Synthesis and Characterization of Coated Gold Nanoparticles with Embedded SERS Tags

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Introduction

Background: Nanoparticles are finding widespread use in many fields such as healthcare and the environment.[1,2] However, they are of particular importance as drug delivery vehicles in biological systems. By tagging them with therapeutic drugs or antibodies and coating them in a phospholipid bilayer they have been found to be biocompatible and enter cells[2].

Surface-enhanced Raman spectroscopy (SERS) is of particular importance as an optical bioimaging technique due to its ability to allow deep and high-resolution volumetric imaging of biological tissues. Moreover, SERS can even allow for single molecule detection[1]. For a drug delivery construct to be monitored in-vivo, a SERS active molecule must be adsorbed close to or on the surface of metal nanoparticles. By using a 532 nm laser, the plasmon resonance of silver is more intense than gold, therefore silver was coated on the Au-NP.

Goals: In this study, gold nanoparticles (Au-NPs) have been synthesized using a modified seed-mediated method,[1,3] coated with *para*-mercaptobenzoic acid (pMBA) (Figure 1), a SERS active molecule, followed by the addition of silver to allow detection from the Raman spectrophotometer to provide greater SERS enhancement, and finally a phospholipid bilayer to promote uptake of the particles into biological systems.
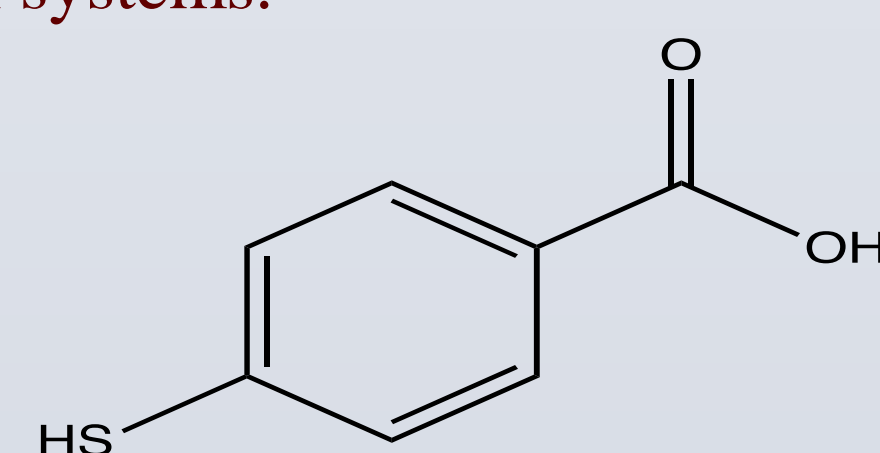
Figure 1. *para*-mercaptobenzoic acid (pMBA)

Materials and Methods

- *Chemicals:* Gold(III) chloride hydrate, sodium citrate tribasic hydrate, 4-mercaptobenzoic acid (pMBA), silver nitrate, cetyltrimethylammonium chloride (CTAC), ascorbic acid, and poly(allylamine hydrochloride) (PAH) were purchased from Sigma-Aldrich (St. Louis, MO). 1-Palmitoyl-2-oleoyl-*sn*-glycero-3-phospho-L-serine (POPS), and lysophosphatidylcholine (LPC) were purchased from AvantiPolar Lipids(Alabaster, Alabama). All the solvents and reagents were analytical grade.

- *Au-NP synthesis:* All glassware was cleaned with Aqua Regia (3:1, conc. HCl:conc. HNO_3) then rinsed with deionized water. Chloroauric acid (3.0 mL, 0.01 M) and deionized water (88 mL) were refluxed until boiling. Sodium citrate (1%, 5.0 mL) was added and the solution was refluxed for an additional 30 minutes until the solution turned a light red color and the solution was cooled on ice.

- *Silver coating:* Au-NP (3 mL) and pMBA (15 μL, 2.5 mM, ethanol) were sonicated and silver chloride (1.2 mL. 1 mM), CTAC (3 mL, 0.1 M) and ascorbic acid (150 μL, 1 M) were added to the Au-NP and pMBA solution.

- *PAH and lipid coating:* PAH (200 μL, 10 mg/mL) and sodium chloride (100 μL, 0.1 M) were immediately added to 1 mL of the solution and purified. POPS/LPC lipid solution (0.5 mL, 1:1 w/w) was added to the solution, incubated, and purified.

- *Spectroscopy analysis:* Au-NP were characterized using a Hitachi UV-vis spectrometer, NanoSight LM10HS particle size analyzer, and the extinction spectra were recorded. Spectra of coated BRIGHTs containing pMBA were obtained with a custom-built Raman spectrometer using a 532 nm laser and 50 μm slit width. Data was acquired using KestrelSpec at 10 second acquisition times with an automatic background subtraction.[5]

Results

Table 1. Approximate diameter of Au-NP at various stages of synthesis.

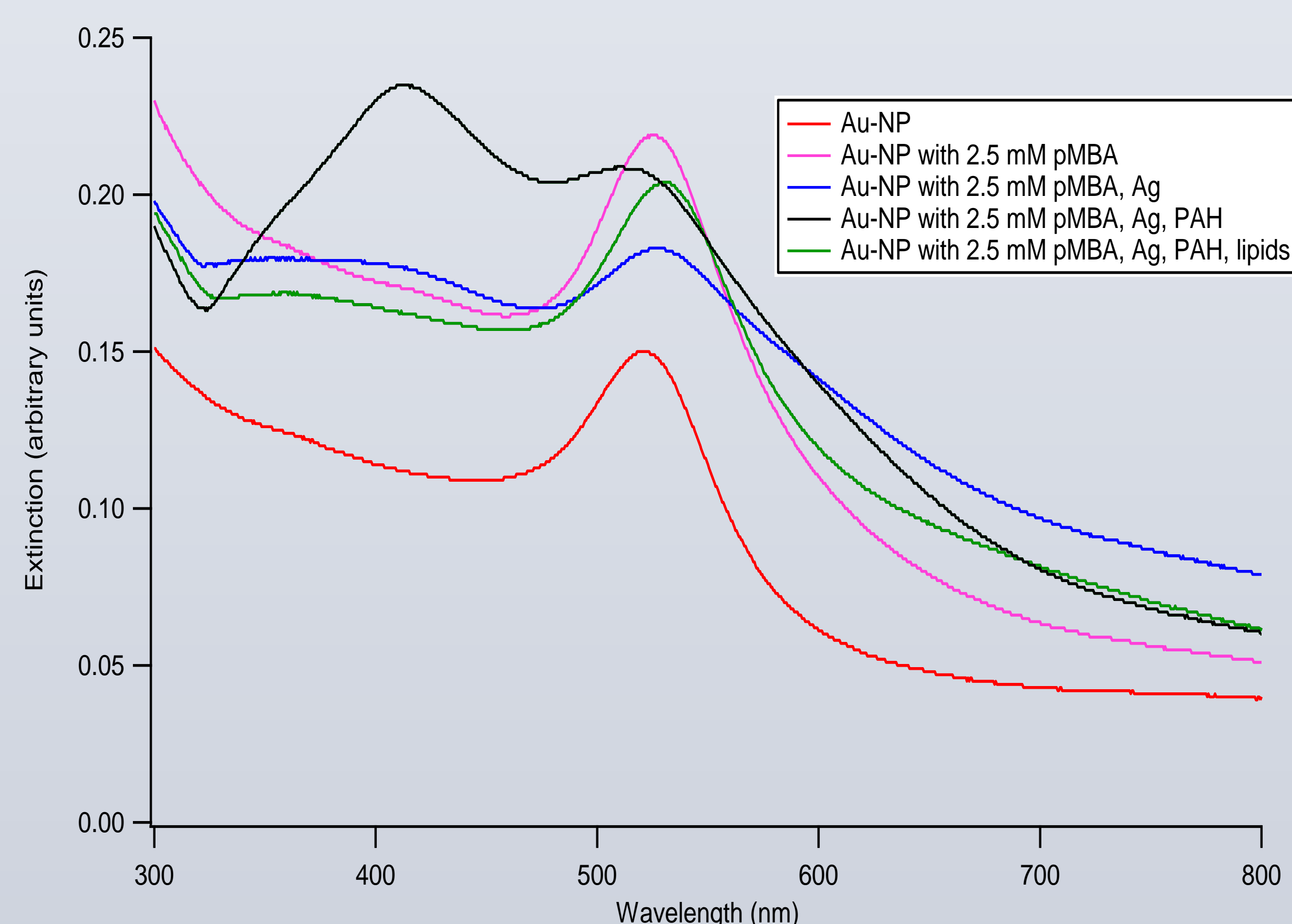| Gold Nanoparticle Solution	Diameter (nm)	Gold Nanoparticle Solution	Diameter (nm)
Stock Solution Au-NP	16	With 0.30 mM Ag shell	60
With pMBA pre-purified	29	With PAH post purification	75
With pMBA post-purified	81	With Lipids post-purification	125
With 0.28 mM Ag shell	55		

Figure 1. UV-vis extinction spectra of Au-NP with 2.5 mM pMBA, 0.28 mM Ag, PAH and lipid coatings.
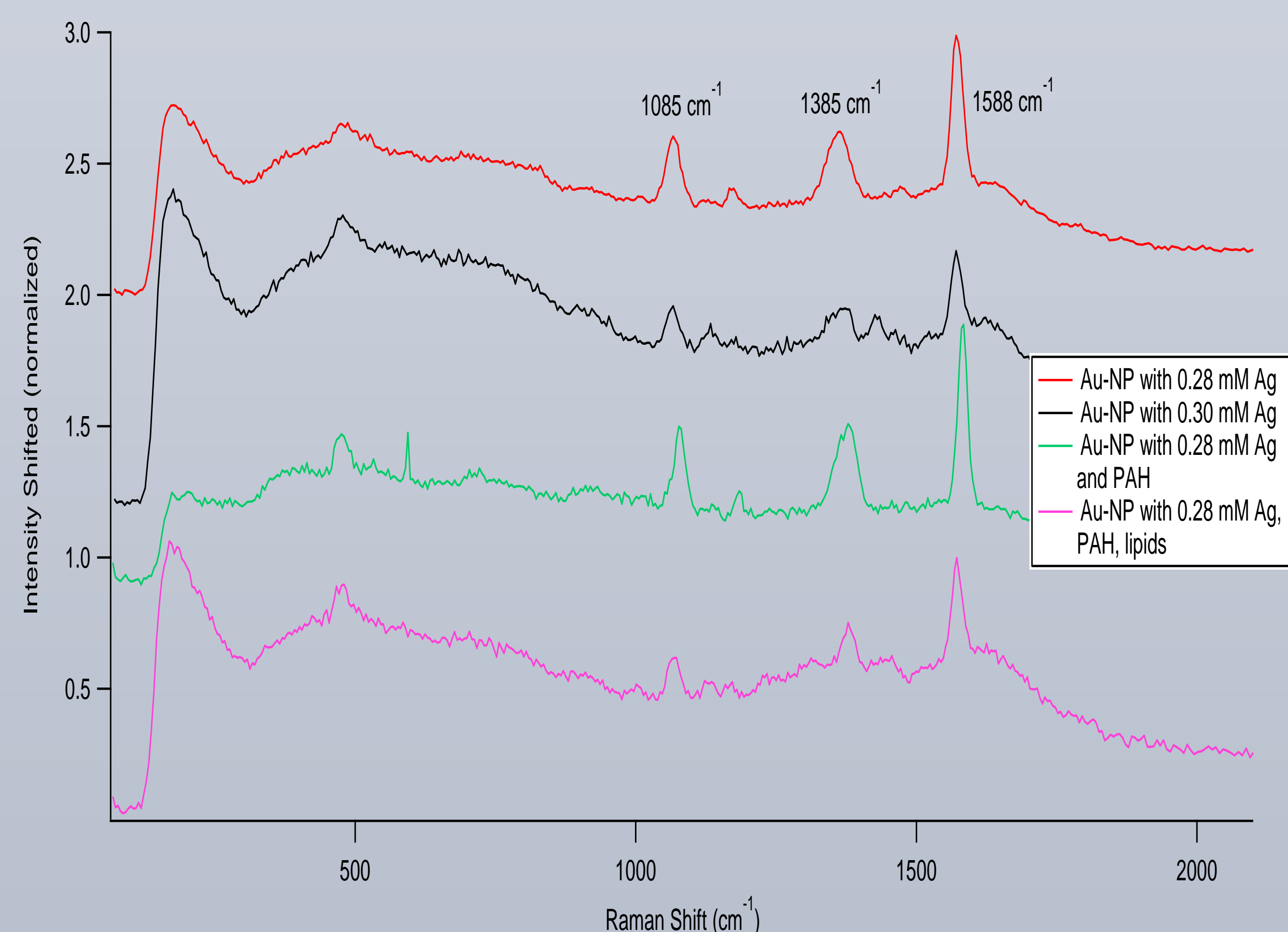

Figure 2. Offset of normalized SERS spectra of Au-NP with 2.5 mM pMBA, Ag (0.28 and 0.30 mM), PAH and lipid coatings.

Discussion

The diameter of the Au-NPs increased with each additional coating (Table 1). Small increases in diameter indicated successful coating of Au-NP. Silver coated BRIGHT's greatly increased in size when purifying directly after silver coating indicating aggregation. This aggregation was reduced by immediately coating with PAH after silver.

Figure 1 shows UV-vis spectra of Au-NPs throughout the coating process. Initial Au-NPs showed a single peak at 521.50 nm and experienced a red shift with each additional coating of the particles. Two peaks are observed in the spectra of particles containing silver indicating that the silver successfully coated the Au-NPs.

Figure 2 shows SERS spectra of pMBA throughout the coating process once the silver shell was synthesized. Characteristic peaks of pMBA can be seen at 1588 and 1085 cm^{-1} signaling aromatic ring vibrations.[4] Another distinct peak can be seen at 1385 cm^{-1} corresponding to the carboxylate group of pMBA.[4]

Conclusions

Small increases in diameter, red shifts in the UV-vis spectra and the constant characteristic SERS peaks of pMBA suggest that coating of the Au-NP with pMBA, Ag, PAH, and lipids was successful while still being able to observe the SERS spectra of pMBA.

Further research will include:
- Increasing the stability of BRIGHTs by altering the concentrations of silver and PAH
- Monitoring cellular uptake in yeast cells
- Coating Au-NP with antibodies to control uptake into biological systems and target specific tissues

References

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