Current Journal of Applied Science and Technology

40(39): 21-28, 2021; Article no.CJAST.74579
ISSN: 2457-1024
(Past name: British Journal of Applied Science & Technology, Past ISSN: 2231-0843, NLM ID: 101664541)

Virtual Docking of VBP1 with HBX and NFκB Protein to Study the Activation of NFκB in Regulatory Mechanism of Liver Cancer

Mamta Sagar [a*], Padma Saxena [a, b], Suruchi Singh [c], Ravindra Nath [d] and Pramod W. Ramteke [d]

[a]Department of Bioinformatics, University Institute of Engineering & Technology and Institute of Biosciences & Biotechnology, Chhatrapati Shahu Ji Maharaj University, Kanpur, Uttar Pradesh, India.
[b]Department of Zoology, TRKM Aligarh, Dr BR Ambedkar University Agra, India.
[c] Department of Computer Science and Engineering, University Institute of Engineering & Technology, Chhatrapati Shahu Ji Maharaj University, Kanpur, Uttar Pradesh, India.
[d] Department of Molecular Biology & Genetic Engineering, RTM Nagpur University: Rashtrasant Tukadoji Maharaj Nagpur University, India.

Authors' contributions

This work was carried out in collaboration among all authors. Authors MS and PS designed the study, performed the computational work and wrote the protocol and first draft of the manuscript. Authors SS and RN performed Data analysis, managed the analyses of the study and literature searches. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/CJAST/2021/v40i3931593

Open Peer Review History:
This journal follows the Advanced Open Peer Review policy. Identity of the Reviewers, Editor(s) and additional Reviewers, peer review comments, different versions of the manuscript, comments of the editors, etc are available here: https://www.sdiarticle5.com/review-history/74579

Original Research Article

Received 07 October 2021
Accepted 14 December 2021
Published 16 December 2021

ABSTRACT

Molecular docking is an efficient way to study protein-protein and protein-ligand interactions in virtual mode, this provides structural annotations of molecular interactions, required in the drug discovery process. The Cartesian FFT approach in 'Hex' spherical polar Fourier (SPF) uses rotational correlations, this method is used here to study protein-protein interactions. Hepatitis B virus (HBV) X protein (HBx) is essential for virus infection and has been used in the development of therapeutics for liver cancer. It can interact with many cellular proteins. It interferes with cell viability and stimulates HBV replication. The von Hippel-Lindau binding protein 1(VBP1) has an important role in HBx-mediated nuclear factor kappa B (NFkB) stimulation. VBP1 and HBx function as coactivators in the activation of NFκB binding. Docking results revealed that HBx and NFkB bind with VBP1 at the common site on amino acids positions Arg 161, Glu 92, and Arg 82, which may

**Corresponding author: E-mail: mamtasagar@csjmu.ac.in, mamta10602@gmail.com;*

have a role in HBx-mediated NFκB activation. Lowest energy complex VBP1- NFkB1 was obtained at -883.70 Kcal/mol. The amino acids involved in interaction among HBx, VBP1, and NFκB proteins, may be involved in transcriptional regulation and has significance in normal and abnormal regulation. These amino acid interactions may be associated with the manifestation of Liver cancer.

Keywords: Hepatitis B virus (HBV) X protein (HBx); nuclear factor kappa B activity (NFkB); von hippel-lindau binding protein 1(VBP1); docking; Spherical Polar Fourier (SPF); liver cancer.

1. INTRODUCTION

Although proteins are flexible, many protein docking algorithms assume the proteins as rigid bodies and use geometric hashing [1] or the Fast Fourier transform (FFT) method [2] to find a relatively small number of putative docking orientations using re-scoring for the refinement process. Several Fast Fourier Transform, based docking algorithms are used in the development of ClusPro [3], GRAMM-X [4] and ZDOCK [5] docking web servers which are available online. The FFT-based approaches also assume that the proteins are rigid, but all possible rigid-body orientations in the 6-dimensional search space are considered significant. Most FFT-based approaches use 3D Cartesian grid representations of the proteins, these can only compute translational correlations, by multiple rotations repeatedly to find all possible conformations in 6D search space. This makes Cartesian grid-based FFT docking algorithms, a computationally expensive approach. To solve this problem, the Cartesian FFT approach in 'Hex' Spherical Polar Fourier (SPF) uses rotational correlations [6] to reduce execution times [7]. In this research, Virtual Docking of VBP1 with HBX and NFκB proteins is performed to study the activation process of NFκB in the regulatory mechanism of Liver Cancer, which uses Spherical polar Fourier (SPF) to predict interactions between proteins.

Millions of people were affected by chronic infection of hepatitis B virus (HBV) which causes liver diseases, including cirrhosis and hepatocellular carcinoma [8]. The hepatitis B virus (HBV) X protein (HBx) has stimulated HBV for transcription and replication in hepatocytes *in vivo*. The function of HBx may be crucial to its stimulatory effect on HBV transcription and replication [9]. HBx has been reported to be capable of activating several signal transduction pathways, such as mitogen-activated protein kinase, Ras-Raf-mitogen-activated protein kinase, and JAK/STAT signaling pathways to affect several cellular processes, including proliferation and differentiation **[**10]. HBx also participates in inducing cell death and affects mitochondrial microenvironment to mediate apoptosis [12-15]. Moreover, HBx plays an important role in tumor dispersion by enhancing cellular migration through upregulation of MMP-9, MMP-3, MT1-MMP and COX-2 [16-19]. VBP1 is reported to be localized in the cytoplasm, especially in the perinuclear region and skeletal muscle, heart, brain, kidney, spleen, lung, and liver [20]. The NFκB associated with tumorigenesis by inflammation, anti-apoptosis, and cell proliferation. HBx is a promiscuous protein containing various functions by interacting with a multitude of cellular proteins. Since VBP1 is a bona fide cellular protein interacting with HBx, the reciprocal effects of each protein in the regulation of other protein need to be tested. The docking interaction between different models of HBx & VBP1 and between VBP1& different types of NFkB has been predicted here.

2. MATERIALS AND METHODS

The protein sequence of HBx has been retrieved from NCBI (The National Center for Biotechnology Information, www.ncbi.nlm.nih.gov/). Geno3d was used for modeling (http://geno3d-pbil.ibcp.fr/); four sequences were modelled (ABR68892.1, Q4R1S1.1, P0C685.1 and Q99HR6.1). The modelling of proteins refers to constructing an anatomic-resolution model of the target protein from its amino acid sequence. Three dimensional X ray crystallized structure of Von Hippel-Lindau protein (VBP1, PDB: 4AJY) were downloaded from the protein data bank [21]. The protein was taken as receptor and ligand protein and the most suitable site was predicted using q site finder [22] ligand binding site prediction (http://www.modelling.leeds.ac.uk). The crystal structure of nuclear factor-kappa B ligand NFkB1 [23], NFkB2 [24], NFkB3 [25] , NFkB4 [16] were downloaded from the protein data

bank. These structures were docked to analyse amino acids interactions using the Hex docking server. HexServer (http://hexserver.loria.fr/) is the first Fourier transform (FFT)-based protein docking server which was powered by graphics processors. first Fourier transform (FFT)-based docking methods use the comparable resolution and scoring functions. Protein binding sites information was used to emphasise and reduce the calculation and time of docking. All Hex docking correlations use SPF shape–density representations to polynomial order N ¼ 20 to generate a list of up to 25 000 candidate solutions. The top 3000 orientations always include some close-native orientations but pruning of good candidates is avoided, then re-scoring is done using higher order shape-only or shape plus electro-static correlations which use polynomials to order N ¼ 25 or N ¼ 30 as selected by the user [26].

Using the CUDA (Common Unified Device Architecture) development tools (http://www.nvidia.com/object/cuda_home. htm), Hex algorithm is further adapted to exploit enormous modern graphics processor units (GPUs; in preparation). For typical Hex docking calculations, a single high-performance GPU can evaluate 170 million trial orientations/second. However, high performance GPUs are faster and relatively expensive than conventional Cartesian grid-based FFT docking approaches, HexServer a web interface (http://hexserver.loria.fr/) for Hex is developed, to make GPU-accelerated docking tool freely available. The Hex SPF algorithm has been validated using CAPRI (Critical Assessment of PRedicted Interactions) blind docking results [27], Hex predictions have been included within the top 100 orientations in recent CAPRI scoring sections. Thus, HexServer provides a very fast method to generate high quality docking predictions. In the process of the SPF approach to protein–protein docking, rotations for both the proteins were computed in 3D and 6D grids. In Sampling Protein Properties. Cartesian(x,y,z) coordinates samples are converted to SPF(r, θ,φ). [28,29]. For priority of site for docking, maximum zone of HBx & NFkB and between NFkB & VBP1 have been selected for docking. Structures of HBx were docked with VBP1 and structures of VBP1 were docked with different types (NFkB1- NFkB1) protein structures. The binding site cavity detection was performed using q site finder [22], a ligand site prediction tool. Docking results show different types of energies; these are E total, E shape, E force and the number of H-bond and interaction between amino acid residues of receptor HBx, NFkB and VBP1, which indicates the process of formation of stable complex among ligand and receptor molecule. Root-mean-square deviation (RMSD) shows the distance between atoms. MVV visualizer (http://molexus.io/molegro-molecular-viewer/)[34] is used for visualisation of structures & their interactions [30,31].

3. RESULTS AND DISCUSSION

The comparative results were obtained from (using hex-docking server) docking simulation with different models of HBx & VBP1(table 1) and between VBP1& different types of NFkB (table-2). The interactions have been analysed to find out the residues that are involved in binding site residues and the number of hydrogen bonds is involved in interaction among them. Docking energy for HBx model 1 was found to be favorable for VBP1, which shows that these compounds can get stuck due to positive interaction. The energy bound conformation with lower value shows hydrogen bond interactions are given in (Table-3, Fig 1).

Table 1. Comparative docking simulation results of HBx modelled structure with VBPI structure using hex-docking server

S. No	ligands	E Total (Kcal/mol)	E Shape	E Force	RMSD
1.	Model-1	-855.10	-855.10	00	-1.00
2.	Model-3	-738.20	-738.20	00	-1.00
3.	Model-4	-789.31	-789.31	00	-1.00
4.	Model-5	-472.83	-472.83	00	-1.00

Table 2. Comparative docking simulation results of NFkB1, NFkB2, NFkB3 & NFkB4 with VBPI hex-docking server

S. No	ligands	E Total	E Shape	E Force	RMSD
1.	NFkB1	-870.15	-870.15	00	-1.00
2.	NFkB2	-768.11	-768.11	00	-1.00
3.	NFkB3	-883.70	-883.70	00	-1.00
4.	NFkB4	-758.74	-758.74	00	-1.00

Table 3. VBPI residues interact with HBx structure using hex-docking server; highlighted residues are involved in H-bonding interactions

S. No	ligands	Interacting residues of receptor VBPI	No. of H-bond interaction
1.	Model-1	Arg 161, Glu 92 and Arg 82	3
2.	Model-2	Arg 161, Gln 11, Ala 14, Ile13, Glu 92 and Arg 82	5
3.	Model-3	Arg 161, Glu 92 and Arg 82	3
4.	Model-4	Arg 161, Glu 92 and Arg 82	3

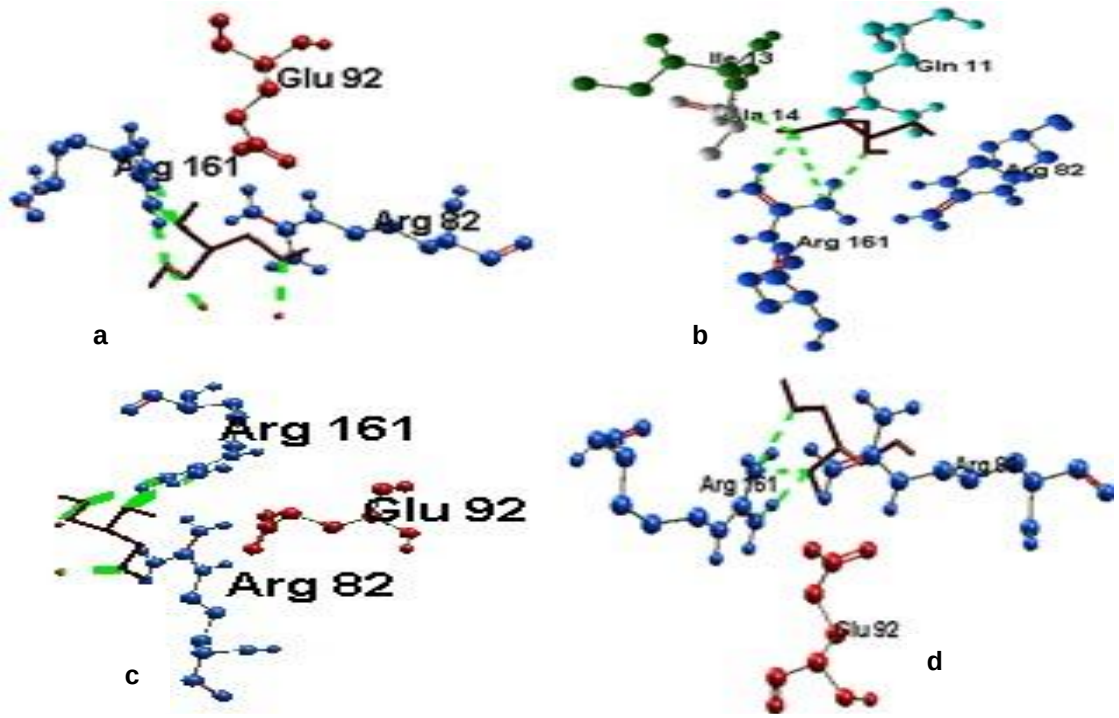

Fig. 1. Docked conformation of hydrogen bonding view of VBPI residues interact with structure model of HBx protein at the active site cavity

The model 1, 2 and 3 show low energy on binding with VBP1 and shows favourable interactions with Arg 161, Glu 92 and Arg 82 residues of VBP1. Among all these, model 1 has -855 Kcal/mol.10 Kcal/mol energy, which is the lowest docking energy. All these residues involved in binding belong to cavity-1. It forms 3 hydrogen bonds with Arg 161 (Table -3 and Fig. 1a). While, the model 2 also shows very high binding energy on binding with VBP1 and it interacts with Arg 161, Gln 11, Ala 14, Ile13, Glu 92 and Arg 82 residues of VBP1. All these residues involved in binding belong to the cavity-1. It forms 5 hydrogen bonds with Arg 161, Gln 11, Ala 14 (Table -3 and Fig. 1 b). Hydrogen bonding is very significant for the interaction of biomolecules. The VBP1 shows -870.15 binding energy on docking with NFkB1 and this interacts with Arg 161, Glu 38, Glu 92 and Arg 82 residues of VBPI. This forms 4 hydrogen bonds with

NFkB1 protein (Table-4 and Fig. 2 a), which shows maximum interactions among all NFkB proteins. Docking of NFkB 3 with VBP1 shows lowest energy -883.70 Kcal/mol but comparatively lower interactions than NFkB1. The VBP1shows very high binding energy to bind with NFkB3 & NFkB4 and it interacts with Arg 161, Glu 92 and Arg 82 residues of VBPI Fig. 2 c & Fig. 2 d respectively. All these residues are involved in binding. They form 3 hydrogen bonds with Arg 161 (Table -4).

NFkB2 interacts with Arg 161, Lys 91, Glu 92 and Arg 82 residues of VBPI. All these residues involved in binding, belong to the cavity-1. It forms 3 hydrogen bonds with Arg 161 (Table -4 and Fig. 2 b) but docking of VBP1 with NFkB2 and NFkB4 shows -768.11, -758.74 respectively less negative energy in comparison to NFkB1 and NFkB3 which have -870.15 and -883.70 Kcal/mol energy.

Protein (VBP1) interacts with von Hippel-Lindau protein (VHL), Complex of these two proteins binds with HBx [32] Tsuchiya H, Tokuhiro I seda]. Recently it has been reported that VHL is associated with the regulation of NFκB [33,34]. Amino acids involved in the interactions are revealed and reported here. The hydrogen bonding are very significant for the interactions of biomolecules.

Table 4. VBPI residues interact with NFkB1, NFkB2, NFkB3 & NFkB4 ligands, using hex-docking server; Highlighted residues are involved in H-bonding interaction with ligands

S. No	ligands	Interacting residues of receptor VBPI	No. of H-bond interaction
1.	NFkB1	Arg 161, Glu 38, Glu 92 and Arg 82	4
2.	NFkB2	Arg 161, Glu 92 and Arg 82, Lys 91	3
3.	NFkB3	Arg 161, Glu 92 and Arg 82	3
4.	NFkB4	Arg 161, Glu 92 and Arg 82	3

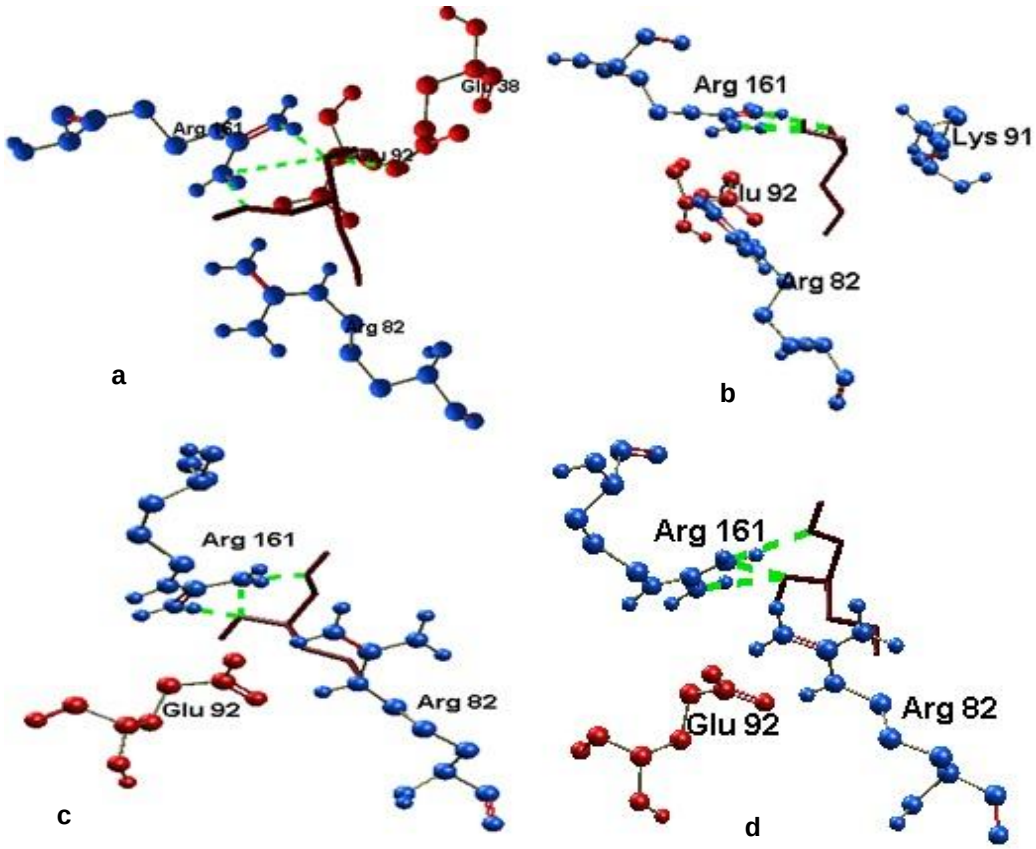

Fig. 2. Docked conformation of hydrogen bonding view of VBPI residues interact with NFkB protein structure at the active site cavity

4. CONCLUSION

It is reported in the literature that VHL binding protein (VBP1) binds to HBx and VBP1 binds with NFkB. Virtual Docking of VBP1 with HBX and NFκB protein helps us to study the activation of NFκB

in the regulatory mechanism of Liver Cancer. Docking results of this study indicate that VBP1 might be involved in the regulatory mechanism of HBx in the activation of NFκB. HBx protein shows interaction with Arg 161, Glu 92 and Arg 82 amino acids of VBP1 protein with -855.10 Kcal/mole energy. VBP1 and NFκB docking revealed NFκB 1 interacts with Arg 161, Glu 38, Glu 92 and Arg 82 amino acids of VBP1 protein with four (4) Hydrogen bonds, which shows maximum interactions. The VBP1 shows comparatively lower binding energy on docking with NFkB2 and NFkB4, but is higher than NFkB1 and NFkB3. The lowest energy complex is VBP1- NFkB1, obtained at -883.70 Kcal/mol. These amino Acids interactions play an important role in the manifestation of Liver cancer. The amino acids involved in interaction among HBx, VBP1 and NFκB proteins, may be involved in transcriptional regulation and may be significant in normal and abnormal regulation. The interactions may be analysed further to understand the modular function of these proteins.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Bachar O, Fischer D, Nussinov R, Wolfson HJ, A computer vision based technique for 3D sequence-independent structural comparison of proteins. Protein Eng., 1993;6:279–288.
2. Katchalski-Katzir E, Shariv I, Eisenstein M, Friesem, AA, Aflalo, C and Vakser I A () Molecular surface recognition: determination of geometric fit between proteins and their ligands by correlation techniques. Proc. Natl Acad. Sci. USA. 1992;89:2195–2199.
3. Comeau SR, Gatchell,DW, Vajda S and Camacho CJ. ClusPro: A fully automated algorithm for protein-protein docking. Nucleic Acids Res. 2004;32:W96–W99.
4. Tovchigrechko A. and Vakser IA. GRAMM-X public web server for protein-protein docking. Nucleic Acids Res.2006;34: W310–W314.
5. Chen R, Li L and Weng Z. ZDOCK: An initial-stage protein-docking algorithm. Proteins: Struct. Func. Bioinf. 2003;52,80–87.
6. Ritchie DW, Kemp GJL. Protein docking using spherical polar Fourier correlations. Proteins: Struct. Func. Genet. 2000; 39:178–194.
7. Ritchie D, Kozakov D and Vajda S. Accelerating and focusing protein-protein docking correlations using multi-dimensional rotational FFT generating functions. Bioinformatics. 2008;24:1865–1873.
8. Parkin DM, Pisani P, Ferlay J. Estimates of the worldwide incidence of 25 major cancers in 1990. Int. J. Cancer. 1999;80:827–841.
9. Gong D, Chen E, Huang F, Leng X, Cheng X, Tang H. Role and functional domain of hepatitis b virus x protein in regulating hbv transcription and replication *In vitro* and *In vivo*. Viruses. 2013;5(5), 1261-1271.

   DOI: 10.3390/v5051261
10. Bondar T, Ponomarev A, Raychaudhuri P. Ddb1 is required for the proteolysis of the Schizosaccharomyces pombe replication inhibitor Spd1 during S phase and after DNA damage. J. Biol. Chem. 2004;279:9937–9943.
11. Bontron S, Lin-Marq N, Strubin M. Hepatitis B virus X protein associated with UV-DDB1 induces cell death in the nucleus and is functionally antagonized by UV-DDB2. J. Biol. Chem. 2002;277:38847–38854.
12. Bressac B, Galvin KM, Liang TJ, Isselbacher KJ, Wands JR, Ozturk M. Abnormal structure and expression of p53 gene in human hepatocellular carcinoma. Proc. Natl. Acad. Sci. USA 1990;87:1973–1977.
13. Chen HS, Kaneko S, Girones R, Anderson RW, Hornbuckle WE, Tennant BC, Cote PJ, Gerin JL, Purcell RH, Miller RH. The woodchuck hepatitis virus X gene is important for establishment of virus infection in woodchucks. J. Virol. 1993;67:1218–1226.

14. Colgrove R, Simon G, Ganem D. Transcriptional activation of homologous and heterologous genes by the hepatitis B virus X gene product in cells permissive for viral replication. J. Virol. 1989;63:4019–4026.

15. Dandri M, Petersen J, Stockert RJ, Harris TM, Rogler CE. Metabolic labeling of woodchuck hepatitis B virus X protein in naturally infected hepatocytes reveals a bimodal half-life and association with the nuclear framework. J. Virol. 1998;72:9359–9364.

16. Doitsh G, Shaul Y. HBV transcription repression in response to genotoxic stress is p53-dependent and abrogated by pX. Oncogene. 1999;18:7506–7513.

17. Doria M, Klein N, Lucito R, Schneider RJ. The hepatitis B virus HBx protein is a dual specificity cytoplasmic activator of Ras and nuclear activator of transcription factors. EMBO J. 1995;14:4747–4757.

18. Duflot A, Mehrotra R, Yu SZ, Barraud L, Trepo C, Cova L. Spectrum of liver disease and duck hepatitis B virus infection in a large series of Chinese ducks with hepatocellular carcinoma. Hepatology. 1995;21:1483–1491.

19. Ganem D, Schneider R. Hepadnaviridae: the viruses and their replication, p. In D. Knipe, Howley P, Griffin D, Lamb R, Martin M, Roizman B, Strauss S. (ed.), Fields virology, 4th ed. Lippincott Williams & Wilkins, Philadelphia, Pa. 2001;2923–2969.

20. Brinke A, Green PM, Giannelli F. Characterization of the gene (VBP1) and transcript for the von Hippel-Lindau binding protein and isolation of the highly conserved murine homologue. Genomics. 1997;45:105-112

21. Van Molle I, Thomann A, Buckley DL, So EC, Lang S, Crews CM, Ciulli A. Dissecting fragment-based lead discovery at the von Hippel Lindau protein: hypoxia inducible factor 1α protein-protein interface. Chem.Biol. 2012;19:1300.

22. Jackson, R.M., et al. 1998 Rapid refinement of protein interfaces incorporating solvation: application to the docking problem. J. Mol. Biol. 276 265–285

23. Ta HM, Nguyen GTT, Jin HM, Choi JK, Park H, Kim NS, Hwang HY, Kim KK. Structure-based development of a receptor activator of nuclear factor-kappaB ligand (RANKL) inhibitor peptide and molecular basis for osteopetrosis. Natl. Acad.Sci.USA. 2010;107:20281-20286

24. Liu C, Walter TS, Huang P, Zhang S, Zhu X, Wu Y, Wedderburn LR, Tang P, Owens RJ, Stuart, DI, Ren J, Gao B. Structural and functional insights of RANKL-RANK interaction and signaling. J.Immunol PubMed: 2010;20483727.

DOI: 10.4049/jimmunol.0904033

25. Nelson CA, Warren JT, Wang MW, Teitelbaum SL, Fremont DH. RANKL employs distinct binding modes to engage RANK and the osteoprotegerin decoy receptor. Structure. 2012;20:1971-1982

26. Gary Macindoe, Lazaros Mavridis, Vishwesh Venkatraman, Marie-Dominique Devignes and David W. Ritchie, HexServer: an FFT-based protein docking server powered by graphics processors, Nucleic Acids Research.2010; Vol. 38: Web Server issue W445–W449

DOI: 10.1093/nar/gkq311

27. Mendez R, Leplae R, De Maria L. and Wodak SJ. Assessment of blind predictions of protein-protein interactions: current status of docking methods. Proteins: Struct. Func. Genet. 2003;52: 51–67

28. David W. Ritchie, 2003, Evaluation of Protein Docking Predictions Using Hex 3.1 in CAPRI Rounds 1 and 2, PROTEINS: Structure, Function, and Genetics 52:98 – 106 (2003)

29. David W Ritchie, Dima Kozakov and Sandor Vajda. Accelerating and focusing protein–protein docking correlations using multi-dimensional rotational FFT generating functions. 2008;Vol. 24: no. 17 2008;1865–1873

DOI: 10.1093/bioinformatics/btn334

30. Thomsen R, Christensen MH. MolDock; a new technique for high-accuracy molecular docking. J Med Chem. 2006;49(11):3315–3321

31. Saxena P. Computational Prediction of Binding of Methyl Carbamate, Sarin, Deltamethrin and Endosulfan Pesticides on Human Oxyhaemoglobin. Jordon Journal of Biological Sciences. 2013; 6(4):320–323.

32. Tsuchiya H, Tokuhiro Iseda, Okio Hino. Identification of a novel protein (VBP-1)

binding to the von Hippel-Lindau (VHL) tumor suppressor gene product. Cancer Res. 1996;56(13):2881-5.

33. An J, Rettig MB. Mechanism of von Hippel- Lindau protein-mediated suppression of nuclear factor kappa B activity. Mol. Cell. Biol. 2005;25:7546-7556

34. http://molexus.io/molegro-molecular-viewer/

Peer-review history:
The peer review history for this paper can be accessed here:
https://www.sdiarticle5.com/review-history/74579