More than 110,000 protein structures have been determined by X-ray crystallography. This provides a wealth of information that is exploited for many goals, such as the understanding of the molecular bases of biological events, the development of drugs, the homology design of unknown proteins, and the simulation of protein dynamics. However, crystallographic “artifacts”, including conformational selection and radiation damages, may affect the quality of structural determinations, thus posing serious questions on their relevance. In order to address these issues, spectroscopic methods have been applied to protein crystals in order to tightly couple function to structure. These methods are uv-vis spectrophotometry, spectrofluorimetry, IR, EPR, Raman and resonance Raman spectroscopy. Some of them have been implemented with on-line instrument at X-ray synchrotron beamlines providing a fourth dimension during X-ray data collection. We will report on representative studies carried out in our laboratory by single crystal polarized absorption uv-vis microspectrophotometry, the most applied technique, on hemoglobins, coenzyme-dependent enzymes and green fluorescent protein. Key biological issues were investigated and straightforward structure-function correlations were obtained.