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Complementary 2.2Å Neutron and 0.8Å X-Ray Diffraction Studies Reveal a Catalytic Proton Pathway in Fully Deuterated Human Aldose Reductase. A. Podjarny¹, A. Mitschler¹, M. Blakeley², F. Ruiz¹, S. Ginell³, M. Haertlein⁴, I. Hazemann¹, F. Meilleur⁴, A. Joachimiak³ and D. Myles², ¹IGBMC, CNRS, ULP, INSERM, Illkirch, France, ²EMBL Grenoble Outstation, ILL, Grenoble, France, ³SBC, ANL, Argonne, IL 60439 USA, ⁴ILL, Grenoble, France.

The enzymatic mechanism of human aldose reductase (h-AR) includes a hydride donation from the coenzyme NADPH and a proton donation from the enzyme. Neutron Laue diffraction data from the fully deuterated protein (h-AR(D), ILL, Grenoble) complexed with the inhibitor IDD594 and NADP⁺, were collected to a resolution of 2.2 Å at room temperature with a small crystal (0.15 mm³). The neutron density maps increased the overall observation rate of H(D) atoms from 54 % (0.66 Å X-ray data) to 61 % (neutron data). This increase is most evident for the mobile H(D) atoms (B > 5 Å² at 100K). Furthermore, the identity between the complexes of h-AR(D) and of h-AR(H) was demonstrated by a helium-cooled X-ray structure of h-AR(D) (C α rms difference = 0.1Å; 15K; SBC-19ID, resolution 0.8Å, mosaicity 0.2°, R-merge 2.3 %, R-Factor 11.5%). The h-AR(D) X-ray structure suggested a catalytic proton pathway W-Asp43-Lys77-Tyr48, which could be clearly confirmed by the neutron structure.