ND*nano* Summer Undergraduate Research
2016 Project Summary

1. Student name: **Joseph Sawyer**

2. Faculty mentor name: **Dr. Ryan Roeder**

3. Project title: **Immunotargeted Nanoparticles Bind to Cancer Cells**

4. Briefly describe any new skills you acquired during your summer research: **During my summer research, I learned how to:**

- **Take care of cell cultures by seeding, changing media, splitting, fixing, counting, passaging/trypsinizing, freezing in cryo, etc.**
- **Make Gold Nanoparticles, rinse them, functionalize them, link proteins to them, and run experiments with them**
- **Use inductively coupled plasma optical emission spectroscopy to determine concentrations of solutions.**
- **Use sterile technique when working with cells**
- **Use fluorescence microscopy**

5. Briefly share a practical application/end use of your research: **My research was a preliminary step in quantifying specifically how quickly gold nanoparticles bind to cancer cells. In the future, this quantification will tell doctors exactly how many nanoparticles will be needed to detect a tumor given the number of cells in the tumor.**

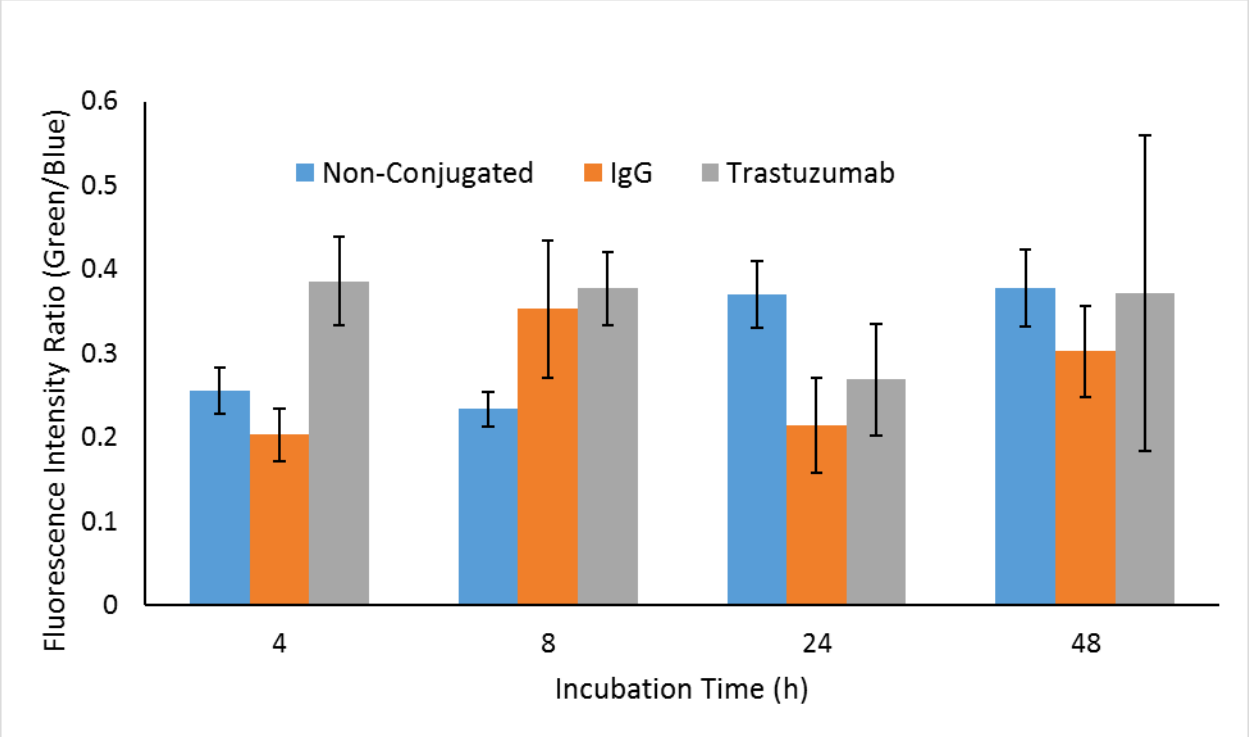
Begin two-paragraph project summary here:

Immunotargeted gold nanoparticles (Au-NPs) have been prepared to enable contrast-enhanced detection of tumors by computed tomography (CT) by researchers in the past. However, in order to determine efficacy and dosing, the Au-NP binding kinetics and isotherm for targeting cancer cells needed be characterized. This means that the actual quantification of how quickly nanoparticles bind to individual cells has yet to be determined. Therefore, the objective of this study was to verify the specificity and measure the binding kinetics of immunoconjugated Au-NPs for HER2+ breast cancer cells.

As the first part of my project, I synthesized the nanoparticles that were to be used in the experiments. Au-NPs were encapsulated with a silica shell, which was volume-loaded with Fluorescein isothiocyanate (FITC) for fluorescence imaging, and immunoconjugated with either trastuzumab for targeting HER2+ cells, or immunoglobulin G for a negative control. While synthesizing the nanoparticles, I was also working with cell cultures. I would grow the cells, change the growth medium every two days, split the cell cultures when the flasks became confluent, and froze down the cells in liquid nitrogen when too many flasks were built up in the incubator. For the experiment with Au-NPs, SKBR-3 cells were incubated with Au-NPs for up to 48 hours, and the rate at which immunotargeted Au-NPs bound to HER2+ cells over time was

compared to non-targeted Au-NPs (negative control). Au-NP binding to cells was analyzed at each time point by using both fluorescence microscopy (Figure 1) and using inductively coupled

Figure 1. *Intensity of FITC fluorescence is normalized by intensity of DAPI fluorescence to give a value correlated to the FITC fluorophores per cell. Data is largely inconclusive, but targeted trastuzumab did bind faster at 4 h than the untargeted IgG.*



plasma optical emission spectroscopy (ICP-OES) to measure the concentrations of gold in solution before and after the cells were incubated with the Au-NPs. Fluorescent microscopy showed greater binding of targeted Au-NPs compared with untargeted Au-NPs. ICP-OES showed an increased concentration of gold binding to cells over time for the targeted Au-NPs, whereas the untargeted Au-NPs remained at a constant concentration over the 48 hours. The results, however, were not statistically conclusive, and more testing needs to be done using higher concentrations of Au-NPs.

Publications (papers/posters/presentations):

Detection of Tumors Using Immunotargeted Nanoparticles For Contrast-Enhanced CT

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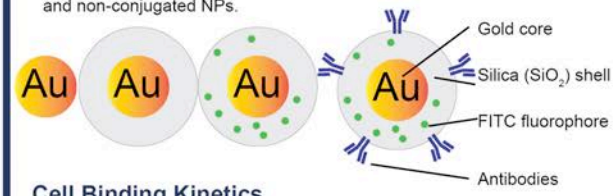
Introduction

- Immunotargeted gold nanoparticles (Au-NPs) can enable contrast-enhanced detection of tumors by computed tomography (CT) [1] and targeted drug delivery [2].
- In order to determine efficacy and dosing, the Au-NP binding kinetics and isotherm for targeting cancer cells must be characterized.
- Therefore, the ***objective*** of this study was to verify the specificity and measure the binding kinetics of immunoconjugated Au-NPs for HER2+ breast cancer cells.
- The ***hypothesis*** was that immunoconjugated targeted NPs would have increased binding compared to non-conjugated, non-targeted NPs.

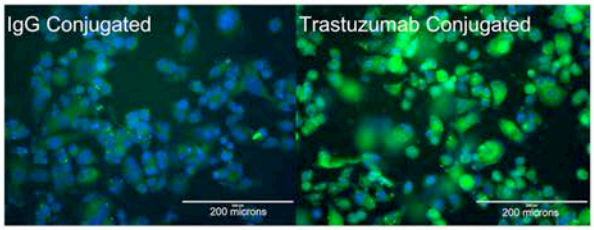
Nanoparticles

- Au-NPs were synthesized by the Turkevich method [3].
- The Au-NPs used were encapsulated with a silica shell volume-loaded with fluorescein isothiocyanate (FITC) to enable fluorescence imaging [4].
- The silica shell was immunoconjugated with trastuzumab to enable immunotargeting.
- Control groups included NPs conjugated with immunoglobulin G (IgG) and non-conjugated NPs.



Cell Binding Kinetics

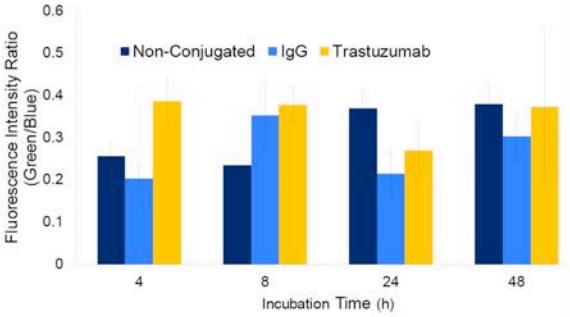
- SKBR-3 cells were incubated in McCoy's 5A medium at 30 °C with Au-NPs at a concentration of 2 μM for 4, 8, 24, or 48 h.
- The rate at which immunotargeted Au-NPs bound to HER2+ cells over time was compared to that of non-targeted Au-NPs and non-conjugated Au-NPs (negative controls).
- Au-NP cell binding was analyzed at each time point by using both fluorescence microscopy and using inductively coupled plasma optical emission spectroscopy (ICP-OES).



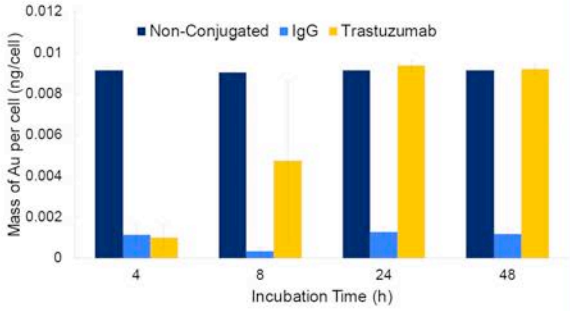
Breast cancer cells incubated with Au-NPs exhibit green fluorescence from FITC and blue fluorescence from DAPI.

Results

- Fluorescence microscopy showed greater binding of targeted Au-NPs compared with non-targeted Au-NPs.



- ICP-OES showed an increased concentration of gold binding to cells over time for the targeted Au-NPs, whereas the non-targeted Au-NPs remained at a constant concentration over the 48 hours.



Discussion

- Au-NPs immunoconjugated with trastuzumab were verified to target HER2+ breast cancer cells faster than non-targeted NPs conjugated with IgG.
- Further testing is required to evaluate the immunoconjugated NPs compared with the non-conjugated NPs.

Acknowledgments

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References

[1]J. Hainfeld, *The British Journal of Radiology,* 2011, **84**, 526-533.
[2]L. Carpin, *Breast Cancer Res Treat* 2010, **125**, 27-34.
[3]J. Turkevich, *Discuss. Faraday Soc.* 1951, **11**, 55.
[4]P.D. Nallathamby, *J. Mater. Chem. B*, in press.