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Colloque II : Les *X* et le temporel

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Proteins on the move : time-resolved structural studies in crystals and in solution

High resolution X-ray crystal structures of enzymatic intermediates are invaluable for understanding the function of proteins, but it is often ambiguous whether the crystal structure accurately reflects the protein structure in solution. By comparing the spectral properties of protein crystals and solution, one can critically evaluate the validity of crystal structures. One example is the Raman spectroscopic study of iron-peroxide intermediates that are involved in the catalytic cycle of many iron-containing enzymes. Such intermediates were also identified in superoxide reductase (SOR) a non-heme mononuclear iron-enzyme that neutralizes superoxide radicals [1-3]. By diffusing hydrogen peroxide into SOR crystals, we trapped iron(III)-(hydro)peroxo species. X-ray diffraction data and non-resonant Raman spectra recorded in crystallo revealed "end-on" iron-(hydro)peroxo configurations. Raman spectroscopy also monitored the influence of X-ray radiation on the trapped intermediate. The ^{18}O isotopic shifts of the iron-peroxide specific vibration bands confirmed the direct involvement of hydrogen peroxide in the formation of the intermediate. As an alternative to the trapping strategy, light induced structural changes can be followed at ambient temperature in crystals by Laue diffraction in real time. The reaction cycle of photosynthetic reaction center was initiated with a ns laser pulse and the structural changes were monitored with polychromatic x-ray pulse followed by a 1 ms time delay. The crystals grown from lipidic-sponge phase endured the high demands of the time-resolved Laue experiment and several high resolution datasets, both ground and charge separated state, were collected. From this data difference electron density maps revealed a light-driven conformational change of a tyrosine residue analogous to tyrZ of plant photosystem II.

Furthermore transient solution structure of intermediates can also be determined by small- and wide-angle X-ray scattering (SAXS/WAXS). Difference scattering curves accurately reflect the changes in the distribution of interatomic distances, which in turn allows direct structural

comparison to crystal structures. We studied bacteriorhodopsin, a seven transmembrane retinal protein that participate in light-driven energy transduction. Time resolved WAXS data captured the conformational changes followed by light-activation as they occurred. The movement of secondary structure elements could be followed at different time points along the reaction coordinate and compared to the low-temperature crystal structures of photocycle intermediates.