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

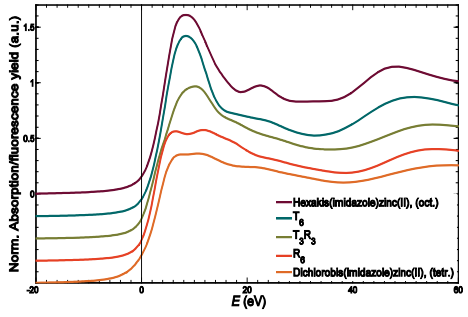
Hexameric insulin exists as an allosteric complex with three well known conformations (T_6, R_6 and T_3R_3) [1–3]. Each hexameric complex contains two divalent metal ions (typically Zn). Both ions are located on the three-fold symmetry axis going through the hexamer and coordinate to three symmetry-related 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*

Inc., Basic Research Program, Argonne National Laboratory, Argonne, IL 60439, (USA). [c]*Department of Crystallography, Faculty of Chemistry, A. Mickiewicz University, Poznan, (Poland).* [d]*Center for Biocrystallographic Research, Institute of Bioorganic Chemistry, Polish Academy of Sciences, Poznan, (Poland).*

Oligomers of the left-handed Z-DNA diffract X-rays to the highest resolution among all crystal forms of DNA. We present the crystal structure of a Z-DNA hexamer duplex $d(CGCGCG)_2$ determined at ultrahigh resolution of 0.55 Å. The structure has been refined to R = 6.77% in the full-matrix anisotropic mode with total absence of stereochemical restraints for DNA, according to the practice of small-molecule crystallography. This way led to very accurate, unbiased values of atomic coordinates and displacement parameters, accompanied by reliable estimates of their standard uncertainties. In consequence, we are able to present a detailed analysis of very fine features of the Z-DNA stereochemistry, not available in the existing literature. Detailed comparisons of the stereochemical library values with the present accurate Z-DNA parameters, shows in general a good agreement, but also reveals significant discrepancies in the description of guanine-sugar valence angles and in the geometry of the phosphate groups.

It should be noted that the crystal structure presented here has a nearly record-breaking resolution in the PDB, where it is surpassed only by crambin (1EJG and 3NIR) determined at 0.54 and 0.48 Å resolution. In the area of nucleic acids, it is currently the highest-resolution model.

Keywords: Z-DNA, ultra-high resolution

MS44.P01

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

Direct Determination of Long-period Stacking/Ordered Structures in $Mg_{81}Zn_8RE_{11}$

Daisuke Egusa, Eiji Abe, *Department of Materials Science & Engineering, University of Tokyo, Tokyo (Japan)*. E-mail: egusa@stem.t.u-tokyo.ac.jp

Mg alloys containing a small amount of Zn and Y, e.g., Mg-1at.%Zn-2at.%Y alloy, reveal excellent mechanical properties with high yield strength ~600Mpa and elongation ~5% at room temperature. One of the remarkable microstructural features is formation of a novel type of long-period stacking/ordered (LPSO) structure [1], which is long-period chemical-ordered as well as stacking-ordered. There are long-period stacking polytypes denoted as 18R, 14H, 10H, 24R, (Fig.1) all of which are composed of a common structural unit represented by local ABCA stacking where B- and C-layers are significantly enriched by Zn and Y (these particular layers are denoted as B'- and C'-layers hereafter). These LPSO structures were firstly identified in Mg-Zn-Y alloys, but at present the LPSO phases have been found in several Mg-Zn-RE (RE: Rare Earth = Dy, Ho, Er, Tm, Gd, Tb) alloys [2].

Looking carefully the electron diffraction patterns taken along the stacking direction (*c*-axis), we find many weak satellite spots that suggest a further ordering within the B'- and C'-layers. In the present work, we investigate the detailed chemical order in the LPSO Mg-Zn-RE phases, using aberration-corrected scanning transmission electron microscopy (STEM). Since the reported compositions of the LPSO phases were different by the nominal compositions of the alloys, we considered that the chemical order in the LPSO phases also changes by them. Therefore we have chosen LPSO phases in the Mg97Zn1Y2 alloy, the Mg97Zn1Er2 alloy and the Mg85Zn6Y9 alloy.

Using the technique of electron diffraction and direct observation by STEM, we determined the chemical order in the LPSO phases. The chemical order was formed by 6-times periodic structures within the basal planes.From the results of direct observations, we found that there were clusters of additional elements which have ordered configurations similar to a $L_1$2-type structure. 6-times periodic structures within basal planes were composed by the periodic arrangements of these clusters. The difference of the nominal compositions of the alloys did not affect the configuration of the cluster, however the densities of clusters in the basal planes were seem to be changed by the nominal compositions. Finally, we constructed averaged models of the LPSO phases from the experimental results. The validity of the model was confirmed by simulations of electron diffraction and STEM image.

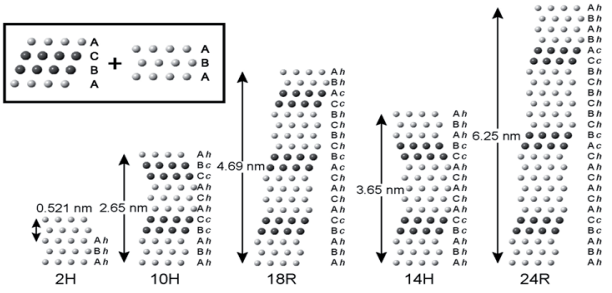


Fig. 1 Stacking polytypes of LPSO phases

[1] E. Abe, Y. Kawamura, K. Hayashi, A. Inoue, *Acta Materialia* **2002**, *50*, 3845-3857. [2] Y. Kawamura, M. Yamasaki *Materials Transactions* **2007**, *48*, 2986-2992.

Keywords: magnesium alloys, crystal structure, electron microscopy

MS44.P02

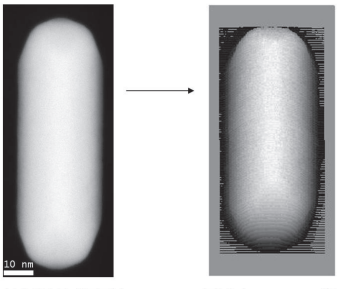
Acta Cryst. (2011) A**67,** C518-C519

A Method to Determine the 3D Morphology and Crystallography of Nanoparticles

Hadas Katz-Boon,[a] Chris J. Rossouw,[a] Matthew Weyland,[a] Alison M. Funston, [b] Paul Mulvaney, [b] and Joanne Etheridge[a] [a]*Departament of Materials Engineering and Monash Centre for Electron Microscopy, Monash University, Victoria, (Australia).* [b]*School of Chemistry and Bio21 Institute, University of Melbourne Parkville, VIC, (Australia).* E-mail: hadas.katz@monash.edu

Nanocrystals have unique properties which are different from the bulk material and strongly dependent on their morphology and crystallography. Characterising the morphology and crystallography is important for understanding the relationship between shape and properties and for determining the mechanism controlling nanocrystal growth.

In this paper we describe a rapid method to measure the thickness profile of a nanoparticle from a single annular dark field scanning transmission electron microscope (ADF-STEM) image [1]. This method deliberately uses ADF-STEM images just below lattice resolution, to enable relatively fast image quantification and measurement but at the expense of spatial resolution. Our focus here is on speed, to provide scientists working on nanocrystals a route to make a meaningful statistical comparison of the shape and properties of a large number of particles in a practical timeframe.



HAADF-STEM image of a gold nanorod

'Thickness profile' image of a gold nanorod

We have applied this