charge density study on this second row metal complex with the aim of providing information about metal coordination, and interatomic bonds and interactions, beyond classical geometrical criteria.

The experimental electron density distribution of the ruthenium complex has been determined from high resolution ($\sin\theta_{max}/\lambda$=1.05Å^{-1}) X-ray diffraction data collected at 100 K. After the conventional spherical refinement, the multipole refinement was performed using Hansen and Coppens model [1] with Mopro software [2, 3]. Electronic configuration of the ruthenium atom and a disorder in the cicloactadiene ligand have been inspected. The deformation density model has been interpreted according to the "Quantum Theory of Atoms in Molecules" [4]. Characterization of the nature of the different metal-ligand interactions in this organometallic complex via the topological properties at the bond critical points will be discussed.

[1] N. K. Hansen and P. Coppens, *Acta Cryst.* **1978**, *A34*, 909-921. [2] B.Gillot, L. Viry, R. Guillot, C. Lecomte and C. Jelsch. *J. Appl.Cryst.* **2001,** *34*, 214-223. [3] C. Jelsch, B.Gillot, A. Lagoutte and C. Lecomte. *J. Appl.Cryst.* **2005,** *38*, 38-54. [4] R.F.W. Bader, *Atoms in Molecules, a Quantum Theory, Internacional Sereis of Monographs in Chemistry, 22; Oxford Science Publications, Claredon Press, Oxford.***1990**

Keywords: Charge density, organometallic, ruthenium.

MS42.P01

Acta Cryst. (2011) A**67,** C516

X-ray absorption spectroscopy studies of copper site in the ubiquinol oxidase

Elena Nazarenko,[a] Jennie Sjöhamn,[a] Linda Johansson,[a] Petra Edlund,[a] Anna Polyakova,[a] Gergely Katona,[a] Alexandr Nasedkin,[b] Santiago Figueroa,[c] Richard Neutze,[a] [a]*University of Gothenburg, Department of Chemistry, Gothenburg, (Sweden).* [b]*Uppsala University, Department of Photochemistry and Molecular Science, Uppsala, (Sweden)..* [c]*European Synchrotron Radiation Facility, Grenoble, (France).* E-mail: elena.nazarenko@chem.gu.se

We present recent experimental and modeling results on membrane protein studied by X-ray absorption spectroscopy (XAS) at the room temperature in solution. Heme-copper oxidases are integral membrane proteins which serve as the final electron acceptor of respiratory chains across all kingdoms of life. These integral membrane proteins accept electrons so as to reduce oxygen to water and thereby exploit the chemical energy released to pump protons. Given the complexity of these processes, it is essential to determine any effect of changes in protonation state of groups near the catalytic iron-copper centre. Such groups are likely to play essential roles in these functionally important conformational changes. Most proposed proton-pumping mechanisms involve CuB site and its histidine ligands. The existence and identity of such reorganization of the CuB geometry caused by protonation/ deprotonation and/or breakage of one of the Cu-N(His) bonds is a difficult matter to either prove or disprove since CuB is spectrally silent. Since the Ubiquinol oxidase consists just one Cu site and two Fe atoms it's ideal candidat to apply X-ray absorption spectroscopy to study what happen near the Cu-site when Cu changes its oxidation state and to study pH dependence of the Cu site.

We report the X-ray absorption near edge spectroscopy (XANES) studies of the copper edge of the cytochrome bo3 quinol oxidase from *Escherichia coli*. Our ab-initio calculations (non muffin-tin FDMNES [1]) and modeling (FitIt software [2]) results indicate that the Cu-site changed its associated ligands for oxidised Cu(II) and reduced Cu(I) states of the protein. However room temperature copper K-edge X-ray absorption spectra remains unchanged in the pH range 6.5-9.5 for both oxidized and reduced forms of copper correspondently, indicating that no structural changes takes place at Cu-site depending on pH.

[1] Y. Joly, *Phys. Rev. B* **2001**, *63*, 125120-125129. [2] G. Smolentsev, *Comp. Matter. Science* **2007**, *39*, 69.

Keywords: XANES, ubiquinol oxidase, Cu K-edge

MS42.P02

Acta Cryst. (2011) A**67,** C516

Ferrocene and fluorescence studies: How can we understand stereochemistry of dilute systems?

Christopher T Chantler,[a] M Tauhidul Islam,[a] Stephen P Best,[b] Feng Wang,[c] [a]*School of Physics, The University of Melbourne, Victoria, (Australia).* [b]*School of Chemistry, The University of Melbourne, Victoria, (Australia).* [c]*Faculty of Life and Social Sciences, Swinburne University of Technology, Victoria, (Australia).* E-mail: chantler@unimelb.edu.au

Ferrocene is a standard for iron X-ray absorption fine structure (XAFS) and X-ray absorption near-edge structure (XANES) especially for comparison to samples in solution, both because of its well-defined structure and its sharp redox properties. Its significance is reflected by award of the Nobel prize to EO Fischer and G Wilkinson in 1973 for the elucidation of its structure [1,2]. We analyse ferrocene data collected in a cold cell at the Australian National Beamline Facility (ANBF) in Tsukuba, KEK, Japan using conventional fluorescence XAFS. A challenge for fluorescence spectra is the determination of information content or significance. With this, critical quantitative hypothesis testing can be pursued. Without this, naive hypothesis testing can be applied for simple systems, becoming fraught for complex systems, even including mixtures of two species or ionization states. We present robust standard errors from such typical datasets and illustrate their potential applied to a subtle and long-standing problem of ferrocene – that of the orientation of the two cyclo-pentadienyl rings.

Similar recent approaches have shown great potential in the comparison of crystallographic parameters to dynamical measurements [3,4,5], measuring dynamical bond lengths [6], solution of local structure in catalytic organometallics [7,8,9] and developing new theoretical and analytical approaches to structure determination [10].

[1] M.D. Rausch, E.O. Fischer, H. Grubert, *Journal of the American Chemical Society* **1960**, *82*, 76-82. [2] J.A. Page, G. Wilkinson, *Journal of the American Chemical Society* **1952**, *74*, 6149-6150. [3] M.D. de Jonge, C.Q. Tran, C.T. Chantler, Z. Barnea, B.B. Dhal, D.J. Cookson, W-K. Lee, A. Mashayekhi, *Phys. Rev. A* **2005**, *71*, 032702-1-16. [4] L.F. Smale, C.T. Chantler, M.D. de Jonge, Z. Barnea, C.Q. Tran, *Radiation Physics & Chemistry* **2006,** *75*, 1559-1563. [5] C.T. Chantler, N.A. Rae, C.Q. Tran, *J. Appl. Cryst.* **2007**, *40*, 232-240. [6] J.L. Glover, C.T. Chantler, Z. Barnea, N.A. Rae, C.Q. Tran, *J. Phys. B* **2010***, 43,* 085001-1-15. [7] J.L. Glover, C.T. Chantler, A.V. Soldatov, G. Smolentsev, M.C. Feiters, *'Theoretical XANES study of the activated Nickel (t-amylisocyanide) molecule,'* 625-627, CP882, *"X-ray Absorption Fine Structure – XAFS13",* B. Hedman, P. Pianetta, eds (**2007**, AIP 978-0-7354-0384-0). [8] J.D. Bourke, C.T. Chantler, C. Witte, *Physics Letters A*, **2007,** *360*, 702-706. [9] J.D. Bourke, C.T. Chantler, *Nuclear Instruments and Methods A* **2010**, *619*, 33-36. [10] J.J. Kas, J.J. Rehr, J.L. Glover, C.T. Chantler, *Nuclear Instruments and Methods A* **2010**, *619*, 28-32.

Keywords: ferrocene, fluorescence XAFS, dynamical bondlengths

MS42.P03

Acta Cryst. (2011) A**67,** C516-C517

Conformational studies of bovine insulin

Christian Grundahl Frankær, Kenny Ståhl, Pernille Harris, *Chemistry*

Department, Technical University of Denmark, Kgs. Lyngby, (Denmark). E-mail: cghar@kemi.dtu.dk

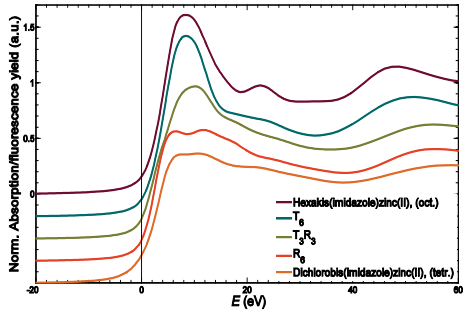
Hexameric insulin exists as an allosteric complex with three well known conformations (T_6, R_6 and T_3R_3) [1–3]. Each hexameric complex contains two divalent metal ions (typically Zn). Both ions are located on the three-fold symmetry axis going through the hexamer and coordinate to three symmetry-related histidine $N^{\varepsilon 2}$ atoms. Octahedral coordination is fulfilled in the T_6 conformation by further coordination of three water molecules and in the R_6 conformation tetrahedral coordination is fulfilled by coordination of one lyotropic anion, which is also located on the three-fold symmetry axis. A dual octahedral/tetrahedral coordination is observed in the T_3R_3 conformation.

In this work we have studied all three conformations of bovine insulin by combining complementary techniques: Single crystal X-ray diffraction (XRD), X-ray powder diffraction (XRPD) and X-ray absorption fine structure spectroscopy (XAFS).

Crystals of T_6, R_6 and T_3R_3 zinc insulin were grown and the structures were solved by single crystal XRD, to obtain good model structures for the XAFS data analysis. For bovine insulin only the structure of T_6 conformation has hitherto been solved [4].

All three conformations form crystals in space group *R*3, and can, however, easily be distinguished by XRPD since the unit cell parameters alter. [5] Using in-house XRPD the conformations were verified before and after XAFS experiments. [6]

The coordination around the zinc sites were studied by XAFS for all three conformations. Furthermore hexameric T_6 insulin crystallized with copper and nickel were studied. Data were collected on beamline 811 at MAX-lab, Lund, Sweden, and are the first protein XAFS experiments carried out on this beamline. Coordination geometry was verified from the near edge region of the spectra (XANES) by comparison with an octahedral and a tetrahedral zinc imidazole complex, see figure. The coordination geometry was in agreement with the extended region of the spectra (EXAFS) and bond distances to the first coordination shell were determined with uncertainties below 0.02 Å.



[1] G.D. Smith, W.A. Pangborn, R.H. Blessing, *Acta Cryst. D* **2003**, *59*, 474-482. [2] E. Ciszak, G.D. Smith, *Biochemistry* **1994**, *33*, 1512-1517. [3] G.D. Smith, E. Ciszak, L.A. Magrum, W.A. Pangborn, R.H. Blessing, *Acta Cryst. D* **2000**, *56,* 1541-1548. [4] G.D. Smith, W.A. Pangborn, R.H. Blessing, *Acta Cryst. D* **2005**, *61,* 1476-1482. [5] C.G. Hartmann, P. Harris, K. Ståhl, Accepted in *Z. Krist. Suppl.* **2011**. [6] C.G. Hartmann, O.F. Nielsen, K. Ståhl, P. Harris, *J. Appl. Cryst.* **2010**, *43*, 876-882.

Keywords: insulin conformations, protein X-ray powder diffraction, X-ray absorption fine structure spectroscopy

MS42.P04

Acta Cryst. (2011) A**67,** C517

XAS/XRD Complementary data on metallodrugs and their proteins complexes

I. Ascone,[a] L. Messori,[b] A. Balerna,[c] C. Castellano,[d] C. Gabbiani,[b] A. Casini,[e] C. Marchioni,[f] A. Congiu Castellano,[f] [a]*ENSCP Chimie ParisTech, LCF; CNRS, UMR 7223, 75005 Paris, (France).* [b]*Dep. of Chemistry,University of Florence,Via della Lastruccia 3,50019 Sesto Fiorentino,Florence, (Italy).* [c]*INFN-LNF,Via E. Fermi, 00044 Frascati, (Italy).* [d]*Dip.di Chimica Strutturale e Stereochimica Inorganica, University of Milan, Via G. Venezian 21, 20133 Milan, (Italy).* [e]*Institut des Sciences et Ingenierie Chimiques, EPFL, 1015 Lausanne, (Switzerland).* [f]*Dip. di Fisica, Univ. Sapienza, P.le A. Moro 2, 00185 Rome, (Italy).* E-mail: isabella.ascone@gmail.com

Ruthenium, gold and iron based complexes form very promising classes of potential cytotoxic and antitumor agents as documented by literature [1] and X-ray absorption may contribute to their structural and electronic characterization [2, 3, 4]. Moreover understanding how metallocomplexes bind to serum proteins is important in evaluating anticancer drug candidates.

We have investigated, by X-ray absorption spectroscopy, several promising antiproliferative agents showing a high propensity to react with proteins: three representative gold(I, III) metallodrugs (i.e., auranofin, $[Au(2,20\text{-bipyridine})(OH)_2](PF_6)$, Aubipy, and dinuclear $[Au_2(6,60\text{-dimethyl-}2,20\text{-bipyridine})_2(l\text{-}O)_2](PF_6)_2$, Auoxo6) and a Ru(III) complex (i.e. NAMI-A, $[trans\text{-}RuCl_4(Im)(DMSO)]$ [ImH] ,where Im is imidazole) and their complexes with two major plasma proteins, namely, bovine serum albumin (BSA) and human serum apotransferrin (apoTf) [2, 3].

XANES and EXAFS, used jointly, allowed us to gain independent structural information on metallodrug/protein systems. The following metallodrug–protein systems were investigated in depth: auranofin/apoTf, Aubipy/BSA, and Auoxo6/apoTf and NAMI-A/BSA. Detailed insight into the gold and ruthenium oxidation state and the local environment of protein-bound metal atoms was achieved.

XANES spectra revealed that auranofin and NAMI-A, upon protein binding, conserve their oxidation state.

In contrast, the reactions of Aubipy with serum albumin and of Auoxo6 with serum apoTf invariantly result in gold(III) to gold(I) reduction. Gold(III) reduction, clearly documented by XANES, is accompanied, in both cases, by release of the bipyridyl ligands; for Auoxo6 cleavage of the gold–gold dioxo bridge is also observed. Gold(III) reduction leads to formation of protein bound gold(I) species, with deeply modified metal coordination environments, as evidenced by EXAFS. These results will be presented highlighting that independent and complementary information may be obtained from XAS and XRD measurements.

[1] C.X. Zhang, S.J. Lippard, *Current Opinion in Chemical Biology* **2003,** *7*, 481-489. [2] I. Ascone, L. Messori, Casini, C. Gabbiani, A. Balerna, F. Dell'Unto, A.C. Castellano,. *Inorg. Chem.* **2008,** *47*, 8629-8634.[3] L. Messori, A. Balerna, I. Ascone, C. Castellano, C. Gabbiani, A. Casini, C. Marchioni, G. Jaouen, A. Congiu Castellano, *JBIC* **2011,** *16*, 491-499.[4] I. Ascone, D. Hamels, P. Pigeon, M. Salome, Y Joly, T. Prangé, A. Vessières, S. Top, G. Jaouen, *Manuscript* in preparation. **2011.**

Keywords: metallodrugs-1, Anticancer-2, X-ray absorption spectroscopy-3

MS43.P01

Acta Cryst. (2011) A**67,** C517-C518

Regularity of $d(CGCGCG)_2$ Z-DNA seen in ultrahigh-resolution crystal structure at 0.55 Å

Zbigniew Dauter,[a] Miroslawa Dauter,[b] Krzysztof Brzezinski,[a] Maciej Kubicki,[c] Mariusz Jaskolski,[c,d] [a]*Synchrotron Radiation Research Section, MCL, National Cancer Institute, Argonne National Laboratory, Argonne, IL 60439, (USA).* [b]*SAIC-Frederick*