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

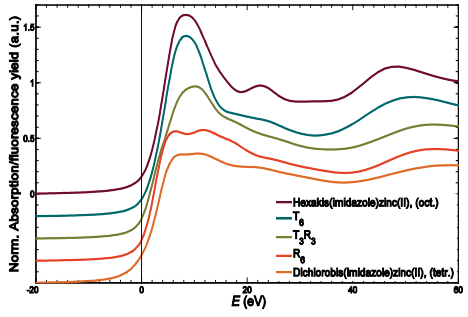
Hexameric insulin exists as an allosteric complex with three well known conformations (T_6, R_6 and T_3R_3) [1–3]. Each hexameric complex contains two divalent metal ions (typically Zn). Both ions are located on the three-fold symmetry axis going through the hexamer and coordinate to three symmetry-related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

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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.**

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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*