In Silico Analysis of Protein-Excipient Interactions: A molecular docking study on therapeutic IL-11

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CANDIDATE'S DECLARATION

I SNEHA hereby certify that the work which is being presented in the thesis entitled Insilco analysis of protein-excipient interactions: A molecular docking study on therapeutic IL-11 in partial fulfilment of the requirements for the award of the Degree of Master in Biotechnology, submitted in the Department of Biotechnology, Delhi Technological University is an authentic record of my own work carried out during the period from January to May under the supervision of Dr. Smita Rastogi Verma. The matter presented in the thesis has not been submitted by me for the award of any other degree of this or any other Institute.

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CERTIFICATE BY THE SUPERVISOR

Certified that Sneha (2k23/MSCBIO/80) has carried out their search work presented in this thesis entitled **"Insilco analysis of protein-excipient interactions: A molecular docking study on therapeutic IL-11"** for the award of Master in Biotechnology from Department of Biotechnology, Delhi Technological University, Delhi, under my supervision.

The thesis embodies results of original work, and studies are carried out by the student herself and the contents of the thesis do not form the basis for the award of any other degree to the candidate or to anybody else from this or any other University/ Institution.

Smita
5/6/25

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Place : Delhi
Date-05.06.2025

Abstract:

Interleukin-11 (IL-11) is a multifariously cytokine of great therapeutic interest, especially for hematopoiesis, inflammation, and management of fibrosis. Recombinant human IL-11 (rhIL-11) has been applied extensively in clinical use for the prevention of thrombocytopenia and other immune disorders. Nevertheless, the stability and bioactivity of IL-11 formulations are affected by their physical and chemical interaction with excipients during formulation. This study investigates the interactions of IL-11 with usual excipients, lactose, sucrose, trehalose, mannitol, and sorbitol using the molecular docking method. Binding affinities and interaction modes were thoroughly investigated to forecast the excipient function in stabilizing IL-11. Findings indicated that sugars such as lactose, trehalose, and sucrose exhibit higher binding interactions (-6.1, -5.8, and -6.0 kcal/mol) compared to sugar alcohols (mannitol and sorbitol), suggesting their viability as more efficient stabilizers for IL-11 in drug formulations. This computational strategy underpins the rational choice of excipients for cytokine stabilization and can inform future formulation design.

Keywords

Excipients; IL-11; In silico analysis; Molecular docking; Therapeutic protein stability

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CHAPTER 1

INTRODUCTION

1.1. Overview of Therapeutic protein Formulation

Improving the storage stability of lyophilized recombinant human interleukin-11 using combinations of disaccharides and hydroxyethyl starch. Protein-based therapies are a priority for most pharmaceutical companies in the contemporary drug discovery (R&D) age. Recombinant biologics have been broadly utilized as the drug of choice for the therapy of diverse diseases, in significant part because of specificity in recognizing and altering disease-related pathways [1]. The first recombinant protein developed for health care application was Insulin, marking a significant milestone since its discovery in the 1970s. Initially, non-antibody protein-based therapies began with insulin, which was extracted from the pancreas of animals such as cows and pigs. In 1922, it was administered to patients with type I and type II diabetes mellitus, offering new hope for managing the disease. It was the 1st paradigm shift. DM-I is an illness whose basic aetiology is reduced level of insulin, which sends signals to the cells in order for the cells to execute functions involved in glucose metabolismDespite its significance, the use of insulin was restricted by factors such as limited availability, high production costs, and the risk of triggering immune responses. It took nearly six decades before the next major breakthrough occurred — the development of Humulin, the first recombinant human insulin produced through genetic engineering, which received approval from the US FDA in 1982.

In pharmacology, protein-based therapeutics are the medications that are emerging the fastest [2]. Recombinant technology helps in making a majority of protein therapies, and more than 170 of them are used in medicine worldwide. However, many more are under clinical trials for a variety of uses, including disease management, immunological disorders, infections, cancer prevention, and diagnosis. These proteins have become an important part of the pharmaceutical business, and research will be done on them to find new, effective treatments [3]. It has been recognized that these treatments make up one-third of the substances approved as novel biological medicines. The emergence of SARS-CoV-2 has led to more than 6 million deaths worldwide, making it the most severe global health emergency since the influenza pandemic of 1918.[4].

1.2 Interleukin-11 (IL-11): Clinical and Pharmaceutical Importance

A cytokine produced from stromal cells, INTERLEUKIN-11 (IL-11), has potential thrombopoietic effects in vivo. [5] [6], Recombinant human IL-11, or rhIL-11, has shown promise in clinical trials as a treatment for thrombocytopenia brought on by chemotherapy. RhIL-11 improves platelet nadirs in patients with breast cancer following chemotherapy and is well tolerated in a phase I trial.MK endoreduplication, Administration of recombinant human IL-11 (rhIL-11) led to an increase in both the frequency of megakaryocytes (MKs) and the proportion of MKs expressing proliferating cell nuclear antigen (PCNA) in the bone marrow of these patients. In two randomized, placebo-controlled phase II clinical trials involving cancer patients with severe thrombocytopenia, treatment with rhIL-11 notably reduced the number of patients requiring platelet transfusions compared to those given a placebo [7] [8]

Additional preclinical research using rhIL-11 in both normal and myelosuppressed animals has demonstrated the cytokine's thrombopoietic capability. Peripheral platelet counts have been demonstrated to rise when rhIL-11 is administered to healthy animals, such as mice, rats, rabbits, dogs, and nonhuman primates. [7] With typical granule production and demarcation membrane system development, the mature MKs that form in the animals' BM appear ultra structurally normal. Mice given rhIL-11 subcutaneously on a regular basis showed an increase in colony-forming unit-MK (CFU-MK) numbers in the spleen and median MK ploidy in the BM. [9]. RhIL-11 therapy enhanced platelet nadirs and sped up platelet recovery in myelosuppressed mice. These effects were accompanied by a significant rise in the number of BM MKs. [10] [11]

RhIL-11's actions on MK-line hematopoietic cells in vitro are consistent with its effects in vivo. It has been demonstrated that rhIL-11 promotes several phases of MK development. [12] [13] [14] RhIL-11 worked in concert with IL-3 or steel factor (SF) to promote growth of human and murine CFU-MK-derived colonies. Human CD34+HLA-DR− BM cells treated with rhIL-11 in addition to IL-3 showed improved colony formation derived from burst-forming unit-MK (BFU-MK). Compared to IL-3 alone, rhIL-11 with IL-3 enhanced MK number and acetylcholinesterase synthesis in murine BM liquid cultures. RhIL-11 alone increased the ploidy and growth of human BM MKs.

IL-11 belongs to the cytokine family, which also includes IL-6, ciliary neurotrophic factor (CNTF), leukemia inhibitory factor (LIF), and oncostatin M (OSM)). These are regulated by

the same signal transducer, gp130, which has ubiquitous expression in many tissues and cell types. [15] The specificity-granting element of the receptor complex is the recently cloned ligand-binding IL-11 α chain. Cytokine stimulation triggers the interaction between the α chain and gp130, leading to the activation of signaling pathways involving the JAK/TYK tyrosine kinases and the MAPK serine/threonine kinases. This activation results in the phosphorylation of transcription factors STAT1 and STAT3. [16].

1.3 Role of excipients in protein based -drug delivery

Excipients can be utilized to minimize surface adsorption, inhibit protein aggregation, stabilize proteins, or just provide normal osmolality. A vast range of compounds are included in the stabilizers, including as sugars, salts, polymers, surfactants, and amino acids. These excipients interactions with the protein, the container surface, and—above all—water is what primarily affect the stability of proteins in solution. [17]While some excipients employ a number of essentially distinct methods via indirect interactions, others use direct binding to stabilize proteins in solution. Since water is no longer present in the prohibited state, any effects that the excipients may have on proteins through their interactions with it are meaningless. Instead, excipients contribute to protein stability by directly interacting with them.

Many proteins change their shape and become unstable when they are in a solution. Because of this, they can be affected by different types of stress during steps like purification, processing, and storage [18**]** [19]. Shear strain, exposure to high pH, surface adsorption, and high temperatures are some examples of these stresses [20]. As a result, a variety of processes may cause protein-based medications to physically degrade (e.g., unfold, aggregate, and form insoluble particles), which may hurt the therapeutic product's safety and effectiveness. The stability of the protein is largely determined by its solvent environment [21]

Studies have shown that specific solvent additives, commonly referred to as osmolytes, can enhance protein stability and thereby reduce the aggregation of proteins with moderate stability. In this context, cosolvents help maintain the native structure of proteins and reduce aggregation by stabilizing the folded state, as protein unfolding typically precedes aggregation [22]. Their effectiveness stems from poor compatibility or repulsive interactions with the protein surface. Conversely, excipients such as proteins, polymers, arginine, and surfactants are commonly used to minimize protein aggregation without necessarily enhancing overall stability [23]. These agents function either by competitively binding to surfaces or interfaces that may destabilize the protein or by forming weak interactions with the protein surface [24].

Certain excipients are also employed to stabilize proteins in their dried form. However, the lack of water in this state alters the underlying mechanisms, rendering excipient-water interaction-based stabilization ineffective. Additionally, since freezing is a critical intermediate step in the lyophilization process, its influence on protein stability in the presence of various additives will also be discussed. Notably, the freezing stage presents a unique physical environment, marked by contact forces within the solution, as water remains initially but is progressively removed during ice crystal formation.

1.4 In Silico Modeling in Excipient Screening

In silico approaches have become increasingly popular in pharmaceutical sciences, notably in protein formulation creation. The stability and efficacy of protein-based treatments depend on the prediction and analysis of protein–excipient interactions, which may be done computationally using molecular docking and molecular dynamics simulations. Excipient selection has traditionally relied on time-consuming, expensive, and tedious experimental trial-and-error techniques. To get over this limitation, current research has focused on computational forecasting of excipient binding sites and interaction architectures with therapeutic proteins. In addition to identifying which excipients should be given experimental priority, these methods offer significant insight into molecular details of how excipients affect protein structure and stability.

Research [25] employed techniques such as hydrogen-deuterium exchange mass spectrometry (HDX-MS) alongside molecular docking to investigate how typical excipients interact with granulocyte colony-stimulating factor (G-CSF). A combined mechanism of selective exclusion and specific binding that can help in protein stabilization is achieved by the Insilco study's identification of hotspot regions on the protein surface where excipients mainly attach. This method showed that the configuration and type of interaction between excipients and protein surfaces may be predicted using in silico docking. To systematically map excipient interactions with different therapeutic proteins, Rathore et al. (2016) [26] conducted docking experiments. These findings revealed that excipients generate non-covalent interactions with certain amino acid residues via hydrogen bonding, electrostatics, and van der Waals forces, which stabilize the protein structure and avoid aggregation. In order to enhance formulation, scientists select excipients that improve product stability while maintaining biological activity.

Their study underlined the need to find interaction hotspots. A computational method was proposed by Sahakyan et al. (2024) [26] in another recent work to predict the impact of

excipients on protein–protein interactions, specifically their ability to change aggregation tendencies. To understand how excipients affect the protein's interaction structure, the study extended in silico screening beyond straightforward protein–excipient docking. It recognizes the relevance of computational modelling for both logical formulation optimization and interaction prediction.

Together, these studies illustrate the growing use of in silico modelling for excipient testing. This highlights how techniques like interaction fingerprinting, energy reduction, and molecular docking are changing excipient selection from an experimental method procedure to a predictive, data-informed strategy. Computational techniques have laid the groundwork for logical excipient design, greatly speeding up the development of reliable and efficient protein-based medicines.

1.5 Molecular Docking as a Computational Tool

Molecular docking is essentially the process of employing computers to simulate ligand-protein interactions within the protein's three-dimensional binding region. The most energetically advantageous binding orientation of a ligand within the protein's binding pocket may be predicted by docking programs using algorithms that take into consideration variables including ligand flexibility, protein conformational variations, and electrostatic complementarity [27].

The quality of the protein structure, the selected scoring function, the inclusion of solvent effects, and the flexibility of the protein are some of the factors that affect the accuracy of molecular docking simulations [28] . Additionally, to evaluate the predicted accuracy of docking algorithms, validation with experimental data, such as crystallographic structures or binding affinity measurements, is crucial.

Molecular docking has emerged as a useful tool in virtual screening attempts to identify potential therapeutic molecules from enormous libraries of chemicals, despite its high processing needs. Scientists may find the most promising candidates for further experimental validation by virtually screening millions of chemicals. Enhance the drug development process and lower the time and expense involved with traditional high-throughput screening techniques.

In silico methods, such as molecular docking, represent a valuable option through the ability to predict binding affinity and contact sites between proteins and excipients with high accuracy

[29]. The present research investigates the interactions of therapeutic IL-11 with typical pharmaceutical excipients through molecular docking simulations. This study utilizes molecular docking to evaluate the binding interactions between IL-11 and a panel of commonly used excipients, namely lactose, sucrose, trehalose, mannitol, and sorbitol, to predict their potential to stabilize IL-11 through favorable interactions.

1.6 Research Objectives and Scope

The core objective of this study to explore and evaluate the interactions between the therapeutic protein Interleukin-11 (IL-11) and selected pharmaceutical excipients using computational molecular docking techniques. The intent is to identify potential excipients that can interact favourably with IL-11, thereby suggesting their stabilizing roles in its formulation.

The specific objectives of this study are:

To retrieve and prepare the three-dimensional (3D) structure: of human IL-11 for in silico docking.

To select and optimize excipient molecules: that are commonly used in biopharmaceutical formulations.

To perform molecular docking simulations: using Auto Dock Vina or a similar tool to predict binding affinities and interaction sites between IL-11 and the selected excipients.

To analyse the docking results: to identify key amino acid residues involved in binding and the nature of non-covalent interactions.

To interpret the implications of these interactions: in terms of protein stabilization, with potential recommendations for formulation design.

This study is limited to the use of in silico methods to investigate the interactions between the therapeutic protein Interleukin-11 (IL-11) and selected pharmaceutical excipients. It does not involve any experimental or laboratory-based work but relies solely on computational tools such as molecular docking to predict potential binding interactions. The excipients chosen for this study are commonly used stabilizers in protein formulations, including amino acids, sugars, and polyols. The primary focus is on identifying the binding affinity and nature of interactions between IL-11 and these excipients, with the aim of understanding their possible roles in stabilizing the protein structure. The docking results are interpreted to highlight key residues involved in excipient binding, which can provide valuable insights into formulation strategies.

However, this study does not assess the functional activity, stability under various storage conditions, or in vivo behavior of the protein–excipient complexes. The findings are intended to support the early phase of formulation development by providing a computational basis for excipient selection, and they serve as a foundation for future experimental validation.

CHAPTER 2

Literature Review

Stability Challenges of Biotherapeutics

Biotherapeutic proteins like monoclonal antibodies, cytokines, and growth factors are extremely sensitive to processing and environmental conditions because they possess large and complex structures. The molecules are prone to different types of degradation mechanisms, like physical instabilities involving aggregation and precipitation and chemical modifications involving deamidation, oxidation, hydrolysis, and disulfide bond reshuffling. Among them, protein aggregation is particularly significant since it can cause loss of activity and can induce harmful immune reactions in patients [31] [33].

Instability may arise in any stage of the product life cycle—from cell culture and purification to formulation, storage, and transportation. All of these may involve various destabilization factors such as temperature variations, exposure to light, pH variations, agitation, and freeze–thaw cycles, which destabilize the conformation of the protein and cause partial unfolding and hydrophobic patch exposure, thus triggering the aggregation process (RSC Advances, 2023; Pharmaceutics, 2022) [34]. In addition, popular surfactants such as polysorbates, even though widely employed to avoid agglomeration, have proven to be limiting as they are prone to oxidation and hydrolysis, leading to the investigation of other excipients (Pharmaceutics, 2022) [34].

To counter these challenges, early evaluation of "stability liabilities" within the drug development stage of protein structure has been suggested. These involve testing regions that are susceptible to oxidation or deamidation with in silico tools and biophysical tests, allowing for rational design of formulation (Sharma et al., 2020) [32]. Stabilization measures can include protein engineering, optimal storage conditions, or the employment of novel stabilizers like amino acids, polyols, and sugars.

Molecular docking is a very potential computer-based tool for predicting the binding interactions between small molecules (ligands) and selected proteins, such IL-11, with high accuracy and efficiency (Morris et al., 2009) [35]. By simulating excipient-protein interactions

within the three- dimensional space of the protein's surface, docking programs can predict the favourable complex confirmation of a ligand, facilitating for development of pharmaceutical and further experimental validation. Despite its computational nature, molecular docking has proven to be a valuable tool in virtual screening campaigns aimed at identifying potential drug candidates from large chemical libraries.

2.1 Excipient-protein interactions: mechanism and implications

The efficacy and stability of biotherapeutic proteins are greatly determined by the type of excipients present in their formulation. Excipients are not inert materials; they actively destabilize or stabilize proteins through various physical and chemical modes of interaction. These interactions are important to inhibit degradation processes such as aggregation, unfolding, and oxidation that can lead to loss of quality and therapeutic efficacy of protein drugs [36].

One of the most prevalent mechanisms is preferential exclusion or hydration. During this action, excipients like sugars (e.g., sucrose, lactose and trehalose) and polyols (e.g., glycerol) are kept out of the protein's immediate surroundings. This thermodynamically Favors the protein's compact, native structure, thus raising its structural stability (Wang, 1999) [37]. As an example, trehalose is commonly incorporated in freeze-dried products for its more effective water replacement and glass transition characteristics that maintain protein structure upon drying [36].

Conversely, certain excipients like arginine and histidine have direct binding interactions with proteins. These involve hydrogen bonding, π-π stacking, or hydrophobic interactions with amino acid side chains on the surface of the protein. These interactions can cover exposed hydrophobic areas and hinder the aggregation tendency of proteins, particularly under agitation or freeze– thawing. However, high concentrations of arginine may sometimes destabilize proteins, highlighting the importance of concentration optimization in formulation design [38].

Additionally, electrostatic interactions with charged excipients and protein surface residues can also influence their conformational stability substantially. For example, incorporation of buffering agents such as histidine not only controls pH but will also bind to specific protein residues and stabilize tertiary structure [36]. Besides, surfactants like polysorbate 20 or 80 are usually added to reduce interfacial stress and surface adsorption, two primary inducers of

protein aggregation in vials and syringes. These surfactants shield proteins from air–liquid interface denaturation, although they are also susceptible to oxidative degradation [39]

Recognition of these interactions enables formulation scientists to design excipients strategically to improve overall stability. For instance, a blend of sucrose and polysorbate will at the same time confer thermal stability and mitigate interfacial stress. But poor choice or undue excipient load may result in unforeseen adverse outcomes such as higher viscosity or solubility problems (Wang et al., 2010) [39]. Thus, detailed biophysical and computational evaluations of protein–excipient compatibility are critical in early-stage formulation.

2.2 IL-11: Structure, Function, and Biomedical Applications

Interleukin-11 (IL-11), a multifunctional cytokine within the IL-6 family, plays a vital role in tissue repair, immune regulation, and hematopoiesis. Structurally, IL-11 is a 19 kDa glycoprotein characterized by a four-helix bundle motif common to all IL-6 family members. This structural feature enables IL-11 to interact with the shared signal-transducing receptor gp130 and the IL-11 receptor α (IL-11Rα), triggering downstream signaling pathways [41].

The main function of IL-11 is to promote the proliferation and maturation of hematopoietic progenitor cells. Let me know if you'd like it expanded into a full sentence or used in a specific context, particularly megakaryocytes, which are the building blocks of platelets. Because of this characteristic, recombinant IL-11 is a useful therapeutic treatment for treating thrombocytopenia, or decreased platelet counts, in patients after bone marrow transplantation or chemotherapy [42]. Furthermore, IL-11 is important in reducing inflammatory and autoimmune illnesses because it modulates cytokine production to provide anti-inflammatory effects [43].

IL-11 is essential for tissue remodelling and repair processes in addition to haematopoiesis. By lowering epithelial cell apoptosis and increasing regeneration, it has been linked to accelerating wound healing and safeguarding epithelial tissues, such as the lungs and gastrointestinal tract [44]. Nevertheless, pathological fibrosis and the advancement of cancer have also been connected to aberrant IL-11 signalling, suggesting the necessity of precise therapeutic control [45].

Because of its numerous biological functions, IL-11 is an attractive target for both therapeutic intervention and drug discovery. Presently, recombinant IL-11 formulations are being used in clinical settings, and research is being conducted to enhance their stability, bioavailability, and

immunogenicity by using sophisticated formulation techniques (O'Neill et al., 2020). Thus, improving the therapeutic efficacy of IL-11 requires the capacity to comprehend and modify it at the molecular level. This emphasizes the significance of in silico research, such as molecular docking analyses, to find excipients that can stabilize IL-11 during formulation.

2.3 Molecular Docking Methods and Applications in Drug Discovery

Docking is a computational approach extensively utilized in drug discovery to forecast how small molecules (ligands) interact with target proteins. Its applications include lead compound identification, optimization, virtual screening, and structure-based drug design. The method involves simulating the geometric complementarity and non-covalent interactions between the ligand and receptor within the binding site, thereby predicting the most energetically favorable binding pose and affinity.

Several molecular docking algorithms have been developed, each with its unique features and strengths. Among the most widely used are Auto Dock, Auto Dock Vina, and DOCK, which employ different scoring functions and search algorithms to explore ligand conformational space and optimize ligand-receptor interactions. These programs incorporate various parameters such as ligand flexibility, protein flexibility, and solvent effects to improve the accuracy and reliability of docking predictions.

Molecular docking has various applications in drug discovery, including lead identification, where it can efficiently screen large chemical libraries to identify potential ligands with high binding affinity and specificity for the target protein. Virtual screening, another key application, involves docking a database of compounds against a target protein to prioritize promising candidates for experimental testing based on their predicted binding energy and fit within the binding site.

Molecular docking plays a crucial role in structure-based drug design by facilitating the prediction of ligand–target interactions guide the rational optimization of lead compounds by systematically exploring the chemical space around the binding site and identifying key interactions driving ligand binding and potency. Docking simulations can also aid in understanding structure-activity relationships and predicting the impact of chemical modifications on ligand binding affinity and selectivity.

Moreover, molecular docking serves as a valuable tool for studying protein- ligand interactions, elucidating the molecular mechanisms underlying drug action and resistance, and guiding the design of novel therapeutic agents targeting various disease pathways.

2.4 Computational approach in Drug Discovery

The development of stable and efficient biotherapeutic formulations is a difficult and resource-intensive process. Computational techniques have become indispensable tools for formulation design, offering a quick and effective alternative to lengthy experimental testing. Enhancing stability, solubility, and bioavailability requires a molecular-level understanding of protein interactions and behaviour with excipients, which these approaches provide [46].

Quantitative structure-activity relationship (QSAR) modelling, molecular docking, and molecular dynamics (MD) simulations are crucial computational techniques for predicting and analysing protein–excipient interactions. In order to guide the selection of excipients that may enhance protein stability, molecular docking helps identify potential binding sites and interaction energies between proteins and small molecules [47]. The dynamic behaviour of protein-excipient complexes over time may also be seen thanks to MD simulations, which display atomic-scale stabilizing interactions and conformational change [48].

Furthermore, methods for machine learning and artificial intelligence (AI) are evolving to predict formulation outcomes through the analysis of large datasets of excipient properties and protein stability. By identifying patterns and recommending optimal excipient combinations, these data-driven approaches reduce the amount of trial-and-error involved in formulation development. Researchers may proactively evaluate the resilience of formulations by using computational modelling to simulate stress elements such as temperature, pH, and mechanical agitation [49].

Combining computational tools and experimental methodologies accelerates the creation of formulations with improved stability and efficacy. The rational design of biotherapeutics tailored to specific clinical needs is made possible by this partnership, which also reduces development costs and time. With continued developments in computer power and algorithms, these technologies are expected to play an increasingly more important role in future biopharmaceutical formulation strategies.

CHAPTER 3

METHODOLOGY

This study utilizes molecular docking to evaluate the binding interactions between IL-11 and a panel of commonly used excipients, namely lactose, sucrose, trehalose, mannitol, and sorbitol, to predict their potential to stabilize IL-11 through favorable interactions. Here, we used PyRx for virtual screening of multiple excipients (ligands) targeting rhIL-11. The following paragraphs detail the approach utilized for ligand selection, protein preparation, molecular docking, and validation procedures.

3.1 Protein structure retrieval and preparation

- The 3D structure of the therapeutic protein interleukin-11 (IL-11) was retrieved

From the AlphaFold Protein structure Database. The predicted structure was produced from the AlphaFold2 deep learning model created by DeepMind and EMBL-EBI. UniProt ID [P20809 for human IL-11] was employed to download the model.

- The prediction confidence for every residue was eliminated using the pLDDT scores provided. Regions with low confidence were visually checked and, if necessary, were removed from docking studies to maintain the reliability of interaction predictions.
- Using Auto Dock Tools, all water molecules and heteroatoms were removed. Polar hydrogens and Gasteiger charges were assigned. The protein was stored in PDBQT format for docking.

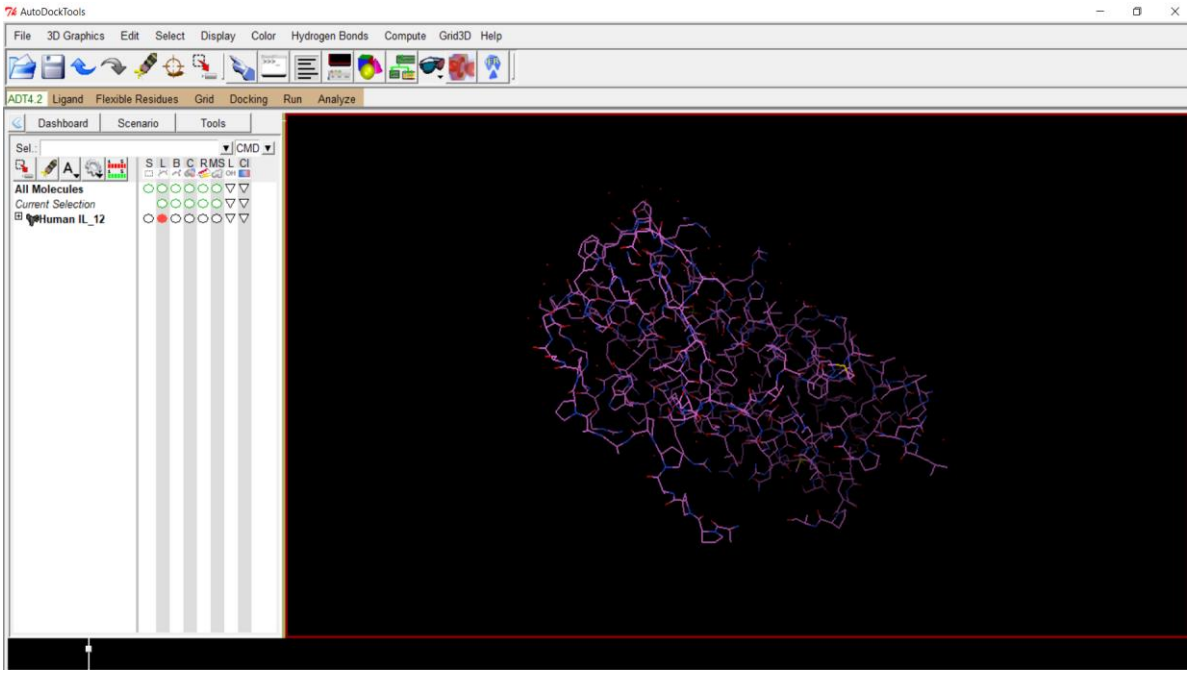


Fig 3.1: Screenshot showing loading of protein in Auto Dock 4.0

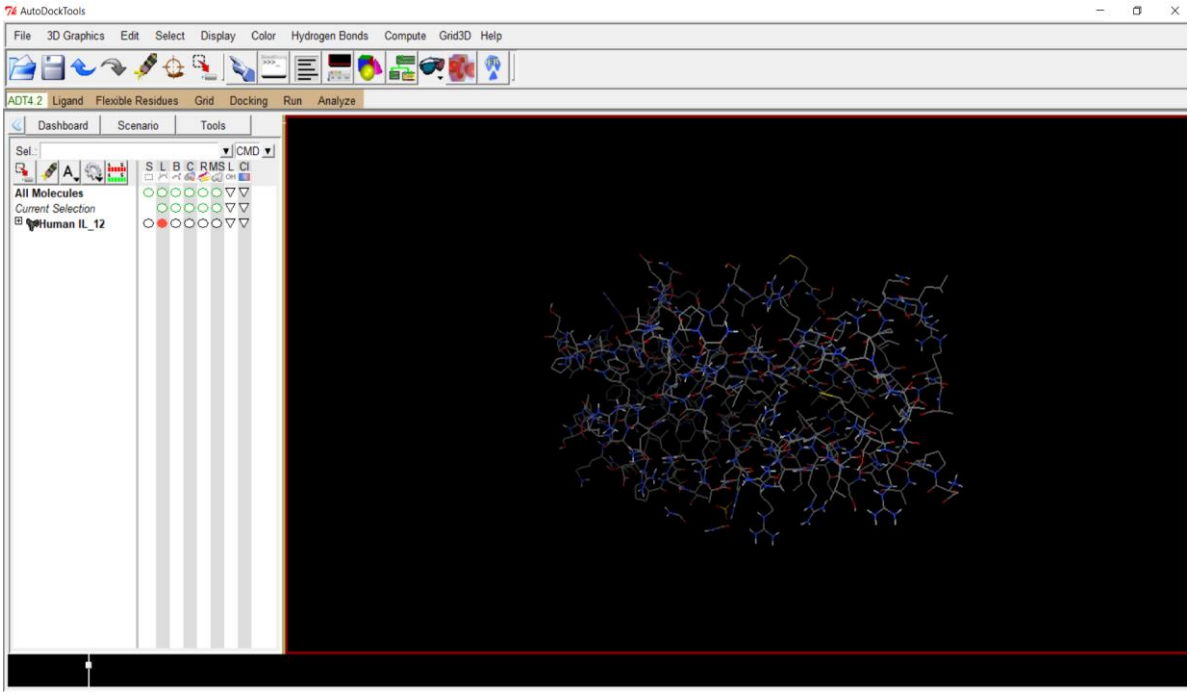


Fig 3.2: Screenshot showing protein after modification

1. Download the ligand

Visit PubChem at www.pubchem.ncbi.nlm.nih.gov.

• 3D Structures of lactose, sucrose, trehalose, mannitol, and sorbitol ligan- ds were fetched from PubChem (https://pubchem.ncbi.nim. nih.gov/in) in SDF format and were converted into PDBQT format using Auto Dock Tools.

• Geometry optimization and torsion tree definitions were applied to prepare flexible ligand

2.Performing docking using pyrx

Autodock Vina will be the docking tool that we use. We use the Vina algorithm to dock it in Pyrx. Launch Pyrx GUI and followed the steps given below:

2.1Protein Loading

• Select "File" -> "Load Molecule" or simply click the first icon in the upper left corner. Choose the protein structure that you downloaded. referred to here as "IL-11_AF"

• Convert pdb format of protein to pdbqt by right clicking on IL-11_AF then on display and now select macromolecule.

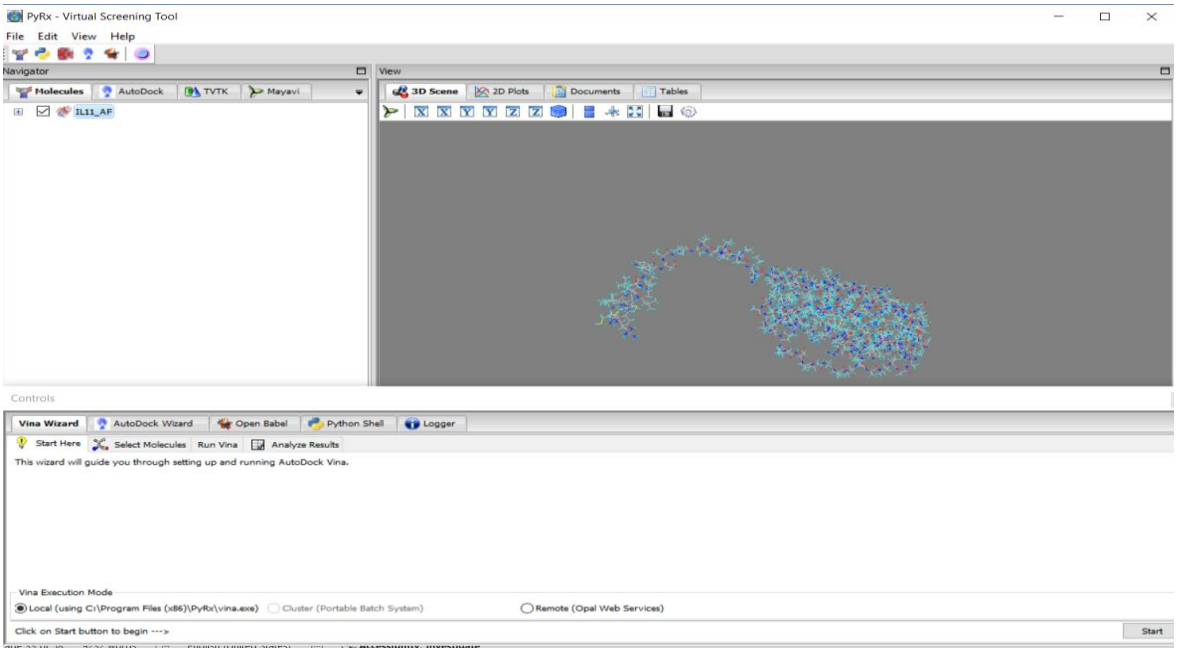


Fig 3.3: Screenshot showing conversion of protein file pdb to pdbqt

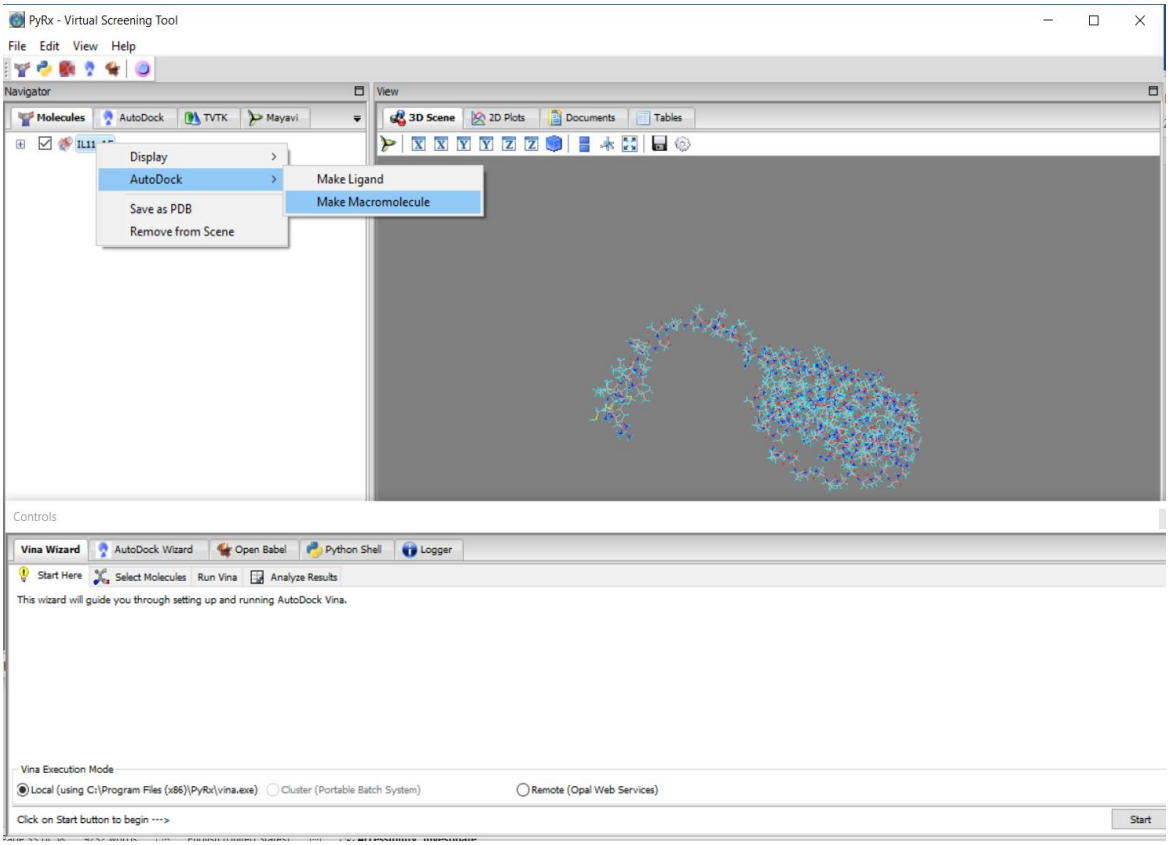


Fig 3.4: Conversion of protein file pdb to pdbqt

2.2 Ligand loading

- In PyRx, click on OpenBabel and select on insert new item present on bottom right corner.

- Now select each ligand from folder one by one and upload it.

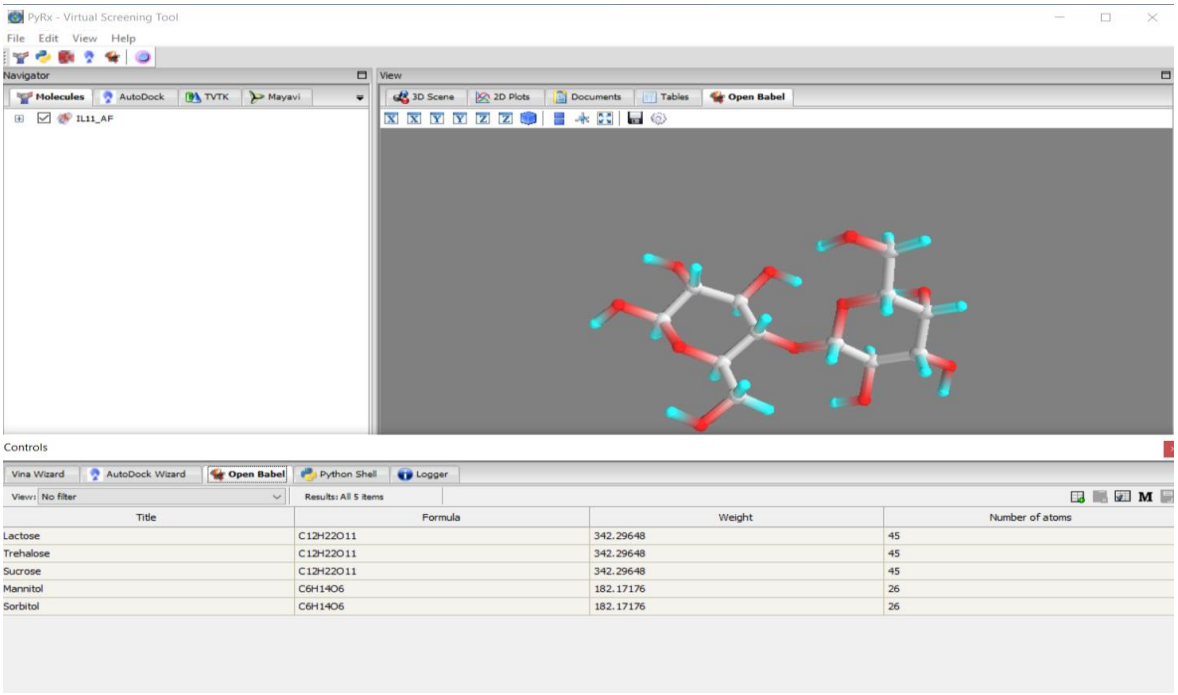


Fig 3.5: Screenshot showing loading of ligands

• After uploading all ligands, right click on ligand and select minimise all to decrease the energy.

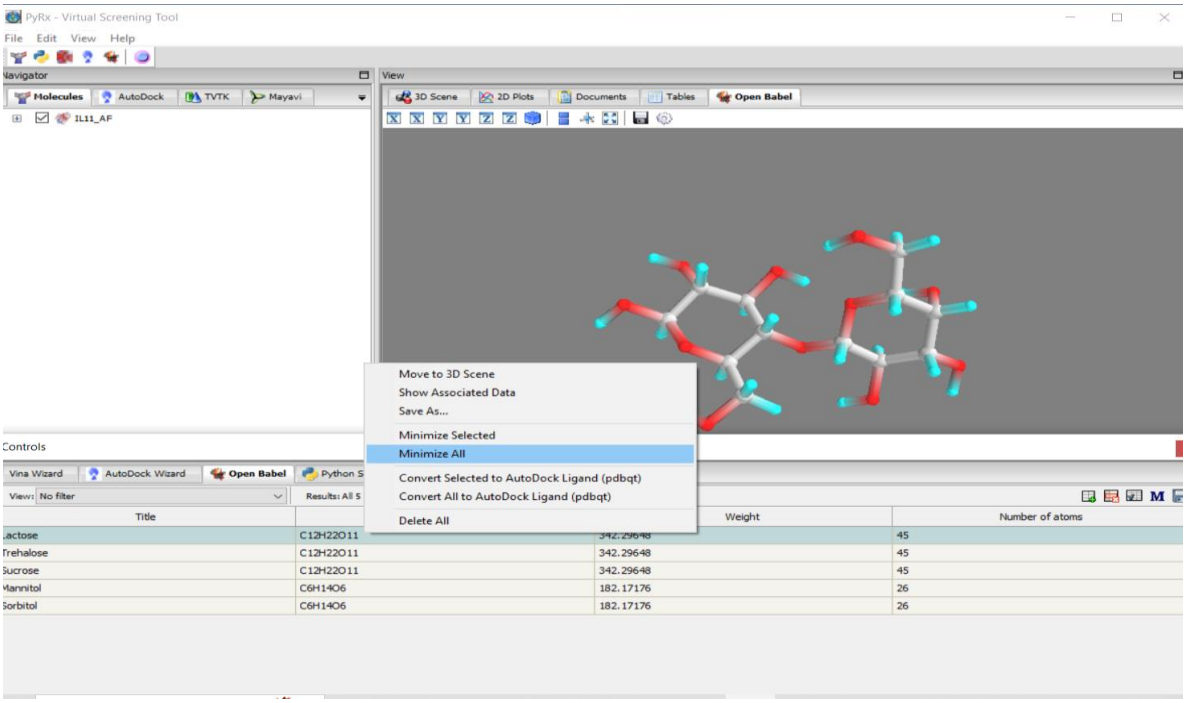


Fig 3.6: Screenshot showing minimization of energy of ligand

• Again right click and select convert all to AutoDock ligand (pdbqt) to convert all ligands to pdbqt format.

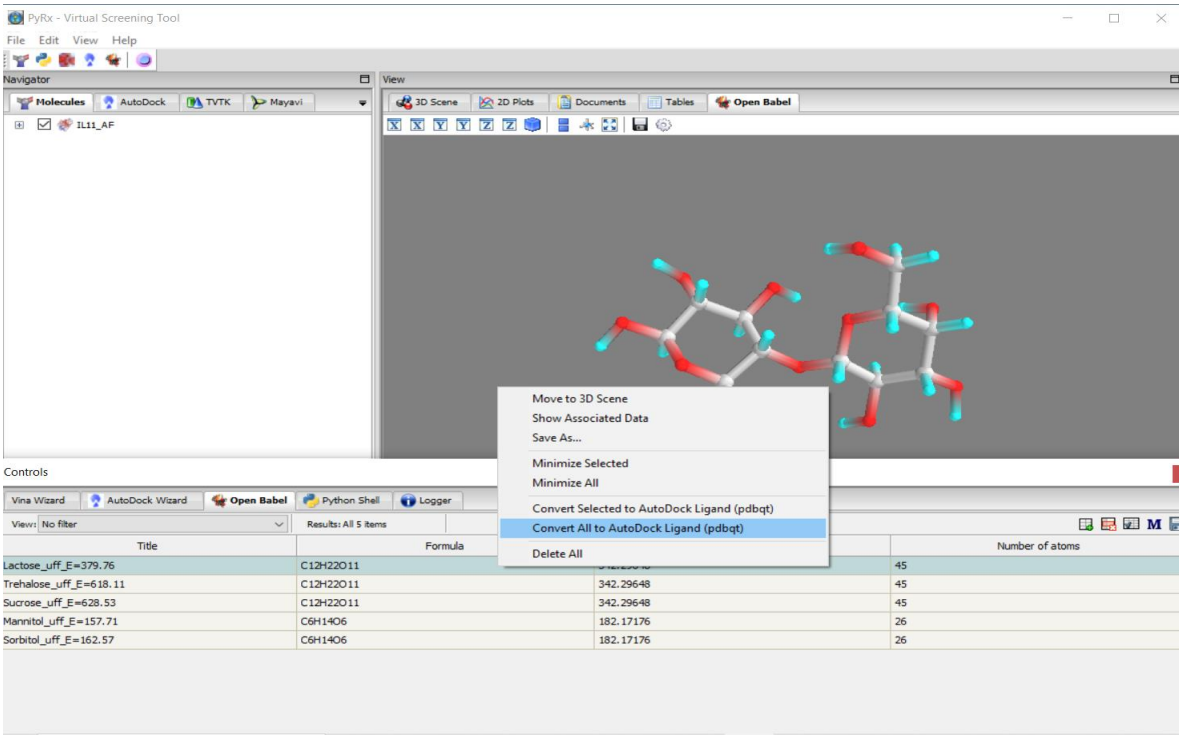


Fig 3.7: Screenshot showing conversion of all ligands to pdbqt

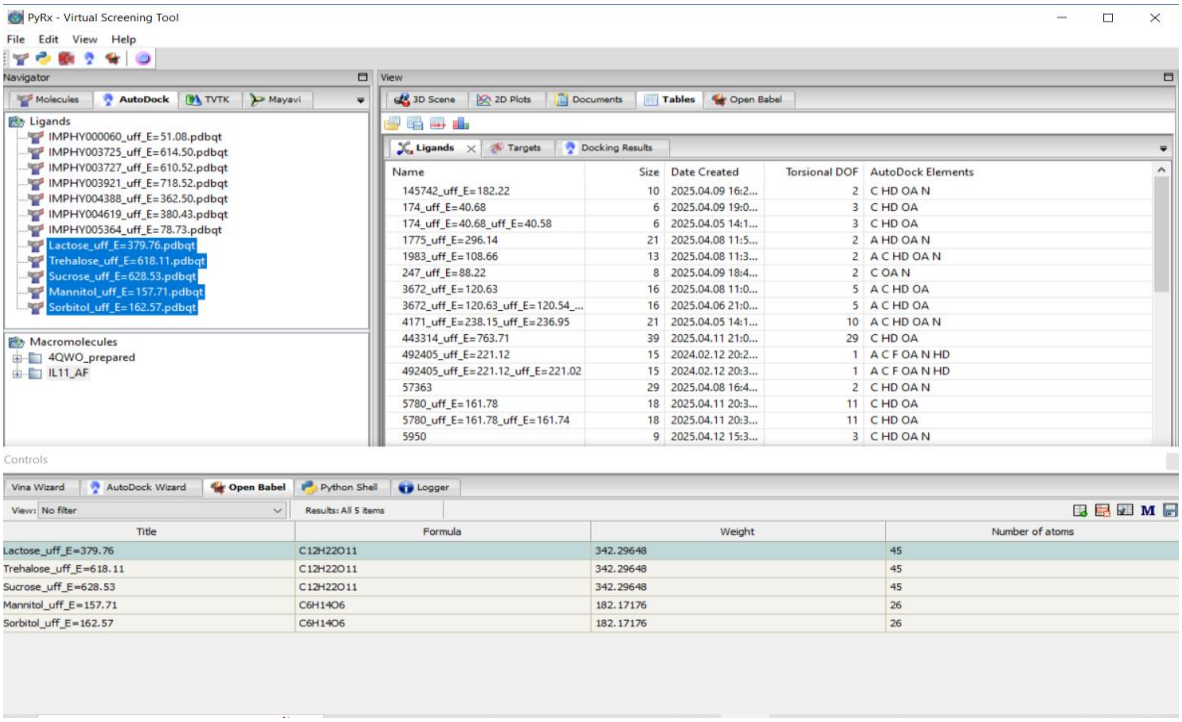


Fig 3.8: Screenshot showing display of all ligands

3 Defining ligands and proteins

The loaded protein and ligand are shown under the "Molecules" tab. It is now necessary to identify which is a ligand and which is a protein. • Right-click on the protein –> “Autodock” –> “Make Macromolecule” to accomplish that.

Perform a right-click on the ligand, select "Autodock," then "Make Ligand."After that, you'll see that it has automatically prepared their PDBQT files under the 'Autodock' page.

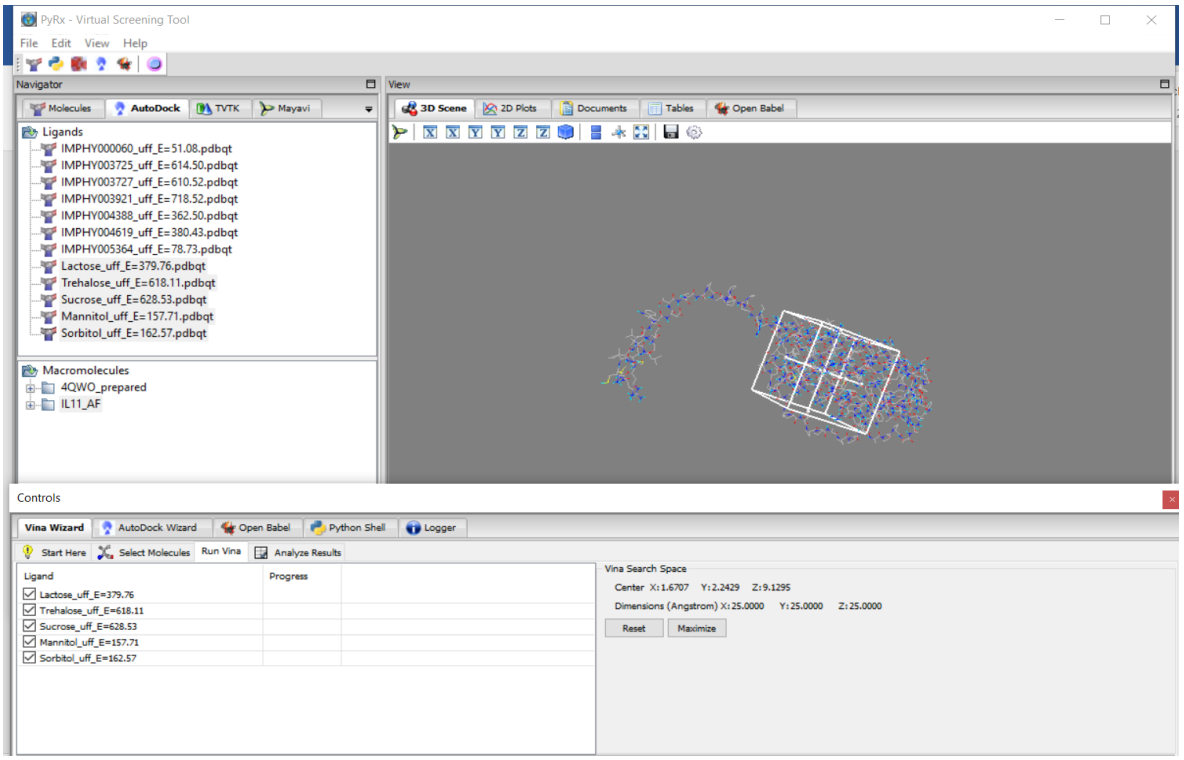


Fig 3.9: Screenshot showing grid box

4 Defining grid box

• Now click on Vina Wizard and select start option on bottom right corner. Start selecting protein and ligand one by one by pressing shift and control button.

• Click on forward. Grid box appears. Return to the 'Molecules' tab located on the right-hand side. Click the loaded protein's "+" symbol.

• All of the residues in the chain will be visible to you. To choose the binding residues, right-click on the residue and choose Atoms, Display, Label, and Atoms. The atoms will start to show up on the protein. Now make the appropriate adjustments to the grid box so that it contains all of the selected residues. The ligand does not need to be enclosed within the grid box.

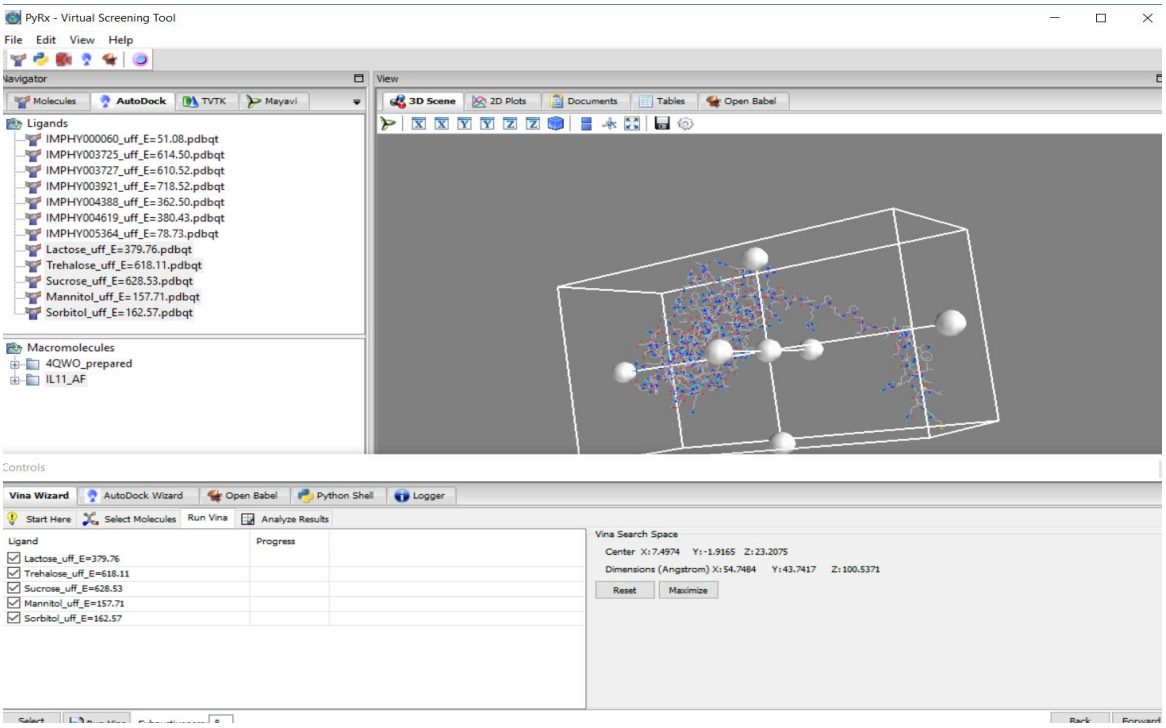


Fig 3.10: Screenshot showing defining of grid box

5 Running vina for docking

• To adjust the exhaustiveness, simply enter the desired number in the box located in the left-bottom corner. Once everything has been adjusted, press the "Forward" button.

• Docking will begin, and the processing will be shown. The bottom panel will display the poses and their binding affinities after the docking process is complete. It will show all poses along with RMSD values. Save your file in excel sheet.

• Now analyze the result and the one ligand which has the highest energy with negative sign is selected. Again, open pyrx tab and click on Auto Dock and select macromolecule and select the ligand with highest binding energy. Now right click and select display then all models of the ligands get displayed and select your desired model and save it in pdb format.

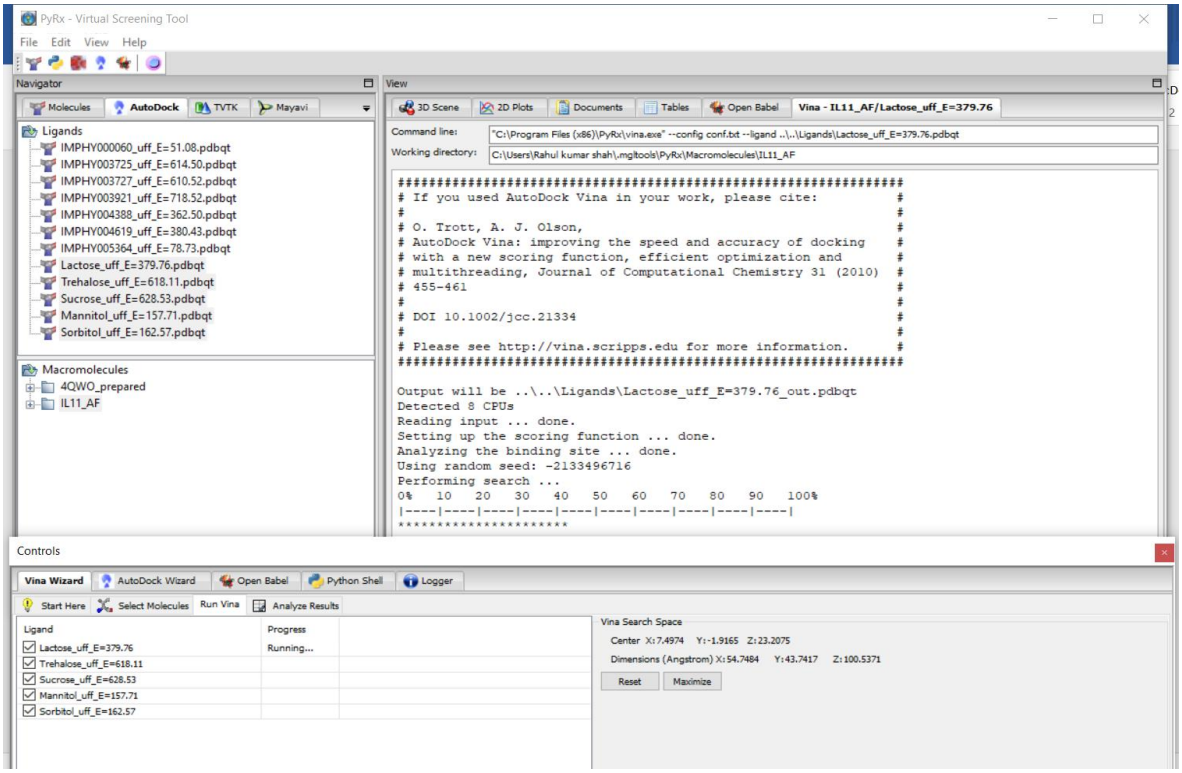


Fig 3.11: Screenshot showing running of vina wizard

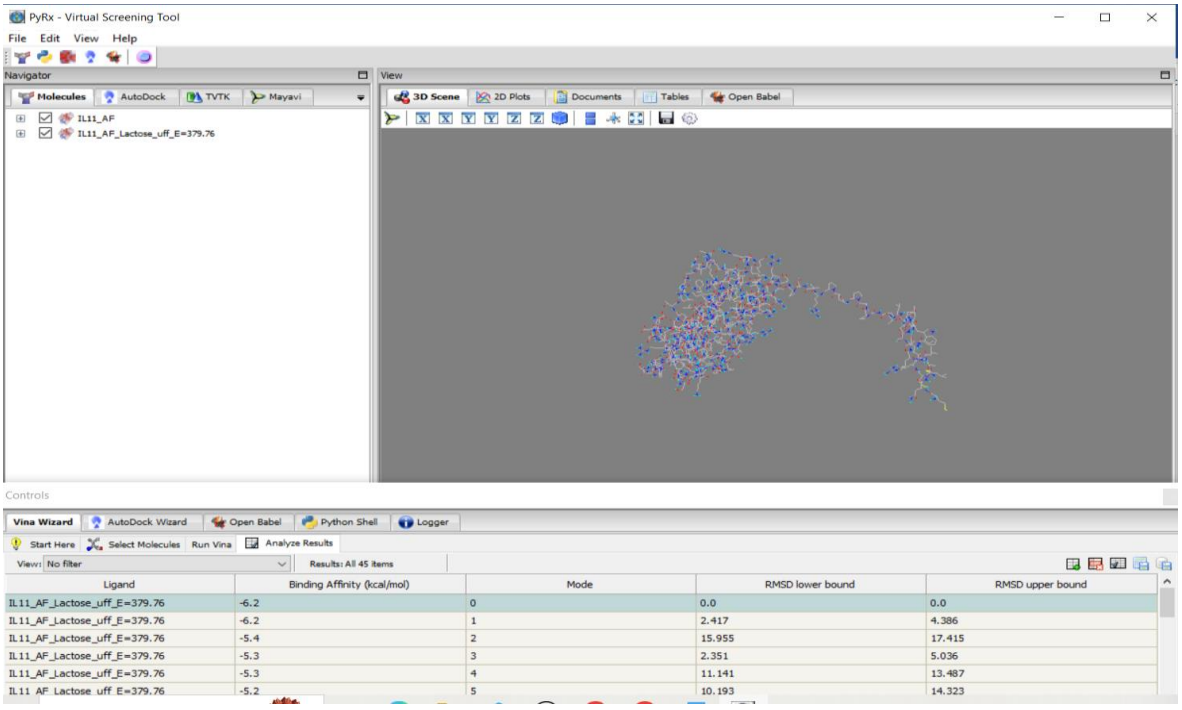


Fig 3.12: Screenshot showing binding affinity of ligands with protein

6 **Open Discovery Studio:** - Start a new project in Discovery Studio.

7 Protein Structure Import:

- Open Discovery Studio and import the structure of the target protein.
- Choose your protein structure file (such as *.pdb) by using File > Open.

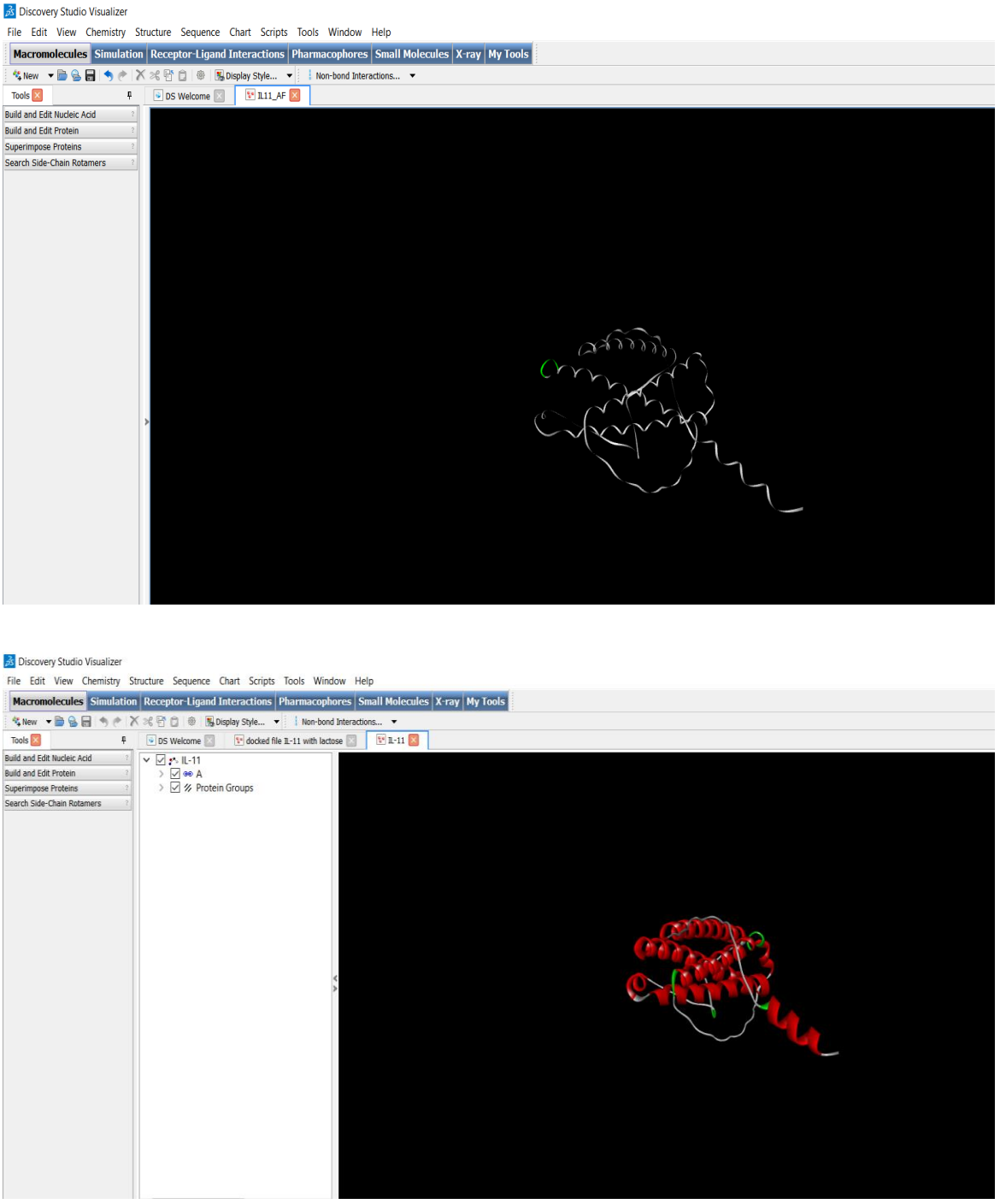


Fig 3.13: Screenshot showing visualization of protein structure in discovery studio

8 Import Docked Ligand Conformations:

- Import the docked ligand conformations.

- Use File > Open and select the converted ligand file (e.g., *.pdb or *.mol2).

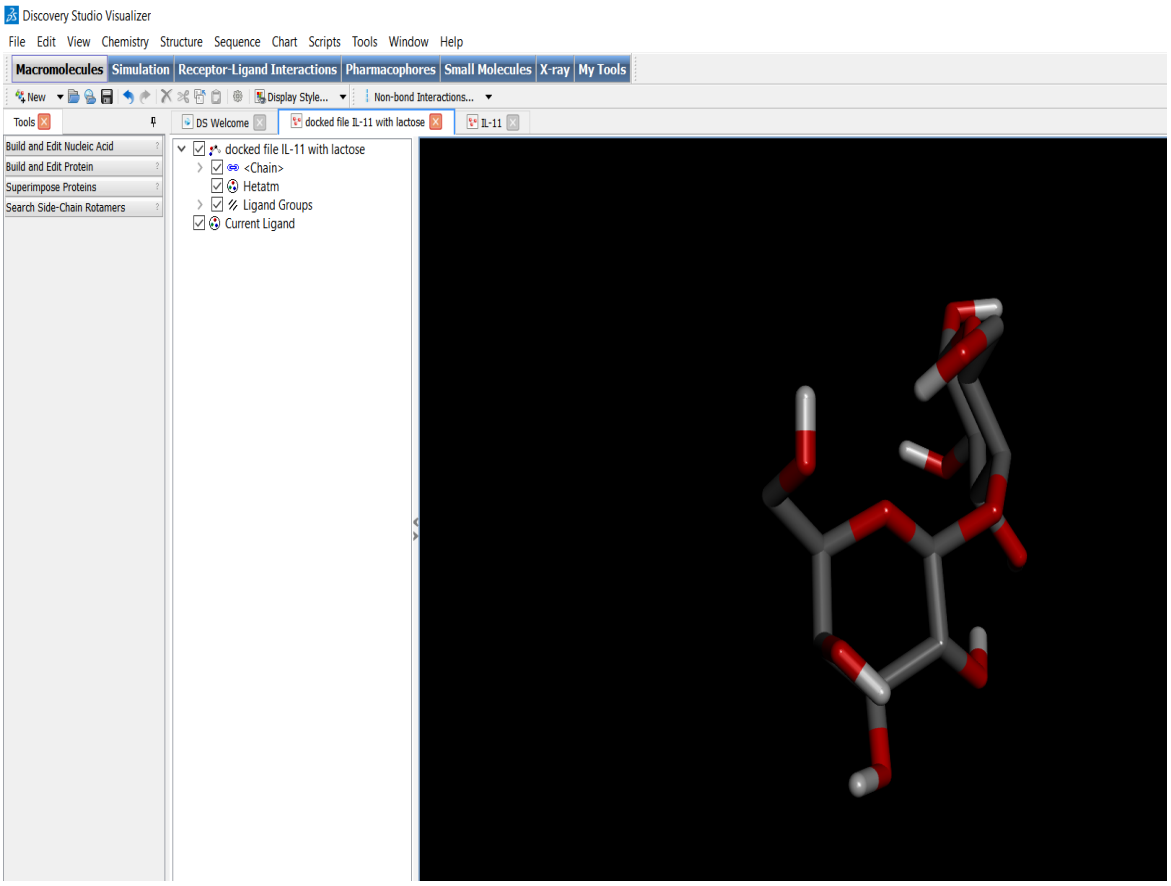
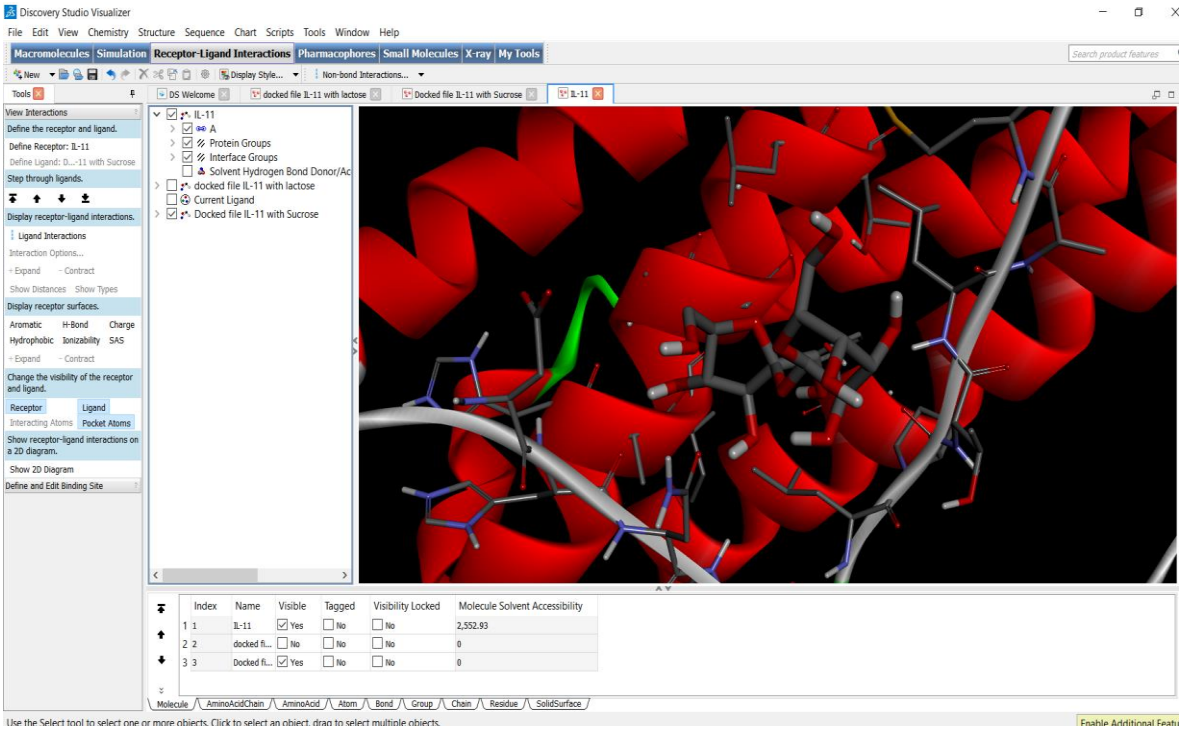


Fig 3.14: Screenshot showing structure of Lactose

9 Visualize Docked Poses:

- Display the protein and ligand together in the 3D workspace.
- Use the View > Sequence panel to ensure the correct structures are loaded.
- Adjust the display settings to show interactions clearly (e.g., stick or surface representations).



10 Analyse Binding Affinities:

- Check the binding affinities associated with each docked pose.
- This information can be found in the PyRx log files or the output summary from PyRx.
- Record the binding affinity scores (typically in kcal/mol) for reference.

11 Examine Binding Interactions:

- Use the Analyze > Receptor-Ligand Interactions tool to identify and visualize key interactions between the ligand and the protein.
- Highlight hydrogen bonds, hydrophobic interactions, salt bridges, and π-π stacking interactions. Check for consistency with known binding sites or important residues in the binding pocket.

12 Evaluate Docked Poses:

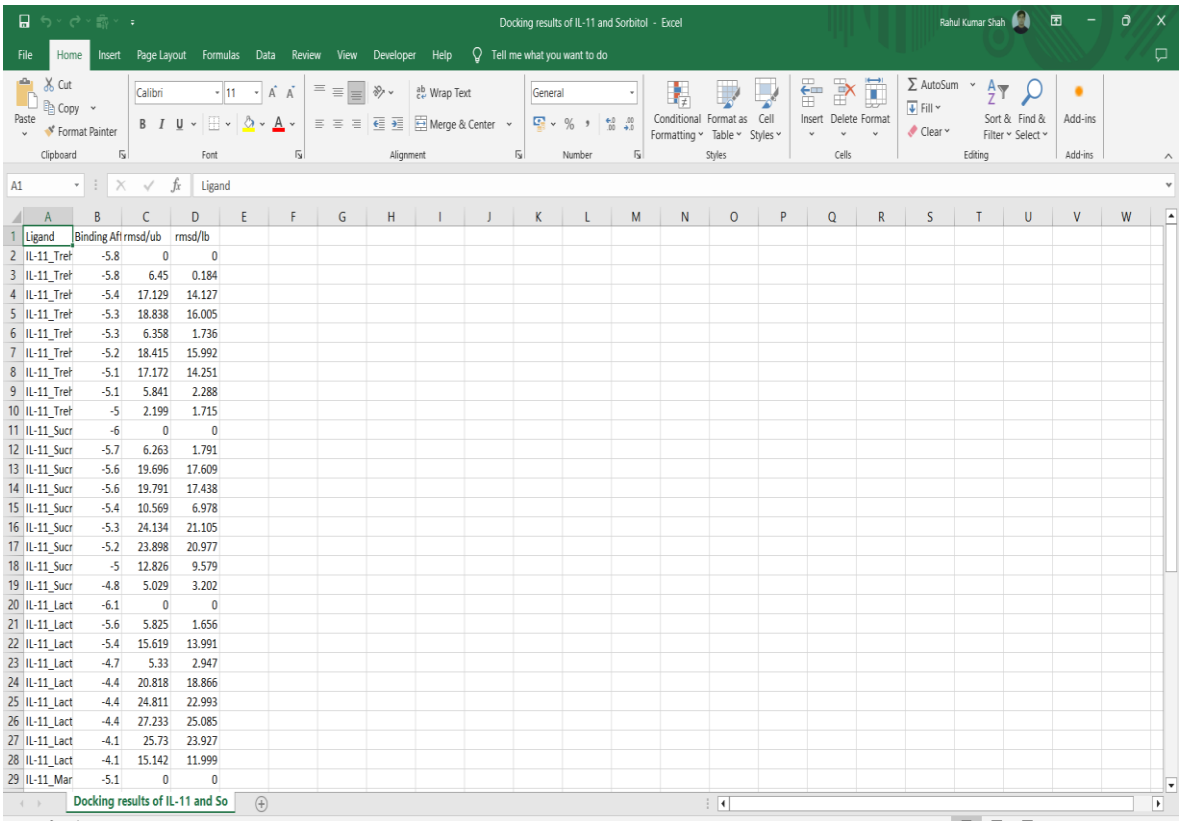
- Compare multiple docked poses to determine if there is a consensus binding mode.
- Use the View > Compare tool to overlay different conformations and evaluate their similarity.
- Consider the Root Mean Square Deviation (RMSD) values to understand the variance among poses.

CHAPTER 4

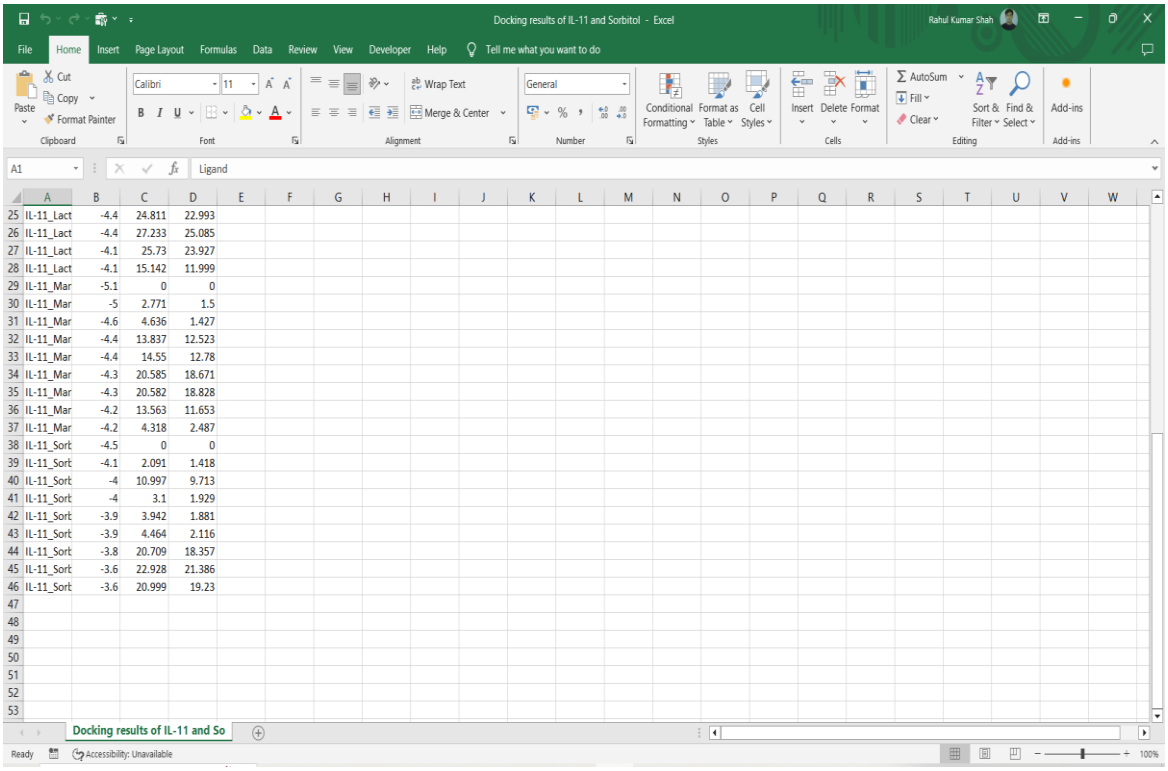
RESULT

The binding affinities (kcal/mol) between IL-11 and various pharmaceutical excipients are summarized in the table below:

EXCIPIENTS	BINDING AFFINITIES (KCAL /MOL)
LACTOSE	**-6.1**
SUCROSE	**-6.0**
TREHALSOSE	**-5.8**
MANNITOL	**-5.1**
SORBITOL	**-4.5**



	A	B	C	D
1	Ligand	Binding Aff	rmsd/ub	rmsd/lb
2	IL-11_Treh	-5.8	0	0
3	IL-11_Treh	-5.8	6.45	0.184
4	IL-11_Treh	-5.4	17.129	14.127
5	IL-11_Treh	-5.3	18.838	16.005
6	IL-11_Treh	-5.3	6.358	1.736
7	IL-11_Treh	-5.2	18.415	15.992
8	IL-11_Treh	-5.1	17.172	14.251
9	IL-11_Treh	-5.1	5.841	2.288
10	IL-11_Treh	-5	2.199	1.715
11	IL-11_Sucr	-6	0	0
12	IL-11_Sucr	-5.7	6.263	1.791
13	IL-11_Sucr	-5.6	19.696	17.609
14	IL-11_Sucr	-5.6	19.791	17.438
15	IL-11_Sucr	-5.4	10.569	6.978
16	IL-11_Sucr	-5.3	24.134	21.105
17	IL-11_Sucr	-5.2	23.898	20.977
18	IL-11_Sucr	-5	12.826	9.579
19	IL-11_Sucr	-4.8	5.029	3.202
20	IL-11_Lact	-6.1	0	0
21	IL-11_Lact	-5.6	5.825	1.656
22	IL-11_Lact	-5.4	15.619	13.991
23	IL-11_Lact	-4.7	5.33	2.947
24	IL-11_Lact	-4.4	20.818	18.866
25	IL-11_Lact	-4.4	24.811	22.993
26	IL-11_Lact	-4.4	27.233	25.085
27	IL-11_Lact	-4.1	25.73	23.927
28	IL-11_Lact	-4.1	15.142	11.999
29	IL-11_Mar	-5.1	0	0



	A	B	C	D
25	IL-11_Lact	-4.4	24.811	22.993
26	IL-11_Lact	-4.4	27.233	25.085
27	IL-11_Lact	-4.1	25.73	23.927
28	IL-11_Lact	-4.1	15.142	11.999
29	IL-11_Mar	-5.1	0	0
30	IL-11_Mar	-5	2.771	1.5
31	IL-11_Mar	-4.6	4.636	1.427
32	IL-11_Mar	-4.4	13.837	12.523
33	IL-11_Mar	-4.4	14.55	12.78
34	IL-11_Mar	-4.3	20.585	18.671
35	IL-11_Mar	-4.3	20.582	18.828
36	IL-11_Mar	-4.2	13.563	11.653
37	IL-11_Mar	-4.2	4.318	2.487
38	IL-11_Sort	-4.5	0	0
39	IL-11_Sort	-4.1	2.091	1.418
40	IL-11_Sort	-4	10.997	9.713
41	IL-11_Sort	-4	3.1	1.929
42	IL-11_Sort	-3.9	3.942	1.881
43	IL-11_Sort	-3.9	4.464	2.116
44	IL-11_Sort	-3.8	20.709	18.357
45	IL-11_Sort	-3.6	22.928	21.386
46	IL-11_Sort	-3.6	20.999	19.23
47				
48				
49				
50				
51				
52				
53				

Fig 4.1: Screenshot showing Binding Affinities

These results indicate that **lactose exhibited the strongest binding** to IL-11, closely followed by sucrose and trehalose. In contrast, mannitol and sorbitol displayed weaker interactions, suggesting a reduced potential for stabilizing the protein.

Molecular docking showed that lactose (–6.1 kcal/mol), sucrose (–6.0 kcal/mol), and trehalose (–5.8 kcal/mol) had stronger binding affinities with IL-11 compared to mannitol (–5.1 kcal/mol) and sorbitol (–4.5 kcal/mol). Disaccharides formed multiple hydrogen bonds with key IL-11 residues, suggesting better surface interaction and potential stabilizing effects. In contrast, sugar alcohols showed weaker interactions, indicating less effectiveness as stabilizers.

Docking of different excipients with IL-11

Docking of IL-11 with lactose

Docking results in binding between IL-11 and Lactose shows

E score=1024.97, binding affinity -6.1 kcal/mol and rmsd value =0

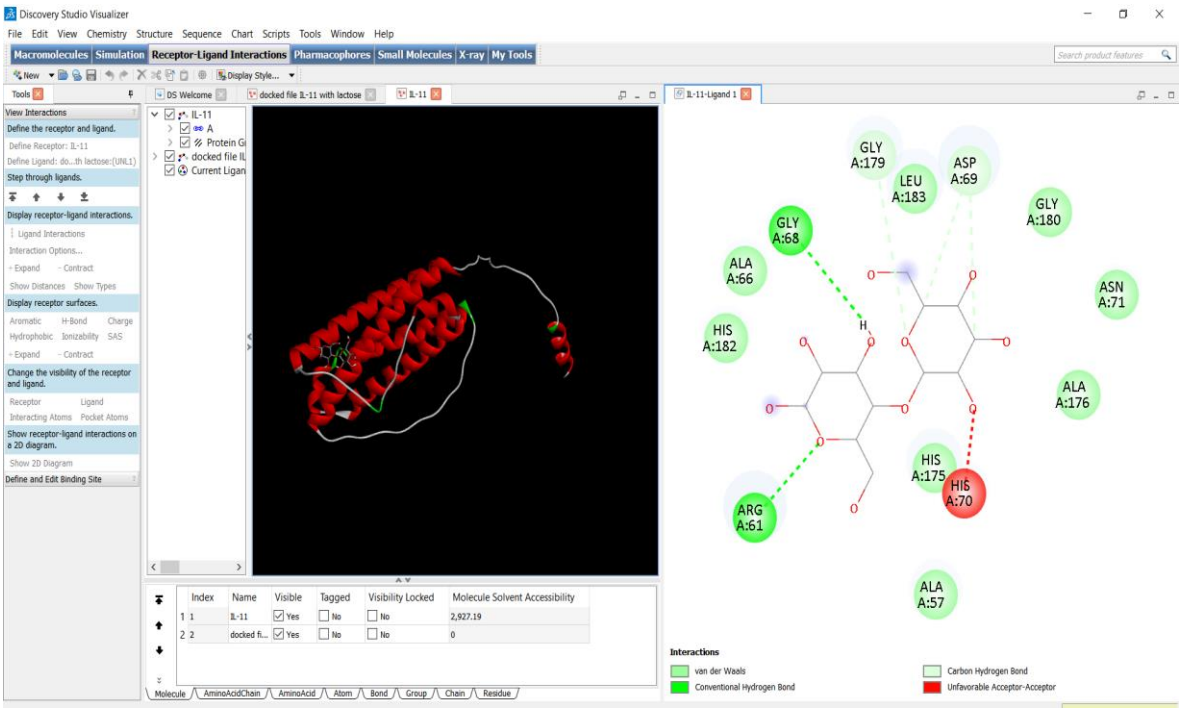


Fig 4.2: screenshot showing binding of excipient with excipient

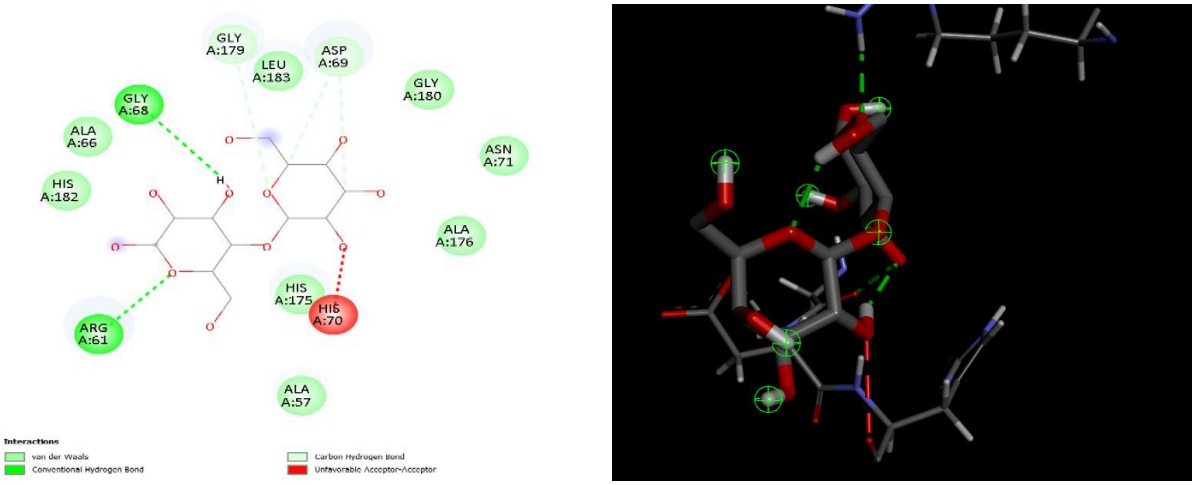


Fig 4.3: 2D and 3D representations of IL–11–lactose complex. Lactose binds to IL-11 via multiple hydrogen bonds (ARG61, GLY68, HIS182) and carbon-hydrogen bonds (ASP69, LEU183), indicating strong surface interaction. One unfavorable interaction with HIS70 is present but does not significantly affect overall binding stability.

Docking of IL-11 with Sucrose

Docking results in binding between IL-11 and Sucrose shows E score= 288.57, binding affinity -6.0kcal/mol and rmsd value 0

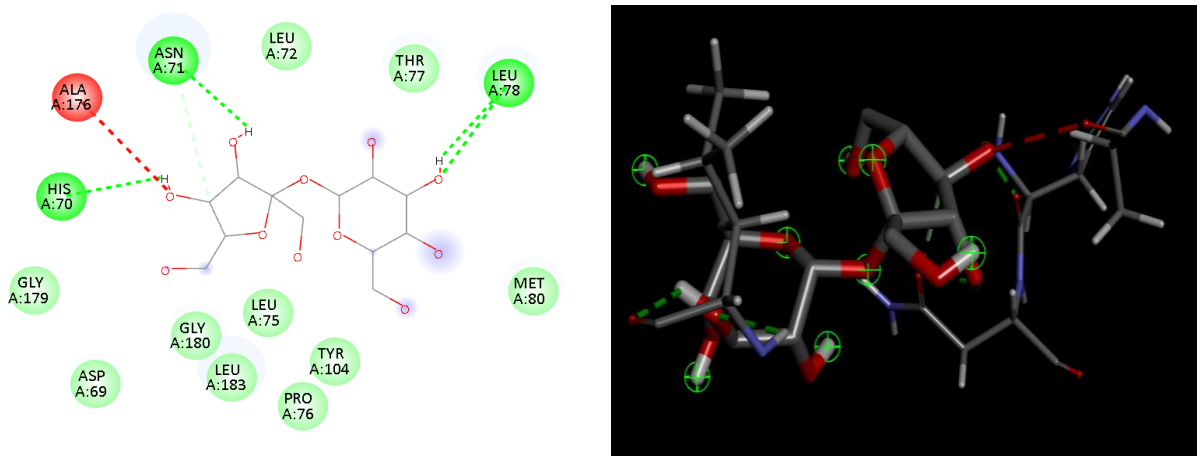


Fig 4.4: 2D and 3D representations of IL-11–sucrose complex Sucrose interacts with IL-11 through hydrogen bonds with ASN71, HIS70, and LEU78, and van der Waals interaction with ASN71 suggesting stable surface binding

Docking of IL-11 with Trehalose

Docking results in binding between IL-11 and Trehalose shows E score= 288.57, binding affinity -5.8 kcal/mol and rmsd value 0

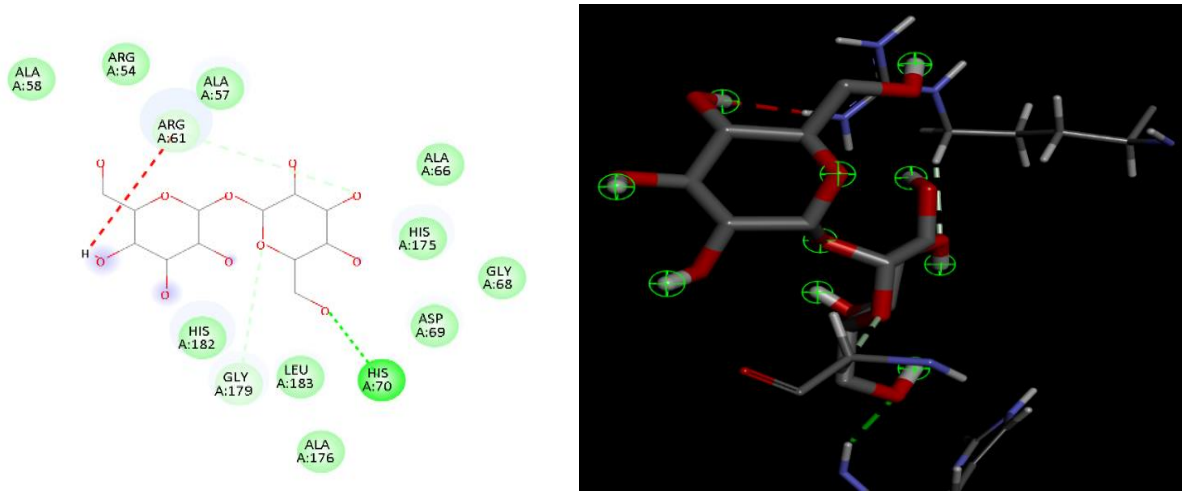


Fig 4.5: 2D and 3D representations of the IL-11–trehalose complex. Trehalose forms a strong hydrogen bond with HIS70, and GLY179 shows van der Waals interaction indicating potential surface stabilization

Docking of IL-11 with Mannitol

Docking results in binding between IL-11 and Mannitol shows E score=407.16, binding affinity –5.1kcal/mol and rmsd value 0

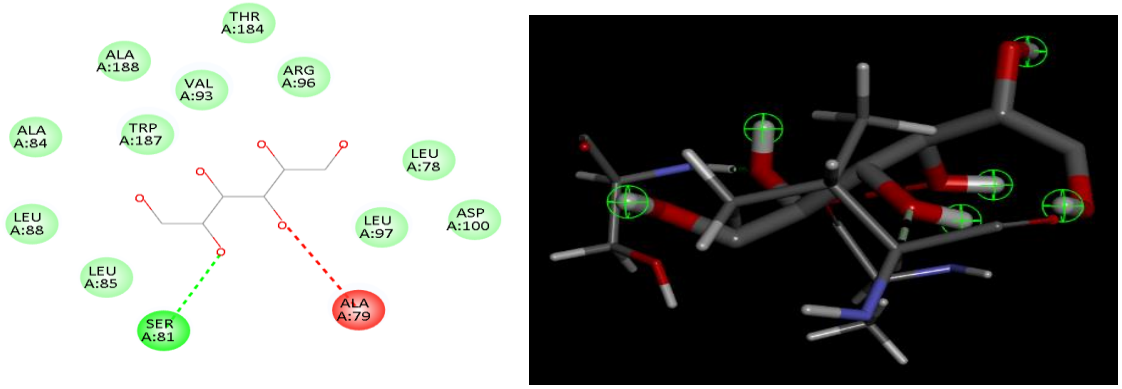


Fig 4.6: 2D and 3D representations of the IL-11–mannitol complex Mannitol forms a conventional hydrogen bond with SER81, suggesting moderate surface interaction

Docking of IL-11 with Sorbitol

Docking results in binding between sorbitol with IL-11 shows E score=1054.96, binding affinity -4.5kcal/mol and rmsd value 0

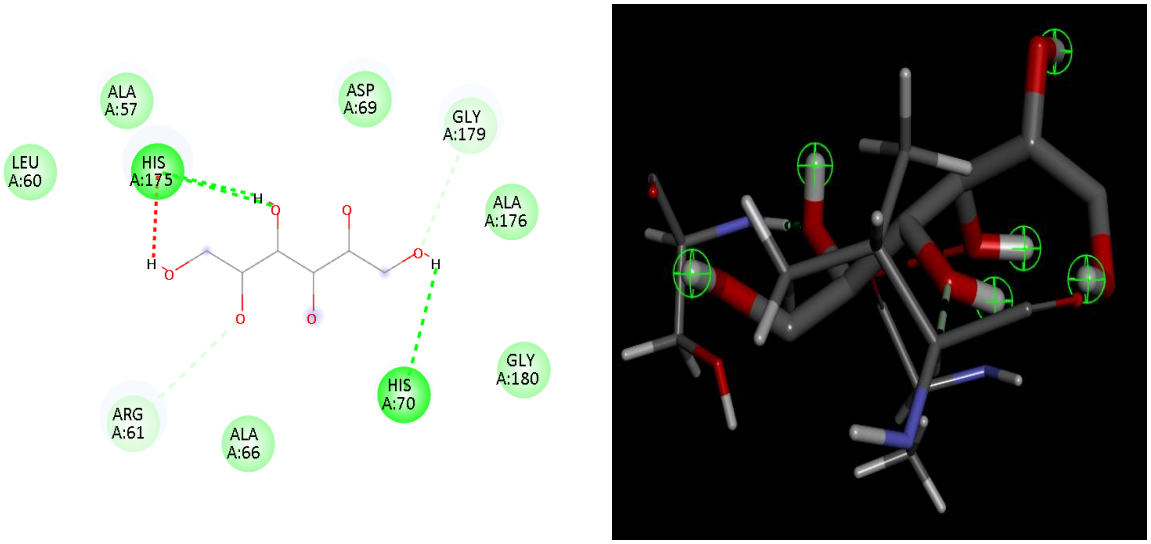


Fig 4.7: 2D and 3D representations of the IL–11–sorbitol complex showing [Sorbitol forms strong conventional hydrogen bonds with HIS70 and HIS175, van der Waals interaction with ARG61

Discussion

Blending sugar excipients with therapeutic proteins can improve their stability, dissolution as well as acceptability by the patients. In the current study, excipient-IL-11 molecular interactions were analyzed computationally using molecular docking. The excipients analyzed included sugar and sugar alcohols. Both sugars and sugar alcohols were capable of forming non-covalent interactions with IL 11, as reflected by their binding affinities (Table 1). However, disaccharides such as lactose, sucrose, trehalose showed more favorable binding energies as compared to sugar alcohols, mannitol and sorbitol. This might be due to the ability of sugars to form multiple hydrogen-bonds with polar and charged protein surface residues, as represented in Fig. 1, 2. The relatively lower affinities of mannitol and sorbitol might be due to their poorer capacity to form extensive hydrogen-bonding networks with the protein surface and being less favourable to IL-11 stabilization. Thus, the formulation stability of IL-11 can be improved by blending it with sugars excipients. A strategy involving the combination of sugar excipient-stabilized therapeutic proteins such as IL-11 with other excipients such as amino acids and polyols could be more effective. Our findings were in corroboration with the stabilization of therapeutic proteins for lyophilization and storage [50]. Shao et al. (2004) showed that disaccharides such as trehalose and sucrose inhibit aggregation and maintain the secondary structure of IL-11. IL-11 formulations in U.S. Patent US6270757B1 also contained stabilizers like sugars and polyols. The concurrence of docking predictions with experimental data confirms the validity of this computational screening method.

Recommendations for Future Research

Interleukin-11 (IL-11), a cytokine of therapeutic relevance is blended with excipients in order to prevent protein aggregation and to enhance stability. Unlike standard practices that deem excipients to be inert substances, the present study explored their molecular interactions with the protein surface. The results established that disaccharides lactose, sucrose, and trehalose bound more favorably with IL-11 than sugar alcohols. This owed to their ability to create stable hydrogen bonds with functional surface residues.

These findings are not only of scientific significance but also of immediate practical utility, as these represent a predictive and economical means of selecting excipients to stabilize proteins. Through the integration of computational methods into the initial stages of formulation, the process has the potential to minimize reliance on high-throughput experimental screening and facilitate the development of stable therapeutic protein formulations. The procedure can be

applied to other biologics as well, thus being a valuable addition to the design of biopharmaceutical formulations. Docking simulations, when integrated with both computational and laboratory-based techniques, help to accelerate drug discovery, minimize research expenses, and improve the effectiveness of lead compound refinement, and spectroscopy techniques would be required to confirm and supplement the computational results.

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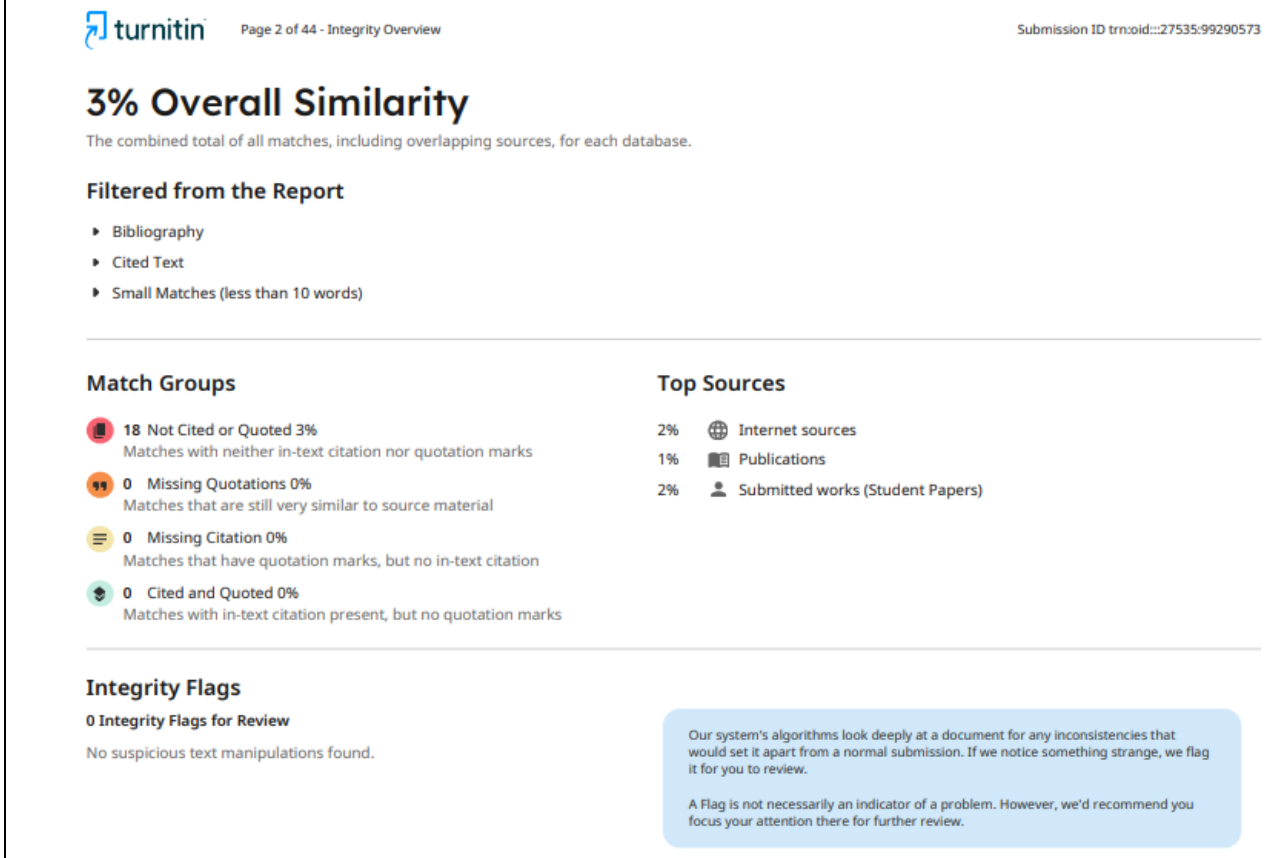

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