

W0087

**Helium-cooled X-ray Diffraction Studies Enhance the Visibility of a Proton Pathway in Human-Aldose Reductase.** A. Mitschler<sup>1</sup>, S. Ginell<sup>2</sup>, A. Cousido<sup>1</sup>, T. Petrova<sup>1,2</sup>, V. Lunin<sup>3</sup>, I. Hazemann<sup>1</sup>, M. Van Zandt<sup>4</sup>, A. Joachimak<sup>2</sup>, A. Podjarny<sup>1</sup>, <sup>1</sup>IGBMC-CNRS-INSERM-ULP, 67404 Strasbourg-Illkirch, France, <sup>2</sup>ANL-SBC, Argonne, IL 60439 USA, <sup>3</sup>IMPB, Puschino, Russia, <sup>4</sup>IDD,CT,USA

Human aldose reductase (h-AR) is involved in severe diabetic complications, and is a clinical target for drug design. Its enzymatic mechanism is based on the transfer of a hydride from coenzyme NADPH and of a proton from the h-AR. The crystal structure (0.66Å/100K) of the ternary complex (h-AR-NADP<sup>+</sup>-inhibitor IDD594) indicated that the visibility of H atoms in difference density maps is correlated to the B values of the corresponding bonded atoms (in the active site, 77% of H are seen when  $B < 5 \text{ \AA}^2$ ). X-ray diffraction data sets were collected at resolutions  $< 0.9 \text{ \AA}$ , at APS-SBC ID19, from He-cooled (15K) crystals of h-AR complexed with different inhibitors. A comparison with the 100 K structures indicates an overall decrease ( $1.7 \text{ \AA}^2$ ) of B values in the ordered parts of the protein. Difference density maps show clearer densities, *e.g.*, for a double conformation of the carboxylate of inhibitor IDD676 and for the double conformations of D-atoms in a proton pathway along water-Asp43-Lys77-Tyr48 for the complex h-AR(D)-NADP<sup>+</sup>-IDD594.

This work is supported by the U.S. Department of Energy, OBER, under Contract W-31-109-ENG-38.