Molecular Dynamic (MD) Simulation and Modeling the Bio-molecular Structure of Human UDP glucose -6-dehydrogenase Isoform 1 (hUGDH) Related to Prostate Cancer

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Abstract

Computational methods were used to investigate both the physical and chemical properties of UDP-glucose 6-dehydrogenase (hUGDH). Secondary structure analysis of the query model was done using the Self-Optimized Prediction method With Alignment (SOPMA), the secondary structure predictions comprise of 40.69% Alpha helixes (Hh), 17.61% Extended strand (Ee), 7.69% Beta turn (Tt) and 34.01% of Random coil (Cc) with aliphatic index of 90.00 and instability index of 33.26 which classify the protein model to be thermally stable irrespective of it environment. Comparative modeling was used to predict a reliable tertiary structure for hUGDH and the obtained 3-dimensional model was validated using DOPE score profile, Ramachandran plot, and the QMEAN Z-score. The DOPE score profile shows a high similarity between the model and the template as little or no disparity was found in the profile patterns. Ramachandran plot of the model also shows that 92.5% of the amino acid residues were found at the most favored regions which make it stereo-chemically stable. The QMEAN z-score of UDP-glucose 6-dehydrogenase was predicted to be -0.15. The superimposed structure of the model and the template also gave RMSD of 0.125. All this shows that the predicted model is of good quality. An RMSD and Rg run via molecular dynamics (MD) simulation equally shows that the protein model attained stability at around 10ns. Protein – Protein interaction (PPI) network was also generated for the model with a high confidence score from UDP-glucuronic acid decarboxylase 1 (UXS1) when interacted with the other twenty proteins. In addition, the docking studies of the model and 3PRJ receptors with two prostate cancer drugs i.e. Apalutamide and Darolutamide gave similar binding affinity ranging between 6.0kcal/mol – 8.0kcal/mol for the most favored binding of the two drugs. Hence, the model can serve as a molecular target for designing new inhibitors for prostate cancer.

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1. Introduction

Prostate Cancer is the most commonly identified tumor in men and equally the second death related diseases from cancer [1]. The frequent occurrences of these diseases have been reported to be highest among the African American males and the Caucasian compared to white men. The Caribbeans and Black men from Europe were also suggested to have a genetic background prone to the risk of contacting the ailment. About 1.3 million global cases of prostate cancer were reported in 2018 and Guadeloupe in France had the highest reported cases of approximately 189.1 per 100,000 men. Due to the availability of screened prostate specific antigen (PSA) in men, larger numbers of elderly men are prone to prostate cancer as the risk of being infected increases with age, this has been widely diagnosed among men aged 65 and older [2, 3]. Its symptoms include frequent urination, blood in urine, urge to urinate frequently at night etc. In proteomics, the protein encoded by UGDH (UDP-glucose 6-dehydrogenase) plays a key role in the conversion of UDP-glucuronic acid to UDP-glucuronate which further involved in the biosynthesis of glycosaminoglycans which are hyaluronan, heparin and chondroitin sulfate. All these compounds are components of the extracellular matrix which participates in other cellular processes such as migration of cells, signal transduction and proliferation of cancer cells [4]. Many of the tissues in human expresses UGDH, but the expression is strongly pronounced in prostate and liver [5]. The presence of hyaluronan in high levels during biosynthesis promotes epithelial cancer growth and it has been reported that its limitation at the level of UDP-glucuronic acid also lowers tumor growth. In addition, UGDH has been recently suggested as a new biomarker for prostate tumor cells [6]. Hence, the presence of UDP-glucuronic acid could be restricted in prostate and other cancer cells lines by inhibition of human UGDH which then serve as a drug target for prostate cancer therapy [7]. This work is devoted to predict a new bio-molecular protein structure using comparative modeling. Physicochemical, binding potential and structural properties of the modelled protein were also determined computationally along with its protein–protein interactions.

2. Methodology

2.1 Sequence Retrieval

The amino acid sequence of human UDP glucose -6-dehydrogenase Isoform 1 (hUGDH) used for the comparative modeling was retrieved from Genbank protein database with Accession no

NP_003350.1. It is made up of amino acid residues of 494. And the modeling was performed using MODELLER 9.24.

2.2. Prediction of Primary Structure

The primary structure of the protein structure was analyzed using Expasy's ProtParam tool [8]. Physicochemical properties such as molecular weight, isoelectric point (PI), number of positive and negative residues, grand average hydrophaticity (GRAVY), instability and aliphatic index, extinction coefficient were calculated.

2.3 Secondary Structure Analysis

In other to predict the secondary structure of hUGDH, Self-Optimised Prediction method With Alignment (SOPMA) was used. Analyzed secondary properties include Extended strand, Bend region, Beta turns, Pi helix, Random coil, Isolated β-bridge [9].

2.4 Protein Template Selection

Basic local alignment search tool (BLAST) is highly important in comparative modeling of a protein. This is done in order to compare the sequences of the protein with those in the database to find regions of any similarity between the protein sequence. During the process, protein data bank (PDB) is considered as the database for template selection. The PSI-BLAST threshold was set at 0.0001. Convergence of the BLAST search results occurs after three different iterations [10]. Protein structures with PDB codes 3PRJ, 5VR8, 6C4J and 6C58 were considered as template structures with percentage identity greater than 80.

2.5 Protein Modeling

A comparative modeling of human UDP-glucose 6-dehydrogenase isoform 1 (hUGDH) model was performed using MODELLER 9.24 [11]. This is a homology modeling program that calculates a three-dimensional model of a protein sequence based on sequence alignment and template been supplied. Once the query and the template protein provided are related, a model of high quality is produced but a low-quality model is obtained with sequence identity lower than 30%. During the process, the best template structures for modeling were selected based on their DOPE (Discrete Optimized Protein Energy) and GA341 score [12]. The lowest DOPE score and the closest to 1 GA341 score will indicate the best template for homology modeling [13].

2.6 Protein – Protein Interaction

This predicts interactions that occur between different known and predicted protein structure. Here, we used STRING [14] database to identify interaction network between UGDH and related proteins from database. STRING database compromises approximately 5,214,000 proteins obtained from more than a thousand different organisms [15]. The residue interaction network of UDP-glucose 6-dehydrogenase was analyzed with RING (Residue Interaction Network Generator) server and then visualized with the aid of Cytoscape v 3.1.0 [16].

2.7 Molecular Docking Study

Molecular docking study was performed on the modeled protein to determine the binding affinities of two drugs to the active site of the protein using Autoduck Vina [17]. A grid box dimension was set for the protein structure. A grid box set was (Box Size: 100 x 100 x 112 Å and Box center: -5.797 x 12.232 x 57.505 for x, y and z respectively). Nine binding modes were obtained from the docking results of the modeled protein with prostate cancer drugs. The docking results were visualized by Discovery studio and Pymol [18, 19].

2.8 Molecular Dynamics (MD) simulation (hUGDH)

The preparation of the native hUGDH protein for molecular dynamics (MD) studies was done using GROMACS package version 5.1 (release version 2020.3) for a total time of 10 ns [20]. The main aim of this simulation was to assess the structural stability of the protein and then consequently analyze its functionality. For the creation of the topology file, pdb2gmx function was used, following by the placement of the protein in a cubic box. Afterwards, energy minimization was done using native OPLS force field of GROMACS and solvation was done via TIP3P water model. Moreover, the equilibration for the constant number of particles, volume and temperature (NVT) as well as for constant number of particles, pressure and temperature (NPT) was done for 200 ps. The final MD run was then carried out for 10 ns having time frame set to 2 fs [21]

3. Result and Discussion

Primary structure of a protein is made up of a unique amino acid in which its stability and functions could be predicted from it computed properties. The primary structure of hUGDH was analyzed and the physico-chemical properties (Table 1) were obtained with ProtParam tool. The

predicted isoelectric point (PI) of the protein (hUGDH) was 6.73. The point at which the total charge of a protein is zero or at neutral point and this is important in protein purification [22]. The PI of hUGDH suggested that the protein contains more positive charge residues present within the protein molecule. Thermal stability of the protein can be predicted from its instability index, where instability index above 40 shows that the protein is not thermally stable [23]. From the results, the instability index of hUGDH was 33.26 indicating that the structure is stable. The calculated protein aliphatic index is 90.00, and this shows that the protein overall aliphatic side chains (valine, leucine, alanine and isoleucine) is very high and these also add to the protein thermo stability. Hydrophobic and hydrophilic nature of a protein could be suggested from their hydrophobicity (Gravy), higher positive score indicate that the protein would interact better with water and negative score predict greater hydrophilicity [24], from the result the Grand average hydrophaticity (GRAVY) is -0.224, showing that it is a globular protein and hydrophilic in nature.

Table 1: Physico-Chemical Properties of UDP-glucose 6-dehydrogenase isoform 1(hUGDH)

1	Molecular Formular	$C_{2448}H_{3895}N_{667}O_{732}S_{20}$
2	Molecular Weight	55024
3	Isoelectric Point (PI)	6.73
4	Extinction Coefficient (Abs 0.1% (=1 g/l) 0.920, assuming all pairs of Cys residues form cysteine)	50600
5	Extinction Coefficient (Abs 0.1% (=1 g/l) 0.906, assuming all Cys residues are reduced)	49850
6	Instability index	33.26
7	Aliphatic Index	90.00
8	Grand average of hydropathicity (GRAVY)	-0.224
9	negative charged residues (Asp + Glu)	63
10	positive charged residues (Arg + Lys)	62

The secondary structure of the protein sequence hUGDH was investigated through SOPMA prediction server as presented in Figure 1, the percentage prediction of Alpha helix (Ah), Beta turn (Tt), Extended strand (Ee) and Random coil (Cc) were 40.69%, 7.69%, 17.61% and 34.01% respectively and other secondary structure elements such as 3_{10} Helix, π helix, Isolated β-bridge and Bend were not found in the protein structure. Therefore, the query protein sequence possesses high Alpha helix structures.

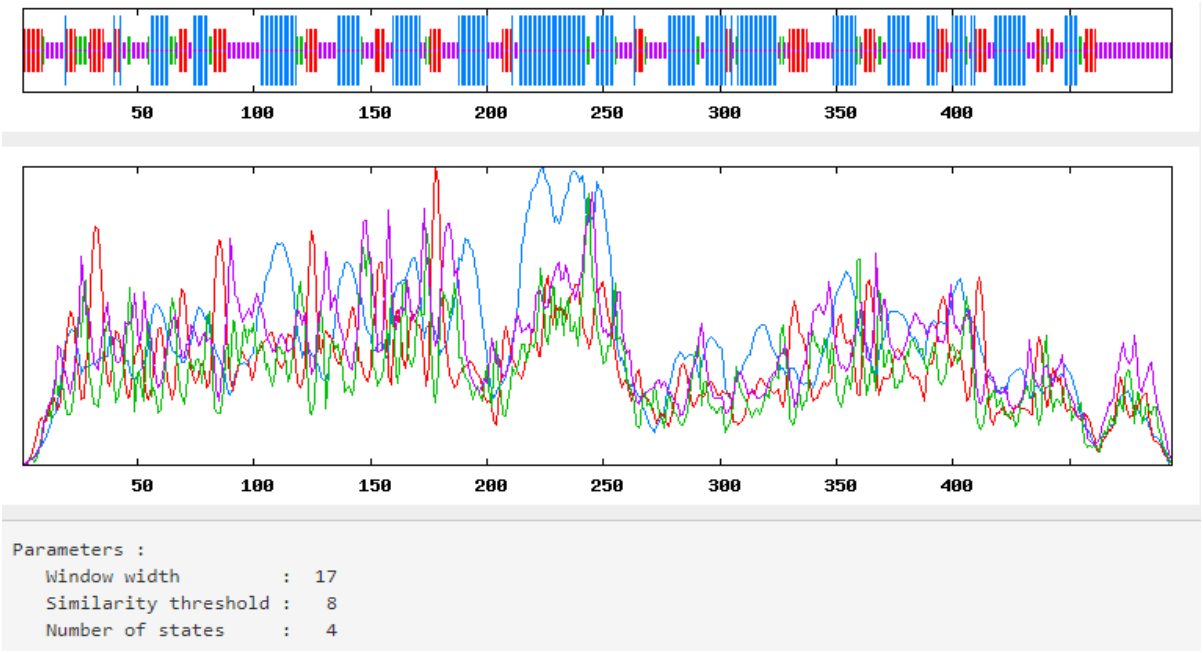


Figure 1: Secondary Structure Prediction by SOPMA server.

The secondary structure prediction and the score curves for all predicted state of the protein sequence with 8 similarity threshold and 4 number of states can be seen in Fig. 1. The structural alignment and the 3D model constructed via comparative modeling using Modeler 9.24 are shown in Figs. 2 and 3 respectively.

```
 _aln.pos         10        20        30        40        50        60
3PRJA     MFEIKKICCIGAGYVGGPTCSVIAHMCPEIRVTVVDVNESRINAWNSPTLPIYEPGLKEVVESCRGKN
UGDH      MFEIKKICCIGAGYVGGPTCSVIAHMCPEIRVTVVDVNESRINAWNSPTLPIYEPGLKEVVESCRGKN
 _consrvd ************************************************************************

 _aln.p   70        80        90       100       110       120       130
3PRJA     LFFSTNIDDAIKEADLVFISVNTPTKTYGMGKGRAADLKYIEACARRIVQNSNGYKIVTEKSTVPVRA
UGDH      LFFSTNIDDAIKEADLVFISVNTPTKTYGMGKGRAADLKYIEACARRIVQNSNGYKIVTEKSTVPVRA
 _consrvd ************************************************************************

 _aln.pos  140       150       160       170       180       190       200
3PRJA     AESIRRIFDANTKPNLNLQVLSNPEFLAEGTAIKDLKNPDRVLIGGDETPEGQRAVQALCAVYEHWVP
UGDH      AESIRRIFDANTKPNLNLQVLSNPEFLAEGTAIKDLKNPDRVLIGGDETPEGQRAVQALCAVYEHWVP
 _consrvd ************************************************************************

 _aln.pos    210       220       230       240       250       260       270
3PRJA     REKILTTNTWSSELSKLAANAFLAQRISSINSISALCEATGADVEEVATAIGMDQRIGNKFLKASVGF
UGDH      REKILTTNTWSSELSKLAANAFLAQRISSINSISALCEATGADVEEVATAIGMDQRIGNKFLKASVGF
 _consrvd ************************************************************************

 _aln.pos       280       290       300       310       320       330       340
3PRJA     GGSCFQKDVLNLVYLCEALNLPEVARYWQQVIDMNDYQRRRFASRIIDSLFNTVTDKKIAILGFAFKK
UGDH      GGSCFQKDVLNLVYLCEALNLPEVARYWQQVIDMNDYQRRRFASRIIDSLFNTVTDKKIAILGFAFKK
 _consrvd ************************************************************************

 _aln.pos         350       360       370       380       390       400
3PRJA     DTGDTRESSSIYISKYLMDEGAHLHIYDPKVPREQIVVDL-------SDQVSRLVTISKDPYEACDGA
UGDH      DTGDTRESSSIYISKYLMDEGAHLHIYDPKVPREQIVVDLSHPGVSEDDQVSRLVTISKDPYEACDGA
 _consrvd ************************************        *******************

 _aln.p  410       420       430       440       450       460       470
3PRJA     HAVVICTEWDMFKELDYERIHKKMLKPAFIFDGRRVLDGLHNELQTIGFQIETIGKK----------
UGDH      HAVVICTEWDMFKELDYERIHKKMLKPAFIFDGRRVLDGLHNELQTIGFQIETIGKKVSSKRIPYAPS
 _consrvd ************************************************************

 _aln.pos  480       490
3PRJA     ----------------V
UGDH      GEIPKFSLQDPPNKKPKV
 _consrvd                 *
```

Figure 2: Alignment of the template and query protein sequence

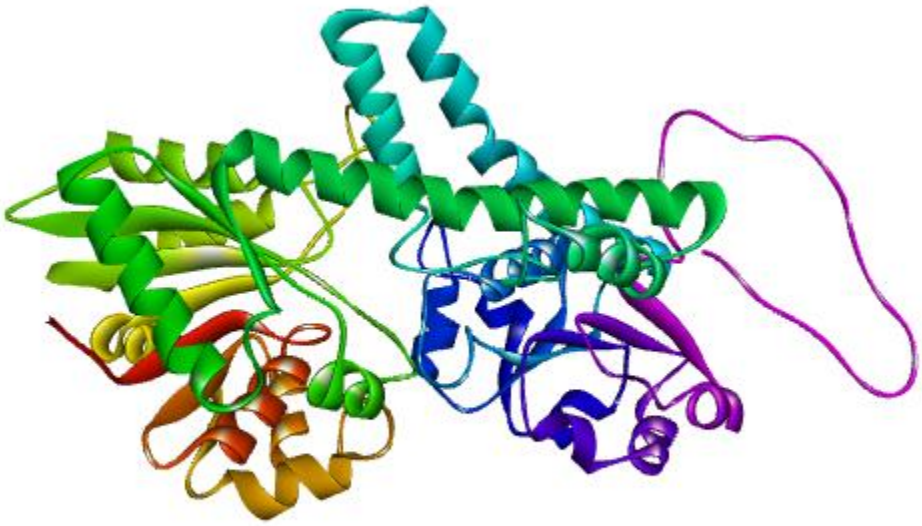


Figure 3: 3D model of UDP-glucose 6-dehydrogenase (UGDH)

The model was validated using DOPE score profile, Ramachandran plot and RMS value of the template and query model alignment of the protein structure. In the process of modeling the query protein sequence, the lowest DOPE score determines the best template. Template with codes 3PRJ has the lowest dope score and was used as our template. In order to determine the quality of the alignment used, DOPE score profile (Fig 5) was generated using assess dope

function of MODELLER, we could observe from the profile that the whole range of the amino acid residues in the protein structure converges with no or little deviations in their patterns from the DOPE score of the template and the model. Also, the DOPE score profile shows no errors in any of its region. Hence, the generated model is of a good quality.

The phi and psi distribution of Ramachandran plot (Fig 6) of the model shows that 92.5% of the residues were found at the most favored regions, 6.4% occurred at additional allowed region, we can then conclude that the model is stereo-chemically stable and comparable with the template model. Superimposed structure of the model and the template is presented in Figure 7, the RMSD value is 0.125. Since the RMSD value of the model is closer to zero, therefore the model is of good quality.

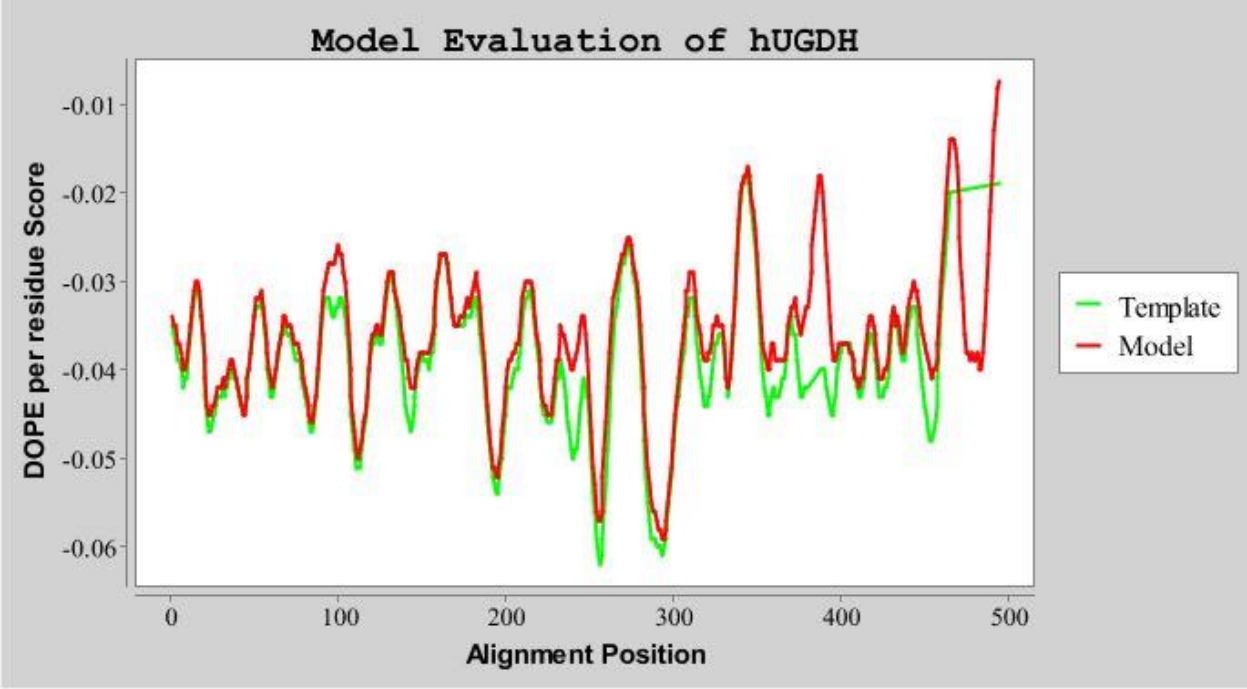


Figure 5: DOPE Score Profile of the model (UDP-glucose 6-dehydrogenase) and the Template

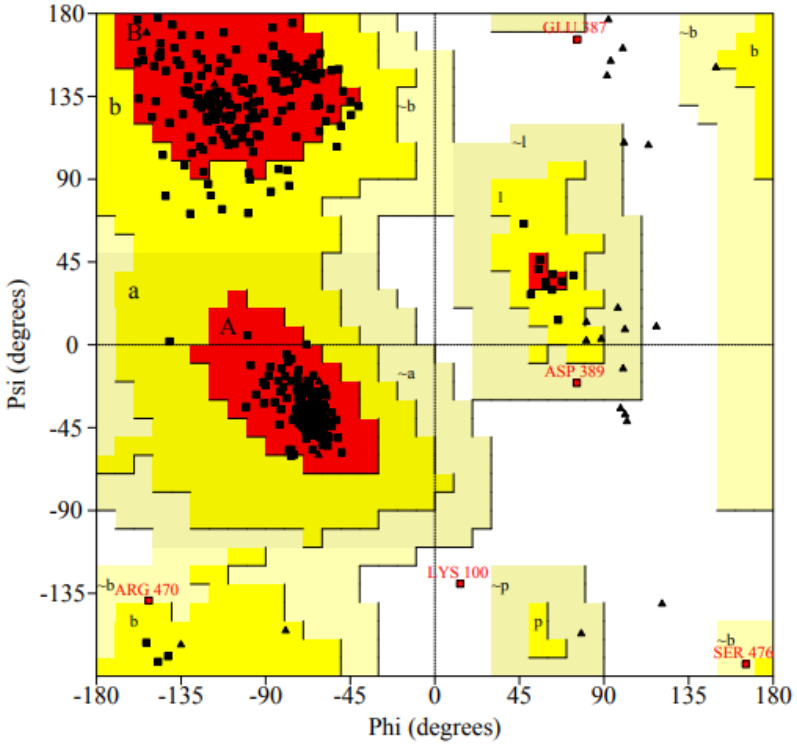


Figure 6: Ramachandran plot of hUGDH protein model, red, yellow and white region indicate favored, allowed and disallowed region respectively

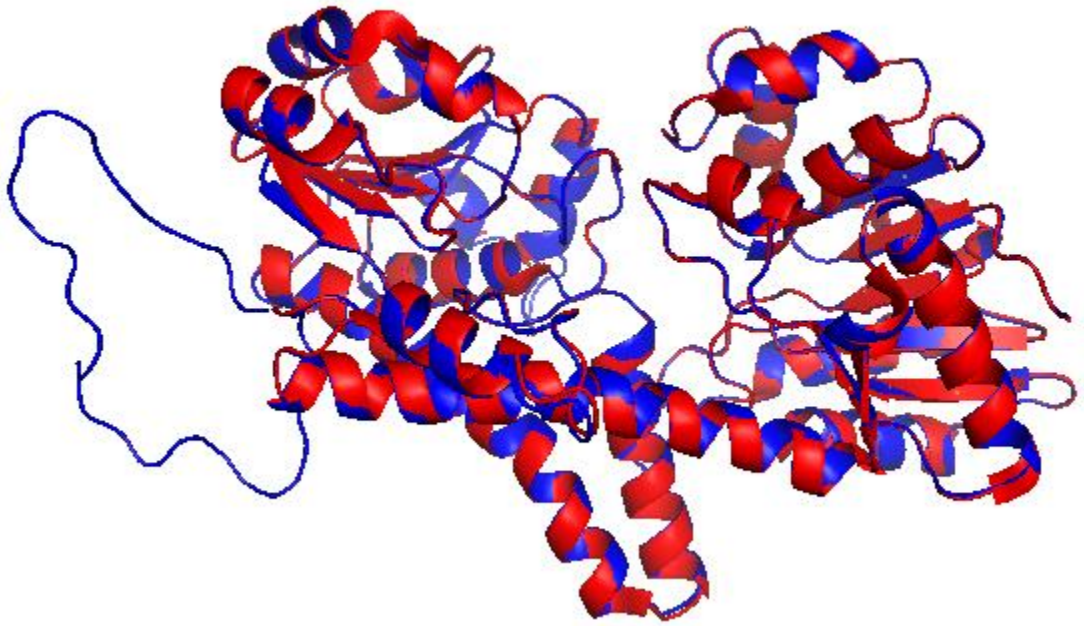


Figure 7: Superimposed structures of the Model and the Template (3PRJ) view with Pymol. Calculated RMSD= 0.125.

Further verification of the protein model was done by calculating the Qualitative Model Evaluation Analysis (QMEAN) using QMEAN server. This relates our model with sets of non-redundant protein structures of high resolution and similar sizes, the QMEAN score determines and shows the extent of the degree of nativeness of the structure given [25]. For a high-resolution model, the average score must be zero. Our query model shows a QMEAN z-score model of -0.15 which is lower than the standard deviation of 1 and the mean value of 0 for a quality model. Also from the graphical presentation (Figure 8), the dark zone indicates that the model has a z-score lower than 1, the red asterisk shows our generated model found in the dark zone and this can be regarded as a good and high quality model according to its position in the dark zone region. Hence our result predicted a quality model of high resolution and we can say that it lies in the range of other protein crystal structures. In modern biomedical research, Protein – Protein interaction (PPI) networks have become an important tool for predicting protein functions and for identifying modulators of disease growth. An interaction of protein equally gives important effect in studying various human diseases with their signaling pathways [26]. Protein-protein interaction of UDP-glucose 6-dehydrogenase obtained from STRING server has shown in (Figure 9), this predicts a confidence score including 3D structures of protein domains and functions of interacting proteins. Interaction networks shows that UDP-glucose 6-dehydrogenase interacts with other twenty proteins with a high confidence score from UXS1 (UDP-glucuronic acid decarboxylase 1), this catalysis NAD-dependent decarboxylation of UDP- glucuronic acid to UDP-xylose. This is useful in biosynthesis of tetrasaccharide present in glycosaminoglycan biosynthesis.

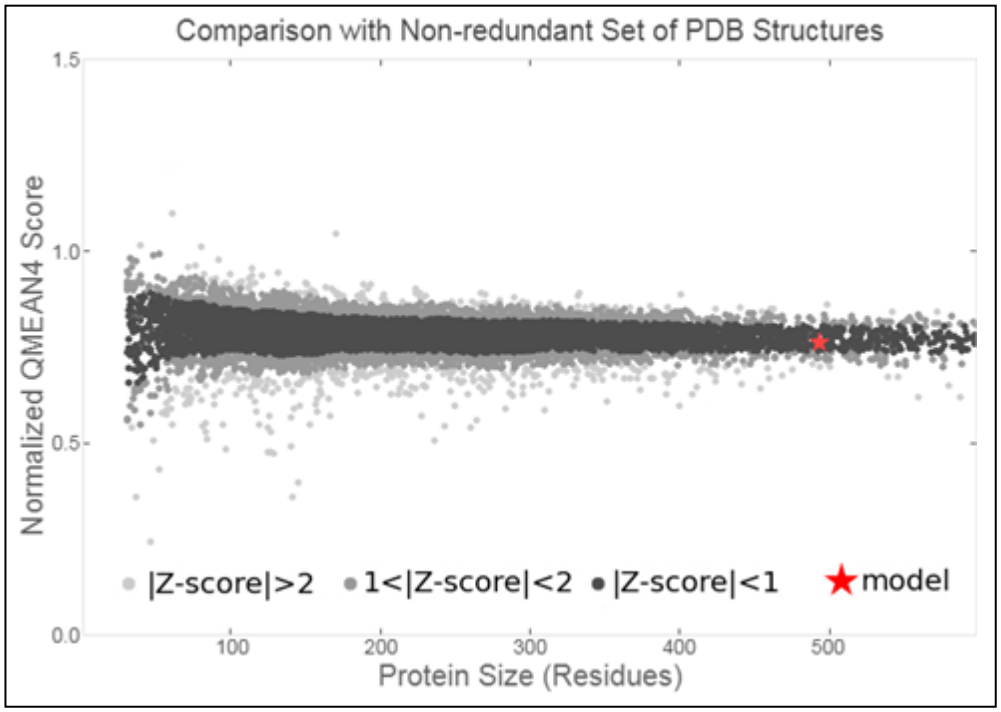


Figure 8: QMEAN z-score Graphical presentation of UDP-glucose 6-dehydrogenase.

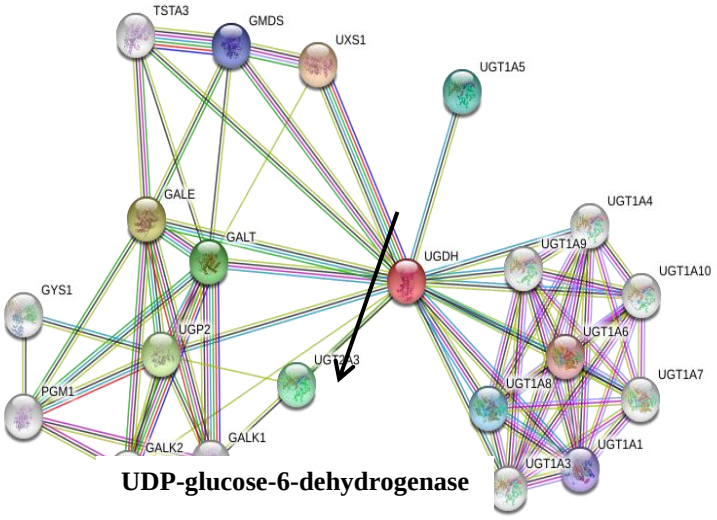


Figure 9: Protein–Protein Interaction Network of UDP-glucose-6-dehydrogenase.

Table 2: Docking Result of the two Prostate Cancer Drug and the model (hUGDH) and 3PRJ.

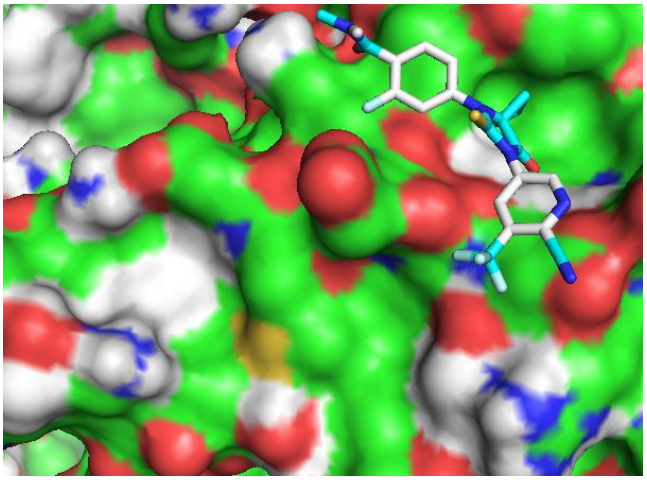
Receptors	Prostate Drugs	Binding Affinity (kcal/mol)	Hydrogen Bond Interaction	Hydrophobic Bond Interactions
Model (hUGDH	Apalutamide	-6.9	TYR-425	ASP-424, Leu-448, GLU-422, LYS-421
	Darolutamide	-6.5	GLN-155, LEU-154, LEU-152	ASP-183, GLU-187
3PRJ	Apalutamide	-6.3	TYR-367, ASP-424	PRO-401, GLU-422, TYR-402, MET-419, PRO-369, TRP-417
	Darolutamide	-7.9	ASN2-224, PHE-265	PHE-277, ALA-164, CYS-276, LEU-227, ILE-231, LYS-267, LYS-339

Docking studies was performed on our built model (UDP-glucose-6-dehydrogenase) and the receptor with pdb code 3PRJ with the aid of autodock tools. Two different approved drugs for prostate cancer (Apalutamide and Darolutamide) were used for the studies. The docking results (Table 2) shows nine binding conformations and the best binding conformation possesses the lowest negative value measured in kcal/mol which is believed to be the best binding affinity between the drugs and the protein receptors.

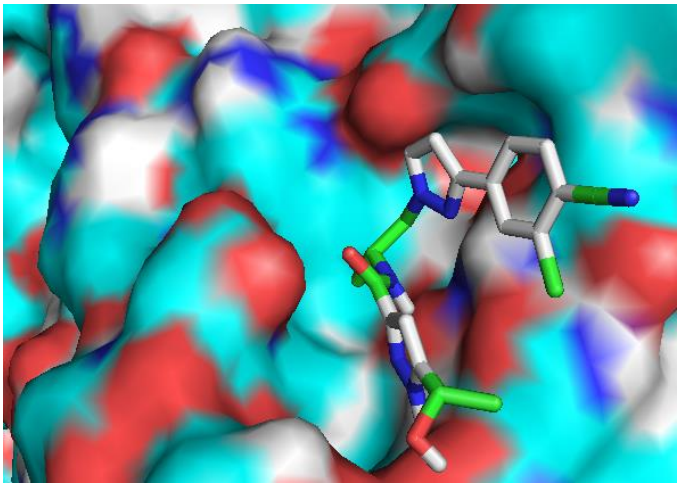
The binding affinity for Apalutamide is -6.9 kcal/mol and -6.5 kcal/mol for Darolutamide when docked with our new model (hUGDH), and -6.3kcal/mol and -7.9kcal/mol for Apalutamide and Darolutamide respectively when docked with 3PRJ receptor. Hydrogen bond and other hydrophobic interaction plays important role in structure based drug designing and also determines the energetic stability of ligands when bind to protein receptors. One hydrogen bonding was found between Apalutamide and hUGDH and this occurs between the residue Tyr-

425 and four other bonds were found. The residues involving in this bonding process are Asp-424, Leu-448, Glu-422, Lys-421. On the other hand, Darolutamide used residues Gln-155, Leu-154, Leu-152 in forming three hydrogen bonding with the hUGDH and Asp-183, Glu-187 to form another bonding type.

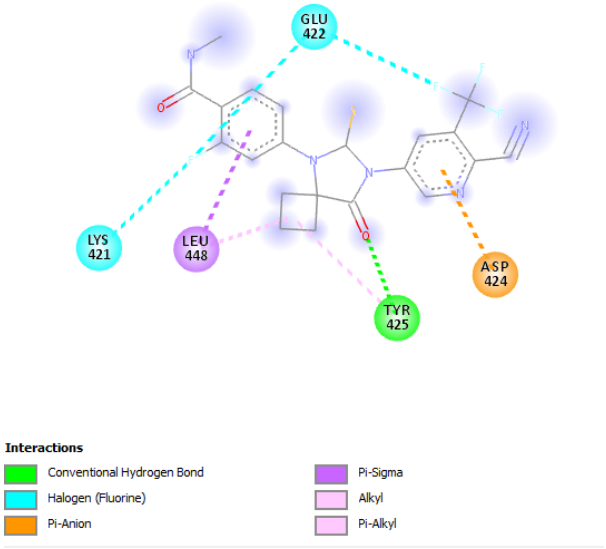
On the other hand, Tyr-367, Asp-424, Asn2-224, Phe-265 are the residue part of the receptor 3PRJ that formed hydrogen bonding with both Apalutamide and Darolutamide as shown in figure. Other amino acid residues that involve in the binding activities with the two drugs are Pro-401, Glu-422, Tyr-402, Met-419, Pro-369, Trp-417, Phe-277, Ala-164, Cys-276, Leu-227, Ile-231, Lys-267 and Lys-339. The two drugs also bind to the active site of hUGDH with almost the same binding affinity and better binding of Darolutamide was observed with 3PRJ receptor. Therefore, the model can serve as a better drug target for prostate cancer. The docking result visualization (2D and surface representation) were also shown in Figure 10 and 11.



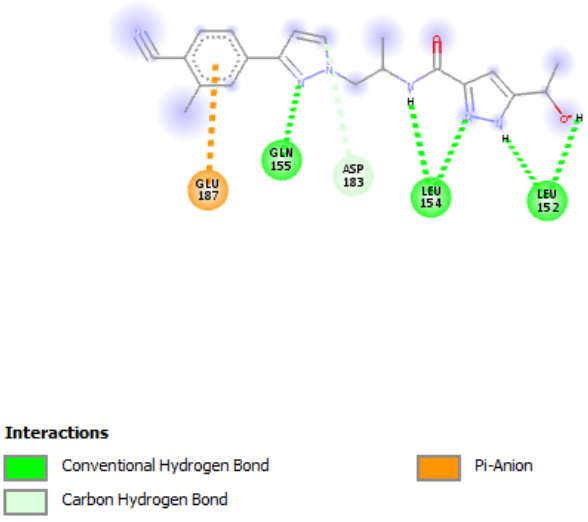
Apalutamide – hUGDH Interaction (A)



Darolutamide – hUGDH Interaction (B)

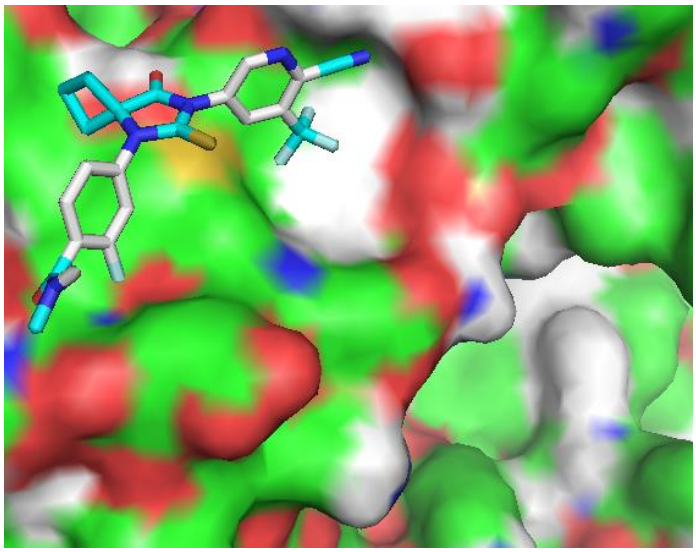


Apalutamide – hUGDH Interaction (C)

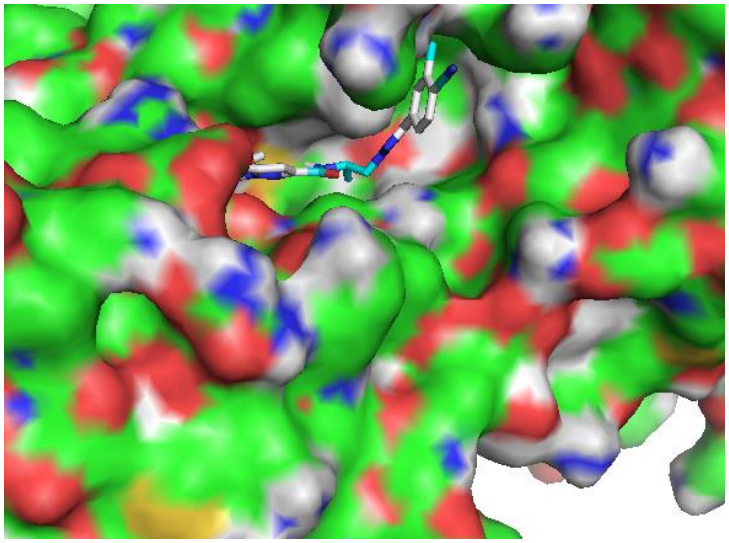


Darolutamide- hUGDH Interaction (D)

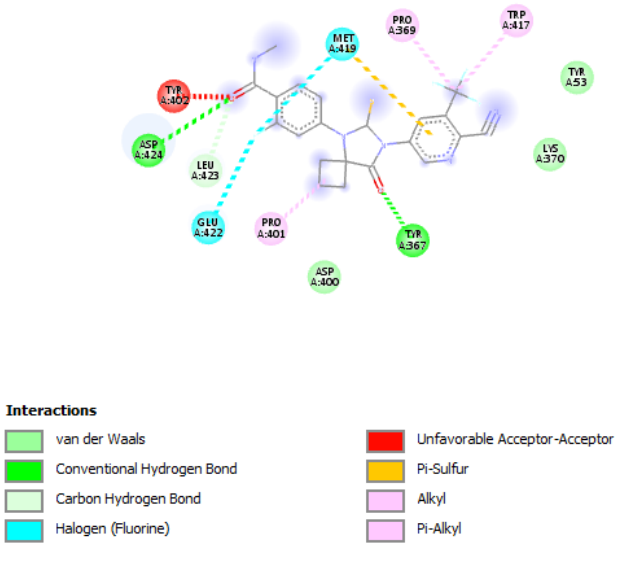
Figure 10: Interaction View of the Drugs at the active sit. (A and B) Surface Representation, (C and D) 2D Representation.



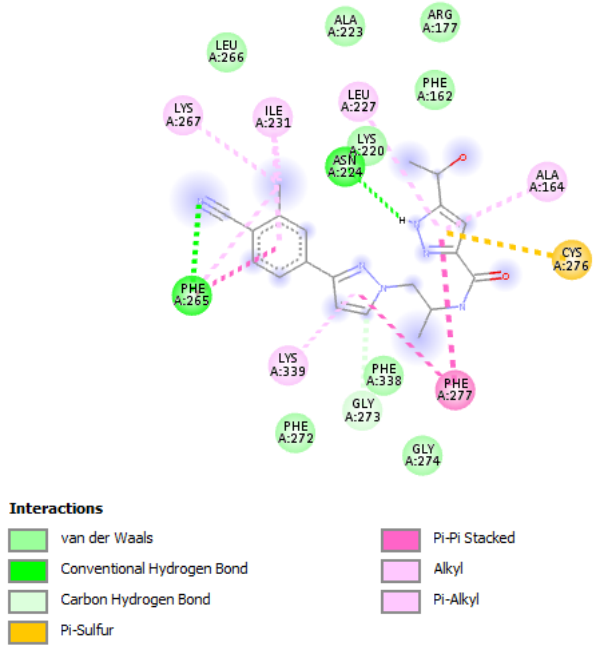
Apalutamide – 3PRJ Interaction (A)



Darolutamide – 3PRJ Interaction (B)



Apalutamide – 3PRJ Interaction (C)



Darolutamide- 3PRJ Interaction (D)

Figure 11: Interaction View of the Drugs at the active sit. (A and B) Surface Representation, (C and D) 2D Representation.

For proteins whose structures have been predicted due to lack of crystallized structures, MD simulation studies are most often performed to see how the proteins can behave in physiological environments via analysis of their stabilities in a simulation environment. Similarly, in this study, hUGDH was simulated and RMSD analysis showed that from 3 ns to 8 ns, the protein is showing rather stable behavior after which its dynamic behavior has been observed.

To further see the overall spread of our protein molecule during the period of 10 ns MD run, Radius of gyration (Rg) was analyses which showed a stable behavior (black line Fig. 12 (b)). Also the value of Rg was low – about 2.6 nm – indicating the structural integrity and better folding behavior of the protein [27]. Looking at the behavior of the Rg at each individual axes, it can be seen that the most dynamicity is being displayed at the z-axis by the protein.

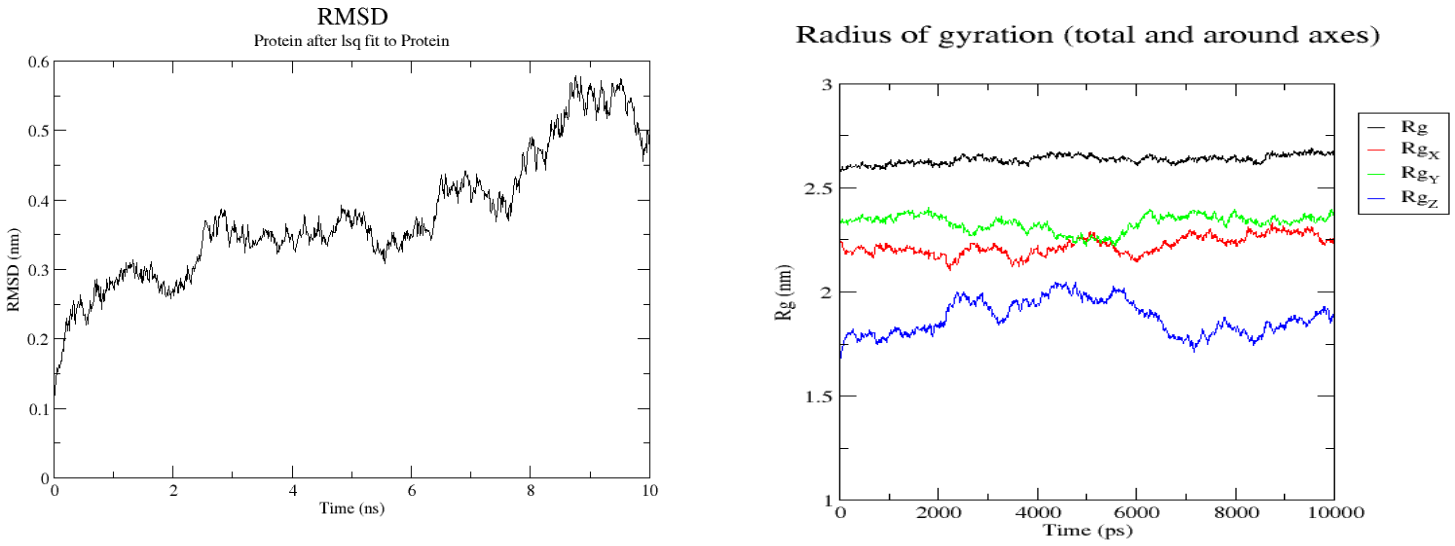


Figure 12: (a) RMSD plot for hUGDH for 10 ns; and (b) radius of gyration of hUGDH, total and around the three axes as well.

4. Conclusions

Human UDP-glucose 6-dehydrogenase (hUGDH) play an important role in the conversion of UDP-glucuronic acid to UDP-glucuronate, this then participate in the biosynthesis of glycosaminoglycans which further involve in cancer growth. Wet experiments supported hUGDH as a new drug target for prostate cancer. Our theoretical studies showcase the binding pocket, critical amino acid residues at the active site of the modeled protein that can be used in designing new inhibitors. This will eventually pave way for further identification and discovery of potential anti-prostate *agents* in the future.

References

[1] M. Terris, A. Rhee, Prostate cancer: Metastatic and advanced disease. EMedicine. 2007 from http://www.emedicine.com/ med/topic3197.htm.

[2] GK. Panigrahi, P.P. Praharaj, H. Kittaka, A.R.Mridha, O.M. Black, R.Singh, R. Mercer, Exosome proteomic analyses identify inflammatory phenotype and novel biomarkers in African American prostate cancer patients. Cancer Med. 13(2019) 22- 38.

[3] F. Bray, J. Ferlay, I. Soerjomataram, R.L. Siegel, L.A. Torre, A. Jemal, Global Cancer Statistics. GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin, in press. The online GLOBOCAN 2018 database is accessible at http://gco.iarc.fr/, as part of IARC's Global Cancer Observatory

[4] E. Sigrid, C. Apirat, L.K. Kathryn, O. Udo, N. Bernd, Structure and Mechanism of Human UDP-glucose 6-Dehydrogenase. The Journal of Biological Chemistry., 286(2011) 23877–23887.

[5] P.S. Nelson, N. Clegg, H. Arnold H, The program of androgen-responsive genes in neoplastic prostate epithelium. Proc Natl Acad Sci U S A 99(2002)11890–5.

[6] Q. Wei, R. Galbenus, A. Raza, R.L. Cerny, M.A. Simpson, Cancer Res., 69(2009) 2332–2339.

[7] D. Huang, G.P. Casale, J. Tian, S.M. Lele, V.M. Pisarev, M.A. Simpson, G.P. Hemstreet, UDP-glucose dehydrogenase as a novel field-specific candidate biomarker of prostate cancer, Inter J. of cancer., 126(2010) 315-327.

[8] E. Gasteiger, C. Hoogland, A. Gattiker, M. Wilkins, R.D. Appel, A. Bairoch, Protein identification and analysis tools on the ExPASy server, In The proteomics protocols handbook Humana press., (2005) 571-607.

[9] Y. Guermeur, C. Geourjon, P. Gallinari, G. Delage, Improved performance in protein secondary structure prediction by inhomogeneous score combination. Bioinformatics., 15(1999) 413–21.

[10] A.S. Alejandro, L. Aravind, L.M. Thomas, S. Sergei, L.S. John, I.W. Yuri, Improving the accuracy of PSI-BLAST protein database searches with composition-based statistics and other refinements, Life Sci Nucleic Acids Res., 29(2001) 2994–3005.

[11] A. Sali, T.A. Blundell, Comparative protein modeling by satisfaction of spatial restraints. J Mol Biol., 234(1993) 779–815.

[12] D. Jurate, O.D. Aisling, D.S Roy, An overview of multiple sequence alignments and cloud computing in bioinformatics. ISRN Biomathematics. 14(2013).

[13] N. Eswar, M.A. Marti-Renom, B. Webb, M.S. Madhusudhan, D. Eramian, M. Shen M, Comparative protein structure modeling with MODELLER. Curr Protoc Bioinformatics., 15(2006) 55–63.

[14] B. Snel, G. Lehmann, P. Bork, M.A. Huynen, STRING, a web-server to retrieve and display the repeatedly occurring neighbourhood of a gene. Nucleic Acids Res., 28(2000) 3442–4.

[15] A. Franceschini, D. Szklarczyk, S. Frankild, M. Kuhn, M. Simonovic, A. Roth, STRING v9. 1, protein-protein interaction networks, with increased coverage and integration. Nucleic Acids Res., 41(2013) 808–15.

[16] W.B. George, L. Fran, Visualizing networks. Methods Enzymol. 411(2006) 408–21.

[17] O. Trott, A. Olson, AutoDockVina: Improving the Speed and Accuracy of Docking with A New Scoring Function,Efficient Optimization and Multithreading, J. Comput. Chem., 31(2010)455-461.

[18] Discovery Studio visualizer version 17.2.0. 16349 Dassault Systemes Biovia Corp.

[19] PyMOL(TM) 1.7.4.4 Edu - Educational Product Schrodinger, LLC.

[20] M.J. Abraham, T. Murtola, R. Schulz, S. Páll, J.C. Smith, B. Hess B, GROMACS: High performance molecular simulations through multi-level parallelism from laptops to supercomputers. SoftwareX., (2015)1-2:19-25.

[21] G. Bussi, D. Donadio, M. Parrinello, Canonical sampling through velocity rescaling. The Journal of Chemical Physics., 126(2007) 014101.

[22] S.P. Soni, E. Adu-Gyamfi, S.S. Yong, C.S. Jee, R.V. Stahelin, The Ebola virus matrix protein deeply penetrates the plasma membrane: an important step in viral egress. Biophy. J., 104(2013) 1940-1949.

[23] O. Elekofehinti, C. Oluwamodipe, P. Jean, B. Oluwaseun, F. Ayodeji, D. Damilare, I.Opeyemi, I. Yetunde, J. Ige, B. Jao, Discovery of Potential Visfatin Activators Using Insilico Docking and ADME Predictions A Therapy for Type-II Diabete,J. Basic App. Sci.,4(2018)35-43.

[24] J. Kyte, R.F. Doolittle, A simple method for displaying the hydropathic character of a protein, Journal of Molecular Biology., 157 (1982).

[25] P. Benkert, M. Biasini, T. Schwede, Toward the estimation of the absolute quality of individual protein structure models. Bioinformatics., 27(2010) 343–50.

[26] G. Chaurasia, Y. Iqbal, C. Hanig, H. Herzel, E.E. Wanker, M.E. Futschik, UniHI, an entry gate to the human protein interactome, Nucleic Acids Res., 35(2007) 590–4.

[27] R.R Erva, S.B. Rajulapati, C.P. Durthi, M. Bhatia, M. Pola, Molecular dynamic simulations of Escherichia coli L-asparaginase to illuminate its role in deamination of asparagine and glutamine residues. 3 Biotech. 6(2016) 60-77.