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Homology Modeling And Molecular Docking Studies Of Xylanase Enzyme From Bacillus. Substilis

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Abstract

The purpose of this study was To find the interaction between xylan with xylanase in *Bacillus subtilis* by performing homology modelling and *in-silico* based molecular docking analysis.The model of the xylanase protein was constructed with SWISS-MODEL Server. The xylanase gene of the sample was sequenced using Sanger Sequencing Method which was then used for homology modelling and further analysis. The sequence was submitted (Accession No.OM986475) in National Center for Biotechnology Information (NCBI)'The Qualitative Model Energy Analysis (QMEAN Z) and Global Model Quality Estimation (GMQE) scores were used to assess the reliability of the modelled 3D structure, GMQE was found to be 0.90+/-0.05 for the modelled protein structure. Local model per residue score was accessed using the QMEANDisCo scoring function,which is a composite score for a single model quality estimation. The SWISS-MODEL Server was also used to provide the structural validation of the modelled target protein (CTX-M) for stereochemical quality and a Ramachandran plot. The ligand Xylan was obtained from PubChem available at https://pubchem.ncbi.nlm.nih.gov/ and was prepared using the Energy Minimisation tool in UCSF Chimera v.1.16. Molecular docking was performed using Autodock Vina. Visualization of this was done with the help of PyMOL v.2.5.2 and LigPlot+. Effect of xylanase on different substrates like xylan, xyloglucan, glucomannan, galactoglucomannan and arabinogalactan was tested using molecular docking., The xylan showed optimum binding affinity with the binding energy of -6.3 kcal/mol. Further interaction analysis, two H-bonds were hydrogen bonding (H-bonding) and five hydrophobic interactions were found for docking with xylan. Through molecular docking study using AutoDock Vina, amino acid residues relevant in protein-ligand interaction were identified.

Keywords: Homology modeling, molecular docking, SWISS-MODEL Server, Autodock Vina, PyMOL v.2.5.2 and LigPlot+

INTRODUCTION:

Glycoside hydrolase families, which make up one of the largest categories of commercial enzymes, include xylanases. The synergistic activity of two essential enzymes, endo— xylanase and beta-xylosidase, is required to depolymerize xylan molecules into monomeric pentose units. Xylanases vary in their mode of action, substrate specificities, biochemical features, and 3D structure, and they are generated by a diverse range of bacteria and fungi. The cellulose-binding domain of xylanase recognizes the linear polysaccharide xylan and hydrolyzes the beta-1,4-xylan to xylose. To achieve pure cellulose, this procedure breaks down the hemicellulose component and removes the interfering polymer. Some species have been shown to manufacture enzymes both intracellularly and extracellularly. However, because xylanases are used in so many different sectors, it is impossible to fulfil xylanase demand only from plant and animal sources. As a result, bacteria are used to produce xylanase, either utilising natural strains or recombinant microbial strains. Currently, large- scale xylanase production is possible because to the use of genetic engineering tools that allow for the rapid discovery of novel xylanase genes and their genetic variants, making it an excellent enzyme. Due to the depletion of fossil fuels, it is critical to create environmentally benign and long-term energy sources.

Virtual screening and molecular docking studies have made structure-based drug design a viable first step in the discovery of new lead compounds for the treatment of various diseases including bacterial. Molecular docking is a widely used method to predict the non-covalent binding modes of small molecule (ligand) to the macromolecule (target ore receptor). It is a multi-stage process, with each step adding one or more levels of complexity. The procedure starts with the use of docking algorithms to position tiny molecules in the active site. These algorithms are supplemented with scoring functions that evaluate interactions between chemicals and prospective targets in order to predict biological activity. Auto Dock Vina is one of the most popular and frequently used open-source molecular docking software. Protein complexes function as the core of biological processes. Only when the 3D structure of the target macromolecule or protein is known, *in silico* approaches like virtual screening or docking are used to screen a huge number of chemicals accessible in chemical databases for possible inhibitors.

The three-dimensional structures of proteins give a vital insight into their molecular activity and provide a vast range of applications in life science research. For a complete understanding of biological systems, including how the protein complexes and networks operate and how to control them, a comprehensive knowledge of their interactions and the overall quaternary structure is required. Homology modelling has evolved into a critical tool in structural biology, helping to bridge the gap between known protein sequences and experimentally determined structures.

The biological action of a protein is normally carried out in foldings (pockets) known as catalytic active sites. In the catalytic active site, functional pockets bring substrate and catalytic side chains together. These geometric and topological features, like surface pockets, inner cavities, and cross channels, are critical for proteins to perform their tasks. The CASTpserver (Computed Atlas of Surface Topography of Proteins) is an online tool for finding, defining, and quantifying certain geometric and topological features of protein structures. It uses a computer algorithm with low human subjectivity to identify and quantify all the concavities (pockets and voids)in the protein. Both pockets and voids are detected and are measured which can further be used for molecular docking analysis.

The present study is to find the interaction between xylan with xylanase in *Bacillus subtilis* by performing homology modelling and *in-silico* based molecular docking analysis.

MATERIALS AND METHODS:

Homology Modelling and Model Evaluation

The model of the xylanase protein was constructed with SWISS-MODEL Server, available at https://swissmodel.expasy.org,and a suitable template was provided.The xylanase gene of the sample was sequenced using Sanger Sequencing Method which was then used for homology modelling and further analysis. The sequence was submitted (Accession No.OM986475) in National Center for Biotechnology Information (NCBI), which is located in Bethesda, MD, the United States available at *https://www.ncbi.nlm.nih.gov/*. The query for the SWISS- MODEL Server should be provided as an amino-acid or protein sequence and ExPASy Translate tool was used for the purpose, available at *https://web.expasy.org/translate/*. The Qualitative Model Energy Analysis (QMEAN Z) and Global Model Quality Estimation (GMQE) scores were used to assess the reliability of the modelled 3D structure. A QMEAN- Zaround zero indicates that the projected structure is –native-like structure, which was found to be

-0.22 for the modelled protein structure.The GMQE score can goes from 0 to 1, with a higher score indicating more dependability. GMQE was found to be 0.90+/-0.05 for the modelled protein structure. Local model per residue score was accessed using the QMEANDisCo scoring function,which is a composite score for a single model quality estimation. The SWISS-MODEL Server was also used to provide the structural validation of the modelled target protein (CTX-M) for stereochemical quality and a Ramachandran plot.

Target Protein and Ligand Preparation

The homology modelled xylanase target protein was downloaded from the SWISS- MODELServer in protein data bank file format (.pdb format). The target protein was prepared by removing the complexed ligands using UCSF Chimera v.1.16, followed by energy minimization using the Minimize Structure tool followed by the DockPrep tool. Xylan was obtained from PubChem available at https://pubchem.ncbi.nlm.nih.gov/ and was prepared using the Energy Minimisation tool in UCSF Chimera v.1.16. Molecular docking was performed using Autodock Vina. It requires the receptor and ligand representations in the pdbqt file format, which is a modified protein data bank format that includes atomic charges, atom type definitions, and topological information for ligands (rotatable bonds) and was performed using the Autodock Tools package.

Molecular Docking and Visualization

The AutoDock Vina v.1.1.2 was used to simulate the docking of xylan with xylanase, and the docking data and binding score were displayed to examine the molecular interactions. The CASTp v.3.0 program was used to define and measure the volume of the active catalytic site of the target protein. The amino acids which may participate in the docking in the active site were predicteds package.

The global search exhaustiveness was set at 8 and a total of 9 binding modes were present. The weights and terms scoring function was set to default parameters. Furthermore, AutoDock Vina uses a gradient algorithm search method to predict the binding scores and modes of ligands in the active receptor sites. Visualization of this was done with the help of PyMOL v.2.5.2 and LigPlot+.

Effect of xylanase on different substrates by molecular Docking analysis

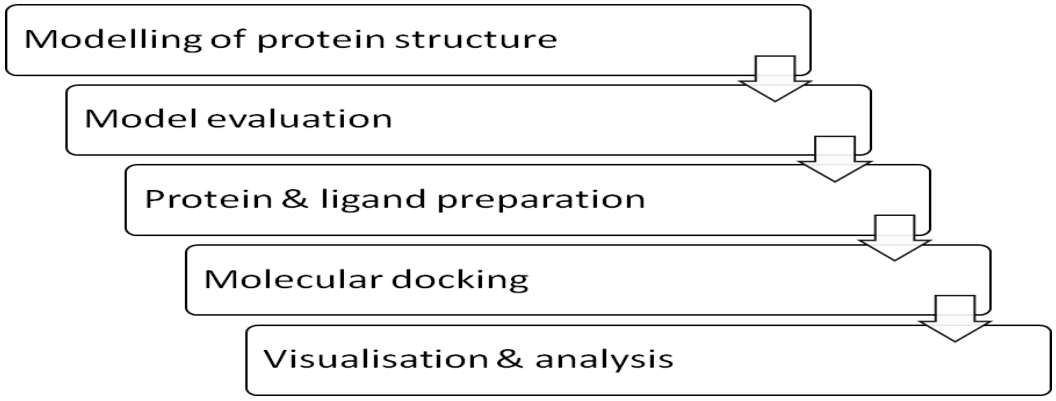
Effect of xylanase on different substrates like xylan, xyloglucan (heteropolymer of D-xylose and D-glucose), glucomannan (heteropolymer of D-glucose and D-mannose), galactoglucomannan (heteropolymer of D-galactose, D-glucose and D-mannose) and arabinogalactan was tested using molecular docking.

Three-dimensional structures of bacterial xylanase were obtained from the Research Collaboratory for Structural Bioinformatics Protein Data Bank (RCSB PDB) (http://www.rcsb.org/pdb, 26.12.19), the United States National Library of Medicine, National Center for Biotechnology Information server PubChem (https://pubchem.ncbi.nlm.nih.gov/, 26.12.19) and Royal Society of Chemistry chemical identifier search database (http://www.chemspider.com/Default.aspx, 26.12.19). Prior to analysis, water molecules (shown as 000, in the software) and other unwanted residues (recognized by characteristic sequence breaks) were removed from all proteins, when necessary, using PyMol (PyMOL™ v2.3.2 - Incentive Product, Schrodinger, LLC). The sequences were then subjected to energy minimization by Swiss-PdbViewer v4.1.0. The enzyme was then docked as protein ligands to different substrates as receptors using PatchDock online docking server (https://bioinfo3d.cs.tau.ac.il/PatchDock). Results were refined and rescored utilizing Firedock (http://bioinfo3d.cs.tau.ac.il/FireDock/php.php, 26.12.19) which provides the global energy for the docked complexes. Usually, ligand- receptor binding energies are calculated using low-energy minima and compared with experimental values. The lowest binding energy/global energy in the solutions table was selected and the polar hydrogens were then added to the models using Biovia Discovery Studio 4.5 64-bit client.

RESULTS

The ligand to receptor docking affinity can be represented by the binding energy and the interactions present. The optimal conformation of the ligand in the active pockets of the target is dependent on the very negative magnitude of binding affinity (lowest binding energy) used to evaluate docking output.

Graph: Graphical abstract of the workflow



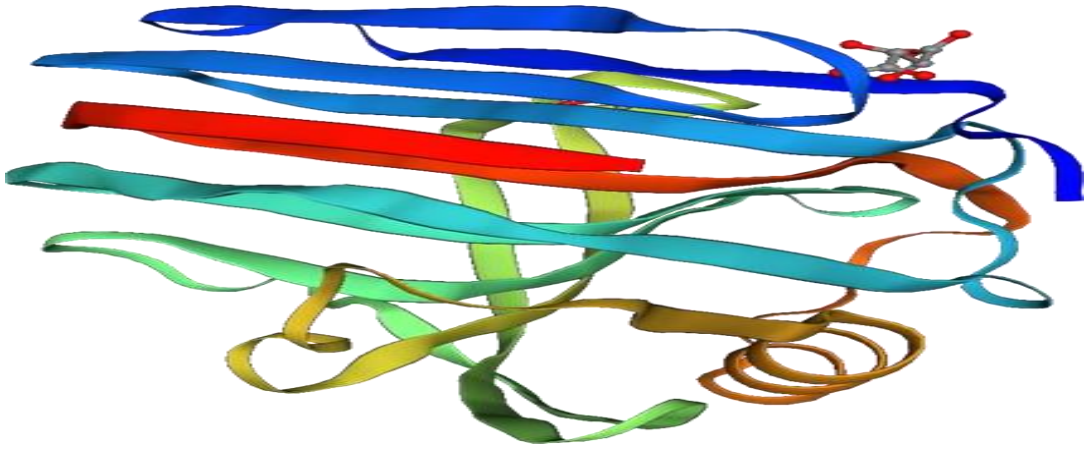


Fig. 1. Showing the 3D representation of the modelled protein structure

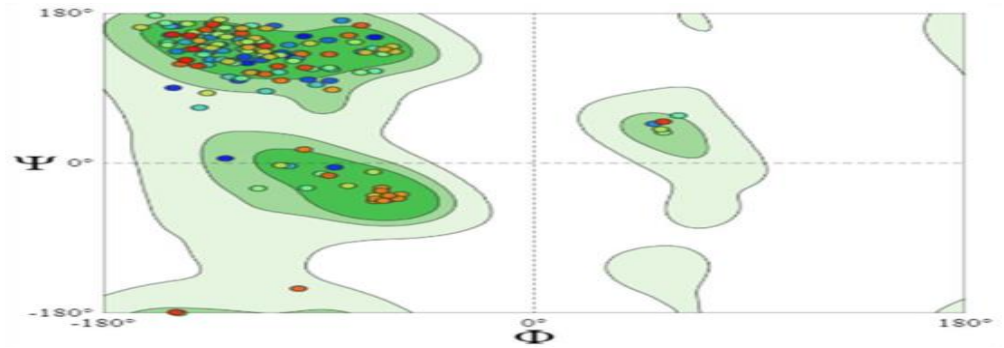


Fig. 2. Showing the Ramachandran plot of the modelled protein structure

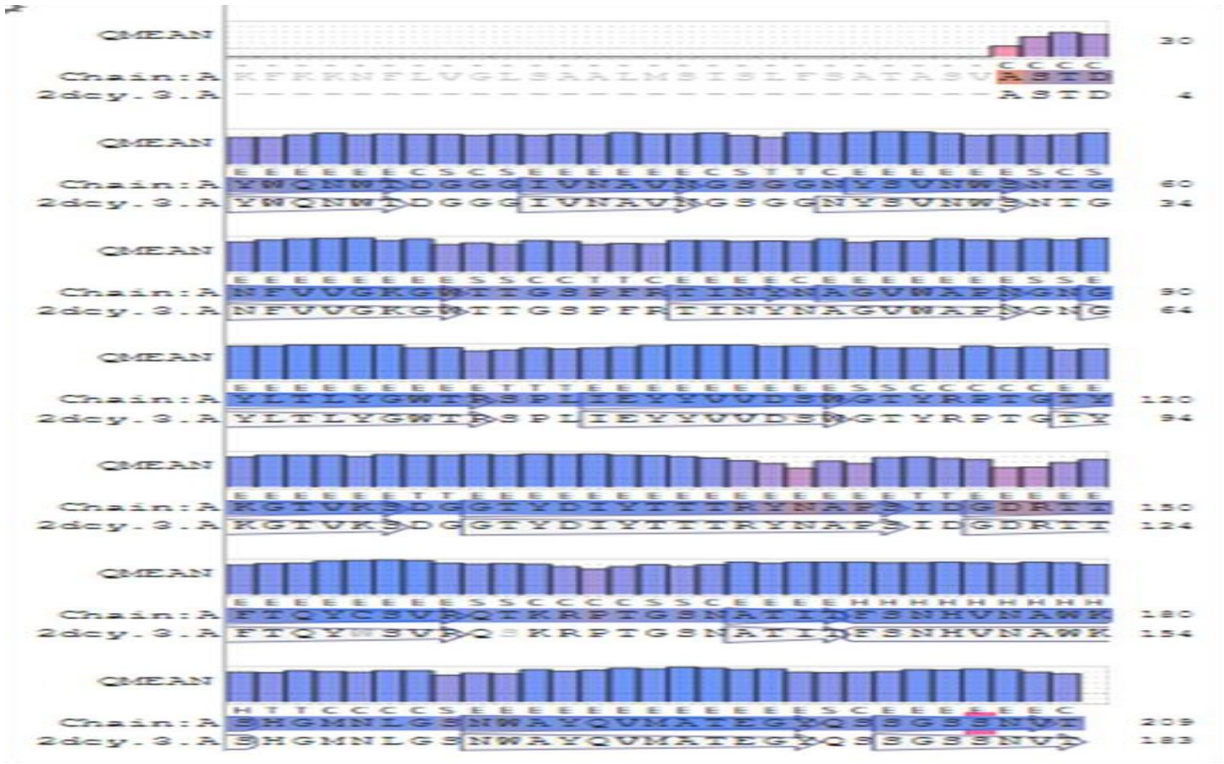


Fig. 3. Showing the alignment of query and template sequence

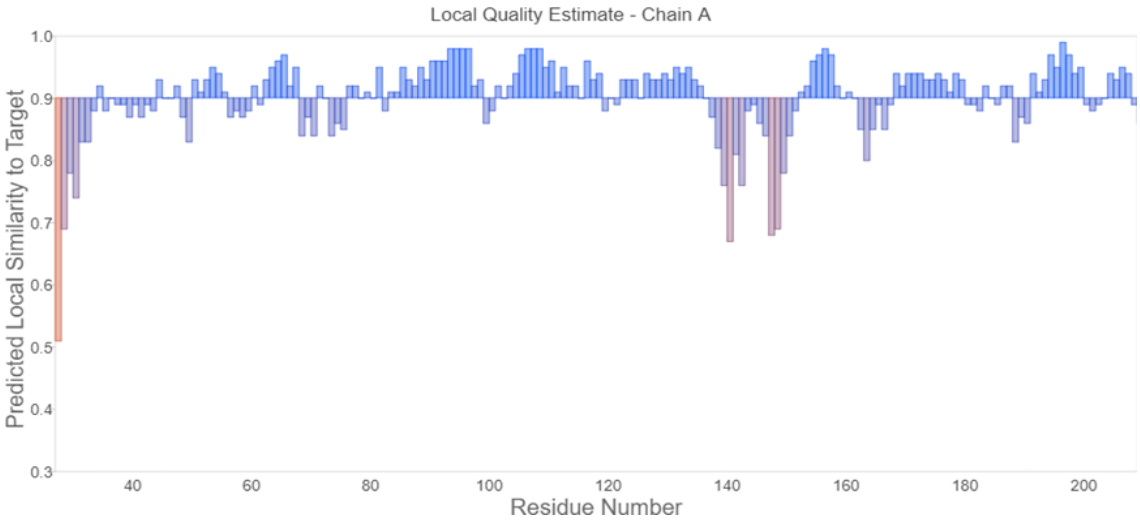


Fig.4. Showing the QMEAN DisCo Local

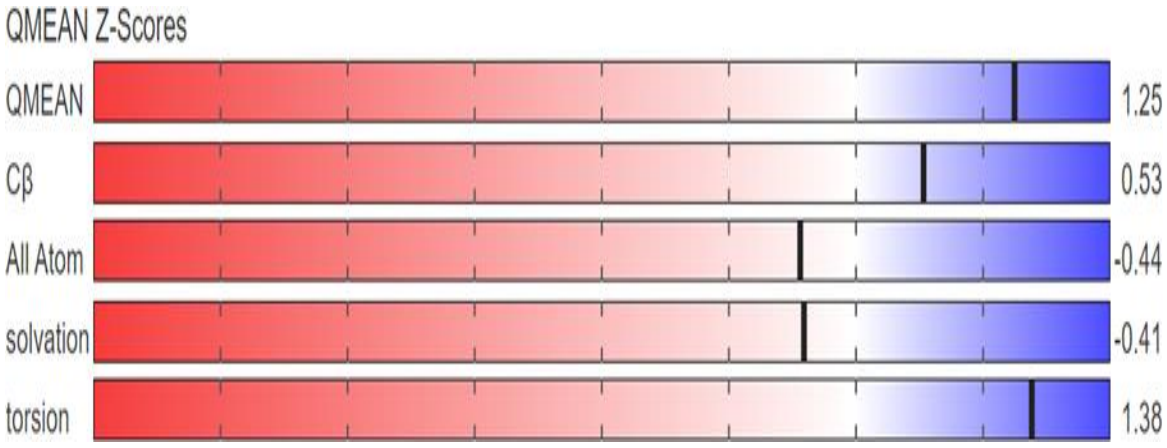


Fig. 5. Showing QMEAN Z-scores

Fig. 6. Showing the comparison plot of QMEAN Z

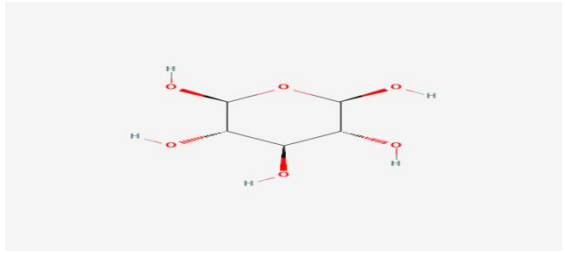


Fig.7. Showing the 2D representation of ligands selected for analysis

Fig. 8. Showing the amino acid sequence (single letter representation) of the xylanase (target protein). Amino acids in the active site have been highlighted blue

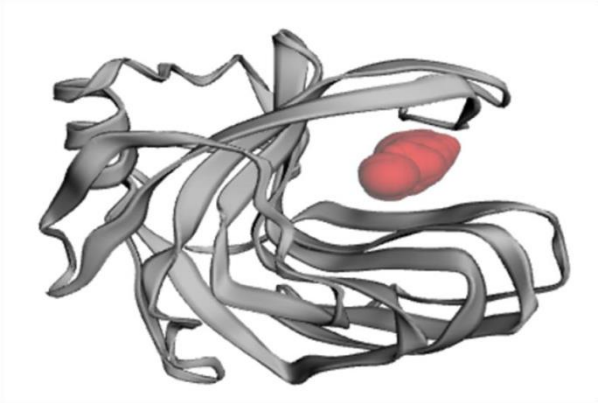
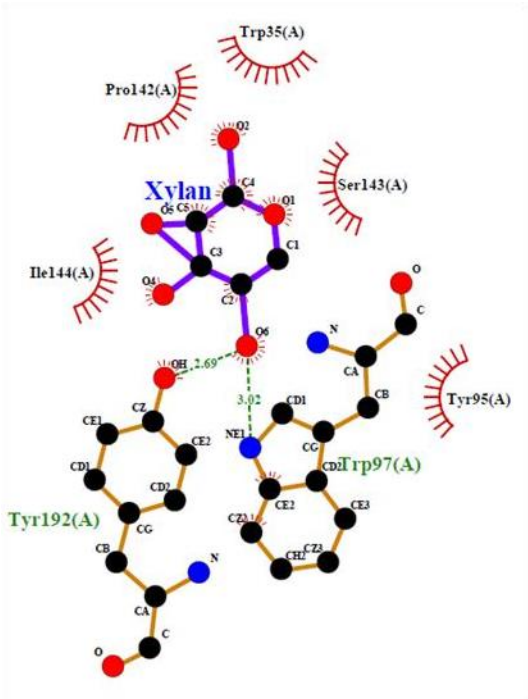


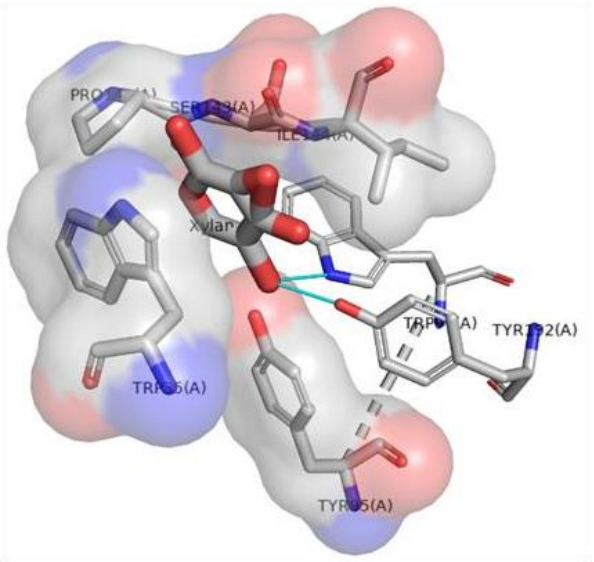
Fig. 9. Showing the binding chamber for the modelled protein

Table 1: Showing details of the ligands selected for analysis

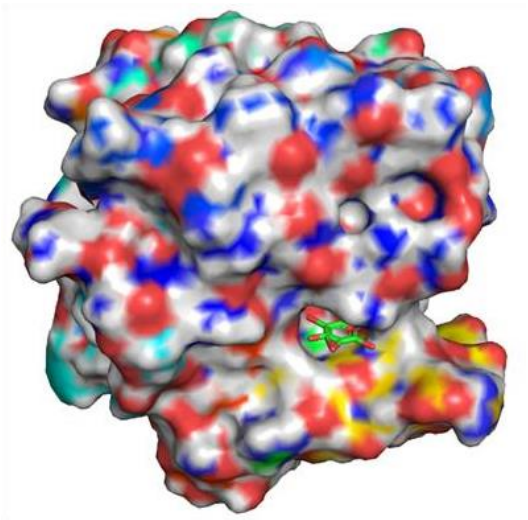
Sl No.	Chemical Compound	PubChem SID
1.	Xylan	111677923

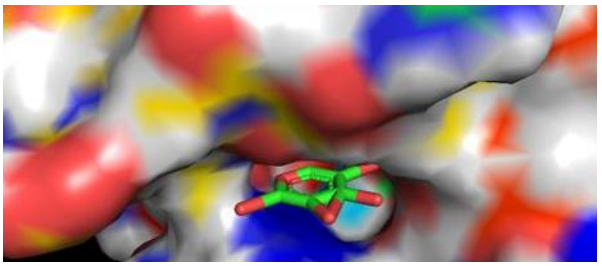


2D representation of H-bonding of xylan with target



3D representation of H-bonding of xylan with target





3D representation of docked pose of xylan with target

Table showing the binding score with the corresponding bonding interaction. Xylan was showing affinity of -6.3 kcal/mol. Further interaction analysis, two H-bonds were hydrogen bonding (H-bonding) and five hydrophobic interactions were found for docking with xylan. The docking interactions and LigPlot representation are delineated in table 4. LigPlot represents the 2D representation of the docking interaction, which includes both H-bonds and hydrophobic interaction. H-bonds are represented in green dashed lines with donor-acceptor distance specified whereas red arcs represent hydrophobic interactions, with spokes spreading towards the ligand atoms. The 3D interactions are represented with the help of PyMOL, where the cyan line represents the H- bond and different elements are colour coded for easy identification for both protein and ligand. When analysing the interaction data, the docking result shows interaction with the amino acids in the active site mentioned in the CASTp server, proving a successful docking.

Table. 2. Showing the visualisation of docking interaction of lig1

SI. No	Chain ID	Sequence ID	Amino acid	Atom
1	A	33	GLN	OE1
2	A	35	TRP	CB, CG, CD1, CD2, NE1, CE2, CE3, CZ2, CZ3, CH2
3	A	61	ASN	OD1
4	A	63	VAL	CB,CG2
5	A	95	TYR	CE1,OH
6	A	97	TRP	NE1,CZ2
7	A	104	GLU	OE1,OE2
8	A	106	TYR	OH
9	A	138	ARG	NE, NH2
10	A	142	PRO	C,O,CB,CG
11	A	143	SER	N,CA,C,O,
12	A	144	ILE	N,CA,CG2
13	A	151	PHE	CZ
14	A	153	GLN	NE2
15	A	192	TYR	OH
16	A	198	GLU	CD,OE1,OE2

CONCLUSION:

In this work, we describe the QMEANDisCo composite score for single model quality estimation. It employs single model scores suitable for assessing individual models, extended with a consensus component by additionally leveraging information from experimentally determined protein structures that are homologous to the model being assessed. By using the found homologues directly, QMEANDisCo avoids the requirement of an ensemble of models as input.

QMEANDisCo has been developed with its application in the SWISS-MODEL homology modelling server in mind. A template search is the first step of any homology modelling pipeline. As this is the computationally most expensive step in QMEANDisCo, its integration into SWISS-MODEL comes at minimal additional computational cost. The low response times are also reflected in CAMEO where QMEANDisCo returns results within a few minutes with most of the time being spent in the template search step. We believe that we provide a valuable tool that can easily be accessed through the QMEAN-Server. We demonstrated state-ofthe- art performance in predicting lDDT scores with a focus on perresidue predictions. Prediction accuracy can expected to further increase given the growing number of experimentally determined protein structures.

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