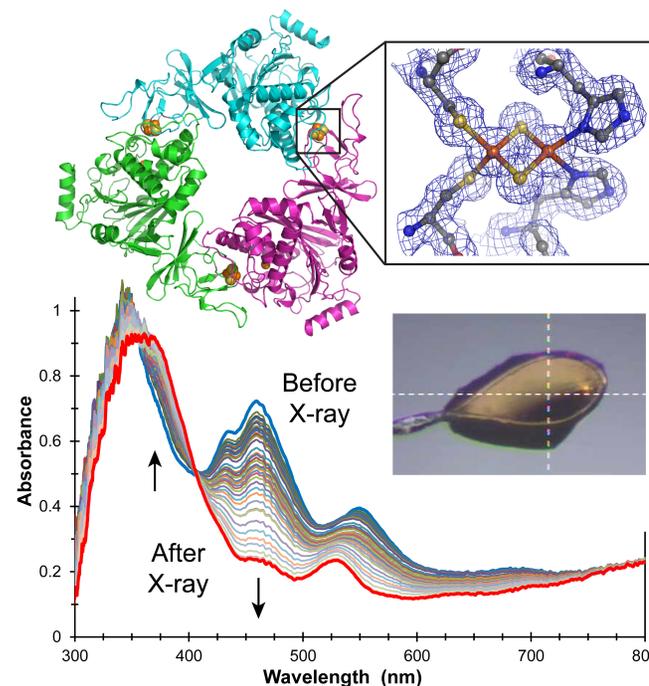


# Combining Crystallography and Visible Spectroscopy To Understand Enzymes

- An enzyme's catalytic function derives from the **atomic**, **electronic** and **vibrational** structures of the atoms participating in the reaction, but information for the latter two is not revealed by x-ray crystallography scattering methods.
- Using NSLS beamline X26-C, the only beamline in the world fully dedicated to single-crystal spectroscopy and x-ray crystallography, researchers have developed a new technology that simultaneously carries out crystallography and UV-visible and Raman spectroscopy to determine the atomic structure of the entire protein as well as the electronic and vibrational structures of metal ions and cofactors within.
- The combined instrumentation has been used to study the process of demethylation of an organic substrate molecule by an enzyme whose active site includes an iron-sulfur cluster.
- The scientists used spectroscopy to follow the change in the oxidation state of the cluster during the crystallography data collection, and to formulate a mechanism for the overall enzyme reaction mechanism.
- Results provide insight into a new demethylation reaction, an important class of phenomena that control cellular behavior.



The research team focused on the stachydrine demethylase from *Sinorhizobium meliloti* 1021. The applications of electronic absorption and resonance Raman spectroscopies, in conjunction with x-ray diffraction, all derive from the same  $\sim 25 \mu\text{m}^2$  region of the crystal (intersection of the cross hairs). The x-ray beam alters the visible spectrum of the crystal with each exposure required to determine the crystal structure of the trimeric enzyme and the Fe-S cluster within each subunit.