J Herbmed Pharmacol. 2026; 15(2): 258-267.

http://www.herbmedpharmacol.com doi: 10.34172/jhp.2026.53240

Journal of Herbmed Pharmacology

# Bioinformatic analysis of the interaction between concanavalin A (ConA) from *Canavalia ensiformis* and the human *ALDH18A1* gene in pediatric gastrointestinal cancers with encephalopathy symptoms

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**ARTICLE INFO**

***Article Type:***
Original Article

***Article History:***
Received: 29 Jun. 2025
Revised: 22 Aug. 2025
Accepted: 22 Aug. 2025
epublished: 1 Apr. 2026

***Keywords:***
Concanavalin A
Encephalopathy
Gastrointestinal cancer
Metabolic homeostasis
Pediatric cancer

**ABSTRACT**

**Introduction:** Pediatric gastrointestinal (GI) cancers with encephalopathy are complex. The role of key genes like ALDH18A1, involved in metabolism and tumorigenesis, remains unclear in these disorders. This study aims to use bioinformatics to explore a potential non-canonical structural interaction between the plant lectin concanavalin A (ConA) and the dysregulated cancer target ALDH18A1.

**Methods:** The amino acid sequences of the two target proteins were retrieved from the UniProt database and aligned using BLASTp. Their three-dimensional structures were then obtained via Protein Data Bank (PDB) data and the SwissModel server, and structural quality was assessed using Ramachandran plot, QMEAN, and QMEANDisCo metrics. Subsequently, protein–protein docking simulation was conducted using the ClusPro server, resulting in 30 proposed binding clusters.

**Results:** No significant sequence similarity was detected between ConA and *ALDH18A1* (Identity<10%, non-significant E-value). Nevertheless, the structural model of ConA demonstrated high quality (Ramachandran outliers: 0.59%, QMEANDisCo: 0.87), while *ALDH18A1*, despite its structural complexity, showed a reliable model (Ramachandran outliers: 2.37%, QMEANDisCo: 0.72). The docking analysis revealed that several clusters—particularly clusters 3, 5, 11, and 15—had highly negative binding energies (as low as –1060.7), suggesting a thermodynamically favourable interaction with potential biological significance.

**Conclusion:** Despite no sequence similarity, a structural interaction between ConA and ALGH18A1 is plausible. This may influence immunity and cancer cell growth indirectly, providing a groundwork for developing targeted therapies for pediatric GI cancers. Further experimental validation is required to confirm these preliminary findings.

*Implication for health policy/practice/research/medical education:*
Bioinformatic analyses revealed a stable structural interaction between ConA and ALDH18A1, supported by docking simulations (lowest binding energy: –1060.7). Despite no sequence homology, molecular dynamics suggest a feasible binding interface. This interaction may influence pathways in pediatric GI cancers with encephalopathy, warranting further experimental validation to elucidate its biological significance.

*Please cite this paper as:* Mohammadi MH, Mohammadkhani Sh, Alkhazali AS, Shafiei Y, Pour Masoumi H, Fazeli-Nasab B. Bioinformatic analysis of the interaction between concanavalin A (ConA) from *Canavalia ensiformis* and the human *ALDH18A1* gene in pediatric gastrointestinal cancers with encephalopathy symptoms. J Herbmed Pharmacol. 2026;15(2):258-267. doi: 10.34172/jhp.2026.53240.

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## Introduction

Gastrointestinal (GI) cancers, particularly those occurring in early childhood, are among the most challenging chronic and life-threatening diseases (1,2). While the incidence of these conditions in pediatric populations is lower than in adults, their aggressive nature, complex diagnostic processes, and unpredictable treatment responses have led to extensive research into the genetic, molecular, and environmental factors contributing to their development. The neurological symptoms, including encephalopathy, are mainly linked to underlying metabolic disorders such as P5CS deficiency, rather than being directly caused by tumor metastasis or paraneoplastic syndromes (3-5).

The *ALDH18A1* gene (Aldehyde Dehydrogenase 18 Family Member A1), which encodes the Delta-1-pyrroline-5-carboxylate synthase protein, is a key gene involved in the biosynthetic pathways of proline, arginine, and glutamate. This mitochondrial enzyme plays a crucial role in maintaining cellular metabolic homeostasis. Impaired function of this gene can lead to the accumulation of toxic metabolites and the induction of oxidative stress, which not only activates inflammatory and oncogenic pathways but may also contribute to serious neurological disorders such as encephalopathy. Furthermore, recent studies have shown the elevated expression of *ALDH18A1* in GI cancer cells, which is associated with drug resistance and poor prognosis. Mutations in the ALDH18A1 gene are associated with pyrroline-5-carboxylate synthetase (P5CS) deficiency (OMIM #138250), a rare autosomal recessive disorder characterized by neurodevelopmental delay, encephalopathy, and connective tissue abnormalities. Elevated expression of ALDH18A1 has been associated with drug resistance and poor prognosis in several cancer types, including colon and pancreatic cancers (6).

Concanavalin A (ConA), a well-characterized plant lectin extracted from *Canavalia ensiformis* (jack bean), exhibits strong affinity for recognizing and binding cell surface carbohydrates. ConA can be internalized by cells through receptor-mediated endocytosis, allowing it to reach intracellular compartments and potentially interact with cytoplasmic or mitochondrial targets. By specifically targeting mannose and glucose residues, ConA has been shown to modulate several signalling pathways such as MAPK, NF-κB, and autophagy-related cascades in various cancer cell types, and its apoptotic and antitumor effects have been demonstrated in several studies (7). Moreover, evidence suggests that ConA can interact at the molecular level with intracellular components, influencing pathways related to cell growth regulation, autophagy, and immune modulation (7).

Advancements in bioinformatics have greatly improved our ability to model three-dimensional protein structures, analyze intermolecular interactions, and accurately identify potential signalling pathways with increased speed. Over the past decade, tools like BLASTp for sequence alignment, SWISS-MODEL for structural modelling, and ClusPro for protein–protein docking have become standard methodologies in functional protein analysis. These tools facilitate the exploration of hypotheses that are challenging to assess through experimental methods alone, such as the potential interaction between ConA and *ALDH18A1* (8).

Given the essential role of *ALDH18A1* in cellular metabolism and the well-established capability of ConA to bind glycan structures and trigger apoptotic signalling pathways, it is hypothesized that a structural or indirect functional interaction may exist between these two proteins, despite the absence of sequence similarity. Since initial bioinformatic analyses reveal no significant sequence homology, the application of 3D modelling and docking tools may provide critical insights into potential non-homologous but structurally feasible interactions. (9). This study aimed to computationally assess the potential structural interaction between the lectin ConA and the metabolic enzyme ALDH18A1 using molecular docking analysis, with implications for pediatric GI cancer biology.

## Materials and Methods

### Protein sequence retrieval

The amino acid sequences of ConA from *C. ensiformis* (Figure 1) and the human *ALDH18A1* protein (Delta-1-pyrroline-5-carboxylate synthase) (Figure 2) were retrieved from the UniProt database (https://www.uniprot.org). The UniProt IDs used were P02866 for ConA and P54886 for *ALDH18A1*. UniProt is recognized as a reliable and standardized resource for accessing comprehensive structural and functional information on proteins (10).

### Comparison of sequences using BLASTp

To evaluate the homology and similarity between the amino acid sequences of the two proteins, the BLASTp (Basic Local Alignment Search Tool for Proteins) tool

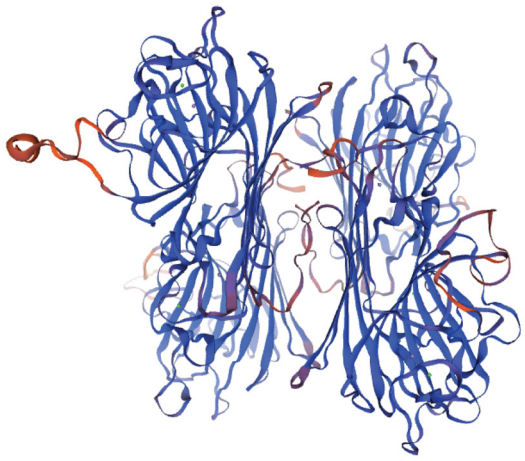

**Figure 1.** 3D structure of Concanavalin A protein (UniProtKB/Swiss-Prot: P02866).

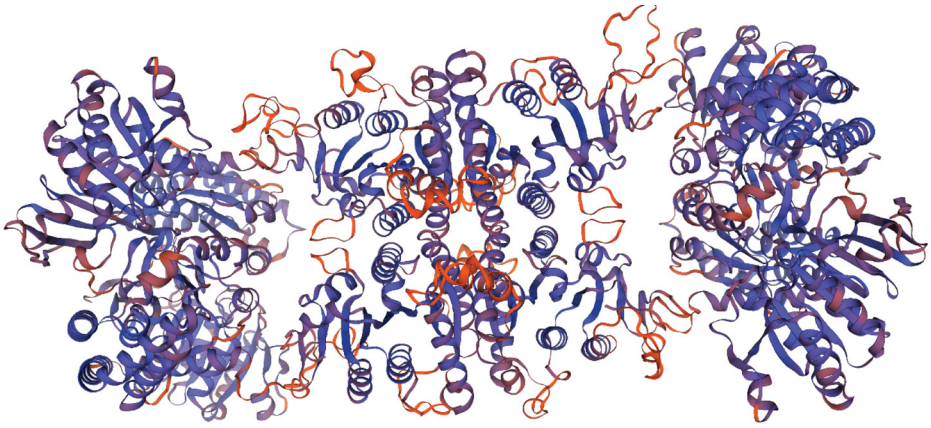

**Figure 2**. 3D structure of *ALDH18A1* protein (UniProtKB/Swiss-Prot: P54886).

from the NCBI database (https://blast.ncbi.nlm.nih.gov) was employed. The alignment analysis was conducted using default parameters, and statistical metrics, including sequence identity percentage, E-value, alignment length, and bit score, were reported. Sequence alignments with identity values below 30% and E-values less than 1e–5 were considered to indicate significant divergence or homology (11).

### Retrieval and evaluation of three-dimensional structures

The 3D structure of ConA from *C. ensiformis* was obtained from the Protein Data Bank (PDB) under the accession ID 1NLS. Likewise, the 3D structure of the human *ALDH18A1* protein was retrieved from the same database with PDB ID 2H5G. Both structures were determined by X-ray diffraction with a high resolution of 1.60 Å and 2.25 Å, respectively. These PDB files were used for structural analyses, molecular docking, and quality assessments (12).

### Quality assessment of 3D structures

To validate the quality of the retrieved structures, structural analysis tools, including Ramachandran plot, QMEAN (QMEANDisCo scores >0.6 were considered to represent reliable structural models for docking), and QMEANDisCo scores were utilized. These tools assess the geometric correctness of the 3D models, dihedral angle distributions, and deviation from experimental structures, and provide local quality estimations for each residue. The results were analyzed to ensure the models' adequacy for subsequent docking studies (13).

### Protein–protein docking simulation

Protein–protein docking was performed using the ClusPro server (https://cluspro.bu.edu), in which ConA (PDB ID: 1NLS) was treated as the receptor, and *ALDH18A1* (PDB ID: 2H5G) as the ligand, based on the exposed sugar-recognition site of ConA. Although ConA is smaller in size and typically considered the ligand in lectin–carbohydrate interactions, we designated it as the receptor to enable docking alignment based on its well-characterized glycan-recognition surface. ALDH18A1, our target of interest, was designated as the ligand for practical modelling. We selected the 'balanced' scoring model, which incorporates van der Waals interactions, electrostatics, and desolvation energies. In the post-docking analysis, clusters were ranked according to their lowest energy scores and sizes. We further analyzed interface residues and potential hydrogen bonding interactions using PyMOL visualization tools (9). All online tools and databases used in this study were accessed between January 2024 and March 2024

## Results

### Docking results analysis

The docking outputs included parameters such as binding energy, the number and positions of hydrogen bonds, van der Waals forces, electrostatic interactions, and surface contact areas between the two proteins. These data were analyzed and visualized using PyMOL software. The top-ranked models in terms of structural stability were selected for further analysis.

### Results of BLAST analysis between *C. ensiformis*, ConA, and Human *ALDH18A1*

To assess the sequence similarity between ConA from *C. ensiformis* and the human *ALDH18A1* protein, the BLASTp (Protein–Protein BLAST) tool was applied. The amino acid sequence of ConA, consisting of 290 residues (query), was compared against the *ALDH18A1* sequence with 795 residues (subject). The summary of alignment results is presented in Table 1.

### Quality assessment of the 3D structure of ConA protein (*C. ensiformis*)

#### *Ramachandran plot analysis*

The Ramachandran plot analysis of the 3D model of the ConA protein (*UniProtKB/Swiss-Prot: P02866*) from jack bean revealed that only 0.59% of the residues were located in outlier regions, which is well within the acceptable

**Table 1.** Summary of BLASTp analysis results between Concanavalin A (Con A) and human *ALDH18A1* proteins

| Parameter | Value | Description/Meaning |
|---|---|---|
| Sequence length (Query/ Subject) | 290 / 795 amino acids | Length of the sequences analyzed for ConA and *ALDH18A1* |
| Sequence identity (%) | No significant similarity | Lack of meaningful matches in aligned regions |
| E-value | No significant value | Alignment lacks statistical significance |
| Alignment length | No specific alignment | Absence of overlapping regions between sequences |
| Bit score | Very low | Indicates a very weak alignment between the two sequences |

range for a high-quality structure. This low percentage indicates proper geometric stability and accuracy of the model. The outlier residues included C164 ALA, B163 ASN, A165 ASP, B155 THR, C158 PHE, and A158 PHE. The presence of such outliers, especially in specific residues such as proline or phenylalanine, may result from angular constraints or local flexibility, which is considered normal in protein modelling. Overall, 94% of residues were found in favoured regions, and 5.41% were in allowed regions, suggesting a reliable and structurally valid model (Figure 3).

### *QMEAN score evaluation (QMEAN Z-score)*

The quality of the 3D model of the ConA protein was evaluated using the QMEAN scoring function. The model's Z-score was calculated at -0.29, which is within the acceptable range for high-quality homologous models. This score suggests that the predicted structure aligns well with experimentally determined structures available in the PDB database. Further analysis of the individual QMEAN sub-scores reinforces the model's geometric validity: the Cβ interaction score was -1.27, the all-atom energy was -0.87, the solvation energy was -0.38, and the torsion angle score was 0.07. Since all these values fall within expected standard ranges, we can conclude that the 3D model displays satisfactory spatial geometry and energetic stability. This confirms the model's reliability for further studies, especially for molecular interaction simulations (Figure 4).

### *Local quality assessment using QMEANDisCo*

There was a strong agreement between the model and experimentally derived protein structures (at 0.87 ± 0.05), reflecting high reliability and overall structural accuracy. Local quality scores, found in the B-factor column of the PDB file, ranged from 0.4 to 0.9. The highest scores, around 0.9, were observed in the central regions of the protein (residues ~120 to 180), suggesting high structural stability in these areas. In contrast, lower quality scores were noted in certain flexible regions, particularly in residues 220 to 260, which may correspond to loops or structurally less stable segments (Figure 5).

## Evaluation of the 3D structure of human *ALDH18A1*

### *Ramachandran plot analysis*

The Ramachandran plot analysis for the predicted 3D structure of human *ALDH18A1* (UniProtKB/Swiss-Prot: P54886) revealed that 2.37% of residues fall within disallowed regions. Although this value slightly exceeds the threshold typically associated with high-quality models, it remains acceptable considering the large size and multi-domain nature of the protein. The outlier residues included amino acids such as Pro, Val, Arg, Glu, Ser, Thr, and Gln, distributed across various segments of the polypeptide chain and spanning chains A through D. The presence of these residues in disallowed regions may result from their

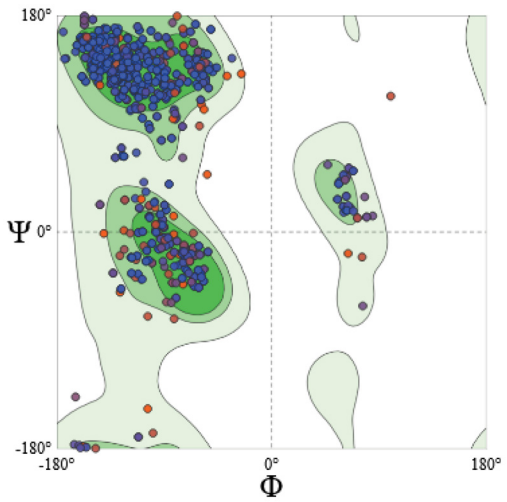


**Figure 3.** Ramachandran chart for 3D structure of concanavalin A protein (UniProtKB/Swiss-Prot: P02866).

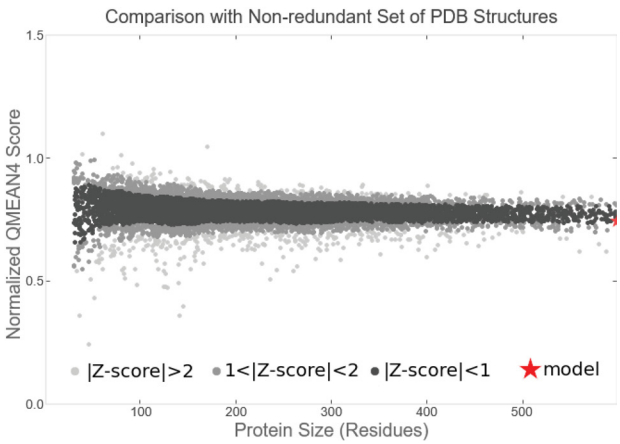


**Figure 4.** QMEAN Z-Scores for 3D structure of concanavalin A protein (UniProtKB/Swiss-Prot: P02866).

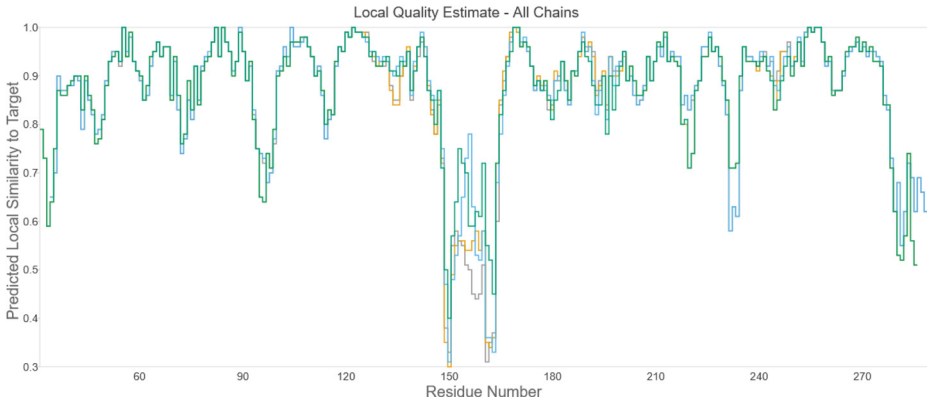


**Figure 5.** QMEANDisCo local for 3D structure of concanavalin A protein (UniProtKB/Swiss-Prot: P02866).

inherent flexibility or specific structural characteristics. Overall, 90.58% of the residues were located in favoured regions, indicating a relatively reliable model in terms of backbone dihedral angles (Figure 6).

### *Assessment of QMEAN score (QMEAN Z-score)*

The overall quality evaluation of the predicted 3D structure of human *ALDH18A1*, based on the QMEAN scoring function, yielded a Z-score of –2.98. Although this value slightly deviated from the optimal range observed for high-resolution experimental structures, it remained justifiable considering the large size of the protein and the presence of multiple structurally flexible domains. Further analysis of the QMEAN sub-scores provided additional insights: the Cβ interaction score was –0.63, the all-atom interaction energy was –0.16, the solvation energy was –0.06, and the torsion angle component showed the highest deviation at –2.80. Although there was a significant deviation in torsional geometry, the other parameters remained within acceptable limits for reliable models. Overall, these findings indicate that the predicted structure, despite minor local imperfections, is sufficiently high-quality for structural analyses, molecular docking simulations, and related studies (Figure 7).

### *Local quality assessment using QMEANDisCo*

The global QMEANDisCo score for the model was 0.72 ± 0.05, indicating a relatively good agreement with experimental structures from the PDB. Analysis of the local quality scores revealed that central regions of the protein, particularly around residues 180, 360, and 540, exhibited high scores (≥0.7), suggesting good structural stability in these areas. In contrast, regions with lower scores (≤0.4) were also detected, especially around residues 270 and 630, which might correspond to flexible loops or poorly structured segments. These insights can help identify regions that may require further refinement (Figure 8)

### *Protein–protein docking simulation results*

To investigate the potential spatial interaction between ConA from *C. ensiformis* and the human *ALDH18A1*

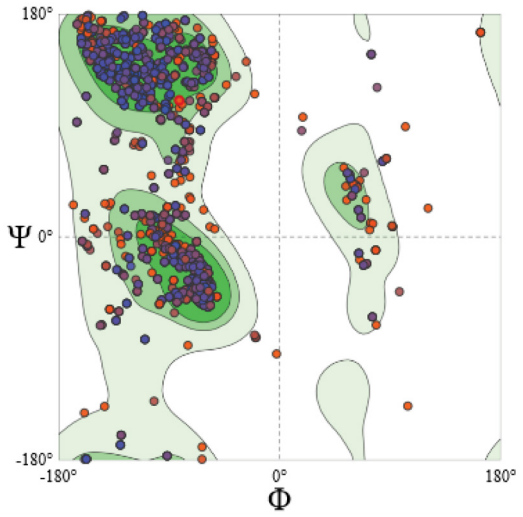


**Figure 6.** Ramachandran plot for the 3D structure of the ALDH18A1 protein (UniProtKB/Swiss-Prot: P54886).

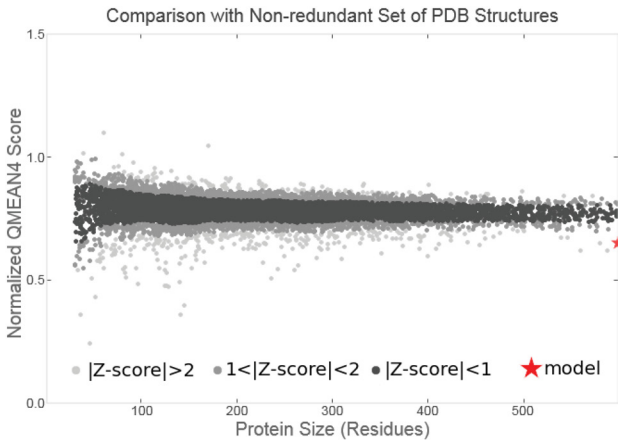


**Figure 7.** QMEAN Z-Scores for 3D structure of ConA Protein of *ALDH18A1* protein (UniProtKB/Swiss-Prot: P54886).

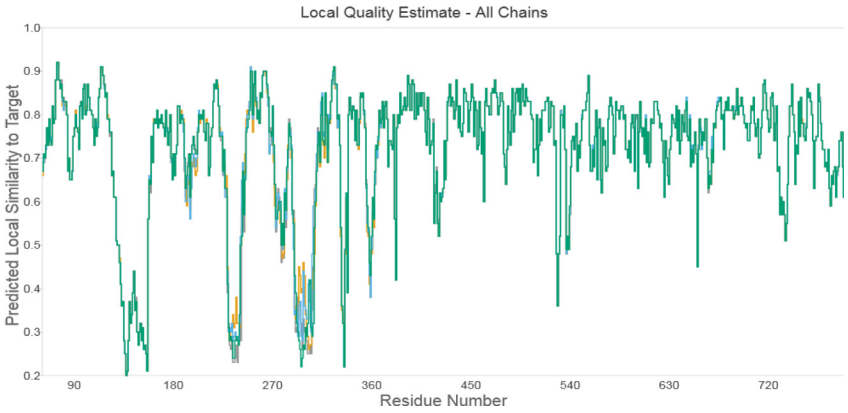


**Figure 8.** QMEANDisCo local for 3D structure of Concanavalin A protein of *ALDH18A1* protein (UniProtKB/Swiss-Prot: P54886).

protein, molecular docking simulations were performed using the ClusPro server. The 3D structures of ConA (PDB ID: 1NLS) and the predicted model of *ALDH18A1* were uploaded to the platform as receptor and ligand, respectively. ClusPro utilized advanced algorithms to calculate intermolecular interaction energies, encompassing van der Waals forces, electrostatic interactions, repulsion, and DARS-based statistical potential modeling. This approach allowed for the generation and classification of clusters of potential docking conformations.

In total, 30 docking clusters were identified as proposed models for the interaction between the two proteins. For each cluster, the number of members, representative energy score, and lowest binding energy were calculated and recorded. Table S1 summarizes the top 30 docking clusters, their energy scores, and the number of members (provided as supplementary material). Cluster 3 exhibited the lowest binding energy among all predicted docking models (Figure 9), with a value of –1060.7, indicating the most stable interaction configuration. This significantly negative energy score reflects a high potential for the formation of a stable complex at the molecular level. Additionally, Clusters 1, 5, 11, 15, and 20 also displayed relatively low binding energies, suggesting the existence of multiple plausible conformations for the interaction between the two proteins. The docking score of –1060.7 reported by ClusPro is presented in arbitrary units (not kcal/mol) and is used only for ranking docking poses based on their clustering and estimated interaction energy. Key interactions, including hydrogen bonds and salt bridges, were visualized using LigPlot+. Notably, residues Asp103, Glu112 (ALDH18A1), Asp208, and His24 (ConA) participated in hydrogen bonding at the interface. The docking interface is located outside the NADP+ binding domain of ALDH18A1, suggesting that the interaction does not disrupt its catalytic site. The obtained results support the hypothesis of a potential structural and non-homologous interaction between ConA and *ALDH18A1*, thereby providing a foundation for further investigations at the level of residue-specific and functional interactions. Currently, there is no experimental evidence supporting

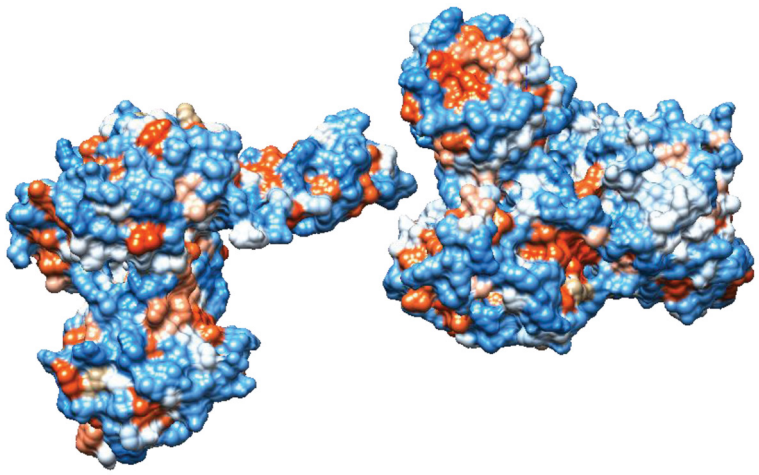

**Figure 9**. Three-dimensional docking model of Concanavalin A (ConA; green, Chain A) and ALDH18A1 (blue, Chain B) derived from Cluster 3. Key interface residues involved in the interaction are highlighted, with hydrogen bonds shown as yellow dashed lines. This model suggests a thermodynamically favorable interaction and was visualized using PyMOL.

the glycosylation of ALDH18A1. The interaction may be influenced by structural compatibility that is not mediated by glycans. We acknowledge this limitation. While there are no experimental glycosylation data for ALDH18A1, we investigated potential glycosylation motifs using the NetNGlyc 1.0 server, which did not identify any significant N-glycosylation sites.

## Discussion

### Functional and structural analysis based on the absence of sequence homology between ConA and *ALDH18A1*

BLAST analysis revealed no significant similarity between the amino acid sequences of ConA and human *ALDH18A1*. This finding indicates that these two proteins are not homologous at the primary sequence level, suggesting an unlikely functional similarity based on sequence similarity alone. However, the lack of sequence homology does not necessarily preclude functional or structural interactions between them (11). BLASTp revealed <15% sequence identity and E-value > 0.1 between ConA and ALDH18A1, supporting the absence of homologous similarity. Although ConA is classically known to bind to cell surface glycoproteins, previous studies have demonstrated its ability to enter cells via receptor-mediated endocytosis and subsequently localize in intracellular compartments. Given that ALDH18A1 is a mitochondrial protein, it is plausible—though not confirmed—that ConA may influence mitochondrial targets either through direct uptake, intracellular trafficking, or via indirect modulation of cellular signaling pathways that regulate mitochondrial enzymes. This hypothesis requires experimental validation.

ConA can enter cells through either clathrin-dependent or clathrin-independent endocytic pathways, a phenomenon previously noted in cancer cells. Additionally, its effects on ALDH18A1 may occur indirectly by modifying upstream regulators or signaling pathways, such as those related to oxidative stress or apoptosis. These changes can subsequently influence the expression or function of mitochondrial enzymes. ConA, a well-known plant lectin, possesses the ability to bind to cell surface carbohydrates and plays important roles in various biological processes such as cell growth regulation, apoptosis induction, autophagy, and immune modulation (16). On the other hand, the *ALDH18A1* gene encodes a key enzyme involved in proline biosynthesis and mitochondrial functions, playing a critical role in amino acid metabolism. Dysfunction of *ALDH18A1* has been linked to GI cancers and pediatric encephalopathy (14). Therefore, despite the absence of sequence homology, it is plausible that these two proteins may form structurally favorable but functionally distinct interactions rather than sequence-based similarity. To further explore this hypothesis, molecular docking and three-dimensional structural analyses are essential.

### Comparative analysis of the structural quality of three-dimensional models of ConA and *ALDH18A1* proteins to assess modeling adequacy for docking studies

In this study, the three-dimensional structures of the two target proteins, ConA and human *ALDH18A1*, were qualitatively evaluated. Structural analyses revealed that both models possessed acceptable quality for docking studies and functional analyses, although differences in their structural validity were observed (15).

The modeled structure of the bean ConA protein exhibited excellent structural quality. Ramachandran plot analysis showed that only 0.59% of residues were located in disallowed regions, well below the typical threshold for experimental models. Additionally, the global QMEANDisCo score was 0.87 ± 0.05, indicating high conformity with experimentally determined structures available in the PDB. Furthermore, QMEAN sub-scores—including solvation energy, dihedral angles, and atomic interactions—were all within normal ranges. These results suggest that the ConA model has high spatial stability and sufficient accuracy for use in functional studies and docking simulations (16). In contrast, the three-dimensional model of the human *ALDH18A1* protein, despite having a complete model and adequate sequence coverage, showed a higher percentage (2.37%) of residues in outlier regions on the Ramachandran plot. Although this value remains acceptable for large, multi-domain structures, it indicates regions with potential flexibility or local modeling errors. Moreover, the overall QMEAN Z-score was -2.98, slightly deviating from the ideal range. This deviation likely reflects the structural complexity, presence of flexible domains, and inherent mobility in certain regions. Nevertheless, the global QMEANDisCo score of 0.72 ± 0.05, along with relatively high local quality scores in central regions (≥ 0.7), still supports the model's validity for structural analyses (16).

The assessment of structural quality shows that the three-dimensional model of ConA is superior to that of *ALDH18A1*. However, both models are reliable for further analyses, including molecular docking and protein–protein interaction studies. Furthermore, identifying low-scoring regions in both structures presents opportunities for refinement and improvement in future research (16).

### Interpretation of binding energy and stability of molecular complexes resulting from ConA–*ALDH18A1* docking

Analysis of molecular docking results between ConA and *ALDH18A1* proteins using the ClusPro server indicated the potential for spatial interaction between these two proteins despite the absence of sequence homology. Based on the binding energy scores, several clusters with significantly negative energies were identified, indicating high structural stability of the complexes formed by the interaction of these molecules. The docking interface does not overlap with the known catalytic or NADP+

binding domains of ALDH18A1, indicating a structurally independent interaction (9). While ALDH18A1 is mitochondrial and ConA primarily binds extracellular glycans, lectins are known to enter cells via endocytosis, raising the possibility of indirect or transient intracellular interactions. Previous docking studies between ConA and known glycoprotein targets such as CD45 or lysosomal enzymes have shown binding energies typically ranging between –850 to –1000 ClusPro units. The observed energy for ConA–ALDH18A1 docking (–1060.7) is within or lower than this range, supporting the potential stability of this complex.

Among the 30 clusters predicted by ClusPro, cluster number 3 exhibited the lowest binding energy of –1060.7, clearly representing the most stable model among the proposed structures. This energy value is considerably lower than that of other clusters, reflecting the formation of a highly stable molecular complex. Binding energies below –900 in ClusPro simulations are generally considered indicative of genuine and effective protein–protein interactions (9). Moreover, the presence of multiple other clusters with relatively low binding energies, such as clusters 1, 5, 11, 15, and 20 with energies ranging from –987.6 to –1015.7, suggests that the interaction between ConA and *ALDH18A1* exhibits spatial flexibility and can occur in multiple conformations. Such diversity in predicted structures is commonly observed in non-covalent interactions, especially when the binding interfaces display high spatial complementarity (9).

From a biological perspective, the docking results suggest a non-classical structural interaction between ConA and *ALDH18A1*. ConA, a plant lectin known for its ability to bind glycoproteins, interacts with host human proteins through hydrogen bonding, van der Waals forces, and electrostatic interactions. In contrast, *ALDH18A1* is an enzyme involved in mitochondrial metabolic pathways and proline biosynthesis, playing significant roles in the pathophysiology of GI disorders and neurological conditions like encephalopathy. Consequently, the potential interaction between these two proteins could indicate indirect regulation of cellular pathways, induction of apoptosis, or modulation of oxidative stress (6).

Overall, the molecular docking analysis in this study indicates that the obtained structural models have a high potential to form stable complexes between ConA and *ALDH18A1*. These findings not only support the hypothesis of a non-homologous structural interaction between the two proteins but also provide a foundation for further experimental studies to investigate the functional role of this interaction in GI and brain tissues in relevant biological systems or model organisms (7).

This study comprehensively investigates the potential interaction between two non-homologous proteins from plant and human sources, which operate in entirely different biological pathways. Despite the lack of sequence similarity between ConA and *ALDH18A1*, spatial structural modeling and docking analyses suggest the possibility of non-covalent and structural interactions between them. The predicted structure of *ConA* was of exceptionally high quality, making it an ideal candidate for functional analyses. Although the structure of *ALDH18A1* exhibited geometric complexities, it still demonstrated sufficient validity. This underscores the ability of modern bioinformatics tools to produce reasonably accurate structures, even for multidomain proteins.

Molecular docking using ClusPro, as a key component of this study, revealed a set of stable conformations between the two proteins. The presence of multiple complexes with highly negative binding energies (such as cluster 3 with an energy of –1060.7) not only indicated the spatial interaction potential between ConA and *ALDH18A1* but also reinforced the likelihood of biological effects stemming from this interaction. These findings pave the way for hypotheses regarding ConA's modulatory roles in pathways related to cancer, autophagy, or metabolic regulation in human cells. Looking ahead, experimental studies (in vitro and in vivo) aimed at investigating the direct or indirect effects of ConA on the expression, function, or stability of *ALDH18A1* could provide deeper insights into the role of this interaction in specific diseases such as GI cancers or neurological disorders. Moreover, this study underscores the power of bioinformatics tools in predicting non-homologous interactions that are otherwise undetectable at the sequence level. Ultimately, this research may serve as a foundation for the design of targeted therapeutics based on plant lectins like ConA in treating complex, multifactorial human diseases, especially within the field of pediatric oncology. Although the docking results suggest a thermodynamically favorable interaction, binding energy values alone cannot establish functional relevance. To strengthen this hypothesis, experimental validation using immunoprecipitation or co-immunolocalization assays would be required. Furthermore, previous studies have shown that lectins such as ConA can modulate enzyme activity or localization, such as in the case of CD45 and glycosylated lysosomal enzymes, supporting the possibility of similar interactions with ALDH18A1

This study relied exclusively on in silico predictions, and the findings need further validation through experimental methods, such as in vitro binding assays or cellular studies, to establish the biological relevance of the predicted ConA–ALDH18A1 interaction. Molecular dynamics (MD) simulations were not conducted due to resource limitations. However, future research will incorporate MD simulations (≥300 ns) to assess the conformational stability and flexibility of the predicted ConA–ALDH18A1 complex. Additionally, this study did not include control docking with unrelated proteins to assess interaction specificity. Future work will involve control docking

with proteins that lack structural or functional similarity to ALDH18A1, to confirm the selectivity of the ConA–ALDH18A1 interaction.

Considering the subcellular localization, direct interaction between ConA and mitochondrial ALDH18A1 in a live cell remains speculative. However, ConA internalization via endocytosis has been reported, and once internalized, it may influence mitochondrial proteins either directly or through modulation of intracellular signaling pathways. This potential intracellular access highlights the need for experimental studies to clarify whether such interactions are biologically plausible. Such interaction may influence mitochondrial metabolism, oxidative stress regulation, or apoptotic signaling, warranting further investigation.

## Conclusion

This study investigates the interaction between plant-derived ConA and human *ALDH18A1*, even though they do not share sequence similarity. Structural modeling and docking analyses demonstrated the formation of stable complexes with strong binding energies, indicating possible biological effects. The findings underscore the importance of bioinformatics in predicting non-homologous interactions, which could facilitate future experimental studies and therapeutic applications, especially in pediatric oncology. However, it is important to note that this study is purely computational and lacks experimental validation. Nonetheless, it may provide insights for lectin-based targeting strategies or mitochondrial modulation in pediatric GI cancers.

## Acknowledgement

This article was achieved based on the material and equipment of the Research Institute of Zabol, for which the authors thank it.

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## Conflict of interests

All authors declare no conflict of interest.

## Ethical considerations

Ethical issues (including plagiarism, data fabrication, and double publication) have been completely observed by the authors. The authors have adhered to ethical standards, including avoiding plagiarism, data fabrication, and double publication.

## Consent for publication

All authors read and approved the final manuscript for publication.

## Availability of data and material

All the data are embedded in the manuscript.

## Funding/Support

This study received no fund, but technically was supported by Research Institute of Zabol.

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