The Cryobench: A Tool For In Crystallo Spectroscopy

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Protein crystallography provides near-atomic views of complex macromolecules. However, when taken alone, these views are often insufficient to explain the subtle functional machinery of biological molecules, because links to the biochemical and cellular contexts are missing. Furthermore, crystallographic structures might be altered by the X-ray beam. To address these fundamental questions, there is a growing interest in complementing structural data with spectroscopic data recorded in the crystal. One reason is that spectroscopy is generally non-invasive and can be applied equally well to the different phases of matter.

The Cryobench laboratory (1) offers the possibility to record UV/visible absorption, fluorescence (steady state or time-resolved), and Raman spectra on nano-volumic samples, at room or cryo-temperatures. Recently, the laboratory has been rebuilt as a satellite building of the macromolecular crystallography beamlines ID23, making *in crystallo* spectroscopy a permanent facility of these beamlines, open to users as a platform.

The methodological developments carried out offline at the Cryobench laboratory can be adapted to online instruments that can be used directly on the ESRF MX beamlines (2). In particular, optical fibers will soon link the laboratory to the ID23 experimental stations, making online in crystallo Raman spectroscopy easily available, to be used for example as a metric for assessment of radiation damage.

Through some examples, principally in the field of kinetic crystallography (3,4), the Cryobench facility will be presented and some ways of combining the near-atomic views provided by macromolecular crystallography and the local sub-atomic views provided by spectroscopy will be described.



Fig. 1 - The Cryobench microspectrophotometer

- (1) Bourgeois D et al. Journal of Applied Crystallography (2002) 35(3): 319-326
- (2) McGeehan JE et al. (2007) Journal of Synchrotron Radiation, 14, 99-108
- (3) Adam V et al. Structure (2004) 12(9): 1729-1740
- (4) Katona G et al. (2007) Science, 316(5823): 449-453

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