Computational biology approaches in drug discovery against hepatitis-B

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Abstract

Hepatitis B virus (HBV) infects 250 million people worldwide, resulting in nearly one million deaths annually. HBV reactivation due to persistence of HBV genomic reservoirs is the major clinical limitation for currently available anti-HBV agents. There is a need, therefore, for understanding persistence mechanisms of HBV and to develop novel antiviral agents against HBV. Computational biology approaches like homology modeling, molecular dynamics and bioinformatics are being employed in recent years to understand the mutations of drug resistance and persistence in HBV and to study the DNA and RNA protein sequences of viruses. In the present review we discuss recent studies on discovery of anti-HBV agents using these computational approaches.

1. Introduction

Chronic hepatitis B virus (HBV) affects 250 million people worldwide and is the major cause for liver cancer and cirrhosis [1]. Current treatment with oral nucleos(t)ides entecavir or tenofovir provide sustained suppression of HBV. However, relapse of HBV after drug discontinuation is the major limitation. Persistence of genomic HBV reservoirs as episomic cccDNA and chromosomic integrated HBV-DNA is responsible for the rebound HBV. Researchers, therefore, focused on drug development to prevent HBV persistence and relapse. Computational chemistry incorporates the ideas of theoretical chemistry into computer programs, which are then able to simulate chemical structures, chemical properties, and molecular interactions [2, 3]. In recent years, advances in computational chemistry led to the development of novel therapeutics for major viruses [4]. Computational chemistry tools can be divided into (i) Bioinformatics, the combination of computer science, statistics, mathematics, and engineering to study biological data to study genetic basis for disease [5] and (ii) molecular modeling, the series of techniques that use mathematics, physics, and chemistry to simulate the behavior of complex chemical systems. Molecular modeling can be further sub divided into three major techniques: homology modeling, docking, and molecular dynamics. Homology modeling is used to construct atomic resolution protein models based on their amino acid sequences and structural similarities. Docking is used to analyze ligand–protein interactions, and to determine where on the protein the ligands are most likely to bind. Finally, molecular dynamics is used to simulate the movement and behavior of molecules in their natural environment. All of these modeling techniques and bioinformatics methods have contributed significantly to viral research, including the field of hepatitis B, and towards the development of new therapies. The present review makes an effort to discuss these computational approaches.

2. Computational biology approaches

2.1. Homology modeling

Homology modeling is a comparative modeling for prediction of protein structure prediction by alignment of amino acid sequence of target protein and evolutionarily related protein with a known structure to produce an approximate 3D model structure of an unknown protein [6]. Homology modeling provide a basis for structure-based drug design and can be useful in reaching qualitative assumptions about the protein. However, low sequence identity, flexible loops, and errors in side chain packing lead to imperfect models. There is a variety of

software available for use in creating, refining, and validating homology models including, MODELLER [7-10], HHPred, SWISS-MODEL, Rosetta, RapotrX, COMPOSER and CONGEN. Some molecular modeling suites that are used in homology modeling such as Prime, distributed by Schrödinger [11] and SYBYL-X, developed by Tripos/Certara are commercially available for purchase and download. MODELLER and SYBYL, have been the most widely used packages in creating homology models of hepatitis B viral proteins. There are a number of freely available programs used for homology model verification that generally fall under one of two categories. Programs such as the widely used PROCHECK, WHATCHECK, and WHATIF, all validate models based on accurate stereochemistry of the local atomic details. Other programs, such as VERIFY3D, validate models by assigning scores for residues in their current environment and evaluating the fitness of sequence to structure [6].

Reverse transcriptase (RT) domain of HBV DNA polymerase (HBV-pol), is a major target for drugs against HBV. Das et al., have built the protein structure of HBVpol with MODELLER & SYBL using the crystal structures of HIV-1 RT–DNA–dNTP (PDB ID: 1RTD) and murine leukemia virus (MuLV) RT domain as templates for the model Comparative models retain accuracy only within and near the active site of HBV-pol where sequence similarity is high than in other areas of the model. In this study, it was found that, HIV and MuLV, posses high conservation of functionally important amino acid residues, especially in the active site, validating their use as template structures. The developed 3D model was used to study the mutations responsible for drug resistance in HBV[12]. It was reported that, V173L, enhances viral replication efficiency in conjunction with mutations L180M and M204V In this study, V173L was positioned near the active site of HBV-pol directly under the template strand of the viral nucleic acid. The study suggested that the leucine mutation effects viral replication capacity by repositioning the template nucleic acid or, due to the close proximity of V173L and F88, altering the environment of regions that undergo conformational change during polymerization, leading to enhanced polymerization efficiency [13].

2.2. Molecular docking

Molecular docking predict the most likely occurring orientation of a compound when bound to a protein and reports an energy for the pose based on a scoring function. Molecular

docking is a valuable tool for virtual screening. Molecular Docking helps in understanding the conformation of binding and is useful as a starting point for structure-based refinement. Some commonly used docking software include Autodock [14], GOLD[15], UCSF DOCK [16] and GLIDE [17, 18]. Some docking web servers include SwissDock [19] and DockingServer [17, 20]. Docking has been frequently used in HBV studies to analyze interactions between potential anti-HBV drugs and drugs approved for HBV therapy. Pathak tested plant derived natural compounds for their anti-HBV potential by using flexible docking to dock these compounds into the HBx protein, showing rutin as a potent anti-HBV agent[21]. Arooj et al., have tested various ligands for their affinity towards the HBV target protein GIPC2 using in silico rigid docking with AutoDock [22]. The protein ligand conformations with the lowest, most favorable, score were tested for bioactivity using quantitative structure activity relationship and helioxanthin analog 1–8 was identified as the lead compound. Sixteen residues were found with five angstroms of GIPC2's active site and shown to have a large part in determining ligand binding. It was also reported that lamivudine had better affinity for GIPC2 than adefovir for GIPC2 protein based on molecular docking study [23]. Shahabadi and Falsafi have docked adefovir with reverse transcriptase DNA sequence using in silico flexible docking in Autodock [24]. It was found that, Adefovir binds to the minor groove of DNA within A–T regions with mainly hydrophobic interactions. Ismail et al., have obsereved that mutation of I233V does not alter the binding site and also affinity of Adefovir with HBV-pol. Ismail also docked entecavir into HBV-pol using Autodock and related the results to human subjects that developed no antiviral resistance mutations to entecavir The V173L mutation was shown to not change the entecavir binding site and the binding efficiency of entecavir [25].

2.3. Molecular dynamics

Molecular dynamics evaluate the protein–ligand motions between molecules, polypeptide folding, partitioning between solvents, and membrane or micelle formation. The main application of molecular dynamics, for HBV research, is the conformation of the HBV mutant polymerase strains and the binding of NNRTI drugs and other inhibitors [26-28]. Molecular dynamics is being used in binding free energy simulations (free energy perturbation, thermal integration, etc.), a calculation not done with docking. Energy minimization is done prior to molecular dynamics simulations, and after the simulations are

complete, the stabilization of the molecule is determined and is used to test the conformational rearrangements of the molecule. Multiple programs have been used to perform the molecular dynamics simulations and are free or commercial programs. Program examples include: GROMACS, a free program used for high performance molecular dynamics simulations; NAMD, another free program used in fast parallel molecular dynamics; VMD, Visual Molecular Dynamics, a free program used to visualize the molecular graphics; METAGUI, an extension of the VMD program, it is a tool for analyzing metadynamics based simulations; VLifeMDS; USCF Chimera, another free program that is used to analyze or setup the molecular model; and Desmond, a free program for universities and non-profits, but requires a license for commercial applications. A critique for MD is that it takes a long time, a large amount of computational power, and is not held up to the same criteria as other methods. Chong et al. reported the mechanism behind the binding of clevudine to the HBV-pol RT domain using energy minimization, MD, and Monte Carlo conformational search [26]. It was reported that the L-FMATP can act as a competitive inhibitor of the HBV-pol in HBV polymerase:DNA:L-FMAU-TP complex. This results of this study suggests L-FMAU-TP occupies the catalytic site of the polymerase, which inhibits the priming of the HBV-DNA chain elongation, thus inhibiting replication of the HBV.

Coarse-grained simulations are a type of MD simulations that require less computational processing power, as they consider the protein domains as single units thus not treating each atom individually. Roos et al. used coarse-grained MD simulations to measure the mechanical responses of viral capsids, specifically HBV, and compare them with theoretical descriptions [29]. Li et al. used MD simulations to generate the data to create and test the binding affinity for adefovir [30]. The simulations were trajectory analysis, binding free energy decomposition, and alanine scanning, which were used to determine the contribution of specific residues in the complex, to establish a stable conformation for molecular docking studies.

2.4. Bioinformatics tools

Bioinformatics is the study of DNA, RNA, and protein sequences with the help of computational software. It focuses on the sequences themselves, rather than the structures that they form. Several online sequence databases serve as sources of reference sequences, as well as aggregators of user-submitted data. The National Center for Biotechnology

Information (NCBI) in Bethesda, MD hosts GenBank. As of fourth quarter 2014, GenBank was the home to nucleotide sequences for over 300,000 species. These sequences are collected from submissions from authors and sequencing facilities [31]. The NCBI website also contains a broad array of bioinformatics tools and resources accessible for free: http://www.ncbi.nlm.nih. gov/guide/all/ [32]. The Europea Bioinformatics Institute and the DNA Data Bank of Japan also host databases and tools similar to GenBank's [33]. Several bioinformatics tools are available for genotyping the HBV genome, as well as looking for drug resistance (Table 2).

3. Future directions

Comparitive modeling can be used to study novel target proteins of HBV

HBV-pol has been the primary target of comparative modeling studies. However, in recent years researchers have also focused on studying other HBV proteins of potential interest. In 2007, Potenza et al. developed a comparative model of HBV RNase using crystal structures from Escherichia coli and Bacillus haloduransRNase as templates [34]. In a recent study, E. coli RNase H was utilized as a template to produce a model of HBV RNase and identified the viral protein as a potential drug target [35]. It was concluded that HBV RNase H is a type 1 RNase and that treatments effective against other type 1 RNases should be tested against HBV, supporting experimental research, in which several drugs targeting HIV RNase H retained high inhibition when tested against HBV RNase [36]. These initial models of HBV RNase H will need to be improved before they can find general use in antiviral research. Although template-based comparative modeling is a very useful tool in producing 3D models of proteins with unknown structures, new techniques such asab initio/de novofolding and threading methods have proven to be very useful in protein structure prediction, especially when there is low or no sequence homology with a protein whose structure is known. Programs such as QUARK, and I-TASSER, which are free to download use a combination of ab initio folding and threading techniques and were deemed the best structure prediction programs in CASP 10 [37]. Major advances in software, genome sequencing, algorithms, computational power, as well as crystallization of more viral proteins with higher sequence identify may allow us to build viable 3D models of such proteins, greatly accelerating the movement towards a cure for HBV.

Molecular Docking can be used in hit identification and lead optimization

Similarly for HBV, docking can be used to screen compounds for allosteric inhibitors that target HBV-pol and could be used in combinations with NNRTIs. For further discovery of anti-HBV drugs, docking may be used in (i) hit identification, where large databases of potential drugs are screened, in silico, for molecules that might bind to the target protein and (ii) lead optimization, where docking helps determine which molecules might have better enthalpic interactions and thus an increased biological effect. Advances in molecular docking programs have focused on problems with adequate protein–ligand pose sampling, docking scoring algorithms and receptor flexibility for ligand binding [38]. As more 3-D models of HBV proteins, or better homology models, become available and accuracy of docking increases, molecular docking will become more useful for anti-HBV drug discovery.

Molecular dynamics and combination therapy of HBV

Emeriging body of evidences suggest that combination drug therapy should be utilized to maximize replication suppression, which will decrease the emergence of drug resistance. Future HBV research will experiment in determining the interactions between novel drugs, and the possible directions for development of drugs combinations to more efficiently and specifically inhibit HBV. This specificity will allow for more effective medication that will reduce the mutational resistance, as well as better assist the patient. MD could aid in the understanding of how two drugs binding to the same target interact and change the protein conformation. Homology modeling is inter-related to MD, because the homology models can be validated and refined with MD. Langlet et al. performed MD simulations in order to equilibrate a dNTP binding site on a molecular model of HBV-pol/DNA complex [39]. This was done to observe the inhibition of HBV-pol when exposed to entecavir.

There are multiple constraints that can be applied to MD simulations, with examples such as: the restriction of interactions of residues that are within a certain distance from the active site and the stability of the molecule [26]. This improvement in simulation software will be beneficial in HBV research by increasing the accuracy of the data and revealing structures that were previously hidden, due to the condensed timeframe. The use of coarse-grain MD simulations has been used to study viral associations, specifically in the assembly/disassembly properties of the capsid. Arkhipov et al. performed experiments that

utilized coarse-grain MD simulations to impair HBV with indentations on the viral capsids. The data suggested that the deformation was plastic and capsid geometry can undergo smooth transitions. The data further concluded that determining viral mechanics will be useful in future research, which could be applied to HBV-drug interactions. A future step would be the construction on an all-atom model of the entire hepatitis B virus, as performed by Zhao et al. in relation to HIV. This would further the understanding of drug-virus interactions, and mutations on viral assembly/disassembly, not simply single protein interactions. It would also assist in the understanding of the timing of the capsid opening, an essential element for the degree of virulence in HBV. However, this is beyond the current computational analysis available, but Moore's Law will change this over the next 5– 10 years, allowing for a better understanding of different virus interactions with their host [3].

Linking databases for better drugs against HBV

Moriconi et al. points out it also may be beneficial to link pathogen-specific databases to host-specific databases to find host-virus interactions. This database linking as well as the creation of new tools and databases will necessitate the creation of standardized methods to manage the large amounts of new data. Linking other databases, such as medicinal plant databases may also be beneficial. [40]. Network pharmacology could help lead the way to finding new drugs, as over 80% of new drugs bind to targets associated with previous drugs [41]. As more data is collected, databases will grow and provide a better understanding of the HBV virus, leading to better diagnostic tools. This combined approach of predicting drug resistance as well as creating new drugs through the use of improved bioinformatics tools and databases will allow for better treatment options as well as improved patient outlooks.

4. Conclusion

Although the importance of computer tools and simulations are not stressed enough, they are valuable tools in antiviral drug discovery. In recent years, Computational biology, as a whole, has allowed us to progress dramatically in the field of virology by allowing us to simulate and analyze scenarios which otherwise would be too hard or costly to examine experimentally.

Table1. Bioinformatics in anti-HBV research

Sl.No	Purpose	Exp.Results	Citation
1	For a comparative analysis of antiviral resistance mutations between HIV-RT and HBV-pol	several key resistance mutations in HIV-RT were highly conserved in similar locations within HBV-pol	[12, 42]
2	To investigate the interaction of HBV-pol and certain analog drugs used to treat HBV. One study built HBV-pol homology models with HIV1-RT (1RTD) as a template in order to explore how entecavir (ETV) might function differently than other nucleoside analogs. Multiple models were developed to represent wild type HBV RT, and compare it to HBV RT with lamivudine resistance, adefovir resistance, and ETV substitutions.	The models produced in this study suggested that high potency of ETV is a result of an optimal fit of ETV into a novel pocket in the rear of dNTP binding site of HBV-pol, whereas other analogs, such as lamivudine and adefovir might distort the pocket when bound	[39]
3	To investigate efficacy of adefovir and how it is affected by the I233V mutation using HIV-1 RT (1RTD) as the template structure	The model showed that the mutation is located far from the drug binding site, suggesting that the I233V mutation does not independently reduce antiviral effectiveness of adefovir, but may be more important in combination with other mutations.	[43]

Tool	Website	Description	Citation
HepSEQ	www.hepseq.org	tools to analyze data capable of genotyping, identifying mutations, and searching for homologies	[44, 45]
HBVdb	hbvdb.ibcp. fr/HBVdb/	makes use of an automated procedure to annotate all HBV sequences from the European Nucleotide Archive based on a reference set of genomes. also allows for the annotation of user input sequences, as well as genotyping and screening for potential drug resistance	[46]
HBVregDB	http://lancelot.otago.ac.nz	serves as a source of annotated HBV genomes as well as hosting comparative analysis tools	[47]
SmallGenomeTools	http://hvdr.bioinf.wits.ac.za	a suite of tools to help with the HBV bioinformatics workflow	[48]

References

[1] Soriano, V.; Barreiro, P.; Benitez, L.; Peña, J.M.; de Mendoza, C., New antivirals for the treatment of chronic hepatitis B. *Expert Opinion on Investigational Drugs*, **2017**, (just-accepted).
[2] Jorgensen, W.L., The many roles of computation in drug discovery. *Science*, **2004**, *303*, (5665), 1813-1818.
[3] Morgnanesi, D.; Heinrichs, E.J.; Mele, A.R.; Wilkinson, S.; Zhou, S.; Kulp, J.L., A computational chemistry perspective on the current status and future direction of hepatitis B antiviral drug discovery. *Antiviral Research*, **2015**, *123*, (Supplement C), 204-215.
[4] Sliwoski, G.; Kothiwale, S.; Meiler, J.; Lowe, E.W., Computational methods in drug discovery. *Pharmacological reviews*, **2014**, *66*, (1), 334-395.
[5] Qi, H.; Wu, N.C.; Du, Y.; Wu, T.-T.; Sun, R., High-resolution genetic profile of viral genomes: why it matters. *Current opinion in virology*, **2015**, *14*, 62-70.
[6] Vyas, V.; Ukawala, R.; Ghate, M.; Chintha, C., Homology modeling a fast tool for drug discovery: current perspectives. *Indian journal of pharmaceutical sciences*, **2012**, *74*, (1), 1.
[7] Šali, A.; Blundell, T.L., Comparative protein modelling by satisfaction of spatial restraints. *Journal of molecular biology*, **1993**, *234*, (3), 779-815.
[8] Arnold, K.; Bordoli, L.; Kopp, J.; Schwede, T., The SWISS-MODEL workspace: a web-based environment for protein structure homology modelling. *Bioinformatics*, **2006**, *22*, (2), 195-201.
[9] Kaufmann, K.W.; Lemmon, G.H.; DeLuca, S.L.; Sheehan, J.H.; Meiler, J., Practically useful: what the Rosetta protein modeling suite can do for you. *Biochemistry*, **2010**, *49*, (14), 2987-2998.
[10] Li, H.; Tejero, R.; Monleon, D.; Bassolino- Klimas, D.; Abate- Shen, C.; Bruccoleri, R.E.; Montelione, G.T., Homology modeling using simulated annealing of restrained molecular dynamics and conformational search calculations with CONGEN: Application in predicting the three- dimensional structure of murine homeodomain Msx- 1. *Protein Science*, **1997**, *6*, (5), 956-970.
[11] Nayeem, A.; Sitkoff, D.; Krystek, S., A comparative study of available software for high- accuracy homology modeling: From sequence alignments to structural models. *Protein Science*, **2006**, *15*, (4), 808-824.
[12] Das, K.; Xiong, X.; Yang, H.; Westland, C.E.; Gibbs, C.S.; Sarafianos, S.G.; Arnold, E., Molecular modeling and biochemical characterization reveal the mechanism of hepatitis B virus polymerase resistance to lamivudine (3TC) and emtricitabine (FTC). *Journal of virology*, **2001**, *75*, (10), 4771-4779.
[13] Delaney, W.E.; Yang, H.; Westland, C.E.; Das, K.; Arnold, E.; Gibbs, C.S.; Miller, M.D.; Xiong, S., The hepatitis B virus polymerase mutation rtV173L is selected during lamivudine therapy and enhances viral replication in vitro. *Journal of virology*, **2003**, *77*, (21), 11833-11841.
[14] Morris, G.M.; Huey, R.; Lindstrom, W.; Sanner, M.F.; Belew, R.K.; Goodsell, D.S.; Olson, A.J., AutoDock4 and AutoDockTools4: Automated docking with selective receptor flexibility. *Journal of computational chemistry*, **2009**, *30*, (16), 2785-2791.
[15] Jones, G.; Willett, P.; Glen, R.C., Molecular recognition of receptor sites using a genetic algorithm with a description of desolvation. *Journal of molecular biology*, **1995**, *245*, (1), 43-53.

[16] Lang, P.T.; Brozell, S.R.; Mukherjee, S.; Pettersen, E.F.; Meng, E.C.; Thomas, V.; Rizzo, R.C.; Case, D.A.; James, T.L.; Kuntz, I.D., DOCK 6: Combining techniques to model RNA–small molecule complexes. *Rna*, **2009**, *15*, (6), 1219-1230.
[17] Chen, Y.-C., Beware of docking! *Trends in pharmacological sciences*, **2015**, *36*, (2), 78-95.
[18] Friesner, R.A.; Banks, J.L.; Murphy, R.B.; Halgren, T.A.; Klicic, J.J.; Mainz, D.T.; Repasky, M.P.; Knoll, E.H.; Shelley, M.; Perry, J.K., Glide: a new approach for rapid, accurate docking and scoring. 1. Method and assessment of docking accuracy. *Journal of medicinal chemistry*, **2004**, *47*, (7), 1739-1749.
[19] Grosdidier, A.; Zoete, V.; Michielin, O., SwissDock, a protein-small molecule docking web service based on EADock DSS. *Nucleic acids research*, **2011**, *39*, (suppl_2), W270-W277.
[20] Bikadi, Z.; Hazai, E., Application of the PM6 semi-empirical method to modeling proteins enhances docking accuracy of AutoDock. *Journal of cheminformatics*, **2009**, *1*, (1), 15.
[21] Pathak, R.K.; Baunthiyal, M.; Taj, G.; Kumar, A., Virtual screening of natural inhibitors to the predicted HBx protein structure of Hepatitis B Virus using molecular docking for identification of potential lead molecules for liver cancer. *Bioinformation*, **2014**, *10*, (7), 428.
[22] Arooj, M.; Sattar, M.; Safdar, M.; Kalsoom, S.; Shahzad, S., In silico molecular docking and design of anti-hepatitis B drugs. *IOSR-JPBS*, **2012**, *3*, 41-55.
[23] Daga, P.R.; Duan, J.; Doerksen, R.J., Computational model of hepatitis B virus DNA polymerase: molecular dynamics and docking to understand resistant mutations. *Protein Science*, **2010**, *19*, (4), 796-807.
[24] Shahabadi, N.; Falsafi, M., Experimental and molecular docking studies on DNA binding interaction of adefovir dipivoxil: Advances toward treatment of hepatitis B virus infections. *Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy*, **2014**, *125*, 154-159.
[25] Ismail, A.M.; Sharma, O.P.; Kumar, M.S.; Eapen, C.E.; Kannangai, R.; Abraham, P., Virological response and antiviral resistance mutations in chronic hepatitis B subjects experiencing entecavir therapy: an Indian subcontinent perspective. *Antiviral research*, **2013**, *98*, (2), 209-216.
[26] Chong, Y.; Chu, C.K., Understanding the unique mechanism of L-FMAU (clevudine) against hepatitis B virus: molecular dynamics studies. *Bioorganic & medicinal chemistry letters*, **2002**, *12*, (23), 3459-3462.
[27] Doo, E.; Liang, T.J., Molecular anatomy and pathophysiologic implications of drug resistance in hepatitis B virus infection. *Gastroenterology*, **2001**, *120*, (4), 1000-1008.
[28] Ghany, M.; Liang, T.J., Drug targets and molecular mechanisms of drug resistance in chronic hepatitis B. *Gastroenterology*, **2007**, *132*, (4), 1574-1585.
[29] Mamaeva, V.; Niemi, R.; Beck, M.; Özliseli, E.; Desai, D.; Landor, S.; Gronroos, T.; Kronqvist, P.; Pettersen, I.K.; McCormack, E., Inhibiting notch activity in breast cancer stem cells by glucose functionalized nanoparticles carrying γ-secretase inhibitors. *Molecular Therapy*, **2016**.
[30] Li, W.; Cowley, A.; Uludag, M.; Gur, T.; McWilliam, H.; Squizzato, S.; Park, Y.M.; Buso, N.; Lopez, R., The EMBL-EBI bioinformatics web and programmatic tools framework. *Nucleic acids research*, **2015**, *43*, (W1), W580-W584.
[31] Elumalai, P.; Gunadharini, D.; Senthilkumar, K.; Banudevi, S.; Arunkumar, R.; Benson, C.; Sharmila, G.; Arunakaran, J., Induction of apoptosis in human breast cancer cells by nimbolide through extrinsic and intrinsic pathway. *Toxicology letters*, **2012**, *215*, (2), 131-142.

[32] Geer, L.Y.; Marchler-Bauer, A.; Geer, R.C.; Han, L.; He, J.; He, S.; Liu, C.; Shi, W.; Bryant, S.H., The NCBI biosystems database. *Nucleic acids research*, **2009**, *38*, (suppl_1), D492-D496.
[33] Kosuge, T.; Mashima, J.; Kodama, Y.; Fujisawa, T.; Kaminuma, E.; Ogasawara, O.; Okubo, K.; Takagi, T.; Nakamura, Y., DDBJ progress report: a new submission system for leading to a correct annotation. *Nucleic acids research*, **2013**, *42*, (D1), D44-D49.
[34] Potenza, N.; Salvatore, V.; Raimondo, D.; Falanga, D.; Nobile, V.; Peterson, D.L.; Russo, A., Optimized expression from a synthetic gene of an untagged RNase H domain of human hepatitis B virus polymerase which is enzymatically active. *Protein expression and purification*, **2007**, *55*, (1), 93-99.
[35] Hayer, J.; Rodriguez, C.; Germanidis, G.; Deléage, G.; Zoulim, F.; Pawlotsky, J.-M.; Combet, C., Ultradeep pyrosequencing and molecular modeling identify key structural features of hepatitis B virus RNase H, a putative target for antiviral intervention. *Journal of virology*, **2014**, *88*, (1), 574-582.
[36] Tavis, J.E.; Cheng, X.; Hu, Y.; Totten, M.; Cao, F.; Michailidis, E.; Aurora, R.; Meyers, M.J.; Jacobsen, E.J.; Parniak, M.A., The hepatitis B virus ribonuclease H is sensitive to inhibitors of the human immunodeficiency virus ribonuclease H and integrase enzymes. *PLoS pathogens*, **2013**, *9*, (1), e1003125.
[37] Zhang, Y., Interplay of I- TASSER and QUARK for template- based and ab initio protein structure prediction in CASP10. *Proteins: Structure, Function, and Bioinformatics*, **2014**, *82*, (S2), 175-187.
[38] Humphrey, W.; Dalke, A.; Schulten, K., VMD: visual molecular dynamics. *Journal of molecular graphics*, **1996**, *14*, (1), 33-38.
[39] Langley, D.R.; Walsh, A.W.; Baldick, C.J.; Eggers, B.J.; Rose, R.E.; Levine, S.M.; Kapur, A.J.; Colonno, R.J.; Tenney, D.J., Inhibition of hepatitis B virus polymerase by entecavir. *Journal of virology*, **2007**, *81*, (8), 3992-4001.
[40] Moriconi, F.; Beard, M.R.; Yuen, L.K., Bioinformatics methods for the analysis of hepatitis viruses. *Antiviral therapy*, **2013**, *18*, 531-539.
[41] Lagunin, A.A.; Goel, R.K.; Gawande, D.Y.; Pahwa, P.; Gloriozova, T.A.; Dmitriev, A.V.; Ivanov, S.M.; Rudik, A.V.; Konova, V.I.; Pogodin, P.V., Chemo-and bioinformatics resources for in silico drug discovery from medicinal plants beyond their traditional use: a critical review. *Natural product reports*, **2014**, *31*, (11), 1585-1611.
[42] Bartholomeusz, A.; Tehan, B.G.; Chalmers, D.K., Comparisons of the HBV and HIV polymerase, and antiviral resistance mutations. *Antiviral therapy*, **2004**, *9*, (2), 149.
[43] Ismail, A.M.; Sharma, O.P.; Kumar, M.S.; Kannangai, R.; Abraham, P., Impact of rtI233V mutation in hepatitis B virus polymerase protein and adefovir efficacy: Homology modeling and molecular docking studies. *Bioinformation*, **2013**, *9*, (3), 121.
[44] Gnaneshan, S.; Ijaz, S.; Moran, J.; Ramsay, M.; Green, J., HepSEQ: international public health repository for hepatitis B. *Nucleic acids research*, **2006**, *35*, (suppl_1), D367-D370.
[45] Myers, R.; Gnaneshan, S.; Ijaz, S.; Tedder, R.; Ramsay, M.; Green, J.; Committee, H.S., HepSEQ--an integrated hepatitis B epidemiology and sequence analysis platform. *Euro surveillance: bulletin Européen sur les maladies transmissibles= European communicable disease bulletin*, **2008**, *13*, (19).
[46] Hayer, J.; Jadeau, F.; Deléage, G.; Kay, A.; Zoulim, F.; Combet, C., HBVdb: a knowledge database for Hepatitis B Virus. *Nucleic acids research*, **2012**, *41*, (D1), D566-D570.

[47] Panjaworayan, N.; Roessner, S.K.; Firth, A.E.; Brown, C.M., HBVRegDB: annotation, comparison, detection and visualization of regulatory elements in hepatitis B virus sequences. *Virology journal*, **2007**, *4*, (1), 136.
[48] Bell, T.G.; Kramvis, A., Bioinformatics tools for small genomes, such as hepatitis B virus. *Viruses*, **2015**, *7*, (2), 781-797.