s1.m7.p23 **Neutron diffraction structure of fully deuterated Aldose Reductase: A necessary complement to X-Ray ultra-high resolution structures.** A Podjarny[a], A. Mitschler[a], I. Hazemann[a], T. Petrova[a], F. Ruiz[a], M. Blakeley[b], M.T. Dauvergne[b], F. Meilleur[b], M. Van Zandt[c], S. Ginell[d], A. Joachimiak[d] and D. Myles[b#]. [a]*IGBMC, CNRS, 1 rue Laurent Fries, 67404 Illkirch, France,* [b]*EMBL Grenoble Outstation, ILL, 6 rue Jules Horowitz, 38042 Grenoble, France,* [c]*IDD, 23 Business Drive, Branford, Connecticut, USA,* [d]*Bioscience Division, Structural Biology Center and Midwest Center for Structural Genomics, Argonne National Laboratory, 9700 South Cass Avenue, Argonne, Illinois,USA. #: Current address: Oak Ridge National Laboratory, Tennessee,USA. E-mail: podjarny@titus.u-strasbg.fr*

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Human Aldose Reductase (AR), an enzyme in the polyol pathway belonging to the aldo-ketoreductase family, is implied in diabetic complications. Its ternary complexes (AR-coenzyme NADPH-selected inhibitor) provide a good model to study the inhibition and enzymatic mechanisms. Indeed, X-ray electron density maps solved at very high resolution of AR complexes with different inhibitors (IDD-594, 0.66 Å; IDD-552; IDD-393; Fidarestat, 0.90 Å) show within the active site crucial protonation states. In some cases, different protonation states appear simultaneously, each with a partial hydrogen atom occupation. Therefore, we have started neutron diffraction experiments. First trials based on H_2O/D_2O exchange, using crystals of 0.1 mm^3, showed neutron diffraction up to only ~4.5 Å. New crystallisation trials, with fully deuterated protein (EMBL,Grenoble) complexed with the inhibitor IDD-594, succeeded. X-Ray diffraction from these crystals, measured at the SBC-APS, achieved a resolution of 0.8 Å at 10K. Neutron diffraction measured on LADI (ILL,Grenoble) achieved a resolution of 2.5 Å at room temperature, despite a rather small crystal volume of only 0.14 mm^3. Growth of larger crystals is being planned. Even with the current data, the refined model shows information about the protonation states of the inhibitor and active site residues. The results will be presented at the meeting. This work is supported by the U.S. Department of Energy, Office of Biological and Environmental Research under contract No. W-31-109-ENG-38, the Centre National de la Recherche Scientifique (CNRS), by collaborative projects CNRS-CERC and ECOS-SUD, by the Institut National de la Santé et de la Recherche Médicale and the Hôpital Universitaire de Strasbourg (H.U.S), the ILL, the EMBL, and by the Institute for Diabetes Discovery, Inc. through a contract with the CNRS.

s1.m7.p24 **Crystal structure of BclA, a protein of the exosporium of the spore of *Bacillus anthracis.*** Stéphane Réty,[a] Sylvie Salamitou[b] and Anita Lewit-Bentley[a], [a]*LURE, Centre Universitaire Paris-Sud, BP 34, 91898 Orsay Cedex, France, and* [b]*Institut de Génétique et de Microbiologie, Centre Universitaire Paris-Sud, Bât 409, 91400 Orsay, France. E-mail: Stephane.Rety@lure.u-psud.fr*

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Bacillus anthracis, the causative agent of anthrax, is mainly found in the environment (or released as a weapon) as a spore, a dormant cell type of the bacilli characterized by a strong resistance to stress factors such as heat, drought or chemicals. The spore is surrounded by a thick multilayered structure named the coat, the outermost layer of which is named the exosporium. Electron microscopy has revealed that it is composed of a paracrystalline basal layer with an hexagonal lattice structure and a hair-like outer region. It is surmised that the exosporium serves as a primary permeability barrier, as a source of spore surface antigens and that it makes initial contact with the host. Crude exosporium contain at least 12 major protein components. The most prominent was coined BclA, encoded by the *bclA* gene [1]. BclA is glycosylated and is the immunodominant antigen of the spore surface. It was shown to be the structural constituent of the exosporium filaments. The length of *bclA* differs between strains of *B. anthracis*, encoding proteins with different size. All these proteins possess a N-terminal collagen-like region (CLR) of GXX repeats which include a large proportion of GPT triplets. The number of repeats varies between strains: the CLR of the different BclA proteins contains 17 to 91 GXX repeats. It was shown that the length of the BclA CLR is responsible for the variation in filament length [2]. Recombinant BclA containing 19 GXX repeats, produced in *E. coli*, crystallizes in space group $P6_322$ (a=b=68.3Å, c=163.9Å). No sulphur atom is naturally present in BclA protein. Structure was thus determined with heavy atom derivatives. Native and derivatives data were collected with an in-house X-ray source. Phasing an automatic tracing of the model was done using AutoSHARP with the data from a single halide derivative (KI, 1M). Though the protein is not cleaved in the crystal, the N-terminal collagen-like domain is absent from the structure (R=17%, Rfree=21%). The C-terminal part is folded in a jelly-roll domain and shares a very high structural homology with the globular part of the complement protein C1q, despite the lack of sequence similarity between these two proteins. The fact that BclA mimics C1q structure raises new perspectives for the role of exosporium proteins during the infection and the germination of the spore in the host organism.

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