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Proton Transfer In Human Carbonic Anhydrase II: Effects Of Ph And Position Of The Proton Shuttle On The Transfer Rate. Zoë Fisher¹, Jose Hernandez¹, Chingkuang Tu², David Duda¹, Craig Yoshioka¹, Haiqian An², Lakshaman Govindasamy¹, David N. Silverman², Robert McKenna¹, ¹Depts. of Biochemistry & Molecular Biology, ²Pharmacology & Therapeutics, College of Medicine, Univ. of Florida, Gainesville, FL, 32610.

The catalysis of CO₂ hydration/dehydration reaction by human carbonic anhydrase II (HCAII) is rate limited by proton transfer steps leading from the active site to the bulk solvent. These steps involve an ordered water network and the acid-base function of the proton shuttling residue His64. To evaluate the effect of pH and position of a proton shuttling residue on proton transfer and water network organization, the structures of wild type HCA II and a double mutant (His64_Ala and Thr200_His; H64A/T200H) at various pH were determined by X-ray crystallography. The catalytic rate of the hydration/dehydration reaction, from pH 5.0 to 9.0, has also been measured using the ¹⁸O-exchange method. Crystal structures of wild type HCA II (at pH 5.1 to 10.0) and of H64A/T200H (at pH 6.0, 7.8 and 9.3) are presented along with catalytic data for the wild type a double mutant. Structural and functional comparisons of the crystal structures to the measured rates of proton transfer are discussed in terms of the requirements for efficient proton transfer in an active-site environment.