Cluster Innovation Centre

Enhancing the discovery and refinement of aptamers with superior binding affinities tailored for precise targeting of diverse diseases.

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Abstract

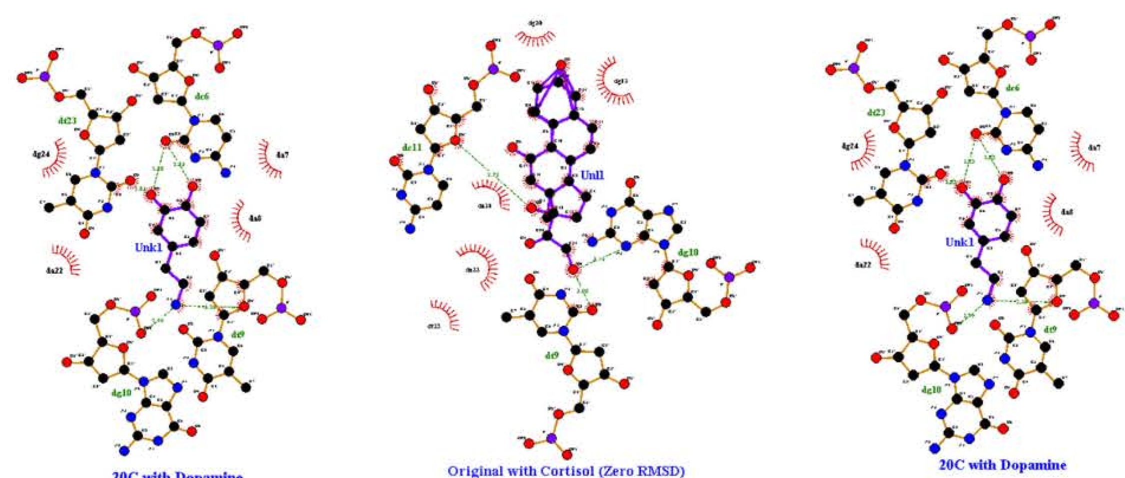
Aptamers are single-stranded oligonucleotides that bind various biological targets. DNA aptamers, more stable than RNA aptamers, excel as surface sensors and selective inhibitors for protein interactions. Traditional aptamer selection methods like SELEX are labor-intensive, time-consuming, and costly, with limitations in stability and specificity. **This study introduces a novel methodology for identifying and selecting DNA aptamers by mutating pre-reported aptamers, generating 2D and 3D structures, performing molecular docking with selected ligands, and evaluating binding affinities and RMSD values, aiming to enhance the efficiency of the selection process.**

Introduction

Aptamers are single-stranded oligonucleotides that bind a wide range of biological targets. Developed through SELEX, aptamers offer several advantages over traditional antibodies: they are easier and cheaper to produce, less likely to trigger immune responses, and can regain function after disruption. DNA aptamers, in particular, are stable and versatile, with significant potential as biosensors and selective inhibitors for protein interactions. However, traditional SELEX methods are labor-intensive, time-consuming, and costly, limiting aptamer screening and resulting in challenges such as instability in vivo and unintended binding. Our study presents a novel methodology for aptamer identification by mutating pre-reported aptamers, generating 2D and 3D structures, and evaluating binding affinities via molecular docking. This approach aims to streamline the selection process, enhancing efficiency and applicability in diagnostics and therapeutics.

Discussion

The molecular docking of ligands with the receptor aptamer using AutoDock Vina provided nine poses per ligand, from which the best pose (highest binding affinity and 0.0 RMSD) and the lowest non-zero RMSD pose were selected for further analysis. These poses were visualized in LigPlot to examine molecular interactions in detail, revealing specific hydrogen bonds, hydrophobic contacts, and other significant interactions between ligand and receptor atoms.

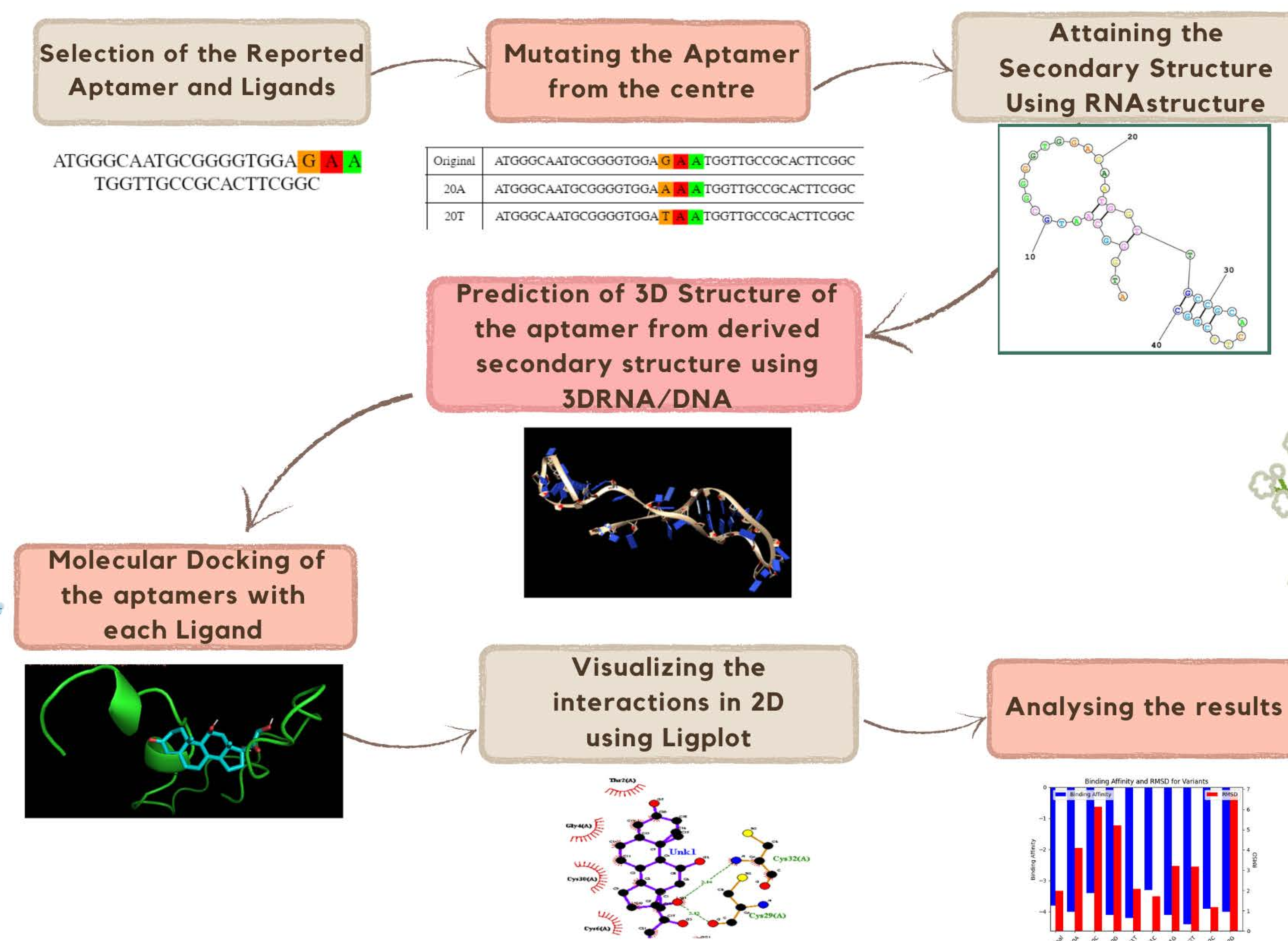


This analysis is particularly valuable as it provides insights into the precise atomic interactions that are not observable in wet lab experiments, thereby enhancing our understanding of the binding mechanisms and stability of the ligand-aptamer complexes.

Conclusion

In our study, we successfully identified ssDNA aptamers with optimal binding affinity by computationally screening and mutating pre-reported aptamers. By generating secondary and tertiary structures of the aptamers in 2D and 3D and performing molecular docking against Serotonin, Cortisol, and Dopamine, we discovered aptamers with optimal binding affinity than the original. Our findings demonstrate that single nucleotide changes can significantly alter aptamer structures and their binding affinities. 21G and 22T have shown the best comparative results from our aptamer pool.

Methodology

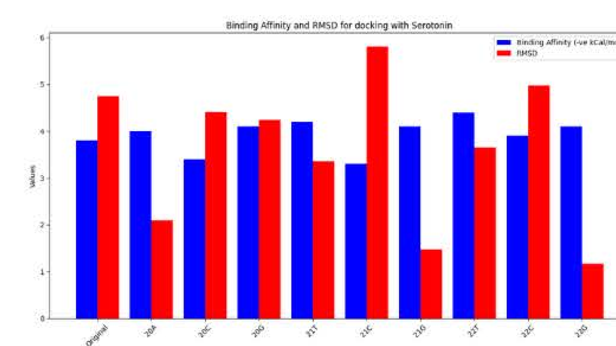


Results

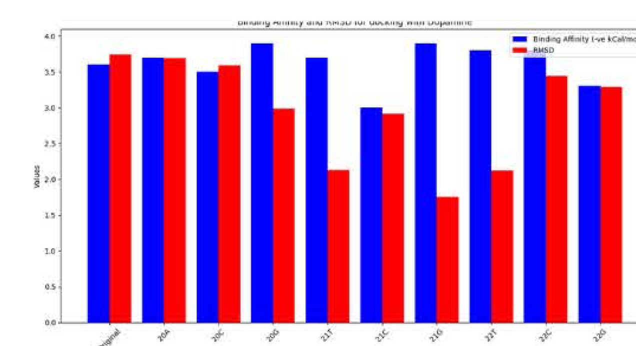
Name	Aptamer Sequence	Tertiary Structure
Original	ATGGGCAATGCGGGGTGGA GAA TGGTTGCCGCACTTCGGC	
21G	ATGGGCAATGCGGGGTGGA GGA TGGTTGCCGCACTTCGGC	
22T	ATGGGCAATGCGGGGTGGA GAT TGGTTGCCGCACTTCGGC	

Figure (a): Comparison of Structure on a single change

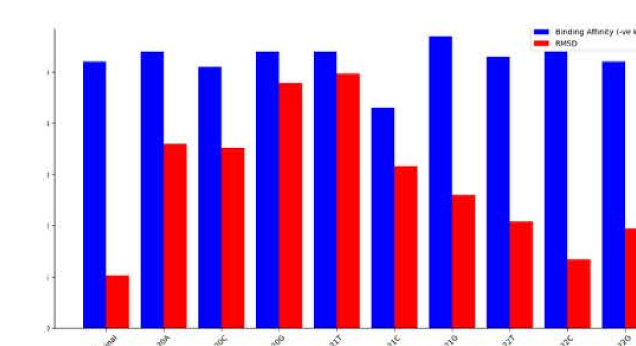
The table demonstrates that single nucleotide changes significantly alter aptamer structures, affecting their binding affinities. The adjacent bar graphs compare the binding affinities and RMSD values of all aptamers with our three target ligands. Notably, 21G and 22T exhibit the best comparative results.



Figure(a) : Binding Affinity and RMSD values of Docking with **Serotonin**



Figure(b) : Binding Affinity and RMSD values of Docking with **Dopamine**



Figure(c) : Binding Affinity and RMSD values of Docking with **cortisol**

Acknowledgments

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Future Work

We plan to develop software that automates the entire process, streamlining the workflow and enhancing efficiency. Additionally, we aim to integrate AlphaFold3 into our research, leveraging its advanced capabilities in aptamer structure prediction to gain deeper insights and improve accuracy.

References