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Status of the Cryobench in crystallo spectroscopy laboratory of the ESRF

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The Cryobench laboratory of the ESRF has been founded in 1999 in order to record UV-visible absorption and fluorescence emission spectra of protein crystals [1]. The possibilities of recording Raman spectra and fluorescence lifetime histograms were later added, while the laboratory was rebuilt and expanded [2,3]. In the context of the ESRF upgrade programme, the Cryobench was rebuilt in 2009 in the vicinity of the structural biology beamlines ID29 and ID30A, opening up the possibility of routine on-line spectroscopy experiments. The so-called 'in crystallo'spectroscopy techniques allow to verify the state of a crystalline protein in comparison to solution (redox state, photoactive state, nature and state of ligand) and, in combination with crystallographic data, to correlate structure and function. It can also serve to monitor the extent of radiation damage or the progress of a reaction. Over the years, Cyan Fluorescent Proteins (CFPs) have become a favourite topic at the Cryobench laboratory. In this talk, I will review several projects that took advantage of complementary techniques to understand the underlying mechanism of efficient fluorescence by CFPs [4], which in fine led to the development of a fluorescent protein with an almost-perfect quantum yield [5].

References

[1] J. Appl. Cryst. 35, 319 (2002). [2] J. Appl. Cryst. 40, 1105 (2007). [3] J. Appl. Cryst. 40, 1113 (2007). [4] Biochemistry 48, 10038 (2009). [5] Nature Comm. 3, 751 (2012).

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