Designing L-RNA-Based Spiegelmers Against Ecto-GPR37: A Novel Approach for Parkinson's Disease Biomarker Detection

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Parkinson's disease (PD) is a neurological condition that progressively deteriorates the nervous system over time. One potential biomarker associated with this disease is Ecto-GPR37, the extracellular portion of the GPR37 protein. To enable its detection, we propose the use of spiegelmers—synthetic mirror-image oligonucleotides composed of L-ribose instead of the naturally occurring D-ribose found in RNA and DNA—making them inherently resistant to nuclease degradation. The research aimed to design and evaluate L-RNA-based spiegelmers targeting ecto-GPR37 as stable molecular probes for the early detection of PD. We hypothesize that spiegelmers can bind to the predicted binding site of ecto-GPR37 and serve as molecular probes for its detection. Moreover, the conversion of nucleotides from the D-form to the L-form is expected to enhance their stability by preventing enzymatic degradation. To investigate this, we employed AlphaFold 3 to generate a high-confidence three-dimensional structure of the ecto-GPR37 protein. In parallel, RNA sequences were initially modeled using RNAfold and subsequently converted into 3D structures using SimRNA. These D-form RNA structures were then transformed into their L-form counterparts via an in-house script. Following this, the spiegelmers were docked onto both ecto-GPR37 and RNase H1 using the HDOCK software to assess their binding potential and nuclease resistance. The resulting docked complexes were further analyzed using the PLIP web server to characterize the molecular interactions involved. Our analysis revealed that RNA A1 exhibited strong binding affinity to the ecto-GPR37 protein and was therefore selected for downstream evaluation. Overall, the findings from this study support the design and application of nuclease-stable spiegelmers as a novel approach for the detection of PD.

1. INTRODUCTION

Parkinson's disease (PD) is a neurodegenerative movement disorder that progressively damages the nervous system over time, Figure 1.(1) Initial symptoms of PD begin with tremors of appendages and progress into dysfunctional motor control and bradykinesia as well as speech difficulties.(2) Factors that influence the development of PD consist of genetic and environmental backgrounds.(1) With this neurodegenerative condition, changes in the brain result in the presence of Lewy bodies—with presence of Alpha-synuclein protein found within—along with altered mitochondria.(3) Risk of PD increases with age (typically proceeding 50 years), genetic relation to affected individuals (first-degree relatives), male sex, and exposure to chemicals like certain herbicides and pesticides.(4) Due to symptoms being subtle and often associated with other conditions during early stages of PD, early diagnosis is challenging. Although lacking a cure, PD is treated with Deep Brain Stimulation (DBS) to alleviate symptoms regarding motor functions.(4)

Due to symptoms commonly being mistaken for those of other conditions, PD is often difficult to diagnose in the early stages. A popular test to assist in the diagnosis of PD is DaTscan; it is an imaging test that utilizes radioactive tracers in the bloodstream to highlight the signals from the striatum.(5) Magnetic resonance imaging (MRI) can also be used for diagnostic purposes along with biomarker tests.(3) To formulate tests to assist with the diagnosis of PD, biomarkers—a measurable indicator for biological conditions, like ecto-GPRT7—are utilized. Ecto-GPR37 is the extracellular fragment of the membrane protein GPR37, primarily found in the central nervous system (dopaminergic neurons).(6) The primary function of GPR37 includes dopamine signaling, neuron survival, and the regulation of parkin. The misfolding of protein GPR37 is an indicator of

PD, and the cleaved buildup of ecto-GPR37 is used as a potential biomarker to indicate PD.(6)

Spiegelmers—a synthetic oligonucleotide (specifically L-nucleotides as opposed to naturally occurring D- nucleodies)—that bind to biological targets without risk of degradation by enzymes.(7) Due to resistance against damaging enzymes, spiegelmers can be used as a biosensor to detect biomarkers with increased stability and specificity compared to other alternative methods.(7) Argerich et al. have found that GPR37 can be a potential biomarker of PD and could be used in the early detection of the disease, Figure 2. We hypothesize that spiegelmers can bind to the predicted binding site of ecto-GPR37 and serve as molecular probes for its detection. In this research, we have performed computational simulations to identify compounds that can not only be used for the detection of GPR37 but also prevent nuclease degradation. The results show that S2 formed the strongest interaction with the GPR37 protein and could be used in its detection. The current results will aid in the design of novel methods for detecting and treating PD.

2. METHOD

To obtain the amino acid sequencing of protein ecto-GPR37, UniProt was utilized (ID: P08195).(8) Uniprot is an extensive database with comprehensive information regarding protein sequence and function. We used this resource to extract the complete amino acid sequence, which was then used for structural modeling and visualization. To predict the 3-dimensional structure of ecto-GPR37, we used AlphaFold 3, an AI modeling tool that predicts 3-dimensional structures of proteins along with accurate biomolecular interactions that are simultaneously involved.(9) The tool enabled us to model the tertiary structure of ecto-GPR37, which is essential for understanding how it may interact with other molecules and function biologically. For visualizing the membrane of ecto-GPR37, we utilized PROTTER, an interactive web-based tool that allows for visualization of protein sequences and their subsequent features.(10) PROTTER allowed us to map extracellular, transmembrane, and intracellular domains of protein GPR37, offering insight into how the protein is positioned in the plasma membrane.(10) To further showcase the subcellular localization of GPR37, we used the Human Protein Atlas, a research resource focused on mapping all human proteins in six main fields: tissue, cell, pathology, blood, brain, and metabolism.(11) Through this platform, we confirmed dopaminergic neurons express GPR37; this supports its relevance to potential relation to PD. In addition to protein modeling, Spiegelmers were designed as synthetic mirror images of RNA aptamers composed of L-oligonucleotide as opposed to naturally occurring D-ribose.(7) Spiegelmer's high resistance to enzymatic degradation and high target-binding specificity make it ideal for utility as a diagnostic biosensor. Three Spiegelmer sequences were selected for modeling, as shown in Table 1. Each sequence was submitted to mFold to generate 2-dimensional models of RNA and obtain CT files.(12) We then converted the CT-files to dot-bracket notations using the RNAstructure ct2dot server.(13) The dot-bracket notation and the primary sequence are input into web-based platforms SimRNAweb and Rosetta FARFAR2 to predict the 3-dimensional structure of the RNA.(14)

The table shows the RNA sequence and the dot-and-bracket sequence of the RNA utilized in this study. The dot-and-bracket sequence was obtained by using RNA structure software.

3. RESULTS

The location of protein GPR37, as shown on the left portion of Figure 3, is to be located in the transmembrane portion of the cells; the ecto-GPR37 portion is considered to be the extracellular region of protein GPR37. In the subcellular level, as shown in the right portion of Figure 3, protein GPR37 is shown to be located on the cellular plasma membrane as well as inside the nucleoplasm of the cell.

As showcased by Figure 4a, the protein was simulated into a 3D model utilizing RNAstructure software to convert animo-acid sequence into 2-dimensional models then utilized with the red coloration indicating its associated binding site. Figures 4b, c, and d represent the three different models of RNA and their associated molecular binding structure with protein GPR37 in a 3-dimensional format. In the next step, we performed a detailed protein–spiegelmer interaction analysis using the Protein–Ligand Interaction Profiler (PLIP) web server. As shown in Figure 5, the S3 spiegelmer demonstrated the strongest and most stable interaction profile among all the candidates. Specifically, S3 formed 23 hydrogen bonds and 7 salt bridges, indicating an exceptionally tight and energetically favorable binding mode. In addition to these interactions, PLIP analysis revealed multiple hydrophobic contacts and π–cation interactions, which further stabilized the complex and contributed to specificity. The presence of several water-mediated bridges also suggests a well-organized binding interface that enhances structural complementarity between the spiegelmer and the protein. Overall, the rich interaction network—particularly the high number of hydrogen bonds and salt bridges—supports that spiegelmer S3 has a high binding affinity and strong conformational fit. Based on these detailed interaction patterns, S3 was selected as the most promising candidate for use as a protein detection tool, enabling sensitive and specific recognition of the target biomarker.

4. DISCUSSION

Since this research study employed computational tools to model the structure and membrane positioning of ecto-GPR37 and design potential spiegelmer-based aptamers, it is essential to acknowledge the limitations inherent in this approach. As a fully computational paper, the findings are predictive and require experimental validation, which can be conducted through laboratory methods to confirm the study's integrity. Along with the complete computational aspect, only three spiegelmers sequences were modeled; this represents a limited poll of candidates for targeting ecto-GPR37. The small sample size restricts the potential aptamer-protein interactions that could be explored. In a future study, an increased number of aptamers, along with the integration of wet-lab experiments, would be essential to convert these computational findings into practical applications in the biomedical field.

5. CONCLUSION

In the current research, we have performed computational modeling of a spiegelmer that can target Ecto-GPR37, a promising biomarker for PD. Protein modeling and docking identified spiegelmer S3 as the most promising candidate, forming strong and stable interactions with ecto-GPR37. The use of L-form nucleotides provides intrinsic nuclease resistance, enhancing stability for diagnostic applications. While experimental validation is still needed, these results highlight spiegelmers as a novel and durable approach for early PD detection.

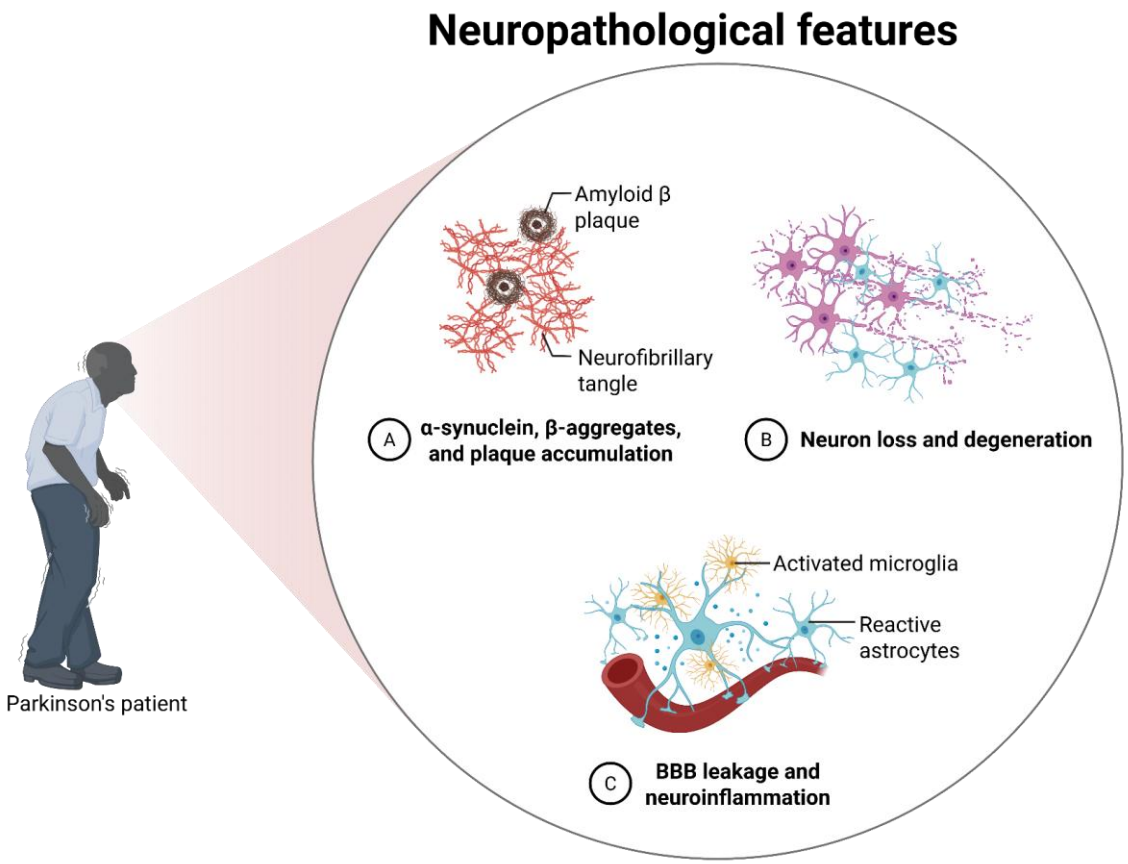

Fig. 1. Figure showcases the neuropathological features of PD. Synuclein proteins clump together, causing the formation of plaque. Neurons begin to degenerate and decline in numbers. This results in an increase in neuroinflammation, which leads to further damage to neurons in the brain. The figure was generated by using Biorender.com.

Table 1. The table shows the RNA sequence and the dot-and-bracket sequence of the RNA utilized in this study. The dot-and-bracket sequence was obtained by using RNA structure software.

Seq. No.	RNA Sequence	Dot-Bracket Notation
1	GGUACGUUAGCGUACGCUGAUCCAGUGGAUCGUACGUUAGCU	.....((((((((((...((((((...)))))))))))))))).
2	ACCGUAGCUUGACGAUCGGUAGGCUAGCUUGACCGUACGUAGUGCUAGACG	....((((...(((..(((((((....)))).)))..)))...))))....
3	UGGAUCGUAGCUAGUCCGAUCGUAGGCUUACGUAGUCCGAUCGGUACUAGUCGU	.........((((((((((((..((((.....))))))))))).))))))...

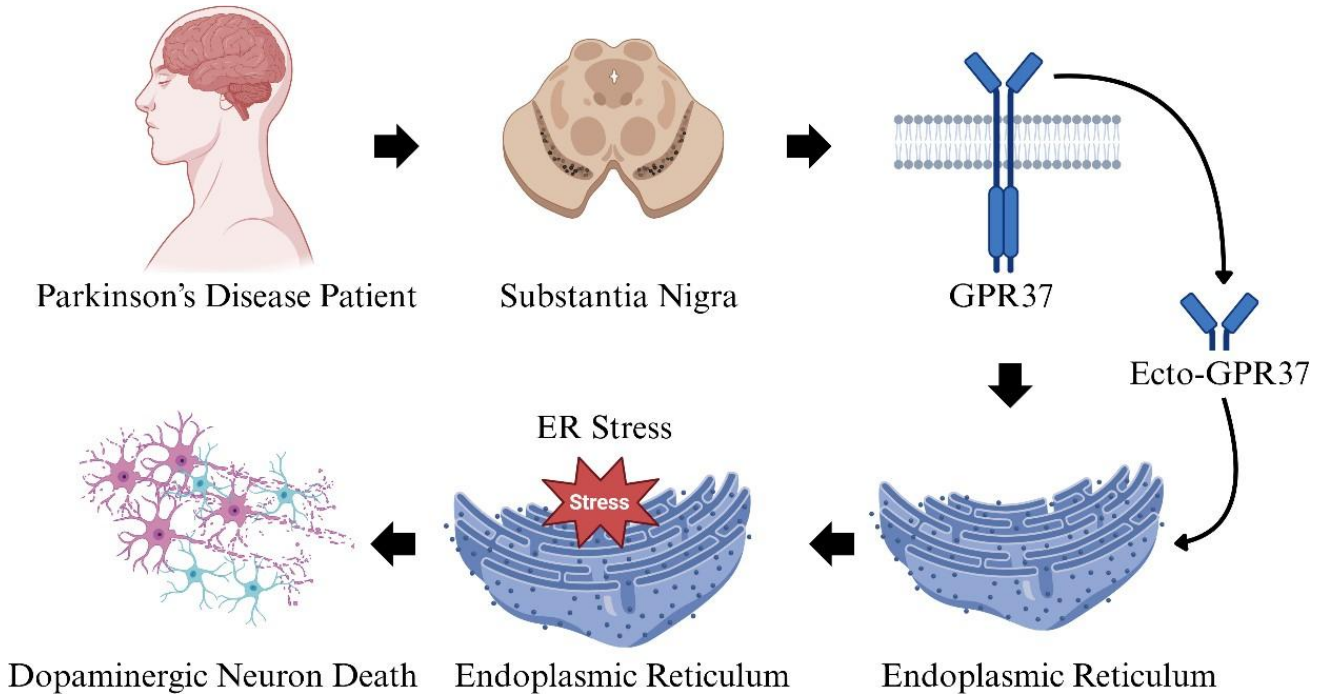

Fig. 2. This figure showcases the nerve degeneration in PD patients. In the affected individual's substantia nigra, the overexpression of the GPR37 receptor (located on the surface of neurons) leads to the excess release of ecto-GPR37. Ecto-GPR37 travels to the endoplasmic reticulum, leading to increased stress and the consequent death of dopaminergic neurons. The figure was generated by using Biorender.com.

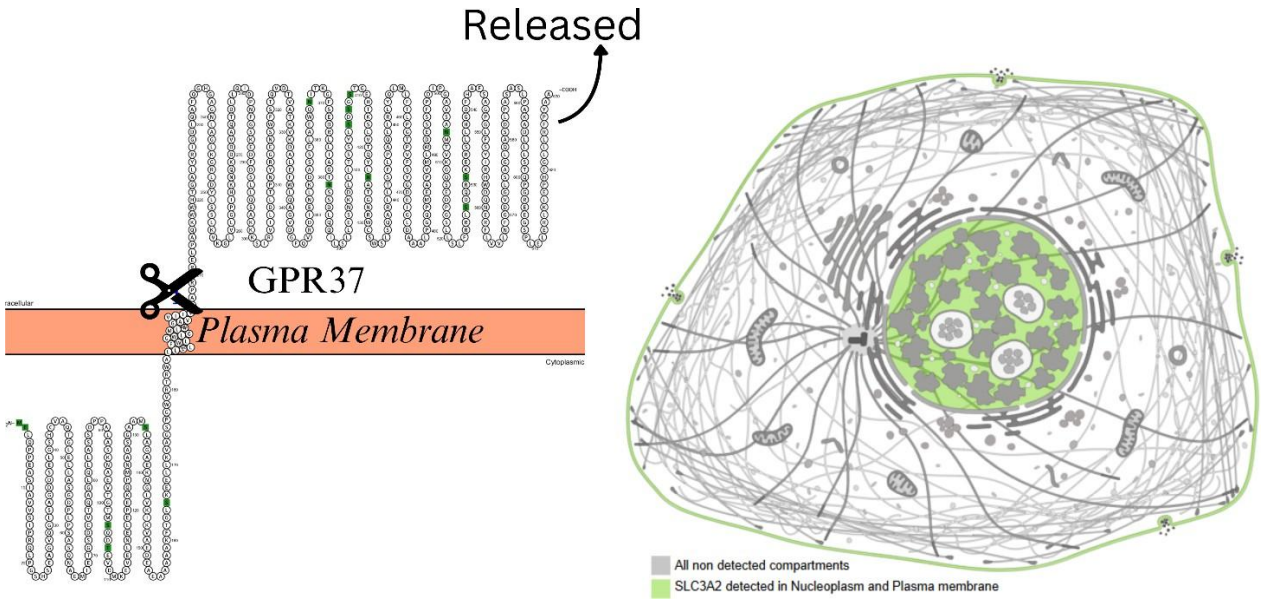

Fig. 3. Protein GPR37 is present on the cell surface, and a protein image within the cellular membrane is obtained through the use of PROTTER. Protein GPR37 was also identified to be located in the cell surface and nucleus, as showcased by the image obtained from The Human Protein Atlas.

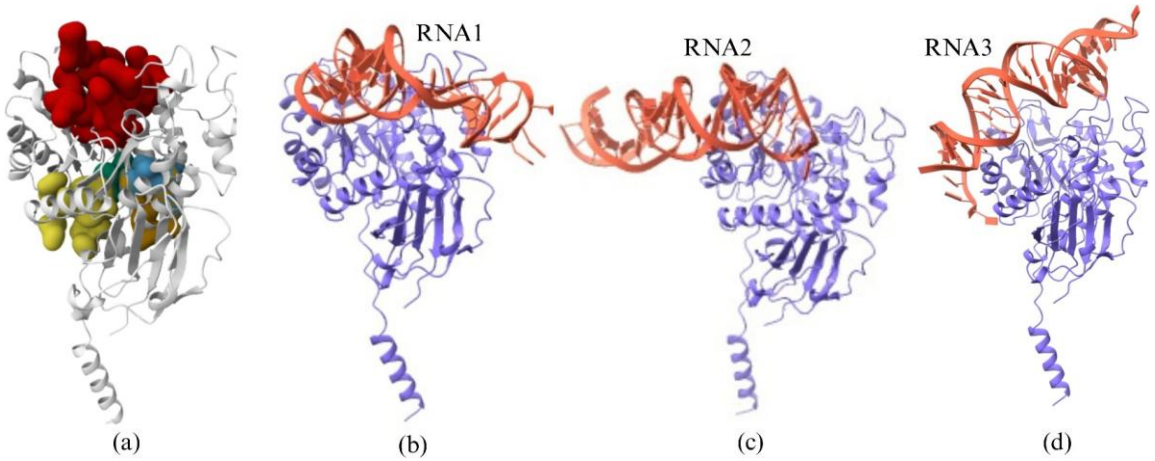

Fig. 4. (a) shows the binding site on the surface of the protein; the red colored portion is the most prominent region for the RNA-spiroglimers to bind with the protein. Figure (b), (c), and (d) depicts the molecular docking structure of ecto-GPR37 and the three RNAs utilized in the study. The docking structures were obtained using the HDOCK software.

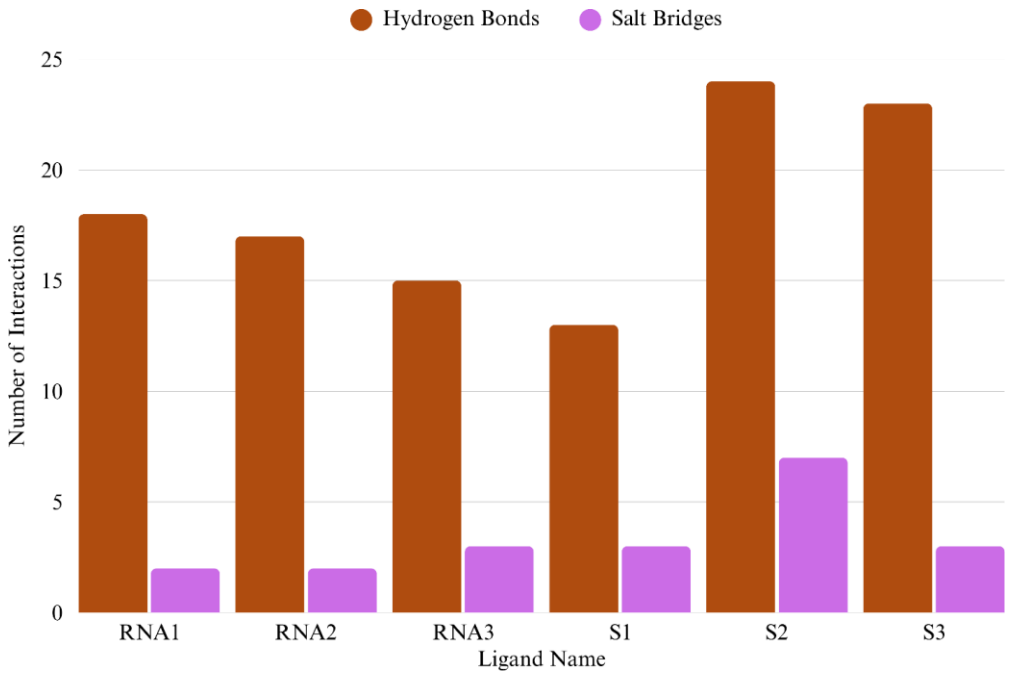

Fig. 5. Illustrates the types and numbers of bonds between the RNA and protein. Salt bridges and hydrogen bonds were the two types of bonds observed. According to this study, S2 formed the highest number of bonds and was identified using the PLIP server.