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In-situ Microspectrophotometry for Macromolecular Crystallography

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Many proteins contain a chromophore or metal centre with absorption bands in the UV/VIS spectral region. The details of the absorption bands report on the state of the protein, like different oxidation or conformation states. In X-ray crystallography, the absorption may even reflect radiation damage during data collection. Especially in high-resolution data collection, alterations in the protein structures are still a severe problem. In-situ microspectrophotometry is a valuable tool to monitor alterations in the UV/VIS absorption of a protein and enables the characterisation of a state of a protein during data collection. In the HZB MX-group a setup for an in-situ UV/VIS microspectrophotometry was build up recently and will be installed for on-line experiments. It consists of a focussing optics directly mounted on the sample stage of the diffractometer. Optical fibres are used to illuminate the protein crystal from a UV/VIS light source and to connect to a compact spectrometer for data recording.

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