

W0033

**Effect of Hydrophobic Mutations on Proton Transfer and Active Site Structure in Human Carbonic Anhydrase II.** S.Z. Fisher<sup>§</sup>, D. Bhatt<sup>¶</sup>, C.K. Tu<sup>¶</sup>, M. Agbandje-McKenna<sup>§</sup>, D.N. Silverman<sup>¶</sup>, R. McKenna<sup>§</sup>. <sup>§</sup>Dept. of Biochemistry and Molecular Biology, <sup>¶</sup>Dept. of Pharmacology and Therapeutics, College of Medicine, Univ. of Florida, Gainesville, FL, 32610.

The mammalian  $\alpha$ -carbonic anhydrases ( $\alpha$ -CAs) are ubiquitous zinc metalloenzymes that catalyze the reversible hydration carbon dioxide to form bicarbonate. The rate-limiting step in catalysis by human CA II is the transfer of a proton from the Zn-H<sub>2</sub>O to bulk solvent via the proton-shuttling residue, H64. The intra-molecular proton transfer proceeds through a well-ordered chain of H-bonded solvent molecules. The solvent molecules are coordinated through H-bonds by several active site residues and other solvent molecules. We have constructed, three single site CA II mutants, replacing 3 polar residues that assist in the solvent coordination with hydrophobic residues (Y7F, N62L, and N67L). We report the crystal structures of all 3 mutants (1.6 – 1.8 Å resolution) at different pHs (6.0- 10.0), and discuss the observed conformational changes in the active site architecture, solvent order, and subsequent measured increases and decreases in proton transfer efficiencies, as compared to wild type CA II.

