NDnano
Notre Dame Nanoscience and Technology

NDNano Summer Undergraduate Research
2023 Project Summary

1. Student name & home university: Charlie Desnoyers, University of Notre Dame

2. ND faculty name & department: Dr. Kaiyu Fu, Chemistry & Biochemistry; Dr. Thomas O' Sullivan, Electric Engineering

3. Summer project title: Development of an Aptamer Based Gold Nanoparticle Biosensor for the Detection of Kanamycin

4. Briefly describe new skills you acquired during your summer research:

Throughout the summer, I have adopted skills from both a synthetic and analytical chemistry toolbox. I have learned how to synthesize citrate-capped gold nanoparticles and functionalize them with dye-linked kanamycin aptamers. To functionalize these dye-linked aptamers, I used an NHS-amine ester coupling reaction. I applied these aptamer-linked gold nanoparticles with UV-Vis and fluorescence spectroscopy.

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

These aptamer-linked gold nanoparticles can be used as a solution technique to detect various analytes (depending on the availability of the corresponding aptamer) without the need for separation in complex matrices, immobilized in a membrane for wastewater analysis, or immobilized onto a metal surface for laser spectroscopy analysis.

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

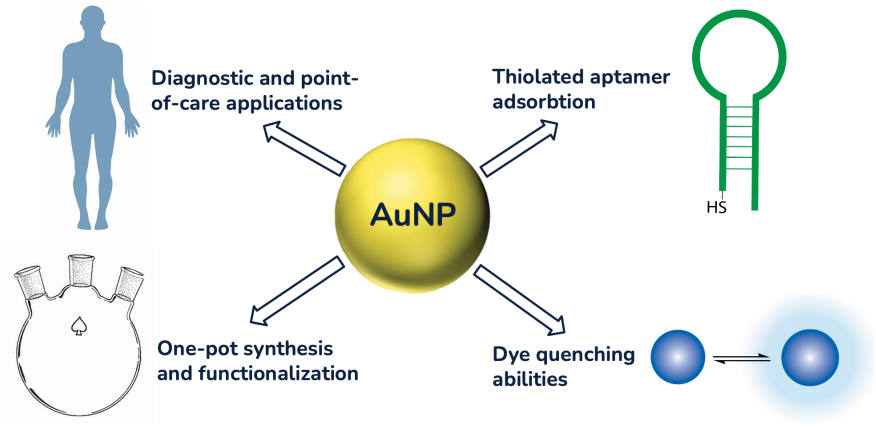
I have developed an aptamer-linked gold nanoparticle (Apt-AuNP) biosensor that allows for the detection of kanamycin via fluorescence measurements. By using gold nanoparticles as a quenching agent, fluorophore conjugated aptamers can be attatched to AuNP which allows for a sensitive signal to be seen. I synthesize, characterize, and measure the Apt-AuNP to understand their behavior with and without the addition of kanamycin. While a linear range was not achieved, signal was seen with nanomolar levels of kanamycin.

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

1. Ellington, A., Szostak, J. In vitro selection of RNA molecules that bind specific ligands. Nature 346, 818–822 (1990)
2. FRENS, G. Controlled Nucleation for the Regulation of the Particle Size in Monodisperse Gold Suspensions. Nature Physical Science 241, 20–22 (1973)
3. *Anal. Chem.* 2007, 79, 11, 4215–4221
4. Liu, J., Lu, Y. Preparation of aptamer-linked gold nanoparticle purple aggregates for colorimetric sensing of analytes. Nat Protoc 1, 246–252 (2006)
5. Langmuir 2019, 35, 41, 13461–13468

Scope of Project

My project goal was to create aptamer-linked gold nanoparticles (Apt-AuNP) for biosensing applications. Specifically, the sensor I developed is used to detect kanamycin. Aptamers are since stranded oligonucleotides that selectively and structurally bind to one target analyte.[1] The aptamers I worked with are thiolated (ending with an -SH group), which has a high affinity for the gold nanoparticle (AuNP) surface, making the functionalization of the Apt-AuNP relatively easy and via a one-pot method. Additionally, gold nanoparticles have fantastic fluorescence quenching properties, meaning their abilities to "turn-off" the fluorescence when a dye is near the AuNP surface. Lastly, AuNP have a long history of use in medicine and point-of-care applications, such as in surgery and immunoassay testing strips.



Synthesis, Functionalization, and Characterization of Aptamer-linked Gold Nanoparticles

I synthesized the bare AuNP via the Frens-modified Turkevich method, which involves reacting chloroauric acid ($HAuCl_4$) with trisodium citrate (Na_3-Cit), to reduce the gold atoms from Au(III) to Au(0), which forms various sizes of gold nanoparticles depending on the volume and concentration of Na_3-Cit used.[2] The UV-Vis spectra of AuNP made with various volumes of Na_3-Cit (1mL, 0.6mL, 0.3mL, and 0.16mL) were taken. The absorbance at the surface plasmon resonance peak and at 450nm can be analyzed to calculate the diameter and concentration of AuNP.[3] The UV-Vis of the dye (methylene-blue and fluorescein)-modified aptamer can be taken to calculate the concentration of the dye-aptamer conjugate.

To functionalize the AuNP into Apt-AuNP, a protocol was followed and modified[4] to suit our concentration of AuNP. In brief, TCEP is used to cleave the disulfide bonds the thiolated aptamers form, which turns the disulfide bonds into thiol groups, which easily adsorb to the AuNP surface. Acidic TRIS buffer (pH = 6) is used to deprotonate the thiol groups and stabilize the final Apt-AuNP. After an incubation period, the Apt-AuNP solution is centrifuged and the supernatant is removed, then redispersed in water, which was found via the optimization tests.

To calculate the number of aptamers immobilized per AuNP, I used mercaptohexanol (MCH) to displace the aptamers from the AuNP into solution. The literature reports that MCH, when in excess, can fully displace all aptamers from a gold nanoparticle.[5] After incubating the Apt-AuNP with MCH, the AuNP were centrifuged out and the solution's fluorescence was measured and compared to a calibration curve. The figure below donates all characterizations of the MB Apt-AuNP used for sensing.

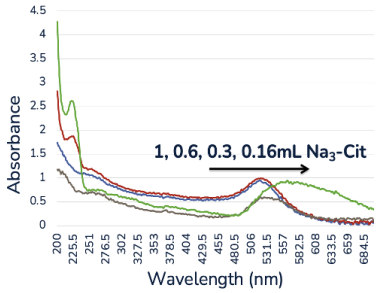


Figure 1. UV-Vis of synthesized AuNP with various volumes of Na_3-Cit

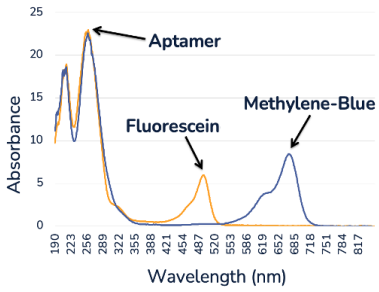


Figure 2. UV-Vis of FAM and MB functionalized aptamer

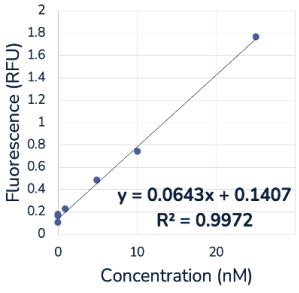


Figure 3. Concentrations of MB versus fluorescence signal

Concentration (AuNP/mL)	(7.55 ± 1.26) x 10^{11}
Concentration (nM)	1.25 ± 0.21
Aptamer per AuNP (N)	38.0 ± 5.00
Diameter (nm)	18.5 ± 1.11

Figure 4. Characterizations of AuNP used for sensing

Optimization of Sensing Conditions

To make the Apt-AuNP into a sensor, I had to optimize the fabrication of the sensor (buffer used, concentration of aptamer, and volume of redispersed Apt-AuNP) and sensing conditions (volume of kanamycin and time after measurement).

Optimizing the sensor involved various trials of different sensing conditions and measuring the fluorescence values with and without kanamycin at a 50µM concentration. A bar chart depicting the % increases under various conditions, and a chart detailing the sensing conditions used, is found below.

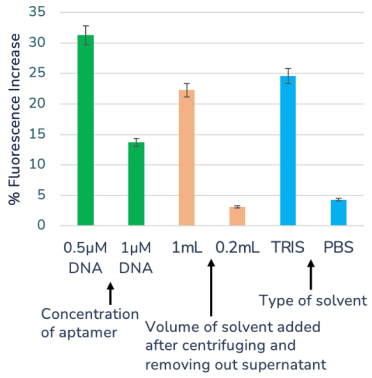


Figure 5. Chart of the % increase in fluorescence of blank vs. spiked solutions of Apt-AuNP at 50µM under various conditions for optimization

Volume of Kanamycin	4µL
Solvent	Water
Aptamer Concentration	0.5µM
Time before measurement	45 minutes
Volume of Apt-AuNP Sensor	1.4mL

Figure 6. Chart detailing the sensing conditions used for the data reported here.

Measurements using Apt-AuNP

Using a benchtop fluorometer, measurements were taken with the Apt-AuNP solution with various concentrations of kanamycin. In brief, our methylene-blue Apt-AuNP sensing solution, water, and kanamycin were vortexed, then incubated at 4°C for 45 minutes, then measured on a benchtop fluorometer which emits at 635nm and collected the maximum fluorescence from 671-693nm. The first experiment was to test the sensor over many ranges of magnitude to find where a true linear range falls.

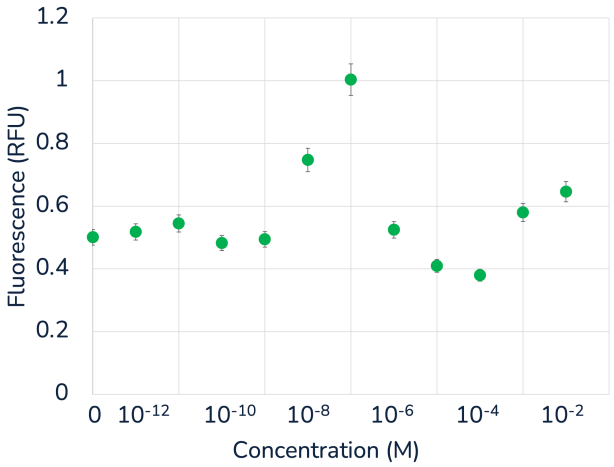


Figure 7. Graph of MB-Apt-AuNP fluorescence at various concentrations of kanamycin. Sensor consists of: 100µL Apt-AuNP, 4µL Kanamycin, 96µL Water

This graph does not look like a standard sensing curve – one would expect 3 sections to the curve: (i) Below LoD (ii) Linear Range (iii) Saturation of signal. However, the first two sections can be seen, but after a potential linear range, the signal returns to and at, and below, the blank value. To make sure the increase seen from the 10^{-9} to 10^{-7} range is not just a random occurrence, I tested more concentrations in this range of interest (nM).

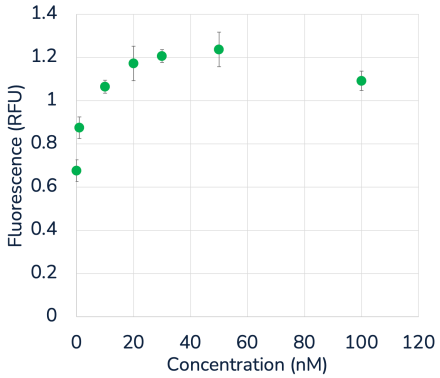


Figure 8. Graph of MB-Apt-AuNP fluorescence versus concentration at specific concentrations in determined range of interest (0-100nM)

This increase in fluorescence agrees with the increase in fluorescence seen when tested over several ranges of magnitude. The decrease in signal can be seen at 100nM as well, signalling this is an issue regardless of the batch (new functionalization) of Apt-AuNP.

A problem with the data from the Apt-AuNP fluorescence measurements is that the fluorescence values are in a small range of eachother (0.4-1.2 RFU) and that there is no linear range. Data from the optimization tests suggests that when the fluorophores are too concentrated (in both the concentration added for functionalization and Apt-AuNP in solution), self-quenching is responsible for the decrease in fluorescence, and potentially the difficulty finding a linear range for the sensor.

Future Directions

Potential remedies to these issues can include using less concentrated aptamer, more dilute Apt-AuNP solution, and a fluorophore with a greater quantum yield. These potential fixes work to decrease the concentration of fluorophore or to increase the fluorescence value, in hopes the AuNP will not quench the signal as much as we see under the current sensor conditions. Additionally, using fluorescein could get greater signal as the dye emits at the AuNP's surface plasmon resonance frequency, which would cause the AuNP to emit a signal as well. Preliminary data shows this increase in signal the case, but the signal is not proportional.