Protein function in the crystalline state, Andrea Mozzarelli[a], Stefano Bettati[b] and Stefano Bruno[a], *[a]Department of Biochemistry and Molecular Biology, and [b]Department of Public Health, University of Parma, 43100 Parma, Italy. E-mail: biochim@unipr.it*

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The number of known protein 3D structures is constantly increasing, opening new avenues for the understanding of the molecular basis of complex biological processes. However, the determination of protein structures in the crystalline state provides only a static picture and just a hint on dynamics-based protein action. Thus, structure-function correlations derived from functional properties observed in dilute solution. and structures obtained in the crystalline state are somewhat arbitrary. Therefore, it is of paramount relevance to assess for each individual protein which functional properties are associated with the determined structure and which functional properties are maintained in the crystal with respect to solution. This information can be obtained by single crystal polarized absorption microspectrophotometry [1], that allow i) to measure uv-vis spectral properties, ii) to determine ligand kinetic and binding parameters, iii) to characterize ligand-induced changes of conformational distribution and equilibrium distribution of catalytic intermediates, iv) to define the experimental conditions for the isolation and accumulation of metastable species, eventually suitable for x-ray analysis, and v) to assess potential radiation alterations in protein cryocrystallography. Microspectrophotometric measurements have been carried out on single crystals exploiting the chromophoric properties of pyridoxal 5'-phophate-dependent enzymes, flavin-dependent enzymes, NAD^+-dependent enzymes, heme- and copper-containing proteins, and natural or chemically modified reporter group-containing proteins. Microspectrophotometers on line with x-ray sources at either synchrotron facilities or home labs have been developed for a direct assessment of diagnostic spectral features. A major issue, still unsolved in spite of considerable effort, is the synchronized triggering of a reaction throughout all protein sites in the crystal. Photo-activation is the key strategy but was proved to be successful on a few selected proteins, including MbCO, photoactive yellow protein, photosynthetic reaction center and bacteriorodopsin [1,2]. Pre-diffused photochemically activated caged compounds, originally proposed to be of general applicability in protein kinetic crystallography, have been used in a few cases, exhibiting limitations. We will discuss a few cases that emphasise i) striking differences between crystal data interpretation and functional properties determined in the same physical state, as observed for oxygen binding stoichiometry and allostery in T quaternary state hemoglobin crystals, ii) the subtle role of lattice forces in altering protein function in the crystal, as observed in crystals of *O*-acetylserine sulfhydrilase, and iii) the relevance of measuring functional properties of structurally determined conformations, as observed in crystals of cystathionine beta-synthase, cystine lyase and GABA aminotrasferase.

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