Insilico Docking Analysis of Nitrogen Containing Bisphosphonate with Human Fernasyl Pyrophosphate Synthase

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ABSTRACT

Bisphosphonates are currently the most important class of antiresorptive agents used in the treatment of metabolic bone diseases, including tumor-associated osteolysis and hypercalcemia. These compounds have high affinity for calcium ions and therefore target bone mineral, where they are internalized by bone-resorbing osteoclasts and inhibit osteoclast function. Nitrogen-containing bisphosphonates (N-BPs) are currently used as clinical inhibitors of bone-resorption diseases. It target osteoclast farnesyl pyrophosphate synthase (FPPS) and inhibit protein prenylation. FPPS, a key branchpoint of the mevalonate pathway, catalyzes the successive condensation of isopentenyl pyrophosphate with dimethylallyl pyrophosphate and geranyl pyrophosphate. In this study the docking accuracy and scoring reliability for the docking of nitrogen containing bisphosphonate with human FPPS using Auto Dock 4.0 has been presented the most potent drug for the treatment of osteoporosis.

Keywords

fernasyl pyrophosphate synthase, osteoclast, docking, bisphosphonates, hypercalcemia.

1. INTRODUCTION

Osteoporosis is a disease of bone that leads to an increased risk of fracture. Osteoporosis is characterized by low bone mineral density (BMD), disrupted micro-architecture of bone tissue, and altered amount and variety of proteins in bone. On the basis of measurement of BMD World Health Organization (WHO) has suggested a definition for osteoporosis in women. According to WHO, a women is osteoporotic when bone mineral density is 2.5 standard deviations less than peak bone mass of young healthy white women. The term "established osteoporosis" includes the presence of a fragility fracture. [1] Osteoporosis commonly occurs in women after menopause, in this condition it is called postmenopausal osteoporosis. The disease may also progress in men, and may appear in anyone in the presence of particular hormonal disorders, chronic diseases and as a result of wrong or over medications, specifically glucocorticoid [where the disease is called steroid- or glucocorticoid-induced osteoporosis (SIOP or GIOP)]. In the midst of its influence, the disease increase the risk of fragility fracture significantly affect life expectancy as well as the quality of life. Farnesyl pyrophosphate synthase (FPPS) is a key regulatory enzyme in the mevalonate pathway. In mammals this pathway provides important lipid molecules, such as cholesterol and isoprenoids. Isoprenoids is essential for prenylation of small GTPase signaling protein which is essential for normal cellular function (2). The blockade of key enzyme of this pathway is important therapeutic target for various disease, like inhibition of hydroxymethylglutaryl-CoA reductase with the drug statins reduces cholesterol biosynthesis, and inhibition of FPPS with the drug nitrogen-containing bisphosphonates (N-BPs) inhibit protein prenylation in osteoclat. Due to its unique bone cell targeting properties, N-BPs selectively inhibits FPPS and prevents loss of prenylated proteins in osteoclasts, thereby stops the bone- damaging function of these cells (3). FPPS catalyzes the sequential condensation of isopentenyl pyrophosphate (IPP), to dimethylallyl pyrophosphate (DMAPP) and then to geranyl pyrophosphate (GPP) which results in the formation of C15 farnesyl pyrophosphate (FPP). FPP acts as a substrate for geranylgeranyl pyrophosphate synthase, and produce the C20 isoprenoid geranylgeranyl pyrophosphate (GGPP). Posttranslational prenylation of small GTPases with FPP or GGPP is crucial for their correct subcellular localization and as risedronate (RIS) and zoledronate (ZOL) (4). These agents are currently used to treat postmenopausal & steroid-induced osteoporosis, and other condition like, function (3). It is now clear that FPPS is the major enzyme target of N-BPs, such hypercalcemia, and osteolysis associated with multiple myeloma and metastatic cancers (5, 6). Because of their ability to bind calcium ions, bisphosphonates (BPs) accumulate rapidly in bone cells, where they inhibit the activity of bone-resorbing osteoclasts.. Molecular docking is a frequently used tool in computer-aided ligand-based rational drug design. It evaluates how small molecules called ligands (substrates, inhibitors, drugs or drug candidates) and the target macromolecule (receptor, enzyme or nucleic acid) fit together. Auto Dock (http://autodock.scripps.edu/) is an automated docking tool and used to predict how small molecules bind to a target protein of known 3D-structure. Besides generating binding energies in these docking studies, the position of the ligand in the host's binding site can be visualized. It can be useful for developing better drug candidates and also for the understanding the nature of the binding. In this experiment the comparative docking analysis of five ligand and target protien, fernasyl pyrophosphate synthase has been performed.

2. MATERIAL AND METHODS

Several automated docking programs have been developed [7-9]. to obtain information about the position and energy of binding between an inhibitor and the corresponding protein. Recent improvements in search algorithms and energy functions, computational docking methods have become a valuable tool to probe the interaction between an enzyme and its inhibitors. These procedures can add considerably to the understanding of structural and energy basis of enzyme-substrate interactions [10-12]. The set of ligand molecules studied in this work include Alendronate, Zolendronate, Ibandronate, and Pamidronate. The structure of these compounds is shown in Figure1. The three dimensional structure of human farnesyl pyrophosphate synthase (PDB ID: 2F7M) was obtained from Protein Data Bank (PDB) [13-14]. In order to carry out the docking simulation, Auto Dock 4.0 suite has been used as molecular-docking tool [15]. It is appropriate for performing automated docking of ligands to their macromolecular receptors. Normally, the ligands are substrates or drug candidates and the macromolecule is a protein of known three dimensional structures. In this docking simulation, semi-flexible docking protocols have been used in which the target protein FPPS was set aside as rigid. The ligands being docked were kept flexible, in order to discover random number of torsional degrees of freedom in addition to the six spatial degrees of freedom spanned by the translational and rotational parameters

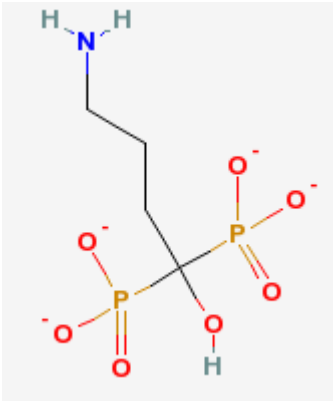

Figure 1A: Two dimensional molecular structures of Alendronate ligand

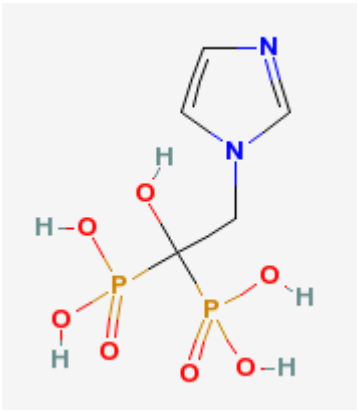

Figure 1B: Two dimensional molecular structures of Zoledronate ligand

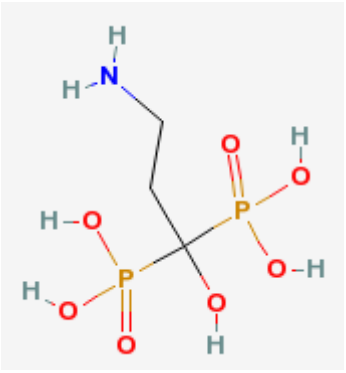

Figure 1C: Two dimensional molecular structures of Pamidronate ligand

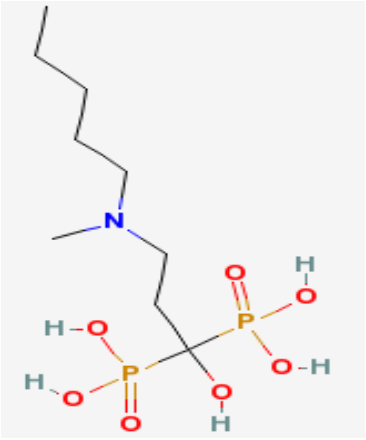

Figure 1D: Two dimensional molecular structures of Ibandronate ligand

2.1 Preparation of Ligand and Target Molecule

Auto Dock Tool was used to prepare ligand and receptor, then it was run, and analyze the docking simulations. Kollman united atom charges, solvation parameters and polar hydrogens were added into the receptor PDB file for the preparation of protein in docking simulation. This FPPS enzyme structure does not have ligand molecule but it has water and phosphate molecule which has been removed from its PDB file and make a free receptor. As ligands are not peptides, Gasteiger charge was assigned and then non-polar hydrogens were combined. Instead of selecting physically, the rigid roots of each compound were define automatically. The amide bonds were made non-rotatable. All rotatable dihedrals in the ligands were assigned with the help of auxiliary program Auto-Tors and were allowed to rotate freely.

2.2 Docking Procedure

Pre-calculated grid maps are required by Auto Dock, for each atom of the ligand being docked and it stores the potential energy arising from the interaction with macromolecule. The grid box size was set at 40, 40, and 40 A° (x, y, and z), though it was changed depending on the ligand size. Auto Grid 4.0 Program, supplied with Auto Dock 4.0 was used to produce grid maps. The distance between grid points was 0.375 A°. The Lamarckian Genetic Algorithm (LGA) was chosen to search for the best conformers. For each ligand, a maximum of 10 conformers was considered during docking. The size of

the population was set to 150 and the individuals were initialized arbitrarily. Maximum number of energy estimation was set to 2500000, maximum number of generations 27000, maximum number of top individual that automatically survived was set to 1, mutation rate of 0.02, crossover rate of 0.8, Step sizes were 0.2 Å for translations, 5.0° for quaternions and 5.0° for torsions. Cluster tolerance 2.0A°, external grid energy 1000.0, max initial energy 0.0, max number of retries 10000 and 10 LGA runs were performed. The program LGA and pseudosolis and wets method were applied for minimization using default parameter. (Table-1)

2.3 Evaluation of Results of Docking

In docking crystallographic position of ligand on the target is unknown and no further experimental information is available. One can only assume that the minimum energy represent the best ligand-protien complex. Autodock 4.0 uses a semiemperical free energy force field to evaluate conformation during docking simulations. The force field was parameterized using a large number of protein-inhibitor complexes for which both structure and inhibition constants and Ki are known. The force field evaluates binding in two steps. The ligand and protein start in an unbound conformation. In the first step, the intermolecular energetic are estimated for the transition from these unbound states to the conformation of the ligand and protein in the bound state. The second step then evaluates the intermolecular energetic of combining the ligand and protein in their bound conformation.

The force field includes six pair-wise evaluations (V) and an estimate of the conformational entropy lost upon binding (ΔSconf) is computed as

$$\Delta G = (V^{L-L}_{bound} - V^{L-L}_{unbound}) + (V^{P-P}_{bound} - V^{P-P}_{unbound}) + (V^{P-L}_{bound} - V^{P-L}_{unbound} + \Delta S_{conf})$$

Where L refers to the "ligand" and P refers to the "protein" in a ligand-protien docking calculation.

Each of the pairwise energetic terms includes evaluation for dispersion/repulsion, hydrogen bonding, electrostatic and desolvation.

$$V = W\sum_{i,j}\left(\frac{A_{ij}}{r_{ij}^{12}} - \frac{B_{ij}}{r_{ij}^{6}}\right) + W_{hbond}\sum_{i,j}E(t)\left(\frac{C_{ij}}{r_{ij}^{12}} - \frac{D_{ij}}{r_{ij}^{10}}\right) + W_{elec}\sum_{i,j}\frac{q_i q_j}{\varepsilon(r_{ij})r_{ij}} + W_{sol}\sum_{i,j}\left(S_i V_j + S_j V_i\right)e^{\left(-r_{ij}^2/2\sigma^2\right)}$$

	Docking statistics (RMSD-tolerance of 2.0 Å)				
Molecules	Number of cluster	Cluster rank	Number of cluster in conformation	Lowest binding energy (kcal/mol)	Number of run
Alendronate	2	1 2	8 2	-7.59 -7.48	6 2
Zoledronate	3	1 2 3	2 6 2	-5.65 -5.35 -5.15	9 4 10
Pamidronate	2	1 2	8 2	-7.47 -6.60	3 5

Table1: Docking parameter for all four ligands into protein fernasyl pyrophosphate synthase

		1	3	-2.58	2
Ibandronate	4	2	5	-2.17	4
		3	1	-0.37	7
		4	1	-0.19	10

3. RESULTS AND DISCUSSION

3D structure of protein FPPS (PDB ID:2F7M) was taken from protein data bank. Ligands have several possible torsional degrees of freedom and they are composed of functional groups and bonds. Target protein fernasyl pyrophosphate synthase (2F7M) with 349 residues were selected for docking. Identifying the ten different conformation of each ligand and best conformation has been chosen for docking analysis on the basis of their binding energies (table of binding energies). It is general observation that in docking calculation if a ligand molecule cover large piece of protein surface to find its proper location then probability of finding the energy minimum is much less. The most important requirement of docking calculation is its ability of distinguishing the real binding site on the protein from nonspecific or energetically unfavorable ones. To verify the accuracy of the Auto Dock 4.0 results, some top clusters of conformations/orientations were also considered in addition to the best scored one. The docking accuracy was evaluated in terms of the root mean square deviation (RMSD) between the docked position and the experimentally determined position for the ligand. In this molecular docking study, prediction was considered successful if the RMSD value is less then 2.0 Å for the best-scored conformation [16]. Lipinski's rule of five was calculated for all the four ligand molecules that satisfy the 'rule-of-5' and it was found that all the ligand molecules satisfied the rule for potent inhibitors (Table 3).

3.1 Docking of Alendronate into FPPS

Docking simulation of alendronate into the active site of the FPPS produced two clusters of conformers using RMSD-tolerance of 2.0 Å out of 10 docking runs (Table 1). The conformation of the 1 ranked cluster was favored in that structure and repeated six times out of 10 runs. The lowest binding energy of the docked complex was -7.59 kcal/mol. In this docked complex, NH group of LYS57 and OH group of alendronate acts as hydrogen bond donar. The bond distance between donors and acceptors atoms of hydrogen bond was approximately 1.8Å (Table 2). Interaction of alendronate and FPPS is shown in Figure2.

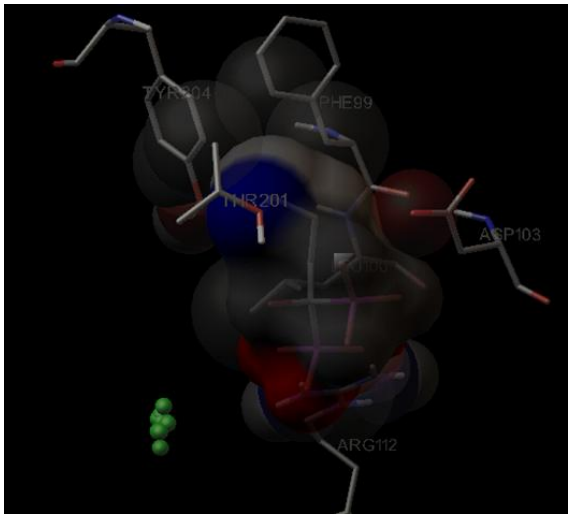

Figure2: Docking of alendronate into FPPS as obtained by Autodock4.0

3.2 Docking of Pamidronate into FPPS

Docking simulation of pamidronate into the active site of the FPPS produced two clusters of conformers using RMSD-tolerance of 2.0 Å out of 10 docking runs (Table 1). The conformation of the 1 ranked cluster was favored in that structure and repeated three times out of 10 runs. The lowest binding energy of the docked complex was -7.47 kcal/mol. In this docked complex, NH group of LYS257,ARG60 and OH group of pamidronate acts as hydrogen bond donar. The bond distance between donors and acceptors atoms of hydrogen

bond was approximately 1.9Å (Table 2). Interaction of pamidronate and FPPS is shown in Figure3.

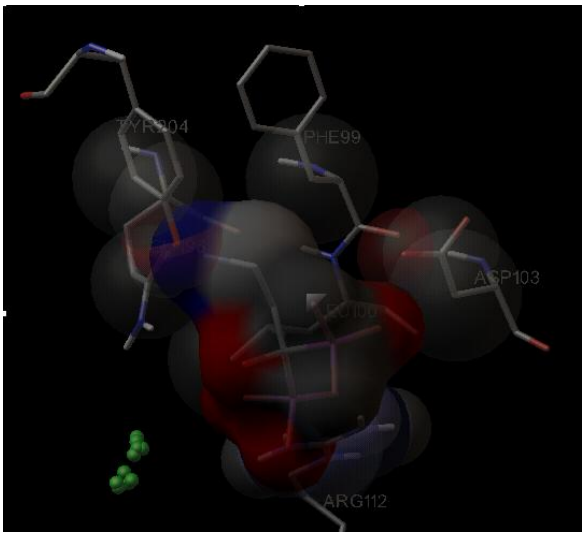

Figure3: Docking of pamidronate with FPPS as obtained by Autodock 4.0

3.3 Docking of Zoledronate into FPPS

Docking simulation of zoledronate into the active site of the FPPS produced three clusters of conformers using RMSD-tolerance of 2.0 Å out of 10 docking runs (Table 1). The conformation of the 1 ranked cluster was favored in that structure and repeated nine times out of 10 runs. The lowest binding energy of the docked complex was -5.65 kcal/mol. In this docked complex, NH group of LYS57, ARG60 ARG113 and OH group of zoledronate acts as hydrogen bond donar. The bond distance between donors and acceptors atoms of hydrogen bond was approximately 1.9Å (Table 2). Interaction of zoledronate and FPPS is shown in Figure4

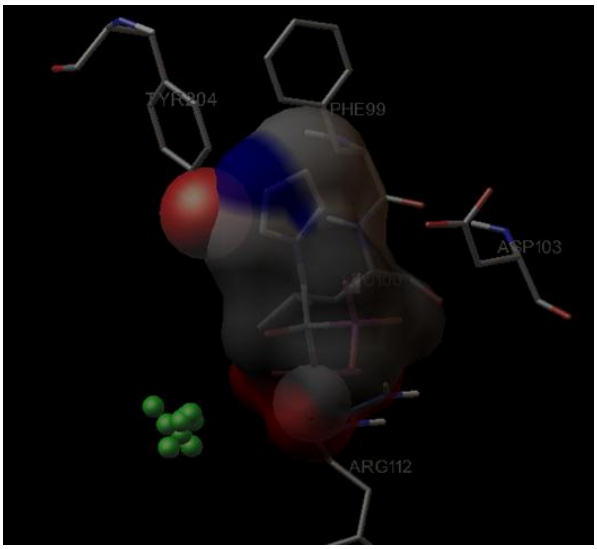

Figure4: Docking of zoledronate with FPPS as obtained by Autodock4.0

Table2: Molecular interactions of all four ligands into protein fernasyl pyrophosphate synthase.

Molecules	Hydrogen bond donor	Hydrogen bond acceptor	Length of hydrogen bond (Å)
Pamidronate	rigid:F:ARG60:HH11 rigid:F:LYS257:HZ2 rigid:F:LYS57:HZ1 Pam:F: N22 rigid:F:ARG60:HH21	Pam:F:O Pam:F:O10 Pam:F:O3,O5 rigid:F:ASP243:O12 Pam:F:O2	1.963 1.894 2.161 1.884
Alendronate	Alen:F:THR201:HG1 rigid:F:LYS57:H22 rigid:F:LYS57:H23 rigid:F:LYS257:H22 rigid:F:LYS257:HZ1	rigid:F:THR201:N Alen:F:O12 Alen:F:O15 Alen:F:O16 Alen:F:O11	1.853 1.883 1.795 1.81 1.961
Ibandronate	Iban:F:THR201:HG1 rigid:F:ARG60:HH21 rigid:F:GLN96:HE22	rigid:F:VAL192:O Iban:FO9 Iban:FO14	1.592 2.019 1.901
Zoledronate	rigid:F:ARG60:HH11 rigid:F:ARG113:HN rigid:F:LYS57:H21 rigid:F:ARG113:HE rigid:F:ARG113:HH22 rigid:F:ARG60:HH21 rigid:F:GLN96:HE21	Zol:F:O12 Zol:F:N17 Zol:F:O10 Zol:F:O15 Zol:F:O16 Zol:F:O11 Zol:F:O16	2.089 2.134 1.89 1.676 2.015 2.181 1.910

Table 3: Lipinski properties of docked ligand

Molecule Name	Molecular weight	Log p	H-bond donor	H-bond acceptor
Alendronate	245.06	-6.9	2	8
Zoledronate	272.08	-4.5	5	8
Ibandronate	319.22	-4.1	5	8
Pamidronate	235.06	-6.9	6	8

3.4 Docking of Ibandronate into FPPS

Docking simulation of Ibandronate into the active site of the FPPS produced four clusters of conformers using RMSD-tolerance of 2.0 Å out of 10 docking runs (Table 1). The conformation of the 1 ranked cluster was favored in that structure and repeated nine times out of 10 runs. The lowest binding energy of the docked complex was -2.58kcal/mol. In this docked complex, NH group of THR201, ARG60, GLN96 and OH group of ibandronate acts as hydrogen bond donar. The bond distance between donors and acceptors atoms of hydrogen bond was approximately 1.8Å (Table 2). Interaction of ibandronate and FPPS is shown in Figure5.

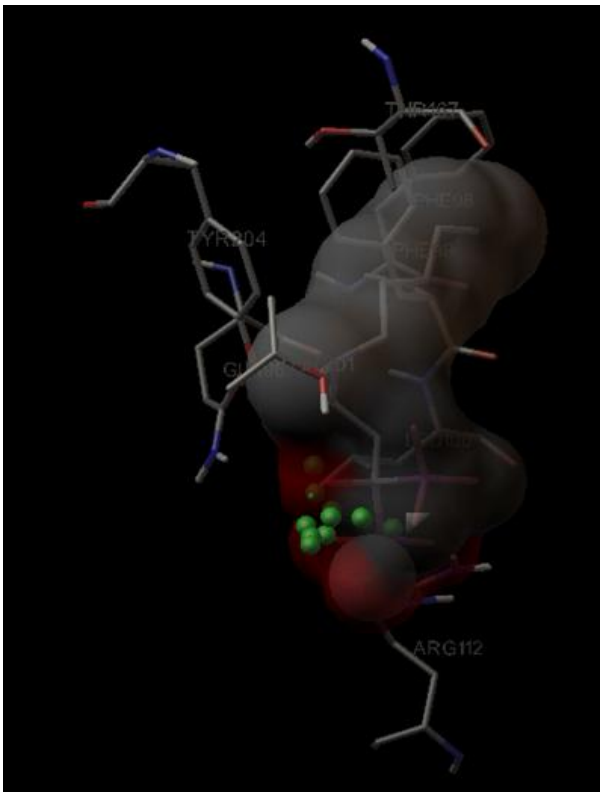

Figure 5: Docking of ibandronate with FPPS as obtained by Autodock4.0

4. CONCLUSION

Molecular docking simulation of alendronate, zoledronate, pamidronate and ibandronate with protein fernasyl pyrophosphate synthase shows that the numbers of clusters with each ligand vary in the range from two to four for FPPS. On the basis of minimum energy concept, it can be concluded that alendronate is most powerful inhibitor of FPPS amongst others considered in the study. It also confirms the Lipinski rule of five. It is depicted from the observation that except ibandronate other three ligand molecule namely alendronate, pamidronate and zoledronate participate in hydrogen bond formation with nitrogen atom of the LYS57. ARG60, ARG113 and LYS257 take part in hydrogen bonding with comparatively higher frequency. The findings of present study may prove to be very useful for design of new drugs for osteoporosis in which potential inhibitors should interact strongly with residues mentioned as above.

5. REFERENCES

[1] WHO (1994). "Assessment of fracture risk and its application to screening for postmenopausal osteoporosis. Report of a WHO Study Group". World Health Organization technical report series 843: 1–129. PMID 7941614.

[2] 2Goldstein J. L., Brown M. S. Nature. 1990; 343:425–430. [PubMed]

[3] Rogers M. J. Curr. Pharm. Des. 2003;9:2643–2658. [PubMed]

[4] Dunford J. E., Thompson K., Coxon F. P., Luckman S. P., Hahn F. M., Poulter C. D., Ebetino F. H., Rogers M. J. J. Pharmacol. Exp. Ther. 2001;296:235–242. [PubMed]

[5] Rodan G. A., Reszka A. A. Curr. Mol. Med. 2002;2:571–577. [PubMed]

[6] Russell R. G., Rogers M. J. Bone. 1999; 25:97–106. [PubMed]

[7] Morris GM, Goodsell DS, Halliday RS, Huey R, Hart WE, Belew RK, Olson AJ. Automated Docking Using a Lamarckian Genetic Algorithm and Empirical Binding Free Energy Function. J Comput Chem. 1998; 19:1639–1662.

[8] Henry AG, Richard MJ, Michael JES. Modelling Protein Docking using Shape Complimentarity, Electrostatics and Biochemical Information. J Mol Biol. 1997;272:106–120.

[9] Kramer B, Rarey M, Lengauer T. Evaluation of the FlexX incremental construction algorithm for protein-ligand docking proteins. Structure Functions and Genetics. 1999; 37:228–241

[10] Verkhivker GM, Rejto PA, Gehlhaar DK, Freer ST. Exploring the energy landscapes of molecular recognition by a genetic algorithm: 10 analysis of the requirements for robust docking of HIV-1 protease and FKBP-12 complexes. Proteins. 1996;25:342–353.

[11] Sham YY, Chu ZT, Tao H, Warshel A. Examining methods for calculations of binding free energies: LRA, LIE, PDLD-LRA, and PDLD/S-LRA calculations of ligands binding to an HIV protease. Proteins. 2000; 39:393–407.

[12] Pegg SC, Haresco JJ, Kuntz ID. A genetic algorithm for structure-based de novo design. J Comput Aided Mol Des. 2001;15:911–933.

[13] Protein Data Bank http://www.rcsb.org/pdb/home/home.do

[14] Berman HM, Westbrook J, Feng Z, Gilliland G, Bhat TN, Weissig H, Shindyalov IN, Bourne PE. The Protein Data Bank. Nucleic Acids Res. 2000; 28:235–242.

[15] Morris GM, Goodsell DS, Halliday RS, Huey R, Hart WE, Belew RK, Olson AJ. Automated docking using a Lamarckian genetic algorithm and an empirical binding free energy function. J Comput Chem. 1998; 19:1639–1662.

[16] Cole JC, Murray CW, Nissink JW, Taylo RD, Taylor R. Comparing protein-ligand docking programs is difficult. Proteins. 2005; 60:325–332.