Computational Analysis of Aptamer-Based DNA Origami for Targeting Glioblastoma

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Glioblastoma is an aggressive and lethal brain tumor known for its rapid growth and resistance to surgical removal. Its invasive nature and tendency to recur make effective treatment particularly challenging. An aptamer is a short, single-stranded nucleic acid that folds into a specific structure and binds to target molecules, potentially leading to the treatment of diseases. DNA origami is a technique that involves folding DNA into precise nanostructures for medical applications. We hypothesize that these aptamers will bind to the binding sites of the CD19 and CD71 receptors, thereby facilitating the interaction between CAR T-cells and cancer cells. In the current work, we have designed DNA origami-bound aptamers target- ing the CD71 receptors of cancer cells and the CD19 receptors of CAR T cells. First, we modeled the CD19 and CD71 receptors using the AlphaFold 3 software. Next, we modeled the aptamers using the Vfold2D and Vfold3D software. Following this, molecular docking of aptamer on the receptors was performed with the HDOCK server. The docking results show that the aptamer binds to the predicted binding sites of the receptors and could act as a binder to bring CAR T cells closer to cancer cells, potentially result- ing in cancer cell apoptosis. The DNA origami design was created with cadnano software. Finally, the DNA origami was bound with the aptamer and the receptor. This study lays the groundwork for a novel DNA origami-aptamer-based system that could enhance the precision and efficacy of CAR T-cell therapy in glioblastoma treatment.

1. INTRODUCTION

Glioblastoma multiform (GBM) is an aggressive brain cancer and makes up 15% of all brain cancer cases (1). It is highly aggressive and classified as a grade IV tumor by the WHO (1). Originating from astrocytes, GBMs increase, leading to symptoms like headaches, seizures, and cognitive changes (2). Diagnosis typically involves imaging (CT, MRI) and a biopsy (2). Treatment is multimodal, combining surgery, radiation, and chemotherapy (usually temozolomide) (2). Ongoing research explores new therapies like gene therapy, immunotherapy, and personalized medicine (2). About 48–50% of all malignant brain tumors in adults are glioblastoma multiforme (GBM), the most prevalent and aggressive primary malignant brain tumor (3). In the US, there are roughly 3.2 cases of GBM for every 100,000 persons annually. Although pediatric forms such diffuse midline gliomas sometimes occur, it is infrequently seen in children and primarily affects older adults, with a median age at diagnosis of 64 years. The prevalence of GBM is higher in men than in women, with a male-to-female ratio of roughly 1.6 to 1. Together with the substantial age correlation, this gender gap raises the possibility of underlying hormonal and biological factors determining susceptibility. The Figure 1 illustrates the 5-year survival probability across different age groups at the time of cancer diagnosis, with data separated by gender (male and female) and combined totals. (3)

An aptamer is a short DNA or RNA strand that binds specifically to a molecule like a protein or cell (4). Created using SELEX, aptamers are used in diagnostics, therapeutics, and biosensors for their ability to detect molecules, deliver drugs, or inhibit targets (4). They are cheaper, easier to produce, more stable, and can be chemically modified compared to traditional antibodies, making them valuable in medical research and clinical applications (5). DNA origami, a method that shapes DNA into tiny structures for medical use, holds great promise for treatments like cancer and gene therapy despite some manufacturing challenges (6). These structures help deliver drugs directly to target cells, reducing side effects (6). They can release drugs when triggered by specific conditions and protect them in the bloodstream. DNA origami can carry multiple drugs and help with diagnosis (7). The mechanism of the cancer cell proliferation in glioblastoma is shown in Figure 2a and the Schematic of DNA origami is shown in Figure 2b. DNA origami is a nanotechnology technique developed in 2006 by Paul Rothemund at the California

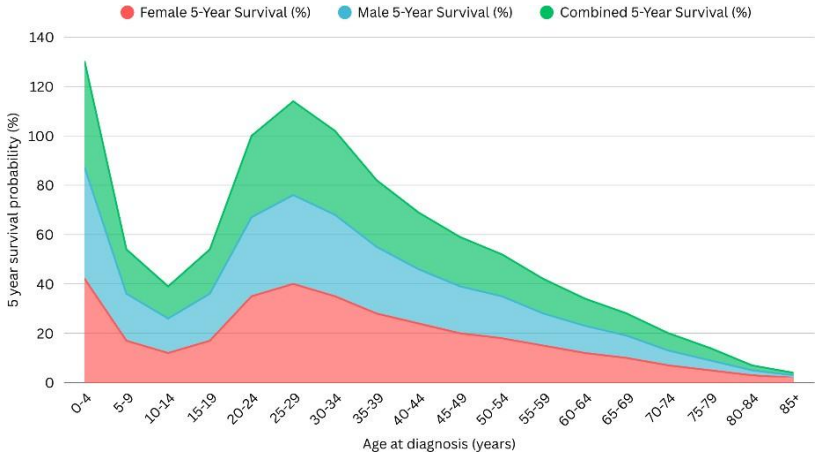

Fig. 1. Five-year cancer survival probabilities by age at diagnosis and sex. The area plot illustrates the 5-year survival rates for females (red), males (blue), and the combined population (green) across different age groups. Survival probabilities are highest in the youngest age group (0–4 years) and steadily decline with increasing age. Females generally show slightly higher survival rates than males across most age groups.

Institute of Technology. It involves folding a long single strand of DNA into specific shapes and structures using hundreds of short "staple" strands that bind to complementary sequences, much like origami with paper. This method revolutionized the field of nanotechnology by enabling the precise design and construction of two- and three- dimensional nanostructures at the molecular level.

CD71, called the transferrin receptor 1 (TfR1), helps cells absorb iron (8). It attaches to transferrin, a protein that carries iron, and brings it into the cell. Inside, iron is released and used by the cell (8). CD71+ glioblastoma cells are fast-growing cancer cells, because they need more iron (8). This makes CD71 a good target for cancer treatments that deliver drugs directly to cancer cells (8). CD19 is found on neutrophils and helps clear out immune complexes and activate neutrophils (9). CD19 is essential for immune responses like eating up invaders and releasing inflammatory signals (9). It is also targeted in cancer treatments to enhance the immune attack on cancer cells, making it a crucial component in these therapies (9).

Recently, Hu et al. developed aptamer-based DNA origami targeting cancer cell receptor CD71 and CAR T-cell receptor CD19 for tumor identification (10). This enables CAR T-cell-mediated tumor detection and apoptosis (10). We hypothesize that these aptamers should bind to a binding site of the CD19 and CD71 receptors and should help in bringing CAR T-cells and cancer cells together. The research involves modeling the 3D structure of aptamers, their docking on specific receptors, and their design on DNA origami structures. This process of designing aptamers based on DNA origami structures involves a series of intricate steps, including identifying the target receptor, creating a 3D model of the aptamer, and the precise docking of the aptamer on the receptor. This is a crucial step towards developing aptamers-based DNA origami for CAR T-cell therapy, as it ensures the aptamer is specifically designed to target the cancer cell receptor.

2. METHOD

I used AlphaFold 3 to model 3D structures of the CD19 and CD71 receptors (11). We followed these steps to convert a DNA aptamer sequence from 1D to 2D and then to 3D. We first went to the fold DNA folding web server and uploaded the DNA sequence, Table 1.(12) After submitting it, we saved the generated PNG image. We additionally download the CT (connectivity table) file, which will be used in the next step. Next, we navigated to the RNA structure web server and uploaded the CT file we obtained from the previous step. The server-generated dot and bracket notations represent the 2D structure of the DNA sequence. Then, we proceeded to the Vfold3D web server (13). We imputed the RNA sequence, dot, and bracket notations into the Vfold3D server (13). We set the number of clusters to 1, submitted it, and waited for the results. DNA origami was designed using these steps. Initially, the TALOS web server was used to get the predefined origami share in *.cando file format (14). The downloaded file was uploaded on the CADNANO web server to download the origami PDB file (7). This PDB file was truncated to obtain a linear origami design. Finally, aptamers were attached to the

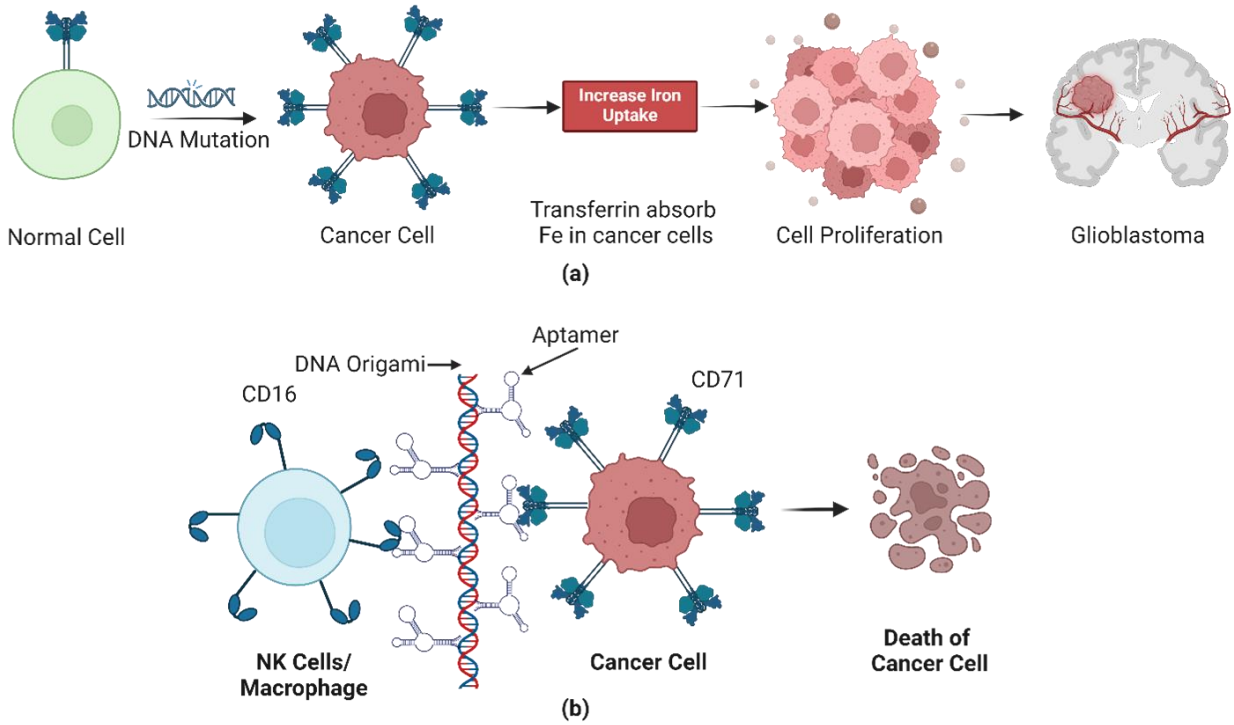

Fig. 2. Schematic representation of transferrin-mediated glioblastoma proliferation and aptamer-based immunotherapy strategy.(a) Normal cells acquire mutations that lead to the formation of cancer cells, which overexpress transferrin receptors (CD71). These receptors facilitate increased iron uptake, promoting rapid cell proliferation and contributing to glioblastoma development; and (b) A targeted immunotherapy strategy utilizing DNA origami scaffolds functionalized with aptamers specific to CD16 (on NK cells/macrophages) and CD71 (on cancer cells). This approach facilitates immune cell recruitment and targeted binding to cancer cells, ultimately leading to cancer cell death.

origami using YASARA software (15). To create DNA origami, we logged in to the Talos DNA Origami platform(10). We then selected the "Submission" option and entered the email ID. After clicking "Submit," an email containing a link was received. We followed the link to download a .zip file. Inside this folder, a file named caDNAno.json was found (7). We clicked on "Convert Structure," and once the conversion was complete, we downloaded the resulting archive. The zip file contained the DNA origami PDB file. The aptamer sequences used in this study. Dot-bracket notation shows the bonding and non-bonding base pairs of aptamers.

3. RESULTS

Using modeling, docking, and structural tools, the aptamers were shown to bind effectively, enabling potential CAR T-cell-mediated tumor targeting. Using CAR T-cell therapy, this work computationally designs DNA origami-bound aptamers targeting CD71 and CD19 receptors for enhanced glioblastoma treatment. We have worked on two receptors, CD71 (present on the surface of cancer cells) and CD19 (located on the surface of CAR T-cells). CAR T-cell induces cancer cell apoptosis, Figure 3. Using the GrASP tool (16), we identified drug binding sites on a protein to understand the interaction between the receptor and aptamers. The binding regions were highlighted in yellow-green (binding sites) and blue (non-binding sites). ChimeraX was used to map the protein's electrostatic potential, helping to align the negatively charged aptamer with the positively charged protein areas (17).

In the next step, we performed 3D modeling of the aptamer, Figure 4. In this process, the primary structure (aptamer sequence) was first converted into dot-bracket notation, representing its secondary structure. This was then used to generate the tertiary (3D) structure of the aptamer. The tertiary structure is essential for the HDOCK molecular docking simulation, as shown in Figure 4a. Figure 4b displays the folding form of the aptamer, where the sequence along with its dot-bracket notation is presented. Green brackets indicate interacting regions, while red dots represent non-interacting regions. The secondary structure, shown in the middle, includes a bulky tail, and the corresponding 3D structure is also depicted. These structural insights are crucial for understanding aptamer folding and are fundamental for accurate molecular docking simulations.

The docking PLIP analysis f the two targets—CD19 and CD71—evaluates their interactions with ligands based on salt bridges, hydrogen bonds, Table 2 (19). The binding energy was computed using PDA-Pred (20). CD19 shows strong interaction with six salt bridges, 19 hydrogen bonds, and a binding energy of -16.46 kcal/mol. CD44 has the strongest interaction with two salt bridges, 20 hydrogen bonds, and the highest binding energy of -19.34 kcal/mol. CD71 has six salt bridges and 15 hydrogen bonds, but its binding energy is unknown. C-met forms five salt bridges, 12 hydrogen bonds, and has a binding energy of -14.63 kcal/mol, indicating a relatively strong interaction, though weaker than CD19 and CD44. The drug binds to the locations identified in yellowish-green in Figure 3, as shown by the druggable site prediction made with the help of the GrASP web server (16). Figure 3b displays the electrostatic surface potential.

Table 1. Aptamer sequences used in this research.

Aptamer ID	Aptamer sequence and dot-bracket notations
XQ-2d(CD71)	ACTCATAGGGTTAGGGGCTGCTGGCCAGATACTCAGATGGTAGGGTTACTATGAGC
	..............(((.....))).....((((.....(((.....)))))))).
CLN0020(CD19)	CCACTGCGGGGGTCTATACGTGAGGAAGAAGTGG (((((((((.....))). .....)))))

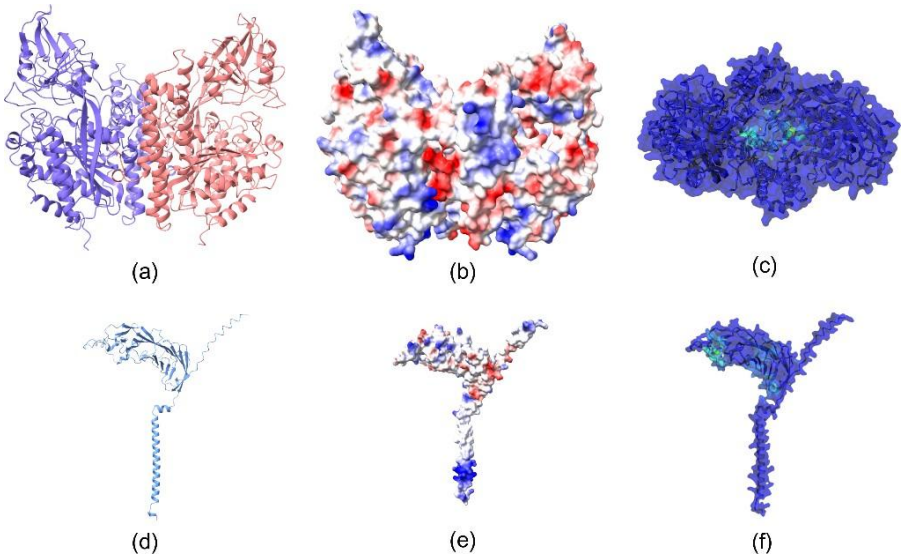

Fig. 3. The structure of receptors used in this study. The CD71 structure is shown at the top (a), and the CD19 structure is shown at the bottom (d). (b) and (e) shows the electrostatic surface potential of CD71 and CD19, respectively. (c) and (f) shows the binding site of the two receptors.

Table 2: The number of interactions formed between the receptor and aptamers.

Aptamer interactions with receptors			
	Hydrogen bonds	**Salt bridges**	**Binding Energy ΔG (kcal/mol)**
CD19	19	6	-16.46
CD71	15	6	-14.32

Finally, we calculated the network of protein-protein interactions, which shows that sister proteins of the CD19 protein and the CD transferrin receptor are involved (Figure 6a. Furthermore, as illustrated in Figure 6b, we examined the interactions between the protein receptors. In this region, the majority of these proteins are found in intracellular, transcellular, and extracellular spaces. Precision medicine design and the creation of patient-specific treatment variations can both benefit from this information. Moreover, it can facilitate the development of artificial receptors or pathways for application in cells that have been created. Finally, we also designed DNA origami using Cadnano software, as shown in Figure 7. The DNA origami can be used to attach the aptamer to the surface and facilitate binding to the respective receptors, as illustrated in Figure 7.

4. DISCUSSION

Recent studies have demonstrated the potential of DNA origami-based receptor-aptamer complexes to enhance in vivo diagnostic and therapeutic capabilities (21). These hybrid nanostructures have improved effectiveness and better efficiency. Additionally, incorporating gold nanoparticles within the DNA origami framework can improve them. The complex tumor microenvironment presents a significant challenge for CAR-T cell therapy for solid tumors, potentially limiting the cells' ability to target and destroy cancer cells effectively (14). Researchers have explored using DNA origami structures to modify the tumor microenvi- ronment and enhance CAR-T cell performance to address this issue.

DNA origami, a technique that uses long single-stranded DNA scaffolds folded into complex nanoscale shapes, holds great promise in biomedical applications, including targeted drug delivery and biosensing. However, potential side effects must be carefully considered. These may include unintended

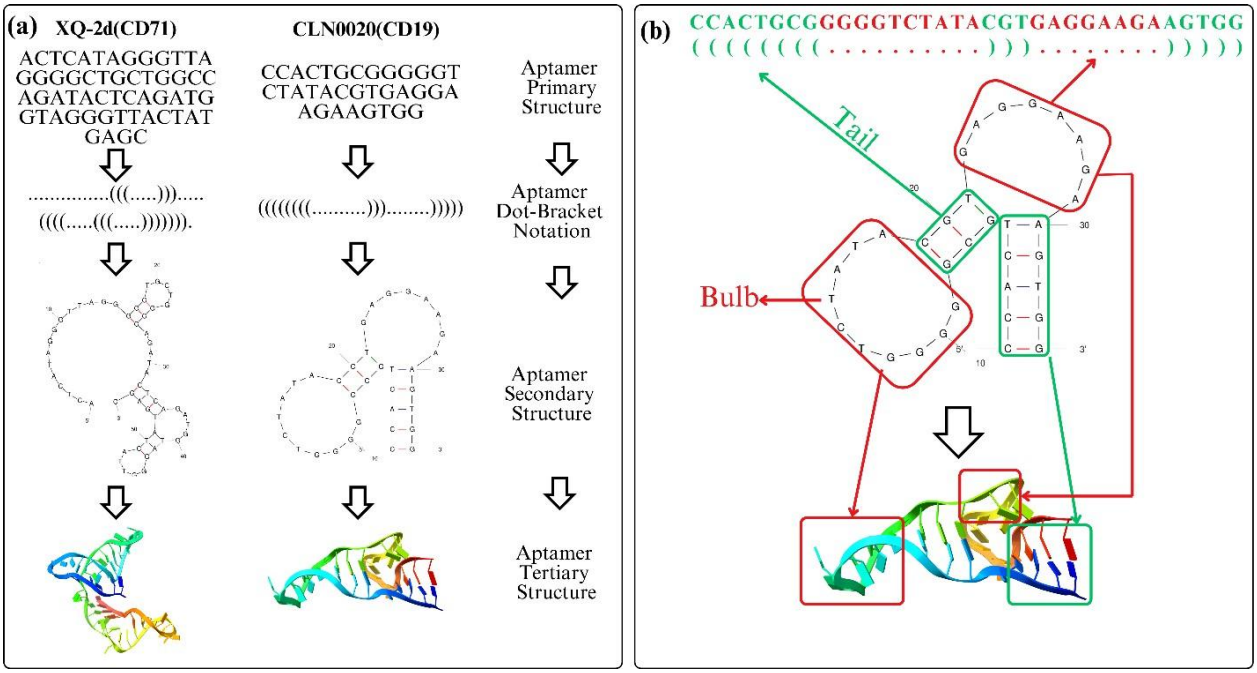

Fig. 4. In silico prediction of aptamer structure from primary to tertiary level. (a) The modeling pipeline used to generate RNA aptamer structures targeting CD71 (XQ-2d) and CD19 (CLN00020). The aptamer sequences (primary structure) are shown at the top, followed by the corresponding dot-bracket notation representing predicted base pairing. Secondary structures are generated using these notations, and the resulting tertiary structures are modeled from them; and **(b)** Detailed annotation of the aptamer's structural features using the predicted secondary and tertiary structures. Key structural motifs such as the bulb and tail are highlighted and color-coded. The boxed regions show correlations between specific nucleotide segments in the secondary structure and their spatial orientation in the 3D folded structure. The tertiary structures of the aptamers were obtained and subsequently used for molecular docking simulations. These simulations involved docking the aptamers onto their respective receptors, which were predicted using AlphaFold. Molecular docking with HDOCK confirmed the aptamer successfully bound to the protein's druggable region, revealing the potential interaction and binding mechanism (18). The docked structures from the HDOCK software are displayed in Figure 5.

immune responses due to the recognition of DNA nanostructures as foreign by the innate immune system, especially if the constructs are not appropriately modified to evade detection. Additionally, concerns about DNA degradation, off-target interactions, and accumulation in non-target tissues may lead to cytotoxicity or interfere with normal cellular functions. The long-term biocompatibility and clearance of these structures also remain under investigation. Therefore, while DNA origami offers exciting possibilities, its clinical translation requires rigorous safety evaluation and optimization.

By attaching specific ligands on the surface of DNA origami, the CAR-T cells ability to recognize and eliminate cancer cells can be increased. This can further help identify cancer cells in the tumor environment. In addition, DNA origami can serve as a vehicle for transporting drugs where the regular drug cannot reach. Computational research shows significant promise in the field of DNA origami. Additionally, aptamers can break down in the body due to enzymes, reducing their stability. This makes them less valuable and may require further modifications to improve their durability. To overcome these issues, future research should focus on thorough testing in live animals to confirm the potential and safety of aptamer-bound DNA origami in CAR T-cell therapy. These studies should aim to assess distribution in the body, targeting accuracy, and overall effectiveness in reducing tumors. Additionally, chemical optimization of aptamer sequences can improve their stability and binding selectivity.

5. CONCLUSION

The study of using aptamer-based DNA origami to target glioblastoma sheds insight on a fresh and exciting way to improve cancer therapy. By computationally creating DNA origami-bound aptamers that target the CD19 receptors of CAR T-cells and the CD71 receptors of glioblastoma cells, the study demonstrates how this technique may improve the immune system's ability to recognize and eliminate tumor cells. Molecular docking provides information about the possible effectiveness of aptamers by validating their successful binding to specific receptors. Though computational models appear promising, further in vivo research is required to confirm the therapeutic efficacy, targeting accuracy, and stability of aptamer-bound DNA origami in natural biological systems. To increase aptamer endurance, future research should concentrate on chemical alterations and investigate experimental applications.

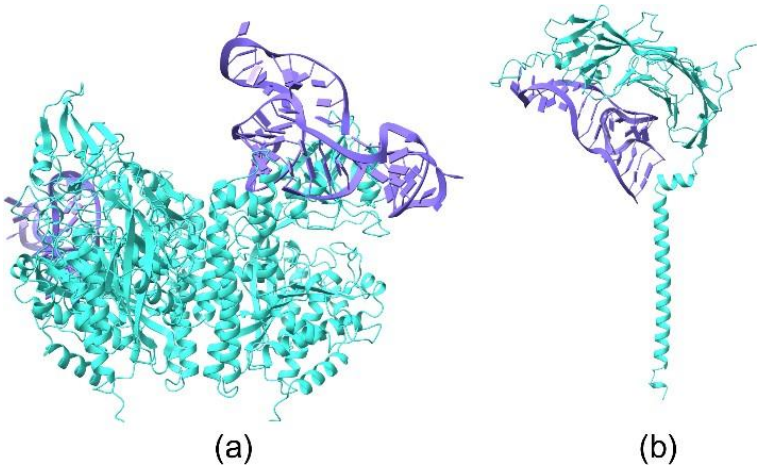

Fig. 5. Structure of Receptor-aptamer complexes. (a) CD19 and (b) CD71 receptors bound the aptamers. The receptors are shown in Cyan and the aptamers are in purple.

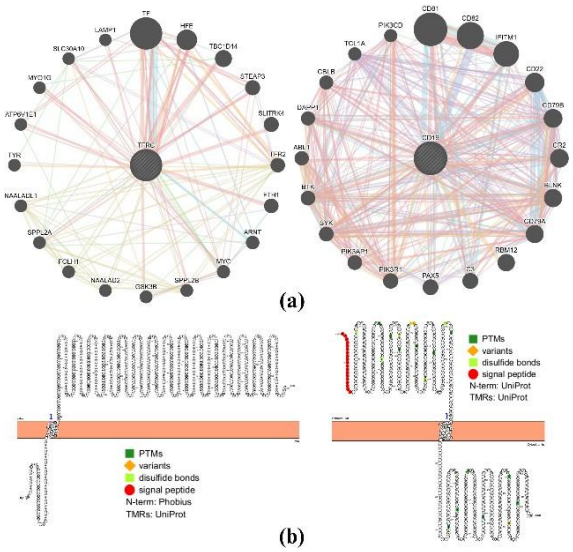

Fig. 6. Structural and interaction network analysis of transmembrane proteins. (a) Protein–protein interaction networks of two transmembrane receptor proteins, showing connections with other proteins. Each node represents a protein, and the lines indicate protein–protein interactions. The color of the lines denotes different interaction types. (b) Topology of the same two transmembrane proteins, illustrating transmembrane regions (TMRs), post-translational modifications (PTMs, green circles), sequence variants (yellow diamonds), disulfide bonds (red lines), and signal peptides (red segments). The topology was derived from UniProt and Phobius data.

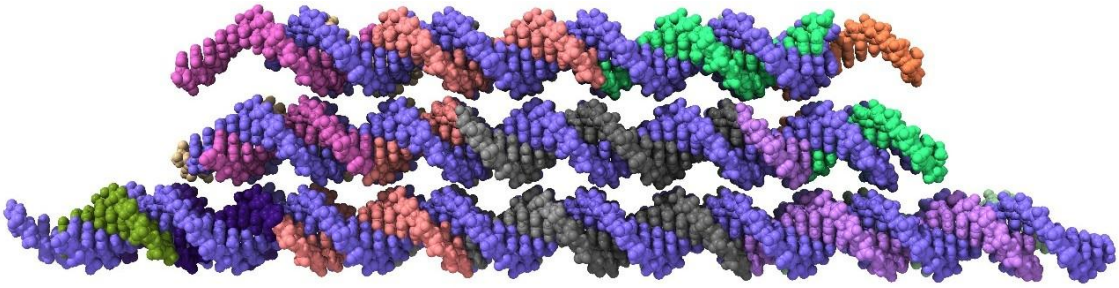

Fig. 7. DNA origami obtained from CadNano software.

6. CONCLUSION

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