Stoichiometric Analysis and Surface Loading of IgG-Gold Nanoparticle Conjugates

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Introduction:

Gold nanoparticles have a wide range of applications, including drug delivery, medical imaging, diagnostics, and biosensing. They are extremely useful because they are relatively inexpensive, chemically stable, and easy to modify compared to other methods. At the nanoscale, gold particles have a large surface area relative to their size, meaning many atoms are available to interact with other molecules. This makes them convenient for attaching chemical compounds.

A method of attaching molecules to gold nanoparticles uses thiols. Thiols act like chemical “glue” because they contain a sulfur group that strongly binds to gold surfaces. One end of the thiol molecule attaches to the gold nanoparticle, while the other end can be chemically modified to carry a desired molecule. This process is called chemisorption. It allows scientists to use gold nanoparticles for specific applications such as targeting, sensing, or binding biological molecules.

Gold nanoparticles also interact with light in a unique way through a phenomenon called localized surface plasmon resonance. When light hits a nanoparticle that is smaller than the wavelength of the light, the delocalized electrons (plasmon) on the gold surface move back and forth in response to the electromagnetic field. When the light frequency matches the natural frequency of movement of the electrons, resonance occurs. This interaction causes the absorption of certain wavelengths of light, giving gold nanoparticles their specific colors. For example, gold nanoparticles around 10–20 nanometers in size absorb green and blue light, causing red light to be reflected, which makes gold colloid solutions appear red.

Immunoglobulin G (IgG) is the most abundant antibody in human blood and plays a major role in immune defense by recognizing and targeting pathogens. Certain bacterial proteins, such as protein A and protein G, naturally bind to IgG antibodies. This interaction is used in antibody purification and the development of biosensors. However, the exact binding strengths between IgG and proteins A and G are not fully understood. Current methods used to measure protein interactions require expensive equipment and specialized facilities. Gold nanoparticles provide a more accessible and cost-effective alternative. Their color-changing properties, which can be measured using UV–visible spectrophotometry make it possible to quantify binding interactions in a simpler and more affordable way. Gaining more data on these binding strengths can improve antibody purification techniques, enhance biosensor design, and contribute to advancements in immunological research.

Engineering Goal:

This protocol establishes a foundational bioengineering workflow for designing gold nanoparticle based biosensors that utilize surface plasmon resonance to detect and quantify biomolecular interactions, such as Immunoglobulin G binding to Protein A or G, through UV–Vis spectrophotometry. By determining binding constants ($K_a = 1/K\_d$), it enables optimization of nanoparticle stability, affinity, and sensitivity. The approach supports diverse applications, including point-of-care diagnostics using rapid colorimetric assays, selective antibody capture for protein purification, targeted drug delivery via Fc-receptor binding, and proteomics research through accessible tools for analyzing binding kinetics and informing advanced plasmonic sensor design.

Procedure:

Part I - Synthesis of Gold Nanoparticles:

1. Dissolve 50 mg $HAuCl_4 \cdot 3H_2O$ in 500 mL DI water (0.25 mM solution).
2. Dissolve 147 mg trisodium citrate dihydrate in 50 mL DI water (1% w/v solution).
3. Heat 100 mL of gold solution to boiling with stirring.
4. Quickly add 10 mL of citrate solution.
5. Continue boiling for 10–15 min until color turns deep red.
6. Cool to room temperature and store at 4°C in the dark.

Expected Result: Wine-red solution with UV–Vis peak at ~520 nm confirming ~20 nm AuNP formation.

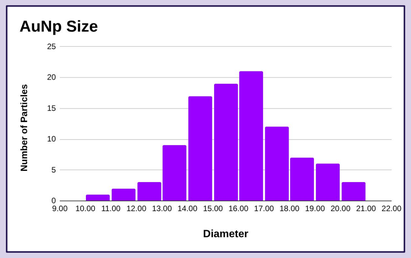
Part II - Immunoglobulin G conjugation via Carbodiimide Coupling:

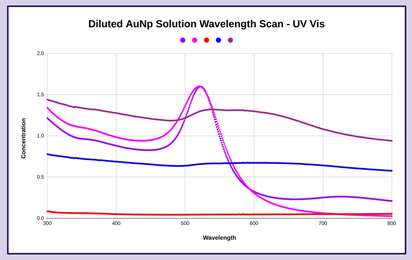
1. Prepare Protein A/G dilution series (0–200 µg/mL) in PBS.
2. Mix 450 µL IgG-AuNPs with 50 µL Protein A/G solution in microtubes.
3. Incubate 30 min at room temperature. Observe color shift (red → purple/blue).
4. Record UV–Vis spectra (400–800 nm).
5. Calculate aggregation index = A_{650}/A_{520}.

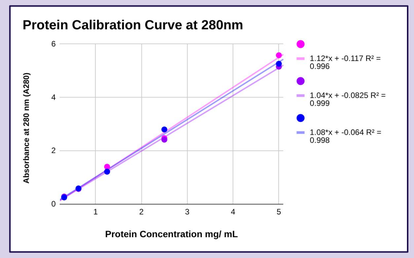
Part III - Protein A/Protein g aggregation Assay:

1. Prepare Protein A/G dilution series (0–200 µg/mL) in PBS.
2. Mix 450 µL IgG-AuNPs with 50 µL Protein A/G solution in microtubes.
3. Incubate 30 min at room temperature. Observe color shift (red → purple/blue).
4. Record UV–Vis spectra (400–800 nm).
5. Calculate aggregation index = A_{650}/A_{520}.P

Data and Results:







AuNP’s and Particle Count:

- Radius r = 8.01 nm = 8.01×10^{-7} cm.
- Volume of one AuNP (sphere): $V = (4/3)\pi r^3 = 2.15 \times 10^{-18}$ cm³.
- Mass of one AuNP: $m = \rho V = (19.30\ g/cm^3)(2.15 \times 10^{-18}\ cm^3) = 4.15 \times 10^{-17}$ g.
- Total number of AuNPs: 0.027108 g / (4.15×10^{-17} g/particle) = 6.52×10^{14} particles.

IgG Quantification:

- Pure IgG mass: 3.723 mg × 0.85 = 3.16455 mg = 0.00316455 g.
- Moles of IgG: 0.00316455 g / 150,000 g/mol = 2.1×10^{-8} mol (21.1 nmol).
- IgG molecules: (21.1×10^{-9} mol)($6.02214076 \times 10^{23}\ mol^{-1}$) = 1.27×10^{16} molecules.

Overview:

- Au mass available: 0.027108 g
- AuNP diameter (assumed): 16.02 nm
- AuNP count: 6.52×10^{14} particles\
- Surface area per AuNP: 806.3 nm²
- Total AuNP surface area: 0.526 m² (≈ 5,261 cm²)
- IgG amount: 21.1 nmol (1.2707×10^{16} molecules)
- Loading: ≈ 19.5 IgG per AuNP
- Predicted Dh (end-on model): ≈ 45.0 n
- Estimated antigen-binding capacity (max): 42.2 nmol antigen (≈ 2.54×10^{16} molecules)

Theoretical Size

- Flat-on: thickness ≈ 4.0 nm
- Side-on: thickness ≈ 8.5 nm
- End-on (vertical): thickness ≈ 14.5 nm
- Dh ≈ core diameter + 2 × thickness = 16.02 nm + 2(14.5 nm) = 45.02 nm (≈ 45.0 nm).

IgG Per AuNp:

- IgG per AuNP = (1.27×10^{16}) / (6.52×10^{14}) ≈ 19.5 IgG per particle.

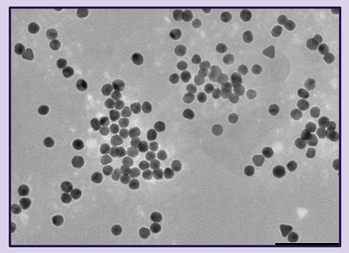
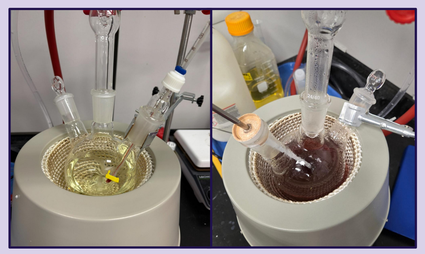
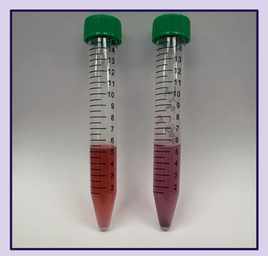


Image of Gold Nanoparticles taken via TEM - Imaging done by Dr. Prakash Nallathamby

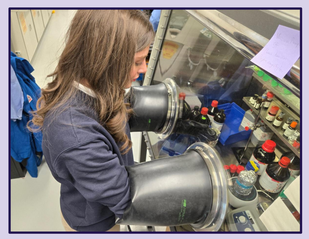
Student Pipettes Gold Colloid Dilutions into vials - Photo taken by Dr. Prakash Nallathamby.



Color difference between gold colloid (left) compared to gold colloid+immunoglobulin G (right) - photo taken by student researcher



Color difference between gold colloid (left) compared to gold colloid+immunoglobulin G (right) - photo taken by student researcher



Student handles Immunoglobulin G inside dry box - Photo taken by Dr.Prakash Nallathamby

Data Analysis:

My engineering goal was to develop gold nanoparticle based biosensors that utilize surface plasmon resonance to detect and quantify biomolecular interactions through UV–Vis spectrophotometry. The high loading of the Immunoglobulin G on Gold Nanoparticles and the use of 85% pure IgG for the conjugation reaction I did not get the standard aggregation response when Protein G was added. This didn’t allow me to calculate the binding constant of Protein G as originally envisioned. However, I ensured high conjugation of Immunoglobulin G to gold nanoparticles and quantified the loading of IgG per Au nanoparticle in comparison to the theoretical estimate which was found to be a good match.

Conclusions:

Although I was unable to complete the aggregation assay for Protein G binding, the high conjugation efficiency and quantification of Immunoglobulin G loading onto the nanoparticles supports several important applications. The validated workflow can be adapted for point-of-care diagnostics, where rapid colorimetric assays enable efficient pathogen detection. Additionally, Immunoglobulin G gold nanoparticle conjugates are useful for immunotherapy applications, including treatments for cancer, and other diseases like Alzheimer’s, where antibody loading is utilized for effective drug design. Lastly, the high-density antibody coverage facilitates protein purification by allowing selective antigen capturing and targeted drug delivery through Fc-receptor binding.

In order to accomplish the objective of determining the binding constants through aggregation I would decrease the IgG loading density so that Protein G can more readily act as a “bridge” between particles. Using higher-purity IgG (>95%) would also reduce interference from other, nonspecific proteins. If I were to continue on with this, I would utilize UV-Vis spectrophotometry to track the spectral peak shifts centered around 520 nm more closely under different concentrations allowing for improved sensitivity of the plasmonic sensor.