Aptamer-Driven Nanovehicles for Targeted Therapy of HER2 Overexpressing Breast Cancer Cells

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Aptamers are single-stranded small nucleotide segments capable of interacting with numerous biomolecules with solid affinity and have gained significant attention in cancer biomarkers and targeted therapy. Recently, a drug delivery nanovehicle incorporating an anticancer compound targeting the HER2 receptor in breast cancer has been developed. This demonstrates how useful aptamer-based nanovehicles are for creating indicators unique to cancer, reducing side effects, and enhancing treatment effectiveness. The interior of the nanovehicle contains a protamine-aptamer complex that binds to epigallocatechin gallate (EGCG: an anticancer medication), while the surface comprises a HER2 affinity aptamer. The nanovehicle is a carrier bound to cancer cells and transports the anticancer medication into them. Our hypothesis is that the HB5 aptamer on the nanovehicle surface interacts with the particular HER2 receptor area to identify HER2+ cancer cells. AlphaFold web server was used for the HER2 receptor 3D structure. We conducted in silico investigations to estimate aptamers' three-dimensional structure and interactions with HER2 and protamine. We discovered that the surface aptamer creates a particular linear shape necessary for HER2 receptor interactions. The inner aptamer is small and forms a patterned structure with the protamine, releasing EGCG into the cytoplasm in the presence of abundant cytoplasmic ATP. These findings would benefit in understanding aptamer-receptor binding, cancer medicine release mechanism, and creating a possible accurate and safe drug-delivery vehicle.

1. INTRODUCTION

HER2 receptors are transmembrane proteins with signal transduction potential due to tyrosine kinase activity [1]. The structure of HER2 receptors contains a ligand-binding domain (extracellular), a single transmembrane helix, a tyrosine kinase domain (intracellular), and a C-terminal tail (intracellular) [1]. HER2, unlike other HER family receptors, does not have a known ligand [2]. Instead, it forms heterodimers with different receptors in the HER family (HER1, HER3, HER4) that are activated by their ligands [3]. HER2 plays a vital part in cell growth, differentiation, and survival. Its heterodimer formation enhances these receptor complexes' signaling potency [4]. This activates intracellular pathways such as the MAPK and PI3K/AKt pathways, helping cell multiplication [5]. However, the overexpression/amplification of the gene controlling HER2 expression is evident in multiple cancers, most notably in breast cancer, causing aggressive tumor growth and poor prognosis [6]. Trastuzumab (Herceptin) and other targeted therapies are designed to inhibit the signaling of the HER2 receptor, affecting the development of HER-2-positive tumors [7]. Although many patients with HER2-positive malignancies respond well to Herceptin (trastuzumab), other people eventually develop resistance to the medication. Furthermore, Herceptin has serious adverse effects that may restrict its usage for some people. These drawbacks emphasize the need for better or alternative treatments to overcome resistance or provide safer alternatives. The normal breast cell and HER2 overexpressed breast cancer cell is shown in Figure 1a.

Docking is a computational technique that predicts interactions between ligands, such as medicines and biomolecules [8]. This approach is critical in drug development and discovery efforts since it allows researchers to find the compounds that can most effectively inhibit/modulate cancer protein activity without actually building and testing the compounds in a lab setting. HER2 receptors are located on the surface of cancer cells, particularly those from breast cancer. Docking allows for the simulation of interactions between HER2 receptors and potentially effective compounds and the development of a method of blocking this receptor, reducing the uncontrolled cell proliferation of cancer cells containing HER2 receptors.

Single-stranded nucleotides called aptamers can bind with particular target proteins [9]. Because of their great specificity and affinity for binding to cancer-specific markers, aptamers are perfect for targeting cancer cells with few off-target effects.

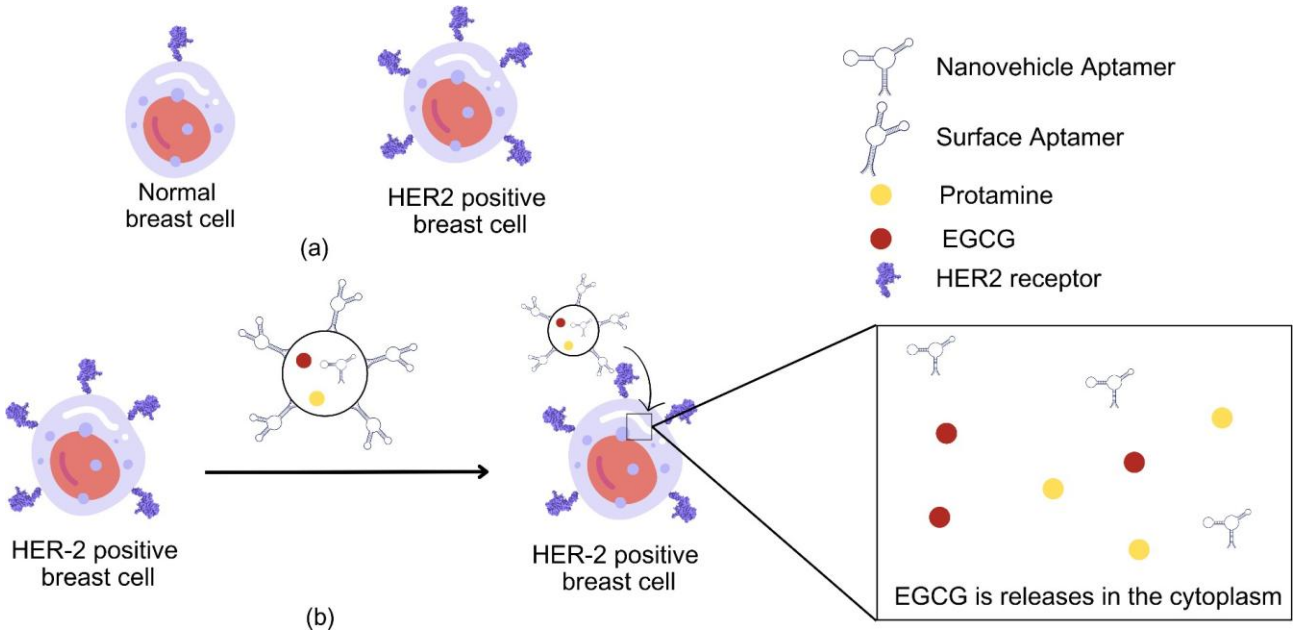

Fig. 1. Schematic of research.(a) The average breast cell has limited HER2 receptors on the surface, while HER2 receptor proliferation occurs on the breast cancer cell surface. These receptors are biomarkers for breast cancer. (b) The nanovehicle is equipped with an aptamer, protamine, and EGCG (an anticancer compound) inside, and a surface aptamer is on the vehicle's surface. The surface aptamer recognizes and binds to the HER2 receptors on breast cancer cells. In the next step, the nanovehicle enters the cancer cell cytoplasm and releases its contents, resulting in anticancer activity against the breast cancer cells.

They are effective and adaptable for both therapeutic and diagnostic applications due to their adaptable structure, which enables precise targeting of distinct cancer cell receptors. These include proteins, molecules, and cells; the aptamer can bind them with high affinity and specificity. SELEX was used to select these synthetic molecules through iterative binding, separation, and amplification rounds [10]. Because aptamers can uniquely fold into 3D shapes, they can interact with the target similarly to antibodies [9]. Due to their chemical modifiability, ease of synthesis, and stability, they are helpful in many applications, such as therapeutic, diagnostic, and research.

Recently, Liang et al. developed a drug delivery nanovehicle incorporating an anticancer compound targeting the HER2 receptor in breast cancer [11]. The nanovehicle comprises a biodegradable polymeric core (e.g., PLGA), encapsulating the drug payload, and a lipid envelope for stability [11]. It is functionalized with cascaded aptamers for HER2 targeting and precision drug release in HER2-overexpressing breast cancer [11]. Therapeutic compounds can be delivered directly to specific cells or tissues using a nanovehicle, which is a nanoscale carrier. By increasing stability, lowering adverse effects, and permitting controlled, site-specific release, it improves drug delivery, increasing therapy effectiveness and limiting harm to healthy cells. The cartoon representation of their drug delivery method is shown in Figure 1b. The interior of the nanovehicle contains a protamine-aptamer complex that binds to epigallocatechin gallate (EGCG-an anticancer medication), while the surface comprises a HER2 affinity aptamer. Protamine is described as a cationic protein that helps condense nucleic acids within the nanovehicle, facilitating efficient cellular uptake. Green tea contains a polyphenol called EGCG, which has antioxidant qualities. It works by stopping cell division and triggering death, especially by focusing on cancer cells' PI3K/AKT and NF-κB pathways. The nanovehicle is a carrier that is bound to cancer cells and transports the anticancer medication into them. We hypothesize that the surface aptamer binds to the specific site of the HER2+ receptor and could help detect cancer cells. Additionally, we suggest that unique interactions between ATP and the aptamer-protamine complex within the cell influence the interior aptamer's anticancer drug release mechanism. We conducted in silico investigations to estimate these aptamers' three-dimensional structure and interactions with HER2 and protamine. We discovered that the surface aptamer creates a particular linear shape necessary for HER2 receptor interactions. The inner aptamer is small and forms a patterned structure with the protamine, releasing EGCG into the cytoplasm in the presence of abundant cytoplasmic ATP. These findings will contribute to studying aptamer-receptor binding and cancer medication release mechanisms and developing a possible medication delivery platform that is biologically safe and has great transport accuracy. Protamine-bound drug formulations use the naturally occurring, positively charged peptide protein protamine to bind and stabilize specific medications, especially negatively charged ones like heparin or nucleic acids [12]. This idea improves medications' administration, bioavailability, or therapeutic profile by taking advantage of the potent ionic interactions between

the negatively charged medicines and the positively charged protamine [12].

2. RESULTS

The term "druggable/binding site" refers to the capacity of medications or ligands to target and bind to particular protein areas effectively. We anticipated that the aptamer would attach to a particular HER2 receptor binding site. To find the universal binding site on the receptor's surface, we employed a graph neural network (GNN) with the Graph Attention Site Prediction (GrASP) web server. The aptamer will most likely attach to this binding site as well. It highlighted the most significant areas of the graph, which also shows the likely site for aptamer binding. Critical atomic interactions and structural properties are learned by the GNN to predict the binding location. To validate this, the binding site on the HER2 surface was estimated using a machine-learning method known as P2Rank [13]. It predicts the ability to bind ligands on the HER2 surface, or ligandability. The binding sites obtained from both methods is displayed in (Figures 2a and b). The term "druggable/binding site" refers to the capacity of medications or ligands to target and bind to particular protein areas effectively. Developing therapies that bind to these macromolecules requires the identification of druggable/binding sites.

The next stage was to predict the 3D structure of aptamers by using the Vfold2d and Vfold3d to make the 3D structure of aptamers clear [14]. This study involved four aptamers: HB5 Aptamer and ATP Aptamer Control inside the nanovehicle and ATP Aptamer and HB5 Aptamer control on the nanovehicle surface. The control aptamer is a nonspecific DNA sequence of equal length to the aptamer for comparison of binding to the HER2 receptor. Table 1 displays the aptamer sequences used in this study. The outer HB5 aptamers were docked on the HER2 receptor to find their interactions. The HB5-aptamer docking was performed using the HDOCK2.4 software, Figure 2d [15]. Their interactions were calculated by the PLIP software, as shown in Table 2. Based on the aptamer structure prediction and aptamer-HB5 binding, the mechanism of aptamer binding and functioning is shown in Figure 3. Amino acids of the HER2 receptor that interact with aptamers are mostly present in the predicted binding site of the HER2 receptor. For instance, Asn416, Gln424, Gly425, His451, His415, His447, and His448 are present in the binding site of the HER2 receptor. On the other hand, only four amino acids, Ser259, Ala276, Cys277, and Gln462 of the HER2 receptor were interacting with the control aptamer. This shows that the HB5 Aptamer binds more strongly to the predicted binding site of the HER2 receptor.

The protamine amino acids involved with the binding to the aptamer were Arg9, Ser29, Arg 24, Cys30, Thr32, Arg33, Met37. The interactions were mainly due to the highly positive nature of the protamine protein. Due to its high specificity for HER2, the HB5 aptamer performs better than the control aptamer, enabling precision drug delivery to breast cancer cells that overexpress HER2. Compared to the non-specific control aptamer, the therapeutic efficacy is significantly improved, and off-target effects are reduced. Similarly, the HB5 aptamer demonstrated a better binding orientation concerning the HER2 receptor in our computational analysis. The HB5 aptamer attaches vertically to the HER2 receptor, whereas the control aptamer binds horizontally, as shown in Figure 2d. Both HB5 and control aptamer sequences were obtained by Liang et al. Vertical binding is preferred over horizontal binding for optimal nanovehicle attachment. This orientation enables more efficient cell-nanovehicle interaction. We did not model the ATP binding and aptamer/drug release mechanisms since they are computationally expensive. Still, we did model the aptamer-HB5 and ATP-protamine complexes, as illustrated in Figure 4. These models, created with AlphaFold3, show how the negatively charged HB5-aptamer attaches to the positively charged protamine protein. We hypothesize that the surface HB5-aptamer binds to the specific site of the HER2+ receptor and could help detect cancer cells. Conversely, negatively charged ATP can attach to positively charged protamine proteins. ATP's high charge density allows it to dislodge the HB5-aptamer from the protamine. These transitions are influenced by ionic strength, pH, and other cell-environmental variables.

We used the CHARMM-GUI web server to model a 200 Å nanovehicle and estimate its drug payload capacity, Figure 5. Our findings show that 20 protamine molecules, each associated with an ATP-aptamer, may fit inside a single nanovehicle of this size. However, due to AlphaFold 3 constraints, which limit the number of protein copies per model, we could only insert 20 protamine-ATP-aptamer complexes. Despite this limitation, the model gives an early estimate of the payload capacity that a nanovehicle can provide. Calculating a single nanovehicle's payload capacity is critical for dose optimization, safety, and therapeutic effectiveness. Knowing how much therapeutic agent may be loaded allows researchers to create nanovehicles that improve medication delivery while limiting potential adverse effects. This information promotes cost-effective production, prevents waste of precious treatments, and assures regulatory compliance for clinical applications. Understanding the payload capacity for the best possible development and use of nanomedicine technology is essential.

3. DISCUSSION

Aptamer-HER2 receptor interactions. The bonds formed between aptamers and the HER2 receptor are shown.

Despite aptamers' popularity in drug delivery and disease imaging, they face several challenges that must be considered [16]. For instance, aptamers are susceptible to nucleases, which degrade them and reduce their half-life and effectiveness [16]. This problem is generally overcome by making reasonable chemical mutations that depend on the important base pairs involved in the protein binding. Moreover, delivering the aptamers to the targeted cell is difficult. It's also challenging to make sure aptamers can interact with the intracellular target through the plasma membrane. A promising approach to overcome all these challenges is using aptamer-conjugated nanomaterials with target-specific binding capacity. Some common nanomaterials are nanoparticles, nanoconjugates, and nanorods. These exhibit unique properties like small size, large surface area, and the capacity to get beyond biological barriers [17] [18]. ATP-triggered drug research is a targeted drug delivery method in which drugs are released in response to elevated ATP levers. Higher ATP levers are common in tumors and inflammatory tissues. The drug is entrapped in a nano vehicle and with an ATP-response element such as aptamers. A higher ATP concentration makes structural changes in the aptamer and releases the drug in a precise location. The nanovehicle-targeted drug transport mechanism heavily depends on the structure of the aptamer. Therefore, we have predicted the aptamer 3D structure for both ATP aptamer (present inside the nanovehicle) and HB5 aptamer (present on the surface). The ATP aptamer is smaller and circular with only 27 base pairs and helps bind

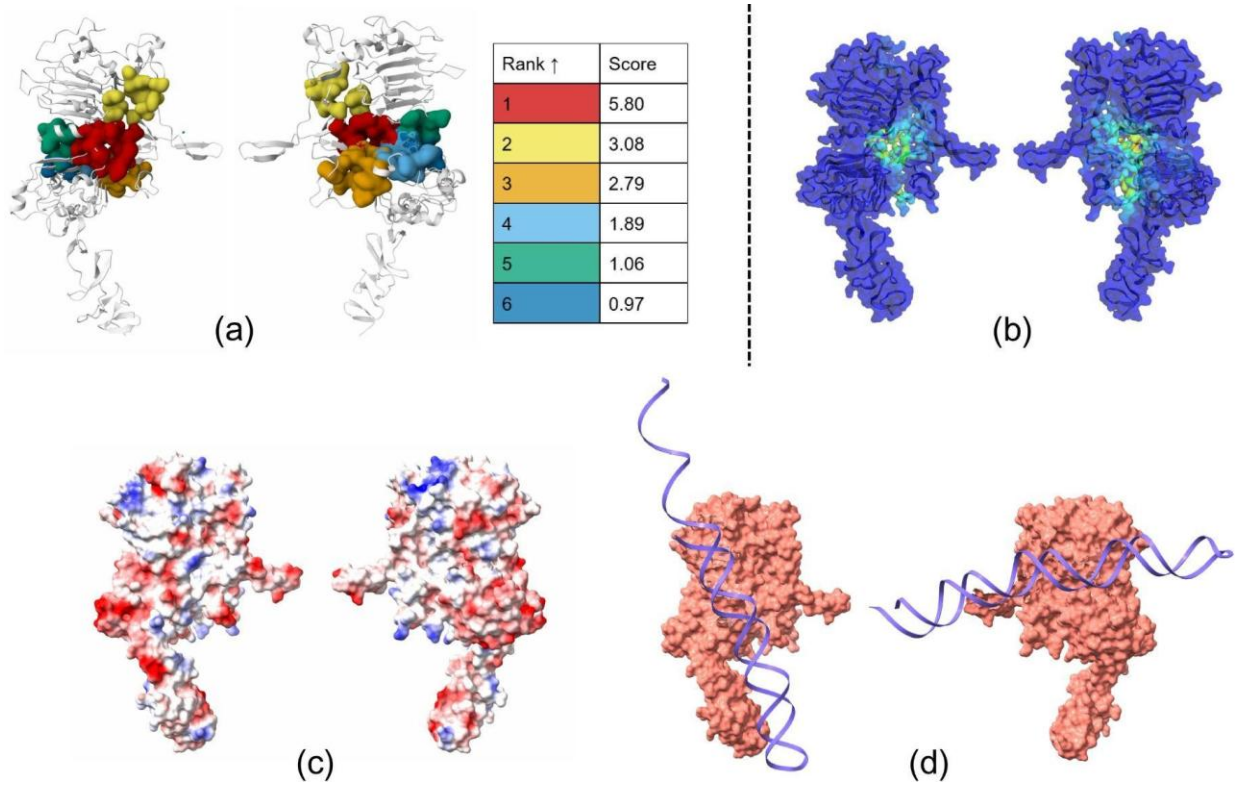

Fig. 2. The HER2 receptor's druggable location.(a) The P2Rank web server identified the druggable site; (b) The GrASP web server forecasted the druggable site, which is displayed in yellow and green; (c) the Electrostatic Surface Potential (ESP) of the receptor which shows the charge on the surface of the protein. In ESP the red is positive charge, blue is negative charge, and white is neutral charge; and (d) the HB5 Aptamer (left) binds linearly and HB5 Aptamer (proper) control binds horizontally to the HER2 receptor.

Table 1: Sequences of aptamers used in this study. The ATP aptamer and ATP Aptamer Control were present in the center of the nanovehicle, while the HB5 Aptamer and HB5 Aptamer Control were present on the surface.

Aptamer Name	Aptamer sequences
ATP Aptamer	ACCTGGGGGAGTATTGCGGAGGAAGGT
ATP Aptamer Control	ACCTTCCTCCGCAATACTCCCCCAGGT
HB5 Aptamer	AACCGCCCAAATCCCTAAGAGTCTGCACTTGTCATTTTGTATATGTATTTGGTTTTTG-GCTCTCACAGACACACTACACACGCACA
HB5 Aptamer Control	AAAAAAAAAAAAAAAACGTGCAGTACGCCAACCTTTCTCATGCGCTGCCCCTCTTAAG-TACGCCAACCTTTCTCATGCGCTGCCCCT

ECGC and protamine. On the other hand, the HB5 aptamer is an 86-base pair long and linear shape aptamer that binds to the HER2 receptors present in abundance on the cancer cell's surface. The linear and long shapes are essential for aptamer to extend and bind to the HER2 receptor. Molecular docking shows that the HB5 interacts with the binding site on the HER2 receptor, further confirming our results. The HB5 receptor's Lenier orientation will let it attach to the HER2 receptor head-on. "Head-on" describes how the HB5 aptamer directly and linearly binds to the HER2 receptor, strengthening the nanovehicle's adherence to cancer cells for efficient drug delivery. For the best possible binding and therapeutic effectiveness, this orientation is essential. Nevertheless, docking simulations demonstrate that the HER2 receptor is vertically bound by the control receptor. As a result, the Her2 receptor and the control receptor are unable to interact correctly. These results also show that a head-on binding between aptamer and HER2 receptor is essential for the nanovehicle binding to the cancer cell's surface.

Another critical factor in designing nanomaterials is the complex's payload or drug-load capacity [17]. A high payload capacity is crucial for effective drug delivery, with higher therapeutic effects and minimum side effects [17]. Because the quality of the models and algorithms that the corresponding software uses for molecular docking rely on them, faulty models may produce false positives. In addition, molecular docking and 3D structure heavily depend on the type of data entered into the system; therefore, experimental validation will be required in future studies. In the future, performing MD simulations in water would be more optimal. In addition, these molecular dynamics simulations will better inform whether or not the aptamers would stay with the HER2. In contrast to docking, which assumes stiff or semi-flexible structures, MD simulations incorporate molecular flexibility, solvent effects, and dynamic interactions throughout time. MD is perfect for researching biomolecular interactions in a physiological setting because it offers a more accurate depiction of binding stability and conformational changes. Furthermore,

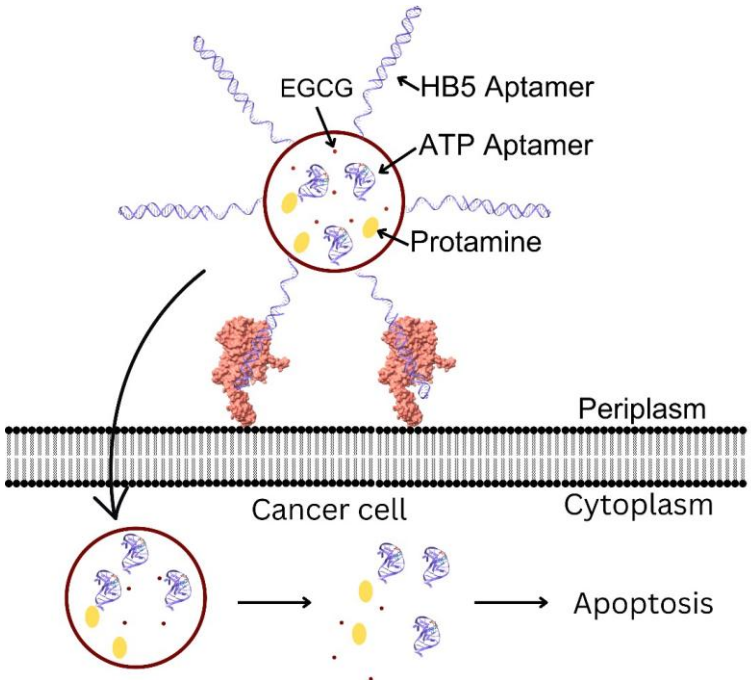

Fig. 3. Mechanism of aptamer binding and functioning. The ATP aptamer, protamine, and EGCG (anti-cancer compound) form a complex inside the nanovehicle. The surface aptamer is located on the surface, and due to its linear shape, it can easily access the HER2 receptor. The nanovehicle reaches the cytoplasm upon binding, and the payload is released to the cytoplasm. Finally, excess cytoplasmic ATP binds the protamine to the ATP molecules, and EGFG is released to the cytoplasm.

the binding energy between the aptamer and HER2 will be determined using MD simulations. To advance the authenticity of the research, experimental methods such as Surface Plasmon Resonance (SPR) or Isothermal Titration Calorimetry (ITC) might be used as a next step after MD simulations [19]. The binding interactions into the HER2 will be explained by these experimental methods.

The study focuses on computational methods for docking HER2 receptors to aptamers. To identify druggable HER2 receptor regions, we used P2Rank and GrASP web service. The HB5 aptamer has been shown to interact substantially with specific amino acids in the HER2 receptor, revealing potential binding sites. Several approaches were used to confirm docking interactions, which were then visualized using molecular modeling tools. The work stresses the value of aptamers in targeted cancer therapy, particularly for HER2-positive breast cancer, and the importance of computational techniques in identifying and validating druggable sites on cancer-related proteins. Future studies will improve docking prediction accuracy and experimentally validate recommended contacts to further the development of aptamer-based nanomedicine.

4. METHOD

The nanovehicle 3D structures were derived using the CHARMM-GUI platform and the following configurations determined by the work of Liang et al. were used: monostearate 57.09%, polyoxyethylene stearate 19.69%, octadecyl amine (ODA) 9.84%, and oleic acid (OA) 13.39% [20]. CHARMM-GUI is used for configuring, modeling, and simulating biomolecular systems. The structures were created by entering the required parameters into the CHARMM-GUI, which subsequently analyzed the data to build precise and detailed 3D models. These models were then utilized for additional research and simulations. The aptamer's fundamental structure (aptamer sequence), which was discovered through earlier research, served as the basis for the structural predictions of the aptamer. This step involves uploading the aptamer base pair sequence to the Vfold2D website to obtain the aptamer's secondary structure [14]. These were useful in figuring out 3D aptamer structure and function. In dot-bracket notation, dots represent non-bonded nucleotides, and a pair of opened and closed parentheses represent bonded base pairs. Vfold3D was used to model the tertiary structure. Several web servers offer the lowest energy structure, or the most stable structure, to validate the structure. HDOCK2.4 simulated aptamer binding to the HER2 receptor [21]. A graph neural network (GNN) was employed to obtain the receptor's binding site, known as GrASP [22]. Additionally, the P2Rank webserver was used for the same objective. By comparing the found binding sites to known HER2 receptor binding regions, the accuracy of binding site predictions made with GNN and P2Rank was evaluated, confirming that predicted locations match the druggable areas shown in experiments. The Protein-Ligand Interaction Profiler (PLIP) webserver was used for the HER2-aptamer interactions [23].

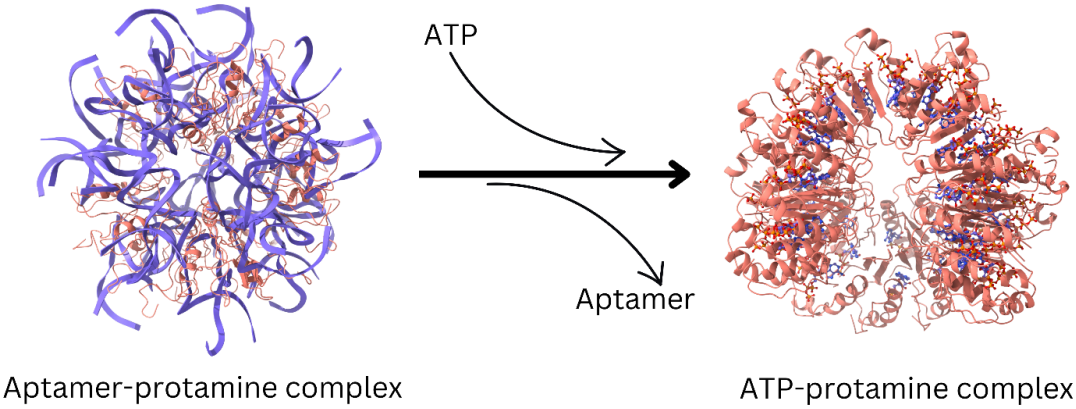

Fig. 4. Proposed structure of Aptamer-protamine and ATP-protamine complex. The highly positive protamine (pink) binds to the highly negative aptamer (purple) and forms a circular complex. In the presence of ATP, the aptamer-protamine complex is replaced by ATP, resulting in the ATP-protamine complex.

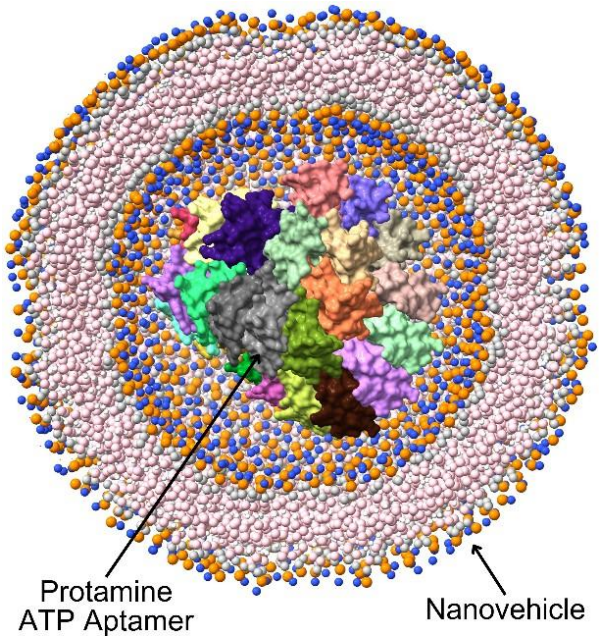

Fig. 5. Model of Nanovehicle. Protamine-bound ATP aptamer is in the middle. In the nanovehicle only 20 protamine-aptamer complexes

Table 2. Sequences of aptamers used in this study. The ATP aptamer and ATP Aptamer Control were present in the center of the nanovehicle, while the HB5 Aptamer and HB5 Aptamer Control were present on the surface.

HB5 Aptamer		
Hydrogen Bonds	Amino Acids	Distance (Å)
	Tyr42	2.26
	Asn416	3.07
	Gln424	3.23
	Gly425	2.35
	His451	2.27
Salt Bridges	Arg332	5.31
	Arg332	3.43
	His415	5.29
	His415	5.18
	His447	4.48
	His448	4.22
	His448	4.88
HB5 Aptamer Control		
Hydrophobic Interactions	Amino Acids	Distance (Å)
	Tyr252	3.48
	Tyr252	3.77
	Pro261	3.28
	Pro261	3.30
Hydrogen Bonds	Ser259	2.21
	Ser259	3.28
	Ser259	2.80
	Ala276	3.17
	Cys277	3.11
	Asp304	2.57
	Asp304	1.55
	Asp304	2.48
	Asp304	1.56
	Gly305	3.28
	Arg308	3.55
	Glu310	3.16
	Lys311	2.17
	Gln462	2.90
Salt Bridges	Lys311	2.53
	Arg536	4.78

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