s3.m1.p7 **Multipolar refinement of aldose reductase at subatomic resolution.** B. Guillot, C. Jelsch, N. Muzet & C. Lecomte *LCM3B-CNRS-UHP Vandoeuvre-les-Nancy FRANCE.* E. Howard, B. Chevrier, A. Mitschler & A. Podjarny *UPR 9004, IGBMC,Strasbourg, FRANCE.* A. Cousson – *ILB, CEA Saclay, FRANCE.* R. Sanishvili & A. Joachimiak – *Biosciences Division, ANL, Argone, Illinois, USA.*

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Aldose reductase is involved in the reduction of glucose in the polyol pathway using $NADP^+$ as cofactor. This reduction leads to complications in diabetic patients like cataract or nephropathies.

The charge density study of biological macromolecules has become of major interest since subatomic resolution is now accessible for proteins. The diffraction data of aldose reductase at 0.65Å resolution [1] allow the charge density refinement.

A database of experimental multipolar parameters for all amino acids [2] has been developed at the LCM^{3}B from charge density studies of peptides. It has been shown that these parameters are transferable to macromolecules [3]. Similarly, the calculation of the multipolar parameters of the aldose reductase cofactor was necessary.

In this work, we present the multipolar refinement of the coenzyme NAD^+ against a combination of very high resolution X-rays and neutron diffraction data. NAD^{+4} is an analogue of $NADP^+$, in which the reactive part (the nicotinamide moiety) is strictly conserved. The resulting density is compared with quantum mechanic results.

The multipolar parameters obtained from this study have been transferred to the $NADP^+$ molecule present in the aldose reductase crystallographic structure, as multipolar refinement starting point. These parameters, and those transferred from the database, are refined against the high resolution data of aldose reductase, in order to determine the electrostatic potential in the active site of the enzyme.

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