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**Neutron Diffraction Structure of Fully Deuterated Aldose Reductase: A Necessary Complement to X-Ray Ultra-High Resolution Structures.** A. Podjarny<sup>1</sup>, A. Mitschler<sup>1</sup>, I. Hazemann<sup>1</sup>, M. Blakeley<sup>2</sup>, M.T. Dauvergne<sup>2</sup>, F. Meilleur<sup>2</sup>, M. Van Zandt<sup>3</sup>, S. Ginell<sup>4</sup>, A. Joachimiak<sup>4</sup>, D. Myles<sup>2#</sup>, <sup>1</sup>IGBMC, CNRS, Illkirch, France, <sup>2</sup>EMBL Grenoble Outstation, ILL, Grenoble, France, <sup>3</sup>IDD, Branford, CT, <sup>4</sup>Argonne National Laboratory, Argonne, IL, USA, #Oak Ridge National Laboratory, Tennessee, USA.

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<sub>2</sub>O/D<sub>2</sub>O exchange, using crystals of 0.1 mm<sup>3</sup>, 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<sup>3</sup>. 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.

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