

DNA Aptamer-Mediated Blood-Brain Barrier Penetration for Targeted Therapeutic Delivery in Glioma

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Glioma is one of the most complicated, lethal, and resistant to treatment tumors. A significant problem with Glioma is that the brain is covered with a protective sheet called the blood-brain barrier (BBB), a barrier of protective cells surrounding the brain. It prevents harmful substances such as toxins and germs from entering and allows nutrients such as oxygen and glucose to enter. However, this prevents essential drugs that may eliminate brain diseases from entering, which can be resolved through aptamers. Aptamers are short, single-stranded molecules of RNA or DNA that can bind to specific targets such as proteins or cells. Aptamers serve as molecular recognition tools, enabling targeted drug delivery across the BBB. We hypothesize that the aptamer binds to the transferrin binding site in the receptor and gets transported across the BBB. AlphaFold3, a software that allows the 3d prediction of a protein's structure, determined the transferrin receptor structure from its amino acid sequence. UNAFold and FARFAR 2 were used to predict RNA secondary structures and interactions, optimizing aptamer-based therapy design. HDock molecular docking identified optimal aptamer-TfR interactions, aiding targeted therapy development and improving receptor-ligand binding insights. The binding energy, which reflects the stability of a nucleus and is equivalent to the energy released during its formation, was calculated using the PDA-Pred tool. The results of the binding energy suggest a strong and thermodynamically favorable interaction between the aptamer and TfR. The aptamer can also bind to the platelet-derived growth factor receptor (PDGFR), preventing the growth factor from activating the receptor. By inhibiting this signaling pathway, the aptamer effectively halts the multiplication of glioma cells, which rely on PDGFR activation for uncontrolled growth. I have incorporated this into my research as well because it poses as an additional therapeutic strategy, aiming to improve treatment effectiveness by disrupting multiple pathways that fuel glioblastoma growth. This research on aptamers seek to create new treatment strategies that improve the precision and effectiveness of therapies for this difficult type of brain cancer.

1. INTRODUCTION

Glioma is an extremely aggressive brain tumor that originates in astrocytes, cells that typically support nerve function.(1) Glioblastoma, a type of glioma, is Grade IV and highly aggressive.(2) Gliomas primarily affect the brain's supportive tissues, causing symptoms such as headaches, seizures, and difficulties with movement or speech, depending on the tumor's location. The tumor develops as a result of genetic mutations that drive uncontrolled cell growth, often involving key genes such as IDH1/IDH2, EGFR, TP53, and PDGFRA, which play critical roles in glioma pathogenesis. (3) Gliomas exhibit highly infiltrative growth, invading surrounding brain tissue by dispersing along white matter tracts, perivascular spaces, and neuronal

pathways, which makes complete surgical removal difficult. (4) Despite treatments like surgery, radiation, and chemotherapy, the prognosis remains poor because of the tumor's aggressive nature and resistance to therapies.(4)

One of the major challenges in glioma treatment is the restricted access of drugs to the brain due to the BBB. The Blood-Brain Barrier (BBB) is a protective layer of cells surrounding the brain, keeping harmful substances in the blood from reaching it.(5) It selectively permits essential nutrients, such as oxygen and glucose, while blocking harmful substances. (5) This barrier is essential for stabilizing the brain's environment and protecting brain cells.(5) However, the BBB also makes it difficult for most drugs to reach the brain because it prevents many large

or water-based molecules from passing through.(5) Most therapeutic compounds struggle to cross the BBB, limiting treatment options for brain diseases. Despite significant advancements, delivering drugs effectively to gliomas remains a major challenge. Nanoparticles, while promising, often face difficulty penetrating the blood-brain barrier (BBB) and may not accumulate consistently within the tumor. Focused ultrasound can temporarily open the BBB to enhance drug delivery, but its effects are short-lived and carry the risk of unintended damage to surrounding brain tissue. Meanwhile, systemic chemotherapy lacks precision, exposing healthy cells to toxicity while delivering only a fraction of the drug to the tumor. These challenges underscore the urgent need for more targeted and efficient treatment strategies. This has led to an increasing focus on finding innovative ways to improve drug delivery across the blood-brain barrier. Finding ways to help drugs pass through the BBB is essential for treating conditions like Alzheimer's, Parkinson's, and brain tumors.

Drug delivery faces major challenges, including poor targeting, stability concerns, and unintended side effects. However, aptamers offer a promising solution with their exceptional specificity, biocompatibility, and versatile functionality. Aptamers are "short, single-stranded molecules of DNA or RNA" that can bind to specific targets, such as proteins, small molecules, or even cells.(6) They function similarly to antibodies in which they can recognize and bind to their target with high specificity but are smaller and easier to produce.(7) Their purpose is to act as molecular recognition tools, making them useful in diagnostics, therapeutics, and, especially importantly, drug delivery. For example, aptamers can be attached to drugs to deliver them directly to specific cells, such as cancer cells. Because of their versatility and stability, they are becoming increasingly important in medical cancer research.

Aptamers' unique ability to bind precisely to specific targets makes them a powerful tool for drug delivery, especially when it comes to overcoming biological barriers like the blood-brain barrier (BBB). Treating brain tumors, such as gliomas, is particularly challenging because the BBB tightly controls what enters the brain. To tackle this, researchers are developing aptamer-based approaches to help transport drugs across the BBB more efficiently, ensuring they reach glioma cells where they are needed most.

Recently, Su et al. designed biomimetic nanocomplexes capable of targeting and binding to glioma's transferrin receptor (TfR).(8) These nanocomplexes contain an aptamer with high specificity for the TfR. We hypothesized that the aptamers would exhibit strong and specific binding to the TfR. Using molecular docking simulations, we predicted the aptamer's binding strength and interaction patterns with the TfR, finding that the aptamers consistently bind strongly to it and supporting its feasibility in crossing the blood-brain barrier (BBB). This research may lead to targeted drug delivery systems for glioma, enhancing treatment effectiveness across the BBB. Aptamer binding to the transferrin receptor (TfR) facilitates blood-brain barrier (BBB) crossing through receptor-mediated transcytosis, a cellular transport mechanism that enables the controlled movement of molecules into the brain. By leveraging this pathway, aptamers can enhance targeted drug delivery to the central nervous system, improving therapeutic efficacy while minimizing off-target effects.

2. METHODOLOGY

To predict the structure of the transferrin receptor (TfR) protein, we began by retrieving the amino acid sequence from the UniProt database.(9) It is a database containing protein amino acid sequences, structure, and properties. The next step uploaded it to the AlphaFold 3 web server to get the predicted structure for the transferrin receptor protein.(10) AlphaFold 3 is the newest AI tool designed to predict the 3D shape of proteins based on their amino acid sequences, providing valuable insights for biological research and drug discovery. For aptamer 3D structure prediction, we began by modeling the aptamer from 1D to 2D. First using UNAFold, we folded the DNA by uploading the aptamer sequence we had found earlier. The resulting .ct file, which contains the predicted secondary structure, was downloaded for further structural conversion. Before moving on, we converted the .ct file into a dot-bracket notation. Before moving on, we converted the .ct (coordinate) file into dot-bracket notation, a standard method for representing RNA secondary structures. The .ct file contains information about the base pairs in the RNA molecule, where each nucleotide's position is listed along with its paired partner, if applicable. To convert this into dot-bracket notation, we used the pairing information to replace each nucleotide in the sequence with either a dot (".") or a bracket. The dot-bracket format helps to easily identify base pairings and unpaired regions. The next step involved converting the 2D model to a 3D structure. We first converted the DNA sequence into its corresponding RNA sequence using Rosetta. Next, we inputted the RNA sequence along with its dot-bracket notation, ensuring that the number of clusters was set to one to optimize 3D structure prediction. Finally, we submitted this information to the VFOLD tool to generate a 3D structure prediction of the aptamer. (11) To perform molecular docking, we used the HDock server by submitting the TfR protein and aptamer ligand data for analysis. (12) HDock was selected for aptamer-protein docking because its hybrid scoring functions enhance accuracy, improving the reliability of docking predictions. Unlike AutoDock, which is primarily designed for small molecules, HDock is well-suited for macromolecular docking, making it a better fit for aptamer studies. Compared to HADDOCK, which requires predefined interaction restraints, HDock performs global docking without prior knowledge of binding sites, making it an essential tool for this research. Following the docking process, we proceeded with interaction analysis using the PLIP analysis web server.(13) PLIP was used to analyze molecular interactions, identifying key hydrogen bonds, hydrophobic interactions, and π - π stacking between the aptamer and TfR.

3. RESULTS

Surface properties of Transferrin Receptor (TfR): We used ScanNet to predict the binding sites' location and identify likely interaction regions. ScanNet is a tool that predicts binding sites using without prior ligand or structure knowledge, which is the primary reason for why ScanNet was chosen over other tools. Unlike tools that rely on sequence conservation (e.g., ConSurf) or docking-based predictions (e.g., COACH), ScanNet provides a more flexible way to identify binding sites, making it essential for modeling aptamer-protein interactions. A binding site is a specific region on a protein where molecular interactions such as hydrogen bonding or hydrophobic interactions, occur with a ligand. The result of the ScanNet was that the predicted binding site for the aptamer is primarily located at the center

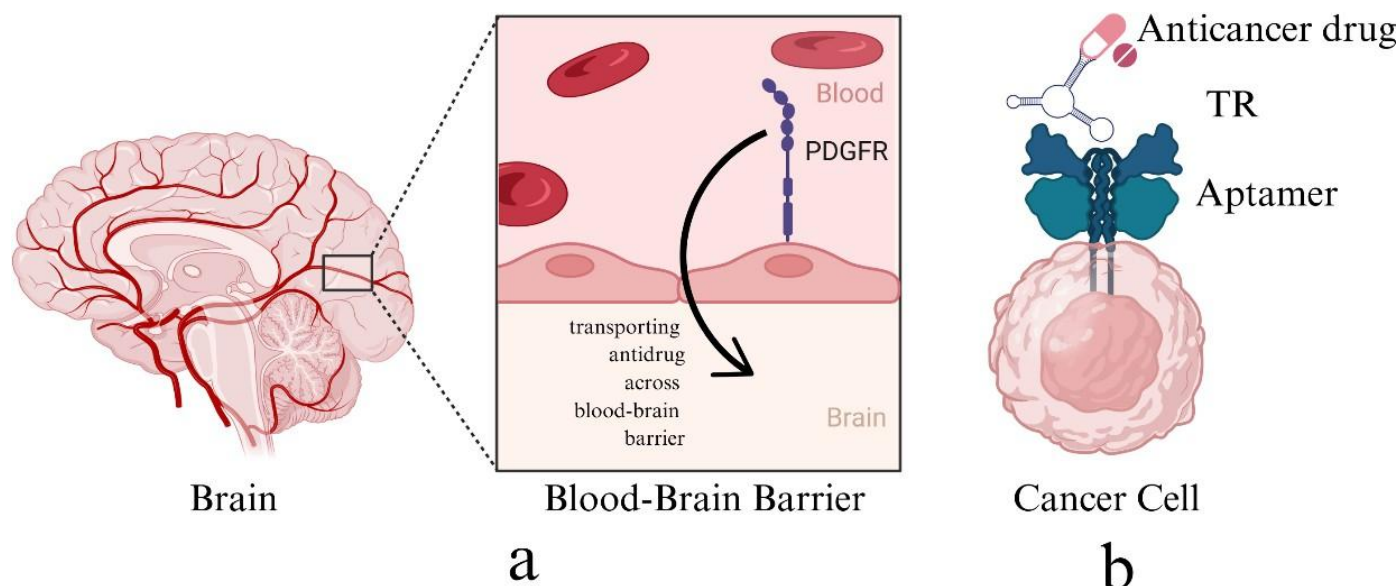


Fig. 1. (a) Receptor Platelet Derived Growth Factor Receptor (PDGFR) can help in crossing drugs across BBB; and (b) the anticancer drug conjugated aptamer binds to the Transferrin Receptor (TfR) and results in the cancer cell apoptosis. Infographic created using Biorender and Canva.

of the protein. Electrostatic surface potential is the distribution of electric charge over a molecule's surface, indicating where positive and negative regions exist on the molecule. From the ESP, we determined that the positive areas on the protein bind to the aptamer frequently, and the overall charge of the TfR is negative. Electrostatic interactions typically occur between oppositely charged molecules, so the positive regions on the TfR attract the negatively charged portions of the aptamer, facilitating binding. This is a common mechanism for aptamers, as their sequences include negatively charged phosphate groups that allow them to interact with positively charged protein surfaces.

Molecular docking simulations: To further investigate the interaction potential of the aptamer with TfR, molecular docking simulations were performed using the HDock server. We used the docking analysis to model and predict how the aptamer interacts with the transferrin receptor, helping assess their binding strength and stability. Based on the docking results, the aptamer binds to the binding site presented on the TfR_FAM protein consistently except for M1. The docked structures are shown in Figure 3. We used PLIP analysis to calculate the TfR-aptamer interactions, allowing us to analyze interactions such as hydrogen bonds, hydrophobic interactions, electrostatic interactions, π -stacking interactions, and salt bridges.

Molecular docking analysis: Binding energy between an aptamer and the transferrin receptor is the energy needed for the

aptamer to firmly attach to the receptor, showing the stability of their bond. We used software known as PDA-Pred or Protein-DNA complex Binding Affinity Prediction. The Protein-DNA Complex Binding Affinity Prediction website offers a tool to estimate the binding strength between proteins and DNA sequences. It aids in predicting how strongly a protein will bind to a specific DNA sequence, supporting the study of molecular interactions in biological processes. The binding energy calculation concluded with a binding energy of -13.67 kcal/mol, which indicates that 13.67 kcal/mol of energy is released when two molecules, like a protein and a ligand, bind together. With a binding energy of -13.67 kcal/mol, the aptamer shows a strong and stable connection to the transferrin receptor (TfR), similar to other well-binding aptamers, which usually fall between -8 and -15 kcal/mol. This suggests that the aptamer has enough strength to cross the blood-brain barrier (BBB) through receptor-mediated transport. The binding interaction between the receptor and aptamer variants is driven by specific molecular recognition, involving hydrogen bonding, electrostatic forces, and hydrophobic interactions, which collectively determine the affinity and specificity of the aptamer-receptor complex.

Platelet-Derived Growth Factor Receptor: The aptamer can also be designed to specifically bind to the platelet-derived growth factor receptor (PDGFR) in the surface of a glioma cell. By binding to domain 2 of the receptor, the aptamer can com-

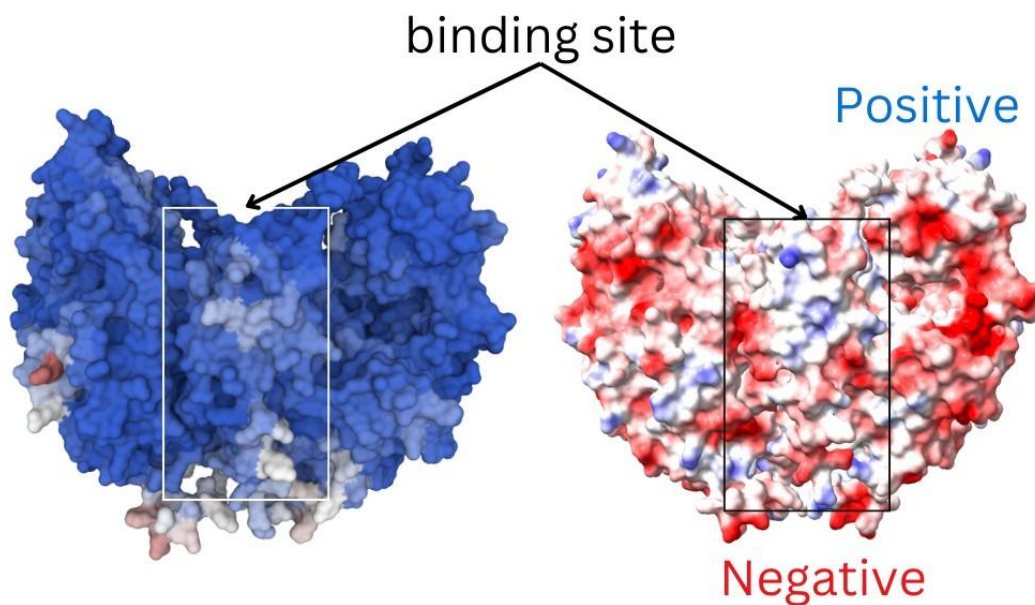


Fig. 2. Surface properties of the Transferrin Receptor (TfR). The left image shows the binding site on the surface, while the right image depicts the electrostatic surface potential (ESP) of TfR. In the right image, red represents negative charge, blue indicates positive charge, and white corresponds to neutral regions.

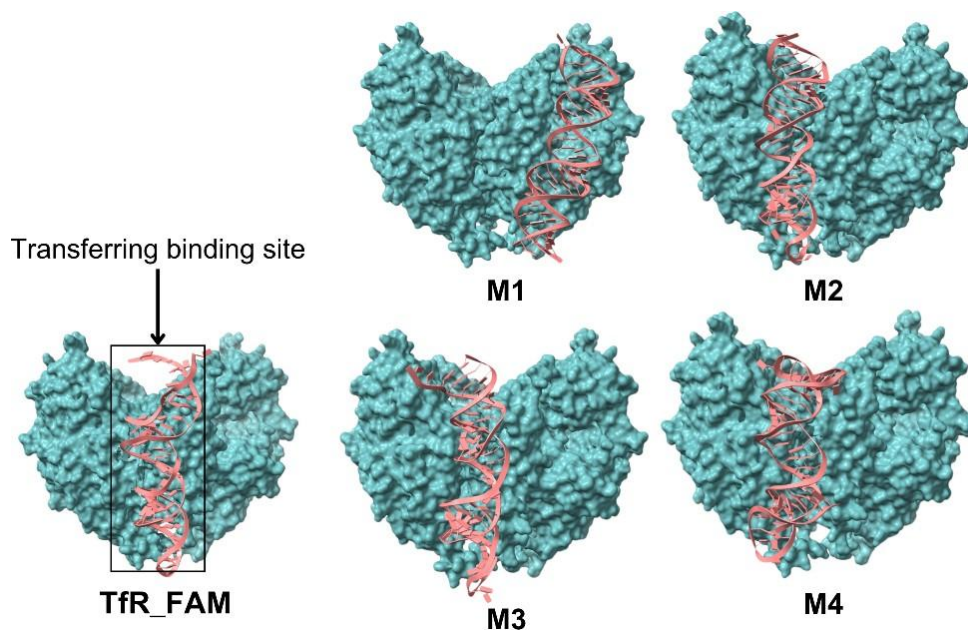


Fig. 3. Docked structure of Transferrin Receptor (TfR) bound with aptamers

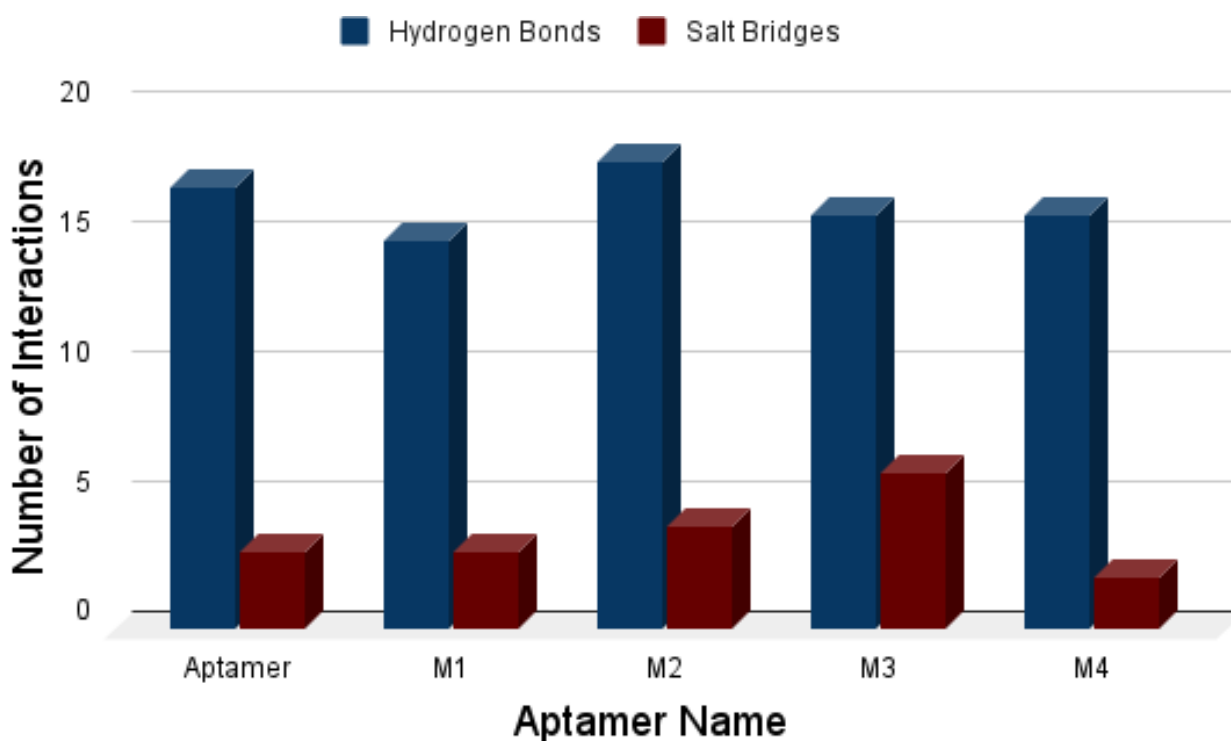


Fig. 4. The number of interactions formed between the Transferrin Receptor (TfR) - aptamer complex.

pletely inhibit the interaction between PDGFR and its natural ligand, platelet-derived growth factor. Without these signals, the tumor loses its ability to multiply as easily, making it more vulnerable. Additionally, blocking the growth factor can lead to the receptor's degradation, further reducing its activity in cell multiplication. This prevents the growth factor from activating the receptor's signaling pathways that aid in glioma cell multiplication. Consequently, the aptamer-mediated blockade of the PDGFR can reduce the growth of the tumor by disrupting the binding of the PDGFR natural ligand.

4. DISCUSSION

RNA and DNA aptamers are vulnerable to degradation by nucleases—enzymes that break down nucleic acids.(14) This limits their effectiveness in clinical and therapeutic settings, especially without chemical modifications to improve their stability. In the context of research using online databases, this issue may not always be fully represented. Databases typically focus on selecting aptamer sequences based on binding strength, rather than addressing how long they remain stable in biological environments like the bloodstream. As a result, aptamers found through database searches might not perform as well in real-world applications unless their stability is confirmed through further testing. To counter degradation, chemical modifications such as 2'-fluoro or 2'-O-methyl substitutions are commonly added to RNA aptamers to increase their resistance to nucleases.(15) PEGylation enhances aptamer stability and prolongs circulation time by attaching polyethylene glycol chains, reducing renal clearance and enzymatic degradation.(16) Locked Nucleic Acids

(LNAs) are modified RNA nucleotides with a methylene bridge locking the ribose ring, increasing binding affinity and resistance to nucleases.(17) Additionally, the aptamer may struggle to function in the tumor's microenvironment, which could affect its binding affinity or accessibility to the PDGFR on glioma cells. While computational methods can suggest these modifications, practical testing is necessary to confirm whether the aptamer remains functional in biological conditions.

Limitations: Although computational analysis is useful for predicting aptamers' structure and binding capabilities, it has some limitations. Factors like pH, temperature, and ion concentration, which affect aptamer structure in real-life conditions, are not always fully accounted for in computer models. Therefore, aptamers predicted in databases may behave differently than expected when tested experimentally. Additionally, computational methods often need help to predict interactions between aptamers and their targets in complex biological systems, especially if detailed information about the target structure is lacking. This can result in less accurate predictions, meaning experimental validation is still essential to confirm the aptamer's true binding performance.

5. CONCLUSION

This study highlights the potential of DNA aptamers in facilitating drug delivery across the blood-brain barrier (BBB) by targeting the transferrin receptor (TfR) in glioma. Computational simulations, including molecular docking and binding energy analysis, confirm a stable and favorable interaction, with a binding energy of -13.67 kcal/mol. The findings suggest that

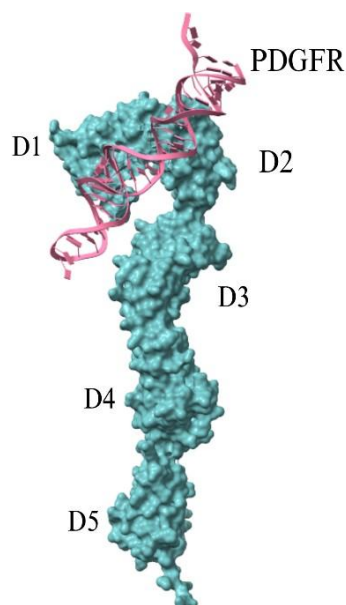


Fig. 5. Aptamer bound to domain 2 of platelet derived growth factor receptor present on the surface of glioma cell.

aptamers can enhance therapeutic precision and effectiveness for glioma treatment. Despite promising results, experimental validation is necessary to confirm aptamer stability and efficacy in biological systems. Future research should focus on chemical modifications to improve aptamer resistance and clinical viability. Additionally, the aptamer can bind to domain 2 of the platelet-derived growth factor receptor and essentially block the growth factor of the glioma cells, eliminating the multiplication of the glioma cells and disrupting a major factor in the glioma's survival and progression. This approach is promising for advancing glioma therapies and overcoming current treatment barriers.

6. REFERENCES

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Table 1. Mutation in Aptamers: We will make changes in the aptamer sequence and check whether the new aptamer is good or bad.

Label	Sequence
h-TG2	ACAAATCCATCAACCGTTCCGAAGGCGTGGTACCACGCTTTCATAT CCTCCCGCTCGTCTC((((((((.....)))))))).
h-TG2_M1	ACAAAUCCAUCAACCGUUCCGAAGGCGUGCUACCACGCUUUCAUA UCCUCCCGCUCGUCUC((((((((.....)))))))).
h-TG2_M2	ACAAAUCCAUCAACCGUUCCGAAGGCGUGGUACCACGCUUUGAUA UCCUCCCGCUCGUCUC((((((((.....)))))))).
h-TG2_M3	ACAAAUCCAUCAACCGUUCCGAAGGCGUGGUACCACGCCUUCAUA UCCUCCCGCUCGUCUC((((((((.....)))))))).
h-TG2_M4	ACAAAUCCAUCAACCGCUCCGAAGGCGUGGUACCACGCUUUCAUA UCCUCCCGCUCGUCUC(((.(((.(.....)).)).)).)...)