

# Azoreductase X-ray crystallography at the X26-C beamline (Simultaneous diffraction, single crystal spectroscopy, and Raman analysis)

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# Outline of talk

- Introduction:
  - History with BNL
  - Broad topic
- Objective and goals of our research
- Oklahoma State University's Native Americans in Biological Science (NABS) program

# History at BNL

- 1<sup>st</sup> year: 2009- (FAST program) Environmental Science, Removal of Pd by *Clostridium perfringens*.
  - Danial Trobare- Choctaw (Pharmacy School)
  - Andre Nixon- Cherokee (Optometry School)
- 2<sup>nd</sup> year: 2010- (FAST program) Ionic liquids and ethanol production via *C. perfringens*.
  - Danielle Nichols- Cherokee (Medical School)
  - Justin Bradley- African American (Medical School)
- 3<sup>rd</sup> year: 2011- (NSF grant) AzoC Crystallography Screening for *C. perfringens*
  - Shelby Rice- Cherokee (Ph.D. Graduate School)
  - Cassandra Camp- Choctaw (MS Graduate School)
- 4<sup>th</sup> year: 2012-(CRTP) AzoC crystallography Optimization
  - Lindsey Berger- Otoa-Missouri (MS Graduate School)
  - Amber Anderson- Cherokee (MS Graduate School)
- 5<sup>th</sup> year: 2014- (NSF grant) AzoC X-ray diffraction
  - Roberta Reed – Muscogee Creek (Pharmacy School)
  - Lauren Thompsom – Cherokee (BS Chemistry)
- 6<sup>th</sup> year: 2015 (NSF grant) AzoC crystallography studies
  - Marla Ichord- Cherokee (BS Microbiology)
  - Zach Ridge – Cheyenne (BS Chemistry and Biochemistry)

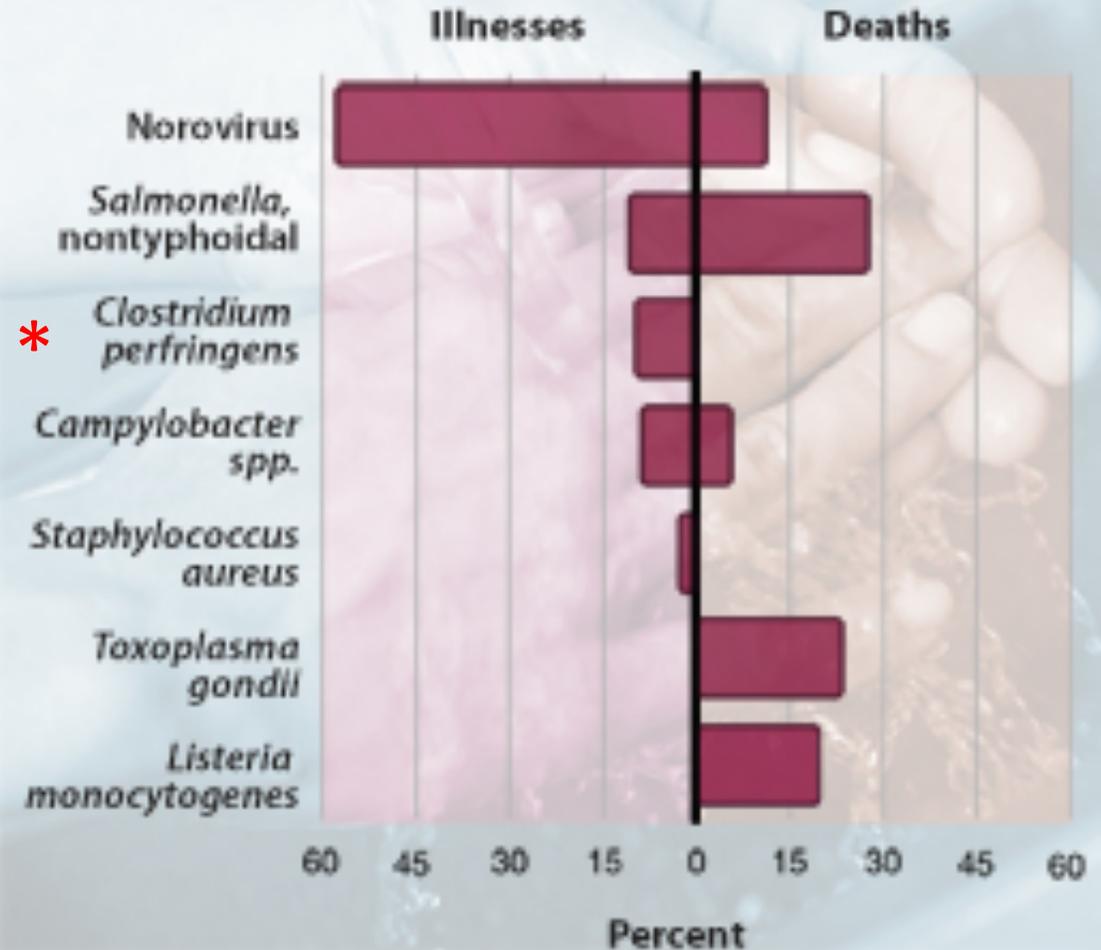
## Foodborne Illness



- 1 in 6 Americans (or 48 million people) gets sick
- 128,000 are hospitalized
- 3,000 die of foodborne diseases

## Center for Disease Control and Prevention (CDC)

### Top pathogens contributing to domestically acquired foodborne illnesses and deaths, 2000–2008



# Wet Gangrene

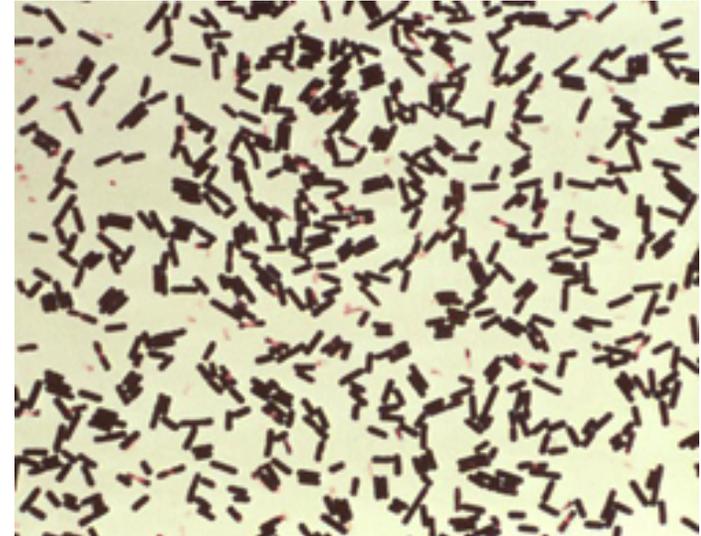


In U.S.

- 30,000-40,000 amputations annually
- *Clostridium perfringens*

# *Clostridium perfringens*

- Common human intestinal bacteria
- Strict anaerobe (no oxygen) bacteria
- Increase in antibiotic resistance

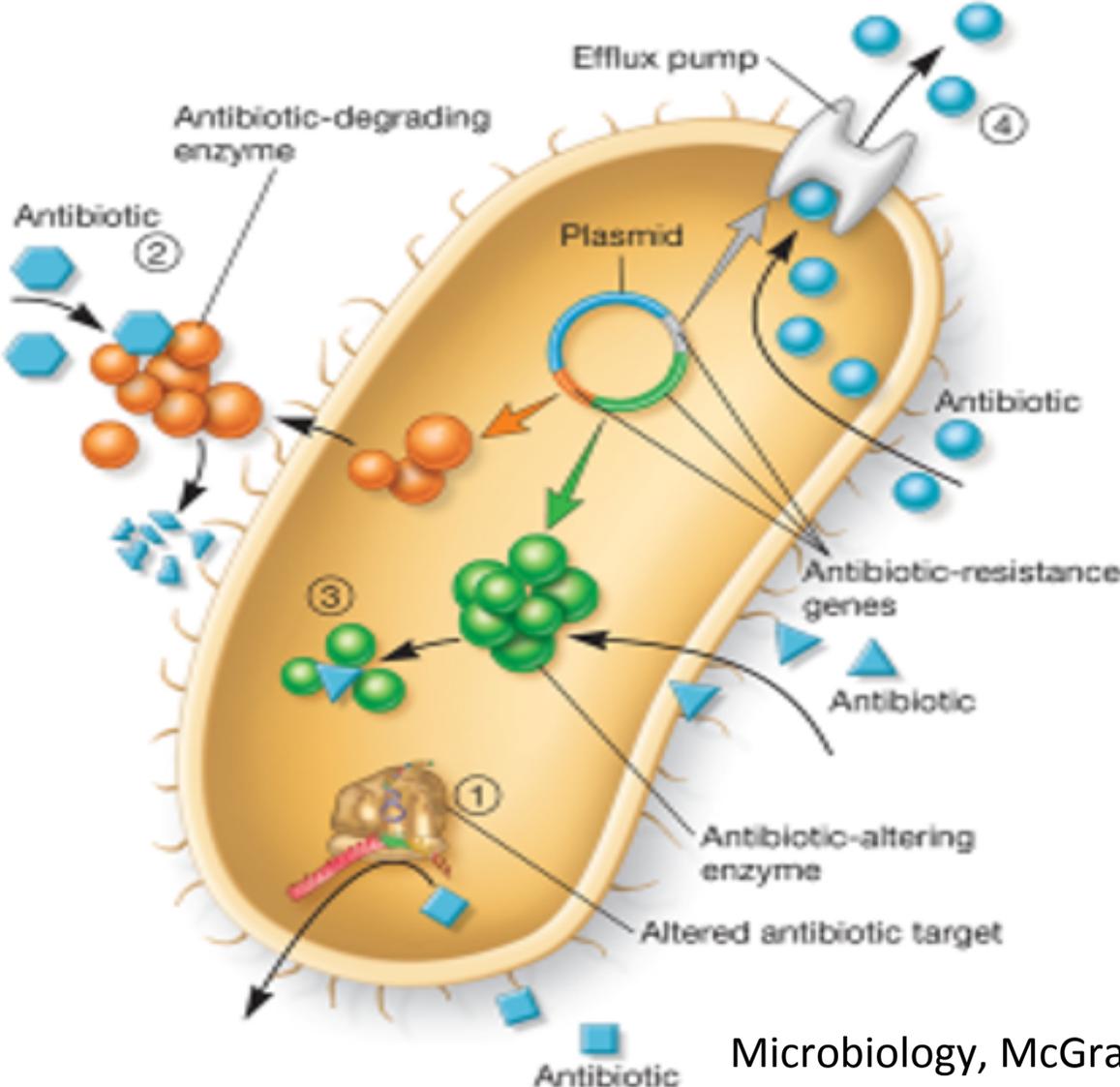


[www.CDC.gov](http://www.CDC.gov)

# Mechanisms of antibiotic resistance

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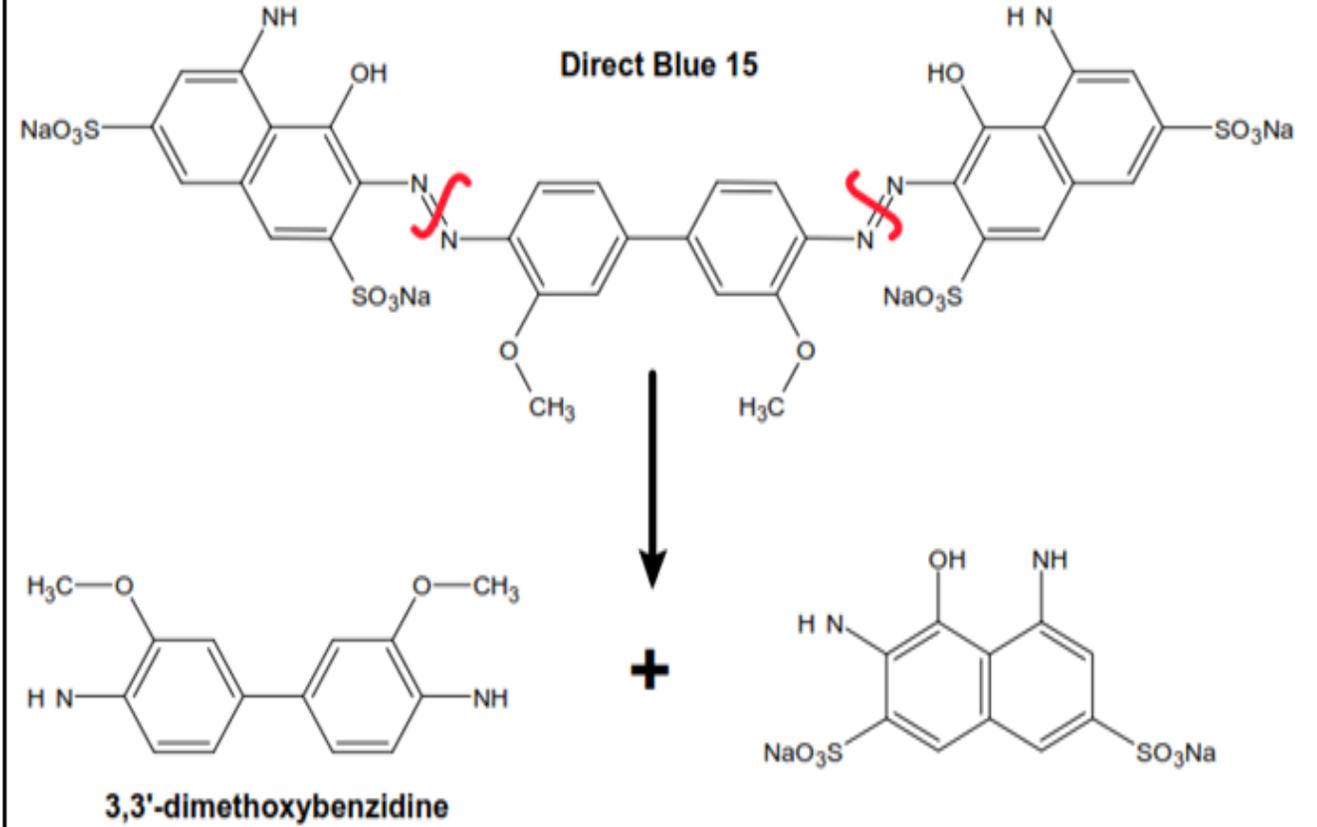
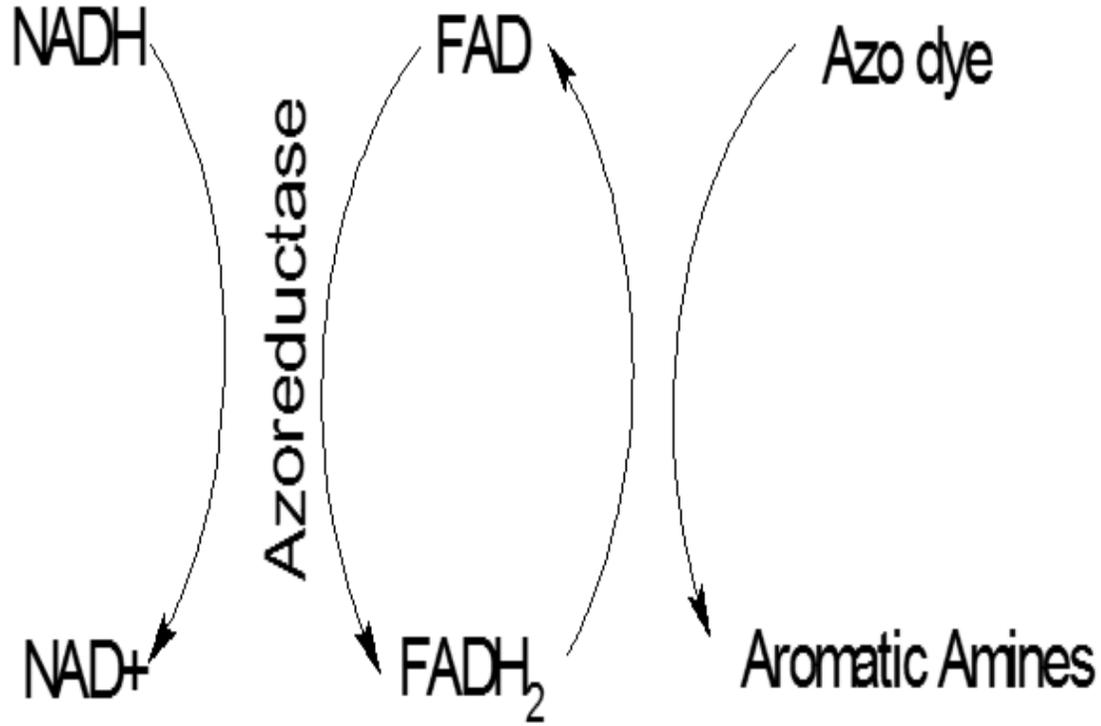
Bacterial survival (growth) is key to drug resistance.



# Azoreductase

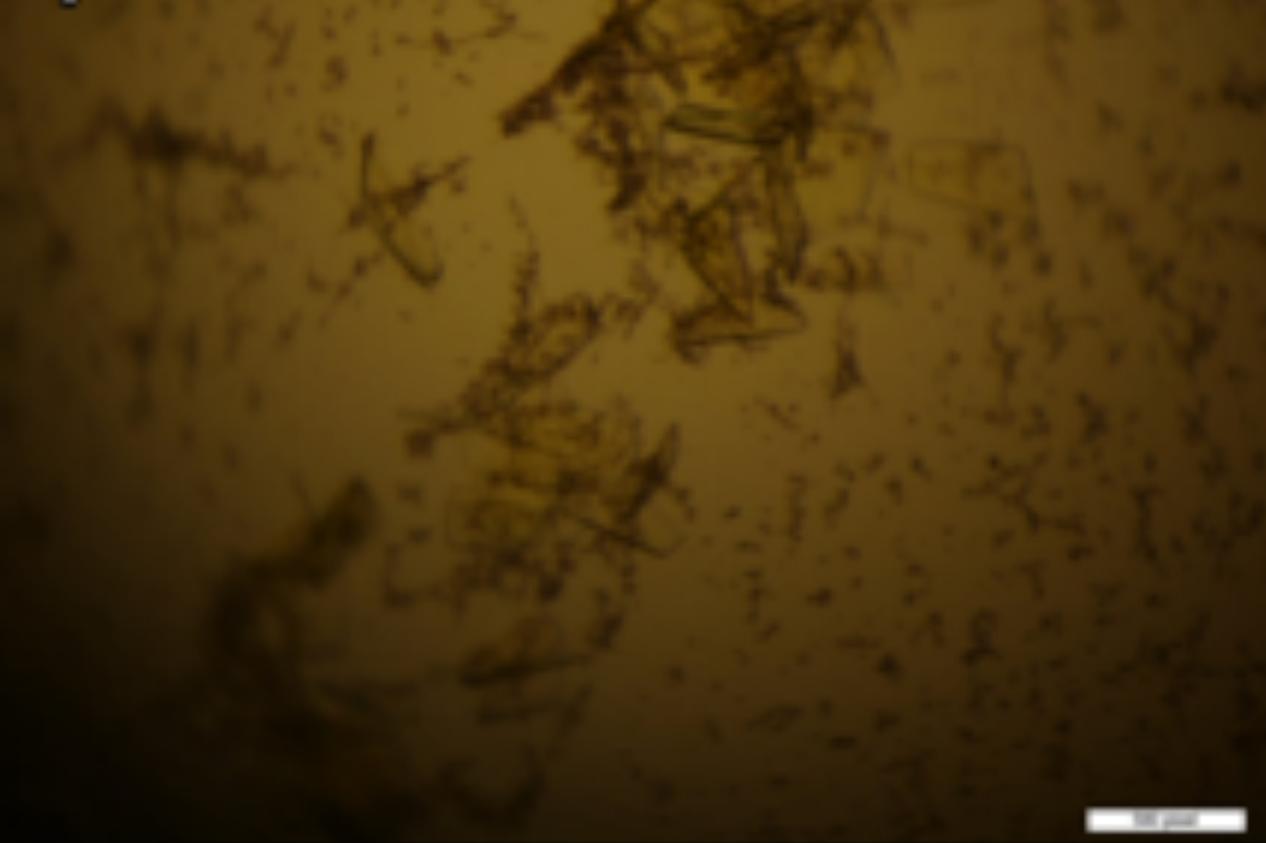
- Found in prokaryotic and eukaryotic cells
- AzoC
  - 206 amino acid (ATCC ) GenBank accession no.
  - Morrison, J.M., Wright,C.M., and John, G.H. (2012). *Anaerobe*, 18(2), 229-34.
- Flavin protein (NADH dependent)
  - FAD cofactor
    - Morrison, J.M., Dai, S.; Taylor, A.; Wilkerson, M.; John, G. and Xie,A. (2013) *Protein Peptide Letters*. 21 (2014) 523-534.
- Low homology compared to other azoreductases

# Reduction reaction



- **Objective:** Understand the structure and function of AzoC, an azoreductase in *Clostridium perfringens*.
- **Why:** AzoC- natural role may be to scavenge oxygen, thereby, increasing the survival of anaerobic *C. perfringens*.

**A) 24% PEG 3350**

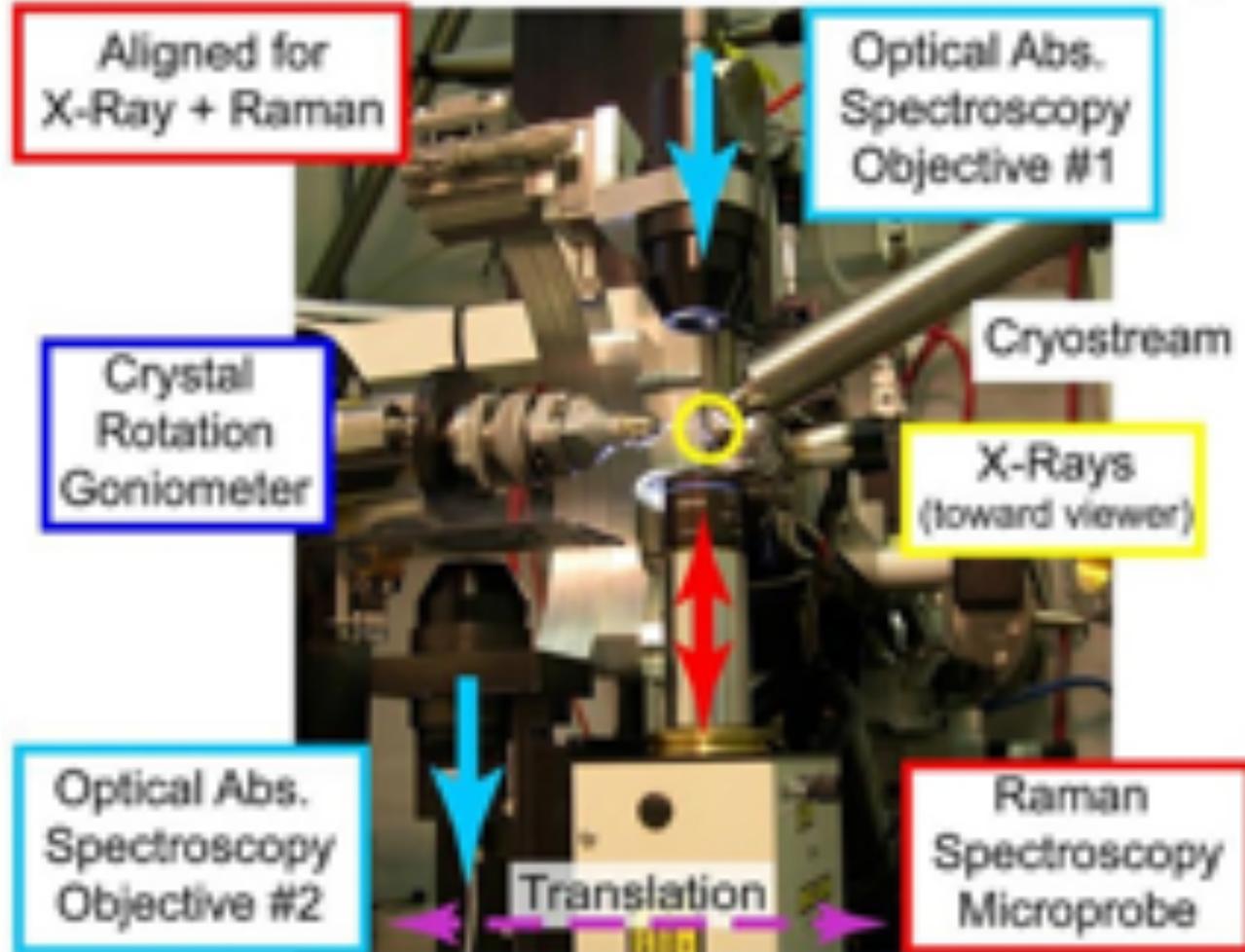


**B) 30% PEG 400**



**Figure 3:** *A)* Crystals growing in the most optimal aerobic condition **P12\_B1** and *B)* the top anaerobic condition **P4\_E6\_D3**.

## NSLS X26-C On-Line Capability w/ X-Ray



X-Ray Crystallography with or without  
(Polarized) Electronic Absorption Spec. (200 – 1000 nm)  
(Polarized) Resonance Raman Spec. w/ Four Lasers  
(785 nm, 633 nm, 532 nm and 473 nm)  
Probe Diameters: 90, 36, 25, or 18  $\mu\text{m}$

## Single Crystal Analysis

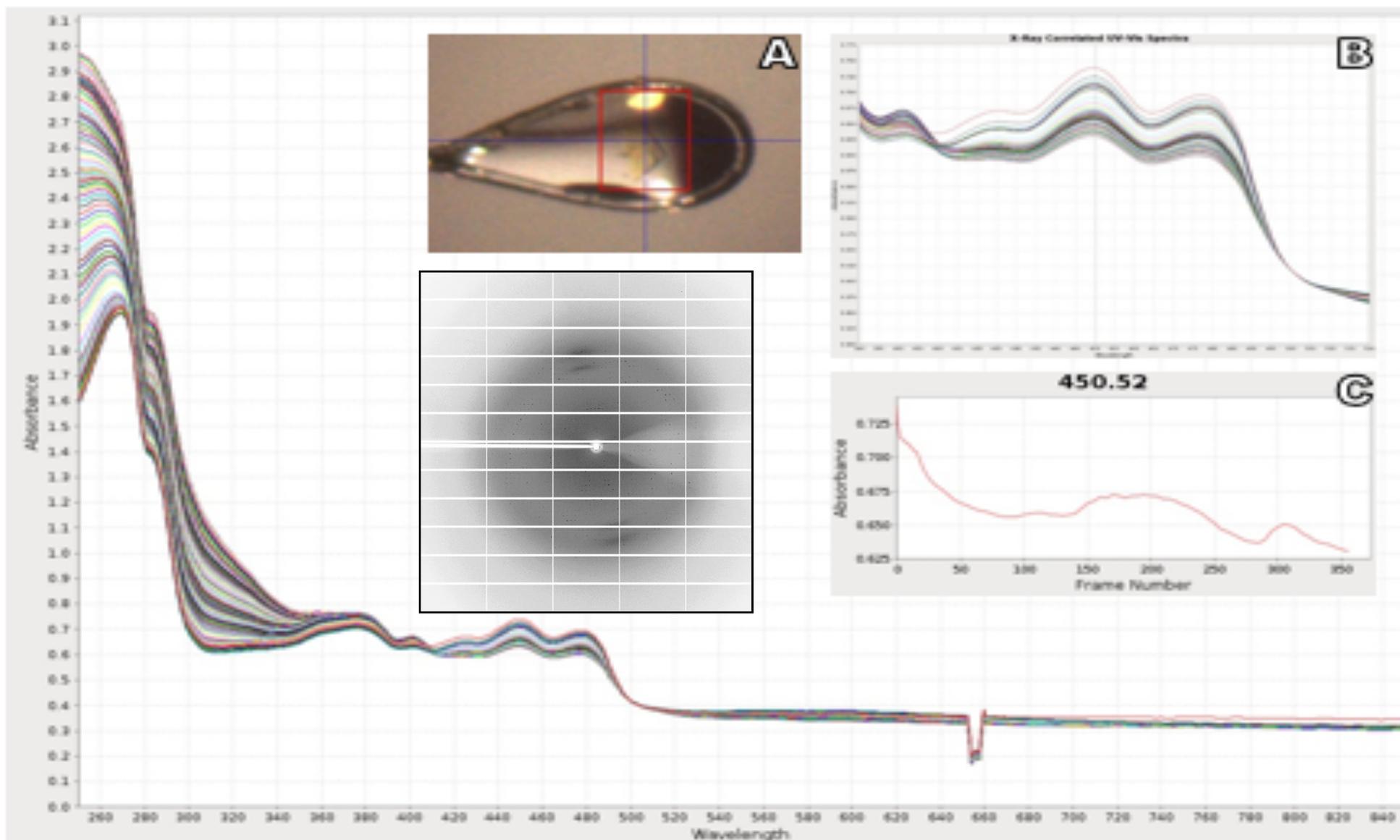
- X-ray diffraction
- Spectroscopy
- Raman

**Quaternary Ammonium Oxidative Demethylation: X-ray Crystallographic, Resonance Raman, and UV–Visible Spectroscopic Analysis of a Rieske-Type Demethylase**

Kelly D. Daughtry,<sup>†</sup> Youli Xiao,<sup>‡</sup> Deborah Stoner-Ma,<sup>§</sup> Eunsun Cho,<sup>‡</sup> Allen M. Orville,<sup>\*,§</sup> Pinghua Liu,<sup>\*,‡</sup> and Karen N. Allen<sup>\*,†,‡</sup>

[dx.doi.org/10.1021/ja2111898](https://doi.org/10.1021/ja2111898) | J. Am. Chem.Soc. 2012, 134, 2823–2834

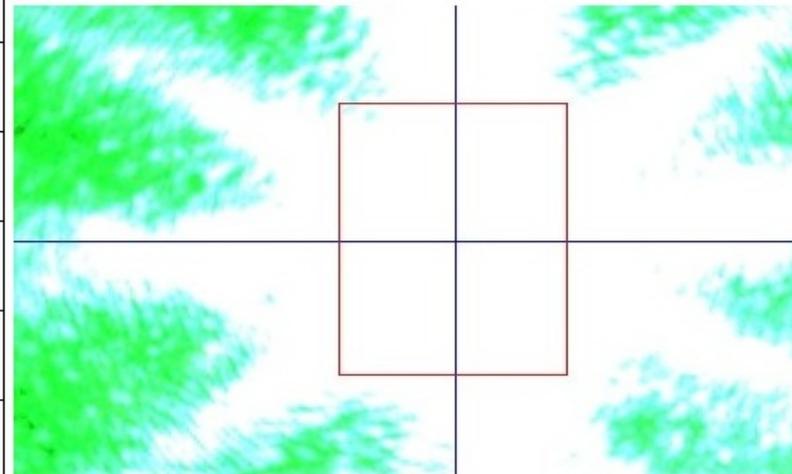
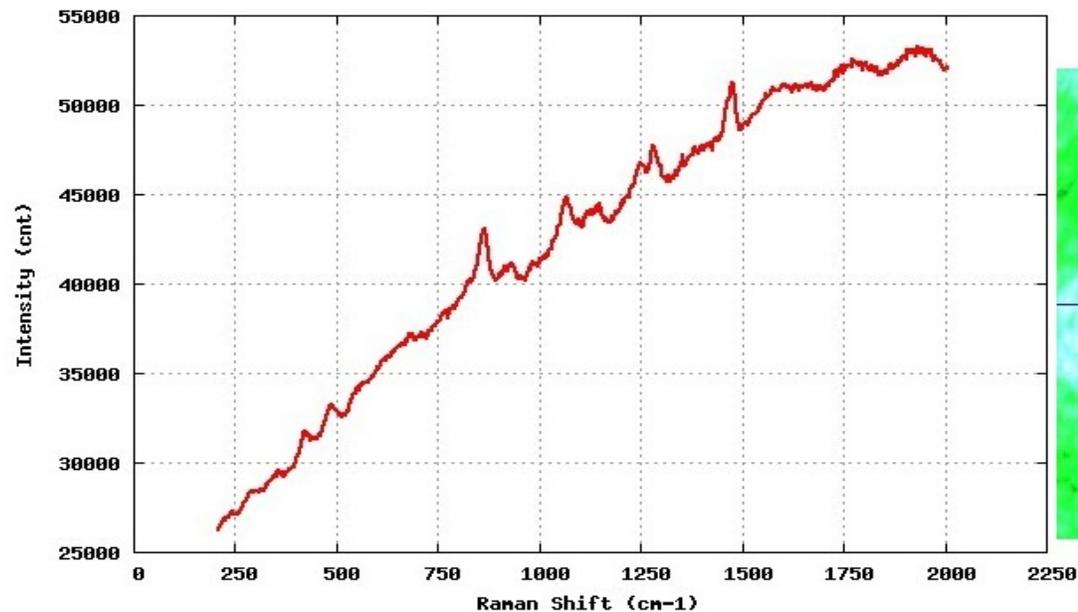
“More than 90% of the X-ray photons that do interact with the sample do not yield a diffraction pattern, but rather deposit energy into the crystal.<sup>68,69</sup> For example, X-ray photons absorbed by the sample liberate two electrons from biological elements (photoelectric effect). They have sufficient energy to induce several hundred further ionization events that can propagate for up to 4  $\mu\text{m}$ <sup>70</sup> and, consequently, may reduce samples in the X-ray beam”.<sup>41,65</sup>



**Figure 4:** UV-Vis spectra of an AzoC crystal taken before, during, and after X-ray exposure at X26-C with data normalized to 500 nm. The small peak at 655 nm is from deuterium lamp. **Inset:** A) AzoC crystal in loop during analysis. B) Spectra showing isosbestic points and C) tracking wavelength at 450 nm.

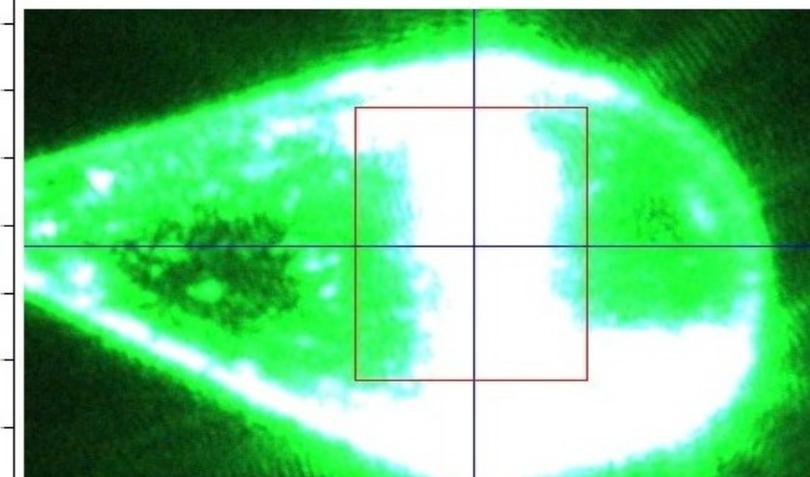
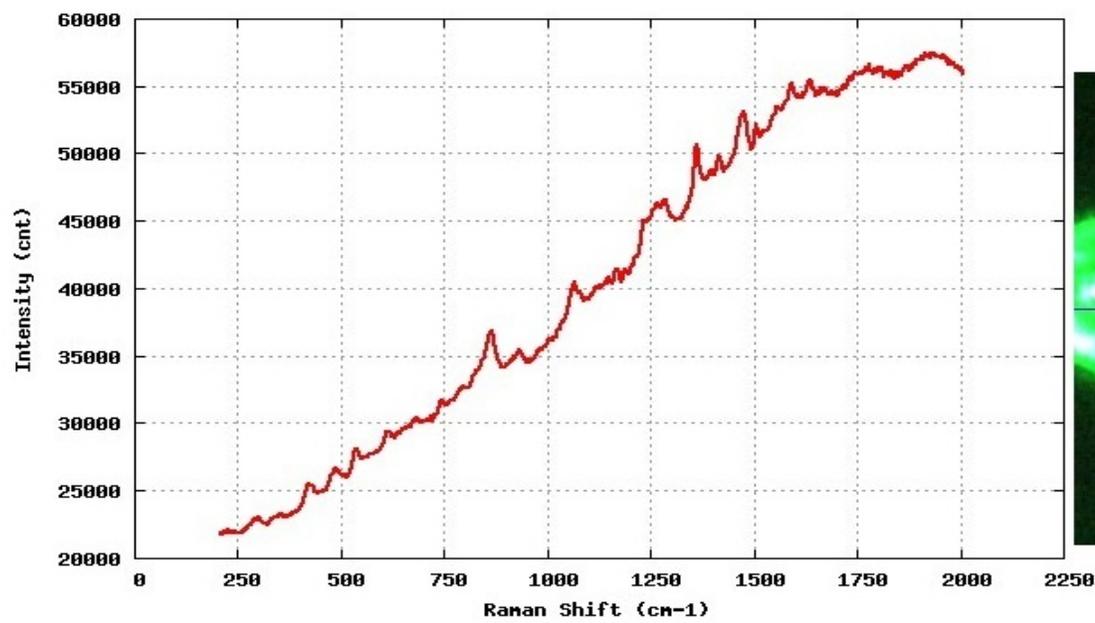
# Raman spectroscopy

ML

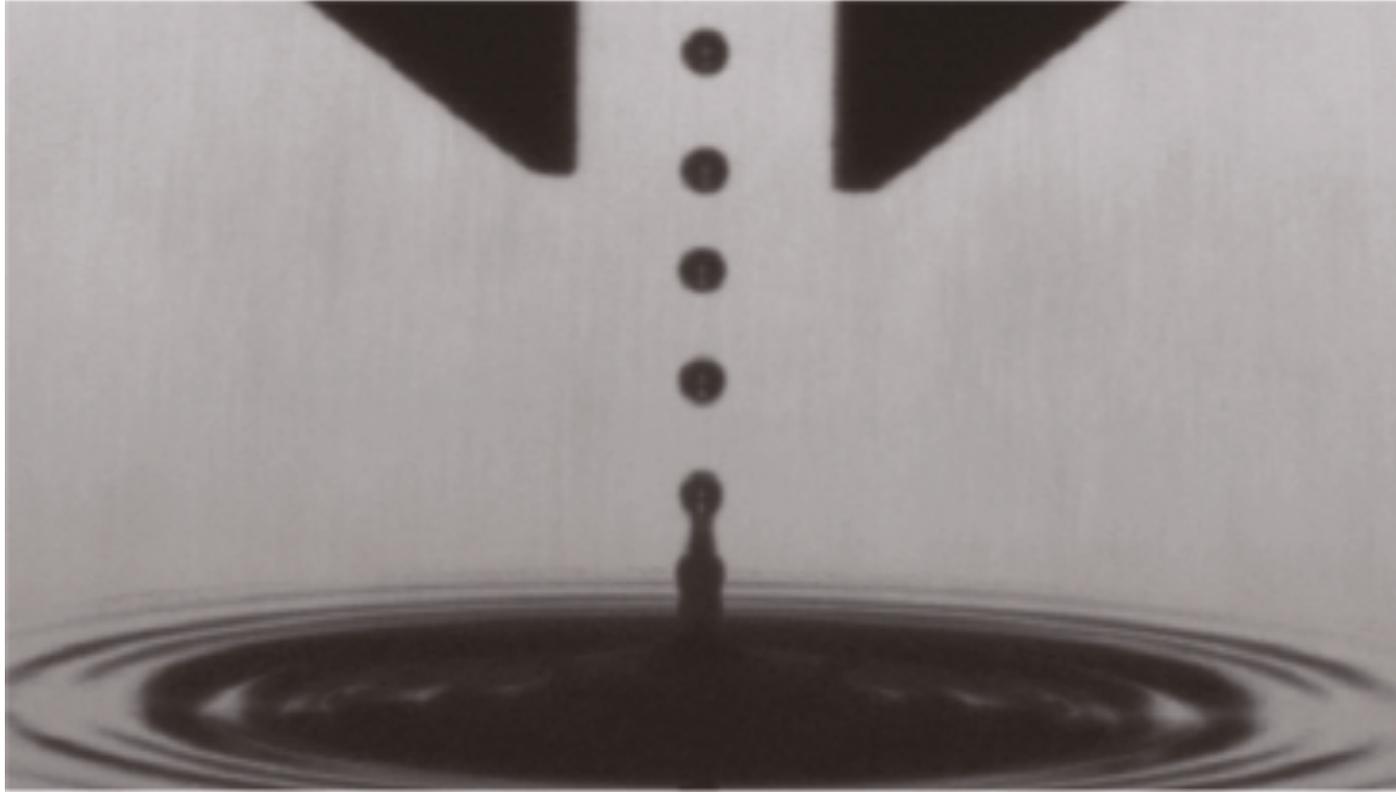


532 nm laser wavelength  
Slit size 1000  $\mu\text{m}$   
Spectro midpt 1152  $\text{cm}^{-1}$   
Grating 600  
Accumulation no. 3  
Taken from 200-2000  $\text{cm}^{-1}$   
Laser power 100%

Crystal



# Acoustic Drop Technology

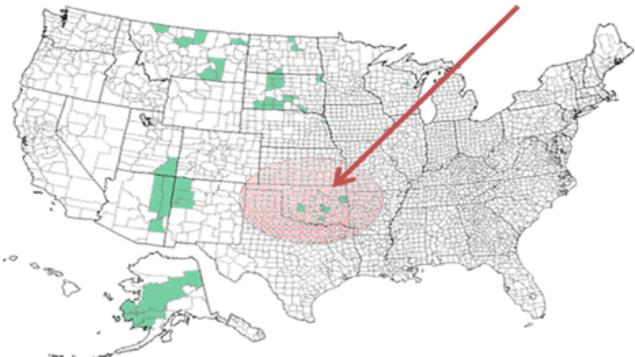


# Conclusion

- Integrated data collection works for our protein (AzoC)
- Acoustic Drop Technology works for AzoC
- Study active site structures and cofactor chemistries

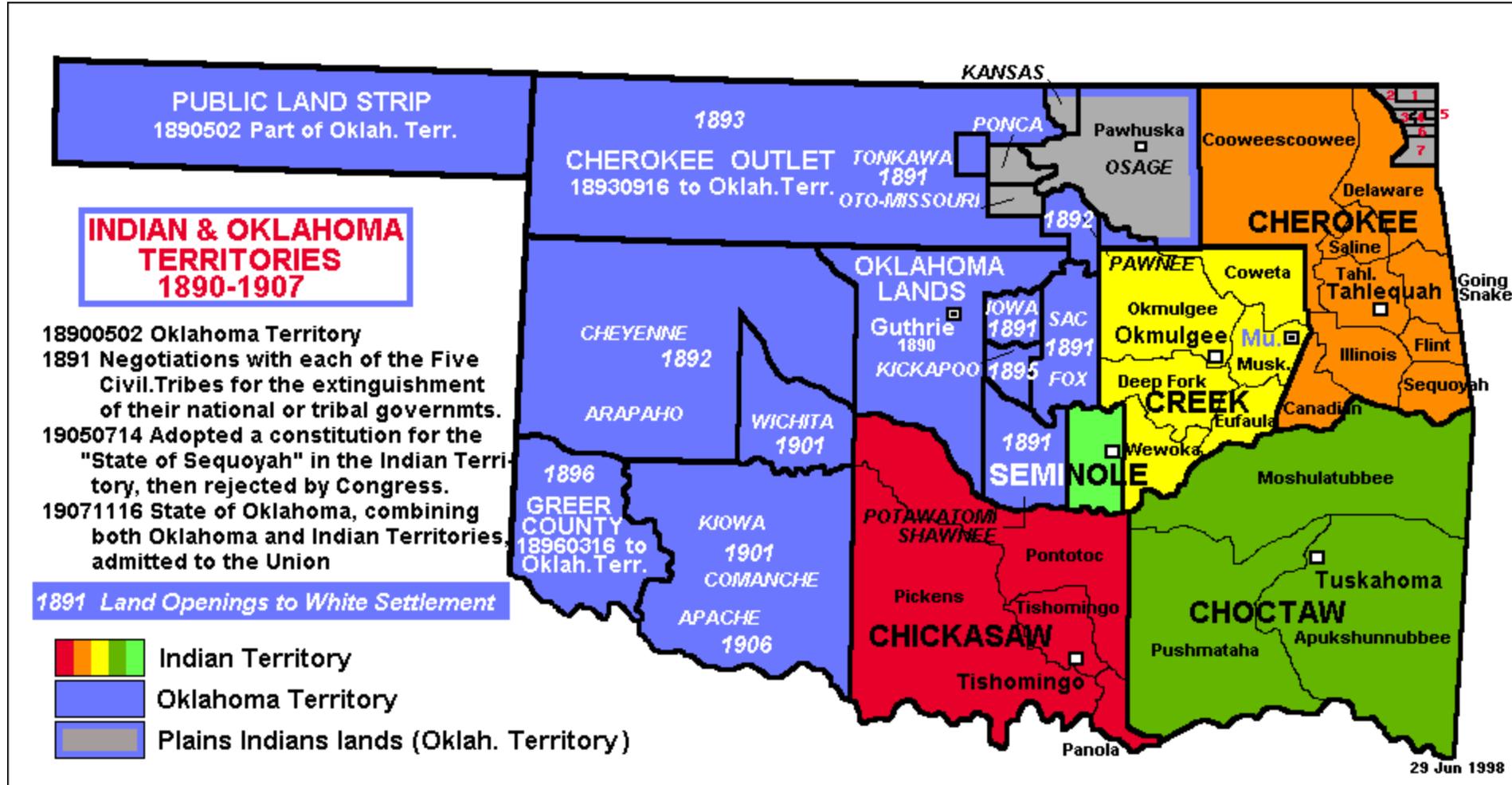
# Native Americans in Biological Science (NABS)



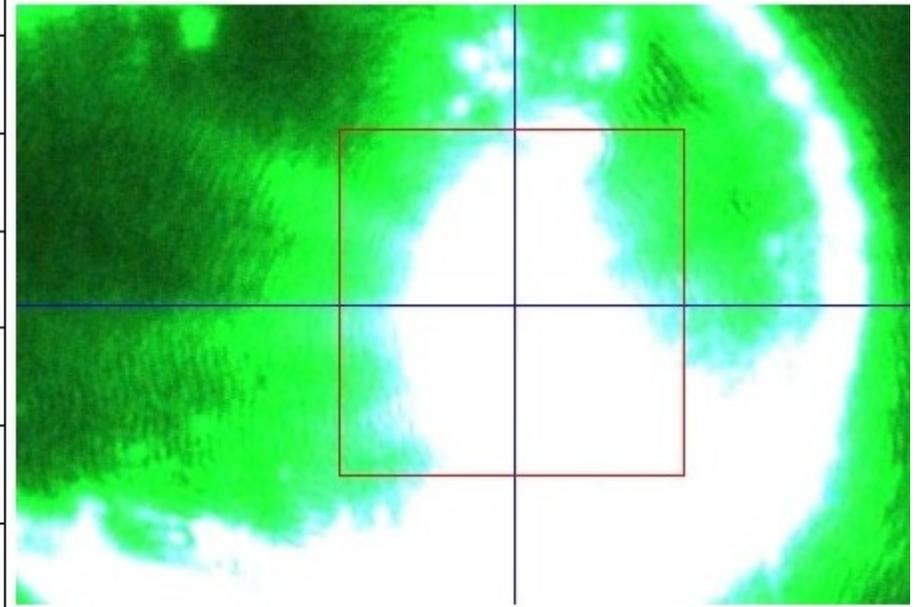
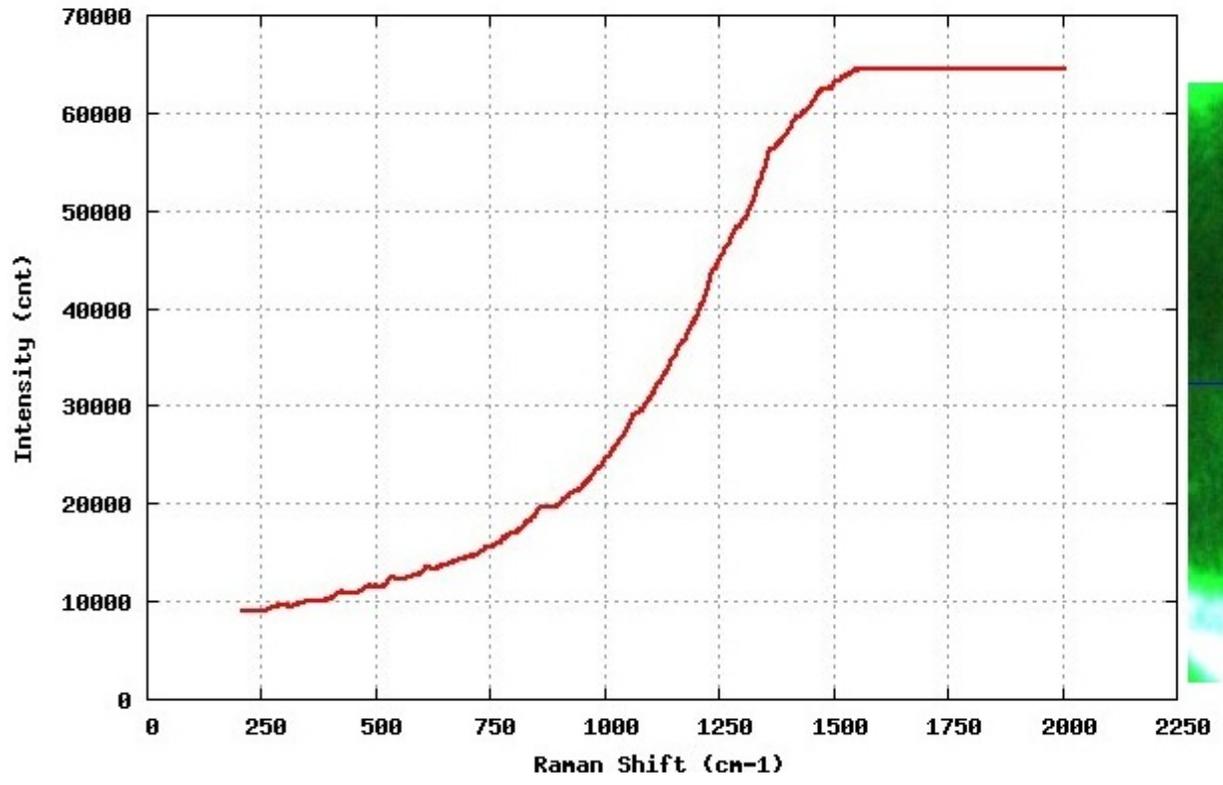


Source: Prepared by ERS using data from the U.S. Census Bureau.

# 39 Tribes



# Single crystal Raman spectroscopy



Excitation laser