MS33-O2 **Surprising Protonation States in Urate Oxidase - Combining X-ray and Neutron Crystallography with QM/MM.** Esko Oksanen,[ab] Matthew P. Blakeley[c], Mohamed El-Hajji[d], Ulf Ryde[e], Bertrand Castro[f], Monika Budayova-Spano[a] *Institut de Biologie Structurale, UMR 5075 CEA-CNRS-UJF, Grenoble, France, [b]European Spallation Source, Lund, Sweden, [c]Institut Laue-Langevin, Grenoble, France, [d]Sanofi-Aventis, Montpellier, France [e]Department of Theoretical Chemistry, Lund University, Sweden [f]CTMM, Institut Charles Gerhardt, University of Montpellier, France*
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Urate oxidase oxidises uric acid to 5-hydroxyisourate and is used as a protein pharmaceutical, but the catalytic mechanism has remained enigmatic, as the protonation state of the substrate could not be reliably deduced. We have determined the neutron structure of urate oxidase in complex with the substrate and the inhibitor chloride at 2.3 Å resolution. A stable form is observed in the crystalline structure showing the substrate as the enol tautomer 8-hydroxyxanthine. We have also determined the neutron structure in complex with the competitive inhibitor 8-azaxanthine at 1.9 Å resolution, showing the protonation states of the K10-T57 catalytic 'dyad'. These neutron structures were refined jointly with X-ray data and, together with atomic resolution X-ray structures and quantum chemical calculations, they allow us to identify a site of the initial substrate protonation and elucidate the inhibition of the enzyme by a chloride anion.

Keywords: protein crystallography; neutron crystallography; enzymatic mechanisms;

MS33-O3 **Determining protonation states in proteins using high-resolution X-ray crystallography.** Fisher, S. J.,[ab] Blakeley, M. P.,[b] Cianci, M.,[c] McSweeney, S.,[d] and Helliwell, J. R.[e] *[a]University of Salzburg, Austria, [b]Institut Laue Langevin, France, [c]EMBL Hamburg, Germany, [d]ESRF, France, [e]University of Manchester, UK.*
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With ever increasing flux at synchrotron facilities and advancements in protein crystallisation techniques, it is becoming ever more possible to collect protein crystallographic data sets to within angstrom resolution. These data sets are usually complete and highly redundant providing an excellent opportunity for determining protonation states of amino acid groups using bond length analysis . Such protonation state information can be crucial for determining enzyme mechanisms that involve proton transfer. Here we extend our previous analyses by assessing a number of other data sets at varying resolutions and completenesses. Furthermore we consider the possibility of determining the protonation state of histidine residues using bond angle analysis, however this requires significantly higher precision than for asp or glu residues . When collecting data to high resolution it is important to consider the effects of radiation damage, especially considering that asp and glu residues are readily radiolysed. Therefore we also conducted an extensive analysis of radiation damage on these data sets.

high-resolution X-ray crystallography, protein crystallography, enzyme mechanisms