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Melamine Induced Inflammation: Fact or Fiction

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Abstract—*Melamine adulteration of food materials is an ongoing problem and melamine induced nephropathy is gradually getting to be recognized as a global phenomenon. Melamine is also known to cause pathology at multiple organs of the human body. How such problems are caused by melamine is currently not understood but there is some preliminary evidence to conclude that melamine per se can cause inflammation. The possibility of above idea is explored using tools of computational biology. It is observed that melamine interacts with various arachidonic acid binding sites of albumin with significant hydrogen bond energy. It is concluded that in chronic melamine exposure possibly melamine can bind with arachidonic acid binding sites of albumin which is difficult to be replaced by arachidonic acid. This can result in generation of free arachidonic acid resulting in formation of more mediators of inflammation. We have supported for experimental validation of our in-silico results.*

1. INTRODUCTION

Melamine adulteration of human and animal food is an ongoing phenomenon which appears to be beyond control by various nations [1-4]. The recent melamine scandal reported from China resulting in life threat to human beings including infants supports the above fact [5]. The chemical affects multiple systems of the living body and its role in precipitation of nephropathy upon chronic exposure is proved beyond any doubt [6-10]. Melamine exposure is now getting recognized as one of the cause of widespread emergence of nephropathy throughout the globe [11]. Apart from crystal formation with uric acid, cyanuric acid etc., melamine is shown to cause chronic inflammation in kidney [12]. Ogasawara et al. observed infiltration of inflammatory cells in melamine exposure associated urolithiasis, a chronic kidney disease [13]. By using human renal proximal tubular cells (Hk-2 cells) it is proved that melamine activates MAPK and NF-kB in a dose dependent manner which may regulate various inflammatory gene expressions [14]. Using Hk-2 cells it is also established that melamine activates ROS production and induces apoptosis [14]. Recently, it has been observed that melamine activates production of COX-2 and PGE-2 in RAW264.7 (a macrophage like cell line) and HEK293 (a human embryonic kidney cell line) cells [15]. So, in presence of chronic melamine exposure inflammation can be due to melamine per se. Therefore in the present work we have explored the relationship of melamine with arachidonic acid which is the common precursor of many mediators of inflammation (including PGE2) using tools of computational biology.

2. METHODS

The three-dimensional structures of melamine and aspirin have been taken from PubChem [16]. The three dimensional structure of human serum albumin has been taken from Protein Data Bank [17] with PDB ID 1GNJ. In human serum albumin seven active sites are located [18-20] and the residues important for ligand binding are mentioned in Table 1. The ligands, arachidonic acid, myristic acid, propofol, halothane, melamine and aspirin have been fitted at the active sites of human serum albumin by docking tool AutoDock4.2.5 [21]. In this docking, combination of genetic algorithm (GA) for global searching and local searching (LS) methods named as Lamarckian Genetic Algorithm (LGA) is used for searching energy minimised conformations of ligand [22]. In AutoDock, the grid center has to be selected. All the known important residues at the active sites have been chosen as grid center individually and the ligand molecule has been docked. In the docked complex, at the active site, the interaction pattern whether hydrophobic or hydrogen bond, have been checked by visual inspection. For hydrophobic interaction, the shortest contact distance between ligand and residue of human serum albumin is 4.5Å [23]. Hydrogen bond interaction has been identified by PEARL [24]. Different types of energy values like van der Waals, electrostatic, hydrogen bond and binding free energy have been calculated by PEARL [24]. The docked conformation which has maximum number of nonbonded interaction with all the active site residues (mentioned in Table 1) has been taken out. Nonesterified fatty acids are the primary ligands of human serum albumin [19]. So arachidonic and myristic acids have been docked at all the active sites as control study. The complex structures of arachidonic acid - human serum albumin and myristic acid–human serum albumin have been solved by x-ray crystallography (PDB code 1GNJ and 1E7C, respectively). Further, for many drugs like anesthetic drugs, propofol and halothane, human serum

albumin is a transport protein [25]. So as control for drug-albumin complex, propofol and halothane have been docked. X-ray structures of propofol-human serum albumin and halothane-human serum albumin have been considered (PDB code 1E7A and 1E7B). Molecular diagrams have been drawn by pymol [26].

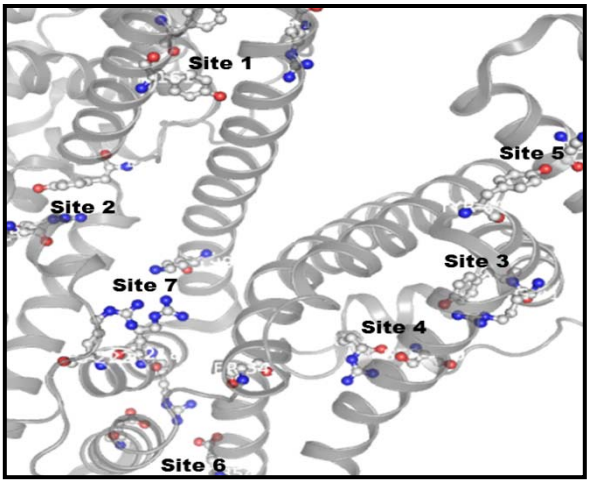

Fig. 1: Cartoon representation of human serum albumin with location of the active sites is shown. Few important residues at these active sites are also mentioned.

3. RESULTS

In human serum albumin, seven active sites are primarily involved in ligand binding [19] and in Fig.1, their location in the protein structure have been represented. X-ray crystallographic structures of arachidonic acid and myristic acid with human serum albumin are known (PDB code 1GNJ and 1E7C). Arachidonic acid has been docked at all the active sites of human serum albumin by following the protocol of Autodock as mentioned in the method section. The hydrogen bonds in the docked complex have been calculated by PEARL bioinformatics tool as well as justified by manual visualization. Shortest nonbonded contacts within 4.5Å between the ligand and active site residues have been identified by visualization and calculating the distance in pymol. The nonbonded interaction pattern in the docked complex has been observed to be similar to the x-ray complex structure of arachidonic acid–human serum albumin complex. In Table 1, the residues of human serum albumin which are in contact with different ligands are mentioned. Propofol and halothane ligands have also been docked at all the active sites of human serum albumin in similar way and nonbonded interaction patterns have been compared with x-ray structures of corresponding complexes. Also in these cases, the nonbonded interaction pattern of docked and x-ray structures have been found to be same. Matching of nonbonded interaction pattern of docked complex with x-ray structures implies the validity of the docking protocol. So in similar way, melamine and aspirin have been docked at the active sites of human serum albumin. In Table 1, the nonbonded distances of active site residues with ligand atoms have been mentioned. It is established that arachidonic acid binds at all the active sites [20] while propofol and halothane bind at specific active sites of human serum albumin [25]. From experimental studies it is known that propofol mainly binds at site 3 and 5 while halothane binds at site 6 and 7. In docking, it has been found that at these mentioned active sites propofol is bound. In Fig 2a, a binding mode of arachidonic acid at active site 5 has been shown. Myristic acid has been observed to bind at active sites 1,2,3,4 and 5 in docked structure as similar in x-ray structure [25]. In docked structure, melamine has been observed to interact at active sites 2,3,5 and 6. Binding mode of melamine at active site 5 has been shown in Fig. 2b. At active sites 1,2,3,4 and 6 aspirin has been shown to interact and in Fig. 3(a,b) the binding mode of arachidonic acid and aspirin at active site 1 are represented.

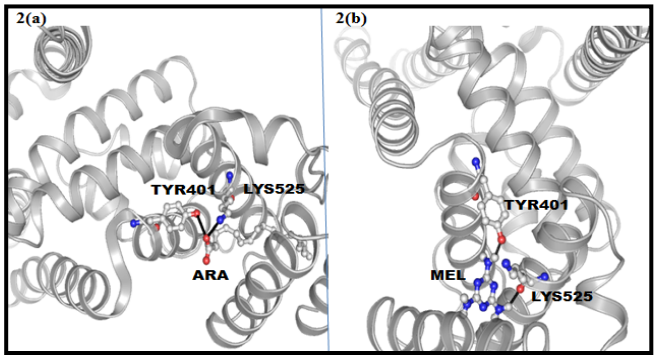

Fig. 2: Cartoon diagram of human serum albumin is represented in black color. At the active site 5, the hydrogen bond interactions of arachidonic acid with Tyr401and Lys525 are shown in Fig 2a. In Fig 2b, the interaction of melamine at the active site 5 is shown. The interacting residues at the active site are represented by ball-and-stick mode."

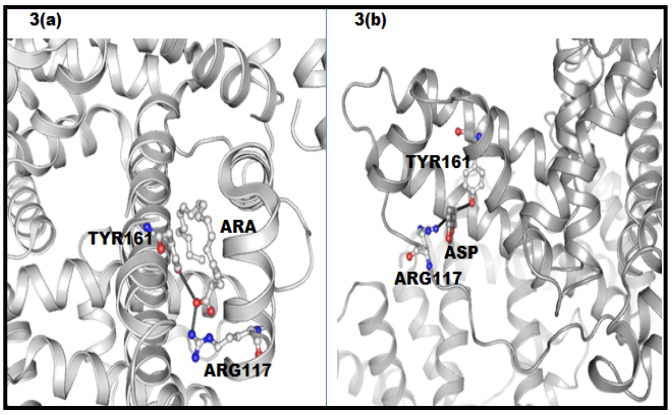

Fig. 3: Cartoon diagram of human serum albumin is represented in black color. At the active site 1, the hydrogen bond interactions of arachidonic acid with Tyr161 and Arg117 are shown in Fig 3a. In Fig 3b, the interaction of aspirin at the active site 1 is shown. The interacting residues at the active site are represented by ball-and-stick mode."

Various types of energies like binding free energy, electrostatic, van der Waals and hydrogen bond as calculated by PEARL software tool are mentioned in Table 2. Binding free energy is the summation of all the energy values– electrostatic, van der Waals, hydrogen bond, conformational entropy [24]. So with increasing the negative values of binding energy, ligand-receptor complex becomes more stable. Here, with all the ligands (arachidonic acid, myristic acid, propofol, halothane, aspirin and melamine) the interaction energy indicates the stability of the complexes. Arachidonic acid and myristic acid are natural ligands of human serum albumin. As a result, binding energy are high. With anesthetic drug, propofol, the binding energy is also in the higher range, however, with another anesthetic drug, halothane, binding energy is not so high. Aspirin has low binding energy with human serum albumin. At active site 1 and 2 of human serum albumin, melamine has high binding energy. At active site 3 and 6, binding energy of melamine is in similar range with halothane. In all ligand-human serum albumin complexes, at all the active sites, van der Waals interaction energy is high. However, in case of melamine-human serum albumin, hydrogen bond energy pattern is different from other ligand–human serum albumin complexes. At the active site 1, 2, 4, 5 and 6, hydrogen bond energy of melamine –human serum albumin complex is higher than other ligand–human serum albumin complexes. Since, melamine has 3 amino groups attached with the benzene ring (Fig. 4), so chance of hydrogen bond formation is higher in melamine–human serum albumin complex in compare to other ligands. As a result, these hydrogen bonds should make melamine–human serum albumin complex stable.

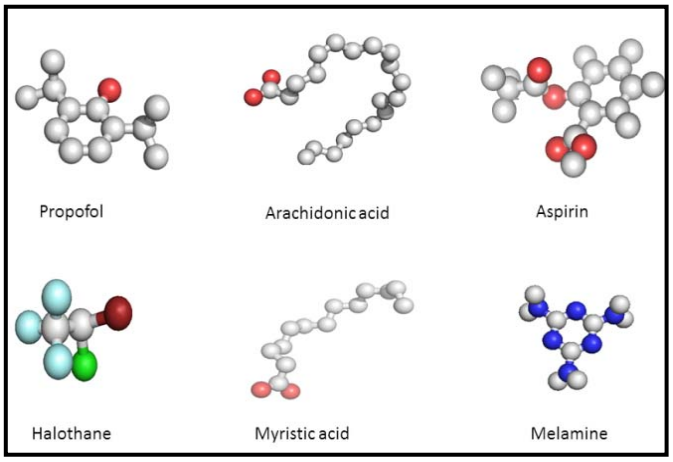

Fig. 4: The ball-stick structures of different ligands are represented

4. DISCUSSION

Arachidonic acid is the precursor of ecosanoid mediators of inflammation. It is circulated in the human body bound with albumin. In case melamine binds with the binding site of arachidonic acid in albumin with significant affinity, there is a possibility of increased free arachidonic acid. This may contribute for synthesis of more ecosanoid mediators of inflammation and that in turn can contribute to melamine induced inflammation in long run. The possibility of the above phenomenon is explored in the present study using tools of computational biology.

By docking study, arachidonic acid is found to bind at all the active sites of human serum albumin. The results are similar to the x-ray crystallographic structures of the complex [20]. Since human serum albumin is the natural carrier of arachidonic acid [19], it is obvious that they should bind at all the active sites of human serum albumin with high free energy of binding. Further, some drug molecules are also carried by human serum albumin. Propofol and halothane are known to bind at specific active sites of human serum albumin [25]. In these cases, the similarity between docking study and x-ray structures has proved the validity of the docking protocol. Like anesthetic drugs, propofol and halothane, melamine and aspirin are bound at specific active sites of human serum albumin (Table 1). The free energy of binding of aspirin to human serum albumin is low except at active site 3 (Table 2) and the structure of aspirin is very small (Fig. 4). It is reported that salicylic acid binds strongly with bovine serum albumin than aspirin and both salicylic acid and aspirin bind strongly with bovine serum albumin than human serum albumin [27]. Further, Aarons et al. [27], have also observed that aspirin bound with albumin is hydrolyzed. This indicates that the binding interaction between aspirin and albumin is not strong. Aspirin is a very common non steroidal anti inflammatory drug [28]. The mechanism for inhibition of inflammation by aspirin is to irreversibly acetylate a serine group at the active site of COX enzyme resulting deactivation of COX proteins and non production of prostaglandins. Binding of aspirin is insignificant for arachidonic acid binding with human serum albumin and its mechanism of anti-inflammatory action is different.

Table 1: Nonbond distances (in Å) of various ligands-Human serum Albumin interactions at the active sites are Given. ACD-Arachidonic acid, MYR-Myristic acid, PFL-Propofol,HLT-Halothane, ASP-Aspirin, MEL-Melamine.

Human serum albumin residues	ACD	MYR	PFL	HLT	ASP	MEL
Site 1 Tyr 161	3.6	-	-	-	2.7	2.1
Tyr 138	-	4.4	-	-	-	-
Arg117	2.8	2.9	-	-	2.9	-
Arg 186	-	4.0	-	-	4.3	-
Ile 142	-	4.3	-	-	-	4.2
Ala 158	-	4.3	-	-	-	-
Site 2 Tyr 150	2.8	2.8	-	-	2.7	2.8
Arg 257	3.8	3.7	-	-	4.3	-
Ser287	2.8	2.7	-	-	3.4	3.9
Leu 251	-	3.8	-	-	-	-
Ala 254	-	4.5	-	-	-	-

Site 3 Arg 410	3.4	-	2.8	-	-	4.3
Tyr 411	3.3	-	-	-	-	2.2
Ser 342	3.0	2.5	-	-	3.9	-
Arg 348	2.5	-	-	-	-	2.0
Arg 485	2.9	-	-	-	2.7	-
Leu 387	-	4.3	-	-	-	-
Gly 434	-	-	3.7	-	-	-
Val 433	-	-	3.5	-	-	-
Asn 391	-	-	3.8	-	-	-
HSA residues	ACD	MYR	PFL	HLT	ASP	MEL
Site 4 Tyr 411	2.9	3.9	-	-	3.4	-
Arg410	4.0	3.1	-	-	-	-
Ser489	2.7	3.2	-	-	-	3.0
Site 5 Lys 525	2.6	2.9	-	-	-	2.1
Tyr 401	3.7	3.1	-	-	-	2.1
Ala 528	-	-	4.2	-	-	-
Leu 532	-	-	3.4	-	-	-
Phe 502	-	-	3.7	-	-	-
Phe 509	-	-	4.3	-	-	-
Met 548	-	3.8	-	-	-	-
Phe 407	-	4.2	-	-	-	-
Site 6 Arg 209	3.6	-	-	3.6	3.3	-
Glu 354	-	-	-	-	2.8	2.2
Asp 324	-	-	-	3.4	-	2.1
Gly 328	-	-	-	3.6	-	-
Ala 213	-	-	-	4.0	-	-
Site 7 Arg 257	4.4	-	-	4.3	-	-
His 242	3.3	-	-	-	-	-
Arg 222	-	-	-	3.0	-	-
Arg 218	-	-	-	2.9	-	-

Table 2: Various energy values (in Kcal/Mol) of ligand-albumin interaction at all the active sites of albumin. ACD-Arachidonic acid, MYR-Myristic acid, PFL-Propofol,HLT-Halothane, ASP-Aspirin, MEL-Melamine."

Energy values in K.Cal/mol	ACD	MYR	PFL	HLT	ASP	MEL
Site 1 Interaction energy	-12.29	-7.64	-	-	-2.80	-5.78
Vanderwaals energy	-9.73	-7.36	-	-	-2.80	-5.99
Hydrogen Bond energy	-0.76	-0.73	-	-	0.00	-0.92
Electrostatics energy	-0.91	0.88	-	-	0.00	-0.03
Site 2 Interaction energy	-12.28	-8.03	-	-	-2.80	-9.05
Vanderwaals energy	-9.45	-6.57	-	-	-2.80	-6.73
Hydrogen Bond energy	-1.35	-1.26	-	-	0.00	-3.02
Electrostatics energy	0.91	0.47	-	-	0.00	-0.06
Site 3 Interaction energy	-11.75	-5.55	-7.44	-	-3.34	-3.91
Vanderwaals energy	-10.04	-7.01	-6.84	-	-3.53	-3.93
Hydrogen Bond energy	-1.69	-1.23	-0.37	-	0.00	-0.69
Electrostatics energy	-0.21	2.69	0.05	-	-0.24	-0.02
Site 4 Interaction energy	-12.34	-6.32	-	-	-2.80	-2.80
Vanderwaals energy	-8.97	-6.34	-	-	-2.80	0.00
Hydrogen Bond energy	-1.29	-1.04	-	-	0.00	-2.80
Electrostatics energy	-0.59	1.16	-	-	0.00	0.00
Site 5 Interaction energy	-12.26	-7.55	-7.36	-	-	-2.80
Vanderwaals energy	-9.90	-7.97	-5.94	-	-	0.00
Hydrogen Bond energy	-0.60	-0.70	0.00	-	-	-2.80
E.E.	-0.02	1.57	-0.13	-	-	0.00
Site 6 Interaction energy	-9.34	-	-	-4.29	-2.80	-4.22
Vanderwaals energy	-8.03	-	-	-5.13	-2.80	-3.97
Hydrogen Bond energy	0.00	-	-	0.00	0.00	-1.19
Electrostatics energy	-0.20	-	-	0.35	-0.00	-0.02
Site 7 Interaction energy	-6.28	-	-	-4.21	-	-
Vanderwaals energy	-6.43	-	-	-5.16	-	-
Hydrogen Bond energy	0.00	-	-	0.00	-	-
Electrostatics energy	0.87	-	-	0.58	-	-

The free energy of binding of melamine is not so high like arachidonic and myristic acid, but the hydrogen bond energy of melamine at active sites 1, 2, 4, 5 and 6 are observed to be much higher than all other ligands studied here. Stability of hydrogen bond energy is greater than electrostatic and van der Waals interaction energy [29] which indicates that more amount of energy will be required to break down hydrogen bond. So from this perspective it can be said that if melamine is bound at specific active sites of human serum albumin, arachidonic acid may not bind at those active sites. As a result, there will be free arachidonic acid inside the cell and this will cause inflammation. The docking result clearly demonstrates that melamine binds with various active sites of albumin with significant hydrogen bond energy. Therefore in chronic melamine exposure the chemical is expected to bind with albumin in a stable manner and is difficult to be replaced by arachidonic acid. This is expected to produce more free arachidonic acid causing generation of mediators of inflammation. Hence we feel melamine per se can cause inflammation by generation of more mediators of inflammation (like PGE2). Keeping in mind high incidence of melamine adulteration of food materials experimental validation of the above findings is warranted.

5. ACKNOWLEDGEMENT

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REFERENCES

[1] X.M. Ding, K.Y. Zhang, L. Wang, S.P. Bai, Toxicity of melamine and cyanuric acid in broilers and residues in tissues, *Hum. Exp. Toxicol.* 31 (2012) 174-184.

[2] W. Phromkunthong, P. Choochuay, V. Kiron, N. Nuntapong, M. Boonyaratpalin, Pathophysiological changes associated with dietary melamine and cyanuric acid toxicity in red tilapia, *J. Fish Dis*. (2014), in press (doi: 10.1111/jfd.12219)

[3] G.L. Newton, P.R. Utley, Melamine as a dietary nitrogen source for ruminants, *J. Anim. Sci*. 47 (1978) 1338-1344.

[4] K. Bischoff, W.K. Rumbeiha, Pet food recalls and pet food contaminants in small animals, *Vet. Clin. North Am. Small Anim. Pract.* 42 (2012) 237–250.

[5] Y. Wei, D. Liu, Review of melamine scandal: still a long way ahead, *Toxicol. Ind. Health* 28 (2012) 579–582.

[6] H.Y. Shen, Y.Q. Liu, J. Gao, H.M. Zhen, N. Zhu, J. Li, In vitro study of DNA interaction with melamine and its related compounds. *DNA Cell Biol.* 30 (2011) 255–64.

[7] M. Vara Messler, D.C. Cremonezzi, E.A. Soria, A.R. Eynard, Nutritional chemoprevention of urinary tract tumors (UTT) induced by lithogenic agents: risk for UTT in children exposed to melamine-contaminated milk formulas. *J. Environ. Sci. Health C Environ. Carcinog. Ecotoxicol.* Rev. 30 (2012) 174–187.

[8] R.H. Yin, X.Z. Wang, W.L. Bai, C.D. Wu, R.L. Yin, C. Li, et al., The reproductive toxicity of melamine in the absence and presence of cyanuric acid in male mice. *Res. Vet. Sci*. 94 (2013) 618–627.

[9] C. Wang, X. Qin, B. Huang, F. He, C. Zeng, Hemolysis of human erythrocytes induced by melamine-cyanurate complex. *Biochem. Biophys. Res. Commun.* 402 (2010) 773-777.

[10] R.P. Dalal, D.S. Goldfarb, Melamine-related kidney stones and renal toxicity, *Nat. Rev. Nephrol*. 7 (2011) 267–274.

[11] V. Bhalla, P.C. Grimm, G.M. Chertow, A.C. Pao, Melamine nephrotoxicity: an emerging epidemic in an era of globalization, *Kidney Int*. 75 (2009) 774-779.

[12] R.L. Melnick, G.A. Boorman, J.K. Haseman, R.J. Montali, J. Huff, Urolithiasis and bladder carcinogenicity of melamine in rodents, *Toxicol. Appl. Pharmacol.* 72 (1984) 292-303.

[13] *H. Ogasawara, K. Imaida, H. Ishiwata, Urinary bladder carcinogenesis induced by melamine in F344 male rats: Correlation between carcinogenicity and urolith formation. Carcinogenesis* 16 *(1995)* 2773*– 2777.*

[14] *T.J. Hsieh, P.C. Hsieh, Y.H. Tsai, et al., Melamine induces human renal proximal tubular cell injury via transforming growth factor-b and oxidative stress, Toxicol. Sci. 130 (2012) 17-32.*

[15] *F.C. Kuo, Y.T. Tseng, S.R. Wu, M.T. Wu, Y.C. Lo, Melamine activates NFkB/ COX-2/ PGE2 pathway and increases NADPH oxidase–dependent ROS production in macrophages and human embryonic kidney cells, Toxicol. In Vitro 27 (2013) 1603-1611.*

[16] http://www.ncbi.nlm.nih.gov/pccompound

[17] http://www.pdb.org

[18] 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.

[19] 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, *Nature Struct. Biol*. 5 (1998) 827-835.

[20] 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.

[21] G.M. Morris, R. Huey, W. Lindstrom, M.F. Sanner, R.K. Belew, D.S. Goodsell, A.J. Olson, Autodock4 and AutoDockTools4: automated docking with selective receptor flexiblity, *J. Computational Chemistry* **16 (**2009) 2785-2791.

[22] G. M. Morris, D. S. Goodsell, R. S. Halliday, R. Huey, W. E. Hart, R. K. Belew, A. J. Olson Automated Docking Using a Lamarckian Genetic Algorithm and an Empirical Binding Free Energy Function, *J. Comput. Chem.* 19 (1998) 1639-1662.

[23] P. Chakrabarti, R. Bhattacharyya, Geometry of nonbonded interactions of planar groups in proteins, *Prog. Biophys. Mol. Biol*. 95 (2007) 83-137

[24] L.Y. Han, H.H. Lin, Z.R. Li, et al., PEARLS: program for energetic analysis of receptor-ligand system, *J. Chem. Inf. Model*. 46 (2006) 445-450.

[25] A.A. Bhattacharya, S. Curry, N.P. Franks, Binding of the general anesthetics propofol and halothane to human serum albumin, *J. Biol. Chem.* 275 (2000) 38731-38738.

[26] http://www.pymol.org/

[27] L. Aarons, P. Clifton, G. Fleming, M. Rowland, Aspirin binding and the effect of albumin on spontaneous and enzyme-catalysed hydrolysis, *J. Pharm. Pharmacol.* 32 (1980) 537–543.

[28] E.H. Awtry, J. Loscalzo, Aspirin, *Circulation* 101 (2000) 1206-1218.

[29] J.M. Berg, J.L. Tymoczko, L. Stryer, *Biochemistry*. 5th edition. New York: W H Freeman; 2002. Section 1.3, Chemical Bonds in Biochemistry. Available from: http://www.ncbi.nlm.nih.gov/books/NBK22567/