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Research Article

# In Silico Evaluation of Microbial Chitinase for Its Anticancer Potential Against Abnormal Glycosylation

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## Abstract

Altered glycosylation is a characteristic feature of cancer cells and is closely associated with oncogenesis, malignant differentiation, tumor growth, and metastasis. To test the hypothesis that chitinase may interfere with abnormal glycosylation through interaction with UDP-NAG and DPAGT1, the anticancer potential of chitinase from *Thermomyces lanuginosus* was evaluated through molecular docking and protein-protein interaction analysis. The chitinase molecule was docked using ligand (UDP-NAG) obtained from the PubChem compound database, and the docking results were analyzed based on docking score, glide score, glide energy, and hydrogen bond interactions. The obtained docking score and glide energy were -5.568 and -53.491, respectively, indicating favorable docking of chitinase with the NAG molecule. In addition, the protein-protein interaction studies between chitinase and DPAGT1 showed favorable structure and score models with balanced, electrostatic-favored, and hydrophobic-favored interactions, suggesting a strong positive interaction. The study concludes that chitinase may be a promising candidate for inhibiting carcinogenesis caused by abnormal glycosylation, either by interacting with UDP-NAG or with DPAGT1, thereby inhibiting the glycosylation process.

**Keywords:** Chitinase; DPAGT1, UDP-NAG, glycosylation; molecular docking, protein-protein interaction

## 1. INTRODUCTION

Glycosylation is a process of post-translational modification where carbohydrates attach to membrane proteins and lipids. The formed glycoproteins or glycolipids consist of ample carbohydrates in the extracellular portion of the cell membrane and have an essential function in the process of cell adhesion, cell trafficking, and cell signaling [1]. In various studies of carcinogenesis, it was observed that the expression of carbohydrates or glycans is distinct in cancer cells compared to normal cells [2,3]. It is because of the altered level of expression of glycosyltransferases. This enzyme forms glycosidic linkages between the glycans, and the glycosidases break the glycosidic linkages [4–6]. The abnormal glycosylation directs the structural changes of the main glycan, such as changed branching and expanded glycan scale, and other chemical composition changes that can cause cancer [7,8]. Consequently, the altered expression of specific glycans, usually structurally altered compared to normal cells, is the trait of cancer cells. It is already reported that altered glycosylation is linked with acute diseases involving oncogenesis, malignant differentiation, and tumor metastasis and growth [9–11]. The presence of 1-6 branching among N-acetyl-D-glucosamine and mannose on N-glycans is perhaps the most widely studied abnormal structure linked to cancer cell surfaces [12,13]. N-glycan biosynthesis has long been inhibited by the destruction of the first dedicated enzyme, DPAGT1 [14,15]. The transfer of an N-acetyl-D-glucosamine-1-phosphoryl unit (NAG-1-P) from UDP-NAG on the dolichyl phosphate (Dol-P) is catalyzed by the dolichyl phosphate N-acetylglucosamine-phosphotransferase (DPAGT1) [10]. The increased branch offers an extra substrate for glycosylation compared to other N-glycans, causing the glycan to increase in size, become structurally complex, and vary in conformity [16–18]. The high occurrence of β1-6 NAG branching is treated as a biomarker in many cancers [6,19,20]. DPAGT1 expression and the occurrence of β1-6 NAG branching have a function in oncogenesis and are related to metastasis and poor prognosis, especially in breast cancer, according to several in vivo studies [21–26], colon-rectum cancer, and in melanoma [22]. The therapeutic effects of preventing the growth of β 1-6 NAG branching in murine and human tumor cells have been discovered through research [23,24]. Despite the importance of N-linked glycans in tumor cell formation from normal cells, immunotherapy targeting N-linked glycans has yet to be established, owing to the inaccuracy of N-linked glycans in distinguishing between normal and malignant cells [4,27]. The Golgi

apparatus produces O- or N-glycan chains through the sequential action of glycosyltransferases[28]. Finding drug-like glycosyl transferase antagonists that inhibit the synthesis of complex branching mechanisms in cancer cells while selectively destroying tumor cells is a difficult task. Chitinase may be further explored as a potential anticancer candidate for the development of protein therapeutics [29,30]. To investigate its anticancer potential, the hypothesis of interaction of chitinase with UDP-NAG and DPAGT1 (dolichyl-phosphate N-acetylglucosamine phosphotransferase 1) at the molecular level was studied through in silico analyses, including molecular docking [31] and protein-protein interaction studies.

## 2. METHODS AND MATERIALS

### 2.1 Software

The Schrödinger Maestro interface (Maestro, version 10.5, Schrödinger, LLC, New York, NY, 2019) was used to perform the in silico docking studies, and the ClusPro web server was used for protein-protein interaction analysis [32].

### 2.2 Retrieval of sequence for the Three-dimensional structure building.

The three-dimensional structure of chitinase from *Thermomyces lanuginosus* was modelled using the primary amino acid sequence (Fig. 1) obtained from the National Center for Biotechnology Information (NCBI, www.ncbi.nlm.nih.gov/ ) under accession number AAY99632.1 [33]

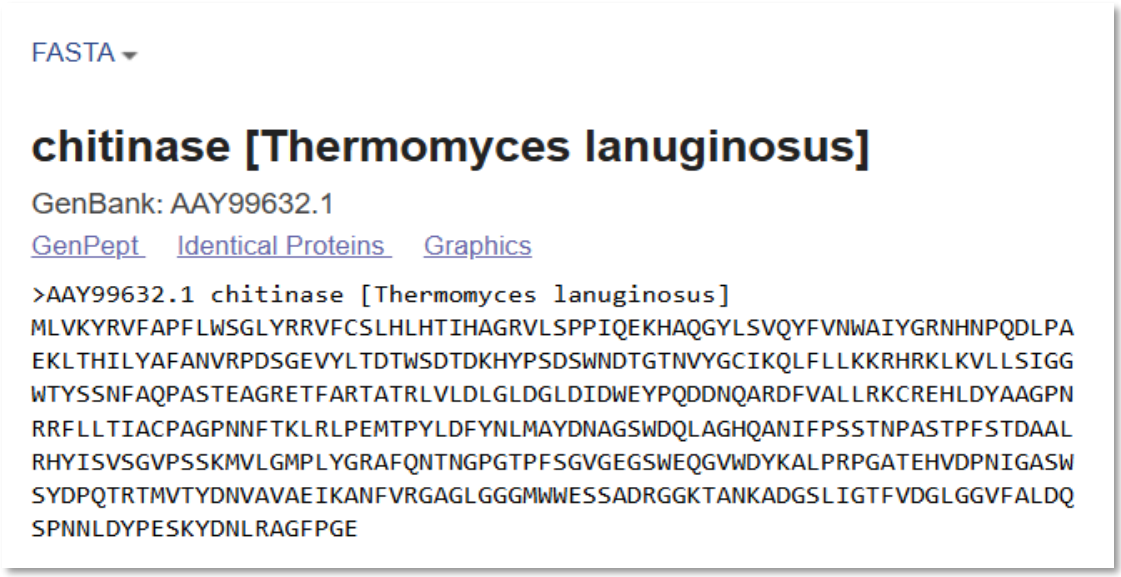



Fig. 1 Amino acid sequence of *Thermomyces lanuginosus* chitinase used to build the Three-dimensional structure.

**2.3 Three-dimensional structure building through homology modeling and its evaluation**

***2.3.1 Three-dimensional Model building of chitinase***

The homology modeling technique was used to build the three-dimensional structure of chitinase from *Thermomyces lanuginosus* using the primary amino acid sequence in FASTA format. The Three-dimensional structure of chitinase from *Aspergillus fumigatus* B1 was used as the template. Homology modeling was performed using the SWISS-MODEL tool (https://swissmodel.expasy.org/ ) [34,35]

**2.4 Validation of the three-dimensional modelled structure through Ramachandran Plot**

The three-dimensional structure of chitinase built through homology modeling was validated using the Ramachandran plot, which evaluates the model based on the phi (ϕ) and psi (Ψ) angles of the amino acid residues [36].

**2.5 Preparation of target/receptor protein molecule for the docking study**

Protein preparation is the first step in docking. A Protein Preparation Wizard (version 4.3) was used to prepare the three-dimensional structure generated by homology modeling [37]. The modeled structure of chitinase from *Thermomyces lanuginosus* was prepared by adding hydrogen atoms, removing atomic clashes, removing water molecules, and performing energy minimization [38]. The energy minimization, a crucial step, was performed using the OPLS_2005 force field at an RMSD of 0.3 Å [39].

### 2.6 Binding Site generation in the target protein molecule

The binding site in the target protein molecule was generated using the SiteMap application (version 3.8), which predicts binding sites by analyzing various factors such as size, tightness, degree of enclosure or exposure, hydrogen-bonding opportunities, and hydrophobic/hydrophilic character [40].

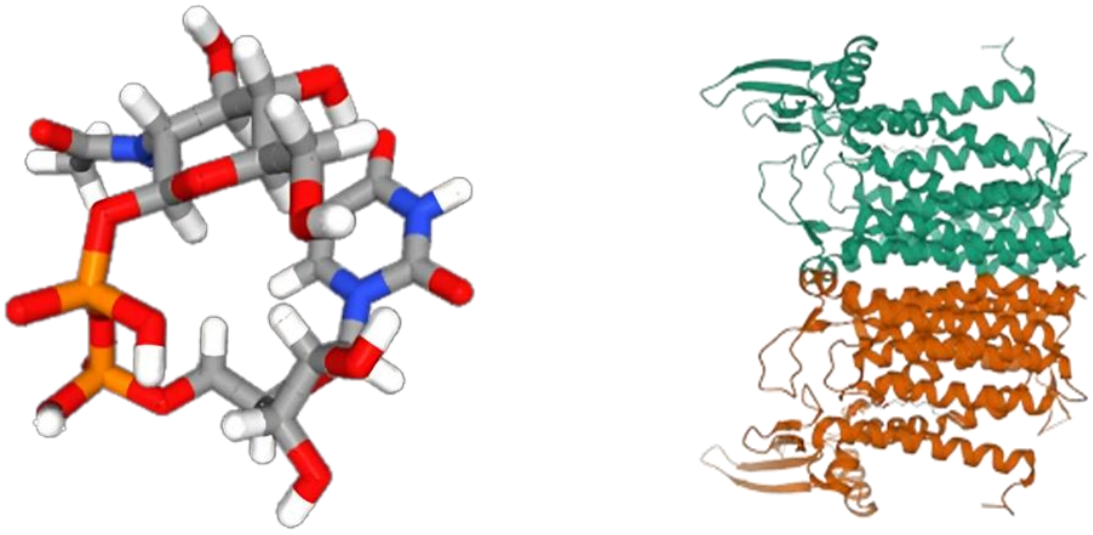
### 2.7 Grid generation and molecular docking studies

#### *2.7.1 Grid Generation in the target molecule*

Grid generation in the target molecule is an important step in molecular docking. It is carried out to determine the size and location of the active or binding site in the target molecule. The grid was generated using the receptor grid generation program with a box size of 20 × 20 × 20 Å. The atoms with a partial atomic charge of less than 0.25 were scaled using van der Waals radii of 1.0 Å [41].

#### *2.7.2 Molecular docking studies of the receptor molecule with the ligand*

The structure of ligand UDP-NAG was obtained from the PubChem compound database (Fig 2(A)). Glide (version 7.0) was used to perform molecular docking between the target molecule and the ligand. The docking was carried out using the standard precision (SP) algorithm[42]. The molecular docking results were reported as docking scores. The docking score predicts the most suitable binding pose between the test ligand and the target protein at the binding or active site [43,44].



**(A)** **(B)**

Fig. 2. Structure of UDP-NAG (A) and DPAGT1 (B) obtained from PubChem and PDB, respectively

### 2.8 Protein-protein interaction between modeled chitinase (ligand) and DPAGT1

The chitinase protein from *Thermomyces lanuginosus* was generated via homology modeling, whereas DPAGT1 was retrieved from the Protein Data Bank under PDB ID 6FM9 (Fig. 2(B)). Protein-protein interaction between the modeled chitinase and DPAGT1 protein was performed using the ClusPro web server. ClusPro (https://cluspro.org) is a commonly used server for protein-protein docking. The results were analyzed based on the top-ranked model and its corresponding coefficient scores [45].

## 3. RESULTS AND DISCUSSION

### 3.1 Molecular Docking Studies of Chitinase

The availability of the three-dimensional structure of chitinase from *Thermomyces lanuginosus* was examined in the Protein Data Bank (PDB). However, no structure of chitinase from *Thermomyces lanuginosus* is currently available in the PDB. Therefore, the required model was built using the homology modeling method.

### *3.1.1 Homology modeling of chitinase*

The SWISS-MODEL tool was used to predict the three-dimensional structure of the chitinase protein. The amino acid sequence of *Thermomyces lanuginosus* obtained from the NCBI database in FASTA format was used as the target sequence to build the homologous model. The crystal structure of *Aspergillus fumigatus* chitinase B1 (2a3c.1.A) was obtained as the best template from the SWISS-MODEL tool to build the homologous model of *Thermomyces lanuginosus* chitinase. Figure, 3(A) shows the alignment between the target protein sequence and the template sequence used for model construction. During modeling, the target sequence and template sequence were aligned (2A) and the sequence of *Aspergillus fumigatus* chitinase B1 showed 62.28% identity. The three-dimensional structure was then built (Fig. 3B).

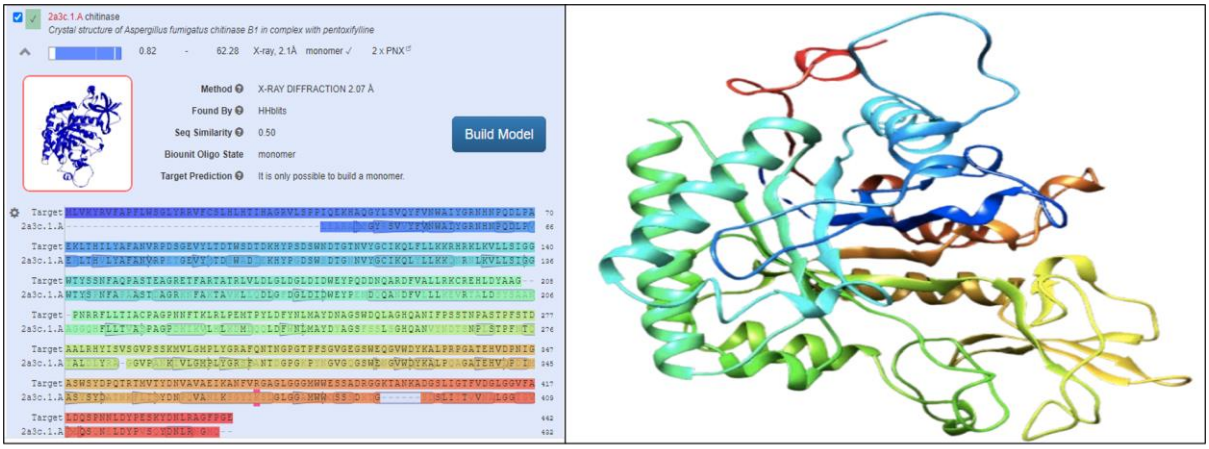



**Fig. 3. (A)** Alignment between the target protein sequence and the template sequence used for model construction **(B)** Homologous 3D Chitinase Model of the target protein (*Thermomyces lanuginosus* chitinase) using *Aspergillus fumigatus* chitinase B1 (PDB ID: 2A3C, chain A) as the template structure.

The modeled structure was validated using the Ramachandran plot (Fig. 4). The plot showed that more than 90% of the amino acid residues were present in the most favored region, whereas less than 10% were located in the additionally allowed, generously allowed, or disallowed regions. These results supported the suitability of the modeled structure for further docking studies. The

modeled structure was initially bound to a ligand, PNX, which was removed using BIOVIA Discovery Studio [46].

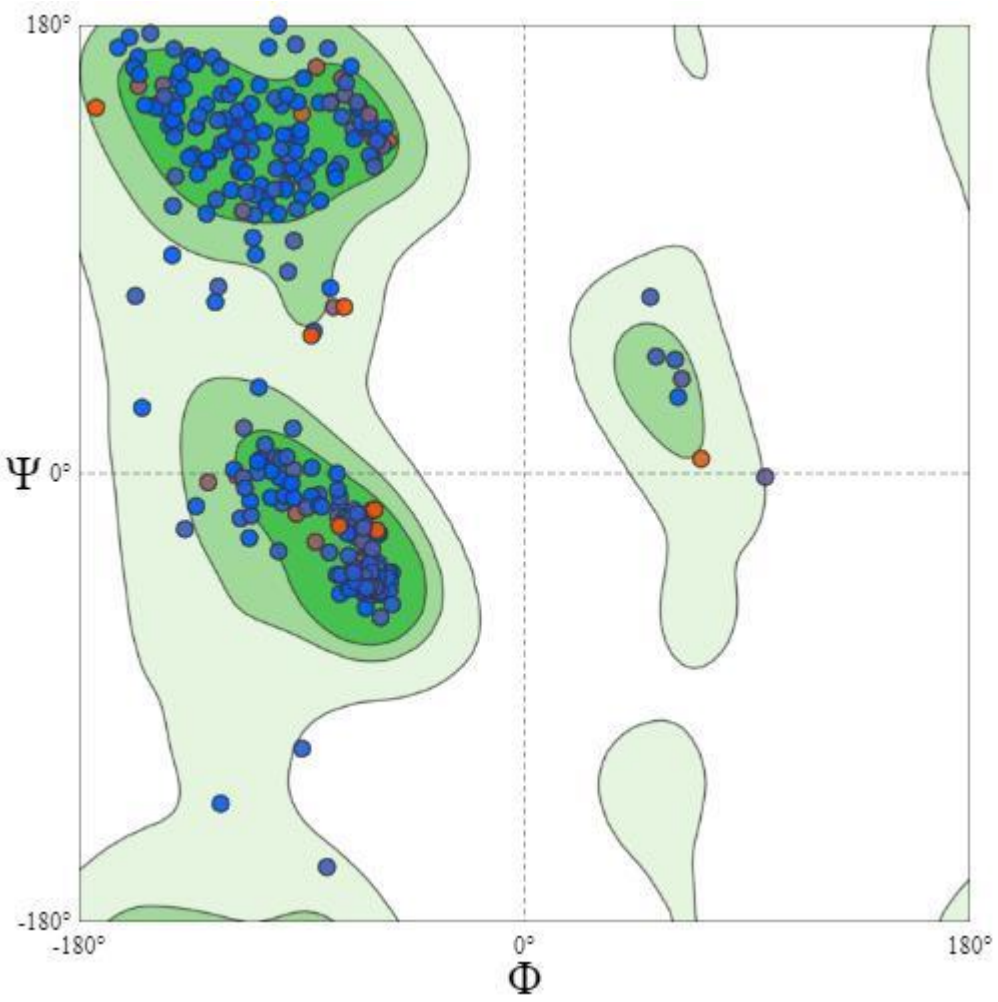



Fig. 4 Ramachandran plot to validate modeled Chitinase.

### 3.2 Docking of chitinase with UDP-NAG

Locating the binding site in a specific protein is the first step before docking. The ligand can be attached to this docking site for the analysis of receptor-ligand interaction. The binding site in the modeled chitinase was generated using the SiteMap application, which predicts the possible binding sites. The "Site Score" was used to determine the most suitable binding site among all the predicted sites. [47] A site score of 1 or higher is considered excellent and is generally used for further docking studies. Sites with a score in the range of 0.8–1 can be partially targeted, whereas sites with a score below 0.8 are generally considered unsuitable for targeting. In the

present study, the highest-scoring binding site in the target molecule was selected (Fig. 5). Once the binding site was identified, the grid was created around it to define the binding region for ligand interaction during docking. Glide was used to assess the binding affinity of chitinase in terms of docking score and glide energy [48]. The interaction was represented by a yellow dotted line indicating the formation of a hydrogen bond. Accordingly, the ligand interaction diagrams (Fig. 5) show the amino acid residues involved in ligand binding. A more negative docking score indicates a more favorable predicted interaction between the protein and ligand[49,50]. The docking score for this interaction was -5.568, and the glide energy was -53.491.

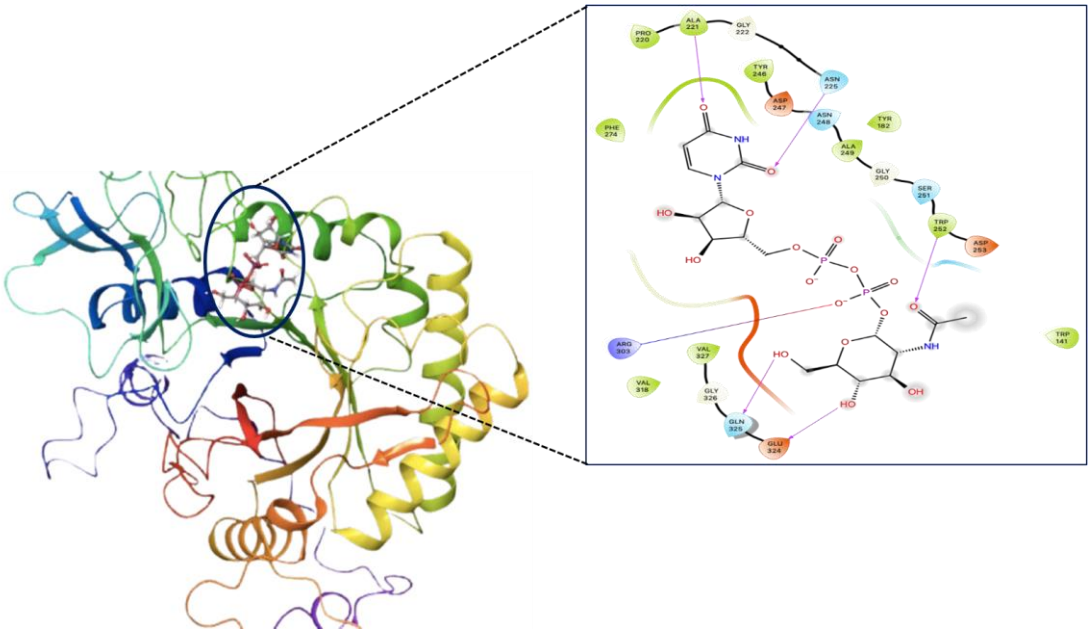



Fig. 5. Molecular docking interaction between Chitinase and UDP-NAG. The microscopic view showing the ligand interaction diagram for the interaction of Chitinase and UDP-NAG.

These findings suggest that chitinase may have the ability to interact with UDP-NAG at the molecular level. Since UDP-NAG is associated with the glycosylation pathway [51,52], the observed favorable docking may indicate the possible involvement of chitinase in interfering with abnormal glycosylation. The hydrogen bond interactions observed in the ligand interaction diagram further support the plausibility of this predicted binding pattern[53]. However, these observations should be interpreted cautiously, as docking analysis provides only a preliminary estimate of molecular interaction.

### 3.3. Protein-Protein interaction between chitinase and DPAGT1

To explore the potential of chitinase against abnormal glycosylation, protein-protein interaction analysis was performed between chitinase and DPAGT1, an enzyme involved in glycosylation. The ClusPro server was used for this interaction study. As a result, the 10 top-ranked models (Fig. 6) were generated with balanced, hydrophobic-favored, and electrostatic-favored weighted scores of center and lowest energies (Table 1). The model with the lowest energy among all the weighted scores was selected as the best model for the interaction (Fig. 7).

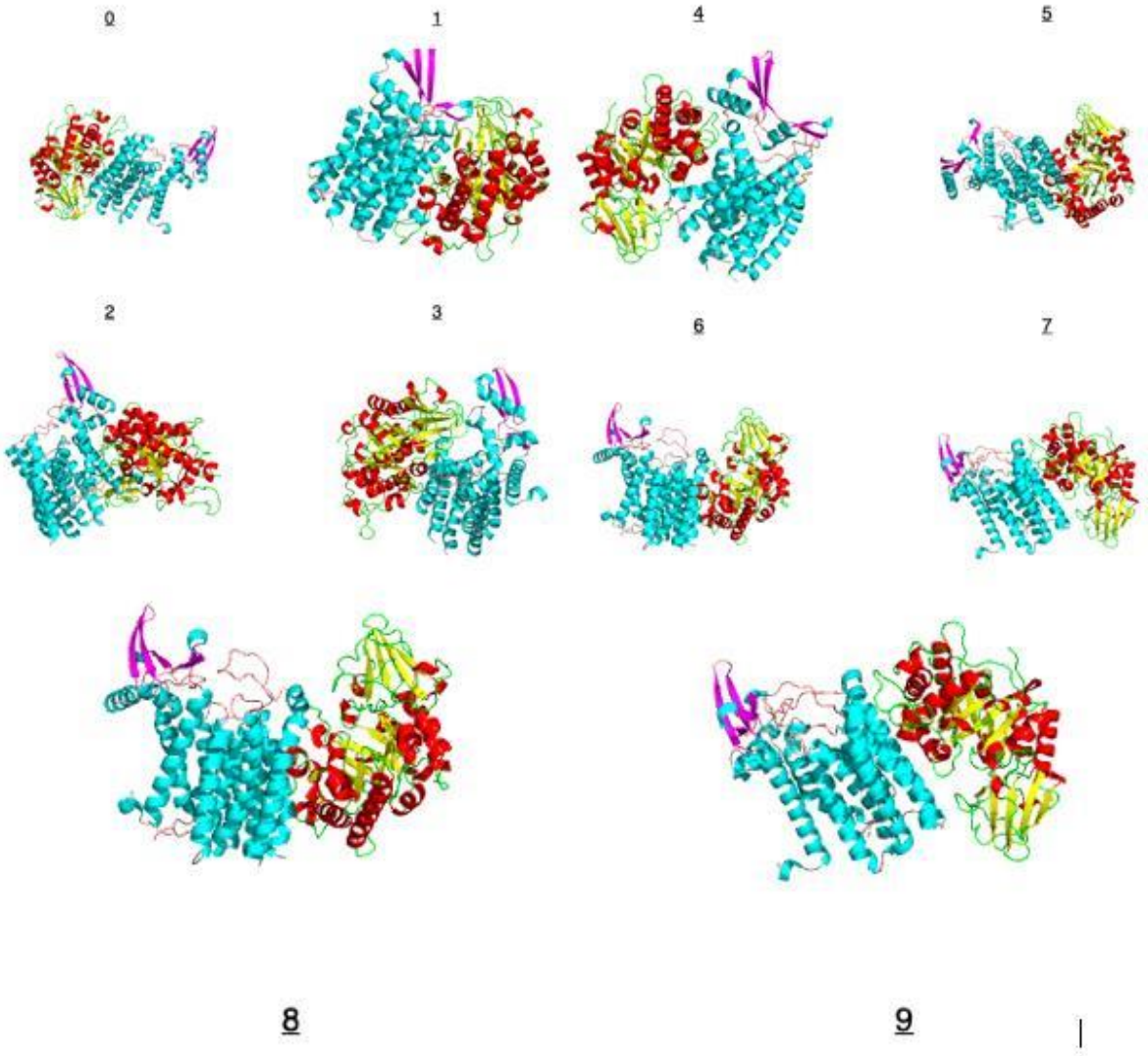



Fig. 6. Generated 10 clusters of protein-protein interaction for Chitinase and DPAGT. Color-coded keys depict the different components of docked protein-protein complexes. Sky blue: α

helix of DPAGT1 (6FM9); Violet: β sheets of DPAGT1 (6FM9); Red: α helix of chitinase protein (2A3C.1); Yellow: β sheets of chitinase protein (2A3C.1)

Table 1: Depicting the generated clusters of protein-protein interaction with the centers and lowest energy levels for balanced, electrostatic favored and hydrophobic favored interactions.

| | | **Balanced** | | **Electrostatic favored** | | **Hydrophobic favored** | |
|---|---|---|---|---|---|---|---|
| **Cluster** | Representative | Members | Weighted score | Members | Weighted score | Members | Weighted score |
| **0** | Center | 73 | -1433.6 | 65 | -1405.2 | 91 | -2404.7 |
| | Lowest Energy | | -1548.4 | | -1541.7 | | -2677.1 |
| **1** | Center | 53 | -1661.9 | 48 | -1731.8 | 61 | -2450.6 |
| | Lowest Energy | | -1799.9 | | -1845.2 | | -2454.0 |
| **2** | Center | 41 | -1333.7 | 45 | -1447.0 | 61 | -2211.4 |
| | Lowest Energy | | -1609.1 | | -1510.0 | | -2477.8 |
| **3** | Center | 39 | -1410.5 | 40 | -1321.6 | 40 | -2054.9 |
| | Lowest Energy | | -1470.3 | | -1612.5 | | -2396.5 |
| **4** | Center | 37 | -1327.1 | 38 | -1288.3 | 40 | -1973.4 |
| | Lowest Energy | | -1446.0 | | -1483.9 | | -2353.4 |
| **5** | Center | 36 | -1294.1 | 36 | -1538.9 | 39 | -2478.2 |
| | Lowest Energy | | -1484.4 | | -1538.9 | | -2609.2 |
| **6** | Center | 36 | -1308.8 | 31 | -1297.8 | 34 | -2363.8 |
| | Lowest Energy | | -1510.4 | | -1502.9 | | -2363.8 |
| **7** | Center | 36 | -1303.0 | 31 | -1505.7 | 32 | -2029.3 |
| | Lowest Energy | | -1473.3 | | -1505.7 | | -2245.6 |
| **8** | Center | 34 | -1492.4 | 29 | -1288.6 | 31 | -2047.0 |
| | Lowest Energy | | -1512.4 | | -1452.0 | | -2130.9 |
| **9** | Center | 33 | -1282.4 | 29 | -1297.4 | 30 | -2255.7 |
| | Lowest Energy | | -1332.7 | | -1462.2 | | -2395.7 |
| **10** | Center | 28 | -1319.3 | 28 | -1264.0 | 29 | -2118.2 |
| | Lowest Energy | | -1506.5 | | -1445.3 | | -2265.1 |

The observed protein-protein interaction between chitinase and DPAGT1 suggests that chitinase may also interact with an enzyme directly involved in the glycosylation pathway. This supports the proposed hypothesis that the anticancer potential of chitinase may be associated not only with

its interaction with UDP-NAG but also with its possible interference with DPAGT1-related glycosylation events. Although the interaction models obtained from ClusPro provide supportive computational evidence, these results should be considered preliminary until validated through additional computational and experimental studies.

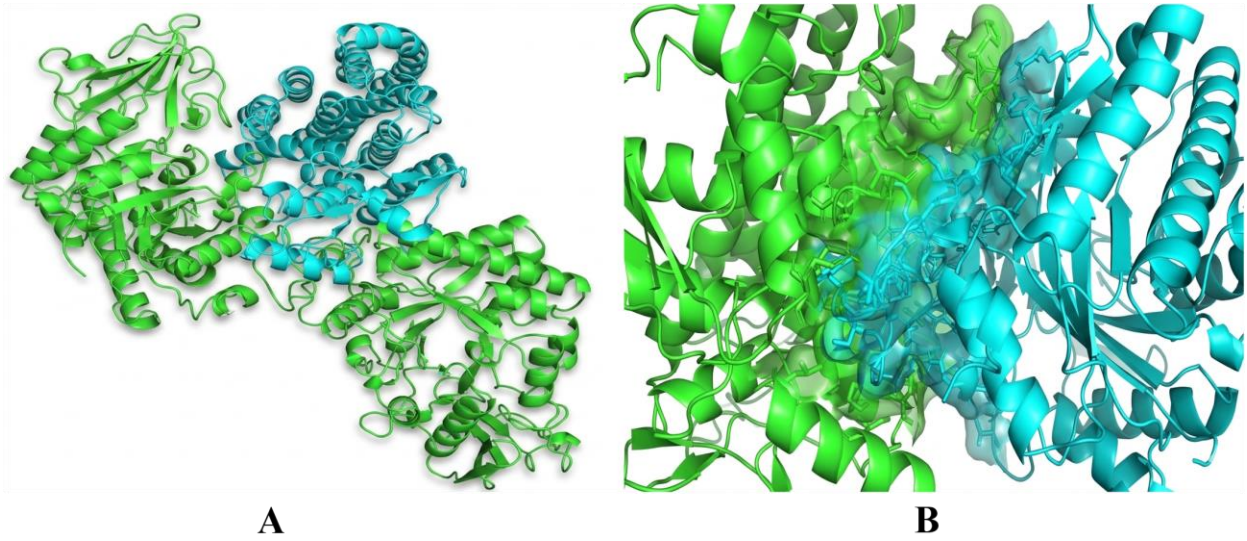



Fig. 7. Protein-protein docked complexes of DPAGT1 (6FM9), with chitinase protein (2A3C.1). Color-coded keys depict the different components of docked protein-protein complexes. Green: Chitinase protein (2A3C.1); Sky blue: DPAGT1 (6FM9).

Taken together, the molecular docking and protein-protein interaction analyses suggest that chitinase may influence abnormal glycosylation through interactions with both UDP-NAG and DPAGT1. Since abnormal glycosylation is closely associated with carcinogenesis and tumor progression [54], these observations provide preliminary in silico support for chitinase's proposed anticancer potential. The combined findings therefore strengthen the hypothesis of the present study, although further validation is required to confirm the mechanistic and biological relevance of these interactions.

The present findings are based on molecular docking and protein-protein interaction analyses carried out under a single computational framework. Therefore, the observed interactions between chitinase and UDP-NAG, as well as between chitinase and DPAGT1, should be interpreted as preliminary in silico predictions rather than definitive evidence of stable binding.

Although the obtained docking score, glide energy, and interaction models suggest favorable binding, further validation using alternative docking protocols, different scoring functions, control systems, molecular dynamics simulations, and experimental studies is required to confirm the robustness and biological relevance of these interactions.

## 4. CONCLUSIONS

Docking and protein-protein interaction studies were performed to evaluate the potential of chitinase against abnormal glycosylation. The molecular docking results were examined in terms of docking score (DS), glide score (GS), glide energy (GE), and hydrogen bond interactions to understand the binding affinity between chitinase and the target molecule. The obtained docking score was -5.568, and the glide energy was -53.491, indicating favorable docking of chitinase with UDP-NAG. Ligand interaction analysis also supported the binding through hydrogen bond formation between the interacting molecules. In addition, the protein-protein interaction studies generated structure and score models with balanced, electrostatic-favored, and hydrophobic-favored interactions, suggesting a favorable interaction between chitinase and DPAGT1. Taken together, these findings suggest that chitinase may have the potential to interfere with abnormal glycosylation either through interaction with UDP-NAG or through interaction with the DPAGT1 enzyme, thereby affecting the glycosylation process. Thus, chitinase may be considered a promising candidate for further investigation as a potential inhibitor of carcinogenesis associated with abnormal glycosylation. However, further experimental validation is required to confirm these in silico findings and to establish their biological significance.

**List of Abbreviations**

- DS: Docking Score
- DPAGT: Dolichyl-phosphate N-acetylglucosaminephosphotransferase
- FASTA: FAST-All
- GE: Glide Energy
- GS: Glide Score
- GLIDE: Grid-based Ligand Docking with Energetics
- OPLS: Optimized Potentials for Liquid Simulations
- PNX: Pentoxifylline
- PDB: Protein Data Bank

- RMSD: Root-Mean-Square Deviation
- SP: Standard Precision
- UDP: Uridine diphosphate-N-acetylglucosamine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## COMPLIANCE WITH ETHICAL STANDARDS


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**Investigations:** Nisha Suryawanshi, J. Satya Eswari, Manisha Yadav,

**Formal Analysis:** J. Satya Eswari, Bikash Kumar; Sushma Chauhan

**Writing – original draft:** Nisha Suryawanshi, Manisha Yadav, Bikash Kumar

**Visualization:** Nisha Suryawanshi, Manisha Yadav, Bikash Kumar

**Writing – review & editing:** Nisha Suryawanshi, Manisha Yadav, Bikash Kumar, J. Satya Eswari, Sushma Chauhan

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## Conflicts of interest

The authors have no conflicts of interest to declare that are relevant to the content of this article.

## Ethical approval

This article does not contain any studies with human participants or animals performed by any of the authors.

## Data Availability

All data generated or analyzed during this study are included in this published article. Protein sequences were retrieved from NCBI, ligand structures from PubChem, and protein structures from the Protein Data Bank (PDB). Homology modeling was performed using SWISS-MODEL. Publicly available software, such as "ClusPro web server," are used."

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