Therapeutic Inhibition of Mutant MnSOD Using Aptamers to Attenuate Mitochondrial Oxidative Stress in Depression

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Depression imposes a substantial burden not only on affected individuals but also on society at large and is characterized by reduced productivity in daily activities, increased healthcare costs, diminished social engagement, and a heightened risk of comorbid health conditions, all impairing an individual's ability to lead a healthy, functional, and fulfilling life. Manganese superoxide dismutase (MnSOD) is an essential mitochondrial enzyme that acts as a protective antioxidant by neutralizing reactive oxygen species (ROS). Mutated forms of MnSOD compromise mitochondrial antioxidant defenses, leading to oxidative stress, neurodegeneration, and mitochondrial dysfunction. Therefore, inhibiting the mutated form of MnSOD could serve as a potential strategy to limit mutation-induced neuroinflammation, ultimately reducing the risk of depression. Aptamers are single-stranded nucleotide molecules that bind specifically to target proteins. We hypothesize that Aptamer 1 is the most suitable candidate for mitigating depression by binding to and inhibiting the MnSOD protein. Computational tools evaluated various aptamers' binding affinity and efficiency to MnSOD. Protein modeling was conducted using AlphaFold3; aptamer structures were modeled using UNAfold and FARFAR2; and aptamer-protein docking was performed using HDOCK. PLIP was used to analyze the molecular interactions between the aptamers and MnSOD. Among the tested aptamers, Aptamer 1 demonstrated the highest number of hydrogen bonds with MnSOD, suggesting superior binding efficiency. As MnSOD is implicated in neuroinflammation and elevated cortisol levels—both contributing factors to depression—Aptamer 1 may offer therapeutic potential by reducing MnSOD-related oxidative stress. This study supports the development of novel aptamer-based therapeutics for depression and related neuroinflammatory conditions.

1. INTRODUCTION

Depression, otherwise known as MDD (Major Depressive Disorder), is a mental health condition portrayed by persistent and long-lasting sad episodes, loss of interest in daily activities, and often these symptoms heavily impact everyday life, unlike typical mood changes/feelings. [1] Though depression can affect anyone, specific demographics are more prone. [2] For instance, young adults between the ages of 18-29, those of Hispanic/African descent, and women are generally more likely to be depressed from their respective demographics of age, race, and gender. [3] This may have to do with a history of oppression in many areas, and the constant stress passes down epigenetically to future generations. [4] Particularly for women, due to their reduced privileges compared to their male counterparts, other unnamed biological aspects, and inherited traits may have significantly influenced this disproportionate gap; women are twice as likely to be depressed as men. Racially, those of Hispanic and African descent are known to have faced barriers in everyday life, such as stigma, poorer health systems, and lower rates of health insurance, and the added stressors of these social and economic disparities, apart from personal circumstances, may have chemically altered these groups' likelihood of depression.

Single-nucleotide polymorphisms (SNPs) are the most common type of genetic variation, with each SNP representing a block that contributes to the formation of the final DNA sequence. [5] They serve as markers of diversity among populations, and though they have no direct effects on health, they are an essential field of study in human health. They are used to track the inheritance of genetic variants associated with complex diseases, such as cancer or heart disease. SNPs also aid in predicting drug response and identifying biomarkers for treatment.

Some methods used to detect SNPs include PCR-based methods and microarray technology, which is the most common. [6] The microarray technology prompts the array-contained probes to bind to specific SNP sites, which are scanned to reveal SNP genotypes and locations. A mutation is a change in the DNA sequence of an organism, often resulting from errors in DNA replication during cell division, infection, or exposure. [7] Mutations can be malignant, harmful, beneficial, or benign, meaning they have no effect. There are 2 types of genetic mutations: gene and chromosomal. Some common chromosomal mutations, though there are many, are deletion, duplication, inversion, and translocation.

MnSOD, Manganese Superoxide Dismutase, is an enzyme in the cell mitochondria. It protects cells from oxidative damage by neutralizing excess oxygen molecules into hydrogen peroxide/oxygen. [8] The primary function of this protein is to convert superoxide radicals, harmful byproducts of mitochondrial respiration, into hydrogen peroxide and oxygen. This process is essential for maintaining cellular redox balance and preventing oxidative damage to mitochondrial DNA, proteins, and lipids. [8] The nuclear SOD2 gene encodes MnSOD and is located in the mitochondrial matrix, where it serves as the primary defense against oxidative injury. [8] Chemical mutations in this protein have been linked with an elevated level of cortisol and neurodegeneration, resulting in depression. Figure 1 illustrates the mitochondrial localization of the MnSOD protein, with the binding site of the protein highlighted in the box. Reactive oxygen species and cell stress cause cell apoptosis, and the MnSOD-mediated ROS neutralization is shown in the box. In addition, MnSOD mediated neuroinflammation and depression pathways is displayed in Figure 2.

We hypothesize that aptamer 1 could be the most appropriate candidate for inhibiting depression by binding to and inhibiting the MnSOD protein. In the current research work, we utilized computational modeling software to generate data, which we analyzed to obtain our final aptamer results. Because Aptamer 1 formed the most hydrogen bonds with MnSOD compared to other aptamers, we concluded that Aptamer 1 was the most effective in this simulation for reducing depression. The current research will help in studies related to depression, searching for solutions to mitigate it as much as possible through new medicines and medical products.

2. METHOD:

To analyze Manganese Superoxide Dismutase (MnSOD), we began with the UniProt database. [9] This web-based database provides information on protein sequences and structures to generate a sequence-structure model of the MnSOD protein. The next step involved using Alphafold 3, a web-based tool for generating accurate biomolecular structure predictions of proteins and their chemical modifications, to predict the 3D structure of both MnSOD and its mutation, given its code. [10] Then, we used PROTTER, an interactive and customizable web-based tool that produces visualizations of predicted and annotated protein sequences, to generate the image of the protein's location in relation to the cell. [11] The last step in the basis of analysis was utilizing the Human Protein Atlas [12], an accessible database and research application aiming to model the complete human proteome, the set of all human body proteins, with PROTTER [11] in tandem to generate the image of the cell to aid in MnSOD protein location. Aptamer Modeling Steps: First, UnaFold, an RNA folding form, was used to process each uploaded DNA sequence into an image (shown in the slide) from 1D to a 2D ct file. [13] Then, the ct file was downloaded for each to produce the dot and bracket notations seen above, correlating with each sequence. Finally, using RosieLabs, the image was processed from 2d to 3d. Each aptamer was downloaded via RosieLab. [14] Then, the download was uploaded to hdock.phys website, as seen below, along with the MnSOD.pdb to determine which aptamer would be received best by the receptor.

1: Aptamer sequences and dot-bracket notations used in this research.

3. RESULTS

To model the protein and aptamer interaction, we needed to obtain the 3D structure of each aptamer. Due to the flexible nature of aptamers, obtaining a 3D structure can be difficult; hence, we used computational tools to model these aptamers. The 1st aptamer, nucleotide sequences, shown in Table 1, were uploaded and converted into 2D structures as shown in Figure 3. The 3D structure of each aptamer is based on the hydrogen bonds formed between the different nucleotide combinations, and Figure 3 shows that different nucleotide sequences have different 2D aptamer secondary structures. Each structure has the following: a bulb, a stem, and a tail.

As shown in Figure 4, once the aptamer 3d structure was obtained, we performed the necessary steps through molecular docking simulation to understand the interaction between the aptamers and the MnSOD protein, shown in Figure 4. These structures were used for further analysis. The molecular docking was performed by using the HDOCK software. These results were downloaded based on the predicted binding site as displayed in Figure 2.

After the molecular docking simulation, the PDB files were downloaded and analyzed for MnSOD protein and aptamer interactions through the usage of the protein-ligand interaction profiler, as shown in Figure 5. Most of the interactions between the aptamer and protein were taken as hydrogen bonds. However, Aptamer 2 also had hydrophobic interactions. Based on this data, Aptamer 1 was selected to be the most efficient and appropriate candidate due to it having the highest number of hydrogen bond interactions with the MnSOD; however, other aptamers also formed similar interactions, just at lower levels.

4. DISCUSSION

Depression is a complex mental health condition that requires a multifaceted approach for effective treatment.(Goldman, Nielsen, Champion, & for the Council on Scientific Affairs, 1999) Popular methods include psychotherapy, drug medication, and changes in one's life patterns. Cognitive behavioral therapy aims to remove negative thought patterns through complex behavioral exercises, while lifestyle changes aim to better the patient's mentality through natural means. However, for this research, we focused on aptamer binding for advancements in drug medication, a popular depression treatment. Most of the methods are external psychological interventions; systemic therapy and depth psychology are some examples. Still, these methods are often coupled with drug prescriptions, so our research remains pertinent to the issue. [15] With all the research, though we were able to produce accurate results, we encountered some problems: the project involved seven aptamers, but ideally, testing many more aptamers would produce more accurate results; there was also a lack of experimental validation as a lab/physical testing

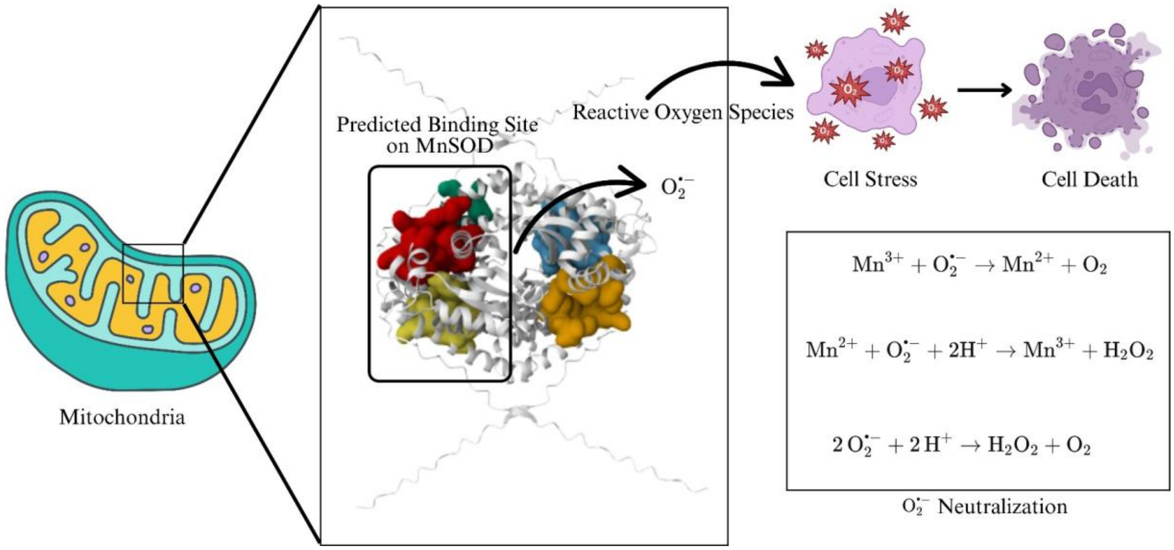

Fig. 1. The MnSOD protein is located inside mitochondria. The predicted binding site for the aptamer on MnSOD was computed using the P2Rank. The ROS formed by the cell can cause cell stress and cell death, which correlate with higher levels of depression. However, when an aptamer binds to the MnSOD protein, which protects the cell from reactive oxygen species, it increases MnSOD production, neutralizes the oxygen produced by ROS, and thus decreases cortisol levels associated with depression.

Table 1. Nucleotide sequences used in this research.

ID	Sequence	Structure
1	GGGAGGACGAUGCGGAUUACAGGUGGAGGCGGAAAUUGCUCUACCGCGU- UGGACCGUUGAAG	....((.((((((........(((((((.((.....)))))))))))))))..)).......
2	GGAUCGCGAGACUACGAGGGUUCGUCGUAGGCUCGGACGAGUAGCUUG- GAUCGUACAGGCCGUA	...(((((((.((((((.......)))))).))))..)))...(((((.......))))))....
3	GGGAAAGACGUAGACUGGUGGCGGAAUCGCGUGUAGGAGUACUCCG- GAGCUAACUAGGGAAGGAUCGU	.............(((((.(((....))).....(((....))).......)))))...........
4	GGAAGGCUAUGGACCUCGGAAGAAUGCUCGGUCGAGUGGAUACGGUCGU- UGGUGGAAGC	.....((((..((((((....))..((((....))))......))))..))))......
5	GGACCGUAGGUGCGUCUACCUCGAUAGGCGGAGAGGCUCUGAUUCGACGU- UAGCUUCCGGAUG	...(((.(((((((((..(((....)))(((((...)))))....)))))..)))).)))...
6	GGGAGCGAUGGAAGCUCGAUGGUGCGUGACCUAAGAGGAUCGGCGUUCG- GAGCUCUGAGGUGCGGCGUAA	..((((.......))))......((((((((((.((((..(((.....)))..)))).)))).).)))))
7	GGUCGAGGAUGCUCUAGCGGAAUUCGGACGGAUUAGGACGAUCG- GAGCUAGGGAUGGAAU	...........((((((((....(((((.((........)).))))))))))))).......

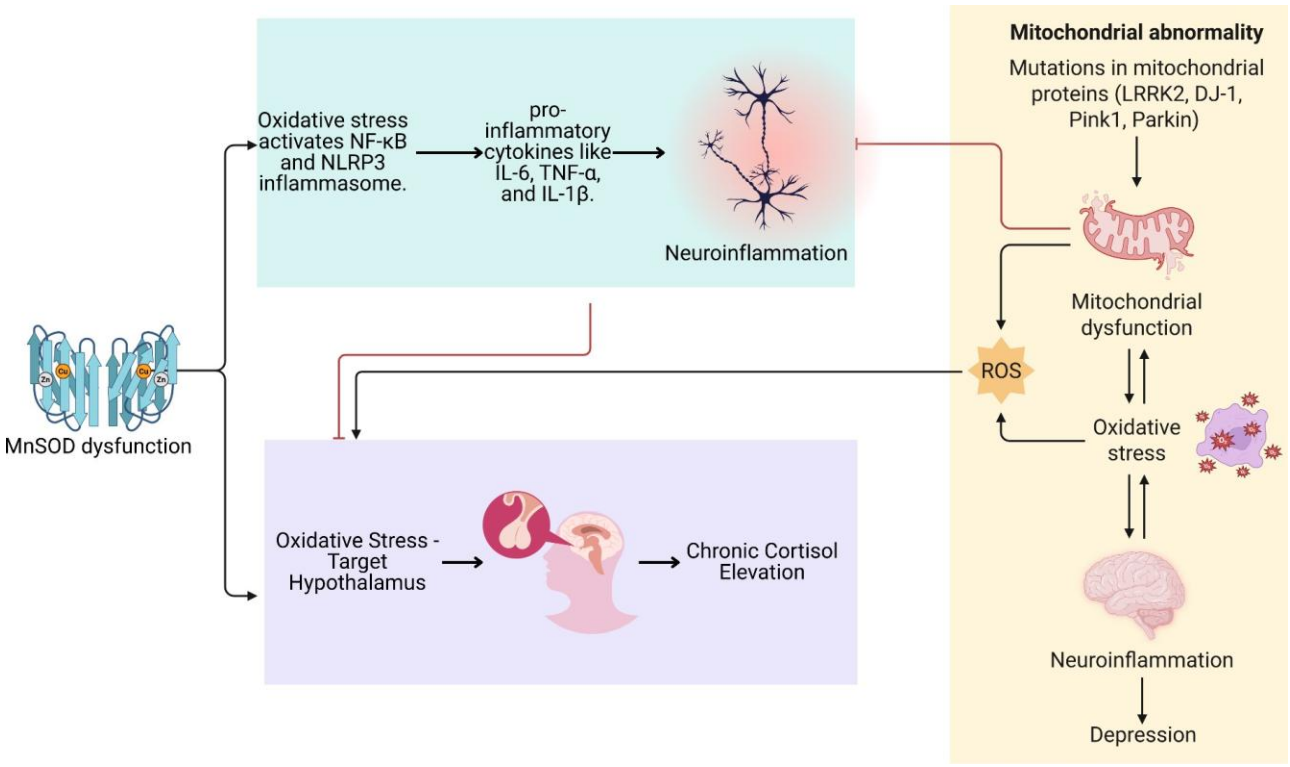

Fig. 2. The MnSOD dysfunction results in oxidative stress that activates the NF-kB and NLRP3 inflammasome. This sends pro-inflammatory cytokines, a group of signaling molecules, which will then activate neuroinflammation. This subsequently causes oxidative stress in the hypothalamus that propels chronic cortisol production, leading ultimately to depression. (Because cortisol is the stress hormone produced by the adrenal glands, often elevated cortisol levels, though not directly impactful, can increase the likelihood of depression through development and intensity.)

center couldn't be accessed and the surface plasmon resonance to experimentally validate the protein-aptamer binding couldn't be secured. Hence, the experiments were conducted using various software that may deviate slightly from real-world scenarios.

5. CONCLUSION

In this research, we found that Aptamer 1 would most likely mitigate the issue of depression due to its number of hydrogen bonds with MnSOD. We had initially hypothesized Aptamer 1 as a strong candidate, and the research supported our hypothesis. This study was important for more effective medicine making and a clearer understanding of the science behind depression. Other aptamers, due to their own number of hydrogen bonds, could also potentially be solutions for depression, though not as efficient as Aptamer 1. In future studies, this aptamer can be used as an efficient aspect of medicine for powerful changes. Perhaps these future studies may explore other aptamer combinations and their binding with MnSOD to find a more efficient candidate for depression mitigation.

REFERENCES

1. M. Fava and K. S. Kendler, "Major Depressive Disorder," Neuron **28**, 335–341 (2000).
2. K. Miller and M. J. Massie, "Depression and Anxiety," The Cancer Journal pp. 12–12 (2006).
3. G. D. Oliveira, R. Cianelli, K. Gattamorta, N. Kowalski, and N. Peragallo, "Social Determinants of Depression Among Hispanic Women," J Am Psychiatr Nurses Assoc **23**, 28–36 (2017).
4. M. J. Lynch, "The power of oppression: Understanding the history of criminology as a science of oppression," Critical Criminology **9**, 144–152 (2000).
5. N. J. Schork, D. Fallin, and J. S. Lanchbury, "Single nucleotide polymorphisms and the future of genetic epidemiology," Clinical Genetics **58**, 250–264 (2000).
6. P. Y. Kwok, "Methods for Genotyping Single Nucleotide Polymorphisms," Annual Review of Genomics and Human Genetics **2**, 235–258 (2001).
7. J. P. Richardson, C. Grimley, and C. Lowery, "gene mutations.GENE MUTATIONS," Proceedings of the National Academy of Sciences **72**, 1725–1728 (1975).
8. A. K. Holley, V. Bakthavatchalu, J. M. Velez-Roman, St, and D. K. Clair, "Manganese Superoxide Dismutase: Guardian of the Powerhouse," International Journal of Molecular Sciences **12**, 7114–7162 (2011).
9. T. U. Consortium, "UniProt: a hub for protein information," Nucleic acids research **43** (2014).
10. J. Abramson, J. Adler, J. Dunger, R. Evans, T. Green, A. Pritzel, . . Jumper, and J. M, "Accurate structure prediction of biomolecular interactions with AlphaFold 3," Nature **630**, 493–500 (2024).
11. U. Omasits, C. H. Ahrens, S. Müller, and B. Wollscheid, "Protter: interactive protein feature visualization and integration with experimental proteomic data," Bioinformatics **30**, 884–886 (2013).
12. F. Pontén, K. Jirström, and M. Uhlen, "The Human Protein Atlas-a tool for pathology," The Journal of Pathology **216**, 387–393 (2008).
13. N. R. Markham and M. Zuker, "UNAFold," in "Bioinformatics: Structure, Function and Applications," J. M. Keith, ed. (Humana Press, 2008), pp. 3–31.
14. A. M. Watkins and R. Das, "RNA 3D Modeling with FARFAR2, Online," in "RNA Structure Prediction," R. K. K. J. Iwakiri, ed. (Springer US, 2023), pp. 233–249.
15. L. S. Goldman, N. H. Nielsen, and H. C. Champion, "Awareness,

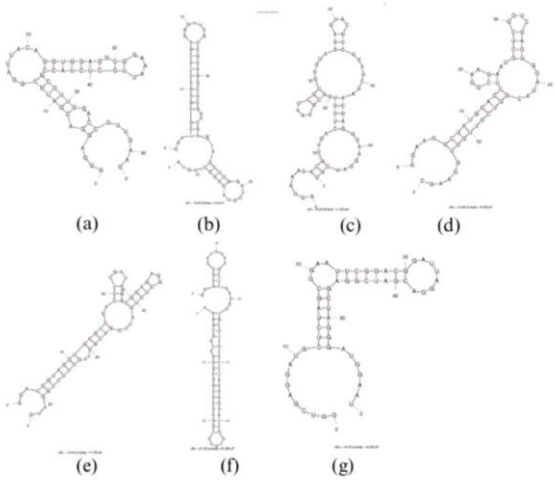

Fig. 3. These are all the 2-dimensional secondary structures of the 8 aptamers used in this research. All different aptamers produce different 2d structures. The aptamer folded structure was obtained by using RNA structure software. This 2d folded form shows the folding/hydrogen bonds formed between the nucleotides (which make up aptamers). Each different sequence folds and interacts in a different way, hence different structures. The circular shape represents a bulb, and the stick-like figure represents a stem.

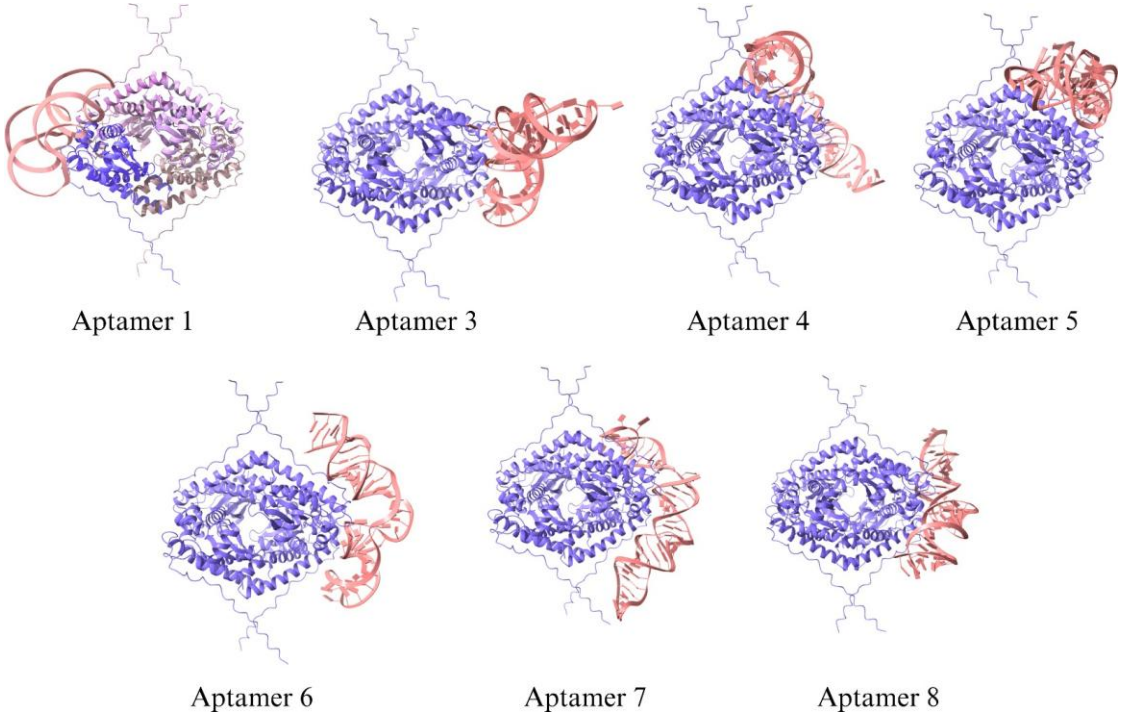

Fig. 4. The final results, shown above, were obtained after completing the docking process for each aptamer and its binding to theMnSOD protein. The pink represents each aptamer, and the purple is the MnSOD protein. (The binding site of all of the aptamers are not relevant to the research.)

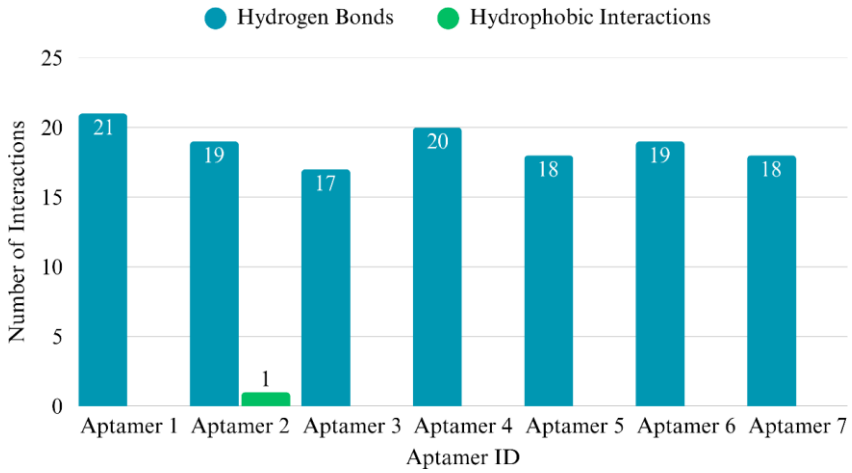

Fig. 5. Protein-ligand interaction formed between the MnSoD and aptamer computed by using the PLIP web server.