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## Gold Nanoparticle Colorimetric Sensing Technology

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# GOLD NANOPARTICLE COLORIMETRIC SENSING TECHNOLOGY

NENG 493

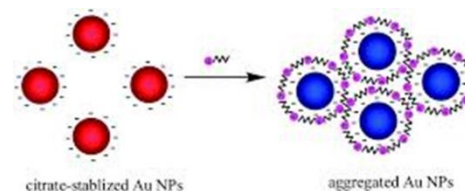
*Eve Borden  
Advisor: Magnus  
Bergkvist*

## 1. Summary

**Problem: Currently, many quick-test sensing technologies are expensive and can involve extensive sample preparation.** Expense of sensors arises from complicated materials being utilized in conjunction with expensive analysis equipment such as electrochemical, microfluidic, or portable imaging systems. Often, to arrive at final detection of an analyte of interest, sensing techniques require many steps of filtering or modifying samples which defeats the purpose of a convenient point-of-care sensor.

**Solution: Utilizing gold nanoparticles to develop a sensing format with the ability to detect targets quickly and inexpensively.** Gold nanoparticles can easily be synthesized in a monodisperse, stable solution (Turkevich method)(Kimling, 2006). These nanoparticles have been studied comprehensively and have a well-known surface chemistry that can be easily modified. In terms of

sensing, the main advantage lies in unique optical properties and obvious color changes depending on the surrounding environment of the nanoparticles (Ahmed, 2016). In addition, gold nanoparticles can be detected in very low concentrations and have high absorption in the UV-visible range.



**Figure 1: Schematic example of colorimetric change that occurs in stable vs. aggregated nanoparticles.**

**Aim 1: Gold nanoparticle synthesis and characterization.** Synthesize gold nanoparticles in a stable and monodisperse solution using a rapid method. Obtain particle size between 10 to 20 nm with repeatable results. Easily determine the size and concentration of gold nanoparticles in solution.

**Aim 2: Evaluate molecule interaction with and adsorption to the gold nanoparticle surface.** Quantify how many protein or antibody molecules adsorb to the surface of a single gold nanoparticle. Determine how this surface interaction affects the stability of the gold nanoparticles in solution.

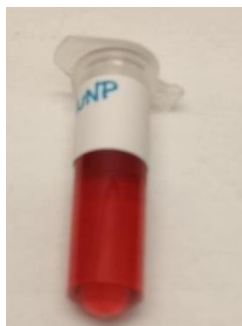
**Aim 3: Immobilization of gold nanoparticles and binding of target.** Determine the necessary concentration of gold nanoparticle to receive a visible signal. Establish binding of the target to the modified gold nanoparticles. Combine all of the components into a useful sensing format.

**Success Criteria:** Repeatable synthesis of monodisperse, 10 to 20 nm gold nanoparticles using a rapid method. Successfully characterize size and concentration of gold nanoparticles in solution. Evaluate and quantify interaction of proteins and antibodies with gold nanoparticles in solution. Obtain confirmation of antibodies on the surface of gold nanoparticles binding with antigen. Complete final sensor format.

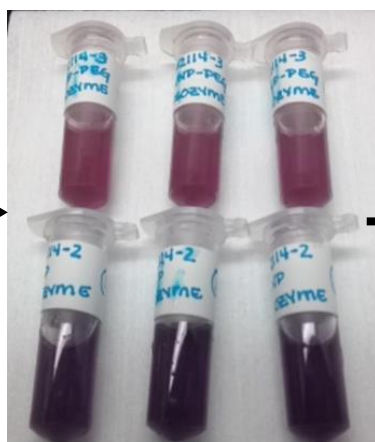
## 2. Introduction

### 2.1 Gold Nanoparticles

Gold nanoparticles are an area of significant interest due to their unique optical properties. Based on the



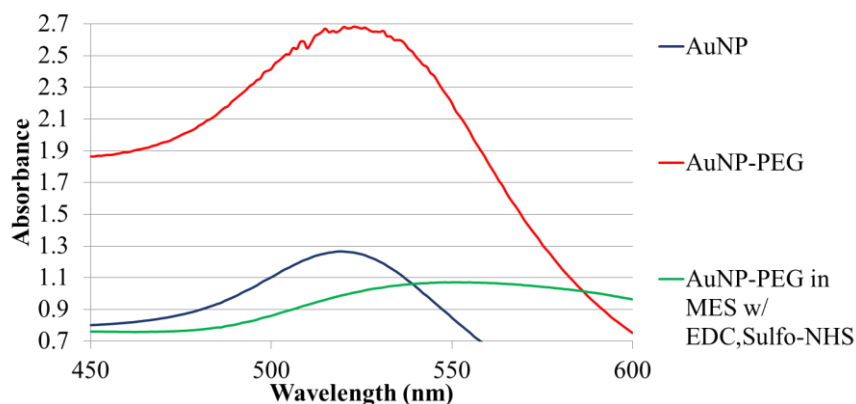
**Figure 2: Visible color change in completely stable gold nanoparticles, slightly aggregated particles, and completely destabilized aggregated particles. (right to left)**



stability of gold nanoparticles in their environment they exhibit visible color

changes that are easily noticed without analysis equipment. Citrate stabilized gold nanoparticles are red in solution and visible at low concentrations (Saha, 2012). Addition of a destabilizing or binding

molecule causes the gold nanoparticles to have a “blue shift” in the UV-Visible spectra and in extreme cases to aggregate into a purple solution. Gold nanoparticles can be used strictly for their high visibility in low concentration, for the color change exhibited when the particles are modified, or for the complete aggregation that occurs when they are destabilized.



**Figure 3: Peak shifts in UV-Visible spectra of gold nanoparticles based on molecules bound to the surface.**

## 2.2 Sensor Design

There are a variety of formats for quick test sensing. They include: lateral flow paper strips, solution based sensing, and molecularly imprinted polymers, among others (Li, 2010). Few sensors used in laboratories today provide results without the use of extensive sample preparation or expensive analysis equipment. There are often cases when a simple positive or negative result could be useful in determining the presence of a molecule or binding activity in solution. One of the most common examples of this type of sensor is used not in a lab setting but in everyday life. Pregnancy tests use a simple binding mechanism in conjunction with a lateral flow strip to reveal a positive or negative result (“Home Pregnancy Test”).

Human chorionic gonadotrophin (hCG) is a hormone secreted during pregnancy that has been used very commonly to determine pregnancy. The pregnancy test strip collects sample on the sample pad and it continues to flow to the conjugation pad where it is mixed with ‘anti-A’ hCG antibody bound to gold nanoparticles which will bind to any hCG present in the sample. In addition Immunoglobulin G (IgG) bound to gold nanoparticles will be picked up in the solution and be used as a control further down the line to confirm the test is working properly. IgG is the most abundant type of antibody present in the body and protects against bacterial and viral infections. It can bind very specifically to each foreign substance. Once the bound hCG and IgG are both in solution the sample travels to the first binding strip. The first binding strip contains

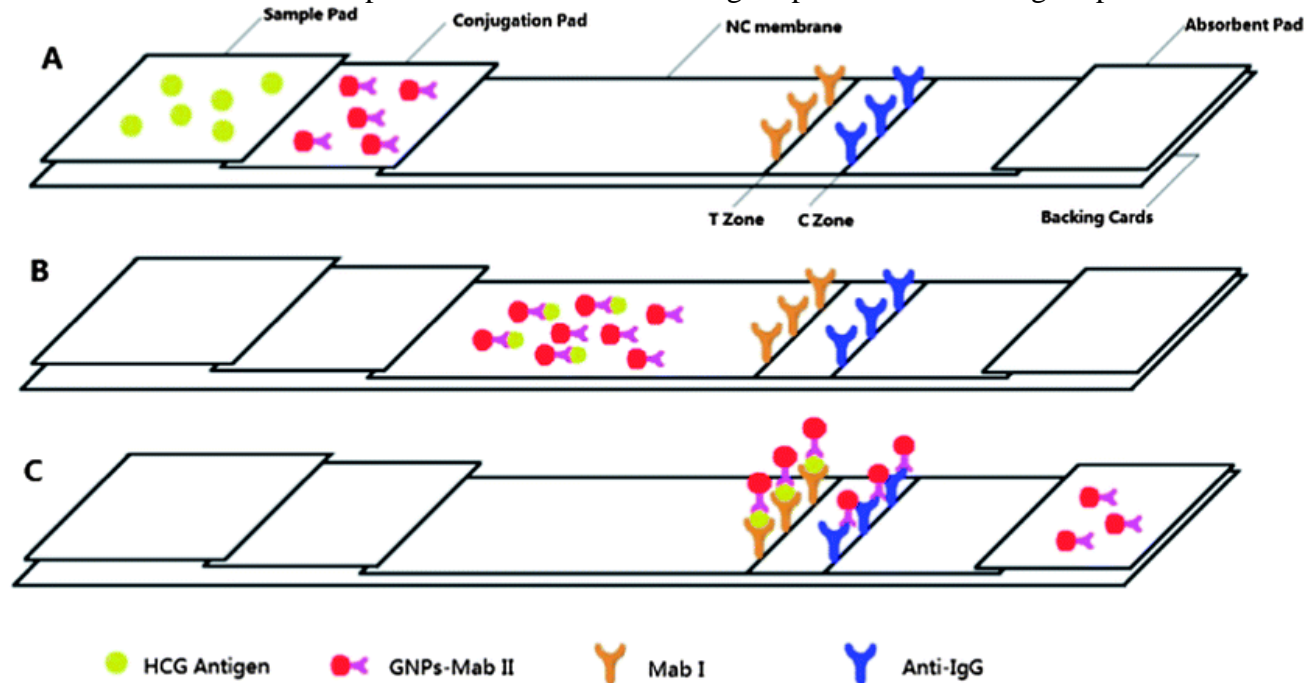
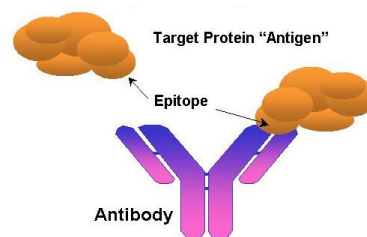


Figure 4: Schematic of how the binding on a typical lateral flow pregnancy test occurs.

‘anti-B’ hCG antibody that will also bind to the hCG-anti-A complex already in solution. The binding activity will produce a red line due to the presence of gold nanoparticles (Su, 2014). As a final control the sample flows to a second binding strip that contains an anti-IgG that will bind

to the IgG picked up by the sample on the conjugate pad. This binding will also create a red line that confirms the test worked properly.

Antibody-antigen binding, as used in a typical pregnancy test, is a highly specific and well-characterized interaction mechanism in which one antibody only binds to a certain antigen. It is an ideal candidate to be used in a rapid sensing format. In general, the lateral flow and binding technique of the pregnancy test can be adapted to molecules besides hCG.



**Figure 5: Schematic of antibody-antigen binding.**

In the example of the pregnancy test, the gold nanoparticles don't exhibit a color based on their environment but instead form a red line as they bind to the sample flowing over the surface. A design could be developed that does not utilize the same anti-A, hCG, anti-B sandwich binding and instead uses only a single antibody-antigen complex. Antibodies can be immobilized on the surface of gold nanoparticles and the presence of an antigen could cause binding to the gold nanoparticles or potentially aggregation of the nanoparticles.

There are many design factors that must be considered in developing this sensing format. First, the uniformity, size, and concentration of gold nanoparticles must be carefully quantified in repeatable experiments. Second, interaction of gold nanoparticles with the antibody of interest must be determined. This includes evaluating adsorption to gold and potential for destabilization or aggregation in solution. Next, actual immobilization of gold nanoparticles onto a paper lateral flow strip must be evaluated. The necessary volume and concentration of gold nanoparticles to create a visible signal needs to be defined. Finally all interactions of gold nanoparticles and antibody, and then also the antigen can be carefully evaluated to determine the sensor arrangement.

Success of the design utilizing gold nanoparticles in a paper strip format will be determined by the ability to synthesize monodisperse, 10 to 20 nm particles using a repeatable method, characterization of the nanoparticles, evaluation of interactions of gold nanoparticles with outside molecules, and immobilization and binding of the gold nanoparticles to a target.

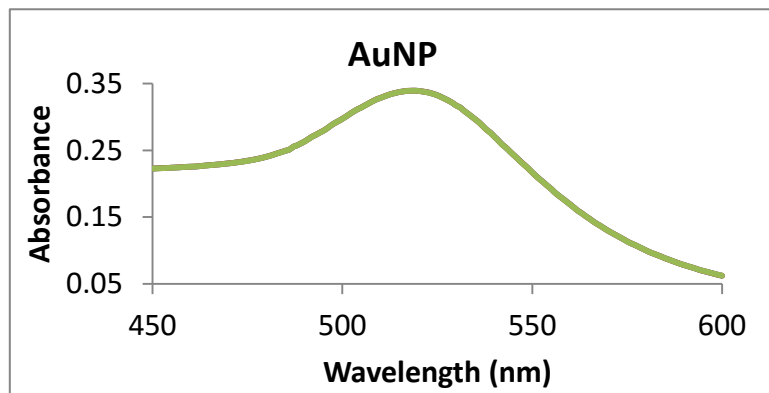
### 3. Results/Discussion

#### 3.1 Gold Nanoparticle Synthesis and Characterization

All glassware used to complete gold nanoparticle synthesis is rinsed with aqua regia (3:1 volume ratio of HCl:HNO<sub>3</sub>) and then 3x with DI water. Gold nanoparticle synthesis has been studied extensively and various methods have been established. Commonly, citrate reduction of gold hydrochloroaurate solution at 100 °C (Turkevich method) is conducted by heating the gold solution to boiling in an oil bath and immediately adding the citrate solution (Kimling, 2006). The combined solution must be maintained at a boiling temperature while flask is occasionally swirled in order to allow the full reaction to occur. Indication that the reaction is completed is indicated by a color change initially from a clear liquid, to a dark purple color, finally to a deep red colored solution. This takes anywhere from 30 to



**Figure 6: Gold nanoparticles in solution after oil bath synthesis.**



**Figure 2: Plot of wavelength vs. absorbance for a sample of gold nanoparticles determined by UV-Vis spectroscopy.**

$A_{spr}/A_{450}$	d / nm	$A_{spr}/A_{450}$	d / nm	$A_{spr}/A_{450}$	d / nm
1.10	3	1.56	12	1.96	40
1.19	4	1.61	14	2.00	45
1.27	5	1.65	16	2.03	50
1.33	6	1.69	18	2.07	55
1.38	7	1.73	20	2.10	60
1.42	8	1.80	25	2.12	65
1.46	9	1.86	30	2.15	70
1.50	10	1.92	35	2.17	75

**Table S-2: Ratio of the absorbance of GNPs at the surface plasma resonance peak ( $A_{spr}$ ) to the absorbance at 450 nm ( $A_{450}$ ) in dependence the particle diameter calculated from Equation 11 using the fit parameters to the experimental results.**

**Figure 8: Supporting info used to determine nanoparticle diameter from UV-Vis spectra.**

passing through the sample of gold nanoparticles to provide both the size distribution and particle diameter contained in the sample. Size distribution by intensity, size distribution by volume, and size distribution by number are all calculated by the DLS software to define what

60 minutes of heating.

Characterization

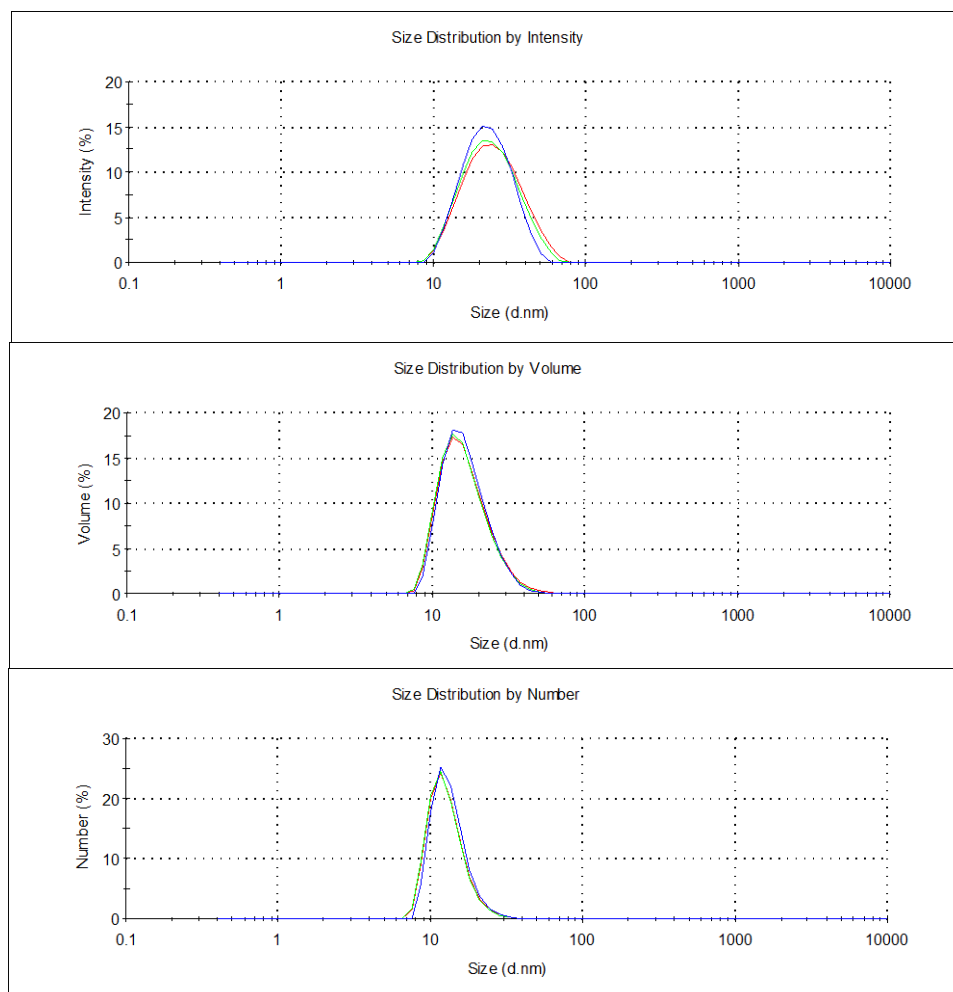
of these gold nanoparticles is completed using UV-Vis spectroscopy and Dynamic Light Scattering (DLS) to give a relative size and concentration. UV-Vis spectroscopy is used to scan the gold nanoparticle sample over a range from 450 to 600 nm. The absorbance at the peak, also referred to as the

surface plasmon resonance (SPR) peak, and the absorbance at 450 nm are recorded. The ratio of the absorbance at the peak to the absorbance at 450 nm can be used with the supporting info of *Determination of Size and Concentration of Gold Nanoparticles from UV-Vis*

*Spectra* (Haiss, 2007) to determine particle diameter. DLS also uses light



size the majority of the particles are and if the sample is monodisperse. Often this method of synthesis would produce



**Figure 3: DLS data for a sample of microwave synthesis gold nanoparticles showing size distribution for three different analysis calculations.**

approximately 15 nm diameter particles but DLS indicated that the solution produced using oil bath synthesis of gold

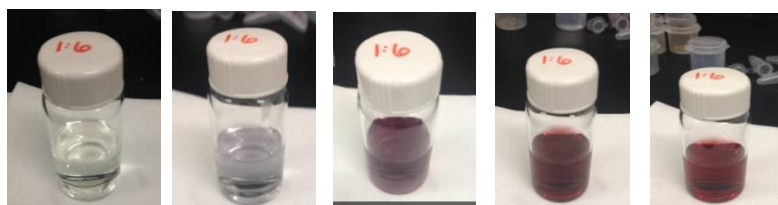
nanoparticles was not monodisperse and a significant size range was present. This led to a study of procedure modification to

ensure a monodisperse solution of gold nanoparticles to be used for sensing.

Experiments were conducted to determine the ideal ratio of citrate to gold in solution.

Seven different molar ratios were synthesized and measured with UV-Vis spectroscopy and DLS. It was found that a 1:6 molar ratio of gold to citrate created the most evenly distributed particles all within the range of about 10 to 20 nm in diameter. In addition, microwave heating was evaluated to regulate the uneven heating that stemmed from use of an oil bath. The gold solution is heated until boiling in the microwave, then citrate is added and flask is swirled to mix. This alternating of heating for about 3 seconds to maintain temperature and swirling the solution

for about 15 seconds is continued for about 15 minutes. The solution will proceed through the normal color changes from clear, to dark purple, to red. Gold



**Figure 4: Color change progression of microwave synthesis gold nanoparticles from clear to purple to deep red.**



nanoparticles synthesized using microwave synthesis instead of oil bath synthesis were significantly more monodisperse and had a much smaller size distribution, therefore this method

## 2. Tabular material for the determination the concentration of uncoated spherical gold nanoparticles in water from UV/vis spectra.

The data of  $\epsilon_{450}(d)$  in Table S-3 can be used to calculate the particle concentration  $c$  in mol per litre from the absorption  $A$  at 450 nm for a standard path length  $l$  of 1 cm according to:

$$c = A_{450}/\epsilon_{450}$$

The data have been experimentally verified in the  $d$  range from 5 – 100 nm (see Figure 8). For particle diameters smaller than 5 nm the values should only be considered as an estimate since surface effects may get increasingly important in this size region.

$d/\text{nm}$	$\epsilon_{450}/\text{M}^{-1}\text{cm}^{-1}$	$d/\text{nm}$	$\epsilon_{450}/\text{M}^{-1}\text{cm}^{-1}$	$d/\text{nm}$	$\epsilon_{450}/\text{M}^{-1}\text{cm}^{-1}$
2	4.25E+05	35	3.21E+09	68	2.50E+10
3	1.49E+06	36	3.52E+09	69	2.61E+10
4	3.62E+06	37	3.84E+09	70	2.71E+10
5	7.20E+06	38	4.18E+09	71	2.82E+10
6	1.26E+07	39	4.54E+09	72	2.93E+10
7	2.03E+07	40	4.92E+09	73	3.05E+10
8	3.07E+07	41	5.32E+09	74	3.16E+10
9	4.43E+07	42	5.74E+09	75	3.28E+10
10	6.15E+07	43	6.18E+09	76	3.40E+10
11	8.27E+07	44	6.65E+09	77	3.52E+10
12	1.09E+08	45	7.13E+09	78	3.64E+10
13	1.39E+08	46	7.65E+09	79	3.77E+10
14	1.76E+08	47	8.18E+09	80	3.89E+10
15	2.18E+08	48	8.74E+09	81	4.02E+10
16	2.67E+08	49	9.32E+09	82	4.14E+10
17	3.24E+08	50	9.92E+09	83	4.27E+10

Figure 5: Tabular material used to determine the extinction coefficient based on particle size, which can then be used to find the concentration of gold nanoparticles.

was used for the remainder of the design project.

In order to calculate the concentration of gold nanoparticles in a stock solution after synthesis additional tabular material from *Determination of Size and Concentration of Gold Nanoparticles from UV-Vis Spectra* (Haiss, 2007) is utilized. This provides the concentration in moles per liter of the dilute concentration used for UV-Vis analysis. To find the number of particles per milliliter or the surface area available per milliliter the density of gold, particle size, and particle

volume must all be defined and used in calculations.

Microwave gold nanoparticle synthesis has been repeated multiple times with very similar results. Nanoparticles are usually 10 to 15 nm in diameter with very good uniformity and a concentration on the order of magnitude of  $10^{12}$  particles per milliliter or around 0.12 mg per mL.

### 3.2 Gold Nanoparticle-Antibody Interactions

The interaction of gold nanoparticles and immunoglobulin G (IgG) were specifically evaluated. First adsorption and interaction of IgG from rabbit was tested. After gold nanoparticle synthesis and characterization a range of six concentrations of IgG from a premade stock solution were added to microcentrifuge tubes each containing 1 mL of stock gold nanoparticles. In parallel, the same six concentrations were added to microcentrifuge tubes each



Figure 6: From left to right - gold nanoparticles before addition of IgG, gold nanoparticles immediately after addition of IgG, and gold nanoparticles 24 hrs after addition of IgG.

containing 1 mL of deionized water to ensure all calculations took in account adsorption of IgG to the plastic tubes. All of the samples were vortexed in order to distribute the IgG throughout the entire solution. The microcentrifuge tubes were left to incubate overnight at

room temperature. As seen in Figure 13, the gold nanoparticles

began to aggregate almost immediately after addition of IgG, as shown by the color change, and had completely aggregated at the bottom of the centrifuge tube after 24 hours. This behavior was identical across all six concentrations evaluated. The gold nanoparticle and deionized water samples were all centrifuged for 15 minutes at 15 g before the supernatant was removed and placed in a separate microcentrifuge tube for analysis. In addition, the supernatant samples were diluted to half of their original concentration with deionized water. A set of 9 standard IgG concentrations is added to a 96-well microplate, each standard filling 3 wells. The supernatant samples are added in the same manner. Working reagent from the BCA assay kit is added to all wells to amplify the absorbance at 562 nm. The microplate is left to incubate for 2 hours at room temperature before the absorbance at 562 nm is measured using the microplate reader. The absorbance data collected can be used to provide a standard curve and calculate: the amount of IgG lost to the plastic tube, the amount of IgG left in solution, and the amount of IgG that adsorbed to the gold nanoparticles. Since a range of concentrations were evaluated, it can be determined at what concentration of IgG does the surface of the gold nanoparticles become saturated and the maximum amount of IgG

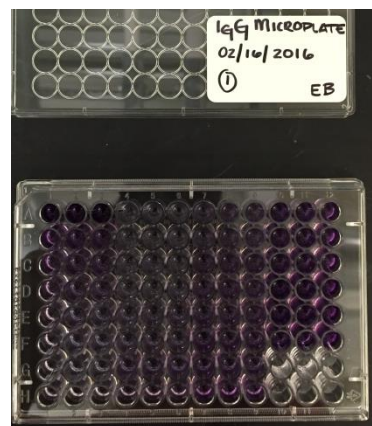


Figure 7: (above) Example microplate for IgG Assay, purple color indicates working reagent reacting with IgG in solution.

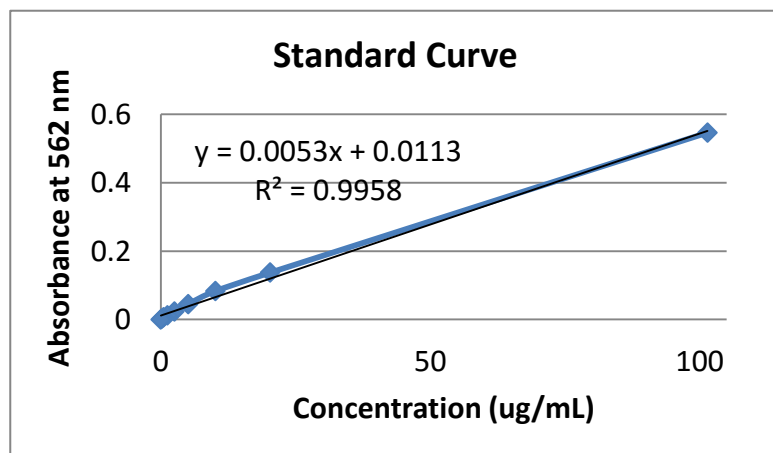


Figure 8: Standard curve with linear fit that can be used to calculate concentration of IgG in unknown samples.

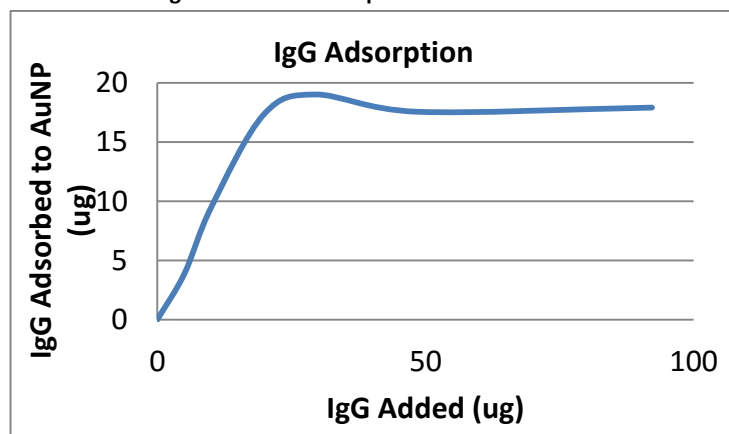


Figure 9: IgG Adsorption curve with the saturation point occurring when 30 ug of IgG are added and ~19 ug adsorb to the surface of the gold nanoparticles.

adsorbed. A simple plot of micrograms of IgG added versus micrograms of IgG adsorbed gives a clear indication of the saturation point. For IgG from rabbit the saturation point occurred when 30 micrograms of IgG were added and

approximately 19 micrograms

adsorbed to the surface of the gold nanoparticles.

From this data, the known size of IgG, and the known surface area of the gold nanoparticles it can be determined that an average of 7 IgG molecules adsorbed to the surface of the gold nanoparticles in solution. This correlates, for an 11 nm

diameter particles, to approximately 50% surface covered with IgG. Due to the aggregation that occurred after the addition and incubation of the gold nanoparticles with the IgG the nanoparticles could not be resuspended in solution. This entire assay was repeated a second time to confirm the results and very similar results were determined.

Adsorption of thiol-modified IgG to gold nanoparticles was evaluated in a similar manner to the IgG from rabbit. For this adsorption, only the concentration shown to yield the highest adsorption to gold was used and no blank water samples were run in parallel (this is due to the small volume of antibody available). The adsorption experiments were run in glass vials and incubation occurred in the 4°C fridge overnight to maintain the functionality of the antibody. This experiment did not reveal similar results. After 24 hours of incubation the gold nanoparticles had not aggregated out of solution. The samples were centrifuged and similar procedure was followed. Upon calculating the amount of the thiol-modified IgG adsorbed to the surface of the gold nanoparticles, the results were inconclusive because more than the original amount of IgG added was left in solution according to the results. Allowing for a margin of error, this result could be determining that none of the thiol-modified IgG had adsorbed to the glass vial or the gold nanoparticles.



**Figure 10: Gold nanoparticles with IgG from rabbit in a glass vial after incubation overnight in the 4 degree C fridge.**

To confirm that the IgG from rabbit would have similar results when incubated in glass vials and in the fridge, the original IgG was retested with the same procedure as the thiol-modified IgG and it was found that approximately 17 ug of IgG adsorbed to the gold nanoparticles which was similar to the initial adsorption experiments. The nanoparticles also aggregated in the bottom of the glass vial in the fridge but they were able to be resuspended after centrifugation.

Although the IgG from rabbit appears to bind to the gold nanoparticles it also destabilizes the surface causes them to aggregate out of solution. On the other extreme, the nanoparticles remain completely stable in the presence of the thiol-modified IgG but almost no adsorption occurs meaning they might have limited use in a sensing application.

### 3.3 Immobilization of Gold Nanoparticles on Test Strip

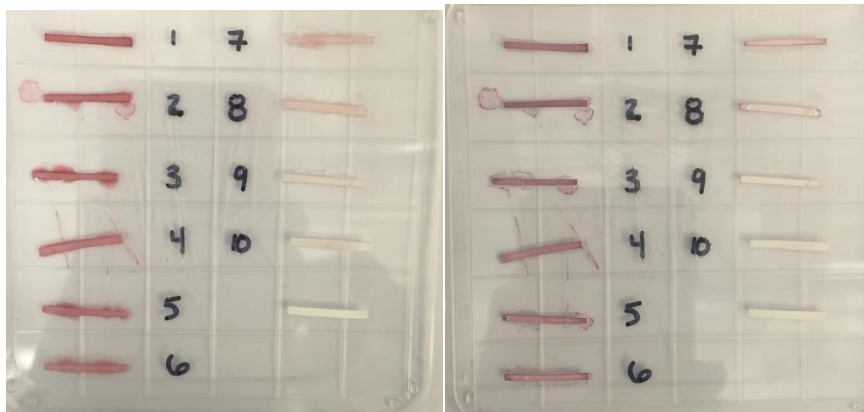


Figure 11: 5  $\mu$ L gold nanoparticle drops containing a range of 10 concentrations. Left is immediately after addition and right is after  $\sim 2$  hours of drying at room temperature.

The most important parameter regarding immobilizing gold nanoparticles on a nitrocellulose paper strip is making sure they are visible. To establish the number of particles necessary for the deep red color of gold nanoparticles to appear bright enough to

work as a sensor, a range of 10 concentrations of a stock solution of gold nanoparticles is pipetted onto dried paper strips. The 5  $\mu$ L drops spread out onto the paper until the last two concentrations are barely visible as seen in Figure 18. The 8<sup>th</sup> concentration is the last clearly visible drop which contains approximately  $1.23 \times 10^9$  gold nanoparticles.

### 3.4 Final Sensor Formatting

To confirm the antibody-antigen binding and to test if the IgG from rabbit immobilized on the gold nanoparticle surface will still bind to antigen, a spotting experiment is run on nitrocellulose paper. Gold nanoparticles with IgG from rabbit, only IgG, and only gold

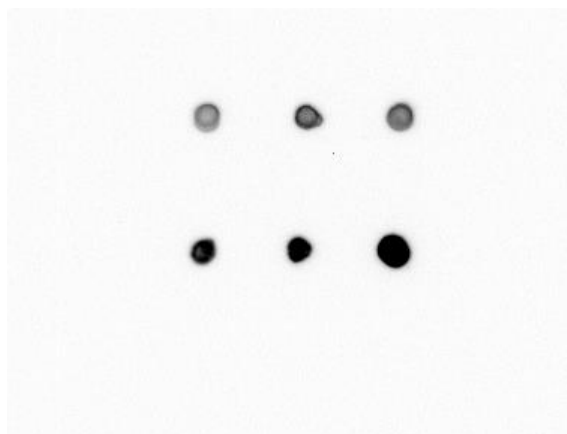


Figure 12: Top - gold nanoparticles with adsorbed IgG showing binding to antigen, Below - control IgG showing binding to antigen, Bottom - space where gold nanoparticles washed away.

nanoparticles are pipetted in 2  $\mu$ L drops and incubated in a solution overnight with the chemiluminescent antigen. The sample is rinsed and then imaged to determine where the antigen is bound. The antigen bound to both the gold nanoparticles with adsorbed IgG antibody and to the control IgG, the control gold nanoparticles were washed away in the rinsing process.

Gold nanoparticles bound to antibody can be immobilized on a paper strip. A solution

containing multiple antigens can be flowed over the strip and the gold nanoparticle-antibody complex will selectively bind to only one antigen.

This could cause aggregation of the gold

nanoparticles or binding of the gold nanoparticles on the visible surface and a distinct color presence. As an alternative gold nanoparticles with bound antibody can remain in solution and

antigen can be spotted on a nitrocellulose paper and the gold solution can be spotted in the same location. After rinsing the paper it can be determined if the gold has bound to the antigen by the presence of the color of gold nanoparticles. In a finalized design there would also be a control to confirm the sensor was working properly.

#### **4. Conclusion**

Gold nanoparticles can be synthesized in a monodisperse stable solution (Kimling, 2006). They have useful colorimetric qualities to be used in a simplified sensing technique. Modification of gold nanoparticles can create an environment in which the particles bind preferentially to a specific molecule of interest. This can be adapted to a sensing format using a lateral flow paper strip to promote ease of use.

Referring back to the previous stated success criteria, gold nanoparticles were repeatedly synthesized in a monodisperse solution containing particles of about 10 to 20 nm in diameter using a rapid method. The gold nanoparticles were also successfully characterized to determine the size and concentration in solution. Adsorption to the gold nanoparticle surface and determination of how this affects the stability of the particles in solution was also effectively completed. Finally immobilization of the gold nanoparticles and confirmation of binding to the target was established. The only missing piece left incomplete in the success criteria is the combination of the components into a final sensor format.

Truly establishing how to expect gold nanoparticles to react when in solution with other molecules became a difficult roadblock in the sensor design. When different antibodies did not cause any aggregation of the particles it was difficult to determine how many antibodies may have adsorbed to the surface of the gold nanoparticles. Too much aggregation of the particles was also an issue. If the particles aggregated completely it was nearly impossible to resuspend them in solution and therefore consider using them for the sensor. Repeated encounters with these issues prevented the completion of the final sensor format.

The basic idea of this sensing technology is that a signal will be evident without the use of analysis equipment. The presence of the color from the gold nanoparticles can determine the binding of a target. In a finalized design there would also be a control present to ensure the test is working properly. It is a technology that could have many applications in and outside of the laboratory whenever a positive or negative result for the presence of a molecule is useful.

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