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Biopython and Nextgeneration sequencing data analysis of Neuroblastoma derived PHOX2B peptide

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Abstract
This work is devoted to computational analysis of the Neuroblastoma-derived PHOX2B peptide bound to HLA-A24:02. PDB ID 7MJA. PHOX2B encodes a homeobox protein that plays a crucial role in the development of the autonomic nervous system. The focus of this study is on the HLA-A24:02 complex, which could be of importance in immune surveillance and targeted therapy. A comprehensive analysis has been performed regarding the structure of the peptide, its functional domains, and interaction with HLA-A24:02. Using Biopython for sequence analysis and a panel of bioinformatics tools such as STRING, MetaGO, COSMIC, DynaMut and CABSdock, the article also looks deep into what implications the known mutations in PHOX2B have on the stability of the peptide and the binding affinity. Our findings define the molecular basis of Neuroblastoma at a new level and reveal targets for therapeutic interventions, including those related to personalized medicine and immunotherapy. Computer-based tools can promote a fine understanding of peptide-MHC interactions, which could be implemented in bioinformatics algorithms and contribute to furthering cancer research.

Keywords: ***Neuroblastoma, NGS, Peptide, PHOX2B, sequenceAnalysis, Mutation Detection, Biopython, Proteomics***

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I. Introduction

Neuroblastoma is a heterogeneous, aggressive pediatric cancer originating from neural crest progenitor cells. The most common extracranial solid tumor in children, it constitutes about 8-10% of all childhood cancers and 15% of pediatric oncology fatalities. The clinical presentation of Neuroblastoma ranges from spontaneous regression to rapid progression with metastatic disease. This clinical variability reflects the apparent complexity of the disease and suggests that molecular pathogenesis is even more complicated than initially thought.The transcription factor, in question, is encoded by PHOX2B (paired-like homeobox 2b), a critical element for the embryonic development of the autonomic nervous system, such as the sympathetic ganglia, the enteric nervous system, and other tissues that are neural crest. PHOX2B mutations are one of the major mutations that take place in the pathogenesis of Neuroblastoma, especially during early onset and familial predisposition. Altogether, these mutations result in the production of aberrant peptides which have the potential to get presented to the immune system and thus make PHOX2B one of the prime targets in cancer immunotherapy research.One of the most studied immune mechanisms in cancer is the presentation of tumor-associated antigens by major histocompatibility complex (MHC) molecules to cytotoxic T lymphocytes. The MHC class I molecule, HLA-A24:02, is very common in Asian populations. There is evidence for the immune response against different tumors in this allele, including Neuroblastoma. This HLA-A24:02-bound PHOX2B-derived peptide (PDB ID: 7MJA) represents a model system to understand the interplay between a tumor antigen and the immune system, particularly its presentation by HLA-A24:02 and recognition by T cells during the development of targeted immunotherapies and vaccines [1, 2, 3, 4, 5].

Cancer biology has been in the last years massively reshaped by high-throughput sequencing technologies and bioinformatics tools. These tools thus enable researchers in the analyses of complicated datasets, identification of new therapeutic targets, and prediction on how patients may react to the treatments. Here, we analyzed the sequence and structure of the PHOX2B-derived peptide in complex with HLA-A24:02 using a comprehensive bioinformatics approach. Therefore, the use of such tools asBiopython, STRING, MetaGO, COSMIC, DynaMut, and CABSdock will help in finding the functional implication of the PHOX2B mutations on Neuroblastoma pathogenesis and investigate their possible targeting by immunotherapy[5, 6, 7, 8].

The importance of the work is in contributing to a development toward a personal approach to treatment of neuroblastoma. Characterization of molecular interactions between PHOX2B peptides and HLA-

A24:02 will hence increase our comprehension of the immunology of tumors and open new possible therapeutic approaches. Furthermore, this paper stresses the usefulness of including bioinformatic tools in investigations on cancer and offers a model for further studies on other malignancies.

II. Materials and Methods

Data Acquisition: A structure model corresponding to the complex of PHOX2B peptide bound to HLA-A24:02 (PDB ID: 7MJA) was retrieved from the Protein Data Bank. This high-resolution structure, newly deposited, was for analyzing the interaction between the PHOX2B peptide and the HLA-A24:02 molecule. The amino acid sequence of the PHOX2B peptide was extracted in Biopython, in order to enable further sequence analysis and manipulations [9, 10].

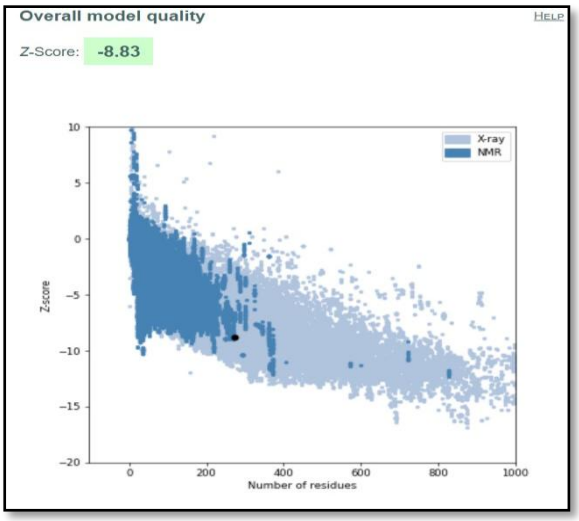
Sequence Analysis: Biopython was used in different sequence manipulations that involved extraction, translation, and alignment of the PHOX2B peptide sequence [11, 12]. MetaGO was used to determine the conserved domains, motifs, and functional sites of the PHOX2B peptide in order to provide a basis for functional annotation towards probable biological activities [13]. STRING was further used to investigate potential protein-protein interactions for which the peptide functionally takes part in cellular pathways that are related to Neuroblastoma [14, 15,25,26,27,29].

Structural Analysis: BioPython was used for the analysis of the three-dimensional peptide-HLA complex structure (7MJA). A structural alignment with the other peptides reported in HLA-A*24:02 was done to appreciate the conformation of the PHOX2B peptide and, therefore, to find the main interaction sites [16]. Structural validation was performed by the ProSA tool for model quality and reliability. Furthermore, the ProSA Web tool was put to use to analyze protein structure in order to pinpoint regions of the model that should be refined on the basis of Z-scores and energy profiles [17, 18,27,23,26].

Mutation and Variant Analysis: The somatic mutations of the PHOX2B gene that are relevant to neuroblastoma were identified and catalogued through COSMIC (Catalogue of Somatic Mutations in Cancer). Such mutation data was integrated into the structural information of the impact of such mutations on the stability and binding affinity of the PHOX2B peptide to HLA-A*24:02 [19, 20]. Furthermore, DynaMut was used to predict the effects of these mutations on protein stability and flexibility to provide an additional level of insight into how these changes would affect peptide–MHC interactions, potentially leading to immune recognition [21].

III. Result and Discussion

1. Structural Insights



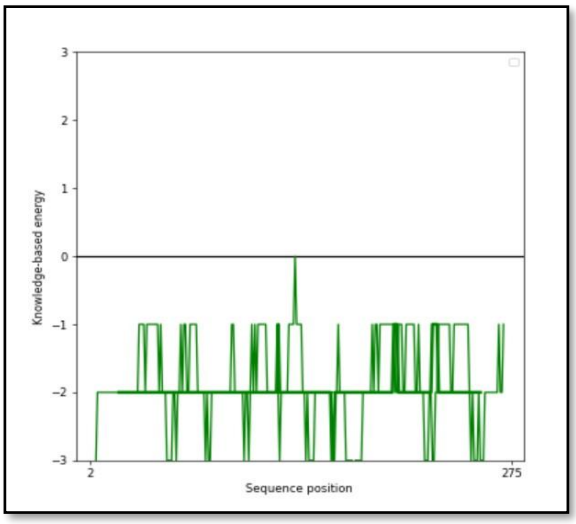


Figure 1: Quality check of proteinBy Figure 2: Sequence analysis by ProSAProSA

The quality of the PHOX2B peptide global model was determined using the ProSA tool, in which Z-scores are compared to a database of known structures. At the top of the plot is listed an extracted Z-score value of -8.83, which falls well within the expected range for native proteins of similar size as shown in this plot. Two colors are plotted: dark blue for X-ray crystallography-derived models and light blue for NMR-derived models. The black dot indicates Z-score of the PHOX2B-HLA model, which was found to fall within the cluster obtained for structures derived from X-ray crystallography. The value of the calculated Z-score is -8.83, which is typical for a good model and testifies that the structure is suitable for further research. The number of residues in the structure (~275 from the x-axis) compares well with that for models from the PDB, and this further

confirms that the PHOX2B peptide structure in complex with HLA is okay. This is illustrated in Figure 2 below, as seen in the images. The ProSA server uses a plot of the energy profile of the protein sequence structure to visualize problematic regions of protein structure. The y-axis values of this graph are the knowledge-based energies of the different sequence positions against the whole length of the protein, from position 2 to 275 residues. Ranging from -3 to 3, favorable regions are represented by values below zero; positive values might indicate structurally unfavorable regions or areas of misfolding. In x-axis, the positions of a sequence are plotted relative to residue 2 to residue 275 of the peptide-proteincomplex.

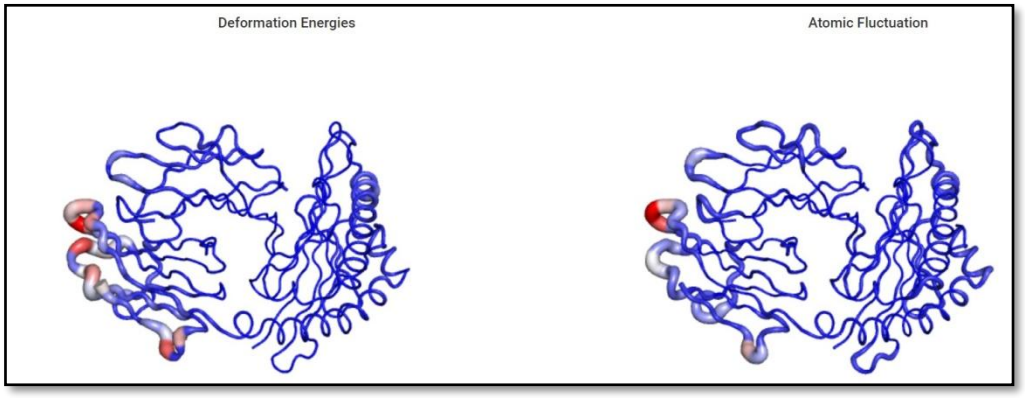


Figure 3: Mutation analysis by DynaMut

The figure includes two analyses, namely Deformation Energy and Atomic Fluctuation, which would grant knowledge of the flexibility in the structure and movement of the PHOX2B peptide-HLA complex. Deformation energy is a measure of the local flexibility of the protein structure, in other words, the amount of energy necessary to make a given region of a protein deform. It can give insight into portions of the protein that show structural rigidity or flexibility. The left image shows the color-coded tube representation with the amount of deformation energy attributed to local regions of a protein. Blue is low in deformation energy and hence rigid, with low flexibility. White and red have a much greater amount of deformation energy—moderate to high—and can be bent or twisted into other conformations.

- Low Deformation (Blue): Such regions will have less deformation and hence are structurally stable and might be involved in the core structure of the protein.
- Moderate Deformation (White): Such regions will show moderate flexibility and might probably represent dynamic binding interactions or structural transitions.
- High Deformation (Red): Those areas with higher deformation energy are more flexible and give way to movement easier. This could be binding areas, loops, or exposed segments that require conformational accommodation for functioning.

Atomic Fluctuation quantifies the absolute movement of atoms within the protein structure. Such analysis provides information on the amplitude of motion, which is a critical component of understanding dynamic behaviors of specific regions in interactions or under external forces. The color coding here is the same as in the deformation energy map:

- Blue is used to indicate the lowest fluctuation, which means that these regions are rigid and stable.
- White shows the medium fluctuation of the regions that have some flexibility.
- Red highlights the highly fluctuating regions that correspond to a dynamical nature.

Regions having large fluctuations are also important in the correct functioning of a protein; for example, binding interfaces, loops, or domains that experience conformational changes need to be flexible. The vast majority of the protein structure in both panels appears blue, which indicates that most of the protein is rather rigid and stable. There are some regions with moderate (white) and high (red) deformation and fluctuation clustered around some loop regions or binding interfaces. These flexible parts would be involved in the PHOX2B peptide being able to change its conformation upon binding to the HLA molecule, or immune recognition feature.

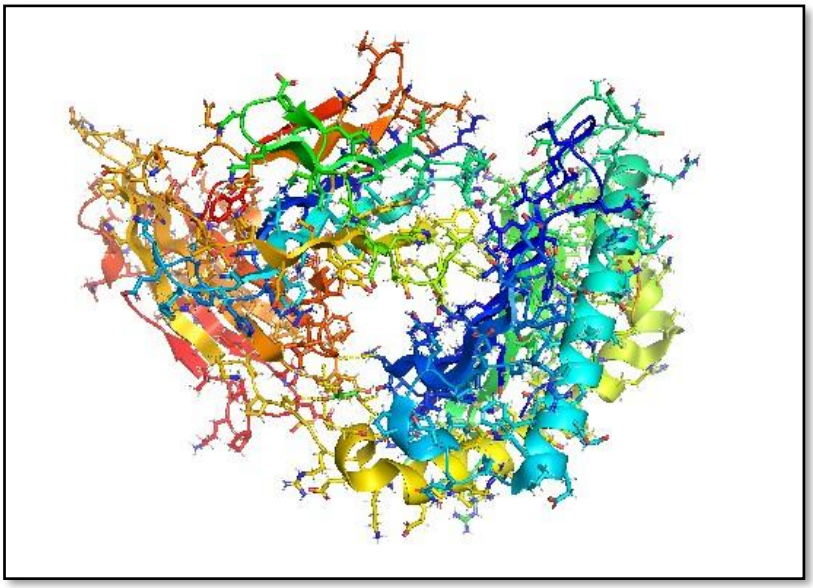


Figure 4:Active site Identification in Neuroblastoma

The visualization identifies the active sites within the PHOX2B peptide, which is critical for understanding its interaction with other molecules, particularly in the context of antigen presentation.

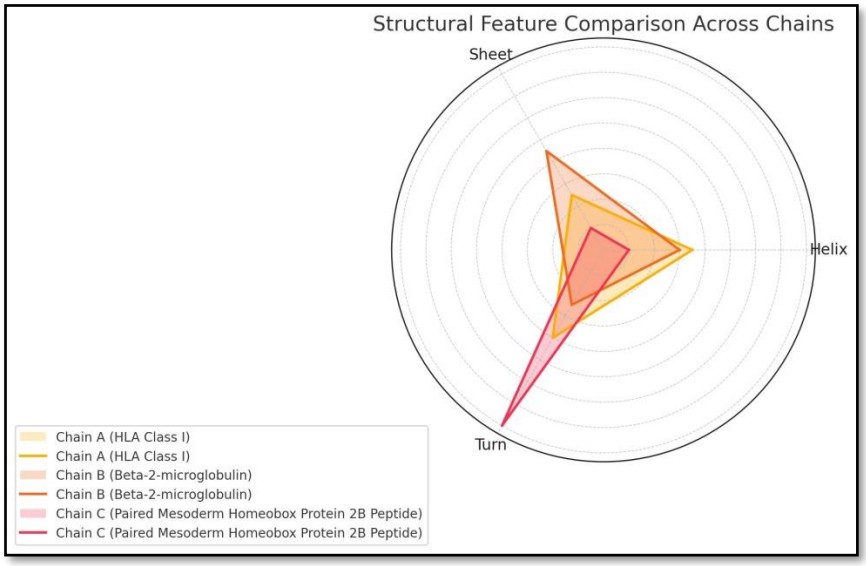


Figure 5: Structural analysis by BioPython

Chain A has a moderate contribution of helices and sheets in its structure, as can be seen by the amount of area marked in yellow in their respective directions. This would present the HLA class I molecule, with the fold and structure typical of this molecule, necessary to perform the function of antigen presentation. There is a significant contribution of turns, indicating flexible loop regions. These regions can be crucial to the binding and presentation of the antigenic peptide.

Chain B is shown in orange, it is of a general similar structure to that of Chain A; however, it has less helices and more turns. This is expected due to the supporting role of the beta-2-microglobulin is stated to have in the HLA complex. It gives more strength in the structure, but it doesn't provide much to the peptide binding site. There is a small amount of sheet structure, which is quite characteristic of the Beta-2-microglobulin fold.

Chain C is shown in red, and it is obvious that it has a significantly higher proportion of turns than the other chains. This demonstrates that the PHOX2B peptide will be structurally flexible to bind appropriately within the groove of the HLA molecule. The absence of helices and sheets in Chain C represents the general structure of peptides in its small size, where most of the secondary structures are not stabilized, like in large proteins.

On the other hand, Chains A and B are major and large, being the active components in the framework HLA complex; hence, they individually contribute major content to both the helical and sheet composition. These further lend the necessary structural rigidity for the presentation of the antigenic peptides. Having more turns in Chain C (PHOX2B peptide) would suggest that this chain is more flexible and thereby can easily provide these turn interactions to the HLA molecule or T cell receptors.

2. Interaction Analysis:

This network analysis identifies key interacting proteins with the HLA-A, Beta-2-microglobulin (B2M), and PHOX2B. The STRING network helps in elucidating the functional relationships and potential pathways these proteins might be involved in, particularly in the context of immune response and cancer biology.

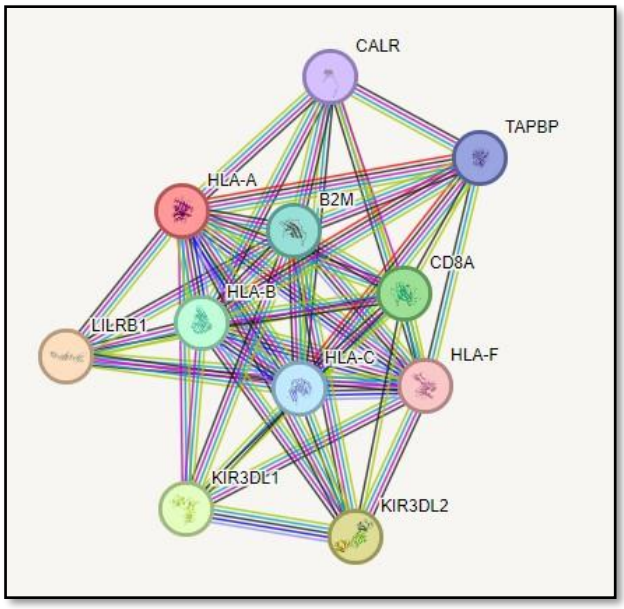


Figure 6: STRING analysis

Table 1: CABSdock result (Visualization of interaction of C chain of protein (shortest chain) with chain A ByCABSdock)

Receptor Residue	Peptide Residue
LEU B 65	PHE D 9
LEU B 55	ARG D 6
ASP B 54	GLN D 1
SER B 53	GLN D 1
HIS B 52	ASN D 3
GLU B 51	ASN D 3
ILE B 36	PHE D 9
ARG A 49	GLN D 1
ASP A 40	THR D 7
ARG A 36	GLN D 1
ARG A 22	PHE D 9
TYR B 68	TYR D 2
LEU B 55	PHE D 9
ASP B 54	ILE D 5
SER B 53	TYR D 2

Table 2: CABSdock result (Visualization of interaction of C chain of protein (shortest chain) with chain A ByCABSdock)

Receptor Residue	Peptide Residue
HIS B 52	ILE D 5
HIS B 52	GLN D 1
VAL B 50	ASN D 3
SER B 34	PHE D 9
ASP A 40	THR D 8
ASP A 38	ARG D 6
ILE A 24	ARG D 6
ARG A 18	THR D 7
PHE B 63	PHE D 9

ASP B 54	ARG D 6
SER B 53	PHE D 9
HIS B 52	PHE D 9
HIS B 52	TYR D 2
GLU B 51	TYR D 2
ASP B 35	PHE D 9
ALA A 41	ARG D 6
ASP A 40	ARG D 6
GLN A 33	GLN D 1
ARG A 22	THR D 8

Several receptor residues from Chain A and Chain B (HLA Class I and Beta-2-microglobulin) form strong interactions with key residues in Chain C, the PHOX2B peptide. For example, LEU B 65 and PHE D 9 show consistent interaction, suggesting a stable peptide binding mechanism. Residues such as LEU B 55, ASP B 54, and SER B 53 anchor the PHOX2B peptide in a multi-residue manner within the HLA binding groove, interacting with residues like ARG D 6, GLN D 1, and TYR D 2.
PHE D 9, ARG D 6, and GLN D 1 are central to many interactions, indicating their importance in the peptide-HLA binding interface. These residues likely contribute to the specificity and strength of the peptide's binding.

3. Sequence analysis:

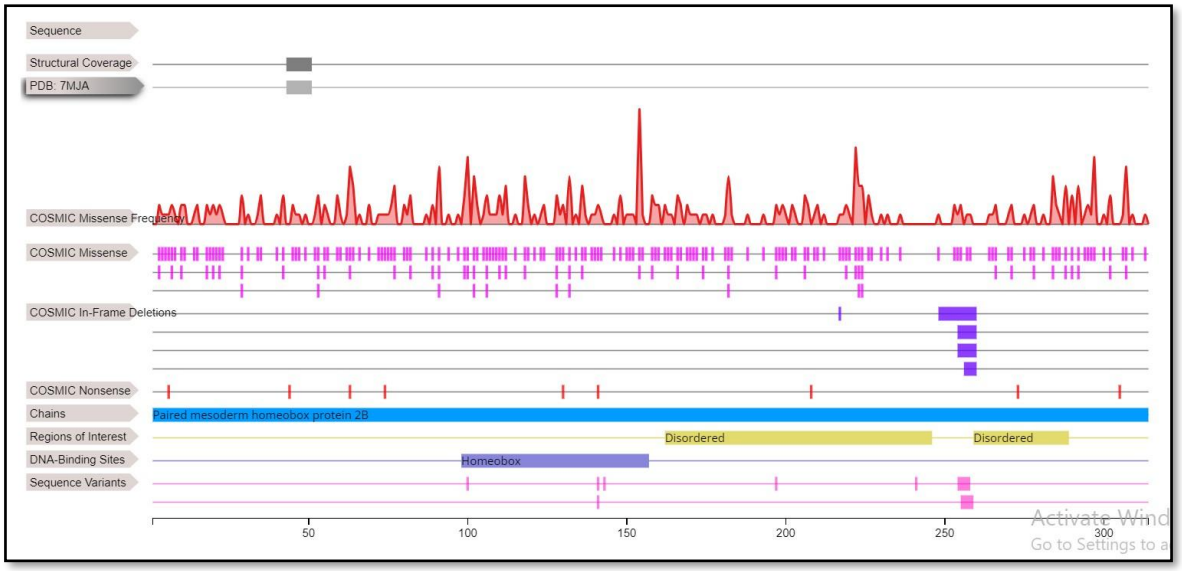


Figure 7: Sequential Mutation analysis by COSMIC

This shows the complete protein sequence length in amino acids; the number of residues is on the x-axis.Structural Coverage bar in grey shows the structural regions covered by the PDB structure (7MJA). The bar highlights which parts of the sequence have been resolved structurally.

The frequency of missense mutation in the protein is shown by red marks, indicating peaks that have a higher mutation rate at certain residues.Pink marks show where missense mutations are located in the sequence, marking it with single lines down the sequence. COSMIC In-Frame Deletions row displays where in-frame deletions are seen within the protein sequence, with purple marks indicating affected residues.

Red ticks represent nonsense mutations that result in premature stop codons, thus shortening the protein.Yellow bars labeled 'Disordered' indicate region(s) in the protein sequence which have been either predicted or observed to be disordered, i.e., lacking a stable structure.The purple box labeled 'Homeobox' represents the homeobox domain of the protein, responsible for interacting with DNA, and it is located around residues 100 to 160.
This visualization helps track structural and mutational features of the PHOX2B protein, giving insights into biological function, mutation hotspots, and potential instability sites in the protein.

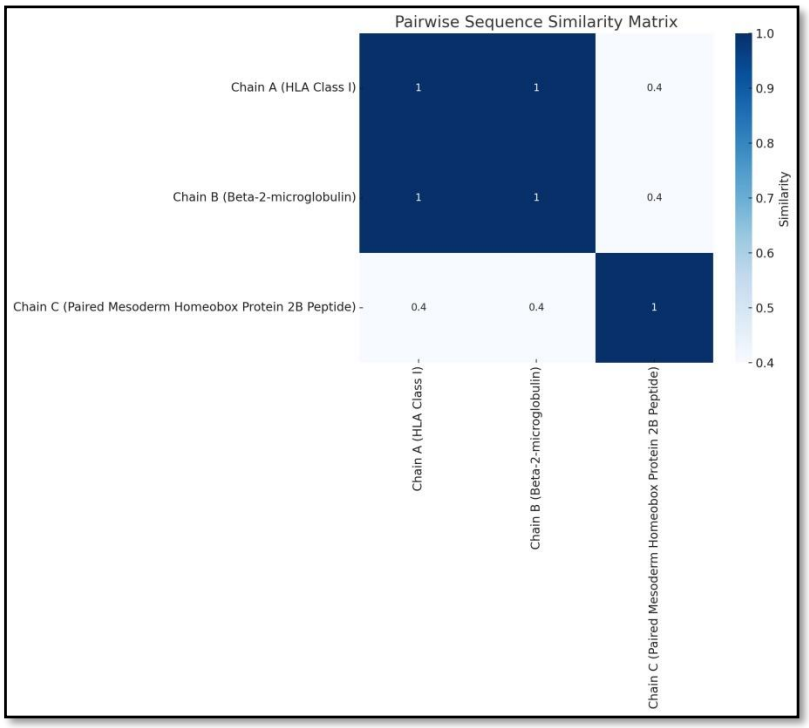


Figure 8: Sequence similarity analysis

The matrix compares the similarity in sequences of three different protein chains: A (HLA Class I), B (Beta-2-microglobulin), and C (Paired Mesoderm Homeobox Protein 2B Peptide). The color intensity shows how similar pairs of chains are to each other. Dark blue represents higher similarities, while lighter shades indicate lower similarity.

Both Chain A (HLA Class I) and Chain B (Beta-2-microglobulin) are 100% similar in sequence to each other, denoted by a value of 1 (dark blue). Chain C (Paired Mesoderm Homeobox Protein 2B Peptide) is only moderately similar to both Chain A and Chain B, with a value of 0.4. Chain C has a self-similarity value of 1, as expected when a chain is compared to itself.

The high similarity between Chain A and Chain B suggests they may contain highly similar or conserved regions in their sequences, likely related to their structural roles within the HLA complex. Chain C, however, shows some similarity to Chain A and Chain B, but its distinct sequence likely reflects its role as a peptide interacting with the HLA complex, without being part of the structurally conserved chains.

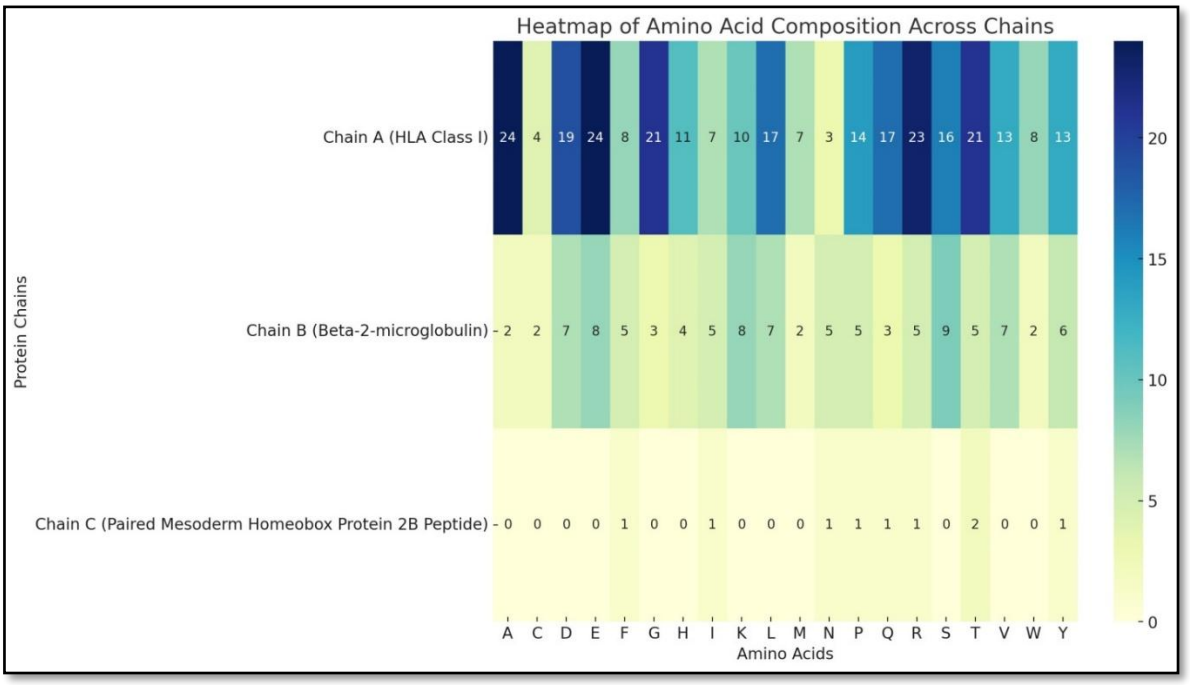


Figure 9:Amino Acid Composition Analysis

These visualizations describe the composition of amino acids across the three chains: A, B, and C. They highlight differences in amino acid occurrences, which might relate to the functional roles of each chain.

The figure is a heatmap across three protein chains: Chain A, Chain B, and Chain C. The x-axis shows various amino acids (A, C, D, E, F, G, H, I, K, L, M, N, P, Q, R, S, T, V, W, Y), while the y-axis shows the three protein chains: Chain A (HLA Class I), Chain B (Beta-2-microglobulin), and Chain C (Paired Mesoderm Homeobox Protein 2B Peptide).

Chain A has a higher abundance of several amino acids, including 24 residues of A, 24 of I, 23 of L, 21 of V, and 19 of F. These residues contribute to the higher intensities of dark blue colors on the heatmap, showing that they occur more frequently in Chain A. This chain is characterized by its larger size and structural complexity, reflected in the significant amounts of amino acids.

Chain B has a more moderate distribution of amino acids, with notable values for A (8), I (8), G (7), and S (9). The amino acid distribution is more spread out, with lighter green shades indicating moderate occurrences for most residues. As expected, Chain B has fewer counts compared to Chain A, reflecting its smaller size or less structured regions.

Chain C has very low counts of amino acids, with S (2) and V (2) being the only amino acids with more than one occurrence. The very light or pale yellow shades reflect this minimal amino acid composition, as expected for a smaller peptide.

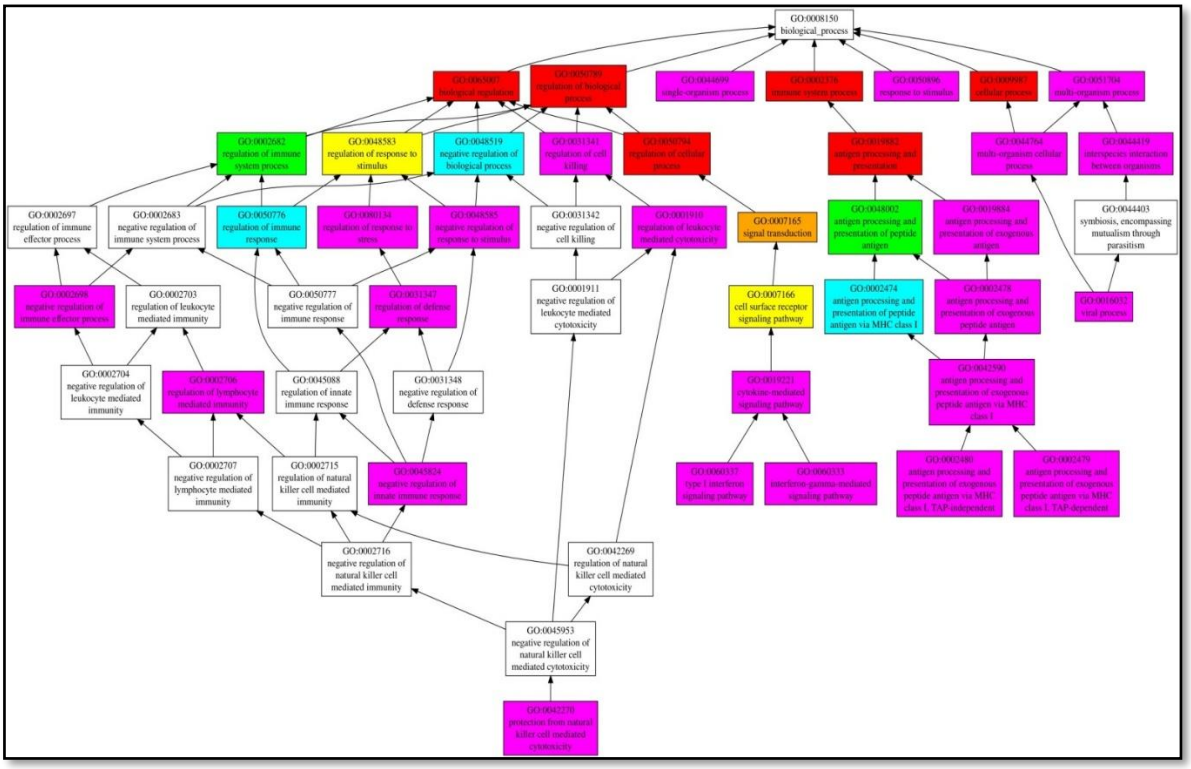


Figure 10:Gene ontology: Biological function prediction by metaGO

The hierarchy of terms reaches from very general biological processes, such as 'biological regulation,' down to very specific immune processes like 'antigen presentation,' 'signal transduction,' and 'regulation of cytotoxic immune cells.' The arrows indicate the parent-child relationship between terms, where those lower in the diagram represent more specific processes derived from their parent terms.

This diagram provides a broad yet detailed view of how various immune processes occur and interrelate in the regulation of high-level biological processes, down to specific immune responses involving antigen presentation and immune cell activation.

Table 3: Protein similarity analysis by CoFactor

Rank	PDB Hit	TM-score	RMSDa	IDENa	Cov.
1	6ujoA	0.99	0.65	0.909	1.000
2	2bckA	0.98	0.90	1.000	1.000
3	8frtA	0.98	0.95	0.909	1.000
4	7bh8A	0.97	1.03	0.777	1.000
5	8gqvA	0.97	1.08	0.690	1.000
6	7edoA	0.96	1.29	0.584	1.000
7	2qriA	0.96	1.30	0.712	1.000
8	1mhcA	0.95	1.86	0.675	1.000
9	7cpoA	0.91	1.82	0.466	0.967
10	1zs8A	0.90	1.49	0.550	0.949

Major hits of this similarity analysis are highly structurally and sequentially similar to the query protein, meaning proteins similar in structure and sequence to the query. For example, 6ujoA, 2bckA, and 8frtA showed very close alignment in both structure and sequence. Other hits, such as 1mhcA and 7cpoA, had much higher RMSD values and lower sequence identity, exhibiting considerable structural and sequence differences.

This analysis helps identify proteins with high structural similarity, which may be involved in similar biological pathways or functions, making it useful for understanding the role of PHOX2B in Neuroblastoma.

IV. Conclusion

The results of this study are important for the development of specific immunotherapies, as understanding the structural and functional characteristics of tumor-related antigens like PHOX2B helps in designing more effective treatments. This research adds to the detailed mechanisms of interaction between the PHOX2B peptide and the HLA molecule, contributing to therapeutic strategies aimed at enhancing immune recognition and response in Neuroblastoma.

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