Research Article **CODEN: IJPRNK** **ISSN: 2277-8713**
Satarupa Roy, IJPRBS, 2013; Volume 2(6): 611-626 **IJPRBS**

INTERNATIONAL JOURNAL OF
PHARMACEUTICAL RESEARCH AND BIO-SCIENCE

MUCOPOLYSACCHARIODOSIS TYPE 1: STRUCTURAL AND FUNCTIONAL ANALYSIS OF THE DISEASE CAUSING PROTEIN USING BIOINFORMATICS TOOLS

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Accepted Date: 03/12/2013; Published Date: 27/12/2013

Abstract: Mucopolysaccharidosis type I (MPS I) is a lysosomal storage disorder caused due to the deficiency of lysosomal human α-L-iduronidase (IDUA) enzyme that catalyzes degradation of glycoaminoglycans (GAG) like heparan and dermatan sulfate. GAG is an important constituent of the extracellular matrix, joint fluid, and connective tissue throughout the body. Progressive accumulation of GAG within the cells of various organs ultimately compromises their function. The major sites of disease differ depending on the specific enzyme deficiency. Due to the absence of three dimensional structure of IDUA, structural-functional relationship of certain mutants leading to various clinical phenotypes are not well known. Totally 82 point mutations in IDUA gene associated with MPS I have been reported. Hence to study the role of IDUA, homology model of various IDUA mutants were constructed by the help of bioinformatics tools using standard protocols. The tool used for homology modeling is the Swiss- Model which is a server for automated comparative modeling of three-dimensional protein structures. Swiss model provides several levels of user interaction through its World Wide Web interface: in the 'first approach mode' only an amino acid sequence of a protein is submitted to build a 3D model. Template selection, alignment and model building are done completely automated by the server. In the 'alignment mode', the modeling process is based on a user-defined target-template alignment. Complex modeling tasks can be handled with the 'project mode' using Deep-View (Swiss-PdbViewer), an integrated sequence-to-structure workbench. Molecular modeling was done to find the binding abilities of the mutant models hence determining the functional aberrations by using 4-methylumbelliferyl α-L-iduronide as the ligand. Also, the interactions of these mutants with the specific ligand were checked using molecular auto-docking tools. Results after carrying molecular docking showed that the severe and the moderate mutant forms caused a major disruption in the structure of the enzyme hence the efficiency or binding ability of the ligand was seen to deteriorate with the severity of the disorder, MPS I while the mild forms showed very minute disruption in the structure of the enzyme. Hence we can conclude that structural-functional characterization of the mutants may help in treatment of Mucopolysaacharidosis Type I by knowing the efficiency of binding of the receptor and substrate.

Keywords: Mucopolysaccharidosis type I, lysosomal storage disorder, human α-L-iduronidase (IDUA), 4-methylumbelliferyl α-L-iduronide, homology modeling (Swiss Model), and Molecular docking.

PAPER-QR CODE

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Satarupa Roy, IJPRBS, 2013; Volume 2(6): 611-626

INTRODUCTION

Mucopolysaccharidosis type I (MPS I,OMIM NO.25280) belongs to the group of metabolic disorders called lysosomal storage disorders (LSDS). MPS I is an autosomal recessive disorder caused due to the deficiency of human lysosomal glycosidase , α-L-idurouidase(IDUA,E.C 3.2.1.76) involved in catabolism of glycosaminoglycans (GAGs) .Examples of GAGs are dermatan sulfate, heparin sulfate, keratin sulfate and chondroitin sulfate. Enzyme deficiency leads to accumulation of unprocessed GAGS substrate. The excessive mass is excreted through the urine. Basically, intra-lysosomal accumulation result in the cell, tissue and organ dysfunction which leads to progressive deformities in patients. There is wide range of clinical phenotype present in MPS I; however there are three subtypes of MPS I such as Hurler (Severe form; MPS IH), Scheie (Mild form; MPS IS),Hurler-Scheie (Moderate form; MPS I H/S) syndromes(Mckusick et al., 1972).It is known that there is a continuous spectrum of clinical involvements observed within MPS I making it difficult to delineate them into three subtypes.[1]

Till now, the effective cure for MPS I is not available, because the currently available treatments are only in a form of supportive management. Now these days, there are some treatments are available for MPS I such as enzyme replacement therapy(ERT) which involves exogenous administration of IDUA enzyme into patients to clear the deposited GAGS in tissues.

Thus to overcome this current scenario in the research related to Mucopolysaccharidosis or IDUA gene, bioinformatics tools have been exploited and a good outcome are been achieved. However, even though many type of mutations leading to different MPS I subtypes have been reported, the outcome of the missense mutation involved in the IDUA gene remains unidentified. Moreover, to explain the impact of missense mutation on the lysosomal enzyme remains a challenge. Based on the substantial inference, with the help of bioinformatics tools we have tried to develop and derive improved IDUA wild homology model using a template which has similar gene sequence as that of the IDUA gene which causes MPS I.[2]

MATERIALS AND METHODS

Computational tools

Swiss Model server web tool (http://swissmodel.expasy.org) accessible via the ExPASy web server, which is a fully automated protein structure homology-modeling server, was used to create the homology wild type model of the IUDA gene. It is a web server for homology modeling of protein 3D structure that generates a reliable 3D protein structure model. Homology modeling methods make use of experimental protein structure (templates) to build models for evolutionary related proteins (targets).

Also, UniProt (Universal Protein Resource) web server was use to obtain the gene sequence of the human IDUA gene in FASTA format. UniProt is a catalog of information of protein sequences.It is a central repository of protein sequence and functional information.It has entries that are obtained from genome sequencing project.[3]

Deep Viewer is a Swiss model application that provides a user friendly interphase which allows to visualize and analyze several protein structure and their conformation at the same time.

MATERIALS AND METHODS

Dataset

Mutations seen in the MPS I patients are obtained from human gene mutation database (HGMD; http://www.hgmd.org). So far, totally 108 missense mutations were reported in IDUA gene, of these point mutation, 15 missense mutations (5 severe, 5 moderate, 5 mild) were selected for the *in silco* study.

Template identification and selection for Homology modeling of IDUA wild type gene:

To create a wild type homology model (target) it is important and necessary to have a gene sequence similar (template) with that of the target. Hence, using the basic local alignment search tool (BLAST; http://blast.ncbi.nlm.nih.gov), the gene sequence obtained from UniProt was searched for a similar sequence and it was found that proteinEndo-beta-1,4-xylanase of the soil microorganism *Cellvibrio japonicas* (PDB ID: 1US3) has gene sequence homology to human IDUA gene. Also, using the T-Coffee software, it was found that both the gene sequence shows 60% homology.

Homology Modeling

IDUA wild type Homology Models were constructed using Swiss Model, and automated protein modeling tool. The human IDUA sequence was obtained in FASTA format from UniProt-Swiss-Prot (http://ca.expasy.org/sprot/; UniProt Accession No. P35475) web server. The suitable template in the PDB file format, homology with the target sequence was obtained using the T-Coffee online sequence alignment tool. And it was found that the protein Endo-beta-1,4-xylanase from *Cellvibrio japonicas*(PDB ID:1US3) has the sequence closest to the target gene and available for homology modeling. Using the template, the initial target-template alignment was generated. Using the optimized target alignment the 15 missense mutant models are being constructed. Finally, the constructed wild type homology model was validated for stereo chemical evaluations using PROCHECK and ProSA web server.[4-8]

RESULTS

Using online homology tools, the following target-template project model was constructed (Figure 1 and Figure 2).

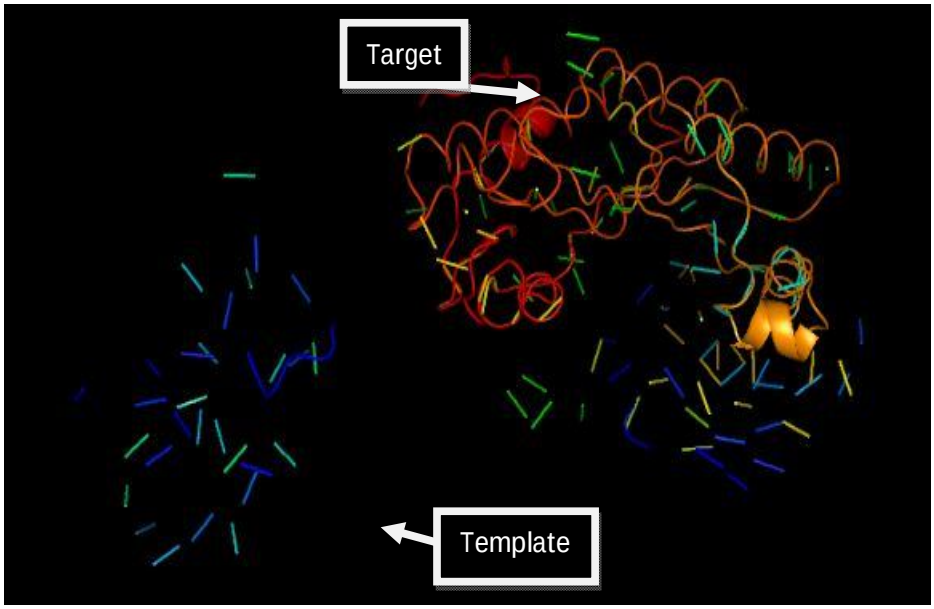


Figure 1: Target-Template homology project model.

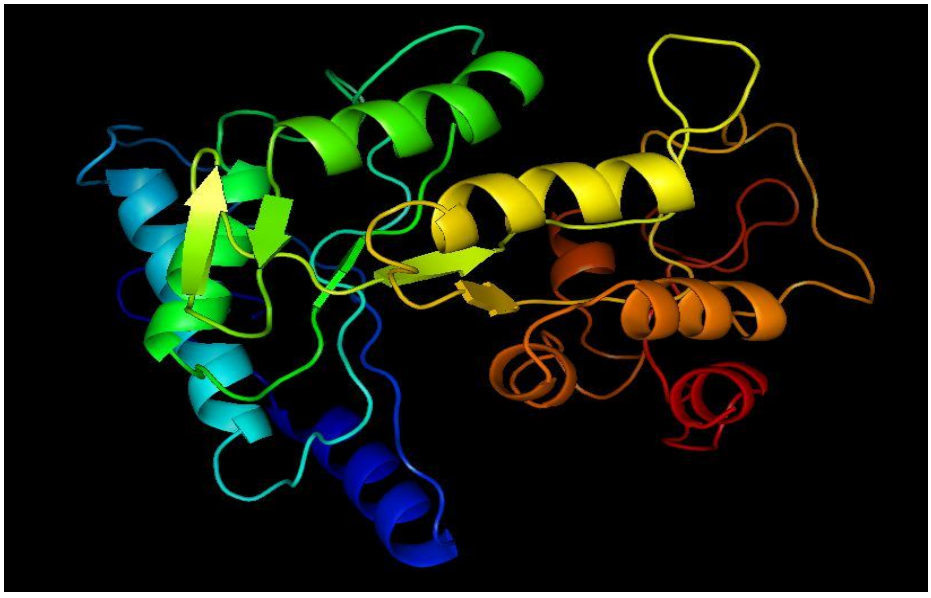


Figure 2: Wild-type IDUA homology model.

Validating the wild type IDUA homology model

The constructed homology model using the template (PDB ID: 1US3) was evaluated for structural and stereo chemical efficiency. Ramachandran phi-psi plot for wild IDUA (Figure 3a) revealed that 75.4% of residues lay in core region (red region), 20.2% in additional allowed region (yellow region), 1.6% in generally region (light yellow) and only 2.8% lay in disallowed region (white region) and the observed G-factor was -0.38.

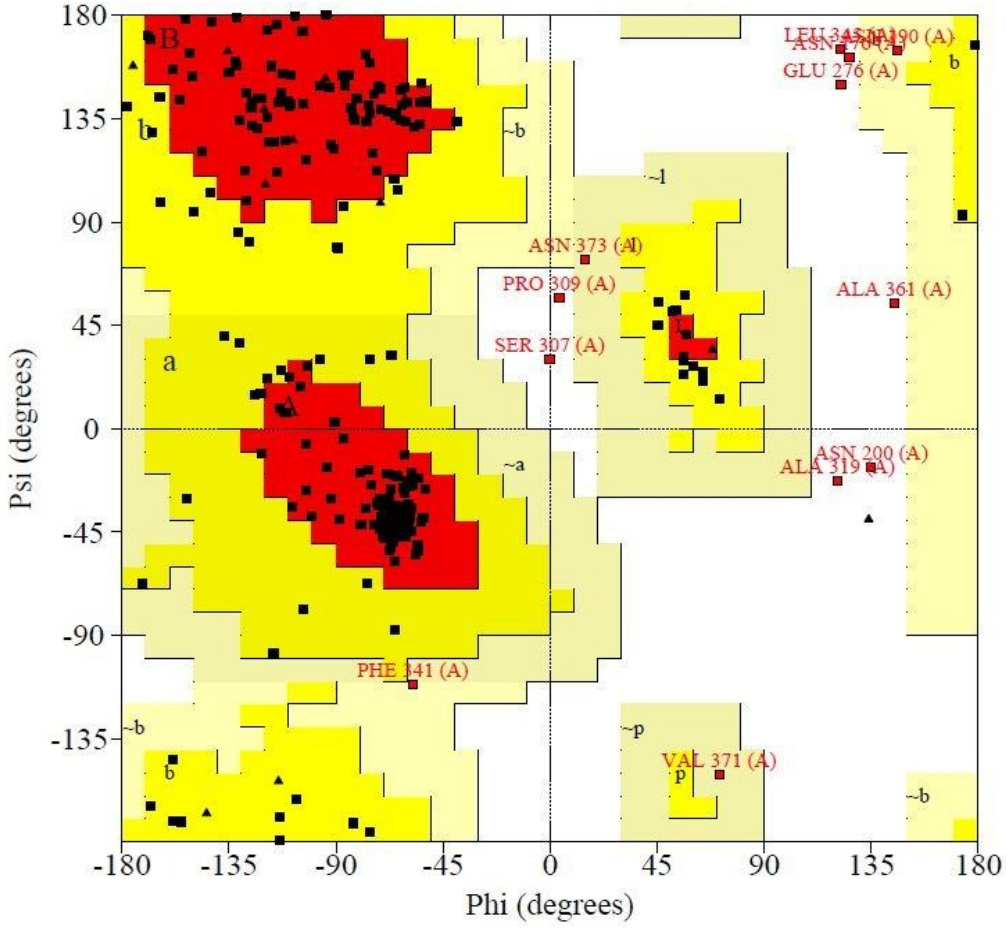
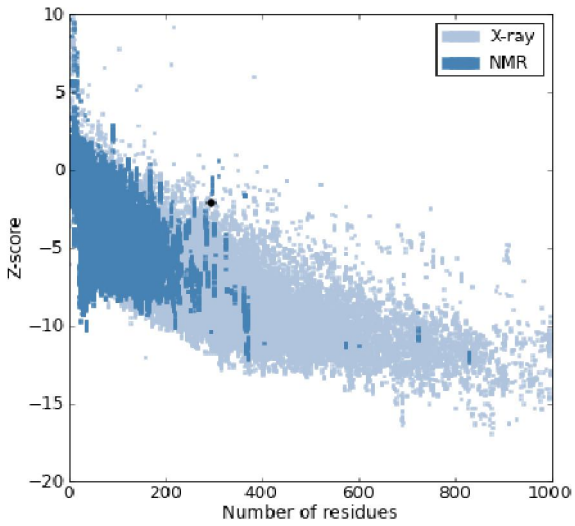


Figure 3a: Ramachandran Plot

Further, the model was evaluated in terms of energy function using ProSA web server. Overall ProSA Z-score evaluated for wild IDUA model (Figure 3b) showed -2.07. This negative Z-score of energy- profile confirm that overall quality of the model was good. Another PROSA energy plot (Figure 3c) indicating the individual energy profile for entire amino acids sequence of protein also showed that majority of residues is within the acceptable range.



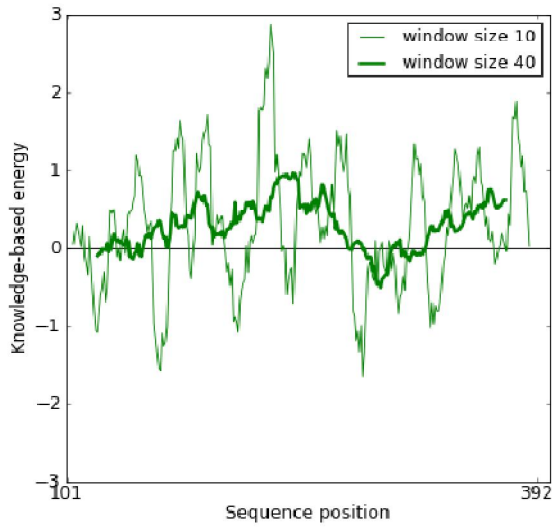


Figure 3b and c: Structural validation of wild IDUA model using ProSA web tool (3b) ProSA overall Z-score -2.07 is indicated in graph as black dot. A negative value of overall energy profile confirmed the reliable structural conformation of wild IDUA. (3c) Energy profile of wild IDUA homology model.

Active binding site prediction

The active site in the constructed wild type IDUA homology model was obtained through Active Site Prediction online web tool (http://www.scfbio-iitd.res.in/). The results obtained showed totally 28 active binding sites in the constructed wild type IDUA homology model as follows (figure 4a and 4b).

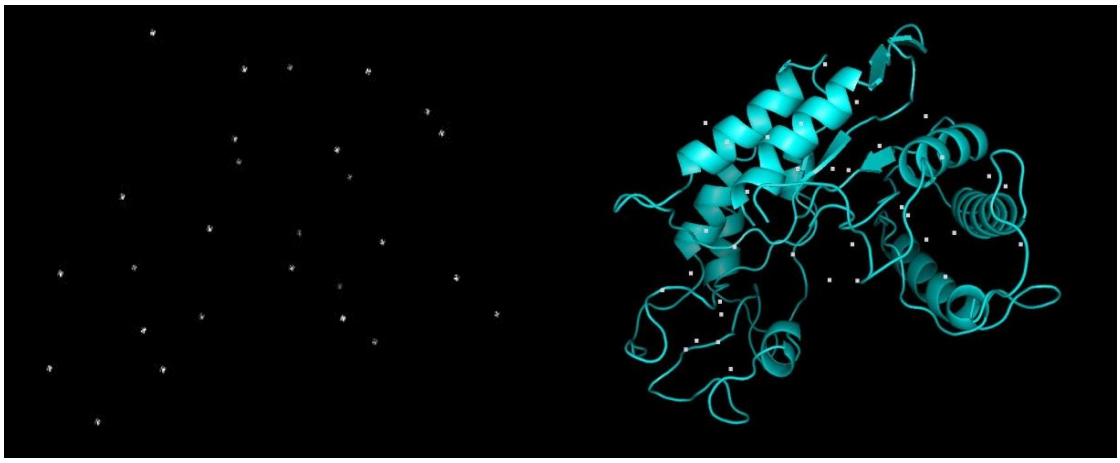


Figure 4a **Figure 4b**

Figure (4a) shows the special arrangement of the 24 active binding sitesfor the drug molecule; whereas **Figure (4b)** shows the wild type IDUA homology model and the active binding sites on the surface of the wild type IDUA homology model.

Inter-molecular hydrogen interaction activity

Using Swiss-model Deep-viewer software the constructed wild type homology model was mutated; inducing point mutations in the structure. The mutations induced were characterized on the basis of severe, moderate and mild form. List of inter-molecular interaction before mutation and after mutating are as follow (Table-1).

Table 1:

Mutation	Phenotype	Wild type form Bond lengh (Å)	Mutant form Bond lengh (Å)
A160D	Severe	6.21	5.62
E182K	Severe	3.12	5.53
G208V	Severe	6.04	52.1
D315Y	Severe	4.61	6.56
D314Y	Severe	5.01	12.4
G151D	Moderate	3.03	4.63

E178K	Moderate	3.79	5.20
A327P	Moderate	6.22	2.45
N200E	Moderate	6.16	6.83
M195I	Moderate	4.25	5.82
D189K	Mild	4.67	4.56
S102K	Mild	5.45	5.68
N110Y	Mild	2.56	1.89
N201D	Mild	7.34	7.98
H327N	Mild	2.34	3.36

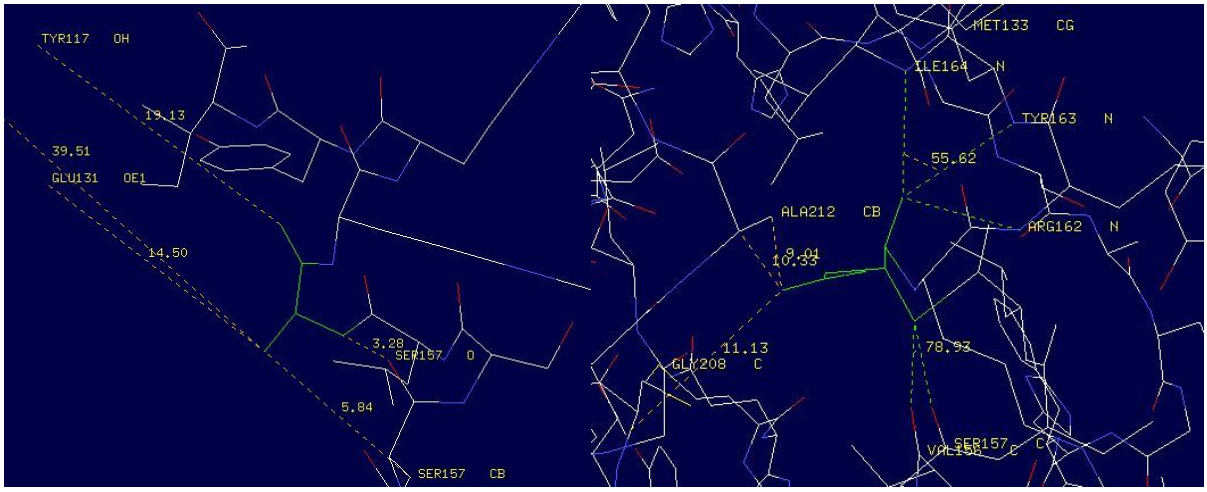


Figure 5a and 5b: The inter-molecular interaction between A160D (severe form) and the surrounding atoms.

Ligand and molecular docking using the wild type homology model of IDUA and the mutant form of IDUA

The constructed wild type homology model of IDUA was docked using Auto-Dock software, with the corresponding ligand molecule. The ligand molecule for docking with the constructed wild type and mutant homology model of IDUA was found for Mucopolysaccharidosis Type I using

the PubChem databases (http://www.ncbi.nih.nlm.gov/Pubchem).Various ligands were found for binding; for example viz; Botulinum Toxin type A, Calcitriol, Serum albumin, 1,2-Diacyl-5n-Glycero-3-Phosphoinesitol, Alpha-phosphoribosylpyrophosphoric acid. However, 4-methylumbelliferyl α-L-iduronide was obtained from the NCBI PubChem database (PubChem Accession. No 128746) which was the adequate ligand for binding.

Molecular docking analysis

The mentioned ligand was docked with the wild type and the mutant type IDUA models at the active binding sites and the following results were obtained (Figures 8).

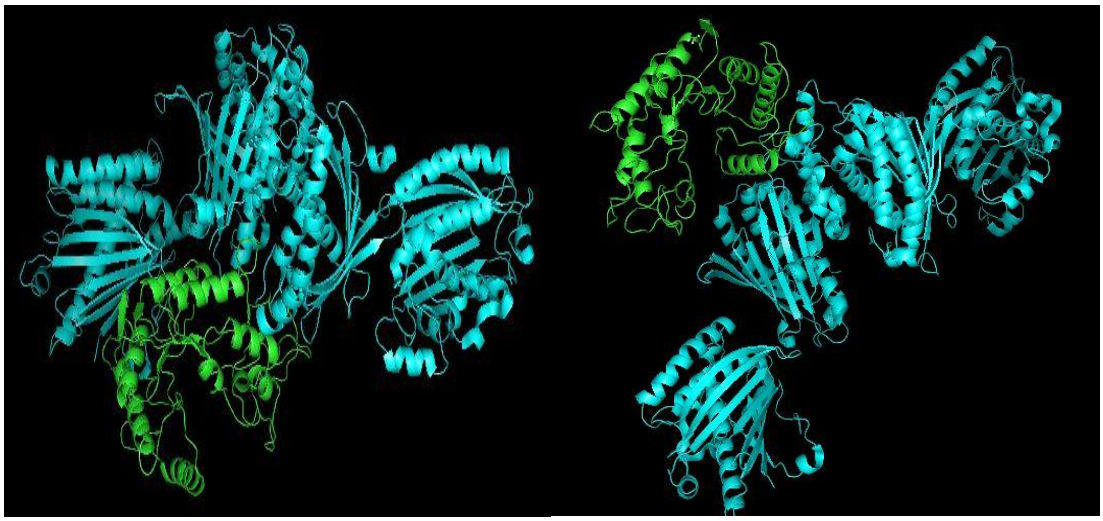


Figure 8a and 8b: The figure 8a (left) is the normal wild type IDUA model and docked with the ligand 1, 2-Diacyl-5n-Glycero-3-Phosphoinesitol (PDB ID:1UW5) and it shows normal binding at the active sites with the binding efficiency of -211.59 whereas the figure 8b (right) is the mutant which is mutated (missense mutation) at the active binding site; hence showing a binding efficiency of 15.45

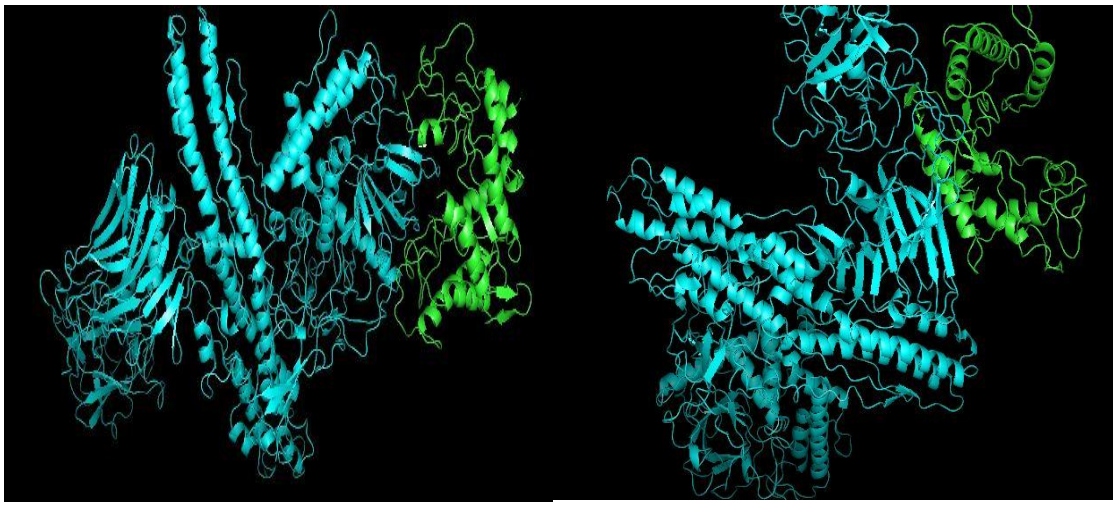


Figure 8c and 8d: The figure 8c (left) is the normal wild type IDUA model and docked with the ligand Botulinum Toxin type A (PDB ID:3BTA) and it shows normal binding at the active sites with the binding efficiency of -195.92 whereas the figure 8d (right) is the mutant which is mutated (missense mutation) at the active binding site; hence showing a binding efficiency of 119.60.

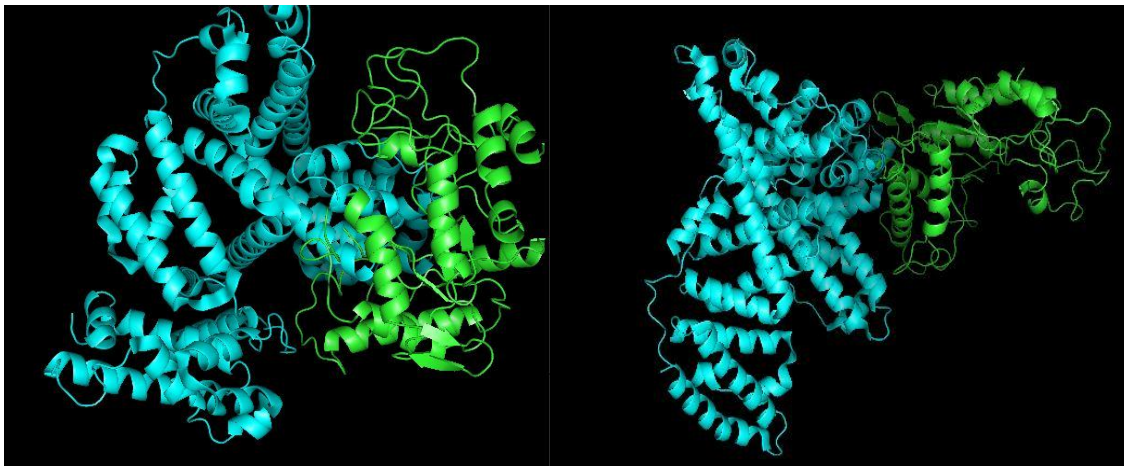


Figure 8e and 8f: The figure 8e (left) is the normal wild type IDUA model and docked with the ligand Serum albumin (PDB ID:1HA2) and it shows normal binding at the active sites with the binding efficiency of -334.24 whereas the figure 8f (right) is the mutant which is mutated (missense mutation) at the active binding site; hence showing a binding efficiency of 82.04.

Discussion

A wild type homology model of IDUA was constructed using Swiss Model and was validated using the Procheck and ProSa software tool. The Ramachandran phi-psi plot for wild type homology model of IDUA revealed that 75.4% of residues lay in core region (red region), 20.2% in additional allowed region (yellow region), 1.6% in generally region (light yellow) and only 2.8% lay in disallowed region (white region) and the observed G-factor was -0.38. Also, the ProSa Z-score value was obtained as -2.07.A negative value of overall energy profile confirmed the reliable structural conformation of wild type IDUA model. Further a comparative study was carried out by inducing missense mutations in the wild type IDUA model. The mutations induced were A160D (severe form), E182K (severe form), G208V (severe form), D315Y (severe form), G151D (moderate form), A327P (moderate form), D189K (mild form), S102 (mild form), N110Y (mild form), N201D (mild form), H355 (mild form). Further the active sites on to the surface of the IDUA model was predicted using online web server for active site prediction.[9-15]

We also evaluated the binding ability of mutant using 4-methylumbeliferyl α-L-iduronide as ligand for molecular docking, with regard to its extensive use in clinical diagnosis of MPS I. In the study, results of docking computations indicated that ligand binding abilities of IDUA followsinto three forms i.e. from severe to moderate to mild; as the severity of disease tend to increase (depending upon the kind of mutation). Generally to hypothesize this phenomenon,analysis of three chief active site mutations *viz*., E182K (severe), A327P (moderate) and H355N (mild) for which either bioinformatics or biochemical characterizations are well-recognized were taken and a comparative study of enzyme-substrate binding energy (G-value) was calculated for the wild type IDUA and the mutant IDUA (Figure 9).[16-20]

Figure 9: The molecular docking interaction of the normal wild type IDUA model (large) with the substrate (ligand) 4-methylumbeliferyl α-L-iduronide (small).

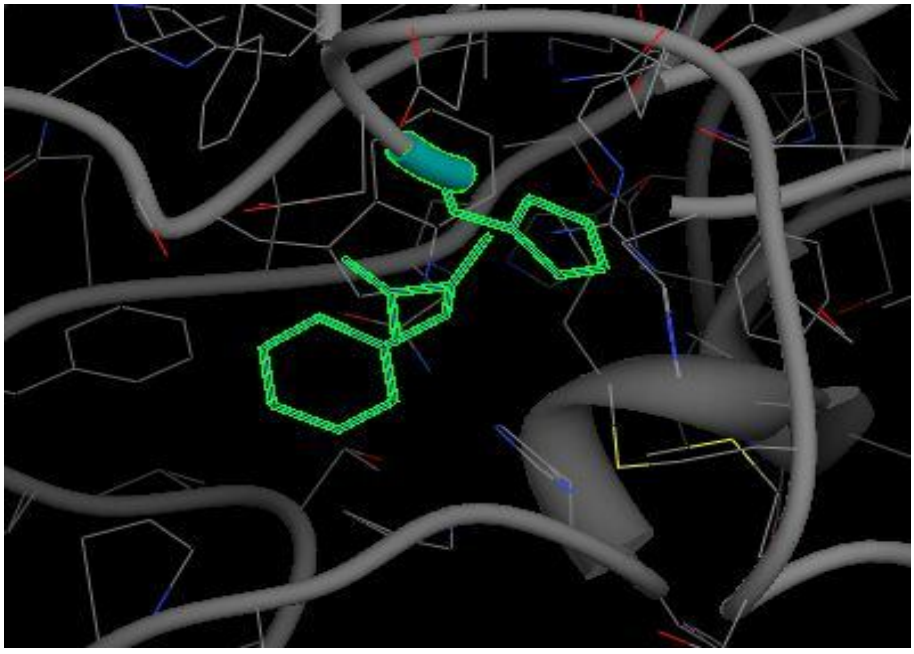


Figure 10: The active enzyme-substrate binding site.

The inter-molecular interaction between the mutant IDUA model active site and the 4-methylumbeliferyl α-L-iduronide ligand was studied on the basis of the bond lengths (table 2).

Table 2:

Mutations	Inter-molecular interaction with the ligand	Bond lengh (Å)
A160D (severe form)	Try153	5.83
	Ala212	4.33
A327P (moderate form)	Val323	3.54
	Lys324	3.88
H355N (Mild form)	Ser302	2.03
	Asn358	1.52

As per the obtained binding energy results and also the bond length values, the MPS type 1 disease is classified into three forms due to the missense mutations on the active site of the IDUA model. For instance, the A160D mutation has a higher bond length when mutated hence the distance between the enzyme-substrate complex is more and thus the enzyme get accumulated due to lesser amount of surface interaction with the ligand and it results in the severe form of MPS type 1. [21-23] Also that the A327P mutation, it has a lesser bond length when mutated that means the substrate has affinity towards the IDUA enzyme and hence has few surface interactions. Hence, the forms a moderate type of disease phenotype. Whereas, the H355N mutation has still less bond length that means the enzyme-substrate interaction is close enough and there is not much of accumulation of the enzyme in the body, thus causing a mild form of MPS type 1 disease phenotype.[24-29]

CONCLUSION

The homology modeling and the insilico analysis of the enzyme IDUA responsible for the disorder, Mucopolysaccharidosis type I (MPS I) was done in order to quantify the structural-functional relationships of mutant IDUA enzymes. Various bioinformatic tools like Swiss model, T-coffee, Uniprot, Procheck, Open babel, Deep view, Auto- dock were used to assess the structural and functional attributes of IDUA. In case of severe forms of MPS I, the mutants show an intense disruption in the structure of the enzyme that normally occurs in the core functional

region of the IDUA structure when compared to other attenuated forms. This change in structure affects the catalytic activity of the enzyme hence leading to very low affinity in the binding of the ligand to the receptor. The insilico analysis was aimed at being the first step in the direction of Pharmacological Chaperon Therapy (PCT) by engineering corresponding corrections in the IDUA missense mutations before assessing clinical therapy to patients suffering from MPS I. This investigation can be anticipated to screen the substitutions producing milder and moderate forms of protein folding in the structure. . Hence we can conclude that structural-functional characterization of the mutants may help in treatment of Mucopolysaacharidosis Type I by knowing the efficiency of binding of the receptor and substrate

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