Electronic Properties of Gold and Silver Nanoparticles Reveal Potential Applications for Medical Treatment

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Abstract: Protein-functionalized nanoparticles introduce a potentially novel drug delivery method for medical therapeutics, including involvement in cancer therapies and as contrast agents in imaging. Gold and silver nanoparticles are of particular interest due to their distinctive properties. Extensive research shows that gold nanoparticles demonstrate incredible photothermal properties and non-toxic behavior, while silver nanoparticles exhibit antibacterial properties but increase toxicity for human use. However, little is known regarding the properties or applications of hybrid silver-gold particles. This study measured the UV-Vis absorbance spectrum for 40 nm diameter Au, streptavidin-conjugated Au, Ag@Au hybrid, Ag nanoparticles, and Transient Absorbance Spectra of Au. Analysis indicates that the hybrid particles exhibit characteristics of both Ag and Au particles, implying potential applications similar to both Ag and Au nanoparticles.

Keywords: nanoparticles, nano-bioconjugates, UV-Vis Spectroscopy, hybrid nanoparticles

Introduction

Current synthetic strategies can be tailored to produce nanoparticles ranging from 1 nm to 100 nm[1]. Among many nanomaterials, metallic nanoparticles have drawn significant attention as they exhibit unique electron interactions known as localized surface plasmon resonance (LSPR)[2]. This interaction occurs when the incident light ray (electric field) excites the valence electrons on the metallic nanoparticles and oscillates electron density. This oscillation will result in a strong light scattering effect, making the different metallic particles have completely different colors than their ordinary states. This property provides potential applications of metallic nanoparticles in cancer therapeutics and medical imaging[3]. Nanoparticles are a rising alternative to enhancing traditional imaging through incorporation into contrast agents for Magnetic Resonance Imaging (MRI). Usage can also be seen in Photoacoustic imaging, which is often used in molecular imaging of cancer and other conditions[4]. Plasmonic nanoparticles are often used as photoacoustic contrast agents, providing high image resolution and greater tissue penetration depths than conventional optical imaging[5]. Nanoparticles are being extensively explored as contrast agents and can potentially benefit imaging modalities, which will in turn, see the rise of nanotechnology in the medical field. Plasmonic metallic nanoparticles also exhibit light scattering and absorbing properties, which some cancer therapies rely on[6]. Traditional photothermal therapy focuses on the absorbing properties of metallic nanoparticles to destroy cancerous tumor cells through hyperthermia therapy from infrared light excitation. Recent research has seen using magnetic nanoparticles to create contrast agents during medical imaging to reduce side effects. Gold nanoparticles

(AuNPs), in particular, are ideal for medical purposes due to their biologically inactive nature[7]. In addition, silver nanoparticles (AgNPs) are also heavily investigated as their strong oxidation tendency makes them popular in antibacterial nanomedicine production. Still, their high cytotoxicity makes it difficult to work with[8].

The current focus on the application of AuNPs aims to utilize their unique absorbance patterns and LSPR in plasmonic photothermal cancer therapeutics (PPTT). AuNPs and modified AuNPs are great for this purpose as their absorbance of light and efficient conversion to heat are properties critical for photothermal therapies[9]. Protein-functionalized AuNPs are common modifications as these proteins can mediate AuNPs entry into specific cells[10]. Combining biotechnology through targeted antibodies in conjunction with nanotechnology involving Au or Ag nanoparticles provides an opportunity to perform cell-specific nano-based medical therapies using the photothermal properties of AuNPs. Including AgNPs in the Au-bionanoconjugates could provide a cell-specific dual therapy utilizing the photothermal properties of Au with the anti-biological properties of Ag[11].

A silver-cored gold hybrid nanoparticle (Ag@AuNPs, Core@Shell) offers an opportunity to study whether the properties of the hybrid nanoparticles are equivalent to Ag or Au nanoparticles or unique to itself. Both AuNPs and AgNPs have significant light scattering and absorption properties and can convert optical energy into heat[12]. AgNPs have a higher photothermal efficiency for radius values lower than 20nm, whereas AuNPs with a larger diameter tend to heat up better.[13]. Nevertheless, AgNPs are not as biocompatible as AuNPs while offering great antimicrobial properties. A hybrid Ag@AuNPs is introduced to combine these properties and study their electronic properties.

Understanding the electron activity as excitation occurs in the molecule is crucial for application development. However, the excitation and relaxation of electrons happen on a femtosecond to an attosecond scale, making studying electron activity especially difficult. Remarkably, Transient Absorbance Spectroscopy (TAS) provides an extraordinary path for studying electron activity. A high-energy pumping laser (~400nm) is generally used to excite the sample, and a snapshot of the sample's absorbance profile will be recorded for differences in absorbance measurements. As the excitation decays within the first few hundreds of picoseconds, a second multiwavelength white light will pass through the sample and further excite some excited electrons to an even higher energy level. By controlling the probing light mirror's position to extend a centimeter scale travel distance, a customizable picoseconds delay is achievable in this manner. Through the analysis on the change in probing light's intensity through log based algorithm, an absorbance change in mOD(Optical Density) can be outputted. A three-dimensional map of electron dynamics is generated based on the Wavelength, Decay time, and Decay energy[14]. From this point, TAS is developed and deployed in many studies of electron activity.

In this paper, the Ultraviolet–visible (UV-Vis) spectrum of nanoparticles with the diameter of 40 nm AuNPs, 40 nm protein Streptavidin-conjugated AuNPs, 40 nm AgNPs, and ~42 nm synthesized Ag@AuNPs (30nm diameter Ag core/6nm Au shell) was obtained and compared. Additionally, TAS of AuNPs and its peak specific wavelengths absorbance decay in time are also collected and analyzed. UV-Vis spectrophotometer is an instrument that measures the amount of light absorbed or transmitted by a sample as a function of wavelength. (Figure1) [15].

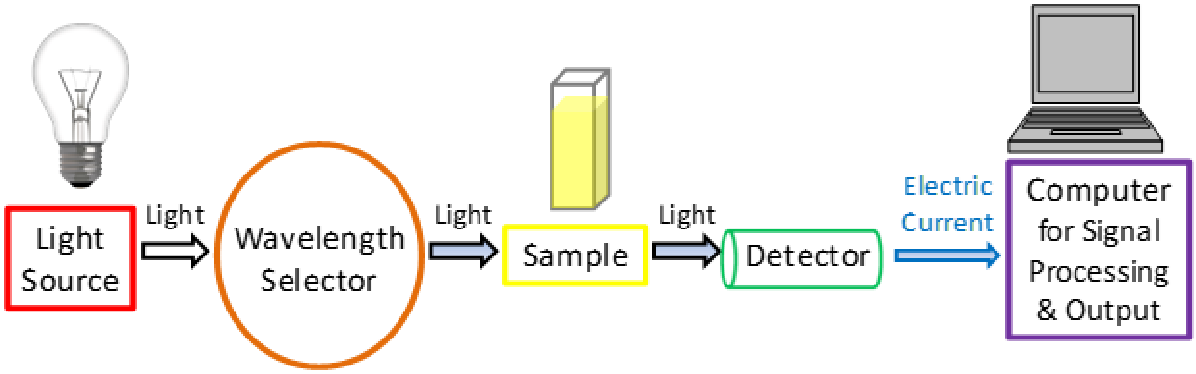

Fig. 1. Schematic of UV-Vis spectroscopy

Methods

UV-Vis spectroscopy measures the absorbance of AuNPs, AgNPs, protein streptavidin conjugated AuNPs, and Ag@AuNPs. Au and Ag nanoparticles were obtained from commercial sources (Sigma Aldrich). Ag@AuNPs were synthesized in Dr. Yadong Yin's lab at the University of California, Riverside[16]. Streptavidin-conjugated AuNPs were obtained from a commercial source (NanoComposix). The colloidal nanoparticles are diluted in distilled water and are analyzed in the UV-Vis Shimadzu UV-2700i Spectrometer from 350 to 650 nm using plastic cuvettes.

The procedure for the conjugation of AuNPs started with pre-wetting the filter device by adding 450 µL of 10 mM potassium phosphate(desalting buffer) to a 2x2 mL housing tube and centrifuging at 5000 RPM for five minutes. The desalting buffer is then aspirated and removed. Next, the antibody solution (Millipore Amicon® Ultra 0.5 mL, 10 kDa, Cat# UFC501096) was added into the filter and concentrated by centrifuge at 5000 RPM for 5 minutes. The filtrate was then removed, and the concentrated antibody was washed five times. In each wash, 350 µL of desalting buffer was added to the filter containing the antibody and centrifuged at 5000 RPM for five minutes. The filtrate was removed and disposed of after each centrifugation. This brings down the antibody concentration to ≥ 1 mg/mL. The antibody solution was conjugated by incubating BioReady™ carboxyl gold with EDC/NHS for thirty minutes at room temperature to activate particles. Activated particles were washed once with reaction buffer, followed by resuspending particles in the reaction buffer and adding antibodies to particles before incubating for an hour. The particles were incubated with hydroxylamine for ten minutes, washed twice with reaction buffer, and resuspended into NCX Conjugate Diluent.

AuNPs were taken to California State University, Northridge (CSUN) for TAS analysis. The data was collected through Femtosecond Laser Spectroscopy. The experimental setup of the femtosecond laser followed a detailed description from Eroglu et al.'s publication [17]. By loading a few drops of AuNPs sample to a well with minor dilution for better signaling, the absorbance change was measured at different time delays between the pump and probe.

Results and Discussion

The UV-Vis spectra from 350 to 650 nm were collected and normalized with respect to their individual maximum measured absorbance for Au, Au-conjugated, Ag, Ag@AuNPs, and streptavidin particles (Fig. 2). The absorbance lambda max (λ_{max}) determined is as 517 nm for Au, 522 nm for Au Conjugate, 404 nm for Ag@Au Hybrid, and 419 nm for Ag. Protein Streptavidin was found to have almost no absorbance for the entire spectrum. The Ag@Au hybrid particles exhibit a broad peak expanding throughout both the ranges of Ag and Au λ_{max} wavelengths, indicating that the Ag@AuNPs have properties inherited from both AuNPs and AgNPs. With a λ_{max} closer to the values of Ag, the properties of AgNPs are expected to be dominant over the properties of Au. However, an increase in the Au peaked absorbance region also shows the influence of the outer gold shell. AuNPs peak at around 517 nm, and Steptavidin-conjugated AuNPs peak at around 522 nm. The small difference between the two λ_{max} demonstrates that the absorbance properties of AuNPs are retained despite the conjugation. This opens the possibility to numerous biomedical applications as protein conjugation can provide selective entrance into cells while keeping the light scattering feature.

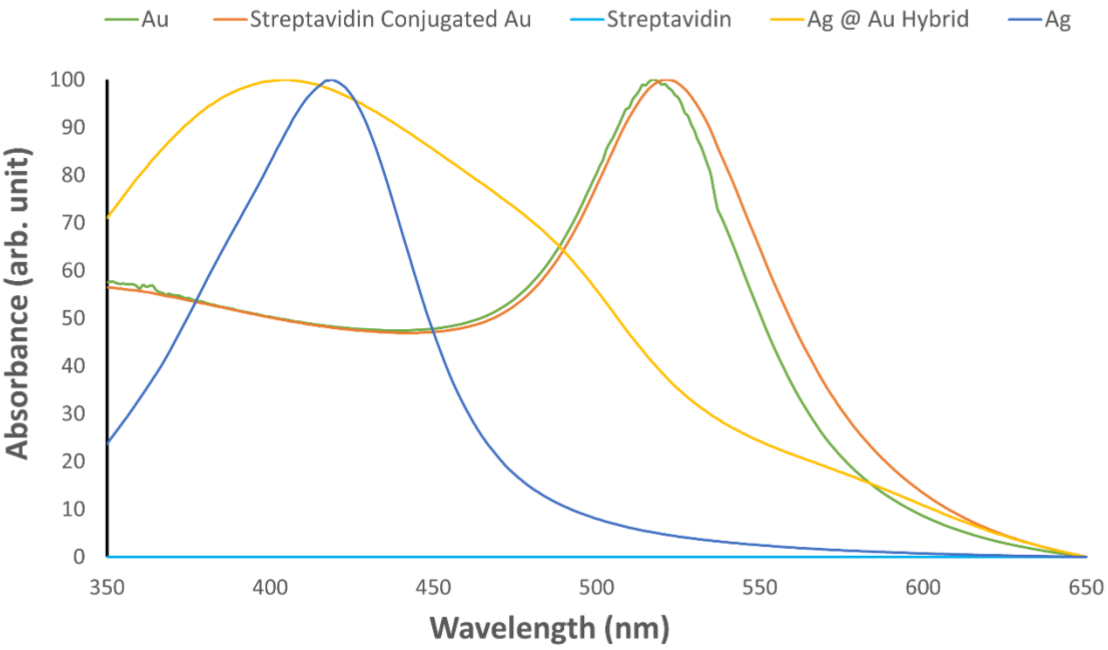

Fig. 2. Normalized UV-Vis absorption spectra of nanoparticles.

The normalized absorption spectra revealed the plasmonic peak of the different nanoparticles. However, the intensity of the light scattering effect for each nanoparticle is a substantial factor in the development of a possible PPTT treatment and effective usage in medical imaging. A simple Mie resonance-based simulation app is used to better understand the light interactions between different nanoparticles. We have used the NFMie program [18], and the results have been plotted in Figure 3. The red plots show the absorption of light impinging upon the nanoparticles, whereas the green plots illustrate the scattering phenomena. The extinction plots account for the total phenomena of absorption and scattering simultaneously. It is obvious that our 40nm diameter particles generate the plasmonic peak locations relatively around the expected wavelength observed in Figure 2 as an outcome of the UV-Vis measurements.

In summary, silver nanoparticles give us a resonance peak around 412nm, whereas gold nanoparticles yield a peak at 527nm (see Figure 3A and 3B). Two different shell thicknesses have been simulated in Figures 3C and D, and it can be concluded that the thinner gold shell lacks the prominent plasmonic peak around 517nm, evident in Figure 3C. Hence, it can be understood that we are losing the gold plasmonic features with the thinner gold shell. In both situations, the silver plasmonic peak still broadens the absorption spectra. In summary, we observe more of the exhibited plasmonic properties from these distinctive metals in Figure 3C.

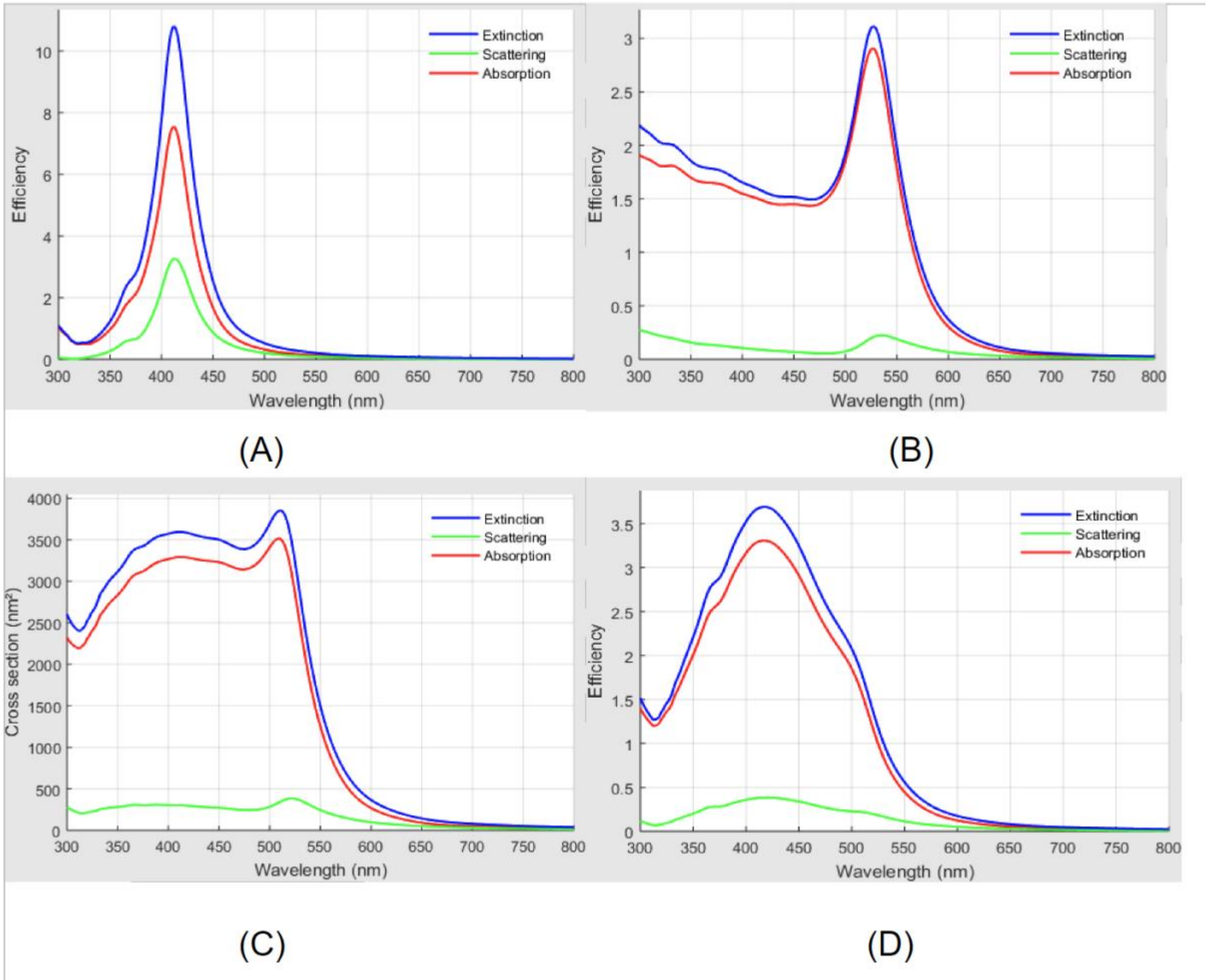

Fig. 3. Simulated Nanoparticles Spectra for (A) 40nm diameter AgNPs, (B) 40nm diameter AuNPs, (C) 30nm-6nm core-shell thickness Ag@AuNPs, and (D) 34nm-3nm core-shell thickness Ag@AuNPs.

The obtained TAS of AuNPs (Figure 4A) presented two induced excited state absorption (ESA) peaks at 475 nm and 571 nm and a ground-state bleaching(GSB) peak at 519 nm. As the pumping laser excites the sample, the ground-state species at the λ_{max} region will experience a decrement in quantity. Thus, as the probing light arrives, since the ground state species at λ_{max} region were depleted by the pump, it is said to leave a hole in this energy level(band). The absorbance measured from probing light will be lower due to decreased absorption activity caused by holes in this band. As a result, a negative peak, referred to as the

GSB peak, will be observed in the difference absorbance spectra[19]. ESA, on the other hand, is the transition of the excited electron to continue to absorb photons and promote to an even higher state.

During the process of electron relaxation, the energy is coupled to phonons, vibrational waves that can be quantized in their interaction with the lattice structure. This coupling is crucial to the understanding of photothermal properties in all metallic nanoparticles. Electron-Phonon interaction will last around the first five picoseconds of the electron excitation[20]. However, as the phonon scattering occurs in the lattice structure, which typically happens after the initial 100 picoseconds, the bonds vibrate to relax, releasing the energy gained in the excitation under the picosecond to nanosecond scale. The energy released during this long-lasting relaxation will express the thermal energy that can be utilized for different purposes, which can be the working principle in the context of PPTT. Thus, TAS can provide a peculiar path for the study of these electron and phonon interactions by measuring the time-resolved energy change (absorption change) within different energy levels.

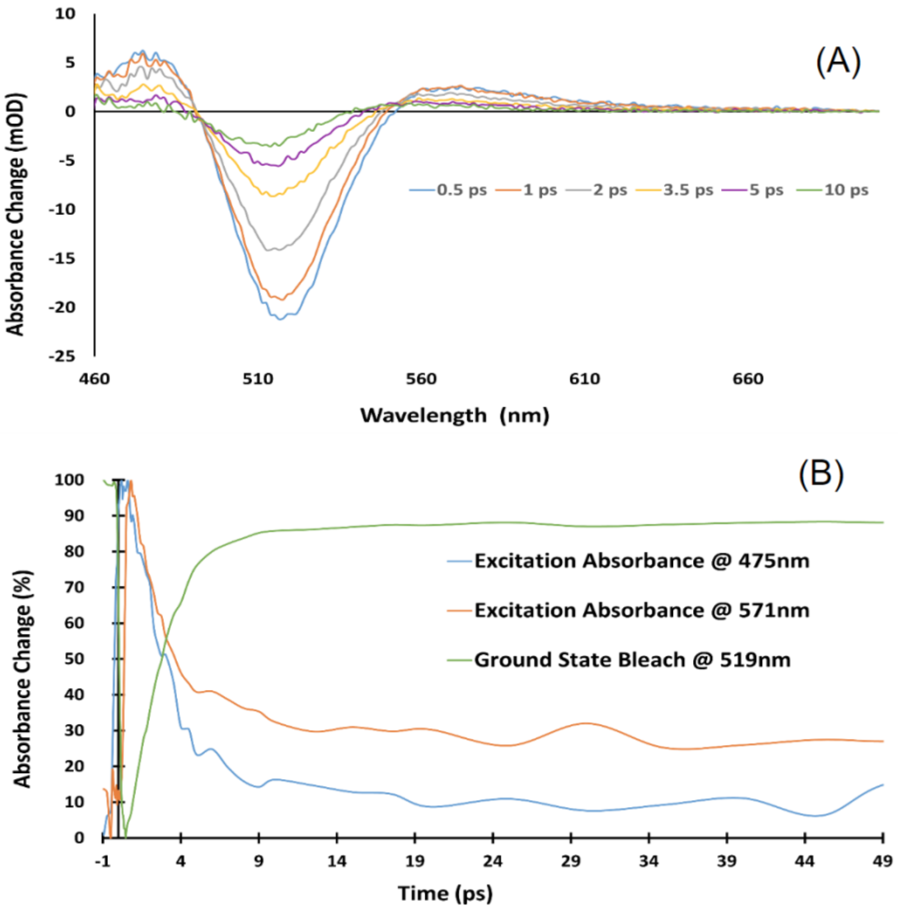

Fig. 4. (A) TAS of AuNPs with different time delays, (B) Time-resolved Peak wavelength absorbance of AuNPs.

A comprehensive comparison of the time-resolved absorption change of all three peaks is presented in Figure 4(B) by comparing the transient absorbance effects in AuNPs at different wavelengths. Similar activities for each peak were also observed in the publication from Zhang[21]. Each peak comprises three stages: initial excitation within one picosecond, rapid energy exchange in different areas due to energy transfer within the initial eight picoseconds, and the long-lasting relaxation caused by the phonon interactions. The ground state bleaching peak shows the fastest response as it corresponds to the direct path for electron-hole recombination and electron-electron and electron-phonon coupling when the AuNPs are excited by the pump. The relaxation finishes within five picoseconds. While the relaxation at 520nm occurs rapidly, a resonance between the excited electron and localized valence electron caused an oscillation of plasmonic nature due to the high energy excitation, thus creating an induced excitation absorption in a broad range of wavelengths between 550nm to 630nm. In the third stage, where long-lasting relaxation takes place, the slow decay of absorption shows a different activity from electron interaction is observed here. The phonon-phonon interaction with the lattice is accountable for long-lasting relaxation. This means the energy from the excitation is coupled and dissipated as heat, which can be utilized in the PPTT or other biomedical photothermal applications. Opposite to the lower energy-induced absorption, the ESA in 480nm shows a more intense energy release. The stronger energy release will result in a faster transition from an excited state to the ground state, reflect as a short phonon-phonon scattering interaction, and reduce the heat generated from this process, which makes it, not an ideal wavelength for the photothermal application as the energy are released more fiercely than the higher wavelength does.

Conclusion

The antibacterial properties of AgNPs and the photothermal properties of AuNPs can be highly beneficial in the nanomedicine industry. Thus, the hybrid particles Ag@AuNPs can potentially provide an alternative path that combines both properties. Meanwhile, antibody-conjugated AuNPs offer a selective cell-entrance advantage over unconjugated AuNPs in medical applications. Studying these nanoparticles through UV-Vis and TAS provides an important understanding of the role electrons play when the system is stimulated through external energy in different scenarios such as PPTT or medical imaging. Through UV-Vis spectra, the hybrid Ag@AuNPs reveal both the properties of AuNPs and AgNPs. The AgNPs core predominates the entire absorbance spectrum, while the AuNPs shell greatly expands the absorption range. The thickness of the shell demonstrates a positive correlation with its Au plasmonic peak region intensity in the absorption spectra based on the Mie scattering simulations. The light extinction effect from the shell can overlay the core and significantly reduce the core absorbance feature as it gets considerably thicker. Compared to AuNPs, streptavidin-conjugated AuNPs demonstrate a similar but shifted absorbance while the general AuNPs absorbance features are retained.

The TAS analysis of AuNPs reveals three different peaks and two different electron coupling mechanisms. The GSB peak demonstrates the formation of an electron-hole pair and its direct interaction with phonons, leading to heat dissipation. On the other hand, the ESA peak displays a rapid and intense relaxation occurring at shorter wavelengths. At higher wavelengths, the relaxation was found to be more gradual, resulting in sustained and enduring heat generation through phonon-phonon scattering while making such longer wavelength energy dissipation channels a better fit for practical applications.

Future research on how the electron activity varies among different nanoparticles has the potential to elucidate a complete image of electron dynamics, establishing a solid groundwork for the utilization of these nanoparticles in actual medical applications. In addition, cell studies on the hybrid Ag@AuNPs will elucidate if the hybrid particles exhibit toxic behaviors more similar to AgNPs or AuNPs.

As an undergraduate research group from a community college, this research experience is definitely a fulfilling and rewarding one. For many of us, this is our very first research experience since PCC does not have an abundance in research resources and opportunities. Throughout this research, we went from learning how to use a micropipette and a centrifuge machine to synthesizing NPs and analyzing the experimental results. There are limitations in a community college lab, such as the lack of proper machines and equipment. In the fortunate scenario where new equipment can be purchased, the operational processes must be mapped out by the group that plans to use it from the very beginning. However, thanks to the MNT-EC led by Dr. Jared Ashcroft and his funding from the NSF, we were able to conduct our experiments and gather significant data using materials and equipment from our partners at CSUN and UC Riverside in a collaborative manner. This development demonstrates how collaboration in academia is extremely important to the success of undergraduate research and how it is necessary to increase research opportunities at the community college level, such as those Dr. Ashcroft offers. From learning how to design an experiment to analyzing data and writing a paper, we learned how these hands-on wet and dry lab skills can benefit and prepare us tremendously. As we prepare for transfer to a 4-year university, we will bring the skills and knowledge that we have obtained throughout this journey. For those of us considering pursuing further education after our bachelor's degree, this experience will propel us toward our academic and future career goals. Since many undergraduates are unaware of the novel nanotechnology research field, these research opportunities at PCC can spark students' interest in studying this field and help pave the way for the next generation of promising and ambitious young scientists. Having the opportunity to perform high-level research with experienced professionals in the field can help develop a confident and inquisitive mindset for budding scientists. Taking the first step into the research field while simultaneously attending a community college can help bridge the institutional gap between 2-year colleges and 4-year universities. More students will be able to explore their interests early on and give themselves a greater chance to flourish brightly in their careers. As students who have benefited from this, we will take the things we have learned with us as we each walk down our chosen paths toward our future.

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Disclosures. The authors declare no conflicts of interest.

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