HORIBA JOBIN YVON

Raman and Resonance Raman Spectroscopy of Enzymes

Introduction

Raman and Resonance Raman spectroscopy have proven to be important research tools to investigate structure-function relationships in enzymes. One such enzyme is DNA photolyase, which is a blue-light photoreceptor and uses flavin adenine dinucleotide in a light-driven, electron-transfer mechanism to repair cyclobutane pyrimidine dimers of DNA. In order to understand the intricate interactions between the FAD cofactor and its protein environment better, it is essential that the assignments of the vibrational modes are well understood. In photolyase, the cyclobutane pyrimidine dimer of DNA binds in close proximity of the FAD cofactor, and one of its carbonyl groups in nearly Van der Waals contact with the C(8) methyl group of the redox-active isoalloxazine ring of FAD (Figure 1). However, the vibrational modes that are associated with the C(8) methyl group and could report on important enzyme-DNA interactions, are yet unknown. A fully integrated HORIBA Jobin Yvon spectroscopy system was used to determine the vibrational modes of flavin that are sensitive to motion of the C(8)-methyl group.

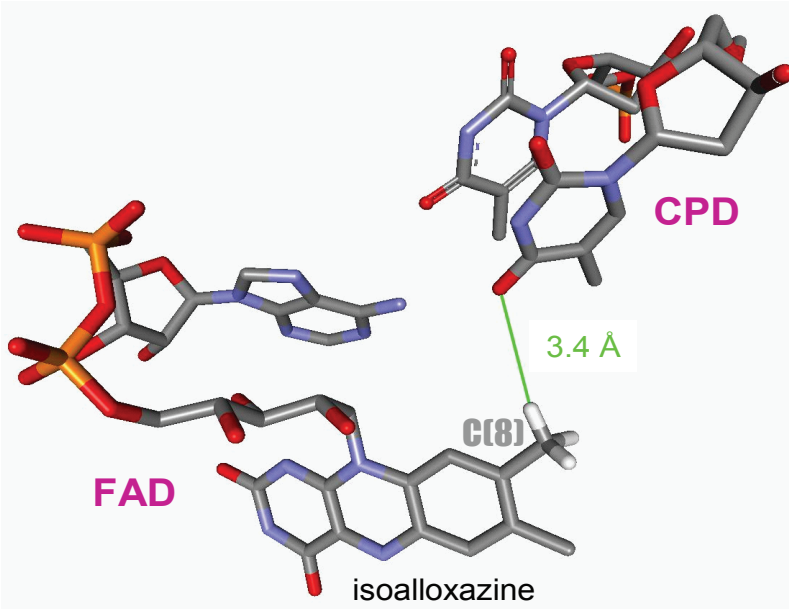

Figure 1. *Molecular structure of DNA photolyase binding in close proximity to FAD cofactor.*

Experimental Setup

Flavin mononucleotide (FMN) was purchased commercially, and deuteration of its C(8)-methyl group (CD3-FMN) was accomplished by following a literature procedure. FMN and CD3-FMN were dissolved in distilled water to a final concentration of 10.0 mM and placed in a quartz cuvette. The 647.1 nm line of a Kr^+ laser was used for excitation in the Raman scattering experiment. The Raman scattered light was collected under a 90 ^{o} scattering geometry and dispersed by a Triax 320 spectrometer with a 1200 gr/mm holographic grating onto a HORIBA Jobin Yvon liquid nitrogen cooled Symphony front illuminated/open electrode CCD detector. A schematic diagram is shown in Figure 2. The data were analyzed with HORIBA Jobin Yvon's SynerJY® data acquisition and analysis software (Origin® 7.0 platform).

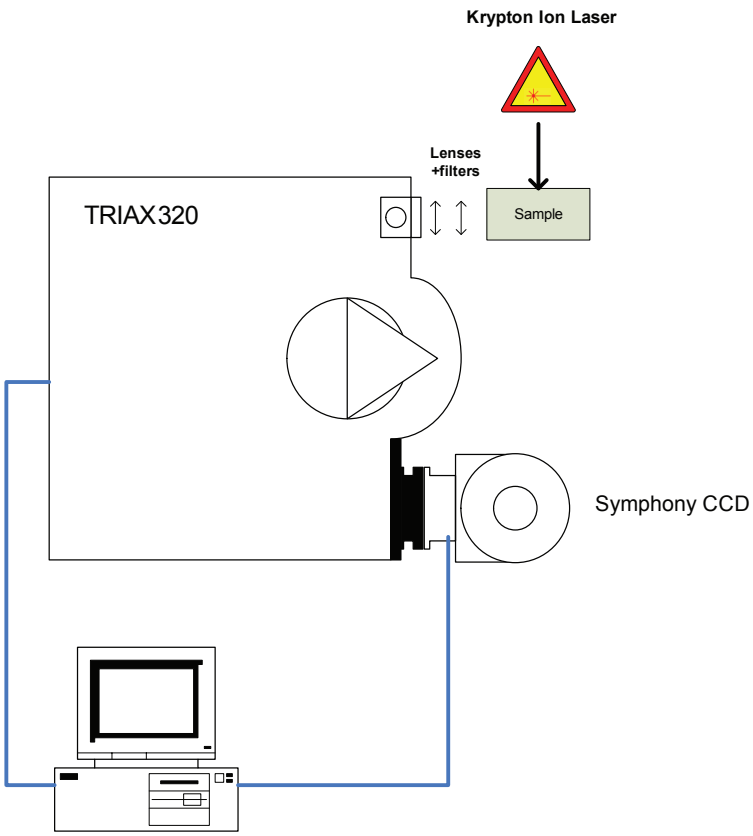

Figure 2. *Schematic drawing of the experimental setup.*

Experimental Results

The Raman spectra of FMN and CD3-FMN in distilled water are shown in Figure 3. Several vibrations are sensitive to the deuteration of the C(8)-methyl group. The vibration at 1228 cm^{-1} is of particular interest. Previous resonanc Raman experiments on a different HORIBA Jobin Yvon system (Triax550 with 2400 gr/mm holographic grating, liquid nitrogen cooled, back illuminated, UV-enhanced SpectrumONE CCD) had already revealed a shift in this vibration in CD3-FMN (Inset B). However, the spectra that were obtained on the new Raman system provide more detail (Inset A). Instead of a simple shift of one Raman band from 1228 to 1236 cm^{-1}, two new Raman bands are observed at 1227 and 1234 cm^{-1}. Furthermore, the Raman intensities in the new spectra that were obtained under non-resonance conditions on the new Raman system are extremely important for comparison to the Raman intensities of vibrational modes that will be calculated by using density functional theory. The fully integrated HORIBA Jobin Yvon spectroscopy system allows for the quick recording of Raman spectra, and allows the user to easily incorporate the optimal laser for the experiment.

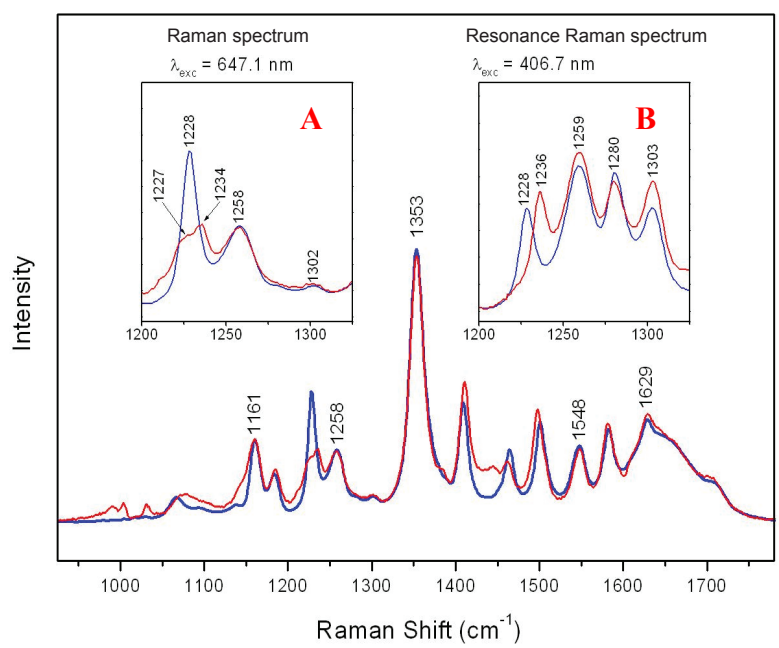

Figure 3. *Raman spectra of FMN and CD_3-FMN in distilled water showing sensitivity to deuteration of C(8) methyl group.*

Conclusions

The Triax and iHR series spectrometers used in Raman system configurations provide superior imaging performance with no re-diffracted light and maximized optical throughput. Coupled to a high-performance Symphony® or Synapse™ CCD detector, these systems provide a high performance spectroscopy platform for the investigation of chemical structures and components.

HORIBA Jobin Yvon's components-based Raman Spectroscopy systems offer full flexibility in designing a Raman detection setup. The systems allow maximum flexibility in implementing collection optics, connections to existing microscopes, and the ability to upgrade or expand existing systems.

The experimental results above demonstrate the ease of data acquisition, and the ability to obtain high resolution and accurate results in a short period of time.

HJY Components	Part Number
Triax 320, 1200 gr/mm x 630 nm holographic grating	Current Mode: iHR320
Symphony CCD 1024 x 256 open electrode, LN_2 cooled	CCD-1024x256-OPEN-1LS, Symphony Solo Fast
SynerJY Software	CSW-SYNERJY

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