FA1-MS11-O1

X-Ray Absorption Spectroscopy as Tool to Study Enzyme Action in Real-Time. Irit Sagi. *Department of Structural Biology, The Weizmann Institute of Scienc. Rehovot, Israel.*
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The one spectroscopic technique that can be used to obtain direct structural information for metalloenzymes in solution is X-ray absorption spectroscopy (XAS). XAS refers to the structured absorption that occurs on the high-energy side of an absorption edge. With careful analysis X-ray absorption spectra can give bond lengths, and coordination numbers of atoms at the nearest environment of metal ion in enzyme active site. Such spectra are also sensitive to the total effective charge of the metal ion and its geometry. One of the key advantages of a XAS spectroscopy is that it can be used in conjugation with rapid mixing apparatus to study reacting systems in real-time. The importance of real-time structural information of dilute enzyme solutions is apparent. In this presentation, I will present the potential of time-dependent XAS to reveal important new insights regarding enzyme mechanisms and active site structural-kinetics [1-4].

[1] Kleifeld, O., Frenkel, A., Martin, J.M.L., Sagi, I. *Nature Structural Biology*, **2003**, 10, 98. [2] Solomon, A., Akabayov, B., Milla, M., Sagi, I. *Proc Natl Acad Sci U S A*, **2007**, 104, 4931. [3] Rosenblum, G., Meroueh, S., Toth, M., Fisher, J., Fridman, R., Mobashery, S and Sagi, I. *Journal American Chemical Society*, **2007**, 29, 13566. [4] Penner-Hahn, J.E. *Nature Structural Biology*, **2003**, 10, 75.

Keywords: X-ray absorption spectroscopy; metalloenzymes; time-resolved

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Protein Dynamics Probed by Time Resolved X-Ray Scattering at the ESRF. Marco Cammarata[a], Michael Wulff[a]. [a]*European Synchrotron Radiation Facility, BP 220, 38043 Grenoble, France.*
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It has always been a dream to visualise the structure of molecules at work to see how they evolve from initial states, through intermediates to final states. Which atoms and molecules take part, are there intermediate states and if so, for how long do they exist? This is a great challenge since molecules change shape and composition extremely fast, from femtoseconds to seconds. With the advent of third generation synchrotrons facilities, intense pulsed beams of hard X-rays are now available that can be used to probe molecular structure with a time resolution down to 100 ps. The ESRF has a dedicated station ID09B for pump-probe diffraction and scattering experiments. A reaction is typically initiated by short laser pulse and the evolving structure is probed by a delayed x-ray pulse from a chopper. The experiments are typically repeated at frequencies between 1-1000 Hz while the x-ray signal is accumulated on a CCD detector. When Fourier transformed maps of the laser induced change are stitched together, the atomic motions are tracked during the reaction. In this talk I will compare films of myoglobin and hemoglobin obtained in crystals (Laue diffraction, [1]) with films in solution (SAXS/WAXS [2]).

[1]F. Schotte, M. Lim, T. A. Jackson, A. Smirnov, J. Soman, J. S. Olson, G. N. Phillips, M. Wulff, and P. A. Anfinrud. Science, 300, 1944-1947, **2003**.[2] M. Cammarata, M. Levantino, F. Schotte, P. A. Anfinrud, F. Ewald, J. Choi, A. Cupane, M. Wulff and H. Ihee. Nature Methods, 5, 881-886, **2008**.

Keywords: protein dynamics; X-ray scattering; SAXS; WAXS

FA1-MS11-O3

A Multi-Spectral Approach to Understanding Catalysis in CU Nitrite Reductase. Michael Hough[a], Svetlana Antonyuk[a], Richard Strange[a], Robert Eady[a], Samar Hasnain[a]. [a]*University of Liverpool, School of Biological Sciences, Liverpool, United Kingdom.*
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The foremost part of our structural knowledge of metalloproteins comes from crystallography using x-rays at SR sources. The impact these intense x-rays have on an active site metal atom must be evaluated during crystallographic experiments and not assessed post hoc. We describe here the development of in situ spectroscopic and x-ray methods and instrumentation that can be applied to characterise the metal sites of a metalloprotein single crystal. The unique capability produced by bringing together these techniques enables accurate monitoring of the oxidation state and spectral properties of metalloprotein crystals. We have applied this combined-methods approach to copper nitrite reductases which contain both a redox type 1 (T1) and a catalytic type 2 (T2) Cu site. In the absence of substrate (nitrite), optical spectroscopy and X-ray absorption data measured from the same crystal prior to and following the crystallographic data collection clearly indicated that the T1Cu site had become reduced to Cu(I) during the experiment while the T2Cu site remained in the Cu(II) state, providing evidence for an ordered mechanism for this enzyme. In the presence of substrate, we have demonstrated the formation of the NO product at the T2Cu site in crystals following radiolytic reduction of the T1Cu site. While only a few studies of this kind have been reported to date, recent and future generations of SR sources will require such a combined approach if proper functional interpretation of the structural data obtained on metalloproteins at these highly intense x-ray sources is to be achieved.

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