Computational Simulation of Phosphoacetylglucosamine Mutase (PGM3) Enzyme for the Detection and Treatment of Glioblastoma Multiforme (GBM)

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Glioblastoma (GBM) is a type of aggressive brain cancer that claims over 200,000 lives in the US alone and has the lowest survival rate of all cancers. The PGM3 protein is a recently identified potential target for treating GBM. We can use antibodies to target the PGM3 protein, which are powerful tools that can bind to and neutralize proteins. We hypothesize that antibody 1A5F will bind strongly to the PGM3 protein. We first used Uniprot to get the amino acid sequence of the PGM3 protein. Then we plugged the sequence into AlphaFold3 to model the PGM3 protein. The protein was then downloaded and converted to the PDB format using ChimeraX software. We then randomly selected antibodies due to limited information on which exhibits the strongest binding affinity. To predict whether the protein and antibody will react, we used HDOCK. We have utilized this software to predict how the PGM3 protein binds to specific antibod- ies. We used PLIP to find the number of interactions between the protein and the antibody. For a deeper analysis, we utilized Prodigy to determine the binding affinity between the two molecules. Based on the above analysis, antibody 2ULY was selected as the most appropriate antibody targeting the PGM3 protein. This research will pave the way for lab-based research, for which the antibody will most efficiently target the PGM3 protein. It can lead to new drug discoveries to treat GBM more efficiently.

1. INTRODUCTION

Glioblastoma Multiforme (GBM) is an aggressive form of brain cancer that occurs mainly in the frontal and temporal lobes of the brain. [1] It originates from astrocytes, cells that support nerve cells, and it grows rapidly, invading and destroying healthy tissue in the brain. The survival rate for this cancer is 6.7%, with over 200,000 cases across the US each year. Some common symptoms of GBM are nausea/vomiting, weakness in parts of the body, double vision, and loss of the ability to understand speech and to speak. [1] Some current treatment methods include radiotherapy, radiosurgery, chemotherapy, drug therapy, and cryotherapy. [2] Drawbacks to these treatment methods include hair loss, sickness, tiredness, nausea, skin irritation, damage to blood vessels, brain swelling, anemia, soreness, seizure, and stroke. In addition to the drawbacks, these current treatment methods are highly complicated. [3] This form of cancer is known to penetrate deep into brain tissue, which makes it extremely hard for it to be surgically removed. Also, it tends to resist therapies such as chemotherapy and radiotherapy because of its genetic diversity and its ability to adapt quickly. [4] Furthermore, the blood-brain barrier is also another obstacle that prevents the effectiveness of most drugs. [5]

The PGM3 protein is a phosphoglucose mutase enzyme involved in glycosylation, vital for the functioning of tissues. [6] The PGM3 gene encodes it. This protein is found in high concentrations in the liver, heart, pancreas, and placenta. This enzyme adds complex chains of sugar to lipids and proteins. This protein is a vital component of the Hexosamine Biosynthesis Pathway (HBP). In HBP, first Glucose is converted to glucose-6P, which is further converted to fructose-6P. Fructose-6p is then converted into a nucleotide sugar called UDP-GLcNAc. For UDP-GLcNAc to be converted into GLcNAc, the PGM3 protein is involved. After, the GLcNac is converted into GLcNAc-1P, which is then used for cell proliferation(cell multiplication). [6]

PGM3 is an essential enzyme which usually converts GlcNAc-6P to GlcNAc-1P in the HBP cycle. Antibodies are a type of protein that is produced by our immune system to neutralize certain substances such as bacterias, viruses, and toxins. It neutralizes certain substances by binding to antigens on the substance. Antibodies are made up of four polypeptide chains, two of which are light chains and the other two are heavy chains. The light chains are made up with around 220 amino acids and the heavy chains are made up with around 440 amino acids. Amino acids

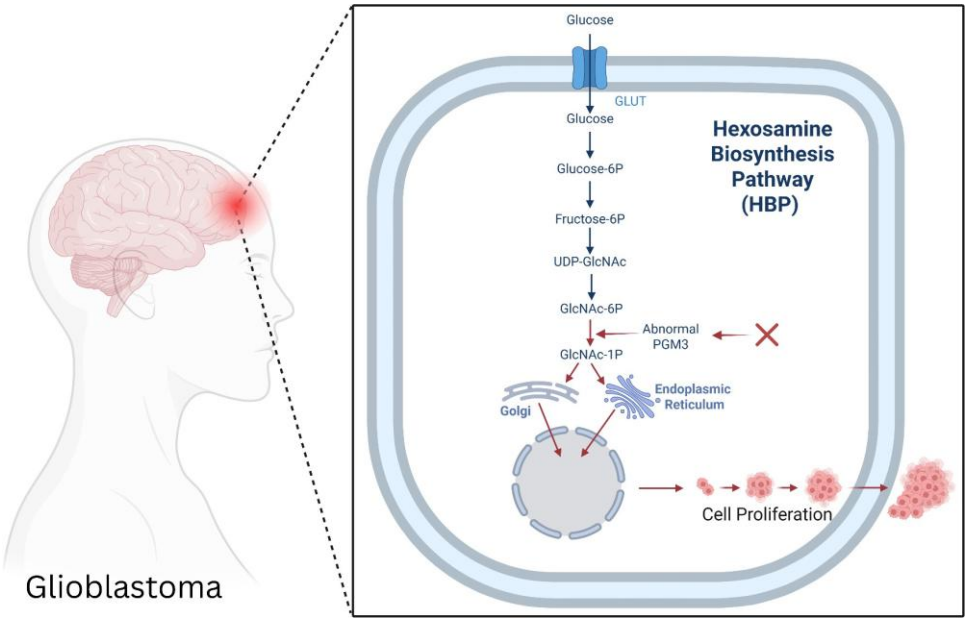

Fig. 1. Hexosamine Biosynthesis Pathway (HBP) involvement in glioblastoma progression. Glucose enters the cell through GLUT transporters and is metabolized through the hexosamine biosynthesis pathway, generating intermediates such as glucose-6-phosphate, fructose-6-phosphate, and UDP-GlcNAc. These metabolites are converted to GlcNAc-6-phosphate and GlcNAc-1-phosphate and further processed in the endoplasmic reticulum and Golgi apparatus for protein glycosylation. Dysregulation of enzymes such as PGM3 alters this pathway, leading to abnormal glycosylation and enhanced cellular signaling that promotes glioblastoma cell proliferation and tumor growth.

are the building blocks of protein with around 20 different types. Antibodies can also be used in the detection of brain cancer, particular in identifying molecular markers on tumor cells.

To identify which antibodies can effectively bind to the PGM3 protein, we employed molecular docking simulations. [7] A molecular docking simulation is a simulation that predicts how two molecules interact with each other, one being a ligand(small molecule) and a receptor(Large Molecule). It simulates how one molecule "docks" into the other at its binding sites. [7–9] Some common molecular docking simulation software are GOLD, Glide, and HDock. [10] We have used HDock to model the antibody reaction with the PGM3 protein. One application of molecular docking simulations is drug discovery. The docking simulation can predict how the drug will interact with a specific molecule.

Recently, Su et al. have demonstrated that inhibiting the PGM3 protein could effectively suppress GBM growth by disrupting the hexosamine biosynthesis. [11] Therefore, PGM3 protein could be a potential target for the inhibition of the GBM. In the current research, we have used computational methods like molecular docking simulations to target PGM3 protein using antibodies. Based on the current research we have selected antibody 2UYL to be the most effective antibody in targeting the PGM3 protein. In the future, this research can provide the groundwork in testing more antibodies and their potential in targeting the PGM3 protein.

2. METHOD

PGM3 protein Modeling: Uniprot was used to get the amino acid sequence of the PGM3 protein (UniProt ID: O95394). [12] UniProt is a free-to-use resource that provides amino acid sequences for thousands of proteins. We have used this resource to find the amino acid sequence of PGM3 to model it on AlphaFold **3.**[13] AlphaFold 3 is an advanced AI model that can accurately predict the structure and interactions of all biomolecules. We used this to make an accurate model of the PGM3 protein. The protein was then downloaded and converted to the PDB format using ChimeraX software to better view the structure of PGM3 protein. [14]

PGM3 protein properties: P2rank is a machine learning based method that can predict the ligand binding site. [15] We have used this method to predict the binding site on the PGM3 protein surface. The binding site of a protein is where a protein binds to a protein. It must bind at a specific site on the PGM3 protein. Similarly, ScanNet is a geometric deep learning model that predicts protein binding sites based on their structure. [16] We have used it to double check the P2rank model. We have also used PROTTER. [17] Protter is a software tool that can help visualize protein sequences, topologies, and annotations. We have used this software to get a membrane protein topology of the PGM3 protein. We have also used the Human Protein Atlas, which is a website that can show where in the cell PGM3 protein is. We have used this to find out where the PGM3 protein specifically is inside the cell.

Computational Simulations: HDock is a protein-protein/DNA-RNA docking software that uses a hybrid algorithm to predict how two molecules will interact. [10] We have used this software to predict how the PGM3 protein will bind with specific antibodies.

Computational Analysis: PLIP analysis is a software that detects and visualizes non covalent interactions between biological molecules such as DNA/RNA and proteins. [18] We have used this to find the number of interactions between an antibody and the PGM3 protein. For deeper analysis between the protein and

the antibody, We have used Prodigy. [19] Prodigy is a server that can predict the binding affinity of two molecules. We have used it to find how strong the bond is between the PGM3 protein and the antibody in kcal/mol.

After using Prodigy, we employed GROMACS to perform molecular docking simulations of the PGM3-antibody complex. GROMACS software is a free-to-use software that performs molecular docking simulations by solving Newtonian equations for each particle. [20] By performing this, we can predict how stable the protein-antibody conjugate really is in a cellular environment.

3. RESULTS

Predicted Binding Site Identification: The molecular docking simulations were first performed to identify the binding interface between PGM3 and different antibody candidates. The predicted binding sites were computed using two independent software tools, P2Rank and ScanNet. As shown in Figure 2 and Figure 3, both tools consistently predicted highly similar binding pockets, thereby validating the reliability of the binding site selection.

Antibody Docking and Selection Criteria: Antibody docking was then carried out to evaluate their interactions with the predicted binding site of PGM3. Candidate antibodies were selected based on their ability to bind directly to the predicted site. Only antibodies that successfully docked at the identified pocket were retained for further analysis.

Protein–Antibody Interaction Analysis: Protein–antibody docking complexes were analyzed using the Protein Interaction Profiler (PIP), as illustrated in Figure 4. Binding affinity was further assessed through binding energy calculations (Figure 5). From these analyses, the 2UYL–PGM3 complex demonstrated the most favorable binding energy and stable interactions, making it the strongest candidate among the tested antibodies.

Molecular Dynamics Simulations: To confirm the docking results, molecular dynamics (MD) simulations were performed on the antibody–PGM3 complexes. The trajectories revealed that the antibody remained stably bound to the predicted binding pocket throughout the simulation time (Figure 6). This stability provides further evidence of strong binding affinity and suggests that the selected antibody can robustly interact with PGM3 under dynamic conditions.

4. DISCUSSION

Application: This research presents a promising new approach to treating glioblastoma (GBM) by targeting the PGM3 protein specifically. By using specific antibodies that can attach to and neutralize PGM3, this could disrupt the HBP cycle that helps tumor cells proliferation. This targeted method could potentially stop or greatly slow down the growth of GBM, which might make it a more effective and precise treatment option compared to the standard therapies we currently have. This research also shows a promising way to treat GBM without the major drawbacks and difficulty of treating GBM. For example, in Fluorescence-Guided surgery, the patient is injected with a fluorescent dye that glows under specific light and binds to the GBM. [3] Then a craniotomy is performed and the doctor has to remove the GBM by following the dye. While this is a somewhat effective method for treating GBM, it is a complicated and lengthy method to treat GBM and only increases the lifespan by a handful of months.

Limitation: One of the significant limitations of this research is that it is based entirely on modeling and simulations. While this can provide accurate results, they must be validated by experimental analysis, as they don't capture the full complexity of living biological systems. We must conduct experiments to make sure that the antibody targeting the PGM3 protein is both safe and effective. In this research, we have used only 20 antibodies. In the future, we must use more antibodies to find potentially more effective antibodies that can be used in treating GBM by targeting the PGM3 protein. Expanding on this research can provide more candidates for treating GBM.

Future application: In the future, we aim to conduct experimental validation to ensure the accuracy of the results from this research. We aim to validate this research by utilizing surface plasmon resonance. This biosensing technique enables real-time detection of biomolecular interactions by measuring changes in the refractive index at the sensor's surface. That will validate that the antibody actually binds to the PGM3 protein.

5. CONCLUSION

Overall, these results demonstrate that PGM3 binds strongly to specific antibodies at a consistently predicted binding site. The 2UYL antibody emerged as the most promising candidate, supported by docking, interaction profiling, binding energy analysis, and MD simulations. These findings lay the foundation for subsequent in vitro and in vivo experimental validation to confirm the therapeutic potential of the identified antibody. This research identifies the PGM3 enzyme as a promising therapeutic target for glioblastoma multiforme (GBM). While further experimental validation is required, these results provide a strong foundation for developing targeted antibody-based therapies against GBM.

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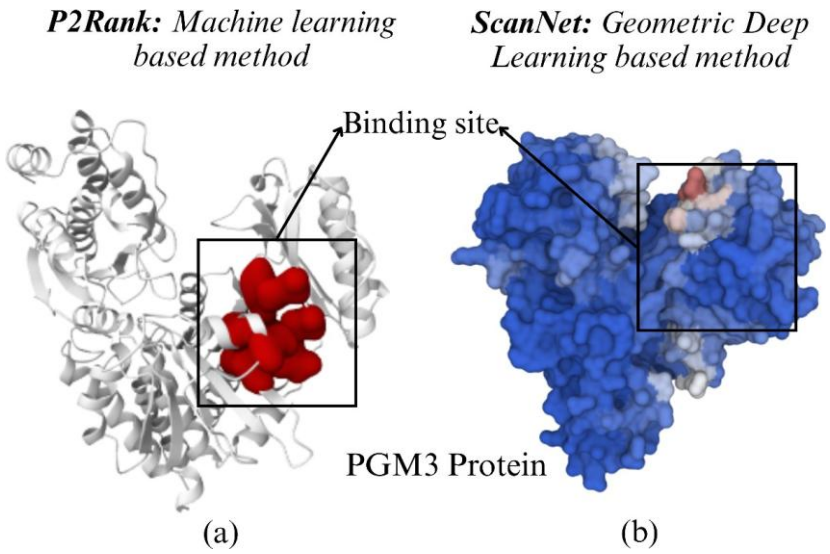

Fig. 2. Binding site prediction of the PGM3 protein using two computational methods. (a) The P2Rank machine-learning–based method predicts potential ligand-binding pockets on the PGM3 protein structure, highlighted in red. (b) The ScanNet geometric deep learning–based method identifies probable binding regions on the protein surface, shown in light-colored patches on the blue surface representation. The highlighted regions indicate potential active or ligand-binding sites used for subsequent docking and drug design analysis targeting the PGM3 protein.

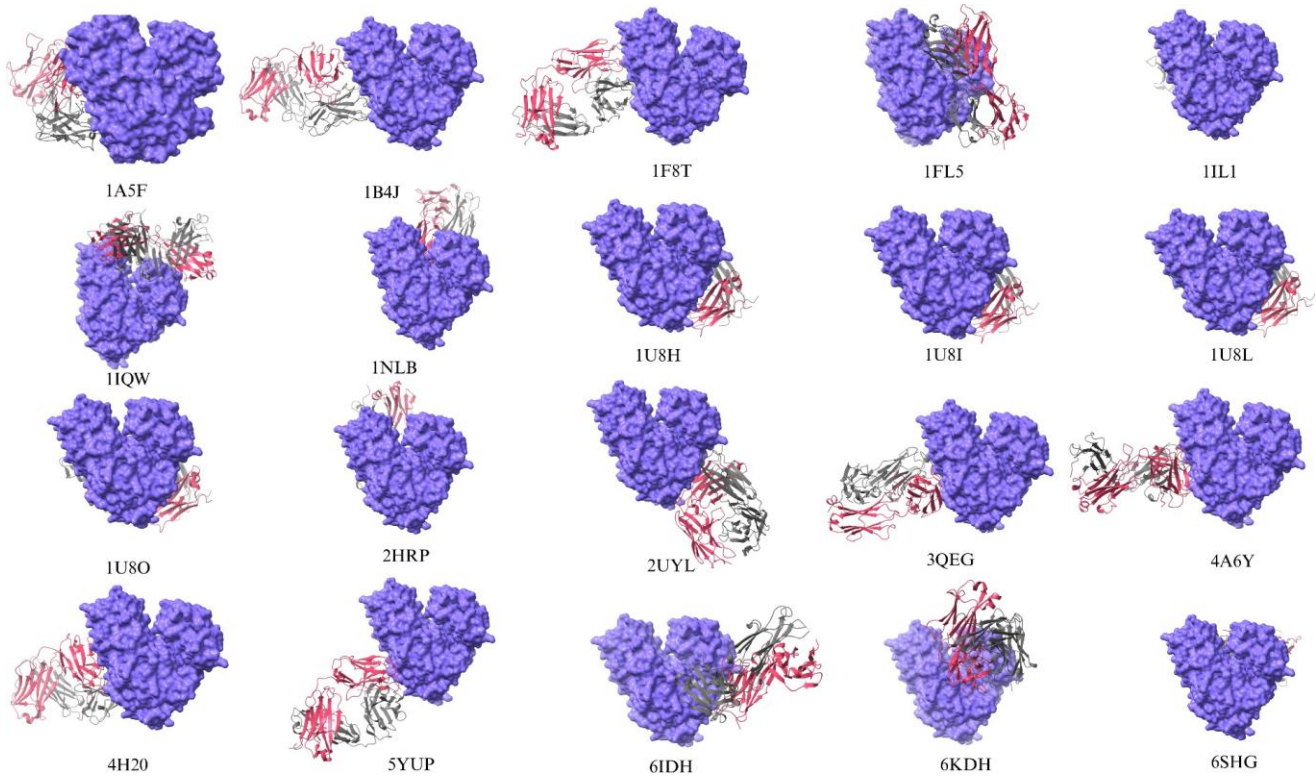

Fig. 3. Docking interactions between the PGM3 protein and multiple antibody structures. The PGM3 protein is shown in purple surface representation, while the antibody structures (gray and red ribbon representations) correspond to different PDB entries. Each panel illustrates the predicted docking orientation between PGM3 and the candidate antibodies, highlighting variations in binding positions and interaction interfaces. These simulations were used to evaluate potential antibody binding regions and identify candidates with favorable interactions for targeting the PGM3 protein.

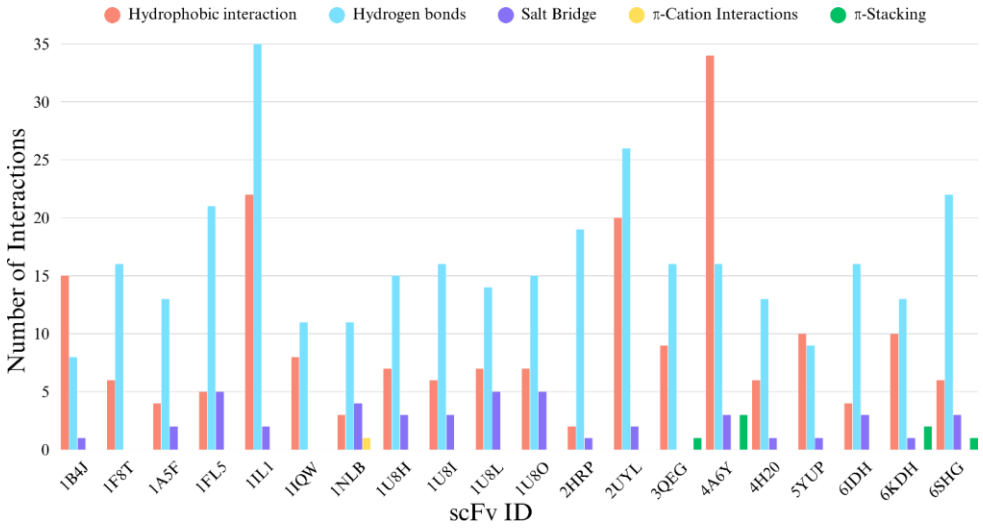

Fig. 4. Interaction analysis between PGM3 and different scFv antibodies. The bar graph compares the number of molecular interactions formed between the PGM3 protein and various scFv antibody candidates. Different interaction types are shown, including hydrophobic interactions, hydrogen bonds, salt bridges, π–cation interactions, and π–stacking. Hydrogen bonding and hydrophobic interactions were the most frequently observed interactions across the complexes, indicating their major role in stabilizing antibody–PGM3 binding. This analysis helps identify antibody candidates with stronger and more stable interactions for targeting the PGM3 protein.

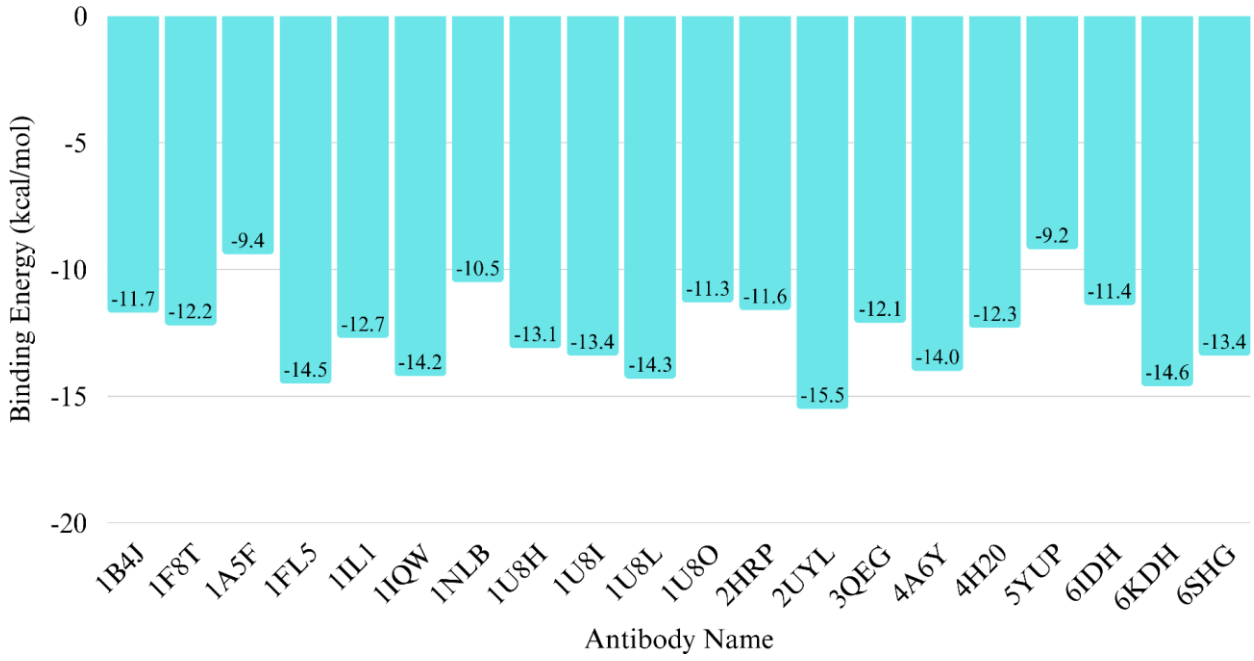

Fig. 5. Binding energy comparison of antibody–PGM3 complexes. The bar chart shows the predicted binding energies (kcal/mol) for different antibodies docked with the PGM3 protein. More negative binding energy values indicate stronger predicted binding affinity. Among the tested antibodies, 2UYL (-15.5 kcal/mol) and 1FL5 (-14.5 kcal/mol) exhibited the strongest binding interactions, suggesting they may be promising candidates for targeting the PGM3 protein in further therapeutic investigations.

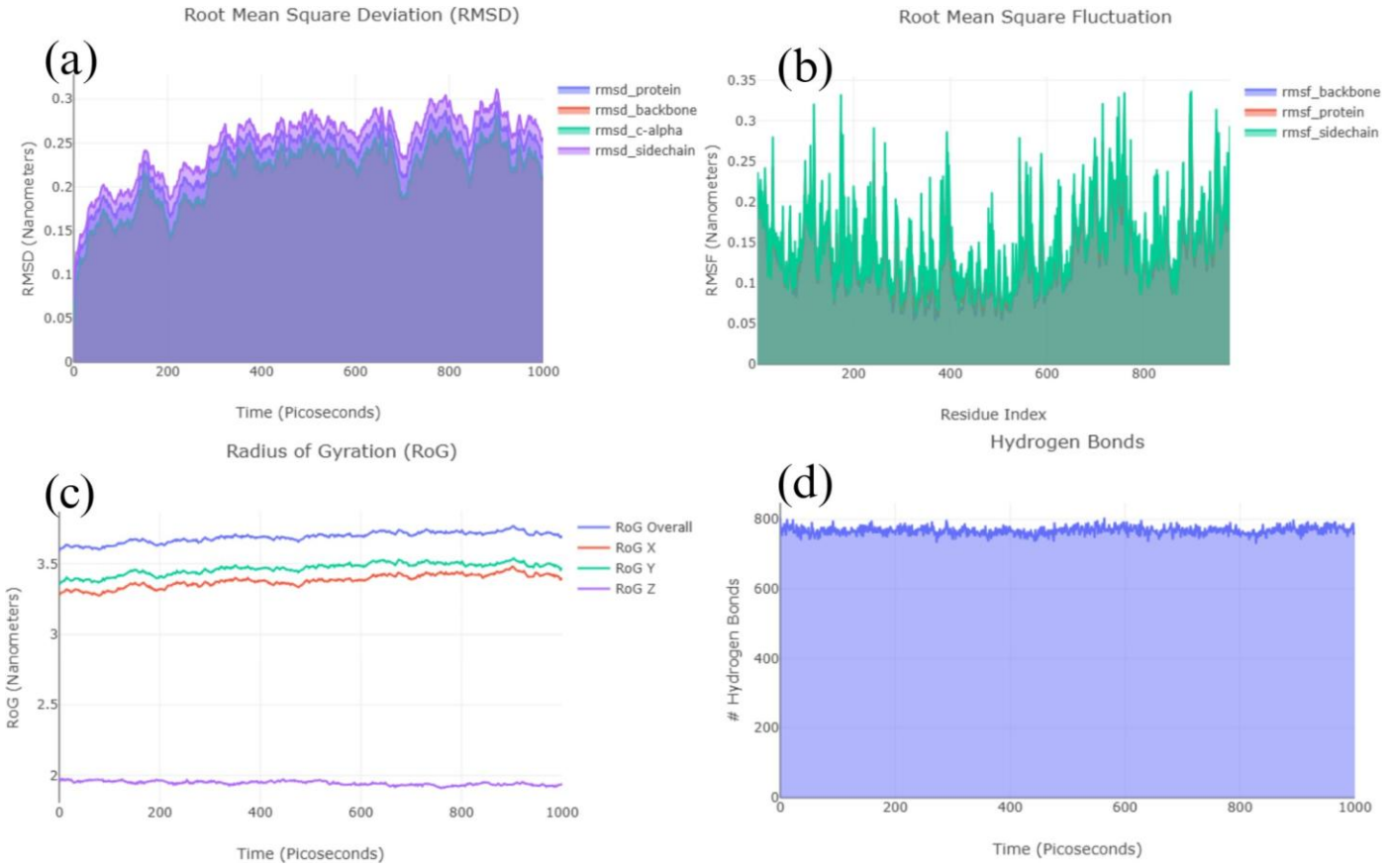

Fig. 6. Molecular dynamics simulation analysis of the PGM3–antibody complex. (a) Root Mean Square Deviation (RMSD) plot showing the structural stability of the protein, backbone, C-alpha, and side chains over a 1000 ps simulation period. (b) Root Mean Square Fluctuation (RMSF) analysis indicating residue-level flexibility within the complex. (c) Radius of Gyration (RoG) demonstrating the compactness and structural stability of the complex throughout the simulation. (d) Hydrogen bond analysis showing the number of hydrogen bonds maintained during the simulation, reflecting the stability of intermolecular interactions in the PGM3–antibody complex.

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