Stoichiometric Analysis and Surface Loading of IgG-Gold Nanoparticle Conjugates

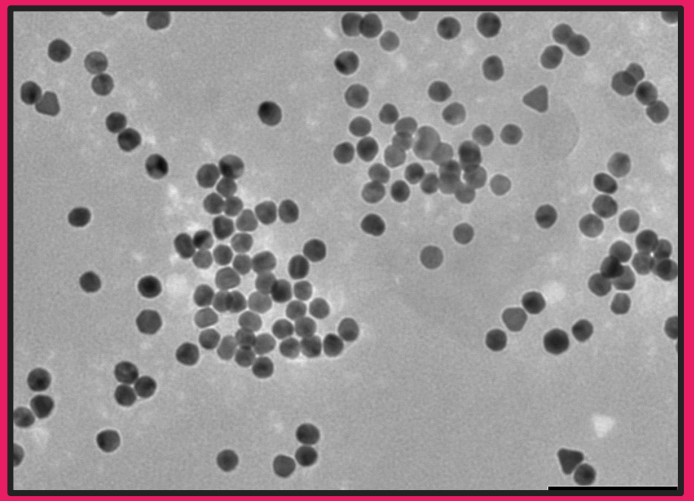


Image of Gold Nanoparticles taken via TEM - Imaging done by Dr. Prakash Nallathamby

Kendall Bradley, Marian High School, Mishawaka, IN, USA

Introduction:

Gold nanoparticles have many different uses like including drug delivery, imaging, diagnostics, and biosensing. Gold nanoparticles are convenient to use because they are inexpensive on a small scale and they are chemically stable making them easy to modify . At the nanoscale, gold particles have a large surface area relative to their size makes them perfect for attaching compounds.

A method of attaching molecules to gold nanoparticles is a process called chemisorption. It uses thiols that 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.

Introduction:

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.

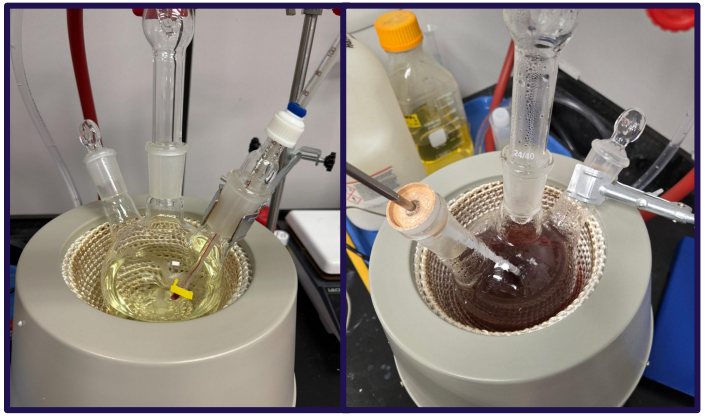
Immunoglobulin G is the most abundant antibody in human blood and plays a major role in immune defense. Bacterial proteins A and G naturally bind to IgG antibodies. The binding strengths between IgG and proteins A and G are not certain. Current methods used to measure protein interactions require expensive equipment and specialized facilities. Gold nanoparticles are more accessible and cost-effective. Their color-changing properties make it possible to quantify binding interactions.

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.

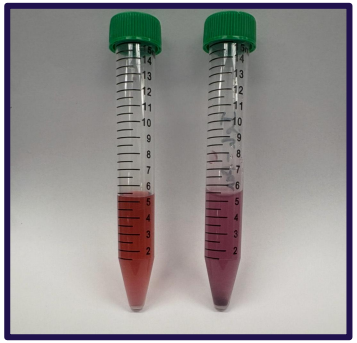
Methods:

- Synthesize gold-nanoparticle conjugates through the boiling of gold + citrate solution.
- Conjugate Immunoglobulin G to gold-nanoparticles through carbodiimide coupling.
- Add protein A and G to perform aggregation assay.

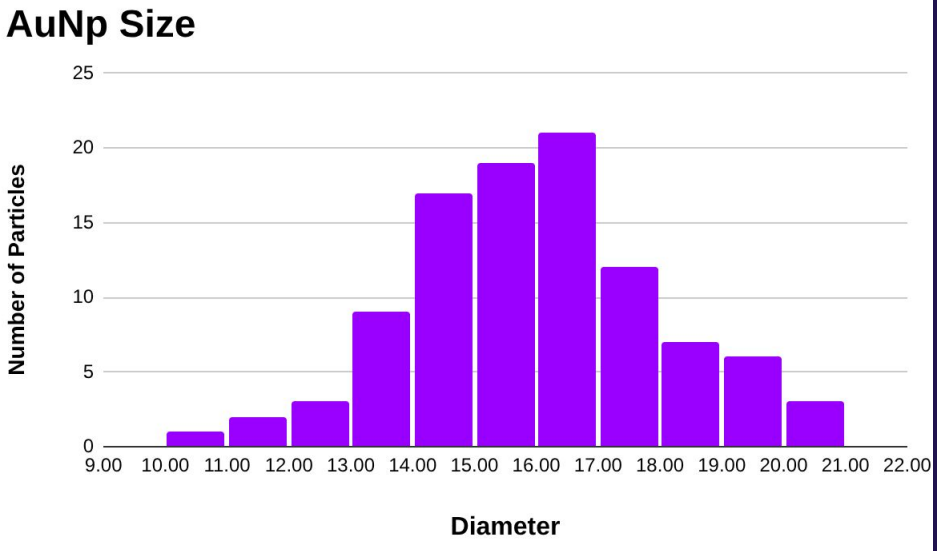


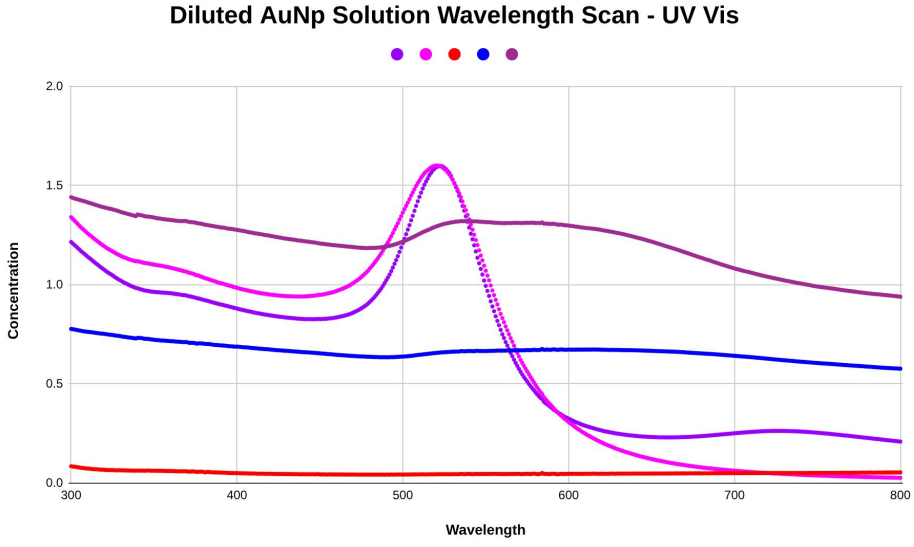
Color difference between gold colloid (left) compared to gold colloid+immunoglobulin G (right) - photo taken by student researcher

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

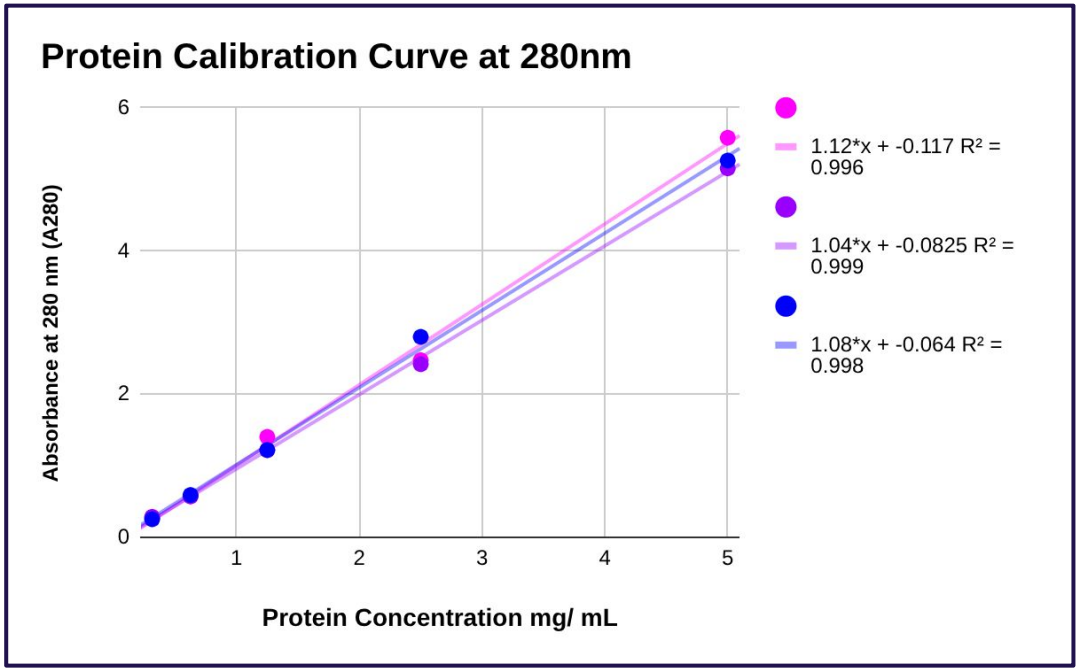


Graphs:


AuNp Size
Number of Particles
25
20
15
10
5
0
9.00
10.00
11.00
12.00
13.00
14.00
15.00
16.00
17.00
18.00
19.00
20.00
21.00
22.00
Diameter


Diluted AuNp Solution Wavelength Scan - UV Vis
Concentration
2.0
1.5
1.0
0.5
0.0
300
400
500
600
700
800
Wavelength

Graphs and Results:



I was able to synthesize gold nanoparticles that were relative to the theoretical size in diameter, mass, and volume. I determined the number of particles and the surface area in which they cover. I calculated the number of IgG molecules and how many were bound to each AuNp. I was lastly able to predict the binding capacity of IgG to AuNps.

Results:

AuNP's and Particle Count:

- Radius r = 8.01 nm = 8.01 × 10^{-7} cm.
- Volume of one AuNP (sphere): V = (4/3) πr^3 = 2.15 × 10^{-18} cm^3.
- Mass of one AuNP: m = ρV = (19.30 g/cm^3)(2.15 × 10^{-18} cm^3) = 4.15 × 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 × 10^{23} mol^{-1}) = 1.27 × 10^{16} molecules.

IgG Per AuNp:

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

Results:

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).

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 nm^2
- Total AuNP surface area: 0.526 m^2 (≈ 5,261 cm^2)
- 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)

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.

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 like treatments for cancer and other diseases like Alzheimer's where antibody loading is utilized for effective drug design.

In order to determine the binding constant 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.

Bibliography:

Brahms, Hero, et al. “Symmetrical Dimethylation of Arginine Residues in Spliceosomal Sm Protein B/B′ and the Sm-like Protein LSm4, and Their Interaction with the SMN Protein.” *RNA*, vol. 7, no. 11, Nov. 2001, pp. 1531–42. *Cambridge University Press*, https://doi.org/10.1017/S135583820101442X.

Hober, Sophia, et al. “Protein A Chromatography for Antibody Purification.” *Journal of Chromatography B*, Polyclonal and Monoclonal Antibody Production, Purification, Process and Product Analytics, vol. 848, no. 1, Mar. 2007, pp. 40–47. *ScienceDirect*, https://doi.org/10.1016/j.jchromb.2006.09.030.

Huang, Tao, et al. “Design and Synthesis of Single-Nanoparticle Optical Biosensors for Imaging and Characterization of Single Receptor Molecules on Single Living Cells.” *Analytical Chemistry*, vol. 79, no. 20, Oct. 2007, pp. 7708–18. *DOI.org (Crossref)*, https://doi.org/10.1021/ac0709706.

Marczewski, Adam W. “Analysis of Kinetic Langmuir Model. Part I: Integrated Kinetic Langmuir Equation (IKL): A New Complete Analytical Solution of the Langmuir Rate Equation.” *Langmuir*, vol. 26, no. 19, Oct. 2010, pp. 15229–38. *DOI.org (Crossref)*, https://doi.org/10.1021/la1010049.

Nallathamby, Prakash D., et al. “Preparation of Fluorescent Au–SiO2 Core–Shell Nanoparticles and Nanorods with Tunable Silica Shell Thickness and Surface Modification for Immunotargeting.” *Journal of Materials Chemistry B*, vol. 4, no. 32, Aug. 2016, pp. 5418–28. *pubs.rsc.org*, https://doi.org/10.1039/C6TB01659F.

Papadea, Christine, and Irene J. Check. “Human Immunoglobulin G and Immunoglobulin G Subclasses: Biochemical, Genetic, and Clinical Aspects.” *Critical Reviews in Clinical Laboratory Sciences*, vol. 27, no. 1, Jan. 1989, pp. 27–58. *DOI.org (Crossref)*, https://doi.org/10.3109/10408368909106589.

Picollo, Marcello, et al. “UV-Vis Spectroscopy.” *Physical Sciences Reviews*, vol. 4, no. 4, Apr. 2019. *www.degruyterbrill.com*, https://doi.org/10.1515/psr-2018-0008.