# In silico analysis of RNA-based aptamers for interaction with CD19 receptor in CAR-positive T-cell enrichment and monitoring

FIZA PACHLOO[1,2] AND GAURAV SHARMA[2]

[1]Baylor University, Waco, TX
[2]Eigen Sciences, Apex, NC
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**The T cells of a cancer patient are extracted and genetically modified during a procedure called CAR T-cell therapy (chimeric antigen receptor T-cell therapy), which can detect a particular tumor-associated antigen. Short, single-stranded oligonucleotides called aptamers tend to have a high affinity and specific binding to target molecules, such as the CD19 receptor. Novel aptamers exhibiting this high affinity and specificity for the chimeric antigen receptor CD19 have been recently engineered. It is assumed that the involved aptamers interact with a particular epitope on the CD19 receptor. In this study, in silico techniques were utilized for the analysis of the interactions between the CD19 receptor and the aptamers. Previous research was used to obtain the nucleotide sequences of the aptamers and used to model their secondary and tertiary structures using VFold2D and VFold3D, respectively. These structures were then docked onto the CD19 receptor using the HDOCK software with its default parameters. The aptamers CARap9s and CARap18s, which bind to the active site of the CD19 receptor through hydrogen and hydrophobic bonding, were further validated by the comparison of the CD19 binding site using electrostatic surface potential analysis and machine learning, such as the Graph Attention Site Prediction (GrASP) web server. Additional experimental work done in a different investigation supported these results. By utilizing CAR-aptamers to enrich and monitor CAR-positive cells, this study aims to facilitate identifying and isolating these cells and offers promising prospects for the advancement of CAR T-cell therapy.**

## 1. INTRODUCTION

Categorized under immunotherapy, CAR-T cells are applied for the management of specific tumors (1). The treatment entails altering the T cells, or the immunological cells, of a patient to manifest a receptor engineered to recognize and bind to tumorspecific antigens (2). Leukapheresis is applied for the extraction of T cells at the initial stage of the procedure from the patient's blood (3). Subsequently, these T cells undergo genetic modification to generate receptors that bind and recognize specific proteins in cancer cells. Until there are enough modified T cells for treatment, they are grown in a laboratory (3). Once there are a sufficient number of cells, the patient receives a second infusion of the redesigned T cells, which can then identify and attach to the cancer cells, causing apoptosis (4). Following infusion, patients are closely watched for side effects such as cytokine release syndrome, otherwise called CRS, which is a common and possibly critical reaction (6). Research is ongoing for the improvement in the efficiency of the CAR-T cell therapy as well as its safety and application to different cancer types (7). Acute lymphoblastic leukemia (ALL) and certain lymphomas are two blood malignancies that have demonstrated great success when treated with this therapy (8).

Aptamers, or short, single-stranded DNA or RNA molecules, selectively attach to various targets, including cells, proteins, peptides, and tiny molecules (9). Aptamers, also called "chemical antibodies," work similarly to antibodies because they bind to their targets with high specificity and affinity (10). Unlike antibodies, they are synthesized and can be chosen based on how well they attach using a procedure known as SELEX (11). Because of this, aptamers have unique benefits that make them valuable tools in research, diagnostics, and therapy (11). These benefits include stability, ease of chemical modification, and chemical synthesis (10).

"Molecular docking" is a computational method used to predict the interactions between a micro molecule, for example, a ligand or medication, and a target protein (12-14). This method

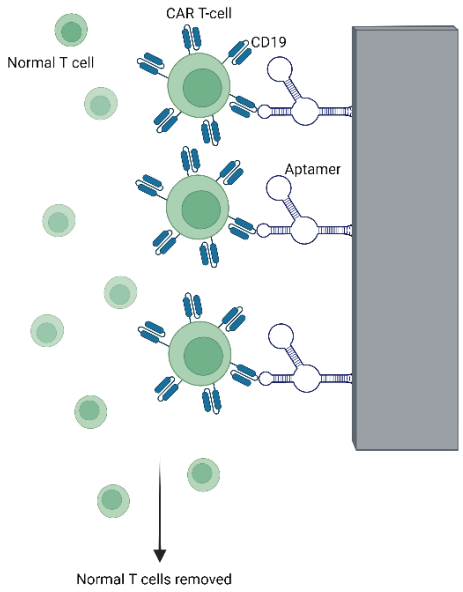


**Fig. 1. The mechanism of CAR T-cell elucidation.** The aptamers bind to the CAR T-cells and help separate them from the normal T cells. In this way, the CAR T-cells are concentrated and injected into the patient's body.

mimics the ligand-protein binding process at the active site to determine the optimal orientation and binding affinity (15). By studying these interactions, researchers may find new avenues for treatment and learn more about the molecular mechanisms underlying biological systems (16). Molecular docking predicts the strength and type of chemical interactions that form among molecules, anticipating the stability and effectiveness of the medication (16). This method is crucial because it allows scientists to swiftly and economically screen large arrays of chemicals (16).

Both CAR-positive (+ve) and CAR-negative (-ve) cells can coexist throughout the manufacturing of CAR T-cell treatments (17). The procedure entails the T-cell introduction of a gene coding a CAR or Chimeric Antigen Receptor (18). CAR-negative cells, usually considered as contaminants, create several problems, including a lack of focused activity, non-specific immune activation, graft-versus-host disease, and dilution of CAR+ cells (19). These problems could cause the patient to react unnecessarily regarding immunity (20). Therefore, effective dosage depends on a large concentration of CAR+ T cells, so the treatment is more efficient and cost-effective, and there is less demand for hospital visits (21). The enrichment of CD19 CAR+ T-cells via aptamers is shown in Figure 1. CD19 CAR-specific aptamers have recently been developed to separate CAR+ T cells from CAR T-cells (22). It is hypothesized that specific amino acids and base pair interactions could cause the aptamer interaction with the CD19 CAR+ T-cells. In addition, we also hypothesize that the CD19 receptor has a flexible structure, and due to this flexibility, it can bind to these aptamers. Here, the 3D structures of these aptamers, which specifically bind to the CD19 receptor, are computationally designed. This work promises to help understand the mechanism of the aptamer binding to the CD19 receptor, which will lay the groundwork for designing novel aptamers for CAR+ T-cell enrichment.

## 2. RESULTS

Using AlphaFold 3, CD19 protein's 3D model was procured (21), as shown in Figure 2a. We hypothesize that on the CD19 receptor, the aptamer attaches to a particular spot, and to find the interaction spot, first AI is used to identify which amino acids can attach with these aptamers. We then employed a Graph Neural Network or GNN using the GrASP, a web server, tool specifically designed to predict the site of bonding on proteins, to indicate this site. Defining the binding site before molecular docking simulations is essential to validate the docking results. The highlighted yellow and green spots in figures 2a and b represent the aptamer binding sites present on CD19 protein. The binding site represents the region of the protein responsible for ligand recognition and binding.

In the next step, the 3D structures of aptamers were obtained for the molecular docking simulations. The following steps involved structure prediction: Primarily, the aptamers 2D structures were obtained using Vfold2D (23), as illustrated in Figure 4. Table 2 presents the dot-bracket representation of secondary structures for each aptamer. Since aptamers are single-stranded, they coil themselves by making intrabase pair hydrogen bond interactions. These interactions determine the overall 3D form of

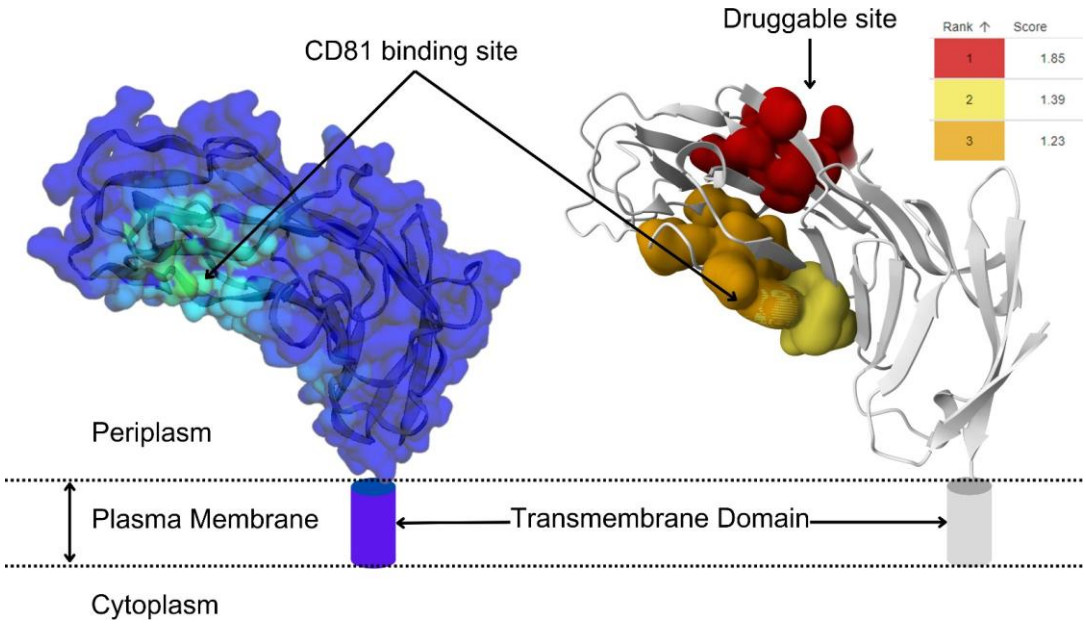


**Fig. 2. Binding site of CD19 receptor.** The binding site of CD19 was predicted using the GrASP web server, which shows the site in yellow and green. Similar results were obtained using the P2Rank web server.

the aptamers, which are also crucial for the binding of proteins. The depiction of base-pair forming hydrogen bonds is done in dot-bracket notation, which uses the dot "." as an unpaired nucleotide and "(or)" as a paired nucleotide. In Table 2 and Figures 3 and 4, the dot-bracket notation is displayed. This dot and bracket notation was used to model the 3D structure of the aptamers in VFold3D.

Subsequently, molecular docking simulations were carried out using the software HDOCK in order to understand binding interaction. Visual inspection of the docked structures shows that out of 12 aptamers, only seven interact with the binding spots as assumed by the GNN. Of the twelve modeled aptamers, aptamers 7, 7s, 9, 9s, 10, 14, 18, and 18s were found to bind at the predicted GNN binding sites. The binding site of these aptamers was mostly neutral, and the bond interactions show that the interactions were mainly due to the formation of hydrogen bonds between CD19 and the aptamers. Experimental studies have also predicted that the aptamers CARap9s and CARap18s have the most robust binding to the CD19 receptor. Figure 5 displays the docked structures of the aptamer binding to the CD19 receptor. Studies using computational and experimental methods demonstrated the high binding capability of CARap9s and CARap18s to the CD19 receptor. Therefore, only CARap9s and CARap18s were chosen for additional assessment. Using PLIP, the interactions between these two aptamers and the CD19 receptor were first calculated. The results indicate that CARap9 forms 3 salt bridges and 16 hydrogen bonds, while, CARap18s only creates 16 hydrogen bonds.

## 3. DISCUSSION

The CD19 receptor is a transmembrane glycoprotein belonging to the immunoglobulin superfamily and is a key target in cancer immunotherapy (23). The CD19 treatment employs chimeric antigen receptor T cells. Tumor-targeted T-cells can be generated by genetically modifying T cells to express CARs. Intracellular signaling, transmembrane, extracellular spacer/hinge, and antigen-binding are the four main domains that CARs comprise. In response to antigen binding, these four domains work in con- junction to activate T cells. The CD19 receptor is a perfect target for the CAR+ T cell treatment as it is found in almost every case of B-cell malignancies, for example, acute lymphoblastic leukemia, non-Hodgkin lymphomas, and chronic lymphoblastic leukemia. Encouraging outcomes, even those of complete re- mission, have been recorded using CAR T cell treatment during clinical trials.

In this research, we have first evaluated the aptamers that help in enriching normal T cells from CAR T cells. The enrichment process is crucial for optimum therapeutic efficacy by increasing the specificity and potency of cancer treatment. It also ensures an elongated persistence of the CAR T cells in the body, assuring more sustained effects. In addition, it increases the therapy response time, including faster antigen recognition. Finally, it reduces the manufacturing time and can lower the overall treatment cost.

Despite its value in drug discovery, molecular docking has several drawbacks. Scoring functions cannot fully represent the intricacy of molecular interactions, which can cause false positive or false negative results. In addition, most docking programs anticipate binding modes incorrectly because they treat proteins as stiff structures and ignore the changes that happen

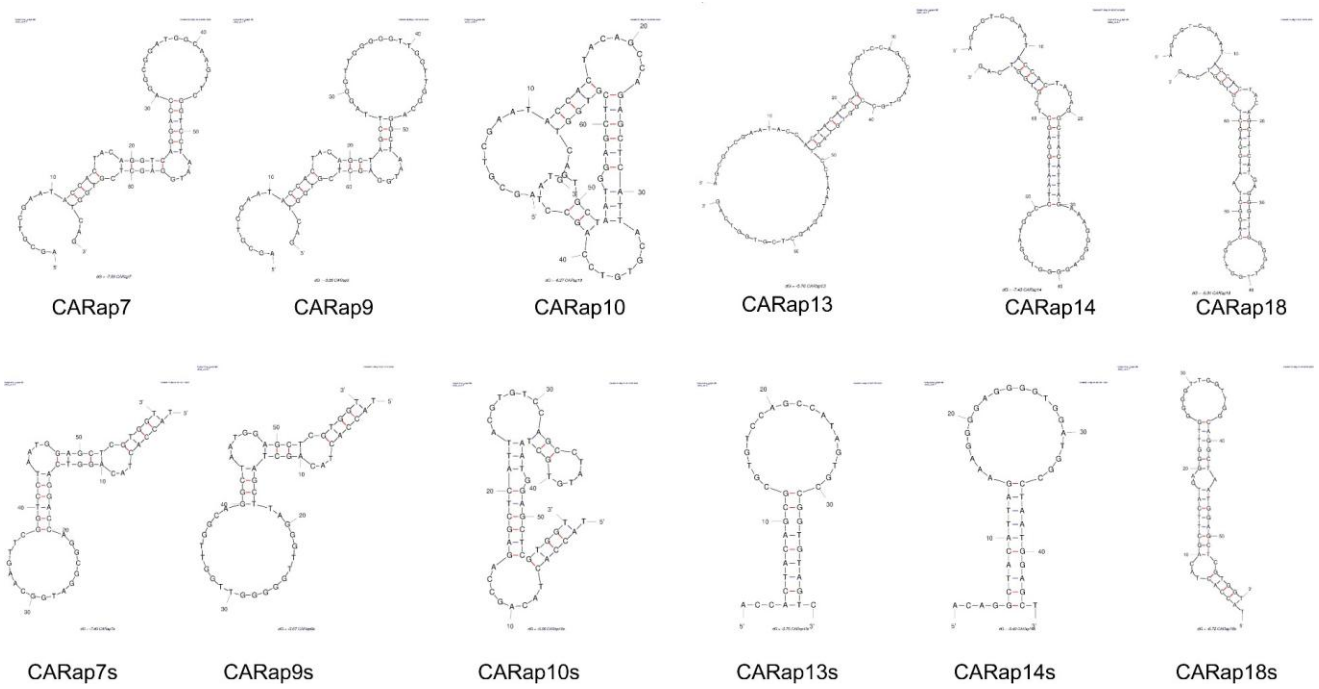


**Fig. 3. Aptamer nucleotide binding.** These images show the base pairs forming hydrogen bonds, which create the 2D aptamer structure.

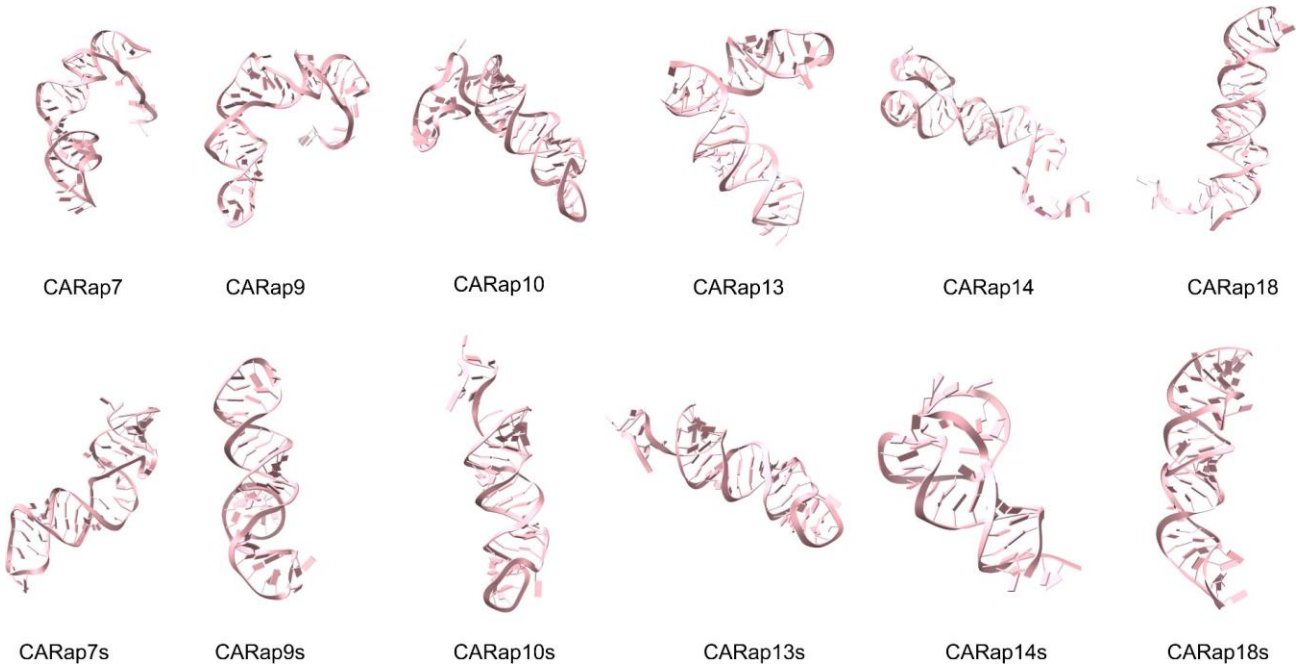


**Fig. 4. Tertiary (3D) structure of aptamers.** Minor base pair changes in the aptamers result in significant structural changes, causing a change in the binding patterns. These images depict the 3D structure of the aptamer.

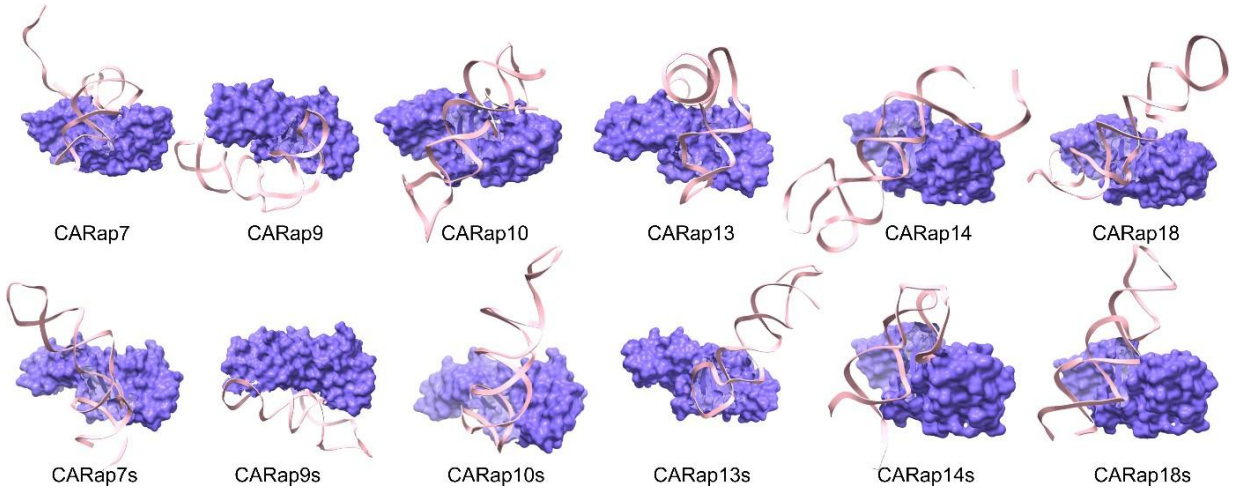


**Fig. 5. Aptamers binding to CD19.** Aptamer binding to CD19 will help enrich the CAR+ T cells containing CD19 protein. Enrichment is a process of separating the CAR+ T-cells from the normal T-cells.

when ligands bind to them. These restrictions emphasize the necessity for other strategies and additional developments in computational methodologies to increase the accuracy of molecular docking data.

This research emphasizes the capability of RNA-based aptamers for the monitoring and enrichment of CAR-positive T cells in connection with their engagement with the CD19 receptor. The in-silico investigation showed that some aptamers, specifically CARap9s and CARap18s, bind to the CD19 receptor efficiently. As predicted and supported, these aptamers form many hydrogen bonds and hydrophobic contacts at the binding area of the CD19 receptor by molecular docking and machine learning approaches. The findings support the hypothesis that these aptamers can be applied to increase the isolation as well as the detection of the CAR-positive T-cells, thus enhancing both the therapeutic efficacy and post-infusion monitoring of CAR+ T-cells. More experimental validation is advised to verify these computational results and investigate the possible clinical applications of these aptamers in cancer treatment.

## 4. METHOD

The 3D structure of the CD19 receptor was predicted using AlphaFold 3 (24). Structural predictions were based on the main nucleotide sequence of the aptamer. The secondary structures of the aptamers were first predicted from their primary nucleotide sequences using Vfold2D (25), followed by the tertiary structure prediction via Vfold3D (26). Then, to perform molecular docking on the HDOCK software, we uploaded the CD19 as a receptor molecule. The aptamers were treated as ligand molecules in the docking simulations (27). The selection of the aptamers was according to their interaction with the binding region of the CD19 receptor. This binding region was predicted by a GNN that was run on the GrASP server (28). This is the same site where the CD81 receptor binds to the CD19 receptor and causes the B cell activation (23).

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**Table 1. Aptamer sequence:** sequence of Aptamers used in this work.

| Aptamer ID | Aptamer Sequence and Dot-and-Bracket-Notation |
|---|---|
| **CARap7** | AGCGUCGAAUACCACUACAGGUCAGGACCAGGCGGAUGGCAAGUUCGGUCCUAAUGGAGC<br>UCGUGGUCAG<br>.....(((((...((.(((((((.....))))))....)).)).)))))... |
| **CARap9** | AGCGUCGAAUACCACUACAGCUAGCUUAGGGUUGGGGGUUGGUUGGCAGGCUAAUGGAGC<br>UCGUGGUCAG<br>.....(((((...(((((((.....))))).....)))).)))))... |
| **CARap10** | AGCGUCGAAUACCACUACAGCCAGAGCUCAUUACGUGUCCAGCCUAUGUGCUAAUGGAGCU<br>CGUGGUCAG<br>((.(((...)))))....((((((((((((((.((..((((...))))))))))).)))))).))))).. |
| **CARap13** | AGCGUCGAAUACCACUACAGCGCGUGUCCAGCCAUAGUGCCGGUGUAGUCCUAAUGGAGC<br>UCGUGGUCAG<br>((((((.....))))))....((((((((((((((((....))))))))))).))))))).. |
| **CARap14** | AGCGUCGAAUACCACUACAGGCUACAUUAGAAAGGGGAGGGGUGGAUGGCCUAAUGGAGC<br>UCGUGGUCAG<br>.....(((((((...((((((((....))...((((((...))))))))))))))))))).. |
| **CARap18** | AGCGUCGAAUACCACUACAGCUUCAUGAGGGUUGGGGGUUGGUUGGCAGGCUAAUGGAGC<br>UCGUGGUCAG<br>.....(((((((..((((((((((((((((.....)))))))..))))))))))))))))).. |
| **CARap7s** | UACCACUACAGGUCAGGACCAGGCGGAUGGCAAGUUCGGUCCUAAUGGAGCUCGUGGU<br>.((((((..((((((((((((((.....))))))))))....)))))))))) |
| **CARap9s** | UACCACUACAGCUAGCUUAGGGUUGGGGGUUGGUUGGCAGGCUAAUGGAGCUCGUGGU<br>.((((((((((((((((.....))))))))))))..(((((...))))))))))) |
| **CARap10s** | UACCACUACAGCCAGAGCUCAUUACGUGUCCAGCCUAUGUGCUAAUGGAGCUCGUGGU<br>.....(((((((((((((.((..((((...))))))))))).)))))).)))) |
| **CARap13s** | UACCACUACAGCCAGAGCUCAUUACGUGUCCAGCCUAUGUGCUAAUGGAGCUCGUGGU<br>.....(((((((((((((.((..((((...))))))))))).)))))).)))) |
| **CARap14s** | ACAGGCUACAUUAGAAAGGGGAGGGGUGGAUGGCCUAAUGGAGCU<br>...((((((((((....))...((((((...)))))))))))))) |
| **CARap18s** | UACCACUACAGCUUCAUGAGGGUUGGGGGUUGGUUGGCAGGCUAAUGGAGCUCGUGGU<br>.((((((..((((((((((((((((.....))))))))..)))))))))))))))) |

**Table 2. CD19-Aptamer bonds:** This table shows that aptamers form multiple bonds with CD19 receptors.

| Hydrogen bond | Residue | Distance (Å) |
|---|---|---|
| | ASN85 | 3.09 |
| | ASP99 | 1.61 |
| | ASP99 | 1.53 |
| | LEU100 | 2.91 |
| | ASP128 | 2.46 |
| | SER135 | 2.76 |
| | THR136 | 2.76 |
| | THR136 | 3.16 |
| | TRP138 | 2.47 |
| | PRO144 | 3.24 |
| | ASP146 | 2.64 |
| | SER149 | 1.78 |
| | SER149 | 3.02 |
| | ARG150 | 3.27 |
| | ASP173 | 3.15 |
| | ALA177 | 2.93 |
| Salt Bridge | His45 | 5.43 |
| | Arg94 | 4.93 |
| | Arg191 | 3.97 |
| Hydrogen Bond | ASN11 | 1.97 |
| | HIS45 | 2.76 |
| | HIS45 | 2.46 |
| | HIS45 | 2.46 |
| | LEU49 | 3.03 |
| | SER56 | 2.15 |
| | SER56 | 2.75 |
| | GLY134 | 1.39 |
| | TRP138 | 3.14 |
| | TRP138 | 1.92 |
| | TRP138 | 2.07 |
| | ASP179 | 2.84 |
| | ARG191 | 2.59 |