POLYDOPAMINE-LIPID INTERFACE FOR SURFACE PLASMON RESONANCE SENSING

The text does not wrap; each field must be entered individually.

By

Chathu Keerthisinghe

A capstone project submitted for
Graduation with University Honors

February 21, 2019

University Honors
University of California, Riverside

APPROVED

_____

Dr. Quan Jason Cheng
Department of Chemistry

_____

Dr. Richard Cardullo, Howard H Hays Jr. Chair and Faculty Director, University Honors
Interim Vice Provost, Undergraduate Education

Abstract

The abstract should not exceed 350 words and should not extend past the bottom of this page. After deleting these instructions, please enter your abstract in Times New Roman, 12 pt. font, double-spaced. As the pdf is not formatted to provide word count or allow for automatic double spacing, please enter your text in a word document with the aforementioned formatting and paste into this field.

The purpose of the project is to design and test a new surface chemistry for use in Surface Plasmon Resonance (SPR) analysis, whereby lipid vesicles that mimic a cell membrane are tethered to a polydopamine surface via a streptavidin bridge. SPR is a surface-sensitive technique that allows real-time, label-free detection of biomolecular interactions by measuring the change of refractive index in the flow medium when biomoleculebinding occurs1. Polydopamine is used because its versatility and provides alternative method of protein-biomolecule conjugation2. Under slightly basic conditions, dopamine will self-polymerize and attach to free amines for a biomolecule attachment. This proves to work efficiently with lipids including 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphocholine (POPC) with Biotin (POPC-Biotin) and POPC alone. In this study, streptavidin is immobilized onto polydopamine coated gold chip under basic conditions (Trizma HCl pH 8.5) offline after which 5% POPC-Biotin and POPC vesicles are injected to SPR channels. In the POPC channel, since there is no biotin therefore no binding to the streptavidin coated polydopamine surface was observed. When the biotin is present in the lipid formation, this interacts with the streptavidin bound to the surface and cause the SPR signal measured in resonance angle shift ($\Delta\theta$) to increase.

To understand further, we studied the effects of incubation time of the polydopamine onto the gold surface and the concentration of lipid vesicle. Using this characterization results, it was concluded that the SPR signal increases with longer incubation time and higher concentration of lipid vesicle. Our results shows highest signal with 45 minutes incubation and 1 mg/ml POPC concentration. Our future work includes to test this surface as a biosensor; for example in the detection of Cholera Toxin (CT).

ACKNOWLEDGEMENTS

This capstone would not have been possible without the mentoring guide and assistance of Professor Jason Quan Cheng from the undergraduate chemistry department at University of California, Riverside. I am especially indebted to Nor Rais, a graduate student at the Cheng Biosensing lab at the University of California, Riverside, for always been very supportive of my educational goals and my passions. I have had great pleasure working with the everyone in the lab.

Furthermore, I am also grateful for everyone who has stayed with me through this journey especially my family and friends. Nobody has been more important and dedicated to my completion of the capstone than the members of my family. I would like to thank my parents for making this opportunity very possible for me.

Chathu Keerthisinghe
February, 2019

TABLE OF CONTENTS

Methods

- **The effect of incubation time of polydopamine on the gold surface**
  - Lipid vesicle was prepared according to the standard protocol[3]
  - Polydopamine (PDA) solution is prepared using 10 mg of Dopamine Hydrochloride and is dissolved it in a 5 mL stock solution of Trizma Hydrochloride Buffer.
  - 1 mL of this solution is added onto the gold chip (Au and Cr layer thickness 30 nm and 2nm).
  - Each chip is respectively stored for 15, 30, 45 minutes to identify and characterize the importance of the effect of the incubation time of the dopamine on the gold to SPR signal.
  - After this incubation period ends, the chips are cleaned thoroughly with nitrogen gas, which has an inert property helps reduce contamination.
  - To create the Polydopamine- Streptavidin bridge, 4 µL of streptavidin is dissolved into 980µL of Tris Buffer.
  - 20µL of the Streptavidin solution is added to each chip and stored overnight in the fridge.

Polydopamine-Lipid Interface for SPR

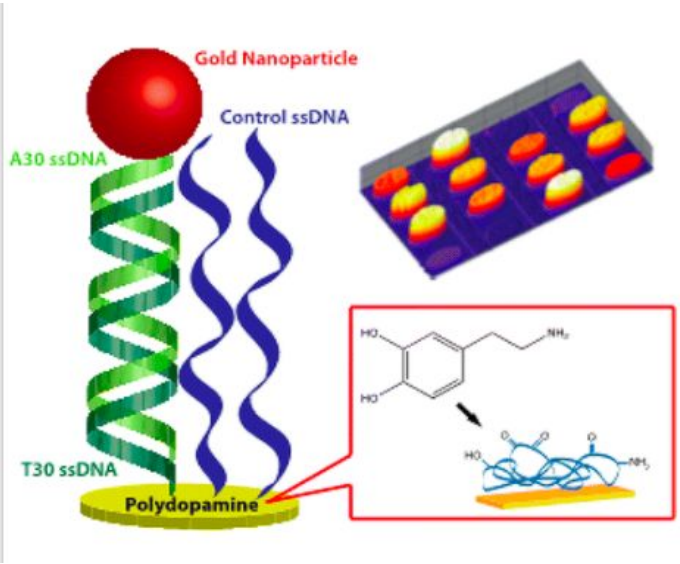


Figure 1: Tethering of Dopamine to Gold Surface[4]

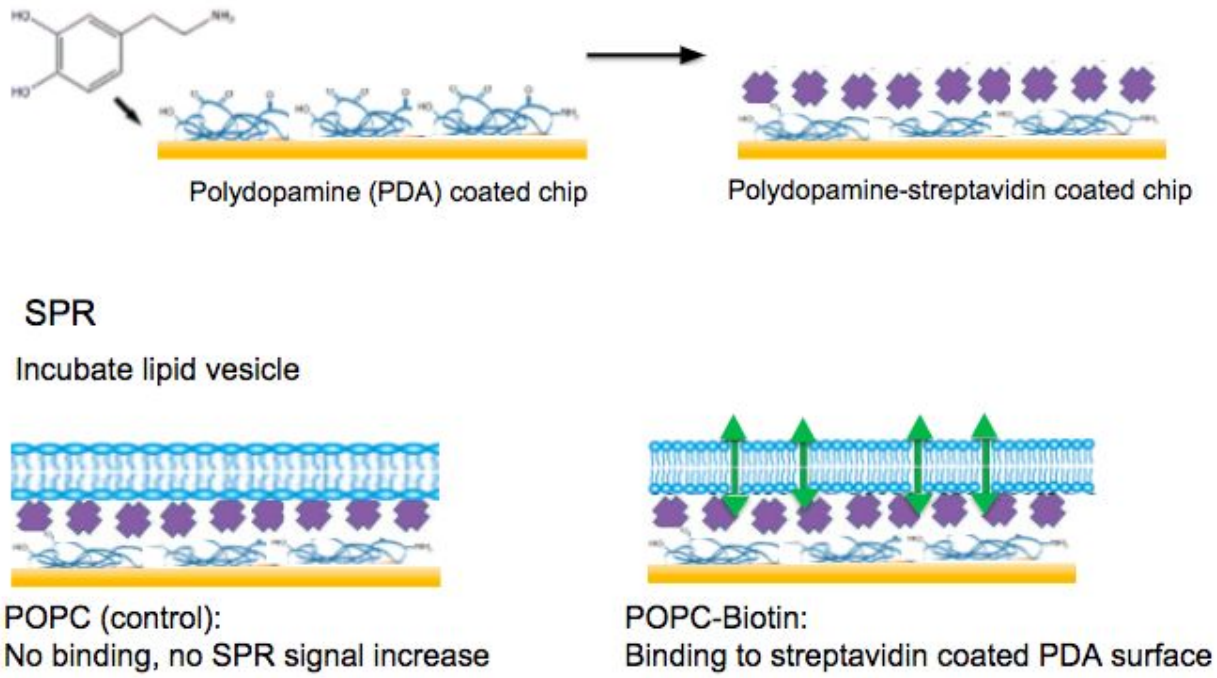
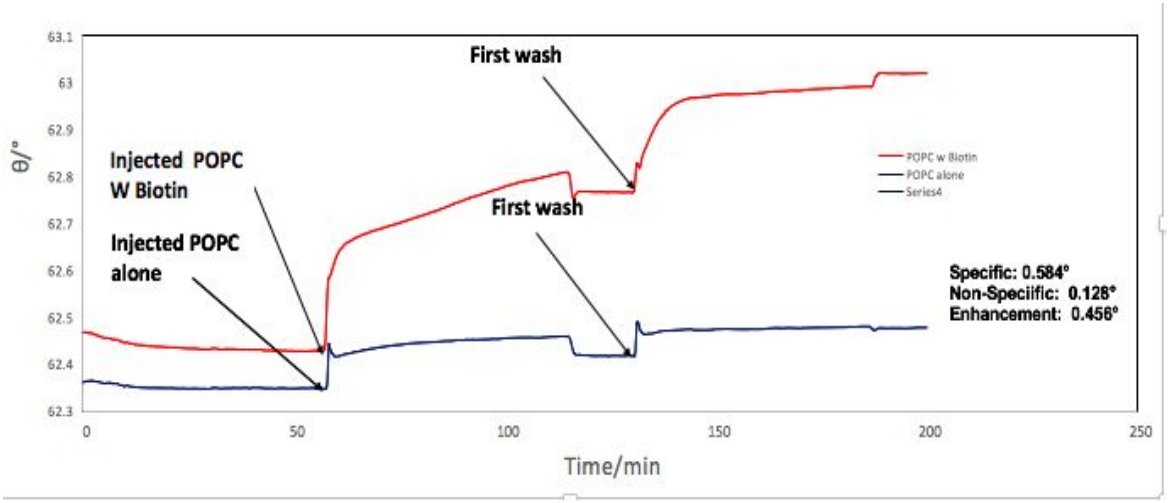


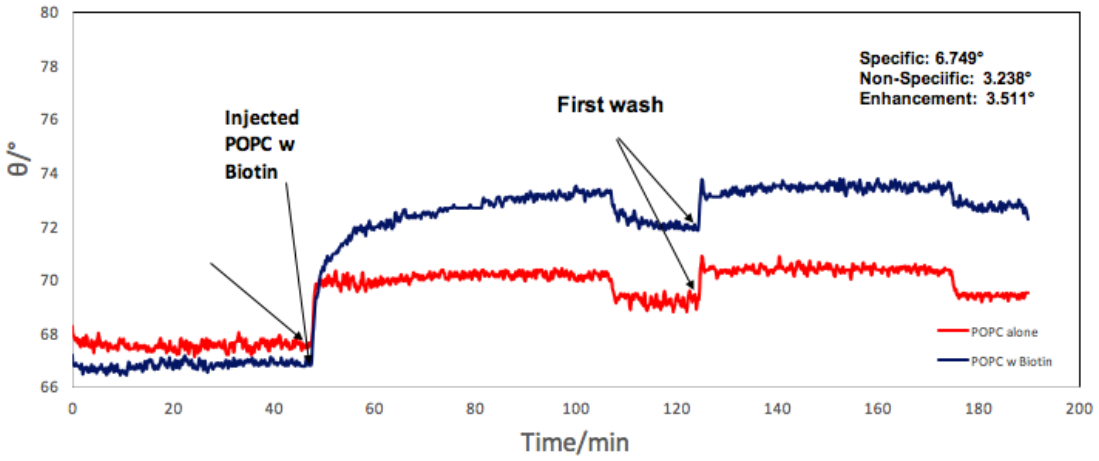
Figure 2: Schematic of surface chemistry on the gold surface. Lipid vesicle was injected online and incubate on the polydopamine-streptavidin coated chip

- **The effect of the concentration of the lipid vesicles**
  - Lipid vesicles were prepared in two concentrations; 0.5mg/mL and 1mg/mL.
  - Concentration of 1mg/mL of POPC-Biotin and POPC were diluted to 0.5mg/mL by utilizing Trizma Hydrochloride Buffer.

Results

How does incubation time of dopamine on the gold surface affect SPR signal?





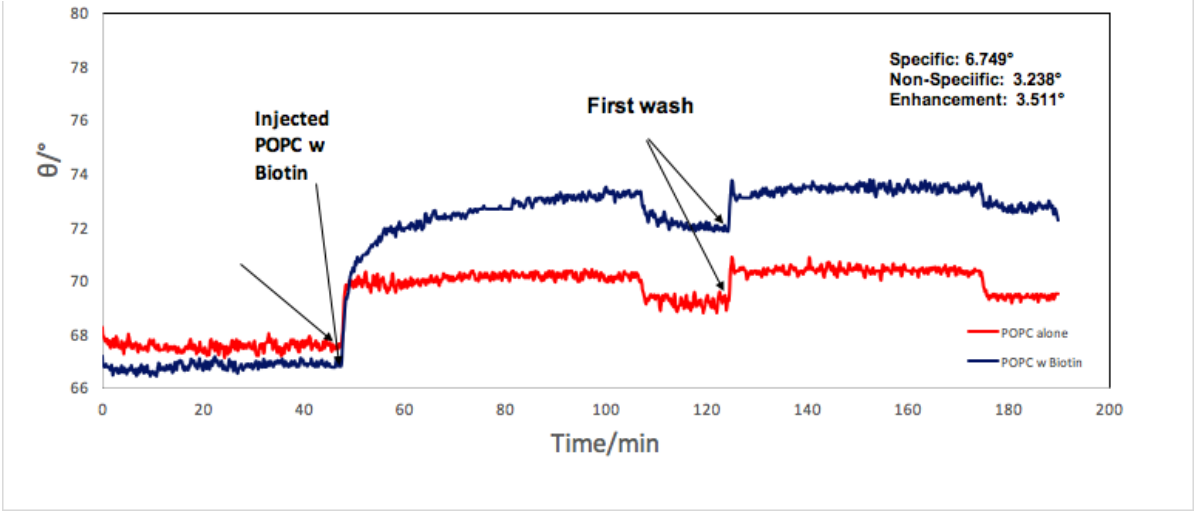
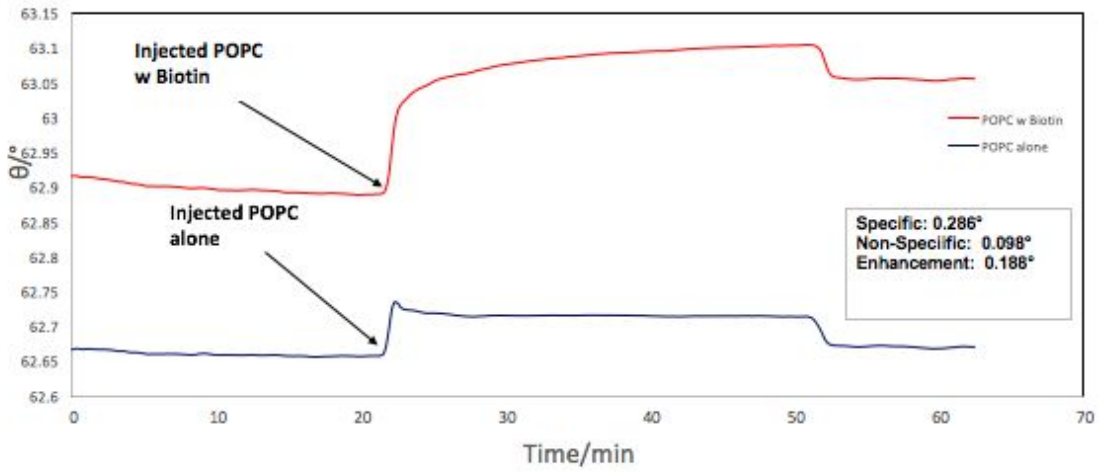


Figure 5: The series of graphs illustrate the changes of SPR enhancement according to different times of incubation. From top to bottom, it shows 45 minutes, 30 minutes, 15 minutes incubation

The above graphs show that the signal increases as the incubation time of dopamine increases . This supported that the longer the incubation period, the better the binding of the dopamine onto the gold surface, thus allowing the Biotin to bind more efficiently. The channel with Biotin-POPC exhibits higher signal in comparison to the POPC alone. The discrepancy was calculated by subtracting nonspecific signal from POPC channel. As a result, POPC alone acts as the control of the study because since there is no biotin, there is no specific binding to streptavidin thus the lower signal. Meanwhile, POPC-Biotin interacts with streptavidin bound to the surface, causing the substantial increase of SPR signal.

How does concentration of lipid vesicles affect SPR signal?



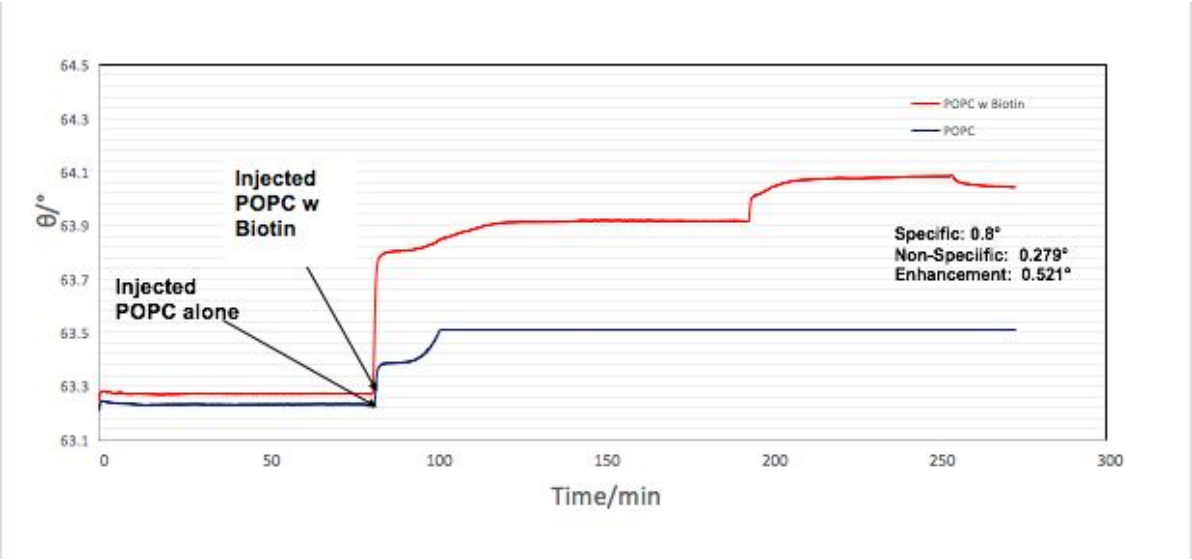


Figure 6: The series of graphs compares the effect of lipid concentration on the changes in SPR enhancement. 1 mg/ml is illustrated by the top graph while 0.5mg/ml is illustrated by the bottom graph.

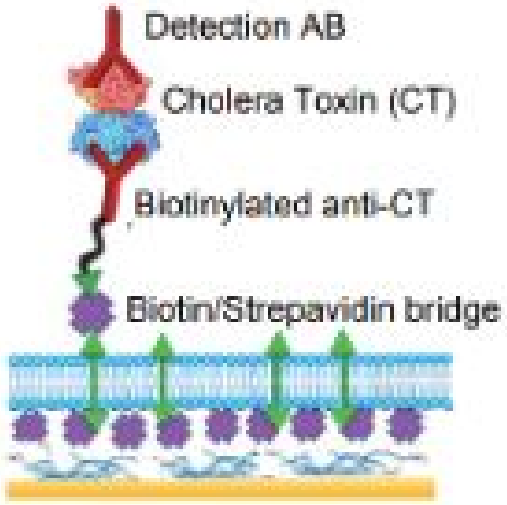
According to the SPR measurements above, there is a higher SPR signal from the 1mg/mL lipid concentration in comparison to the 0.5mg/mL lipid concentration. It can be concluded that higher concentration allows the lipid vesicles to form membrane layer more effectively.

Conclusion

We demonstrated a new surface for SPR whereby lipid vesicles are tethered to a polydopamine surface via a streptavidin bridge. The SPR analysis results shows an increase in SPR signal in the presence of biotin in the lipid formation, while no binding was observed in POPC alone. Characterization results show that the incubation time of dopamine and concentration of lipid vesicles plays a role in efficient polymerization and binding of biotin to streptavidin. As SPR is a very promising method for biomolecular interactions, we will explore this surface for the detection of biomarkers related to medical and diseases diagnostics.

Future Work

As SPR biosensors can be used for many applications such as medical diagnostic, we are exploring this new surface for the detection of biomolecule markers like Cholera Toxin (CT). This study reported that the streptavidin coated polydopamine surface successfully interact with biotin present in the lipid formation. This surface chemistry allows binding of biomolecule targets using biotin-streptavidin bridge, for example biotinylated anti-CT. Figure 7 shows our proposed assay for the detection anti-CT.



References

1. Surface Plasmon Resonance: Material and Interface Design for Universal Accessibility
Samuel S. Hinman, Kristy S. McKeating, and Quan Cheng, Analytical Chemistry **2018** *90* (1), 19-39

2. Fabrication of DNA Microarrays on Polydopamine-Modified Gold Thin Films for SPR Imaging Measurements
Jennifer B. Wood, Megan W. Szyndler, Aaron R. Halpern, Kyunghee Cho, and Robert M. Corn Langmuir **2013** *29* (34), 10868-10873

3. DNA Linkers and Diluents for Ultrastable Gold Nanoparticle Bioconjugates in Multiplexed Assay Development
Samuel S. Hinman, Kristy S. McKeating, and Quan Cheng, Analytical Chemistry **2017** *89* (7), 4272-4279

4. Wood J, et al., *Langmuir* **2013**, 29, 10868-10873