HORIBA Scientific

Raman and Resonance Raman Spectroscopy of Enzymes

OSD-108

ELEMENTAL ANALYSIS
FLUORESCENCE
GRATINGS & OEM SPECTROMETERS
OPTICAL COMPONENTS
FORENSICS
PARTICLE CHARACTERIZATION
RAMAN
SPECTROSCOPIC ELLIPSOMETRY
SPR IMAGING

Vibrational spectroscopy of biomolecules

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 Scientific spectroscopy system was used to determine the vibrational modes of flavin that are sensitive to motion of the C(8)-methyl group.

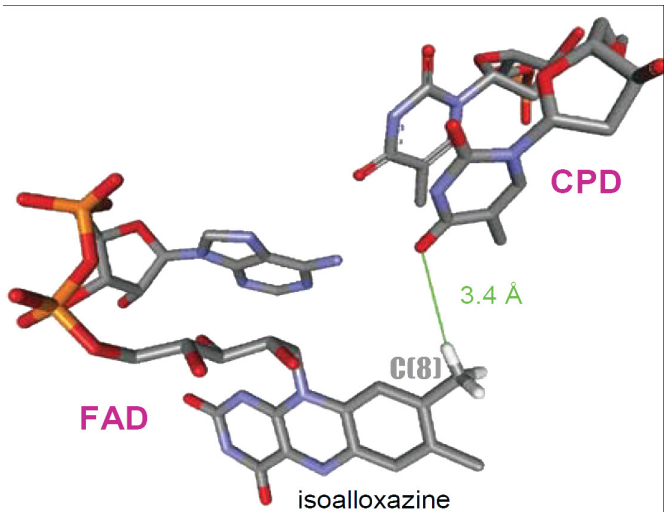

Figure 1. Molecular structure of PNA photolyase binding in close proximity to FAD cofactor.

Experimental setup

Flavin mononucleotide (FMN) was purchased commercially, and deuteration of its C(8)-methyl group (CD_3-FMN) was accomplished by following a literature procedure. FMN and CD_3-FMN were dissolved in distilled water to a final concentration of 10.0 m*M* 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° scattering geometry and dispersed by a TRIAX320 spectrometer with a 1200 gr/mm holographic grating onto a HORIBA Scientific liquid-nitrogen-cooled Symphony® front-illuminated/open electrode CCD detector. A

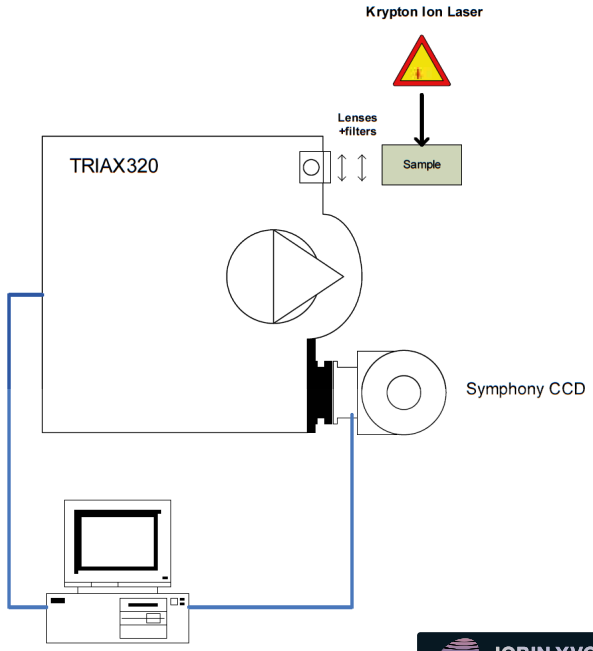

Figure 2. Experimental setup..

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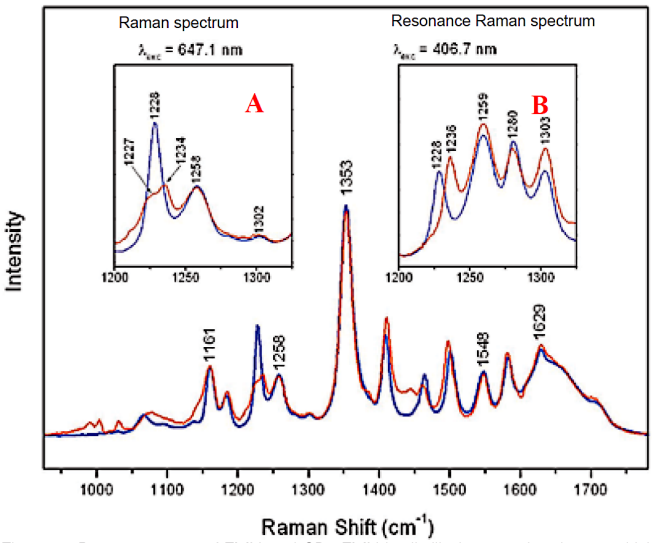

Figure 3. Raman spectra of FMN and CD_3-FMN in distilled water, showing sensitivity to deuteration of C(8)-methyl group.

schematic diagram is shown in Figure 2. The data were analyzed with HORIBA Scientific's SynerJY® data-acquisition and analysis software (Origin® platform).

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 Scientific'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.

HORIBA Scientific components	Part number
Imaging spectrometer, 1200 gr/mm × 630 nm holographic gratingce	Current mode iHR320
Symphony® CCD, 1024 × 256 open-electrode, liquid-nitrogen cooled	CCD-1024x256-OPEN-1LS, Symphony Solo Fast
SynerJY® spectroscopy software	CSW-SYNERJY

Acknowledgements

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