Understanding Electronic Properties of Gold Nanoparticles and Antibody-Conjugated Gold Nanoparticles For Use in Photothermal Cancer Medicine

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Introduction

Gold nanoparticles (AuNPs) are metallic nanoparticles that are widely utilized in plasmonic photothermal therapy (PPTT) to destroy cancerous tumors using infrared light excitation. AuNPs and modified AuNPs are great for this purpose because of their absorbance of light and efficient conversion to heat. Protein-functionalized AuNPs, such as Streptavidin-conjugated AuNPs, can facilitate the delivery of AuNPs into specific cells. We synthesized AuNPs conjugated to the protein Streptavidin so that the absorbance properties and electron activities of AuNPs and the conjugated AuNPs can be analyzed, using the UV-Vis spectroscopy and the ultrafast femtosecond spectroscopy (or transient absorption spectroscopy) in the visible and infrared spectrums to capture the dynamics of energy transfer from nanoparticles to Streptavidin.

Hypothesis

Femtosecond Ultrafast Spectroscopy will reveal a difference in energy profiles of antibody conjugated AuNPs compared to individual AuNPs.

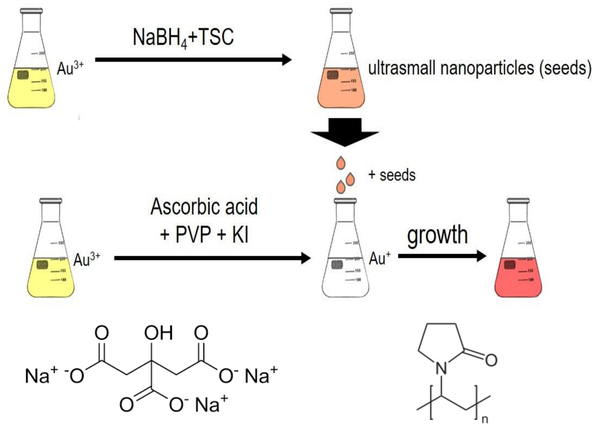
AuNPs Properties

AuNPs are commonly used in nano-based therapies due to their:

- high surface area to volume ratio
- multi-functional surface chemistry
- unique optical properties
- stable, non-toxic, and non-immunogenic nature
- 40-50 nm particles used for biomedical applications

Synthesis of 40-50 nm AuNPs

40-50 nm AuNPs are synthesized using reduction of Au^{3+} using sodium borohydride ($NaBH_4$) in the presence of TSC and PVP (capping agents to control size through electrostatic interactions). The synthesis was done at UC Riverside.

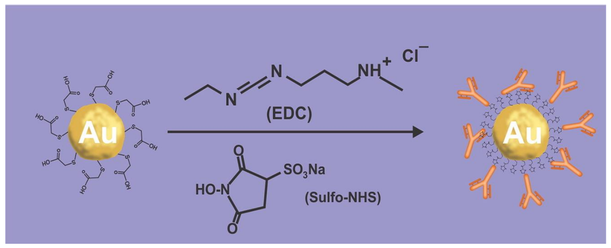


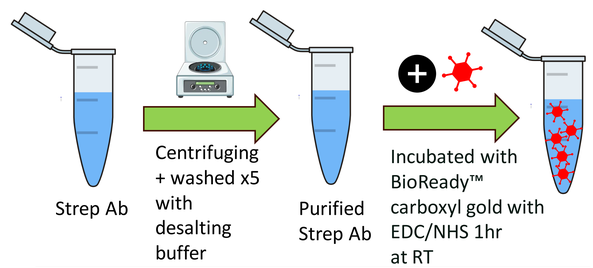
Trisodium Citrate (TSC): Negatively-charged capping agent = smaller Au-NPs

Polyvinylpyrrolidone (PVP): Neutral capping agent = larger Au-NPs

Conjugation of AuNPs to Streptavidin

AuNPs are conjugated to streptavidin through a covalent amide linkage using carboxylic acid derivatized nanoparticle reaction with EDC in the presence of sulfo-NHS





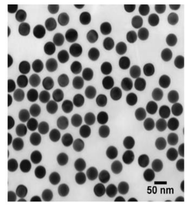
Schematic of Strep Antibody conjugated to AuNPs

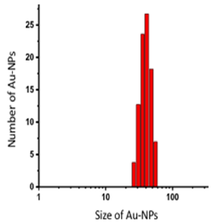
Results

- The maximum absorbance (λ_{max}) shifts from 517 nm for AuNPs to 522 nm for conjugated AuNPs in the visible spectrum.
- The AuNPs and Au conjugates kinetics show electrons absorb to excited state in 0.3 ps and return to the ground state at 5 ps. There is a slight shift showing Au conjugates reach excited state faster than non-conjugated AuNPs.
- Electrons in the conjugated AuNPs leave the ground state and enter the excited state when hit with longer wavelengths of light compared to non-conjugated AuNPs.
- The obtained TAS shows two excited state absorption (ESA) peaks at 475nm and 571nm and a ground-state bleaching (GSB) peak at 519nm.
- The GSB region experiences decreased absorption activity due to a hole made by the pumping laser while the ESA region continues to absorb photons and go to a higher energy state.
- The long-lasting relaxation is caused by phonon-phonon interaction, meaning the energy is dissipated as heat, which can be used in PPTT and other biomedical photothermal applications.

Conclusions

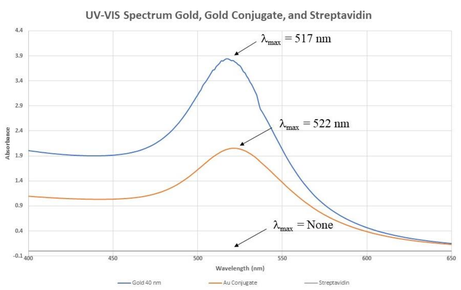
- The data obtained from the UV-Vis spectroscopy and the Femtosecond Laser analysis shed light on the electronic dynamics of AuNPs and Strep-conjugated AuNPs when the system is stimulated by the external energy, such as from PPTT.
- Conjugating proteins to AuNPs affects the electronic properties of the AuNPs, which has potential in using for photothermal medicine cancer treatments.
- Additional TAS analysis of AuNPs reveals different electronic mechanisms regarding the ESA and GSB peak at different timepoints and wavelengths.

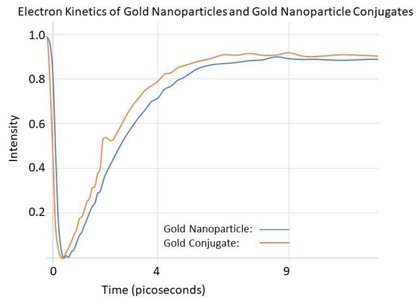


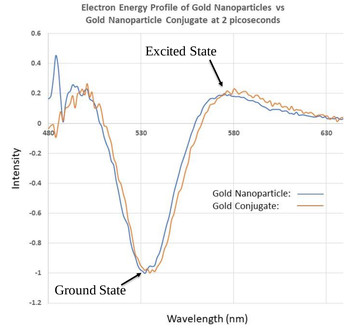


Synthesized (red solution); Scanning Electron Microscope image; and Dynamic Light Scattering showing shapes and sizes of 40-50 nm gold nanoparticles. *Data and images collected at University of Riverside.*

Electronic properties: UV-VIS Spectroscopy and Ultrafast Femtosecond Spectrum of Gold and Gold Conjugates

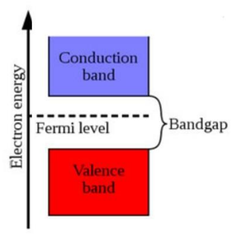






Ultra-Fast Femtosecond Laser Principles

Ultra-Fast Femtosecond Laser applies energy into the gold nanoparticles by shooting light with different wavelengths into them causing their electrons to enter the excited state (conductance band) from the ground state (valence band). Electrons (and thus energy) can then transfer along the conduction band and be released for use in real world applications such as photodetection and in photothermal medicines.



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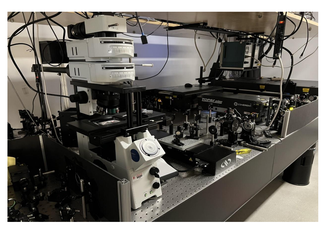
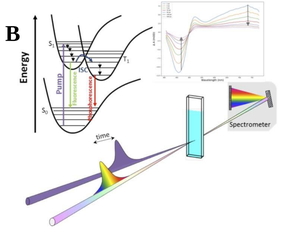
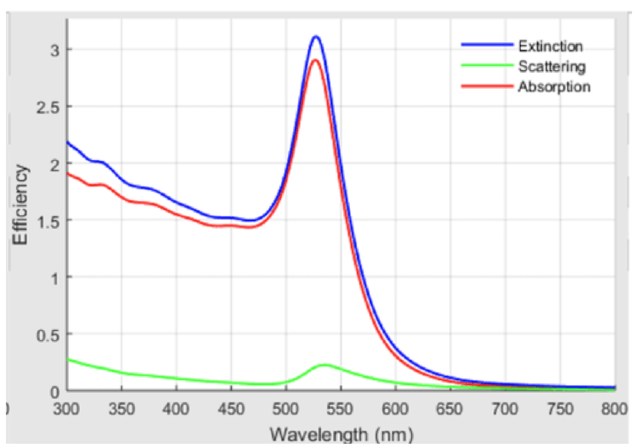
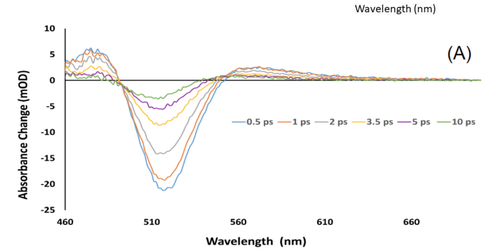


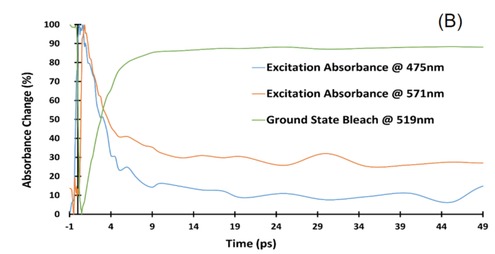


(A) Femtosecond Laser setup at CSUN. (B) Diagram of Femtosecond Transient Absorption Spectroscopy



Simulated Nanoparticles Spectra for 40nm diameter (AuNps) yield a plasmonic peak at 527nm





(A) TAS of AuNPs with different time delay. (B) Time-resolved peak wavelength absorbance of AuNPs

Future Work

AuNPs are suitable for use in photothermal medicines due to their electronic properties and non-toxic behavior. A more advanced Ag@Au hybrid nanoparticle would increase efficacy as a cancer medicine due to a combination of photothermal therapy from the AuNPs and anti-bacterial properties from AgNPs. Future studies will focus on:

- Conjugating Ag-cored Au hybrid nanoparticles to Streptavidin.
- Studying the electronic properties of conjugated and non-conjugated Ag-Au hybrid nanoparticles.
- Performing cell culture to study effects of AuNPs and hybrid nanoparticles on cell viability.

References

1. Azevedo, M. M., Pina-Vaz, C., & Baltazar, F. (2020). Microbes and Cancer: Friends or Faux?. *International journal of molecular sciences*, *21*(9), 3115.
2. Arruebo, M., Valladares,M., González-Fernández, A. (2009). Antibody-Conjugated Nanoparticles for Biomedical Applications, *Journal of Nanomaterials*, vol. 2009, Article ID 439389.
3. Singh, P., et. al. (2018). Gold Nanoparticles in Diagnostics and Therapeutics for Human Cancer. *International journal of molecular sciences*, *19*(7), 1979.
4. Su, C., et. al. (2020). "Antibacterial Properties of Functionalized Gold Nanoparticles and Their Application in Oral Biology", *Journal of Nanomaterials*, vol. 2020, Article ID 5616379.
5. Alibek, K., et. al. (2012). Using antimicrobial adjuvant therapy in cancer treatment: a review. *Infectious agents and cancer*, *7*(1), 33.
6. Amina, S. J., & Guo, B. (2020). A Review on the Synthesis and Functionalization of Gold Nanoparticles as a Drug Delivery Vehicle. *International journal of nanomedicine*, *15*, 9823–9857.
7. Suleiman, E., et al. (2020). Conjugation of Native-Like HIV-1 Envelope Trimers onto Liposomes Using EDC/Sulfo-NHS Chemistry: Requirements and Limitations. *Pharmaceutics*, *12*(10), 979.
8. van de Plas, P., et. al. (2018). GOLD NANOPARTICLE CONJUGATION Adsorption or Covalent Binding? *Aurion, 6, 1.*

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