# RNA's Higher Order Structure and its Effect on Stability and Function For Medical Application

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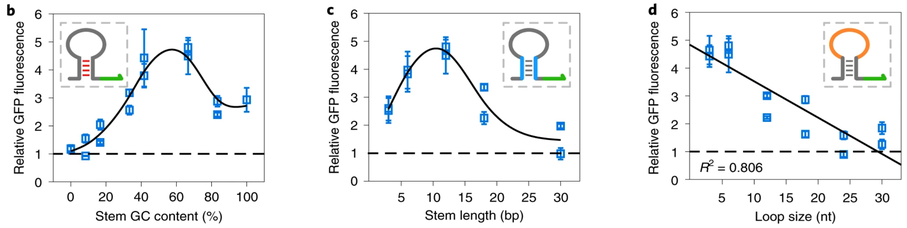
**Research question**: Can we use a specific 5' RNA motif to enhance RNA for medical application?

## Introduction and Importance

-In 2019 a structural motif for RNA(Dt-RNA) was discovered to increase the stability of mRNA in vitro and in E-coli cells(Zhang, Q)

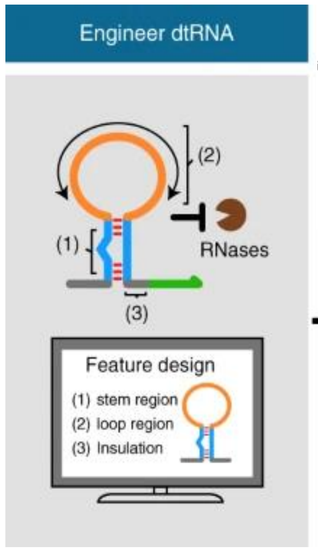



-Given that mRNA of Eukaryotic cells is known to be stable on the 5' end due to capping. We want to see how this structural motif can contribute to MicroRNA(miRNA) Stability.

-miRNA has the potential to continuously circulate in the bloodstream while sending important information to cells without an extracellular vesicle to help treat vascular diseases (Chang and Wang).

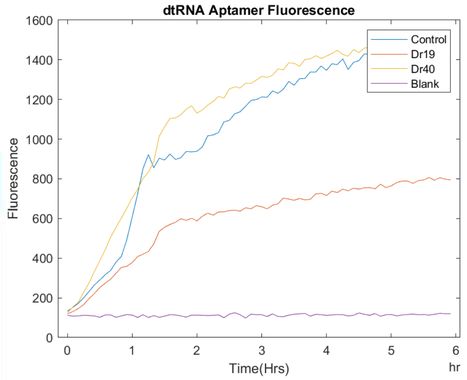
-The goal of this project was to determine whether dt-RNA could increase the stability of miRNA in blood serum, and function with an RNA other than messenger RNA.

- For the first part, of the research, we attached dt-RNA to an aptamer to see if aptamers retain their function. For the second part, we attached dt-RNA to miRNA and tested its stability against regular miRNA in blood serum with different time trials.

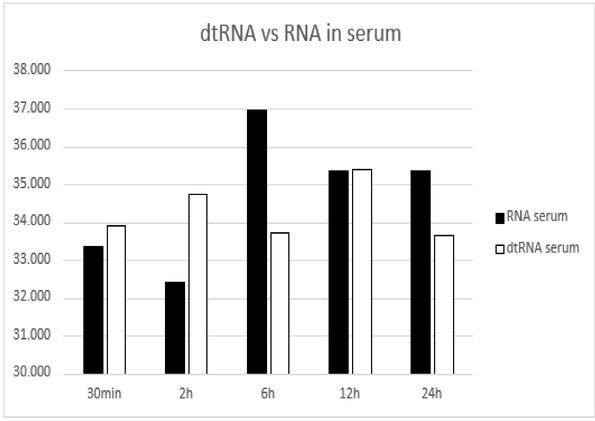



## Results and Discussion

The graph below is the result of Q-PCR using aptamers. Dr19 and 40 represent dtRNA attached to the 5' end of an aptamer. Dr19 and 40 show fluorescence as represented in the graph meaning that aptamer function is retained, and dt-RNA can be used homogeneously.




-The data below represents the qPCR results of RNA that was reverse transcribed into DNA after 5-time points over 24 hours. The results imply that the stability of miRNA is neither impaired nor enhanced.




## Methodology

DNA for miRNA with and without dt motif are transcribed into RNA DNA using AmpliScribe™

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Both RNA types are added to the blood serum at 0-2-6-12 and 24-hour time points

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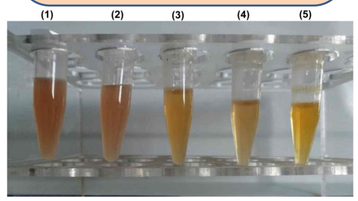
RNA is reverse transcribed into DNA and then measured using qPCR to determine the relative amount of original DNA


CFX384 Touch RT-PCR Detection System

## Conclusion and Future Work

-dtRNA works homogeneously with respect to maintaining the function of attached RNA

-dtRNA shows future promise to increase the stability of RNA in all cell types without impairing its function.

- In the future, we hope to conduct further research using machine learning to test sequences of dt-RNA and determine which motifs work best with miRNA.
We also hope to conduct further research testing the use of the dt motif for miRNA in mammalian cells

## References

- Zhang, Q., Ma, D., Wu, F. *et al.* Predictable control of RNA lifetime using engineered degradation-tuning RNAs. *Nat Chem Biol* **17,** 828–836 (2021). https://doi.org/10.1038/s41589-021-00816-4

-Chang, Y.-J., and Wang, K.-C. (2021). Therapeutic Perspectives of Extracellular Vesicles and Extracellular microRNAs in Atherosclerosis. Curr. Top. Membr.87, 255–277. doi:10.1016/bs.ctm.2021.08.005

-Sean Taylor, Michael Wakem, Greg Dijkman, Marwan Alsarraj, Marie Nguyen, A practical approach to RT-qPCR—Publishing data that conform to the MIQE guidelines, Methods, Volume 50, Issue 4, 2010, PagesS1S5,ISSN10462023,https://doi.org/10.1016/j.ymeth.2010.01.005
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