, and the non-bonded residue interactions of the refined model are within the limits established for high quality structures. Therefore, the refined model of human serum albumin can be reliably used in further structural studies.

### 3.3 Validation of the Performance of AutoDock Vina

One approach to the validation of a docking program is to inspect whether the predicted mode of binding (docked conformation) of a ligand within the receptor is capable of reproducing the bound ligand in the experimental crystal structure. Consequently, in order to evaluate the reliability of AutoDock Vina, the bound ligand indoxyl sulfate was removed from the crystal structure of human albumin (PDB ID 2BXH) and re-docked.

AutoDock Vina had the ability to reproduce the positioning of the ligand within the crystal structure of human serum albumin as shown in Figure 7. Molecular docking of indoxyl sulfate within the crystal structure of HSA resulted in an RMSD value of <2 Å for the top 3 predicted poses (Figure 7). Also, the number and the nature of the interactions of these poses were consistent with those of the co-crystallized ligand indoxyl sulfate (Table 1).

### 3.4 Correlation of Predicted Affinities with Experimentally Determined Association Constants ( $K_a$ )

Many studies have investigated the interactions of human serum albumin with drugs using spectroscopic studies such as fluorescence quenching [26, 51, 34, 42, 22, 36, 47, 5, 13, 30]. These studies have allowed the quantification of equilibrium binding of drugs to purified human serum albumin and provided an estimate of the constant of association ( $K_a$ ). In a comparative analysis, we have subjected some of these drugs to computational docking simulations to investigate whether exists a potential correlation between predicted affinities and experimentally determined association constants.

A highly diverse set of 20 drugs (Figures 2 and 3) was prepared and all molecules docked into the refined model of human serum albumin using the docking protocol described in the methods section. Predicted affinities were then compared with reported experimentally determined association constants (Table 2).

The level of correlation of predicted energy binding and experimentally-determined  $K_a$  yielded by the protocol in which AutoDock Vina was incorporated was high.



The docking protocol that set the sampling to exhaustiveness values of 100 resulted in a correlation coefficient of 0.6136 (Figure 8).

Correlation studies showed that our protocol incorporating AutoDock Vina, with the parameters described, was capable of distinguishing quantitatively strong binders from weak binders, *i.e.* correlated with predicted affinity over a 1000-fold range in  $K_a$  values (Table 2). Therefore, the predicted affinity could be regarded as an index of experimental binding affinity.

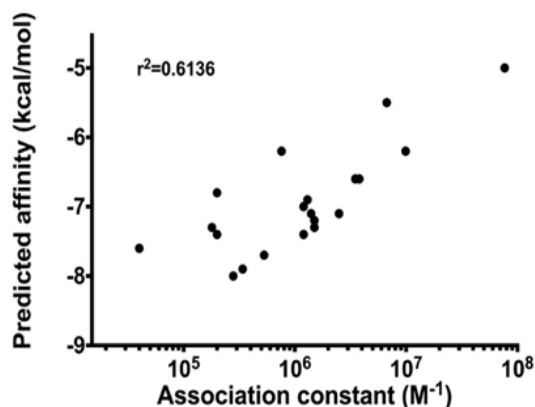
## 4 Discussion

A crucial step in the development of new and effective drugs is the understanding of their ability to bind human serum albumin (HSA), as this protein plays a key role in the Absorption, Distribution, Metabolism, Excretion, and Toxicity, ADMET, of drug molecules, which ultimately determines their biosafety and pharmacokinetics profile. Spectroscopic studies such as fluorescence quenching have successfully been utilized to determine the association constants of drug with HSA. However, these techniques are costly, time consuming, and, fundamentally, they lack the capacity of providing a mechanistic interpretation of the interaction at the molecular level.

In recent years, the meteoric rise in computing power has been harnessed trying to streamline the drug discovery and development process by means of significantly reducing the need of chemical and biological resources, and by filtering or eliminating molecules with undesirable ADMET properties to select the most promising candidates [24].

The availability of the X-ray crystal structure of HSA (PDB ID 2BXH) [15] allowed us to develop a computer protocol in an attempt to characterize, at the molecular level, the interactions of drugs with this protein. Although particularly this X-crystal structure of HSA deposited in the Protein Data Bank possessed a relatively high resolution (2.25 Å), it is very common that atoms, side chains, or even whole residues are not included in the protein structure.

Furthermore, atomic clashes, overall inconsistencies in the folding pattern, and high



**Fig. 8.** Correlation between predicted affinities with experimentally determined association constants ( $K_a$ ) for drug-humans serum albumin (HSA) interactions. Predicted affinity was estimated by docking drugs into the refined model of HSA using AutoDock Vina

potential energies associated to their geometries are common problems in most X-ray structures. To address these issues, and previous to perform molecular docking simulations, we subjected the X-ray model of HSA to a phase of thorough structural refinement using the most advanced computational techniques (see Methods section). We obtained a highly refined structure that represented the typical geometry of the HSA and which has been assumed to correspond to the physiologically relevant state.

The structure of HSA revealed the presence of three homologous domains (I, II and III), which consequently showed similar topology and 3D architecture. Each of the three domains is further divided into subdomains (A and B). The result of various internal and global geometry evaluations, to which the refined model of HSA was subjected, demonstrated its high geometric consistency and robustness.

The finding that 98.9% of the residues of the refined HSA model were located within most favored areas of the Ramachandran plot suggested excellent stereochemical quality. Also, the outcome of the analysis of non-bonded residue interactions yielded an ERRAT score of 94.2%, which is well above the lower limit (85%) accepted for high-quality structures. Altogether, these tests demonstrated the high structural quality of the refined model of HSA.

Although many studies have successfully conducted docking studies in which the search space spans the whole protein receptor [53, 19, 20], to reduce the computational cost, we restricted the search space the regions of HSA reported to be active in the binding of drugs. In all molecular docking simulations carried out in this study, two search spaces were defined in such a way that they covered completely the regions reported to encompass drug-binding site 1 and drug-binding site 2 [15]. Accordingly, drugs were docked into their reported binding site.

Firstly, we attempted to evaluate if AutoDock Vina predicted correctly the binding position of indoxyl sulfate in the experimental crystal structure of HSA (PDB ID 2BXH). This docking program predicted accurately the mode of binding of indoxyl sulfate within the crystal structure and, interestingly, the lowest energy conformations corresponded to the lowest RMSD values. This result suggested that AutoDock Vina could identify reliably the correct pose of a ligand within a receptor and thus may prove useful in molecular docking studies aimed at the characterization of the mechanism of drug-protein interaction.

Spectroscopy fluorescence quenching studies have reported the affinity of a series of drugs towards human serum albumin in terms of their association constants ( $K_a$ ) [51, 52]. To investigate if there is a correlation between predicted affinities (free energy of binding) computationally calculated and experimentally determined  $K_a$  values, a set of 20 drugs were docked into the refined model of HSA.

Our computational studies showed that AutoDock Vina possessed a high capacity to correlate predicted affinities with *in vitro* experimentally calculated association constants. Interestingly, this capacity augmented as the “exhaustiveness” parameter was increased (data not shown). The correlation coefficient reached a maximum of 0.61 when the “exhaustiveness” was set to 100. Similar studies (Table 3), including QSAR (quantitative structure-activity relationships) and protein–protein-docking, have suggested that a coefficient correlation of 0.6, or better, ensures structural and functional validity of models.

The promiscuity of HSA to bind a wide range of drugs is a crucial feature of its biological function. The interaction between small molecules and

**Table 3.** The correlation coefficient values for experimental and predicted binding affinities using *in silico* tools, including molecular docking and protein–protein docking techniques

In silico study reference	Coefficient of correlation
[2]	0.67
[40]	0.72
[9]	0.48
[25]	0.61
[46]	0.42
[50]	0.56

proteins is usually characterized by the value of free energy of binding [26, 2]. The free energy is the thermodynamic quantity directly related to the experimentally measurable value of affinity constant (e.g.  $K_a$ ). Therefore, these results suggested that predicted affinity by AutoDock Vina could be considered as an index of the experimental binding affinity (*i.e.* strength of non-covalent drug-HSA interaction).

Our findings showed consistency between computationally predicted molecular interactions with *in vitro* reported data, thereby confirming the stereochemical robustness, functional validity of the model and a reliable molecular docking protocol. Therefore, our computational protocol may be useful in the conduct of molecular modelling studies and to gain a better understanding of the molecular mechanism of interaction of HSA with drugs.

## 5 Conclusions

Despite the enormous advancements in the development of new drugs, bringing a new drug product to the market is still extremely expensive and time consuming. Interactions of drugs and drug candidates with human serum albumin have attracted great interest in the biomedical sciences. The nature and magnitude of these interactions are key determinants to the safety and efficacy of drugs and therefore they should be considered in

the design process. Although *in vitro* protocols incorporating spectroscopic methods have historically been used to evaluate such interactions, they are economically demanding and also time intensive. Moreover, importantly, spectroscopic methods frequently lack the atomic resolution needed to provide a rational and mechanistic interpretation to the interactions at the molecular level. Therefore, there still remains a pressing need to incorporate new technologies to speed up the evaluation of drug-human serum albumin interactions, and thus to potentially streamline the drug discovery process.

The computer-based method described here incorporated a molecular docking approach to investigate structural aspects of the interactions of human serum albumin with a diverse set of drugs. Using state-of-the-art computational techniques, an exhaustive refinement protocol was applied to obtain a high quality, three-dimensional structure of human serum albumin. The refined structure was used to study drug-human serum albumin interactions using the docking program AutoDock Vina. Our docking protocol, in re-docking experiments, was capable of reproducing accurately the positioning of a co-crystallized ligand within the binding site of the X-crystal structure of human serum albumin. In addition, our protocol was capable of correlating, with high accuracy, predicted affinities (free energy of binding) with experimentally determined association constants.

We believe based on this study, that our computational-based approach incorporating AutoDock Vina may prove useful in understanding the interactions of human serum albumin with drugs. This may contribute to streamline the drug development process.

## Acknowledgements

This work has been funded by *Cátedras CONACYT, proyecto 253*. The authors gratefully acknowledge the computing time granted by LANCAD and CONACYT on the supercomputer Miztli at DGTIC UNAM. Authors also thank the Laboratorio Nacional de Supercómputo del Sureste de México for the computing resources and technical assistance (project 201801055N).

## References

1. **Agarwal, R., Singh, A., & Sen, S. (2008).** Role of Molecular Docking in Computer-Aided Drug Design and Development. *Pharmaceutical Sciences*, pp. 683–710. DOI: 10.4018/978-1-5225-0362-0.ch001.
2. **Badhan, R. & Penny, J. (2006).** In silico modelling of the interaction of flavonoids with human P-glycoprotein nucleotide-binding domain. *European Journal of Medicinal Chemistry*, Vol. 41, No. 3, pp. 285–295. DOI: 10.1016/j.ejmech.2005.11.012.
3. **Berman, H. M., Westbrook, J., Feng, Z., Gilliland G., Bhat, T. N., Weissig, H., Shindyalov, I. N., & Bourne, P. E. (2000).** The Protein Data Bank. *Nucleic Acids Research*, Vol. 28, No. 1, pp. 235–242. DOI: 10.1093/nar/28.1.235.
4. **Bhattacharya, A. A., Curry, S., & Franks, N. P. (2000).** Binding of the General Anesthetics Propofol and Halothane to Human Serum Albumin. *Journal of Biological Chemistry*, Vol. 275, pp. 38731–38738. DOI: 10.1074/jbc.M005460200.
5. **Bi, S., Sun, Y., Qiao, C., Zhang, H., & Liu, C. (2009).** Binding of several anti-tumor drugs to bovine serum albumin: Fluorescence study. *Journal of Luminescence*, Vol. 129, No. 5, pp. 541–547. DOI: 10.1016/j.jlumin.2008.12.010.
6. **Bursulaya, B. D., Totrov, M., Abagyan, R., & Brooks, C. L. (2003).** Comparative study of several algorithms for flexible ligand docking. *Journal of Computer-Aided Molecular Design*, Vol. 17, No. 11, pp 755–763. DOI: 10.1023/B:JCAM.0000017496.76572.6f.
7. **Chang, M. W., Ayeni, C., Breuer, S., & Torbett, B. E. (2010).** Virtual screening for HIV protease inhibitors: a comparison of AutoDock 4 and Vina. *PLoS One*, Vol. 5, No. e11955. DOI: 10.1371/journal.pone.0011955.
8. **Chen, H., Lyne, P. D., Giordanetto, F., Lovell, T., & Li, J. (2006).** On evaluating molecular-docking methods for pose prediction and enrichment factors. *Journal of Chemical Information and Modeling*, Vol. 46, No. 1, pp. 401–415. DOI: 10.1021/ci0503255.
9. **Choi, J. M., Serohijos, A. W. R., Murphy, S., Lucarelli, D., Lofranco, L. L., Feldman, A., & Shakhnovich, E. I. (2015).** Minimalistic predictor of protein binding energy: contribution of solvation factor to protein binding. *Biophysical Journal*, Vol. 108, No. 4, pp. 795–798. DOI: 10.1016/j.bpj.2015.01.001.
10. **Colovos, C. & Yeates, T. O. (1993).** Verification of protein structures: Patterns of nonbonded atomic interactions. *Protein Science*, Vol. 2, No. 9, pp. 1511–1519. DOI: 10.1002/pro.5560020916.

11. Dolinsky, T. J., Nielsen, J. E., McCammon, J. A., & Baker, N. A. (2004). PDB2PQR: an automated pipeline for the setup of Poisson-Boltzmann electrostatics calculations. *Nucleic Acids Research*, Vol. 32, No. 2, pp. W665–W667. DOI: 10.1093/nar/gkh381.
12. Ehrlich, P. (1909). *Experimental researches on specific therapeutics*, by Prof. Paul Ehrlich. New.
13. Epps, D. E., Raub, T. J., Caiolfa, V., Chiari, A., & Zamai, M. (1999). Determination of the affinity of drugs toward serum albumin by measurement of the quenching of the intrinsic tryptophan fluorescence of the protein. *Journal of Pharmacy and Pharmacology*, Vol. 51, No. 1, pp. 41–48. DOI: 10.1211/0022357991772079.
14. Fischer, E. (1894). Ueber den Amidoacetaldehyd III. *Berichte der Deutschen Chemischen Gesellschaft*, pp. 210–217. DOI: 10.1007/978-3-642-51364-0\_42.
15. Ghuman, J., Zunszain, P. A., Petitpas, I., Bhattacharya, A. A., Otagiri, M., & Curry, S. (2005). Structural basis of the drug-binding specificity of human serum albumin. *Journal of Molecular Biology*, Vol. 353, No. 1, pp. 38–52. DOI: 10.1016/j.jmb.2005.07.075.
16. Guha, R., Howard, M. T., Hutchison, G. R., Murray-Rustll, P., Rzepa, H., Steinbeck, C., Wegner, J., & Willighagen, E. L. (2006). The Blue Obelisk-interoperability in chemical informatics. *Journal of Chemical Information and Modeling*, Vol. 46, No. 3, pp. 991–998. DOI: 10.1021/ci050400b.
17. He, X. M. & Carter, D. C. (1993). Atomic structure and chemistry of human serum albumin. *Nature*, Vol. 364, pp. 362–362. DOI: 10.1038/358209a0.
18. Hess, B., Kutzner, C., van der Spoel, D., & Lindahl, E. (2008). GROMACS 4: Algorithms for Highly Efficient, Load-Balanced, and Scalable Molecular Simulation. *Journal of Chemical Theory and Computation*, Vol. 4, No. 3, pp. 435–447. DOI: 10.1021/ct700301q.
19. Hetényi, C. & van der Spoel, D. (2006). Blind docking of drug-sized compounds to proteins with up to a thousand residues. *FEBS Letters*, Vol. 580, No. 5, pp. 1447–1450. DOI: 10.1016/j.febslet.2006.01.074.
20. Hetényi, C. & van der Spoel, D. (2011). Toward prediction of functional protein pockets using blind docking and pocket search algorithms. *Protein Science*, Vol. 20, No. 5, pp. 880–893. DOI: 10.1002/pro.618.
21. Jaghoori, M. M., Bleijlevens, B., & Olabariaga, S. D. (2016). 1001 Ways to run AutoDock Vina for virtual screening. *Journal of Computer-Aided Molecular Design*, Vol. 30, No. 3, pp. 237–249. DOI: 10.1007/s10822-016-9900-9.
22. Johansson, J. S. (1997). Binding of the volatile anesthetic chloroform to albumin demonstrated using tryptophan fluorescence quenching. *Journal of Biological Chemistry*, Vol. 272, pp. 17961–17965. DOI: 10.1074/jbc.272.29.17961.
23. Jusko, W. J. & Gretch, M. (1976). Plasma and Tissue Protein Binding of Drugs in Pharmacokinetics. *Drug Metabolism Reviews*, Vol. 5, pp. 43–140.
24. Kapetanovic, I. M. (2008). Computer-aided drug discovery and development (CADD): in silico-chemico-biological approach. *Chemico-Biological Interactions*, Vol. 171, No. 2, pp. 165–176. DOI: 10.1016/j.cbi.2006.12.006
25. Kemp, C. A., Flanagan, J. U., van Eldik, A. J., Maréchal, J. D., Wolf, C. R., Roberts, G. C. K., Paine, M. J. I., & Sutcliffe, M. J. (2004). Validation of Model of Cytochrome P450 2D6: An in Silico Tool for Predicting Metabolism and Inhibition. *Journal of Medicinal Chemistry*, Vol. 47, No. 22, pp. 5340–5346. DOI: 10.1021/jm049934e.
26. Koch-Weser, J. & Sellers, E. M. (1976). Binding of Drugs to Serum Albumin. *The New England Journal of Medicine*, Vol. 294, pp. 311–316.
27. Krivov, G. G., Shapovalov, M. V., & Dunbrack, R. L. (2009). Improved prediction of protein side-chain conformations with SCWRL4. *Proteins*, Vol. 77, No. 4, pp. 778–795. DOI: 10.1002/prot.22488.
28. Kumar, A. & Jha, A. (2017). Drug Development Strategies. *Anticandidal Agents*, pp. 63–71.
29. Li, H., Robertson, A. D., & Jensen, J. H. (2005). Very fast empirical prediction and rationalization of protein pKa values. *Proteins: Structure, Function, and Bioinformatics*, Vol. 61, No. 4, pp. 704–721. DOI: 10.1002/prot.20660.
30. Lin, J. H. (2007). Transporter-Mediated Drug Interactions: Molecular Mechanisms and Clinical Implications. *Drug-Drug Interactions in Pharmaceutical Development*, pp.159–194.
31. Mao, H., Hajduk, P. J., Randy, B., Craig, R., Borre, T., & Fesik, S. W. (2001). Rational design of diflunisal analogues with reduced affinity for human serum albumin. *Journal of the American Chemical Society*, Vol. 123, No. 43, pp. 10429–10435. DOI: 10.1021/ja015955b.
32. Moore, G. E. (1998). Cramming More Components Onto Integrated Circuits. *Proceedings of the IEEE*, Vol. 86, pp. 56–66.
33. Morris, G. M., Huey, R., Lindstrom, W., Sanner, M. F., Belew, R. K., Goodsell, D. S., & Olson, A. J. (2009). AutoDock4 and AutoDockTools4:

- Automated docking with selective receptor flexibility. *Journal of Computational Chemistry*, Vol. 30, No. 16, pp. 2785–2791. DOI: 10.1002/jcc.21256.
34. **Mote, U. S., Bhattar, S. L., Patil, S. R., & Kolekar, G. B. (2010).** Interaction between felodipine and bovine serum albumin: fluorescence quenching study. *Luminescence*, Vol. 25, pp. 1–8. DOI: 10.1002/bio.1130.
  35. **Mullard, A. (2014).** New drugs cost US\$2.6 billion to develop. *Nature Reviews Drug Discovery*, Vol. 13, pp. 877–877. DOI: 10.1038/nrd4507.
  36. **Naik, P. N., Chimatadar, S. A., & Nandibewoor, S. T. (2010).** Interaction between a potent corticosteroid drug – Dexamethasone with bovine serum albumin and human serum albumin: A fluorescence quenching and Fourier transformation infrared spectroscopy study. *Journal of Photochemistry and Photobiology B*, Vol. 100, No. 3, pp. 147–159. DOI: 10.1016/j.jphotobiol.2010.05.014.
  37. **Nocedal, J. & Wright, S. (2006).** *Numerical Optimization*. Springer Science & Business Media.
  38. **Ouellette, R. G. & Joyce, J. A. (2010).** Pharmacology for Nurse Anesthesiology. *Jones & Bartlett Publishers*.
  39. **Ramachandran, G. N., Ramakrishnan, C., & Sasisekharan, V. (1963).** Stereochemistry of polypeptide chain configurations. *Journal of Molecular Biology*, Vol. 7, pp. 95–99.
  40. **Sapundzhi, F., Dzimbova, T., Pencheva, N., & Milanov, P. (2015).** Determination of the relationship between the docking studies and the biological activity of  $\delta$ -selective enkephalin analogues. *Journal of Computational Methods in Molecular Design*, Vol. 5, No. 2, pp. 98–108.
  41. **Smith, D. A., Di, L., & Kerns, E. H. (2010).** The effect of plasma protein binding on in vivo efficacy: misconceptions in drug discovery. *Nature Reviews Drug Discovery*, Vol. 9, pp. 929–939.
  42. **Steiner, R. F., Roth, J., & Robbins, J. (1966).** The binding of thyroxine by serum albumin as measured by fluorescence quenching. *Journal of Biological Chemistry*, Vol. 241, pp. 560–567.
  43. **Steinkellner, G., Rader, R., Thallinger, G. G., Kratky, C., & Gruber, K. (2009).** VASCo: computation and visualization of annotated protein surface contacts. *BMC Bioinformatics*, Vol. 10, No. 32. DOI: 10.1186/1471-2105-10-32.
  44. **Sudlow, G., Birkett, D. J., & Wade, D. N. (1978).** The characterization of two specific drug binding sites on human serum albumin. *Molecular Pharmacology*, Vol. 11, pp. 824–832.
  45. **Trott, O. & Olson, A. J. (2009).** AutoDock Vina: Improving the speed and accuracy of docking with a new scoring function, efficient optimization, and multithreading. *Journal of Computational Chemistry*. Vol. 31, No. 2, pp. 455–461. DOI: 10.1002/jcc.21334.
  46. **Viswanath, S., Ravikant, D. V. S., & Elber, R. (2013).** Improving ranking of models for protein complexes with side chain modeling and atomic potentials. *Proteins*, Vol. 81, No. 4, pp. 592–606. DOI: 10.1002/prot.24214.
  47. **Wang, C., Wu, Q. H., Wang, Z., & Zhao, J. (2006).** Study of the interaction of carbamazepine with bovine serum albumin by fluorescence quenching method. *Analytical Sciences*, Vol. 22, No. 3, pp. 435–438. DOI: 10.2116/analsci.22.435.
  48. **Williams, C. J., Headd, J. J., Moriarty, N. W., Prisant, M. G., Videau, L. L., Deis, L. N., Verma, V., Keedy, D. A., Hintze, B. J., & Chen, V. B. (2018).** MolProbity: More and better reference data for improved all-atom structure validation. *Protein Science*, Vol. 27, No. 1, pp. 293–315. DOI: 10.1002/pro.3330.
  49. **Word, J. M., Lovell, S. C., Richardson, J. S., & Richardson, D. C. (1999).** Asparagine and glutamine: using hydrogen atom contacts in the choice of side-chain amide orientation. *Journal of Molecular Biology*, Vol. 285, No. 4, pp. 1735–1747. DOI: 10.1006/jmbi.1998.2401.
  50. **Xu, Y., Liu, H., Niu, C., Luo, C., Luo, X., Shen, J., Chen, K., Jiang, H. (2004).** Molecular docking and 3D QSAR studies on 1-amino-2-phenyl-4-(piperidin-1-yl)-butanes based on the structural modeling of human CCR5 receptor. *Bioorganic & Medicinal Chemistry*, Vol. 12, No. 23, pp. 6193–6208. DOI: 10.1016/j.bmc.2004.08.045.
  51. **Yamasaki, K., Chuang, V. T. G., Maruyama, T., & Otagiri, M. (2013).** Albumin–drug interaction and its clinical implication. *Biochimica et Biophysica Acta (BBA)*, Vol. 1830, No. 12, pp. 5435–5443. DOI: 10.1016/j.bbagen.2013.05.005.
  52. **Yang, F., Zhang, Y., & Liang, H. (2014).** Interactive association of drugs binding to human serum albumin. *International Journal of Molecular Sciences*, Vol. 15, No. 3, pp. 3580–3595; DOI:10.3390/ijms15033580.
  53. **Zhou, M., Luo, H., Li, R., & Ding, Z. (2013).** Exploring the binding mode of HIV-1 Vif inhibitors by blind docking, molecular dynamics and MM/GBSA. *RSC Advances*, Vol. 3, No. 22532.

Article received on 15/05/2018; accepted on 17/07/2018.  
Corresponding author is Sergio Mares-Sámamo.