# MICRONAUT: Modeling the Dual Regulation of GFP

Manuel Marques Vilar, Dan Jian, Yuxuan Lin, Maryam Elfeky, Hua Huang, Manas Singh, Wendy Zeng, Genesis Ceron, Fritz Ebner, Varun Gambhir, Nadia Mathew, Max Gazes, Qiongran Dong, Ethan Kim, Mingrui Cui

Undergraduate Biology, Stony Brook University

## Introduction

- Non-Hodgkin's B-Cell Lymphoma is an aggressive form of blood cancer that inhibits the function of white blood cells
- Current detection methods, such as imaging and biopsies, are expensive and/or invasive.
- Upregulated miRNA-326 is associated with non-Hodgkin's B-cell lymphoma
- We aim to equip Escherichia Coli bacteria with a gene circuit to develop a cheaper detection method for clinical use by measuring the levels of miRNA-326
- LacI and L7Ae work as a dual-regulation system to prevent the expression of GFP
- Argonaute2 and miRNA together will inhibit the expression of LacI and L7Ae, allowing GFP to fluoresce, indicating the presence of miRNA-326
- Ordinary differential equations (ODEs) to simulate and compare GFP expression levels under different conditions
- Alphafold to model the integration of the miRNA target site with the LacI protein to prove that it does not interfere with the LacI active sites
- RNAfold to analyze the stability of our mRNA strand with the miRNA target site placed at different locations of the gene sequence

## Methods

### Ordinary Differential Equations

The Ordinary Differential Equations(ODEs) were implemented in MATLAB and solved with the "ode15s" function, a stiff differential equation solver.
Three ODEs were designed: GFP without the Dual-Suppression System (1), GFP with the Dual Repression System (2), and GFP with Dual Repression System, miRNA, and Ago2 (3). In ODE 1, two variables: mRNA and GFP were expressed as a system of ODEs. In ODE 2, 4 variables, mRNA, GFP, LacI, and L7AE were expressed as a system of ODEs. In ODE 3, 8 variables, mRNA of GFP, GFP, LacI, L7AE, mRNA of Ago2, Ago2, RISC were expressed as a system of ODEs.

### Protein Structure Prediction

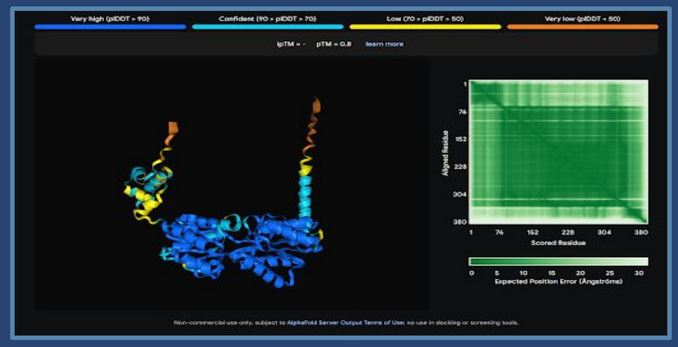
The crystallized protein files (.pdb) was obtained from RCSB Protein Data Bank. Alphafold 3 was used to predict the protein structure of a modified LacI protein with the miRNA target site at the N-terminus and a T2A stub at the C-terminus.

### RNAfold and RBS Calculator

The RNAfold Webserver from ViennaRNA Web Services was used to predict minimum free energy(MFE) of our RNA strands with the miRNA target site at different locations. This MFE was used to check the stability of our RNA strand, providing a quantified predictor of translation initiation rate.

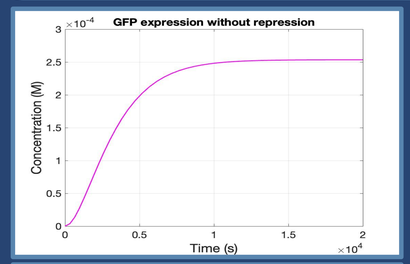
## Acknowledgments

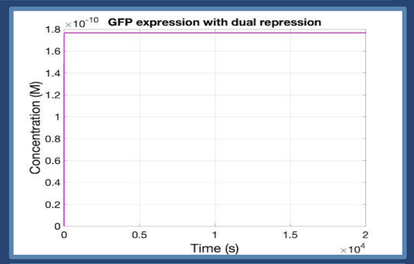
The 2024 iGEM team would like to thank our advisors and mentors Dr. J. Peter Gergen, Dr. Gábor Balázsi, Chris Helenek, and Abhishek Cherath for providing us with ample feedback on modeling. Our project is generously supported by Suny SOAR, PSEG, URECA, the SUNY Career Center, and Denise Loring Award.

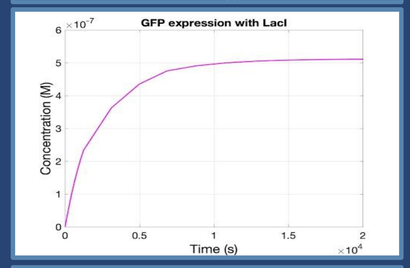


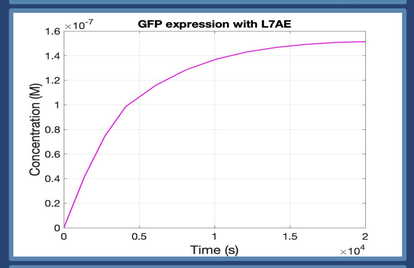

The above schematic represents the LacI protein structure with a T2A and miRNA-326 target site scars simulated in Alphafold.

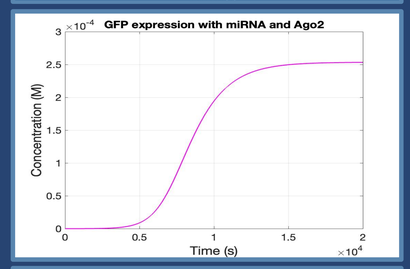
The graphs below show GFP concentration over time in our genetic circuit as modeled by MATLAB.

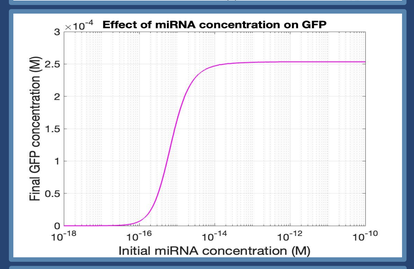


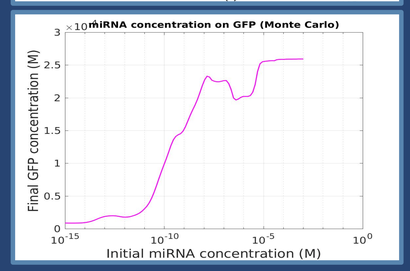



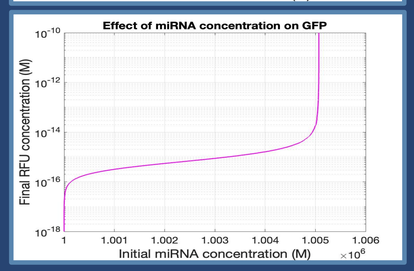




## Discussions

### Ordinary Differential Equations

- The GFP concentration curve without dual repression mimics the expected steady state equilibrium with a final concentration on the order of 1e-4
- The GFP concentration curve with the dual repression also mimics expected results with a sharp peak and quick decline as the dual repression system is transcribed/translated and comes into effect. The GFP concentration bottoms out at a steady state concentration of 0.
- The GFP concentration with dual repression and activation by miRNA still needs to be refined. While a similar peak appears than quickly declines, the final concentration of GFP is 2.43e-16, far too small compared to expected results. A relationship between miRNA concentration, its degradation, and this final GFP concentration is currently being explored with a threshold concentration of miRNA emerging as the breaking point of the system. This concentration is determined by both the starting miRNA concentration, the efficiency of transformation of miRNA into the cell, and the degradation rate of miRNAs in MRE 600 cells.

### Protein Structure Prediction

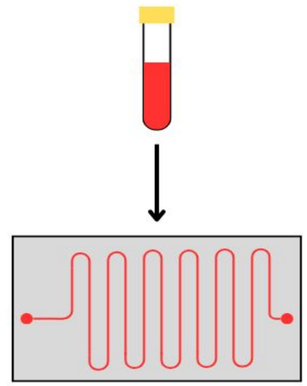
- In our Alphafold model, the predictive value of the miRNA target site and T2A stub have a pLDDT (predicted Local Distance Difference Test) of less than 50. A lower pLDDT indicates a lower confidence interval. However, as the LacI active sites are unaffected, an assumption can be made that miRNA target site attached to LacI site would not affect LacI function.

### RNAfold and RBS Calculator

- RNAfold predicts the minimum free energy(MFE) of the different mRNA strands. A lower MFE indicates greater stability of the mRNA strand. In turn, this inhibits translation rate as the mRNA strand is more tightly folded.
- RBS calculator predicts translation initiation rate(TIR) in arbitrary units. The TIR was obtained for the mRNA strands, and the TIR for the mRNA strand without the miRNA TS shows a greater relative TIR of 1.2x.

## Future Implementations

- GFP ROC curve
  - Create a calibration curve with experimental results
- Umbrella Sampling of Ago2-miRNA binding
- Complete "lab on a chip" implementation
  - Cell-free system on paper strip
  - Microfluidic assay to extract miRNA from blood
  - miRNA then added to paper strip; color intensity tracked to determine risk



## References

Abramson, J., Adler, J., Dunger, J. *et al.* Accurate structure prediction of biomolecular interactions with AlphaFold 3. *Nature* 630, 493–500 (2024). https://doi.org/10.1038/s41586-024-07487-w

Salis HM. The ribosome binding site calculator. Methods Enzymol. 2011;498:19-42. doi: 10.1016/B978-0-12-385120-8.00002-4. PMID: 21601672.