# PROTEIN STRUCTURE AND FUNCTION IN THE TIME-DOMAIN OF VIBRATIONAL SPECTROSCOPIES. THE PROMISING APPLICATIONS OF IR SYNCHROTRON RADIATION MICRO-SPECTROSCOPY

A. Marcelli

*INFN, Laboratori Nazionali di Frascati, P.O. Box 13, 00044 Frascati, Italy*

A protein is characterized by more than 20000 vibrational degrees of freedom, the normal modes of vibration that may be correlated with internal coordinates such as bond lengths and bond angles. Infrared light excites molecular vibrations, as a consequence, IR spectroscopy is a fundamental technique in the study of protein conformation and dynamics. It was applied as early as in 1952 before any detailed X-ray results on proteins were available and nowadays, FTIR spectroscopy allows investigations of proteins in real physiological environments. Actually, because of the large number of normal modes, a vibrational spectrum is extremely complex with many vibrational bands overlapping so that the attempt of spectroscopists to understand from FTIR spectra the protein structure and function is really a hard task. In fact, a protein spectrum is characterized by many features but two main ones: the Amide I and Amide II bands that arise from specific stretching and bending vibrations of the peptide backbone. It is now well established that the frequency of the Amide I band is sensitive to the protein secondary structure, but in the last years, using FTIR micro-spectroscopy, many relevant results have been achieved in term of chemical imaging of living cells and investigations of cellular processes so that the future of this old spectroscopy is promising as ever.

This contribution deals with the most recent advancements of FTIR spectroscopy for the investigation of protein structure and function, which is now possible with unprecedented resolution in space and time by using synchrotron radiation (SR). The high source brilliance of SR (defined as the photon flux or power emitted per source area and solid angle) enables FTIR micro-spectroscopy to be performed within a few minutes at the resolution of just a few microns, a size scale appropriate for investigating individual living cells. A significant capability, since biological cells and tissues are extremely complex structures that vary widely in composition.

When considering the available spatial resolution, three issues should be taken into account. The first one is the acceptable signal-to-noise ratio (S/N), which decreases as apertures are closed to confine the IR beam to small areas. The second issue is the diffraction limit, in fact, using a synchrotron radiation source aperture settings smaller than the wavelength of light can be used. Thus for protein (e.g. at the Amide I band wavelength), the diffraction-limited spatial resolution is approximately 6 micron but experiments up to 3 micron of diameter have been already performed in the MIR region.

Finally, synchrotron radiation has a time structure that ranges from hundreds of ps to ns, e.g., comparable with the time of molecular vibrations and several times faster than conformational changes or protein folding phenomena, so that with new Focal Plane Array detectors the possibility to investigate protein processes in real time and in their native environments seems really possible both in the mid- and far-infrared regions.