Stoichiometric Analysis and Surface Loading of IgG-Gold Nanoparticle Conjugates

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

Research Question:

Introduction:

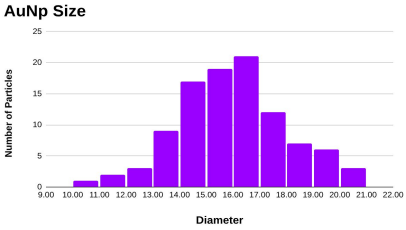
Gold nanoparticles have various application; they are inexpensive, chemically stable, easy to modify, and have unique color changing properties. Immunoglobulin G is the most common antibody in the human blood and binds easily to bacterial proteins like proteins A and G. Insights on binding strengths can improve antibody purification techniques, enhance biosensor design, and contribute to advancements in immunological research.

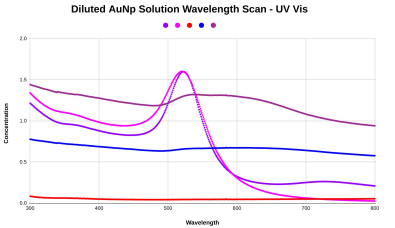
Engineering Goal:

The goal of this project is to design and synthesize gold nanoparticle biosensors that use localized plasmon resonance to quantify biomolecular interaction, such as the binding of Protein A or G to immunoglobulin G, the the use of UV-Vis spectrophotometry.

Data & Results:

- 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)





Methodology:

- Synthesize gold nanoparticles by making a gold citrate solution.
- Conjugating immunoglobulin G to gold nanoparticles.
- Adding protein G to the immunoglobulin G bound nanoparticles.
- Ran on UV-Vis.
- Calculated size, number of gold nanoparticles, amount of immunoglobulin G and how much bound to each nanoparticle.
- Attempted to calculate aggregation of gold nanoparticles.

Conclusions:

My goal was to synthesize gold nanoparticle based biosensors that d quantify biomolecular interactions through UV–Vis spectrophotometry. The high loading of the Immunoglobulin G on Gold Nanoparticles did not allow for an aggregation response when Protein G was added. This didn't allow me to calculate the binding constant of Protein G. However, I had high conjugation of Immunoglobulin G to gold nanoparticles and quantify d the loading of IgG per Au nanoparticle in comparison to the theoretical estimate which was a close match

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. This project has many real world applications such a as point of care diagnostics, immunotherapy protocols, and protein purification processes.