OFFICE OF UNDERGRADUATE RESEARCH
IMMERSIVE RESEARCH INTERNSHIP EXPERIENCE

Portable miRNA Detection with Ligation-initiated Transcription and CRISPR/Cas12a

Jason Berdia, Mina Liao, Michael Scott, Defne Ustundag, Jeremy Vo

FIRE: THE FIRST-YEAR INNOVATION & RESEARCH EXPERIENCE
MOLECULAR DIAGNOSTICS & RAPID DIAGNOSTICS

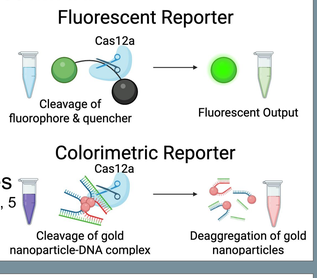
Background

miRNAs and Early Detection:

- Early detection of cancer is important for improving patient prognosis.
- miRNAs are short, non-coding RNA sequences that play a role in gene expression and cancer development.
- miRNAs circulate in peripheral blood and can be used as biomarkers for cancer and other diseases.[1]
- Current detection methods for miRNAs include RT-PCR, microarrays, and Northern blotting.
- However, these methods are time consuming and require expensive equipment.

Using CRISPR/Cas Systems to Detect MiRNA

- Previous assays have used CRISPR-Cas12a's cleavage activity to create miRNA reporting systems.[2, 3]
- Target miRNA in solution initiates Cas12a's cleavage activity.
- Gold nanoparticles (AuNPs) can be functionalized to produce color changes in solution based on their aggregation[4, 5] (**Figure 1**).



Portable Sensor & Heater

Affordable Design: System is built using low-cost, widely available components (thermistor, solid-state relay, heating strip, and RPi microcontroller). Device is 3D-printed, and color capture is achieved with an off-the-shelf camera module.

Color Quantification: Image data is analyzed in Lab* space to enhance sensitivity and reduce ambient lighting effects.[6]

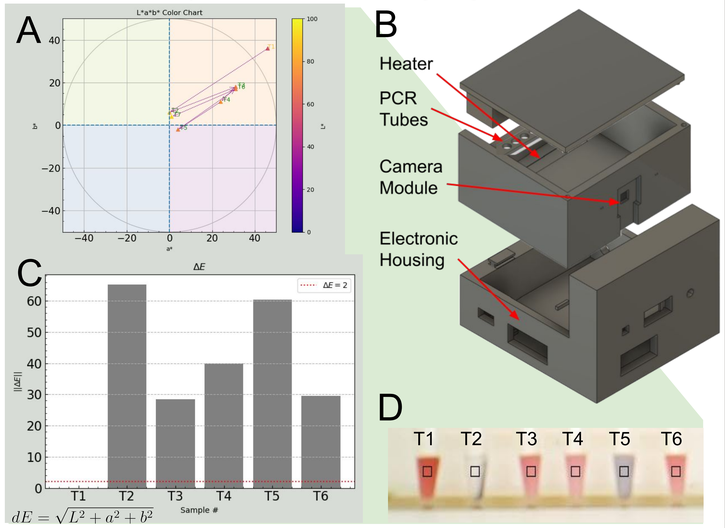


Figure 2. 2A Lab chroma plot visualizing each sample's position in color space, with arrows indicating directional color change from the control (T1). **2B** 3D CAD model of a custom imaging chamber with electronic housing. **2C** ΔE values showing perceptible color differences, with T2 & T5 exhibiting drastic shifts (ΔE > 60). **2D** Results from Figure 6A. Average LAB taken from outlined squares.

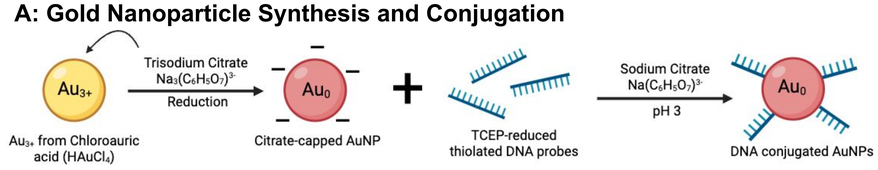
Intent & Motivation

Objective: Develop a colorimetric assay to detect miRNA and a portable detector to incubate assay and quantify its output

Motivation: Increase the accessibility of initial cancer screenings by providing a portable and cost-effective method for the detection of miRNA biomarkers

Methods

A: Gold Nanoparticle Synthesis and Conjugation



B: Ligation Initiated Transcription & CRISPR-AuNP (LTC-AuNP) Assay

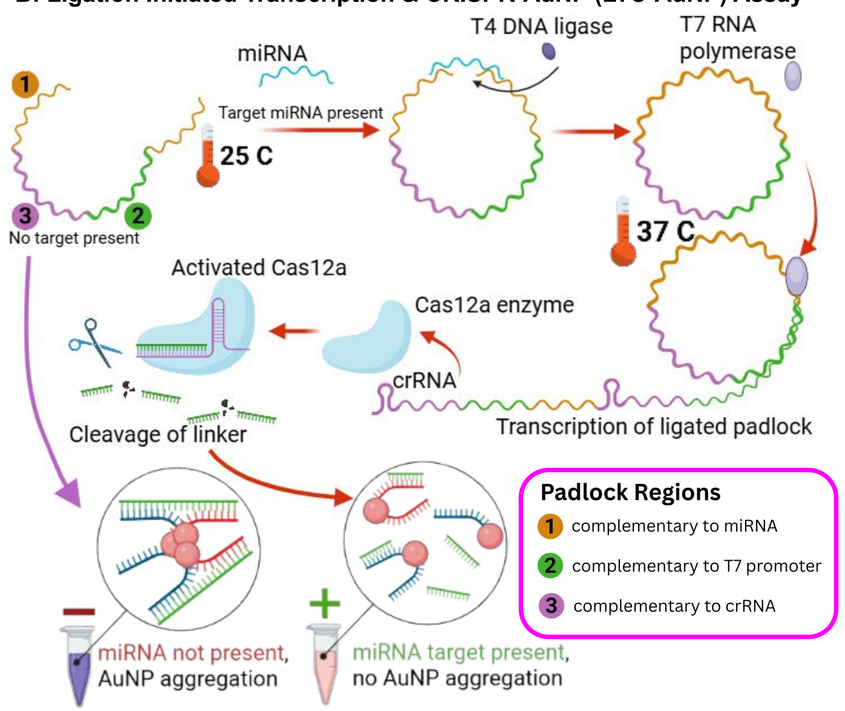


Figure 3. Gold nanoparticles synthesized via the Turkevich method and conjugated with ssDNA using the low-pH method.[7, 8, 9] (**A**) Presence of miRNA initiates ligation of the padlock ends. The ligated padlock is transcribed, creating crRNA repeats. Cas12a/crRNA complex cleaves activator-linker sequence, preventing it from linking and causing deaggregation of the AuNP-DNA conjugates (**B**).

References & Acknowledgements

QR code leads to references and additional portable sensor info.

We would like to express our gratitude to Dr. Catherine Spirito, Dr. Shannon Hilton, Dr. Ian White, and the OUR Immersive Research Internship Experience (IRIE) for their support of our project. Figures 1-3 were made in BioRender.

Results & Discussion

Synthesis of Gold Nanoparticles (AuNPs)

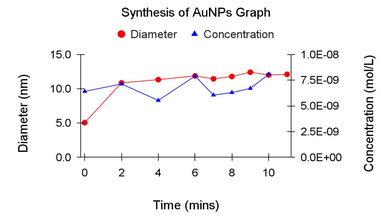


Figure 4. AuNP diameters and concentrations were calculated using data from UV-Vis Spectrophotometry.[10] 12.1 nm AuNPs with a concentration of 8.1 nM were used in all experiments shown in Figure 6.

Confirmation of Ligation, RCT, CRISPR via Gel Electrophoresis

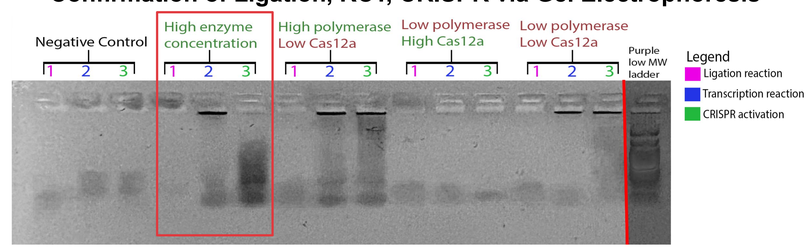
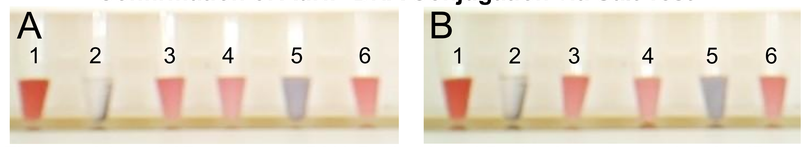


Figure 5. 3% agarose gel stained with Gel Red. High polymerase and Cas12a samples included 0.25 µL of T7 RNA polymerase per 32 µL and 0.25 µL of Cas12a per 34 µL; low enzyme samples were 10X diluted. High enzyme concentrations produced expected results. Negative controls contained no cDNA. Contrast was enhanced separately for the ladder.

Confirmation of AuNP-DNA Conjugation via Salt Test



Figures 6A and 6B represent DNA Probe 1 and Probe 2 conjugated to AuNPs.

1. Unconjugated AuNPs.
2. Unconjugated AuNPs with **200 mM NaCl** in solution (complete aggregation).
3. AuNPs conjugated to probes and washed in water.
4. Conjugated and washed AuNP solution with **200 mM NaCl**.
5. Conjugated and washed AuNP solution with **2 mM $MgCl_2$** in solution. The RCT/CRISPR product of the assay contains about 10 mM $MgCl_2$ from NEBuffer 2.1.
6. Conjugated and washed AuNP solution with **2 mM $MgCl_2$** and **4 mM EDTA** in solution. **200 mM EDTA** was combined with **100 mM $MgCl_2$** before being added to prevent aggregation.

Future Work

- Test and optimize aggregation of AuNPs DNA probes via linker DNA. Combine LTC section of assay with AuNPs to detect miRNA (Figure 3).
- Optimize concentrations of assay components, such as the padlock probe and enzyme concentrations.
- Determine the specificity and sensitivity of the assay with differing miRNAs and concentrations.
- Compare the performance of the assay to other methods of miRNA detection, like RT-PCR and ligation-LAMP.
- Assess if the LTC-AuNP assay can be utilized in a one-pot system.