

NDnano Summer Undergraduate Research 2026 Project Summary

1. Student name & home university: Natalie Moran, University of Notre Dame
2. ND faculty name & department: Professor Saurja DasGupta (Biochemistry) and Professor Kaiyu Fu (Biochemistry)
3. Summer project title: RNA aptamer-based imaging tools for energy metabolites
4. Briefly describe new skills you acquired during your summer research:

During my summer research in the DasGupta Lab, I learned how to perform PCR amplification of single-stranded DNA into double-stranded templates. Using the PCR amplified DNA template, I was able to perform *in vitro* transcription using T7 RNA polymerase to produce RNA. I was then able to perform enzymatic DNase digestion to remove templates, Phenol-Chloroform-Isoamyl alcohol extractions to isolate clean nucleic acids from proteins, and ethanol precipitation to desalt and concentrate RNA samples. Next, I learned how to create and run denaturing PAGE gels with urea matrices to isolate full-length RNA sequences. I also gained hands-on experience analyzing the fluorescence of our samples through AZURE imaging and fluorometry experiments. Ultimately, I was able to present and communicate my work in a lab group meeting and discuss potential next steps with group members.

5. Briefly share a practical application/end use of your research:

The short-term application of this research is to develop an *in vitro* fluorescent sensor that selectively detects oxidized FAD. Ultimately, the data collected this summer will be used to optimize a genetically encoded biosensor, with the long-term goal of quantitatively imaging FAD inside living cells.

6. 50- to 75-word abstract of your project:

RNA is a highly versatile molecule that can function as an aptamer with strong sensitivity and specificity. This summer research project focuses on developing *in vitro* fluorescent RNA biosensors that can selectively detect oxidized FAD, a central redox cofactor present in cellular metabolism. FAD biosensors were constructed and optimized by integrating FAD-binding aptamer domains with fluorescent RNA aptamer modules, providing a foundational strategy for producing a genetically encoded biosensor capable of quantitatively imaging intracellular FAD.

7. References for papers, posters, or presentations of your research:

- Legen, Tjasa, and Günter Mayer. "Modular Approach for Rapid Identification of RNA-Based Sensors." *ACS Sensors*, vol. 9, no. 2, 2024, pp. 753–758.
- Kehrli, Janine, et al. "Fluorogenic RNA-Based Biosensors of Small Molecules: Current Developments, Uses, and Perspectives." *Biosensors*, vol. 14, no. 8, 2024, p. 376.
- Li, Xing, et al. "Imaging Intracellular S-Adenosyl Methionine Dynamics in Live Mammalian Cells with a Genetically Encoded Red Fluorescent RNA-Based Sensor." *Journal of the American Chemical Society*, vol. 142, no. 33, 2020, pp. 14117–24. *ACS Publications*
- Samuelian, John S., et al. "An RNA Aptamer That Shifts the Reduction Potential of Metabolic Cofactors." *Nature Chemical Biology*, vol. 18, no. 11, Nov. 2022, pp. 1263–69. *Springer Nature*

One-page project summary that describes problem, project goal and your activities / results:

Flavin adenine dinucleotide (FAD) is a metabolic cofactor that plays a significant role accepting and donating electrons to power cellular respiration and ensure proper mitochondrial function. Additionally, FAD can act as a protective "cap" on specific RNA molecules, expanding its function beyond classic metabolism. That said, dynamics of FAD can fluctuate and are heavily influenced by genetics, diet, aging, exercise, and pregnancy. While the dysregulation of FAD can be a hallmark of metabolic, neurological, and cardiac diseases, there is a lack of robust methods to quantify its free distribution in cells and cellular compartments. Therefore, the overall goal of our project is to dynamically image free FAD in its oxidized state using RNA as an aptamer. To do so, we integrated FAD-binding aptamer domains with fluorescent RNA aptamer modules using rational design. While we are currently working to develop *in vitro* fluorescent sensors, our long term goal is to quantitatively image intracellular FAD using successful RNA aptamers created.

To contribute to this objective, my research this summer focused on evaluating biosensors engineered with two distinct transducer designs, Transducer 2 (67nt) and Transducer 3 (69nt). The structural design of these biosensors consists of three essential domains: the sensing domain that binds to FAD (X2B2-C14U), the reporting module that binds to a dye and generates a fluorescent signal (Red Broccoli), and the connecting transducer. The transducer is critical because it acts as a mechanical switch, structurally coupling the two modules so that the Red Broccoli domain folds and fluoresces only when FAD successfully binds to the sensing domain.

To evaluate sensor performance, we initially used AZURE imaging to obtain a qualitative assessment of our samples' fluorescence. As a control, we compared FAD against flavin mononucleotide (FMN), a cellular analog with a highly similar structure. We observed that FMN exhibits significantly higher background autofluorescence compared to FAD. This occurs because in solution, FAD naturally folds into a closed conformation, allowing the adenine ring to stack against the flavin ring and quench its fluorescence. On the other hand, FMN lacks this adenine group, and remains unquenched and highly fluorescent. Ultimately, our imaging revealed that the AZURE platform may not be ideal for validating aptamer functionality. Because the fluorescent dye itself (10 ul OBI dye) produced a background signal, control groups containing just the dye and FAD showed fluorescence just as bright as our experimental biosensor groups. Therefore, it became difficult to distinguish the true, aptamer-driven fluorescent signal from independent dye autofluorescence.

We then transitioned to a fluorometer to conduct quantitative fluorometry experiments. As a positive control to validate our experimental setup and dye activation, we utilized an established S-adenosylmethionine (SAM) aptamer sourced from literature. While the SAM control functioned perfectly and demonstrated a clear, target-induced fluorescence increase, our engineered FAD aptamer variants did not show a functional signal. This outcome indicated that the current transducer designs were likely preventing proper folding or structural communication between the FAD-sensing domain and the Red Broccoli reporter module.

Although the initial transducer designs did not yield a functional fluorescent signal, these outcomes provide critical insights that will guide our next steps. Moving forward, the project will focus on engineering and testing a wider variety of RNA sequences. Specifically, we plan to experiment with truncating less of the original sensing domain and directly fusing it to the Red Broccoli reporting module to facilitate more efficient structural communication. Ultimately, the research conducted this summer lays the groundwork for developing successful *in vitro* biosensors. Perfecting these RNA tools is a vital step toward efficiently tracking intracellular FAD dynamics, which will aid in deepening our understanding of cellular health and metabolism.