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Molecular dynamics simulation and docking studies on the binding properties of several anticancer drugs to human serum albumin

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A B S T R A C T

Disposition and transportation of anticancer drugs by human serum albumin (HSA) affects their bioavailability, distribution and elimination. In this study, the interaction of a set of anticancer drugs with HSA was investigated by molecular dynamics and molecular docking simulations. The drugs' activities were analyzed according to their docking scores, binding sites and structural descriptors. The results displayed the ability of cavity 1, located in the cleft between domains I and III, to potentiate as the principal binding site of all tested drugs. This cavity provides a large space without any effective steric hindrance and induces the stability of the drugs in their binding sites by short and long ranged interactions with the accessible residues. Yet, specific structural features may lead some drug configurations to advance stronger interactions with cavities other than cavity 1. Also, the small volume and position of some cavities i.e. cavities 3, 5-10 involve penetration, small molecular volume and specific geometry which consequently force most drugs out of the corresponding binding sites. Therefore, the steric factor seems to play the most important role in the transportation of drugs by HSA.

Key words: Anticancer drugs; Human serum albumin; Molecular dynamics simulation; Molecular docking; Cavities.

INTRODUCTION

Plasma proteins play an important role in the transportation and deposition of substances such as fatty acids, hormones and medicinal drugs in the circulatory system. Therefore, it is important to reveal the interaction between drugs and proteins in the bloodstream, as it may affect the bioavailability, distribution and elimination of pharmaceutical or nutraceutical active compounds. Albumin is the main plasma protein, and its main function is to regulate colloidal osmotic pressure and transport substances in the bloodstream [1]. The interaction of human serum albumin with a wide range of chemically synthesized drugs used in medicine may influence their

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bioavailability and effectiveness. As a result, several studies have recently focused on revealing the molecular details of these interactions [2, 3].

Since the world is expected to see about 20 million cases of various cancers in the next two decades [4], it is essential to study the specific interaction of anticancer drugs with Human Serum Albumin (HSA). As a major drug carrier of the blood stream, HSA may aid in the selective delivery of drugs to a tumor region and, as suggested for the lipoproteins, may facilitate drug access into the cell via receptor mechanisms. On the other hand, the same carrier may cause a decrease in the amount of drug available for the receptor, by its rapid removal from the circulation. The balance between these two activities might differ from one protein to another and also between different drugs [5]. Therefore, understanding the molecular details of the specific interactions of drugs with HSA helps to formulate safe drugs and effective dosages [6].

It has been shown that HSA has three major domains, each with two subdomains. Major binding sites, namely site I and site II, are located at subdomain IIA and IIIA, respectively [7]. Sudlow et al. [8] indicated that site I of HSA has shown high affinity toward warfarin and site II towards ibuprofen. Later studies indicated that binding of some drugs e.g. digoxigenin to HSA is independent of either site I or site II [9], and display high affinity toward site III [10].

In this study, the binding properties of a set of anticancer drugs to HSA was investigated by means of Molecular Dynamics Simulation (MDS) and molecular docking to find out whether any particular property resulted in the interaction with a specific binding site on HSA.

MATERIALS AND METHODS

Data set: The anticancer drugs, retrieved from divert studies on cancer and used in this study are listed in Table 1 [11-15]. The chemical structure of these drugs was constructed by Hyperchem Professional package (Ver. 7.0) [16]. Energy minimizations of the compounds were performed by AM1, semi empirical method, using the Polak-Ribiere algorithm until the root-mean-square gradient of 0.01 kcal mol^{-1}. The resulted geometries were used in the docking study.

Molecular dynamics simulation: MDS was performed by GROMACS 4.0.5 package [17-19] only on chain A of HSA, PDB entry code: 2BXD [20], while the initial structure consisted of two identical chains in combination with warfarin molecules. In the first step, the topology and interaction parameters were generated using the GROMOS96 43a1 force field [21], with the intermolecular potential represented as a sum of Lennard-Jones (LJ) force and pairwise Coulomb interaction. Long-range electrostatic force was also determined by the particle mesh Ewald (PME) method [22-23]. Initial atomic velocities were created on the basis of Maxwellian distribution at the absolute temperature of 310 K [24-25]. Numerical integrations were calculated by the velocity Verlet algorithm [26]. In the next step, the protein was inserted in a cubic water box of 65264 extended simple point charge (SPC) water molecules [27], and the system was then neutralized by the addition of fourteen sodium ions. Energy minimization was performed by 60 ps of the steepest descent method on the system with a cutoff of 7 A° for Van der Waals and Coulomb forces. After this step, the protein and counter ions were fixed and position-restrained for 20 ps to relax the protein. In the last step, the full system was subjected to a 10 ns MD simulation. The number of molecules, pressure and temperature (NPT ensemble) were constant during simulations [28], serving the Berendsen thermostat [29] with coupled pressure at 1 bar. Also, the periodic boundary condition and integration of motion equations were carried out by

the leap-frog algorithm [30] with a time step of 2 fs. The atom coordinates were recorded every 1 ps during the simulation for later analysis.

Molecular docking simulation: The molecular docking of the drug into the quasi equilibrated HSA was established by Molegro Virtual Docker (MVD) software [31]. The energy minimized anticancer drugs and the simulated HSA were imported to the MVD workspace. The structures were refined by assigning the bonds, bond orders, charge and hybridization, creating explicit hydrogen if the software recognized missing data. Flexible torsions of the ligand and HSA were also detected in the initial stage. The potent binding sites with expanded Van der Waals surfaces known as cavities were nominated to extend the grids over the probable binding sites (see Fig. 1). The details of the cavities are represented in Table 2. At a grid resolution of 0.30 A°, the MolDock scoring functions were adjusted as to give 30 final poses. Each pose suggests the best binding conformation, energy and binding site of the drug into HSA in a cycle of runs. All the 30 best poses of each drug were further analyzed.

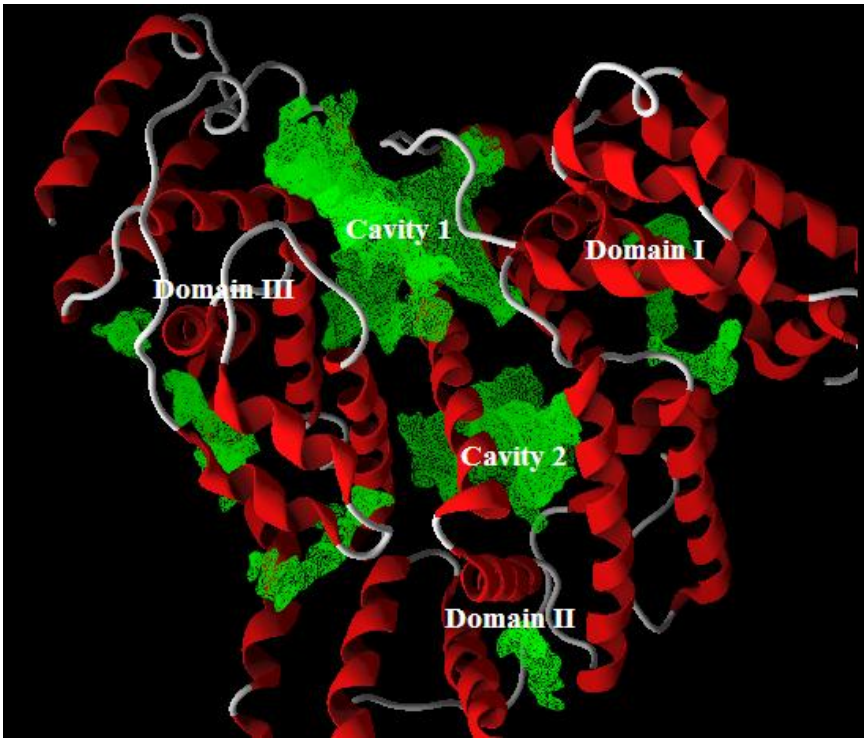

Figure 1: Domains and cavities of HSA

Drug descriptors: Molecular weight (MW, $g.mol^{-1}$), maximum electro-topological variation of negative (MAXDN) and positive charge (MAXDP), spherosity (SPH), polar surface area (PSA) and Moriguchi octanol-water partition coefficient (MLOGP) descriptors of the anticancer drugs were calculated by Dragon software [32]. Molecular volume (MV, cm^3), number of hydrogen bond donors (HBD), acceptors (HBA) and rotatable bond (RB) were also obtained by I-Lab 2.0 server [33]. The results are presented in Table 1.

Table 1: Molecular descriptors of the anticancer drugs and the docking scores

Number	Drug Name	MW	MAXDN	MAXDP	SPH	PSA	MLOGP	MV	HBD	HBA	RB	MDSc	HB
1	Amsacrine	393.5	4.282	4.495	0.98	64.11	2.413	281.4	2	6	5	-132.67	-2.98
2	Anastrozole	293.41	1.862	3.403	0.76	51.99	1.171	270.2	0	5	6	-133.57	0
3	Bicalutamide	430.41	6.111	5.338	0.69	83.38	2.334	282.7	2	6	7	-159.53	-7.86
4	Busulfan	246.34	4.315	3.474	0.9	103.5	-0.5	182.3	0	6	7	-95.883	-4.71
5	Capecitabine	345.37	2.716	6.019	0.9	68.2	0.217	225.3	3	9	7	-138.89	-9.77
6	Cimetidine	252.39	1.163	2.392	0.88	77.24	0.821	198.2	3	6	8	-123.15	-4.07
7	Clodronic acid	244.89	6.028	3.238	0.46	53.76	-0.742	106.1	4	6	2	-70.075	-55.9
8	Clofarabine	303.71	3.07	6.069	0.7	38.36	-1.065	143	4	8	2	-125.36	-6.72
9	Dacarbazine	182.22	2.297	3.752	0.96	60.82	-2.87	122.6	3	7	3	-105.83	-3.69
10	Dasatinib	488.07	1.933	5.693	0.94	76.51	1.108	346.4	3	9	7	-166.4	-7.95
11	Daunorubicin	527.57	3.503	6.663	0.74	78.9	-0.218	339.4	6	11	4	-125.07	-3.3
12	Dexamethasone	392.51	3.23	8.859	0.61	34.14	2.247	296.2	3	5	2	-100.95	-62.9
13	Diethylstilbestrol	268.38	1.383	3.411	0.96	0	3.956	242.2	2	2	4	-110.86	-3.75
14	Doxorubicin	543.57	3.491	6.643	0.72	78.9	-0.935	336.6	7	12	5	-137.12	-5.54
15	Estramustine	440.45	2.062	5.442	0.91	29.54	5.452	351.3	1	4	6	-129.93	-3.04
16	Etoposide	588.61	2.77	6.385	0.75	100.1	0.133	378.5	3	13	5	-188.23	-8.45
17	Exemestane	296.44	1.58	5.399	0.6	34.14	4.042	666.9	0	2	0	-105.27	-1.1
18	Fludarabine	365.25	5.581	5.342	0.81	74.47	-1.506	152.5	6	12	4	-156.6	-12.3
19	Flutamide	276.24	6.11	4.668	0.86	51.21	3.074	-	1	5	4	-83.858	-4.55
20	Gefitinib	446.95	2.17	5.815	0.83	55.65	2.095	337.7	1	7	8	-119.53	-7.95
21	Hydroxycarbamide	76.07	2.606	2.229	1	17.07	-1.859	52.1	4	4	0	-51.412	-4.03
22	Megestrol	328.49	2.35	5.062	0.64	34.14	3.515	279.4	1	3	1	-108.35	0
23	Paclitaxel	860.05	3.609	8.502	0.68	148.6	2.918	630.5	4	15	14	-23.544	-1.79
24	Pamidronic acid	219.09	5.563	3.515	0.71	53.76	-1.501	120.4	6	7	4	-81.684	-6.09
25	Pemetrexed	427.46	2.962	5.29	0.74	96.43	0.685	268	7	11	9	-165.4	-2.84
26	Raltitrexed	462.58	2.91	5.536	0.9	109.2	0.831	306.1	4	10	9	-152.41	-7.42
27	Sunitinib	398.53	2.071	5.703	0.96	68.96	2.105	324	3	6	7	-142.36	-4.57
28	Toremifene	406	0.924	2.371	0.97	12.47	5.402	367.6	0	2	9	-123.96	-1.91
29	Tretinoin	300.48	2.579	3.534	0.93	17.07	4.772	297.1	1	2	5	-124.86	-3.83
30	Vincristine	825.06	3.797	8.268	0.41	130.7	2.592	286.8	3	14	10	-107.34	-1.74

RESULTS AND DISCUSSION

Molecular dynamic simulation: In order to obtain the structure of HSA in a cell-like condition, the protein was subjected to 10 ns MDS in a neutralized water box at 1 bar pressure and 310 K. The root mean square deviation (RMSD) evolution of the HSA structure with respect to the initial one was plotted versus time to determine the time in which the system reaches its equilibrium state. As shown in Fig. 2, HSA has endured an oscillating value of RMSD around the constant average of 5.4 nm from 6 to 10 ns. It can be stated that the protein structure is in a quasi-

equilibrium state and the final coordinate can efficiently resemble its real conformation in the body. Therefore, the final structure was proposed to the docking study (Fig. 2).

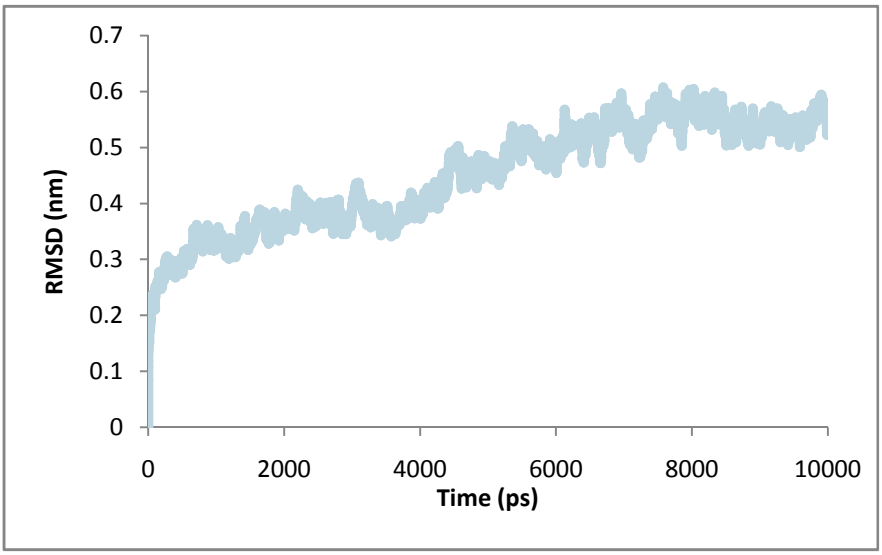

Figure 2: Backbone RMSD evolution of HSA in time.

Molecular docking simulation: Docking studies provide remarkable information on binding sites of drugs, estimating the binding energies of each drug conformation with corresponding scores and functions. In this study, 30 different drug configurations on HSA were clustered for each drug and the MolDock score (MDSc) and hydrogen bonding (HB) for the best drug-HSA complex is reported in Table 1. Their distribution in various cavities is then reported in Table 2. In this table, cavities are determined by their volume (cm^3), constituting residues, and number of negatively charged (NC), positively charged (PC), non-polar uncharged (NPU), polar uncharged (PU) and hydrophobic (HPO) amino acids. If the cavity includes the dominant binding site of the drug poses, the drug's name is shown in bold. If it is not the dominant site but advances the best drug-HSA interaction, the it is shown in italics.

Based on the docking results and the binding sites presented in Table 2, all drugs can bind to more than one site. This is in agreement with the findings of several studies that have demonstrated HSA as a protein capable of binding many ligands in different binding sites [34-37].

All anticancer drugs mostly tend to bind to cavity 1, exceptions being Bicalutamide and Tretinoin. Bicalutamide binds to both cavities 1 and 2, equally. The noticeable value of MAXDN and MAXDP for Bicalutamide (Table 1) in comparison with the other drugs helps it to distribute the partial charges on the structure to overlay on the divert types of residues in both cavities. Comparative high values of SPH and MLOGP for Tretinoin lead it to bind to cavity 2. The long alkyl chain of this drug can relax upon the uncharged and hydrophobic residues of cavity 2, while the oxygen atoms interact easily with the charged amino acids to stabilize the compound with an MDSc value of -124.86.

Several configurations of Anastrozole demonstrate high affinity binding toward cavity 2. The existence of C≡N bonds in the chemical structure of this drug would cause it to prefer interacting with cavity 2. Busulfan structure has two identical tails of $OS(=O)_2CH_3$ connected with an alkyl

chain. Its slightly negative MLOGP verifies the tendency to interact with poor hydrophilic sites, while its large polar surface area (=104) states that the cavity should sufficiently supply polar uncharged and charged residues for the interaction. These characteristics can be found in the nature of cavity 4. However, cavity 1 is also able to display such properties with a larger space. That is why most Busulfan configurations interact with cavity 1.

The existence of considerable numbers of HBD, HBA and RB, doublet and triplet bonds, oxygen, nitrogen and sulfur atoms, which are common properties of these anticancer drugs, cause them to possess hydrogen bondings in addition to spontaneously binding to HSA. Moreover, Paclitaxel, Hydroxycarbamide and Clodronic acid bind poorly to HSA as indicated by a low MDSc. While Paclitaxel has 14 rotatable bonds and 19 hydrogen bond donors and acceptors, the steric factor prevents its large molecular volume to interact effectively. Hydroxycarbamide and Clodronic acid are also small compounds with free vibrating arms that limit binding opportunities.

Table 2: HSA cavities and preferred binding sites of the anticancer drugs

Drugs	Cavity	Site	Volume (cm^3)	Residues	NC	PC	NPU	PU	HPO
1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30	1	A large space between domains I and III	866.304	107-117, 137-148, 184-194, 419-430, 448-462, 520-530	10	12	13	12	23
***2*, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 21, 22, 24, 25, 26, 27, 29, 30**	2	Between domains I, II and III but closer to II	573.952	147-158, 186-194, 234-242, 288-294, 445-451	8	6	11	5	14
-	3	Core of domain I	104.448	17-21, 130-138	2	3	3	1	6
3, 4, 6, 9, 13, 18, 19, 21, 24	4	Between domains II and III, collapsed on III	64	343-354, 446-453, 483-486	3	4	6	1	8
-	5	Outer surface of domain III	51.712	385-390, 484-490	0	3	4	3	3
-	6	Outer surface of domain III (above cavity 5)	36.864	386-392, 400-409, 487-490	1	2	3	7	6
-	7	Domain II (close to surface)	35.84	323-328, 425-428	2	1	2	0	4
-	8	Outer surface of domain III (above cavity 6)	32.256	404-410, 539-544	1	2	3	3	4
-	9	Inner surface of domain III	27.648	420-431, 518-522	3	3	2	4	4
-	10	Domain I (close to outer surface)	27.136	8-23	3	4	2	1	6

The key question is, what leads the drugs to preferring binding sites located in cavity 1? The diversity of descriptor values of the drugs is a limiting factor towards finding a certain relationship between the properties and observations. However cavity 1 with a volume of 866.304 cm3 and divert types of residues can potentially transport all drugs with any specific volume, geometry and moieties. Steric hindrance is too low in cavity 1 and short and long ranges of interaction with residues of domains I and III can significantly promote molecular interactions in order to stabilize any compound in its binding site.

Also, the short volume of cavities 3, and 5-10 as well as their location, limit the drugs to penetrate through. Consequently, most of the drugs are not able to reach these cavities, in spite of their small size, interfering with negligible geometrical hindrances.

In the present study, the binding of a set of anticancer drugs to human serum albumin has been studied by a combination of molecular dynamic and molecular docking simulations. The structural features and descriptors show that most anticancer drugs possess hydrogen bonding since they contain multiple hydrogen bond donor and acceptor centers. All the drugs tend to bind to cavity 1 which lays in the cleft between domains I and III. The main reason is the large space available in this cleft without any effective steric hindrance and the stability induced by short and long ranged interactions with the accessible residues. Specific structural features may lead some drug configurations to advance stronger interactions with cavities other than cavity 1. Also, the small volume of cavities 3, 5-10 involves penetration, small molecular volume and special geometries which forces out most drugs.

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Conflict of Interest: Author has no financial or any non-financial competing interests.

REFERENCE

1. Li Y, He WY, Liu H, Yao X, Hu Z. Daidzein interaction with human serum albumin studied using optical spectroscopy and molecular modeling methods. J Mol Struc 2007; 831:144-150.
2. Varshney A, Sen P, Ahmad E, Rehan M, Subbarao N, Khan RH. Ligand binding strategies of human serum albumin: How can the cargo be utilized? Chirality 2010;22: 77-87.
3. Falé PLV, Ascensão L, Serralheiro MLM, Haris PI. Interaction between *Plectranthus barbatus* herbal tea components and human serum albumin and lysozyme: Binding andactivity studies. Spectroscopy 2011;26:79-92.
4. Abu-Surrah AS, Kettunen M. Platinum Group Antitumor Chemistry: Design and development of New Anticancer Drugs Complementary to Cis-platin. Cur Med Chem 2006; 13:1337-1357.
5. Cohen S, Margalit R. Binding of porphyrin to human serum albumin: Structure-activity relationships. J Biochem 1990;270:325-330.
6. Dezhampanah H, Bordbar AK, Farshad S. Thermodynamic characterization of phthalocyanine–human serum albumin interaction. Spectroscopy 2011;25:235-242.

7. He X M, Carter DC. Atomic structure and Chemistry of human serum albumin. Nature 1992; 358:209-215.
8. Sudlow G, Birkett DJ, Wade DN. Further characterization of specific drug binding sites on human serum albumin. Mol Pharmacol 1976;12:1052-1061.
9. Sjoholm I, Ekman B, Kober A, Ljungstedt-Pahlman I, Seiving B, Sjodin T. Binding of drugs to human serum albumin: XI. The specificity of three binding sites as studied with albumin immobilized in microparticles. Mol Pharmacol 1979;16:767-777.
10. Tian J, Liu J, Xie J, Yao X, Hu Z, Chen X. Binding of wogonin to human serum albumin: a common binding site of wogonin in subdomain IIA. J Photochem Photobio B: Bio 2004;74: 39-45.
11. Radin NS. Meta-analysis of anticancer drug structures - significance of their polar allylic moieties, anti-cancer agents in medicinal chemistry. Anti-Cancer Agents 2007;7:209-222.
12. Watanabe H, Ikesue H, Yoshida M, Yamamoto N, Sakamoto RNS, Koga T, Sueyasu M, Egashira N, Inoshima I, Nakanishi Y, Oishi R. Protection against the extravasation of anticancer drugs by standardization of the management system. Hos Pharm 2008;43:571-576.
13. Yi-Lwern Yap K, Kuo EY, Jun Jie Lee J, Chui WK, Chan A. An onco informatics database for anticancer drug interactions with complementary and alternative medicines used in cancer treatment and supportive care: an overview of the OncoRx project. Supp Care in Cancer 2010;18:883-891.
14. Hurria L, Balducci A. Geriatric Oncology, in: B.S. Mohile S, Nagovskiy N, Balducci L (Eds.), Chemotherapy for the older adult with cancer. Springer USA 2009; pp:201-261.
15. Newman DJ, Cragg GM. The discovery of anticancer drugs from natural sources. Nat Products 2005;3:129-168.
16. HyperChem, Release 7.0 for windows, Hypercube, Inc., 2002.
17. Berendsen HJC, van der Spoel D, van Drunen R. GROMACS: a message-passing parallel molecular dynamics implementation. Comput Phys Commun 1995;91:43-56.
18. Lindahl E, Hess B, van der Spoel D. GROMACS 3.0: a package for molecular simulation and trajectory analysis. J Mol Model 2001;7:306-317.
19. van der Spoel D, Lindahl E, Hess B, Groenhof G, Mark AE, Berendsen HJC. GROMACS: fast, flexible and free. J Comput Chem 2005;26:1701-1719.
20. Ghuman, J, Zunszain PA, Petitpas I, Bhattacharya AA, Otagiri M, Curry S. Structural basis of the drug-binding specificity of human serum albumin. J Mol Biol 2005;353:38.
21. van Gunsteren WF, Billeter SR, Eising AA, Huenberger PH, Kruger P, Mark AE, Scott WRP, Tironi IG. Biomolecular simulation: the GROMOS 96 manual and user guide; Switzerland: 1996.
22. Darden T, York D, Pedersen L. Particle mesh Ewald-an NlogN method for Ewald sums in large systems. J Chem Phys 1993;98:10089-10092.
23. Essmann U, Perera L, Berkowitz ML, Darden T, Lee H, Pedersen LG. A smooth particle mesh Ewald method. J Chem Phys 1995;103:8577-8593.
24. Kennard EH, Earle HK. Kinetic theory of gases: with an introduction to statistical mechanics. McGraw-Hill, New York; 1938.
25. Huang K. Statistical mechanics. Wiley, New York; 1963.
26. Swope WC, Andersen HC, Berens PH, Wilson KR. A computer simulation method for the calculation of equilibrium constants for the formation of physical clusters of molecules: application to small water clusters. J Chem Phys 1982;76:637-649.

27. Berendsen HJC, Postma JPM, Van Gunstetren WF, Hermans J. Interaction models for water in relation to protein hydration. In: Pullman B (ed) Intermolecular forces. Reidel, Dordrecht, Netherlands, 1981;331-342.
28. Andersen HC. Molecular dynamics simulations at constant pressure and/or temperature. J Chem Phys 1980;72:2384-2393.
29. Berendsen HJC, Postma JPM, Van Gunsteren WF, DiNola A, Haak JR. Molecular dynamics with coupling to an external bath. J Chem Phys 1984;81:3684-3690.
30. Van Gunsteren WF, Berendsen HJC. A leap-frog algorithm for stochastic dynamics. Mol Sim 1988;1:173-185.
31. Thomsen R, Christensen MH. MolDock: A new technique for high-accuracy molecular docking. J Med Chem 2006;49:3315-3321.
32. www.talete.mi.it
33. Ilab.acdlabs.com/iLab2/
34. He XM, Carter DC. Atomic structure and chemistry of human serum albumin. Nature 1992;358:209-215.
35. Curry S, Mandelkow H, Brick P, Franks N. Crystal structure of human serum albumin complexed with fatty acid reveals an asymmetric distribution of binding sites, Nature Struct Biol 1998;5:827-835.
36. Curry S, Brick P, Franks NP. Fatty acid binding to human serum albumin: new insights from crystallographic studies. Biochim Biophys Acta 1999;1441:131.
37. Petitpas I, Bhattacharya AA, Twine S, East M, Curry S. Crystal structure analysis of warfarin binding to human serum albumin. J Biol Chem 2001;276:22804.