



# Improving SERS-based readout strategy for biomarker detecting immunoassays

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## Introduction

Detection and monitoring of disease biomarkers increases probability of successful disease treatment. Surface enhanced Raman scattering (SERS) has several advantages over conventional readout strategies utilized in detecting immunoassays. SERS provides a method for chemical characterization based on molecular vibrational spectra. Raman signals are typically weak and need to be enhanced. This can be done using plasmons in nanoparticles of noble metals, we use gold (Au). Molecules with known spectra, called Raman reporter molecules (RRM), can be adsorbed to Au nanoparticles. This enhances the Raman signal of the RRM when illuminated by a laser of optimal wavelength. Adding antibodies to nanoparticles modified with this method can then provide a means for finding hard to detect disease biomarkers.

The focus of this research is the effects to the Raman signal by varying the nanoparticle modification process. We look at the effects of adding PEG molecules to Au nanoparticles and how Raman signals are affected by the laser used to take measurements. RRM, nanoparticle size, and amount of PEG used were varied. Consecutive readings show how the Raman signals change over time due to the laser light used for measurement.

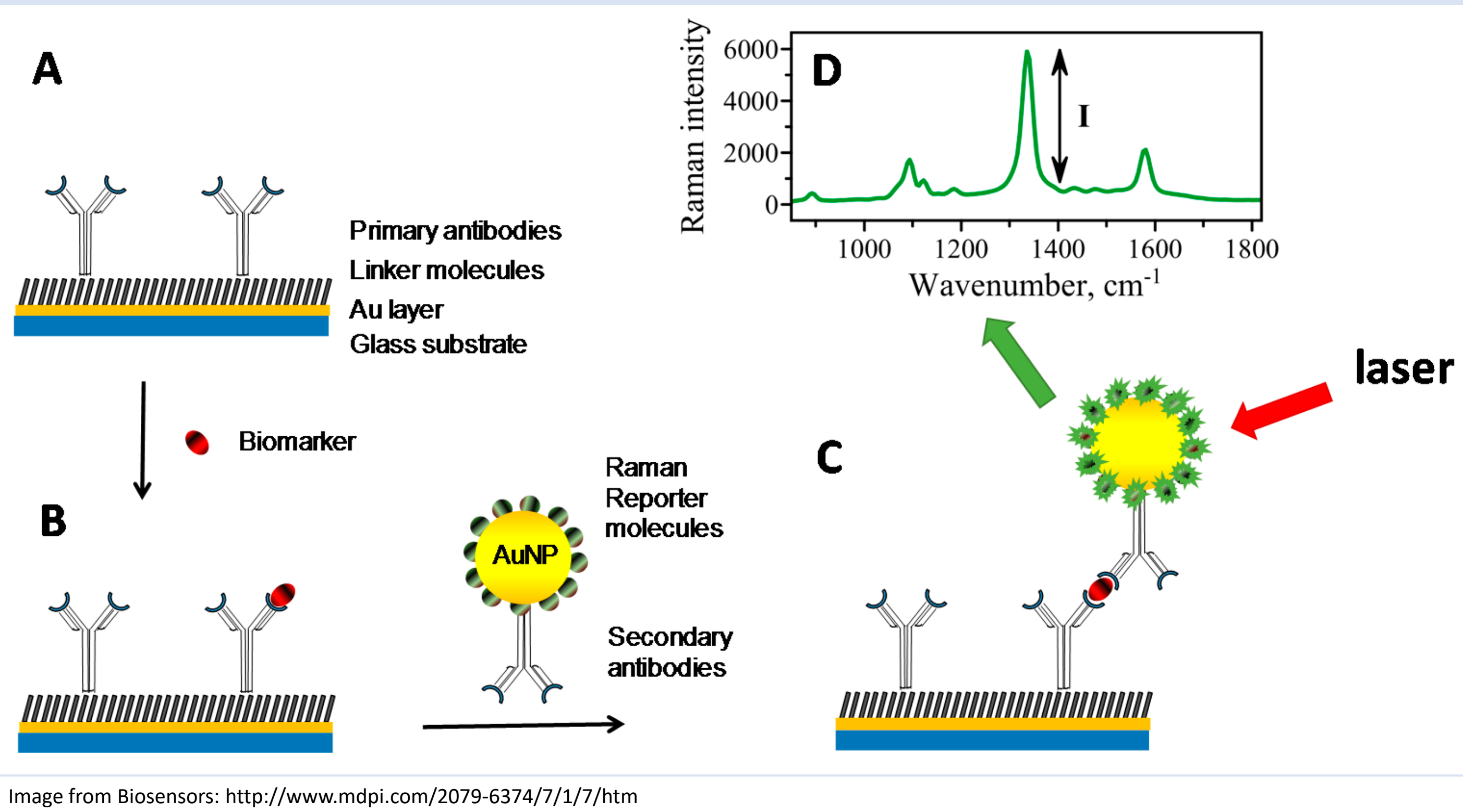


Image from Biosensors: <http://www.mdpi.com/2079-6374/7/1/7/htm>

## Plasmons

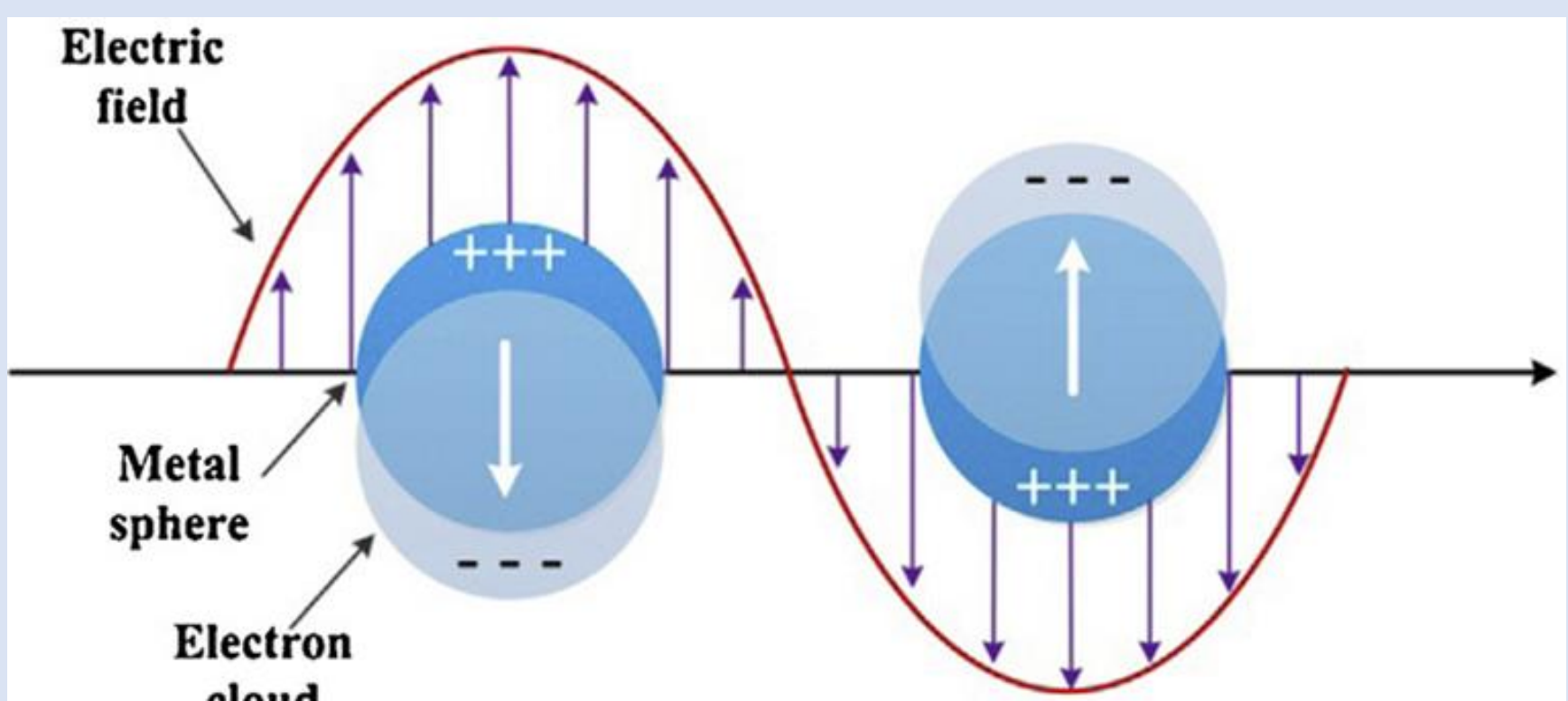
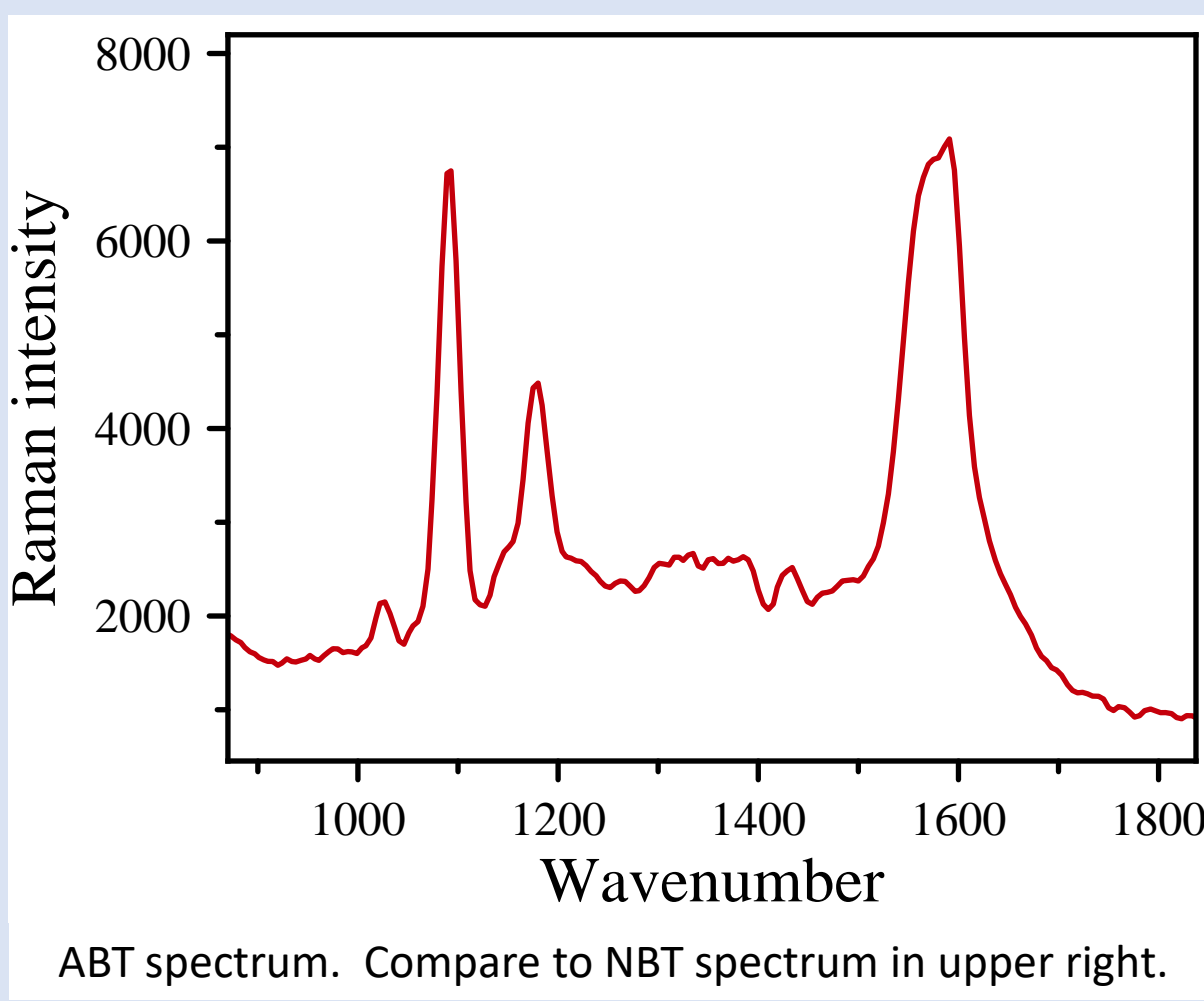


Image from: <http://www.intechopen.com/books/applications-of-molecular-spectroscopy-to-current-research-in-the-chemical-and-biological-sciences/enhanced-molecular-spectroscopy-via-localized-surface-plasmon-resonance>

Metals such as gold, silver, and copper make excellent conductors in bulk because electrons easily move through the metal. In metal nanoparticles, limited space causes the electrons to move as a single cloud called a plasmon. Lasers can be used to excite the plasmon and cause it to oscillate. The oscillating plasmon can increase Raman signals of nearby molecules.

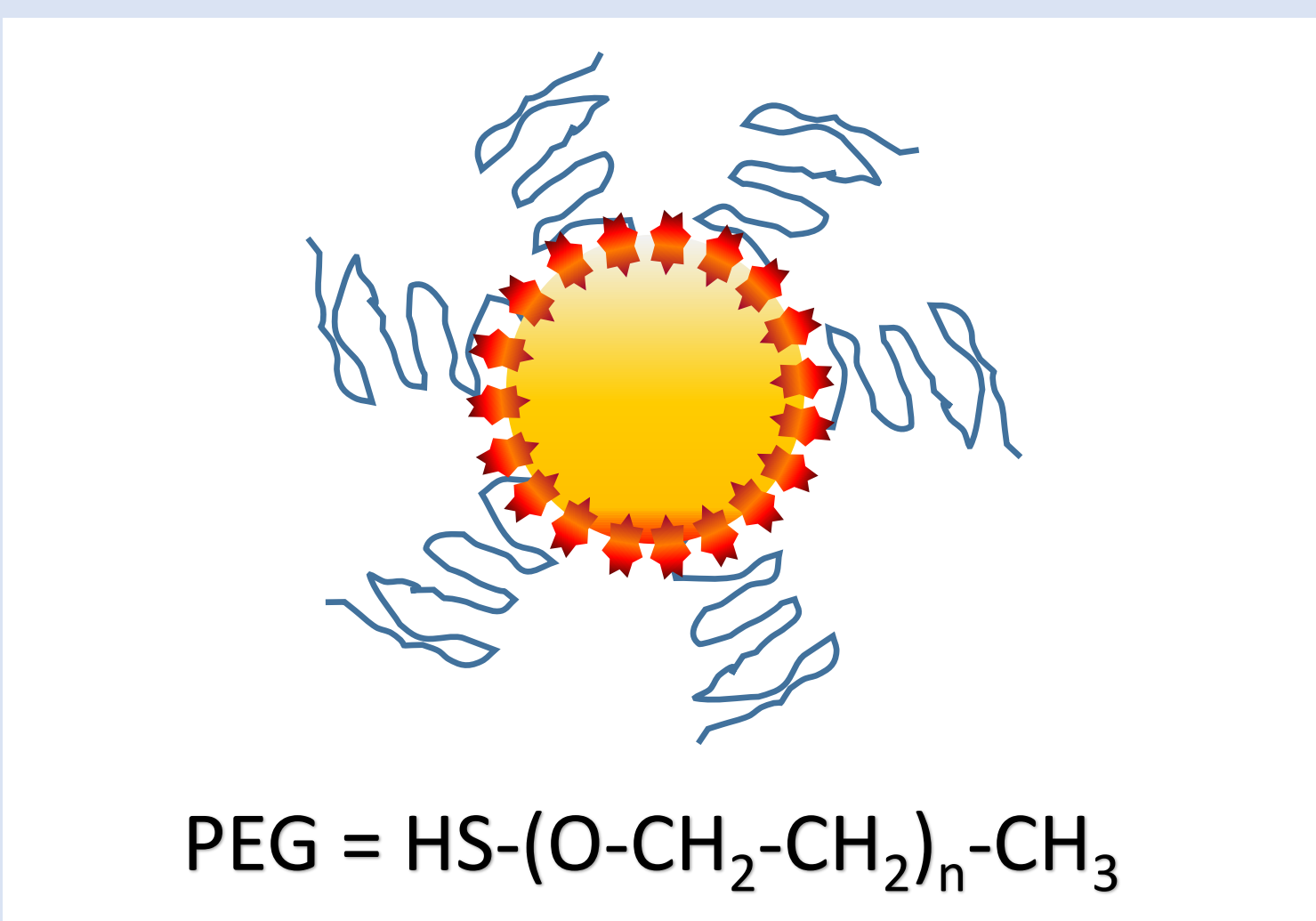
## Raman Signals



ABT spectrum. Compare to NBT spectrum in upper right.

Raman signals vary from one molecule to another. The peaks in the signal correspond to changes in vibrational energy levels of the molecule. Several molecules can be adsorbed onto a gold nanoparticle giving it a strong, easily identifiable Raman signal. The nanoparticles can then be attached to hard to detect disease biomarkers via antibodies. Biomarkers can then be found by the Raman signal of the attached nanoparticle.

## PEG Molecules

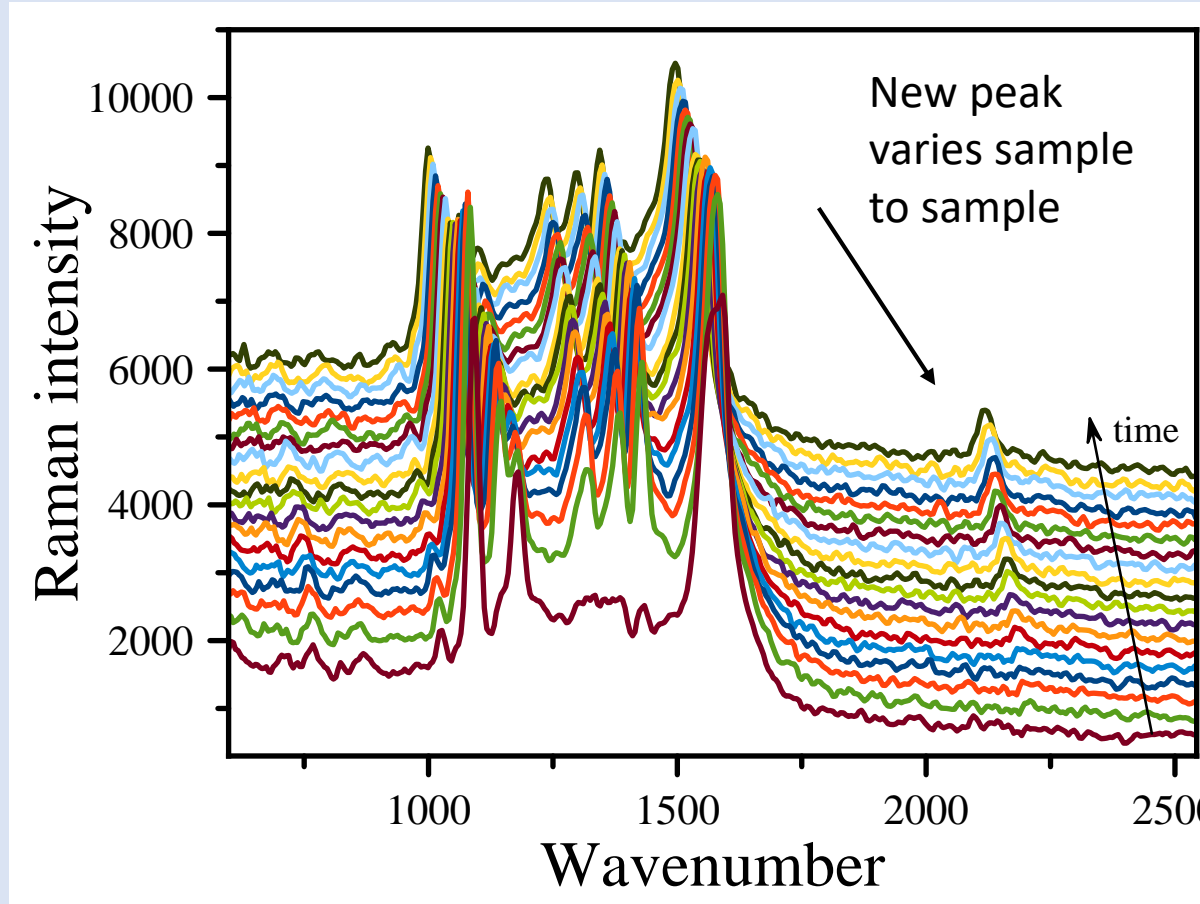
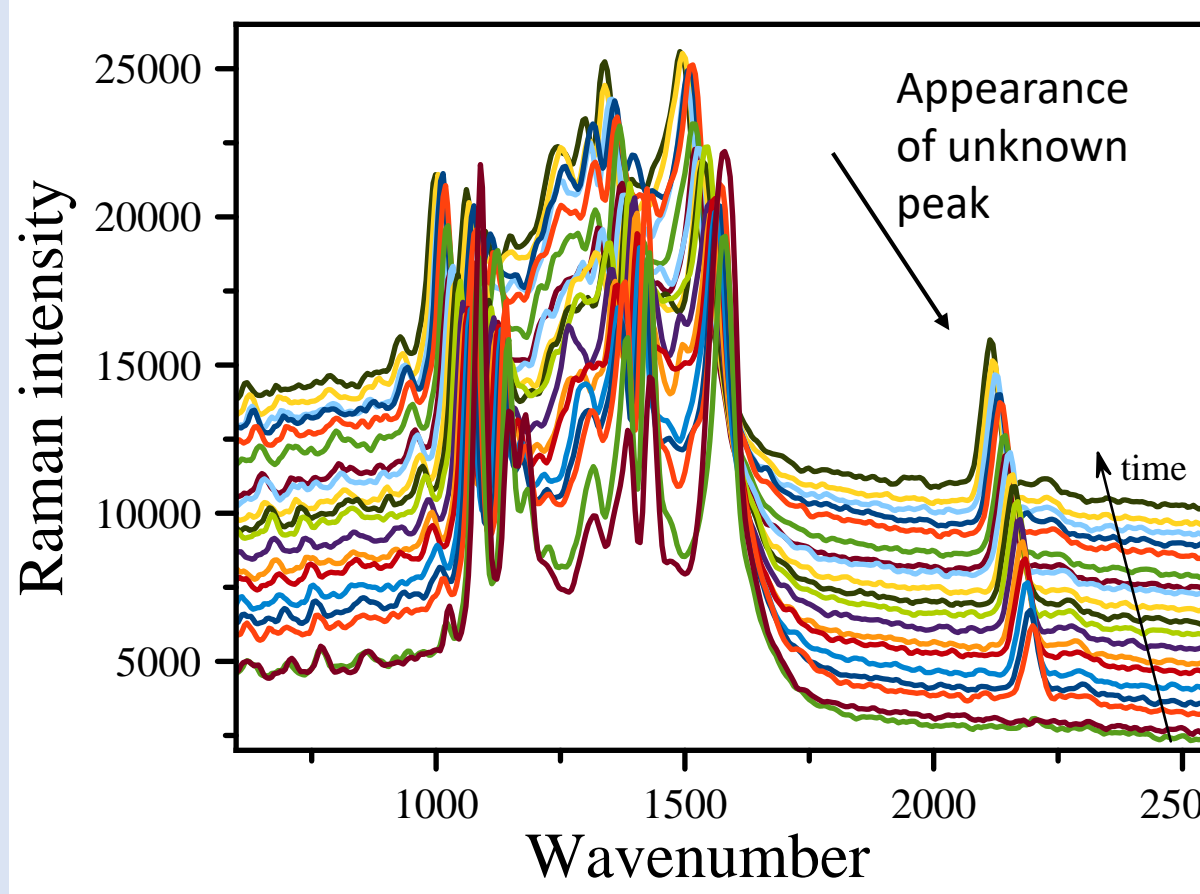
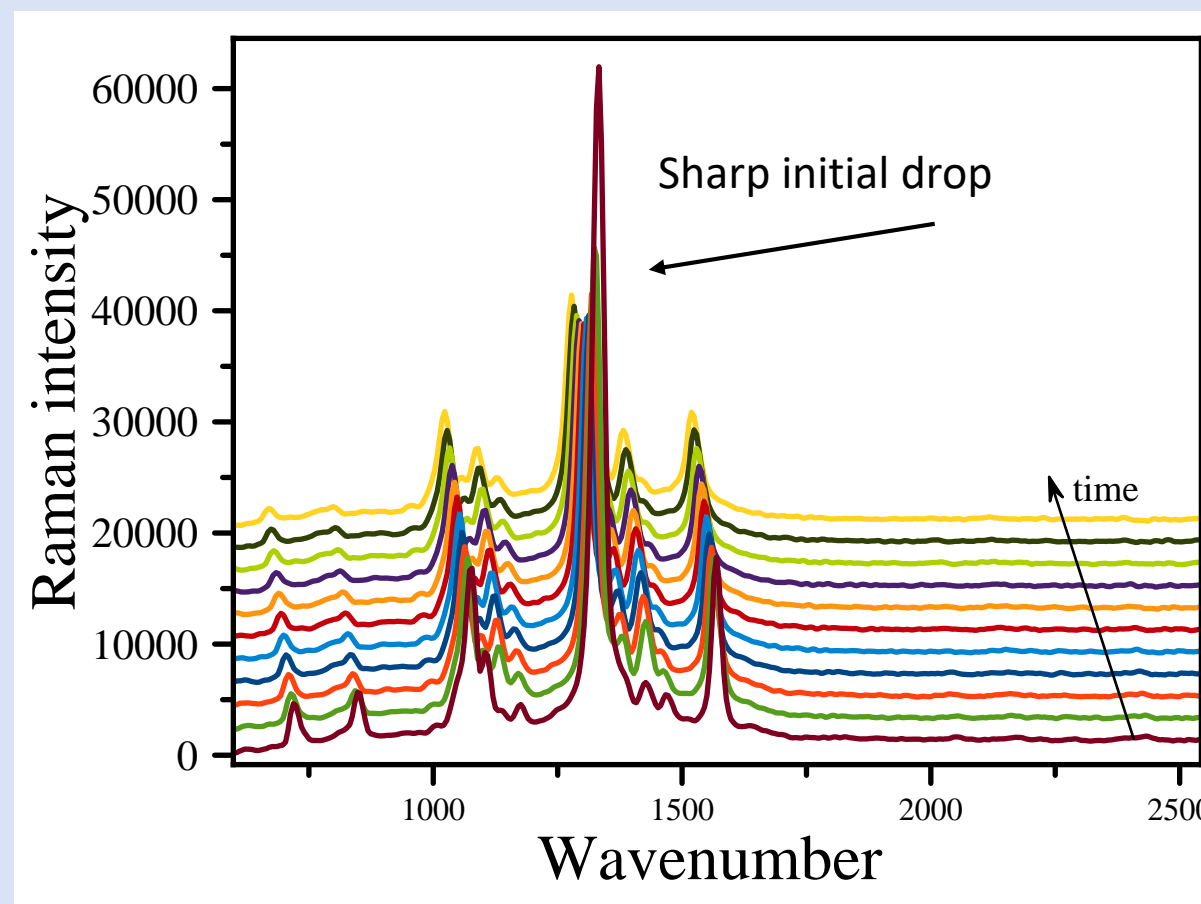
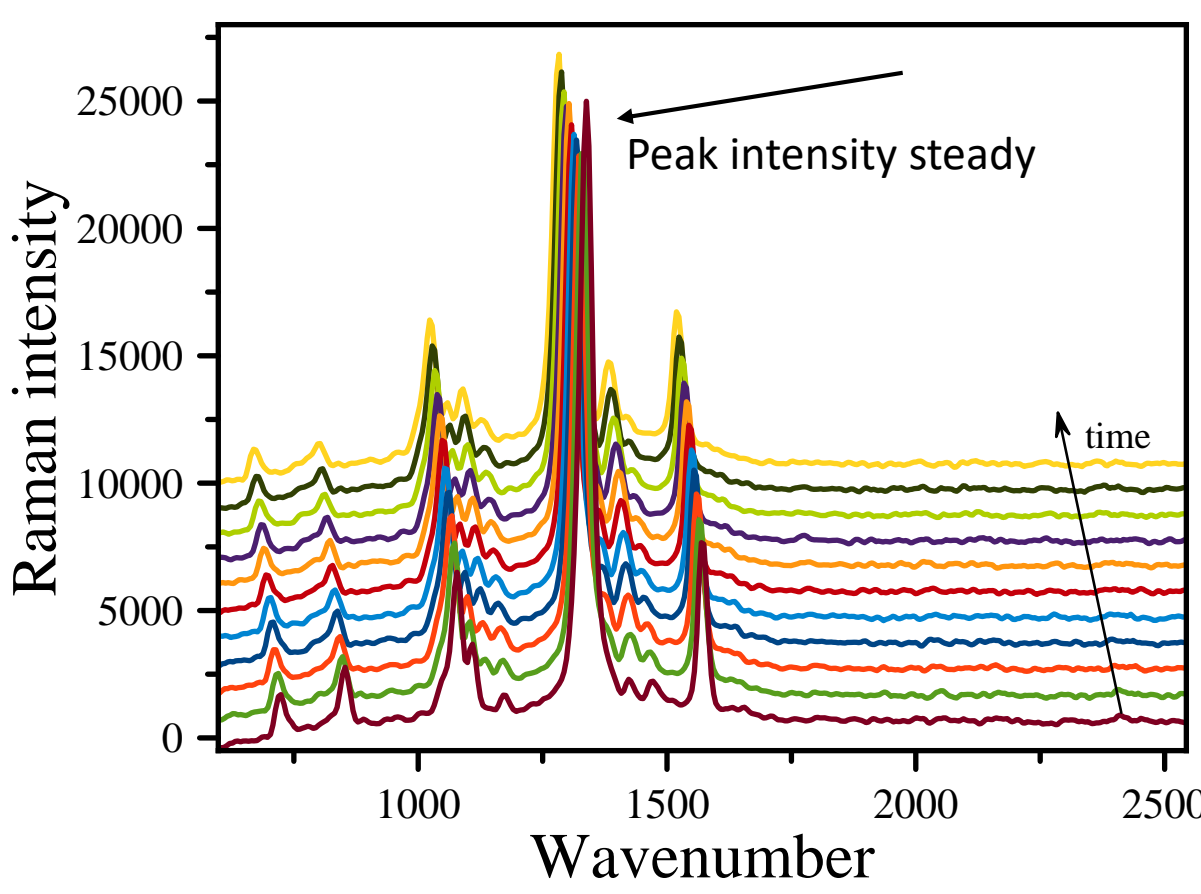
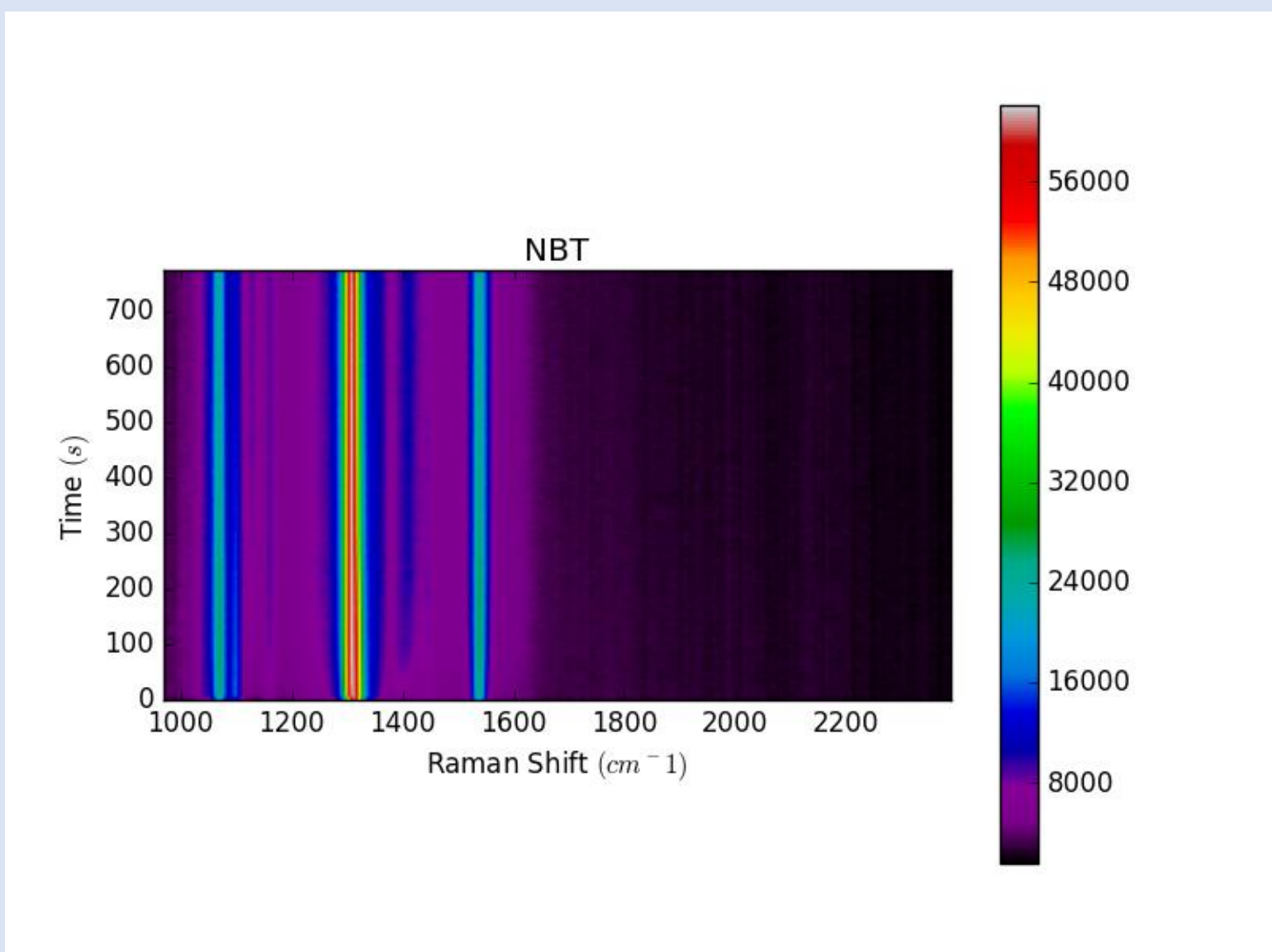


PEG chains are long molecules that readily bind to gold when thiolated at one end. This produces a protective layer around the nanoparticle and the reporter molecules, preventing aggregation of nanoparticles during mixing and affecting how signals change over time.

## Conversion of Raman Signals

Samples were measured using a Spectra Wiz Raman spectroscope with a 632 nm laser. Taking readings at regular time intervals, the signal conversion can be determined.

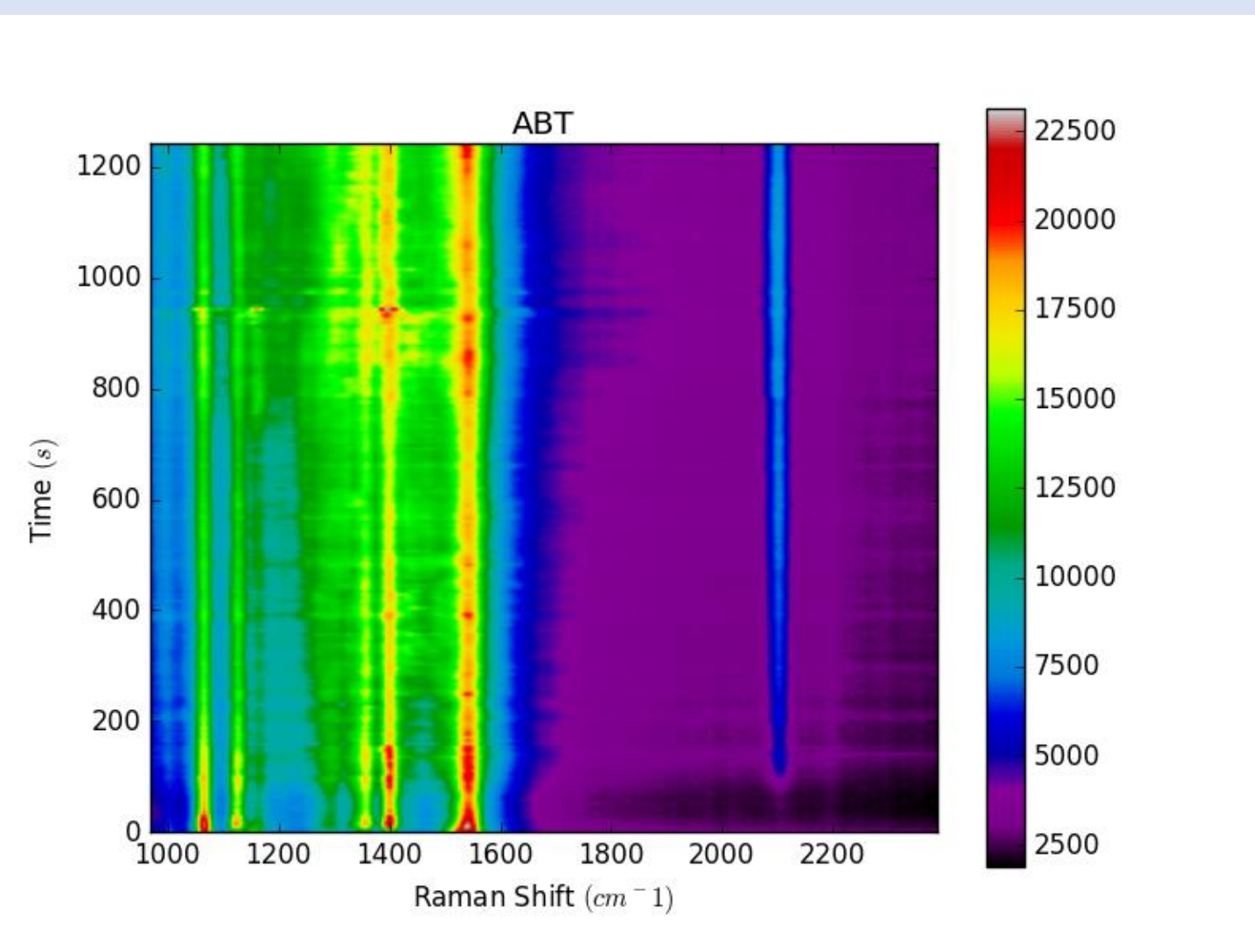
The two images to the right show the effect of PEG on NBT signals. The upper is of 60 nm Au nanoparticles with NBT and no PEG. The lower shows spectra of NBT with PEG added. Below is a spectral heat map of the upper image.



The two images to the left show spectra of ABT modified nanoparticles. ABT molecules tend to cause aggregation of nanoparticles making it unusable without something to prevent this. Adding PEG to gold nanoparticles makes it possible to add ABT without aggregation.

The appearance of a peak at 2130 cm⁻¹ was an unexpected result and is the subject of current research efforts.

Below is a spectral heat map of the upper image



## Results and Conclusions

- PEG molecules work as a stabilizer that prevents aggregation of nanoparticles modified with 4-ABT.
- Adding PEG to NBT modified nanoparticles causes the 1336 cm⁻¹ peak intensity to experience sharp initial decline when exposed to laser light.
- We have interpreted Raman signal changes as plasmon-driven conversion of RRM as well as desorption of RRM from the surface of AuNP and photodamage.
- We also observed catalytic photoconversion of both NBT and ABT to diazobenzene, suggesting that AuNP can act as catalysts in complex reactions.
- We discovered a novel pathway of converting amine substituted benzene to carbon wires as indicated by appearance of the Raman peak at 2130 cm⁻¹.

## Future Research

- Simulation of possible species conversions to explain the changes in Raman signals.
- Determine lengths and bonds of carbon wires that explain the appearance of the 2130 cm⁻¹ peak.
- Test if the 2130 cm⁻¹ peak will appear using 3-ABT or 2-ABT.
- Test other RRM for spectra conversion.
- Use results for future multiplexing experiments.

## Acknowledgements

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