Lipid-Coated Gold Nanoparticle as a Biosensor for Lipid-Protein Interactions

Yuheng Cai
University of Denver

Follow this and additional works at: https://digitalcommons.du.edu/etd

Part of the Biochemistry, Biophysics, and Structural Biology Commons

Recommended Citation
Cai, Yuheng, "Lipid-Coated Gold Nanoparticle as a Biosensor for Lipid-Protein Interactions" (2015).
Electronic Theses and Dissertations. 105.
https://digitalcommons.du.edu/etd/105

This Thesis is brought to you for free and open access by the Graduate Studies at Digital Commons @ DU. It has been accepted for inclusion in Electronic Theses and Dissertations by an authorized administrator of Digital Commons @ DU. For more information, please contact jennifer.cox@du.edu,dig-commons@du.edu.
LIPID-COATED GOLD NANOPARTICLE AS A BIOSENSOR FOR LIPID-PROTEIN INTERACTIONS

A Thesis

Presented to

The Faculty of Natural Sciences and Mathematics

University of Denver

In Partial Fulfillment
of the Requirements for the Degree
Master of Science

by

Yuheng Cai

June 2015

Advisor: Michelle K. Knowles
Abstract

Gold nanoparticles have been intensively studied for their unique optical features. Due to surface plasmon resonance phenomenon, gold nanoparticles can respond to the refractive index change of the environment near the particles. This phenomenon gives gold nanoparticles the potential to become biosensors that detect the biological interactions at or near the particles. In this work, gold nanospheres were coated with phosphatidylcholine as the substrate for lipoxygenase. It is demonstrated, in our work, that lipid coated gold nanospheres can be used to detect the activity of lipoxygenase and provide more information of this reaction than common assays, like conjugated diene assay and TBARS assay. After demonstrating that lipoxygenase activity can be measured with gold nanoparticles, asymmetric gold nanoprisms were synthesized. The purpose of this was to create a more sensitive sensor for future studies of lipid-protein interactions. The long-term goal of the work is to create a versatile biological sensor that can detect enzyme activities under different environments.
Acknowledgements

This work was assisted by Dr. Scott M. Reed and his lab from University of Colorado Denver. Thanks to Dr. Michelle Knowles and every member in Knowles’ lab. Thank my family, especially my parents who support me all the time. Of course I want to thank all of my friends that have helped me and care about me.
# Table of Contents

Chapter One: Introduction ................................................................. 1
   Biosensors ..................................................................................... 1
   Gold nanoparticles .......................................................................... 2
   Surface plasmon resonance .......................................................... 3
   Gold nanoparticle synthesis ........................................................... 6
   Lipid oxidation catalyzed by lipoxygenase ..................................... 7
   Lipoxygenase inhibitors ................................................................. 11
   Traditional methods of lipid oxidation detection ............................ 12

Chapter Two: Gold nanosphere synthesis and lipid coating ......... 14
   Gold nanospheres synthesis and lipid coating .............................. 14
      Materials and Methods ............................................................... 14
      Results ....................................................................................... 16
      Discussion ................................................................................. 20

Chapter Three: Lipid oxidation catalyzed by lipoxygenase ......... 21
   Materials and Methods ................................................................. 21
   Results and discussion .................................................................. 25

Chapter Four: Gold nanopism synthesis and purification ........... 42
   Lipid-coated gold nanopism synthesis and purification ............... 42
      Methods and Materials .............................................................. 42
      Results and discussion ............................................................... 45
   Refractive index sensitivity of gold nanoprisms and nanospheres .. 48
      Methods and Materials .............................................................. 48
      Results and discussions ............................................................. 49

Chapter Five: Conclusion ................................................................. 54

References ......................................................................................... 56
Chapter One: Introduction

Biosensors

A biosensor is a device that detects biological interactions and translates them into measurable signals. Two components are required for a biosensor to accomplish this function. First, a recognition function of a biological interaction is required to detect the changes from this interaction. Second, a transducer is required to translate this change to measurable signals.

There are a wide variety of biosensors used in diagnostics and research. One example of an important biosensor is the one used to measure blood glucose levels, which was proposed by Dr. Leland C. Clark in 1962 (1). The core functional part of this biosensor is an electrode coated with glucose oxygenase. This electrode, now called the Clark electrode, measures the oxygen concentration by polarography. On the platinum cathode, reduced oxygen will cause a current in the circuit, which can be detected by a galvanometer. With the coat of glucose oxygenase, the oxygen electrode is able to monitor the oxygen consumed by the glucose oxidation interaction to estimate the glucose concentration in the solution. In the past decades, many scientists made their efforts on improving this biosensor by removing oxygen dependence, reducing redox interference, increasing electron transferring efficiency and so on (2). Nowadays, the blood glucose detector can be minimized into a pocketsize device for routine monitoring.
of blood glucose with clinically acceptable accuracy. The blood glucose biosensor recognizes the glucose oxidation reaction and transduces it to electronic signal. The sensors, which function in this manner, are classified as electrochemical biosensors. According to different transducing mechanisms, the biosensors can be classified as Optical-Detection biosensors, Thermal-Detection biosensors, Ion-Sensitive FET (ISFET) biosensors and Resonant biosensors (3,3,1). In this work, gold nanoparticles are functionalized and tested as Optical-Detection biosensors because of the unique optical and electronic properties present in nanomaterial.

**Gold nanoparticles**

Gold nanoparticles are particles with an average diameter from several nanometers to hundreds of nanometers. The earliest application of gold nanoparticles is believed to be the Lycurgus Cup, which is a 4th-century Roman glass cup. The addition of gold and silver nanoparticles makes the glass dichroic, which means the color of the glass will be different under reflected light or transmitted light (4). Although this is an ancient technology, modern scientific studies and uses of gold nanoparticles started with Michael Faraday's work in the 1850s. Since then, gold nanoparticles have been studied for biological applications because of their unique properties.

The material properties of gold nanoparticles can be exploited for biosensing in many ways. One of them is the ability of nanoparticles to enhance or quench fluorophores. This can be done in several ways. First, the nanoparticles can affect excited electrons with an optical feature (5). Secondly, with proper conjugated aptamers, gold nanoparticles can aggregate or disaggregate under certain condition. This will change
their absorbance so dramatically that it is observable by naked eyes. A third way is that the localized surface plasmon resonance (LSPR) peak can shift as a response to the analyte behavior, such as protein binding or a deposition of a layer on the nanoparticle (6). In our work, the localized surface plasmon resonance (LSPR) of the gold nanoparticles is utilized to detect protein-lipid interactions.

**Surface plasmon resonance**

Surface plasmon resonance (SPR) is a phenomenon of collective electron oscillation excited by incident light with specific wavelengths. Incident light with the same frequency as the natural frequency of the electrons on metal will be absorbed and then, causes electrons to resonate (Figure 1.1). To create a SPR biosensor, a thin metal film, usually gold, is deposited on a glass prism surface. Light detectors can detect the change in the wavelength of incident light caused by the refractive index change of the environment near the metal film. The SPR spectroscopy has been studied as a label-free method for bimolecular interactions since many interactions are associated with refractive index changes.

Localized surface plasmon resonance (LSPR) is similar to the SPR phenomenon, but occurs when using metal nanoparticles. For the gold nanoparticles, when the electron field of the incident light causes the oscillation of the electrons on the nanoparticles, the electron density is polarized on one side of the particle with the light frequency and the light with the corresponding wavelength is absorbed (Figure 1.1). The LSPR phenomenon is strongly affected by the size and shape of the nanoparticles as well as the environment around the particles (7).
Figure 1 Gold nanoparticle with polarized electron cloud due to the electronic field from incident light. (7)

SPR and LSPR both possess high sensitivity to refractive index changes, which helps lower detection limits for biomolecule interactions. However, the long field decay length of SPR (more than 100nm) makes this method less sensitive to the biomolecules on the surface because the sensing range is much larger than the typical analyte. SPR senses more of the buffer environment instead of just the biomolecules. On the other hand, with shorter field decay from 0.1 nm to 60 nm (8), LSPR is less affected by the environment refractive index changes. This makes gold nanoparticles a powerful biosensor that can sensitively detect refractive index change related interactions or chemical changes while reducing unwanted signals from outside environmental changes.

In one portion of our work (Chapter 2), we focus on changes in the refractive index due to molecular composition changes. With gold nanoparticles of well-defined size and shape, small changes in the refractive index occurring due to change of the environment near the nanoparticles will result in a LSPR signal response. In this work, sodium oleate, phosphatidylcholine and propanethiol are used to functionalize the gold
nanoparticles. The centroid of the LSPR peak changes as those chemicals are added in the nanoparticles (9).

Figure 1.2 TEM pictures of gold nanospheres, nanocubes, nanorods, nanobranches and nanobipyramids. (10)

Once the refractive index of the environment around the nanoparticles increases, the LSPR peak will shift towards red. However, this red shift is strongly affected by the aspect ratio of the gold nanoparticles. Among five types of gold nanoparticles (nanospheres, nanocubes, nanorods, nanobipyramids and nanobranches) (Figure 1.2), the red shifts vary from 44 nm/RIU to 703 nm/RIU (refractive index unit) according to the shape of the gold nanoparticles. Gold nanospheres and nanocubes provide much smaller red shifts per refractive index unit. On the contrary, elongated nanoparticles, for instance,
nanorods or nanobipyramids with large aspect ratios provide larger centroid shifts to the refractive index change. Furthermore, as the apexes of the elongated gold nanoparticles get sharper, the nanoparticles have higher refractive index sensitivity. With the sharpest apexes, nanobranches have the highest sensitivity of 703 nm/RIU among these nanoparticles (10).

**Gold nanoparticle synthesis**

Gold nanoparticles are usually synthesized by reduction of Au (III) ions to Au atoms with different reducing agents. Although, the mechanism of gold nanosphere synthesis remains less well explained, the Turkevich method has been the most common and popular approach to synthesize gold nanoparticles. In this method, tetrachloroauric acid is reduced by sodium citrate with 30 minutes of boiling. Then, gold nanospheres are synthesized and stabilized by citrate ions. (11)

In ancient times, gold nanoparticles were only spheres because they were the easiest gold nano structure to make. In recent decades, the ability to synthesize gold nanoparticles to different shapes and sizes has been developed by controlling the growth rate on every facet. These non-spherical nanoparticles have received intense attention from scientists because they are more sensitive to a refractive index change of the environment than nanospheres. However, non-spherical nanoparticle synthesis is very challenging because the conditions of gold nanoparticles’ nucleation are different from shape control of gold nanoparticles. Monodispersity of gold nanoparticle size is also a challenge. The seed-mediated gold nanoparticle synthesis is a method to balance the
requirements of nucleation and shape control with a good monodispersity of size. First, the seed gold nanoparticles are synthesized rapidly into spheres with small sizes. Then, the conditions are changed to make the seed gold nanoparticles grow on specific facets in order to form different shapes. Since the second step is much slower and controlled, the gold nanoparticles are inclined to grow at similar rates to form gold nanoparticles with similar sizes.

One of the most popular methods for synthesizing gold nanoparticles of obscure shapes and sizes is to use surfactants to change the growth rate on different facets of nanoparticles. Various surfactants are required to change the growth rate differently in order to form different shapes of nanoparticles (12). Among all of the anisotropic gold nanoparticles, the growth of gold nanorods is the most established protocol. By using cetyltrimethylammonium bromide (CTAB, a cationic surfactant) with AgNO₃, the yield of gold nanorods can be as high as 99%.

In our work, gold nanoprisms are synthesized using the method modified by Scott Reed’s lab (13). To make the gold nanoparticles biocompatible and study the lipid-protein interactions, soybean lecithin is used as surfactant to manipulate the shape of gold nanoparticles.

**Lipid oxidation catalyzed by lipoxygenase**

Lipid oxidation is a hydroperoxidation reaction of unsaturated fatty acids catalyzed by a class of non-heme iron enzymes called lipoxygenases (also known as lipoxidases or LOX). There are various types of lipoxygenase with different preferences
of fatty acids as substrates and different reaction pathways. In general, lipoxygenases are named by the possible positions of the hydroperoxy group on arachidonic acid. For example, 15-lipoxygenase means that after the oxidation the product will be a 15-hydroperoxide (Figure 1.3).

Lipid oxidation is essential in many important cellular processes. For example, the oxidized phospholipid on a cell membrane will have a polar tail by the addition of oxygen on it. Unlike the hydrophobic tails of other phospholipids, this oxidized acyl chain preferring the hydrophilic environment on the surface of the cell membrane becomes a ligand for macrophage scavenger receptor CD36, which can trigger the innate immune system in the human body to remove cells expressing oxidized lipids. Understanding how lipoxygenase oxidizes lipids may help reveal the mechanism of many essential biological processes. (14)
Figure 1.3 Arachidonic acid and the hydroperoxides from lipid oxidation catalyzed by 15-lipoxygenase. (15)

In plants, most substrates of lipoxygenase have 18 carbons. When the substrate has 18 carbons, like linoleic acid, the lipoxygenase has a positional preference on carbon atom 9 or carbon atom 13 (16). To simplify the naming system, lipoxygenase from soybean will be referred to as SLO, Soybean Lipoxygenase (17).
Figure 1.4 Crystal structure of SLO-1. Non-Heme active site is the domain on the left with many α-helixes, which is responsible for the oxidation of the lipid. The PLAT domain on the right is believed to help bind to the lipid.

The enzyme used in this work is a lipoxygenase from soybean (SLO-1) (Figure 1.4), while the substrate used in this work is soy phosphatidylcholine (Sigma-Aldrich, product number, P3644). According to the product information from Sigma-Aldrich, the main unsaturated fatty acid is linoleic acid (Figure 1.5). SLO-1 possesses a PLAT (Polycystin-1, Lipoxygenase, Alpha-Toxin) domain at the N-terminal and a non-heme iron active site at the C-terminal. The non-heme iron active site is responsible for the abstraction of hydrogen from the substrate and forms a radical on the carbon chain of the substrate. One model of how the linoleic acid was oxidized in the binding cavity involves
the formation of conjugated diene structure. With the hydrophobic tail of linoleic acid buried in the binding site, the hydrogen atom on pentadiene structure in linoleic acid is close to the iron active site in the binding cavity. The iron hydroxide group will abstract the hydrogen atom from the linoleic acid carbon chain and a radical will be formed on the linoleic acid. (18) Then the radical will be rearranged to form a stable conjugated diene structure on the carbon chain. The next step is the insertion of oxygen to the radical and a proton is added to form a carboxyl group. In this case, the 11-carbon is almost the only site that a proton and an electron can be abstracted from (19,12). With the radical formed at the 11-carbon, a radical rearrangement can transfer the radical to either 9-carbon or 13-carbon on the linoleic acid carbon chain. As a result, the oxygen molecule has two positions to insert, C-9 or C-13, forming either 9-HPOD (9-hydroperoxyoctadecadienoic acid) or 13-HPOD respectively (20).

![Figure 1.5. Structure of linoleic acid (21).](image)

**Lipoxygenase inhibitors**

Many known lipoxygenases can be inhibited by various inhibitors, such as, 5,8,11,14-Eicosatetraynoic acid (ETYA), 3-tert-butyl-4-hydroxyanisole (BHA), nordihydroguaiaretic acid and salicylhydroxamic acid. In our work, two of them, ETYA and BHA, are used to inhibit the lipoxygenase. However, ETYA and BHA act as
lipoxygenase inhibitor with different mechanisms. ETYA is an arachidonic acid analogue. It is reported that ETYA can irreversibly inhibit lipoxygenase and prostaglandin synthetase (22). BHA is an antioxidant commonly used in food, animal food, cosmetics, rubber and petroleum products. With a conjugated aromatic ring, the BHA can stabilize the free radicals and prevent farther oxidation reaction. Considering the mechanism of the lipoxygenase, these two inhibitors should be able to block the enzymatic lipid oxidation reaction, which helps to build negative controls. (23)

**Traditional methods of lipid oxidation detection**

In the past, lipid oxidation reactions were studied by the TBARS (Thiobarbituric acid reactive substances) assay and a conjugated diene assay. Conjugated diene is a delocalization of the pi system caused by two neighboring carbon-carbon double bonds separated by a carbon-carbon single bond. For the hydroperoxide of linoleic acid, it has a conjugated diene around the 11-C position after the abstraction of hydrogen from 11-C and radical rearrangement. The conjugated diene has an absorbance at 234 nm with an extinction coefficient of 29500 L mol\(^{-1}\) cm\(^{-1}\). The oxidation of linoleic acid by lipoxygenase forms this conjugated diene structure and causes an increase of the absorbance at 234 nm. Based on the Beer-Lambert law, the extent of lipid oxidation can be determined by the absorbance at 234 nm. (24) The TBARS assay has been used extensively for lipid oxidation measurements and oxidative stress studies in biological systems since the 1950s. The TBARS assay measures the malondialdehyde (MDA) produced by the oxidation of unsaturated fatty acids to estimate the peroxidation content.
of the lipid (25). In this assay, the MDA will react with thiobarbituric acid when heated up to 90-95 °C with trichloroacetic acid (TCA) and form fluorophores with excitation wavelength at 530 nm and emission around 550 nm. However, the lipid peroxidation is not the exclusive source of MDA, making the result less specific for the enzyme catalyzed lipid peroxidation. Other byproducts of lipid peroxidation can also affect the accuracy of the final results. Time resolved studies, which are useful for measuring kinetics of enzymes that act on lipids, are also not possible with TBARS.

One purpose of our work is to demonstrate that the lipid coated gold nanoparticle is an advanced biosensor to study protein-lipid interactions such as this lipoxygenase-lipid interaction. With the sensitive LSPR phenomenon, the lipid coated gold nanoparticle is expected to response to the refractive index change near the nanoparticle caused by lipoxygenase activity. The ability to track the refractive index in real time also gives lipid coated gold nanoparticle a potential to collect kinetic information of lipoxygenase activity.
Chapter Two: Gold nanosphere synthesis and lipid coating

Gold nanospheres were synthesized (24 nm diameters) and coated with soy phosphatidylcholine (SoyPC) lipids. The lipid coating process is critical because the coating can stabilize the gold nanospheres and provide a substrate for protein binding interaction. After the gold nanosphere synthesis, the lipid coating process was actively monitored by LSPR method.

Gold nanospheres synthesis and lipid coating

Materials and Methods

The source of gold for all nanosphere synthesis was a tetrachloroaauric acid solution. 5 mg chloroaauric acid (HAuCl₄, Strem Chemicals, Catalog NO. 79-0500) was dissolved in 95 mL water (Milli-Q, 18.2 MΩ·cm). 1% sodium citrate solution (Na₃C₆H₅O₇, 34 mM) was prepared by dissolving sodium citrate in water. 16 mg sodium oleate (TCI America, Catalog NO. O0057-25G) was dissolved in 5 mL of water to make a 0.01 M sodium oleate solution. Sodium oleate was made fresh every week and kept away from light because sodium oleate is light sensitive. 10 mM PC₃₀ stock solution (PC₃₀ means the phosphatidylcholine content is ≥ 30%) was made by dissolving phosphatidylcholine (Sigma-Aldrich, 30%, Catalog NO. P3644-25G) in water. This PC stock solution was cup horn sonicated for 1 hour and extruded by nuclepore membrane.
(Whatman, lot#85812) with 100 nm diameter holes. 1 µL of propanethiol (Acros Organics, 98%, Catalog NO. AC13143-0500) was diluted in 10 mL of water. This has to be made fresh every day.

95 mL of chloroauric acid solution was refluxed and 5 mL of 1% sodium citrate solution was added. After boiling for 30 min, the solution was left to cool to room temperature. The average diameter was determined by dynamic light scattering (DLS) to be 24 nm. The gold nanospheres were produced at a large scale and are expected to be stable for months with sodium citrate.

To lipid-coat the gold nanospheres, 5 µL of sodium oleate (0.01 M), 10 µL of PC$_{30}$ stock solution (10 mM) and 30 µL of propanethiol (1 mM) were added to 1 mL of gold nanospheres with 30 min intervals among them (Figure 2.1). By adding these chemicals, the gold nanospheres should be gradually coated by sodium oleate, phosphatidylcholine and propanethiol, meaning the gold nanospheres were successfully assembled as a biosensor and can be used to study the protein-lipid interactions. The gold nanospheres can last for months, but this biosensor should be made fresh each day because the sodium oleate is light sensitive and the PC$_{30}$ can undergo autoxidation with the oxygen in the air.

The lipid coating process was monitored by an LSPR instrument. This is a real-time measurement. The light source (Ocean Optics DH-2000) emits a light beam with full wavelength and the light beam goes through the cuvette with the gold nanospheres. The detector was set to record a spectrum every 1.1 seconds. When the gold nanospheres were coated with lipid, the spectra were analyzed by Matlab (Figure 2.2). Since the refractive
index change causes the LSPR peak to shift towards red, the centroids of every spectrum were extracted by Matlab (Figure 2.3).

**Results**

The procedure we follow for nanoparticle synthesis and coating is depicted in Figure 2.1 (9). First, we started with gold nanospheres reduced and protected by sodium citrate. Second, sodium oleate is added to the gold nanospheres and the sodium oleate will cover the surface of the gold spheres. Then, PC liposomes are added and start to coat the gold nanospheres. Lastly, propanethiol is added and it permeates through the PC coating film and binds to gold nanospheres and stabilizes them from aggregation.

![Figure 2.1 Lipid coating process.](image)

**Figure 2.1 Lipid coating process.** A, gold nanospheres with citrate cap. B, gold nanospheres coated by oleate because of the addition of sodium oleate. C, gold nanospheres coated with phosphatidylcholine because of the addition of PC$_{30}$. D, propanethiol permeates through PC film and coats the gold nanospheres. (9)
We monitor the process of coating the nanoparticles using LSPR. Spectra are measured in time (Figure 2.2) and the change in the location of the peak indicates a change in the surrounding refractive index. By following this in time during the addition of the reagents discussed in Figure 2.1, the synthesis of lipid coated gold nanoparticles can be tracked (Figure 2.3). At the beginning, we started with gold nanospheres reduced by citrate. Then, at around 1800s, sodium oleate is added causes an immediate centroid shift up. 30 min later, at around 3600s, PC30 is added and causing a centroid shift down. After another 30 min, at 5400s, propanethiol is added and the centroid shifts down. These centroid shifts are caused by the change of refractive index at the gold nanoparticle surface.
Figure 2.2 Overlay spectra from LSPR. The x axis is detector number, which can be correlated with wavelength of the spectra. The y axis is optical density with arbitrary unit. This is an overlay of all spectra. The arrow shows that the peak gradually shifts towards red.
Figure 2.3 Gold nanoparticle lipid coating monitored by LSPR. The gold nanoparticles are coated by citrate (a), sodium oleate (b), PC (c) and propanethiol (d) in sequence. The centroid of the LSPR peak responds to the environment’s refractive index changes caused by sodium oleate, PC and propanethiol.
Discussion

Although the exact chemical composition cannot be determined by the LSPR assay, the shift in the centroid indicates that the local surroundings have changed after addition of each reagent. Figure 2.3 shows that the centroid of LSPR peak responds to the addition of sodium oleate, phosphatidylcholine and propanethiol. These red centroid shifts indicate that the sodium oleate, \( \text{PC}_{30} \), and propanethiol change the refractive index near the gold nanoparticles though the exact coating structure is not available directly from LSPR data. However, this synthesis result still provides evidence that gold nanospheres have the potential to be a biosensor with this refractive index detecting ability. This has been well established in the work of others (9).
Chapter Three: Lipid oxidation catalyzed by lipoxygenase

Changes in lipid composition – from a non-oxidized to an oxidized state – result in changes in the index of refraction. The approximate refractive index change that others have seen over time is 0.0025 RIU (26). In theory, these changes in refractive index should be measureable with LSPR techniques using gold nanoparticles. In this work, the 0.0025 RIU will cause 0.37 nm centroid shifts for gold nanoprism and 0.08 nm for gold nanospheres. To test whether it was possible to sense changes in lipid oxidation in the laboratory, the lipoxygenase was added to the lipid coated gold nanospheres. Conjugated diene, TBARS and LSPR assays were used to study this enzyme activity. (24) (25) ETYA and BHA were used as inhibitors to lipoxygenase to create a negative control group. (22) (23)

Materials and Methods

Liposome preparation

The substrate solution was prepared by sonicating 1 mL of PC_{30} stock solution (4 mM) with a cup horn sonicator for 1 hour. Next, the PC_{30} stock solution was extruded with a 100 nm nuclepore membrane 15 times. The liposome size was determined as 24 nm by dynamic light scattering (Zetasizer nano series – S90).
**TBARS assay**

20% Trichloroacetic acid (TCA, J.T.Baker, 0414-01) was prepared by dissolving TCA crystals in water. The 0.67% 2-Thiobarbituric acid (TBA, Sigma-Aldrich, T5500) was prepared by dissolving with water and sonicating 1 hour by cup-horn sonication.

Lipid coated gold nanospheres were assembled as mentioned in chapter two. Then, 100 µL of Tris-HCl buffer was added to maintain the proper pH of the solution for enzyme activity and then 5 µL of lipoxygenase was added to oxidize the lipid coating on the surface of the gold nanospheres. 30 min later, 1.5 mL 0.67% TBA and 1.5 mL 20% TCA were added in and N$_2$ was bubbled in the solution for 10 min to remove the oxygen in the solution. The solution was heated up to 95 °C for 30 min and cooled down to room temperature. At the end, the fluorescence spectrum was acquired with an excitation wavelength at 530 nm and an emission wavelength from 540-560 nm. A parallel negative control was done with the same steps except for the addition of lipoxygenase.

**Conjugated diene assay**

PC$_{30}$ stock solution (10 mM) was prepared as previously described. Tris-HCl buffer (0.1 M, pH 9.0) was prepared to adjust the pH of the environment. To keep the concentration of PC$_{30}$ consistent during the conjugated diene assay, the lipoxygenase in sample group was not diluted in Tris-HCl buffer. 0.0018g 5,8,11,14-Eicosatetraynoic acid (ETYA, Enzo Life Science, BML-ET004) was dissolved in 15 µL of methanol. 0.36g of 3-tert-Butyl-4-hydroxyanisole (BHA, Acros Organics, AC43969) was dissolved
in 1 mL of methanol, making a final BHA concentration of 2 M. The BHA solution was made fresh each day before use.

For the sample group, 0.12 mL of PC$_{30}$ (10 mM) and 0.88 mL of Tris-HCl buffer (0.1 M, pH 9.0) were mixed as the substrate solution for lipoxygenase. Before the addition of lipoxygenase, the spectrum from 200 nm to 300 nm of this substrate solution was scanned by UV-Vis. Then, 1 µL of lipoxygenase (3.6 mg protein/mL, 701,000 units/mg) was added to the substrate solution and mixed for 60 s and a spectrum from 200 nm to 300 nm was taken immediately. After that, the absorbance at 234 nm was measured every second for 60 min on UV-Vis. At the end of this experiment, another spectrum was scanned.

For the ETYA negative control group, 0.12 mL of PC$_{30}$ (10 mM) and 0.63 mL of Tris-HCl buffer (0.1 M, pH 9.0) were mixed as the substrate solution for lipoxygenase. The ETYA/Methanol solution, 1 µL of lipoxygenase (3.6 mg protein/mL, 701,000 units/mg) and 250 µL of Tris-HCl buffer (0.1 M, pH 9.0) were mixed for 30 min. The lipoxygenase should be fully inhibited. The spectrum from 200 nm to 300 nm of the substrate solution was scanned by UV-Vis. The inhibited lipoxygenase was added in substrate solution and the UV-Vis spectrum is scanned after 60 s. After that, the absorbance at 234 nm was measured every second for 60 min on UV-Vis. At the end of this experiment, another UV-Vis spectrum was scanned.

For the BHA negative control group, 0.12 mL of PC$_{30}$ (10 mM) and 0.63 mL of Tris-HCl buffer (0.1 M, pH 9.0) were mixed as the substrate solution for lipoxygenase and a spectrum from 200-300 nm was scanned (Figure 3.5, before BHA). 1 µL of BHA
(2 M) was added to the substrate solution so the final BHA concentration was 2 mM and a spectrum was scanned (Figure 3.5, after BHA). Then, 250 µL of lipoxygenase was added and spectrum was scanned (Figure 3.5, after lipoxygenase 60 s). 60 min later, the reaction was ended and the last spectrum was scanned (Figure 3.5, after lipoxygenase 60 min).

**LSPR assay**

7.5 µL of lipoxygenase (Sigma-Aldrich, Catalog number L-6632, 3.6 mg protein/mL, 701,000 units/mg), 24.25 µL of Tris-HCl buffer (1 M, pH 9.0) and 218.25 µL of water were mixed to make 250 µL of lipoxygenase stock solution (76,000 units/mL).

ETYA and BHA were prepared as previously described in conjugated diene assay. However, the BHA was made with different concentrations, 0.2 M, 8M, and 20 M.

The gold nanospheres were coated with sodium oleate, phosphatidylcholine and propanethiol as described in Chapter Two. 100 µL of Tris-HCl buffer (1 M, pH 9.0) was added to lipid coated gold nanospheres (1 mL) to adjust the pH of solution to 9.0 in order to ensure enzyme function. After stirring on LSPR for 30 min, 250 µL of lipoxygenase stock solution (76,000 units/mL) was added to the gold nanospheres solution. The whole process was done on LSPR under stirring.

For the ETYA negative control, the gold nanospheres were coated with the same steps as mentioned above. However, the 250 µL of lipoxygenase has been mixed with 15 µL of ETYA solution for 1 hour before adding it to the lipid coated gold nanospheres.
For the BHA negative control, the gold nanospheres were lipid coated as described in Chapter 2. 100 µL of Tris-HCl buffer (1 M, pH 9.0) was added to lipid coated gold nanospheres (1 mL) to adjust the pH of solution to 9.0 in order to ensure enzyme function. After stirring for 30 min, a certain amount of BHA was added in the lipid coated gold nanospheres to keep the BHA concentration at 0.2mM, 8mM, and 20mM respectively. The lipoxygenase was also mixed with different concentrations of BHA 1 hour before using.

The LSPR instrument setup includes a deuterium-tungsten halogen light source (Ocean Optics, DH-2000) and a light detector (Ocean Optics, HR 4000). The centroid changes were extracted by Matlab calculation from the LSPR spectra collected by the light detector. (9)

Results and discussion

TBARS assay

To demonstrate the activity of the lipoxygenase, the TBARS assay is used to measure the products from the lipid oxidation. By measuring the amount of malondialdehyde produced from the lipid oxidation, the TBARS assay gives the evidence of lipoxygenase function from products perspective. The malondialdehyde from lipid oxidation can react with TBA (Thiobarbituric acid) to form a fluorophore. By measuring the fluorescence signal, the amount of malondialdehyde can reflect the lipid oxidation content.
With the addition of lipoxygenase, the sample group has a much stronger fluorescence near 550 nm comparing to the control group who has no lipoxygenase. The oxidation products verify the lipoxygenase catalyzed reaction. With the TBARS data, we are able to demonstrate the function of lipoxygenase in our laboratory. (Figure 3.1)

![Fluorescence spectra from a TBARS assay for lipid oxidation.](image)

**Figure 3.1 Fluorescence spectra from a TBARS assay for lipid oxidation.**

*a,* Control group (blue dashed line). *b,* Sample group (red solid line). The control group has no lipoxygenase addition while the sample group is treated with lipoxygenase for 30 min. The excitation wavelength is 530 nm and the emission is collected from 540 to 650 nm.
Conjugated diene assay

With the TBARS assay, we can only measure the products of the lipid oxidation process. The lipid oxidation process cannot be revealed by TBARS assay. However, the conjugated diene assay is using UV-Vis spectroscopy to monitor the absorbance caused by conjugated diene structure. It is an in situ measurement of lipid oxidation process. As the lipoxygenase oxidizes the lipid, the conjugated diene structure was formed in the hydroperoxide of the linoleic acid. This conjugated diene formation would cause the absorbance at 234 nm to rise as the reaction occurs (Figure 3.2). Figure 3.2 has the spectra scanned before the lipoxygenase addition, 60 s and 60 min after the lipoxygenase addition. The addition of enzyme caused a slightly change to the spectra. But, after 60 min, the absorbance near 234 nm clearly increased and formed a shoulder next to the scattering peak, supporting the hypothesis that the lipoxygenase oxidized lipid molecules and formed a conjugated diene structure. Figure 3.3 shows the increasing absorbance at 234 nm along time. The absorbance increases fast at the beginning and gradually reaches a plateau. It appears that the conjugated diene assay can provide some kinetic information for the lipid oxidation reaction.
Figure 3.2 Conjugate diene absorbance spectra of lipid oxidation. *a*, Spectrum of the PC$_{30}$ liposome solution (blue solid line). *b*, Spectrum 60 s after the addition of lipoxygenase (red dash line). *c*, Spectrum 60 min after the addition of lipoxygenase (green dot line).
Figure 3.3 Conjugated diene absorbance at 234 nm for lipid oxidation. This figure is a collection of absorbance at 234 nm for 60 min starting from the addition of lipoxygenase.

With the conjugated diene assay, the lipid oxidation process can be monitored in real time, which gives us a chance to test the enzyme activity under different conditions. In this work, two of the lipoxygenase inhibitors, ETYA and BHA, were tested by the conjugated diene assay. As mentioned in Chapter one, these two inhibitors have different mechanism on lipoxygenase inhibition. If the lipoxygenase was inhibited by ETYA, the binding site of lipoxygenase should be occupied by ETYA, which means that the
lipoxygenase cannot bind phosphatidylcholine or oxidize it. Theoretically, no conjugated diene structure can be formed. However, with the presence of the BHA inhibitor, a radical scavenger, the radical will be protected by BHA from oxygen. This might affect the conjugated diene structure. Since the two inhibitors have different mechanisms, the results from conjugated diene assay are expecting to be different.

**Figure 3.4 Conjugated diene spectra for lipoxygenase ETYA inhibition.**

*a*, Spectrum of the substrate solution (blue solid line). 

*b*, Spectrum 60 s after the addition of lipoxygenase (red dash line). 

*c*, Spectrum 60 min after the addition of lipoxygenase (green dot line).
Figure 3.5 Absorbance at 234 nm for lipoxygenase ETYA inhibition. a, ETYA inhibited lipoxygenase negative control (red solid line). b, non-inhibited lipoxygenase (blue dashed line).

For the ETYA negative control group (Figure 3.4), the spectrum changes after the addition of inhibited lipoxygenase (60 s, red dashed line) when compared to the spectrum scanned before the lipoxygenase addition (blue solid line), because the ETYA has its own absorbance at this wavelength range. But, 60 min later, the spectrum does not appear to change (green dot line), which suggests that the conjugated diene is minimally formed.

From Figure 3.5, the absorbance at 234 nm for the ETYA negative control even decreases
slightly. Considering the mechanism of ETYA inhibition of occupying the lipoxygenase binding site for the pentadiene structure, this result matches the expectation. The formation of the conjugated diene did not occur in the presence of a competitive inhibitor.

**Figure 3.6 Conjugated diene spectra (200-300 nm) for BHA inhibition experiment.**

- **a**, Spectrum of the substrate solution (blue solid line).
- **b**, Spectrum after the addition of BHA inhibitor (red dash line).
- **c**, Spectrum 60 s after the addition of lipoxygenase (green dot line).
- **d**, Spectrum 60 min after the addition of lipoxygenase (purple dash-dot line)
Figure 3.7 Normalized absorbance at 234 nm for BHA inhibition experiment.
a, BHA inhibited lipoxygenase negative control (red solid line). b, non-inhibited lipoxygenase (blue dashed line). Both data are normalized to 1 at 0s.

For BHA negative control group (Figure 3.6), the addition of BHA dramatically changed the spectra (Figure 3.6, before and after BHA), which even increased the absorbance between 210 nm and 240 nm above 1. This might come from the absorbance from BHA itself. But the addition of lipoxygenase after the BHA did not change anything except diluting the solution to 80% of the previous BHA concentration (Figure 3.6, 60 s and 60 min after lipoxygenase). The absorbance at 234 nm nearly remains unchanged.
along the whole experiment (Figure 3.7). Considering the inhibition mechanism of BHA, it is suggesting that the BHA successfully protected the radicals and affects the conjugated diene structure.

The conjugated diene assay, as an in situ assay, is able to collect kinetic information of enzyme activity and monitor the whole lipid oxidation reaction in real time. However, it failed to distinguish the different inhibitors mechanisms. If the lipid coated gold nanoparticles can detect the difference from these two inhibitors by LSPR, it could be a huge advantage when studying the enzyme activity.

**Lipoxygenase activity by LSPR assay**

In this experiment, the LSPR assay was tested with lipoxygenase and its inhibitors. The refractive index of lipoxygenase used in this work remains unknown. However, the protein usually has a larger refractive index than water. If this LSPR assay is sensitive enough, there should be a red shift of the centroid after the addition of lipoxygenase to lipid coated gold nanospheres. With the inhibitors to the lipoxygenase, the LSPR spectra theoretically might not give any response. The lipid oxidation process is first tested by adding lipoxygenase in lipid coated gold nanospheres. As a comparison, the lipoxygenase inhibited by two different inhibitors, was added in lipid coated gold nanospheres in parallel experiments and the whole processes were monitored by LSPR assay.

In the lipid oxidation experiment, after data processing by Matlab, the centroid of the spectra was tracked as in Figure 3.8. As the data shows, the centroid of the LSPR
peak increased dramatically starting the moment the lipoxygenase was added (Figure 3.8, 9000s). This indicates that the lipoxygenase changed the refractive index of the environment around the lipid-coated gold nanoparticles. Combining with the data from conjugated diene and TBARS assay, it is suggesting that the refractive index change is from the lipoxygenase activity.

**Figure 3.8 LSPR data for lipid oxidation catalyzed by the lipoxygenase.** The Tris-HCl buffer (1M, pH 9.0) is added at 7200 s and the 250 μL lipoxygenase is added at 9000 s.
Since the LSPR and conjugated diene assays are in situ measurements of lipoygenase activity, the kinetic information is extracted from the LSPR data and conjugated diene data. Both LSPR and conjugated diene data are plotted by time and fitted into double exponential curves. The equation used in this fitting is:

\[ y = a_1(1 - \exp(-k_1x)) + a_2(1 - \exp(-k_2x)) \]  

Equation 1

The y is the change of absorbance at 234 nm in conjugated diene assay and change of centroid in LSPR assay. The x is time in both assays. The four parameters (a1, k1, a2 and k2) in the equation are calculated by Graphpad Prism and compared in Table 1.
Figure 3.9 Double exponential fitting curves for LSPR and conjugated diene data. A, B are absorbance at 234 nm plotted by time (s) from conjugated diene assay. C, D are change in centroid plotted by time (s) from LSPR assay. Green lines are the actual data and black lines are the double exponential fitting curve from Equation 1 fit in Graphpad Prism.
Table 1. Comparison of parameters from fitting curve for conjugated diene data and LSPR data.

A, B, C and D correlate to the figures in Figure 3.9. There are two sets of data for both conjugated diene assay and LSPR assay.

Table 1. Comparison of parameters from fitting curve for conjugated diene data and LSPR data. A, B, C and D correlate to the figures in Figure 3.9. There are two sets of data for both conjugated diene assay and LSPR assay.

From the double exponential fitting, we can see that there are two rate constants k1 (slow process) and k2 (fast process) for both conjugated diene data and LSPR data. For LSPR data, the k2 (fast process) has the highest k value around 0.01 s⁻¹. The k1 (slow process) has a lower k value around 0.001 s⁻¹. However, the k2 (fast process) from conjugated diene data has a similar k value with k1 (slow process) of LSPR. Since the conjugated diene assay will not be able to show the lipoxygenase binding step, the fast process in LSPR is deduced to be the protein binding step. Besides, considering the slow process in LSPR and fast process in conjugated diene share the similar k value, there is a possibility that LSPR and conjugated diene assays are tracking the same process.
However, with the limited information, it is difficult to interpret the k values from the fitting curve and more experiments are needed to test our working hypothesis of LSPR data.

For the ETYA negative control group (Figure 3.10, b), the inhibited enzyme addition causes a blue shift (0.5 nm average) for the centroid of the LSPR peak. It is not clear why there is a blue shift. This shift could be due to the interaction between ETYA and the lipid membrane. These results suggest that the ETYA inhibits the binding site of phosphatidylcholine and stops lipid oxidation.

For the BHA negative control group (Figure 3.11), the addition of BHA inhibited lipoxygenase still caused a clear red shift for the centroid of the LSPR peak. As the concentration of BHA increases, the red shift increases too, which indicates that the enzyme changed the refractive index around the gold nanospheres. This result suggests that the BHA inhibitor does not affect the lipoxygenase binding process. One possible hypothesis is that the lipoxygenase comes off from the phosphatidylcholine after the oxidation reaction. With the BHA inhibitor, the oxidation reaction is stopped because there is no radical to add the oxygen and form the hydroperoxide. Without the proper substrate structure, which could be the hydroperoxide, the lipoxygenase cannot come off from the phosphatidylcholine. In a macro perspective, the enzymes could get stuck on the lipid coating film and accumulate on the surface of the gold nanospheres. This would explain the increasing trend of red shifts of the centroid with more BHA inhibitors.

However, to demonstrate this hypothesis, more evidence is needed.
Figure 3.10 Two parallel lipid oxidation experiments with and without ETYA inhibition. The x axis means the time starting from the addition of lipoxygenase. The y axis is the change of the centroid. a, lipid oxidation without ETYA (blue and green line). b, lipid oxidation with inhibited lipoxygenase by ETYA (red and purple line).
Figure 3.11 LSPR data for BHA inhibition experiment with different BHA concentration (0, 0.2, 8, 20 mM). The x axis means the time starting from the addition of inhibited lipoxygenase. The y axis is the change of the red shift. As the concentration of BHA increases, the red shift of the centroid is rising too. Before 0 min, same concentrations of BHA are added to the solution.
Chapter Four: Gold nanoprism synthesis and purification

Asymmetric and highly pointed nanoparticles are known to have a higher sensitivity to refractive index changes. Therefore, we began synthesizing and purifying gold nanoprisms. We have successfully synthesized and purified gold nanoprisms with the purpose of creating more sensitive sensors for monitoring lipid reactions and protein-lipid binding events than with nanospheres. The sensitivities of nanospheres and nanoprisms are tested by a sucrose addition experiment and compared.

Lipid-coated gold nanoprism synthesis and purification

Gold nanoprisms are synthesized according to a seed-mediated method to get monodispersity of the gold nanoparticles size (13). Then the gold nanoprisms are purified by sucrose gradient centrifugation and re-purified from the sucrose solution. However, this method can also produce gold nanodisks, nanohexagons, bipyramids and other shapes. To get pure prisms, further research is needed in both synthesis and purification steps.

Methods and Materials

This seed-mediated method contains two main steps, gold nanoseed synthesis and gold nanoparticle synthesis. (13) First, 125 µL of HAuBr₄ (10 mM) was added to 3 mL of
PC$_{30}$ stock solution (10 mM). With vigorous stirring, 300 µL of NaBH$_4$ (10 mM) was added to the previous solution. The reaction was incubated at room temperature for 30 min. Second, 400 µL of HAuBr$_4$ (10 mM) was added into 17 mL of PC$_{30}$ stock solution. Then, 40 µL of gold nanoseeds and 40 µL of ascorbic acid (100 mM) from first step were quickly added to the HAuBr$_4$ and PC$_{30}$ solution with vigorous stirring. Stirring is important to keep the monodispersity of the gold nanoparticles’ size. With rapid color change to purple in several minutes, the gold nanoparticles were successfully synthesized and it is a mixture of gold nanospheres and gold nanoprisms.

To separate these two kinds of gold nanoparticles, a 3 hours (2 hours at 4 °C and 1 hour at 20 °C) of sucrose gradient centrifugation at 2000xg was applied to the nanoparticles. The sucrose gradient was made using five fractions from 80% to 40% sucrose solution in a 15 mL centrifuge tube with 10% increments, and the volume of each fraction is 2 mL (Figure 4.1). After the centrifugation, most of the gold nanoprisms were located at the bottom of the centrifuge tube. The others nanospheres mainly remained in the top sucrose fractions from 40% to 70% depending on their sizes. Then, the sucrose fractions were extracted by pipette separately from top to the bottom. Lastly, pellets containing most gold nanoprisms were collected (usually less than 0.5 mL) out from the centrifuge tube and transfered it to a 1.5 mL eppendorf tube. Deionized water was added to the eppendorf tube to bring the volume up to 1.5 mL. A 10 min centrifugation under 5223xg was applied to the eppendorf tube to precipitate the gold nanoprisms. Next, 1 mL of supernatant was discarded and another 1 mL of deionized water was added and the same centrifugation was done. This process was repeated 3 more times, for a total of 5
times. At the end, the gold nanoprisms should be in 1 mL of water with a sucrose concentration less than 0.3%.

To test the synthesis and purification, the transmission electron microscopy was used to take picture of the gold nanoparticles. One drop of the gold nanoparticle solution was added on the surface of a grid film for TEM and dried overnight. The TEM picture was taken for both raw gold nanoparticles and purified gold nanoparticles. UV-Vis spectra were scanned as support information for TEM pictures.

**Figure 4.1 Sucrose gradient centrifugation.** From the bottom, every 2 mL is one section of sucrose solution with different concentration from 80% to 40%. The raw gold nanoparticles are loaded on the top of the 40% section. This picture is taken after the centrifugation. There is a small pellet at the bottom of the tube and some other gold nanoparticles in sucrose solutions above.
Results and discussions

The sucrose gradient centrifugation is proven to be a purification method of gold nanoparticles in this experiment. Although gold nanosphere and gold nanoprism are identical in density, they still travel at different velocities in sucrose solutions. As a result, time of centrifugation is as important as speed. In this experiment, we successfully separated the prisms from spheres by sucrose gradient centrifugation under 2000xg for 3 hours (2 hours under 4 °C and 1 hour under 20 °C). The gold nanospheres have a peak around 527 nm and the gold nanoprisms have a peak around 710 nm (this varies slightly among our several experiment). The ratio of absorbance peaks between gold nanoprisms and gold nanospheres changed from 0.072 (before purification) to 0.760 (after purification) (Figure 4.2).

In addition, transmission electron microscopy can tell us the relative population of spheres and prisms (Figure 4.3). From the TEM pictures, the amount of the gold nanospheres reduces after the purification process.
Figure 4.2 UV-Vis spectra before and after purification. Combined UV-Vis spectra of both gold nanospheres & nanoprisms (solid) and pure gold nanoprisms (dashed). The 527 nm peak mainly comes from spheres, while the 710 nm peak comes from prisms. The sucrose gradient centrifugation removes most of the spheres and the prisms remain.
Figure 4.3 TEM pictures and respective UV-Vis spectra. A, TEM picture of gold nanospheres and gold nanoprisms before purification. B, TEM picture of purified gold nanoprisms. C, UV-Vis spectra of gold nanospheres and gold nanoprisms. D, UV-Vis spectra of gold nanoprisms.
Refractive index sensitivity of gold nanoprisms and nanospheres

After the purification of gold nanoprisms, the refractive index sensitivity of this prism was tested. The refractive index of the environment changes by altering the sucrose concentration of the solution. The refractive index of water and sucrose solutions are listed in Table 2.

Methods and Materials

In this experiment, 1 mL of the purified gold nanoprisms and nanospheres were monitored by LSPR for 45 min to collect background signals. Then, 80% of sucrose solution was added in the gold nanoprisms gradually with a 30 min interval to change the refractive index. The whole process was monitored by LSPR. The amount of the sucrose solution that was added is also listed in Table 2.
<table>
<thead>
<tr>
<th>80% sucrose solution added (µL)</th>
<th>Sucrose concentration % by weight (w/v)</th>
<th>Refractive index</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0</td>
<td>1.3330</td>
</tr>
<tr>
<td>67</td>
<td>5</td>
<td>1.3403</td>
</tr>
<tr>
<td>76</td>
<td>10</td>
<td>1.3479</td>
</tr>
<tr>
<td>87</td>
<td>15</td>
<td>1.3557</td>
</tr>
<tr>
<td>103</td>
<td>20</td>
<td>1.3639</td>
</tr>
<tr>
<td>122</td>
<td>25</td>
<td>1.3723</td>
</tr>
<tr>
<td>145</td>
<td>30</td>
<td>1.3811</td>
</tr>
</tbody>
</table>

**Table 2.** The first column is the volume of 80% sucrose solution added in gold nanoparticles and nanospheres. The second column is the sucrose concentration of sucrose after every sucrose addition. The third column is the refractive index of the solution after every sucrose addition.

**Results and discussions**

According to the LSPR data, the centroid of the LSPR peak from nanoparticles shifts towards red as the sucrose concentration is increased, which means that refractive index is rising (Figure 4.4 and Figure 4.5). However, the distances of the red shifts from
nanoprisms and nanospheres are different. The centroid shifts are analyzed by Excel and plotted. The correlation between refractive index and centroid shifts is calculated by linear fitting (Figure 4.6). The refractive index sensitivity of nanoprism is 147.29 nm/RIU and the sensitivity of nanospheres is 31.565 nm/RIU. Comparing to the gold nanospheres, the nanoprisms are more sensitive to refractive index changes, which gives nanoprisms the potential to be a better biosensor than gold nanospheres.
Figure 4.4 Centroid shifts from nanoprisms as the sucrose concentration is increasing. A, 0% sucrose. B, 5% sucrose. C, 10% sucrose. D, 15% sucrose. E, 20% sucrose. F, 25% sucrose. G, 30% sucrose.
Figure 4.5 Centroid shifts from nanospheres as the sucrose concentration is increasing. A, 0% sucrose. B, 5% sucrose. C, 10% sucrose. D, 15% sucrose. E, 20% sucrose. F, 25% sucrose. G, 30% sucrose.
Figure 4.6 Centroid shifts of LSRP peak with different refractive index. Blue dots are the centroid shifts from nanoprisms at different refractive index while red dots are from nanospheres. Refractive index is changed by increasing sucrose concentration.
Chapter Five: Conclusion

In our work, we managed to synthesize gold nanospheres and coat them with lipid membranes. From the lipoxygenase catalyzed lipid oxidation experiment, we can see that nanospheres successfully detected the refractive index change caused by lipoxygenase, which shows the potential of gold nanospheres to be a sensitive biosensor. In addition, the lipid coated gold nanospheres are able to differentiate lipoxygenase inhibitors with different inhibition mechanisms. This makes lipid coated gold nanoparticles a better biosensor compared to other traditional assays. Furthermore, with the gold nanoparticle synthesis, the size and shape of the lipid membrane is easily controlled and maintained, which is critical for measuring the curvature preference of enzymes. With the high sensitivity of LSPR phenomenon to refractive index change, protein activity can be monitored with less substrate amount. Since the lipid coated gold nanoparticle is sensing the behavior of the enzyme instead of the enzymatic reaction products, it is more reliable when the reaction leads to many byproducts or a side pathway. In addition, the higher refractive index sensitivity of gold nanoprism makes it possible to build a more sensitive biosensor than gold nanospheres, which can potentially show more information of the lipid-protein interaction.

In the future, the BHA and ETYA inhibitors will be tested by TBARS assay to investigate the difference between these two inhibitors. The gold nanoprism synthesis and
purification need to be modified to increase yield. Based on a reasonable yield, the gold nanoprisms will be tested by lipid oxidation experiments like the nanospheres to see if the prisms can give more information about the lipid oxidation reaction due to its higher refractive index sensitivity.
References


15. Gaffney, B. J. Lipoxygenases: structural principles and spectroscopy. *Annual review*


