



Raman spectroscopy of L-serine single crystals

during X-ray irradiation

Application Note

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Introduction

State-of-the-art 3rd generation synchrotron sources such as the Swiss Light Source (SLS) or PETRA III in Hamburg offer the possibility to focus a large amount of monochromatic photons into spots of less than a micron in size. For these micro- and nanobeam experiments the effect of radiation damage has become a severe limitation for the quality of obtainable X-ray data, especially in case of biological molecules. Radiation damage is often divided into two categories: global radiation damage affecting the crystal as a whole and specific radiation damage which can be observed at specific sites of the molecule. Thus the effects of X-ray damage are ranging from small alterations of specific bonds up to a complete destruction of the overall crystal structure.

One possibility to reduce radiation damage is cooling the samples to cryogenic temperatures during data collection. However, the lifetime of a crystal is limited even at 100 K. For further reduction of radiation damage the underlying processes have to be understood in more detail. One possible explanation for specific radiation damage is X-ray induced hydrogen abstraction. In case of L-serine this is suspected to occur at the methylene side. Our attempt was a combination of Raman spectroscopy with X-ray diffraction techniques to detect the cleavage of such bonds.

Experimental Setup

White beam irradiations were performed at 300 K at F4 at DORIS III in Hamburg. For in-situ Raman measurements the Shamrock spectrograph SR-500i-A and the

Newton CCD detector DU940N-BV from Andor Technology been installed at the beamline. The CCD was calibrated in advance using a Hg-lamp. Spectrograph readout and control were done by the Andor Solis software for Windows.

Figure 1 shows the experimental setup. During the experiment a Raman probe manufactured by Enwave Optronics was connected via a single mode fiber to a 532 nm CW laser. The output power at the probe end accumulated to ~40 mW. A second fiber was used to guide the collection signal into the spectrometer via a fiber adapter. The adapter was optimized prior to the experiment to give a maximum signal input. The crystalline sample was adjusted to the X-ray beam and the Raman probe was aligned in a 90° angle with the incident beam. All optical elements were protected from irradiation with lead covers.

Results and Discussion

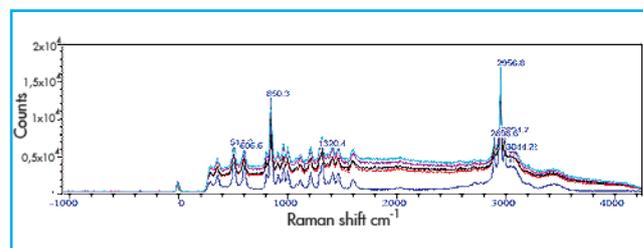


Figure 2: Spectra of L-serine after different exposure times: blue (no irradiation), red (10560 seconds), black (11160 seconds), pink (11760 seconds) and turquoise (13560 seconds).

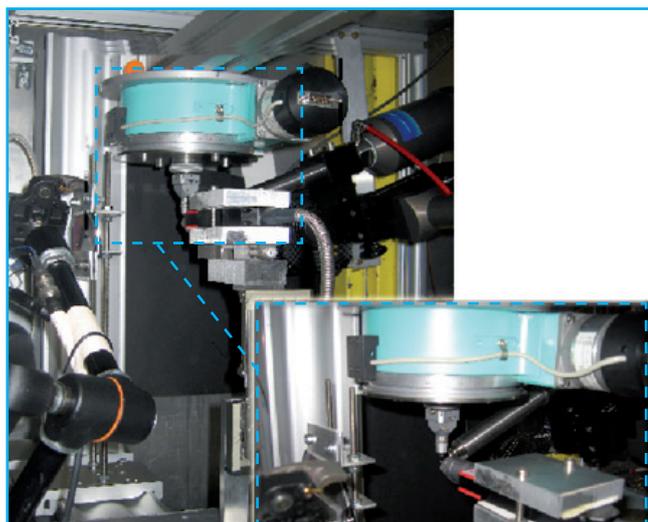


Figure 1: Experimental setup at the beamline (spectrometer not shown).

Figure 2 shows a Raman spectrum of a crystalline sample of unirradiated L-serine (dark blue). The spectrum was acquired using a 300 l/mm grating and with a collection time of 60 seconds. During X-ray irradiation a kinetic data collection of 60 spectra was performed at the suspected Raman shift position with a collection time of 60 seconds/spectrum using a 1800 l/mm grating to monitor alteration of the CH₂ signal. Due to an X-ray induced coloring of the crystal a fluorescence signal was emitted after several runs which interfered with the Raman signal. To obtain conclusive results further measurements have to be performed at a different excitation wavelength.

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Conclusion

For our application an efficient spectrograph with a high-resolution detector is needed. Moreover, the software should be easy to handle and capable of real time data collection. The setup we used fulfilled these requirements. The DU940N-BV CCD detector combined with the possible gratings for the SR-500i spectrograph resulted in a high resolution and a versatile overall setup. The application to in-situ measurements and data evaluation during data collection makes the Shamrock spectrograph together with the Newton CCD detector and the Solis software an excellent tool for Raman measurements during X-ray experiments.

References

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