Supporting Information

Water Networks in Fast Proton Transfer During Catalysis by Human Carbonic Anhydrase II[†]

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Table S1: Steady-state constants for the hydration of CO_2 and dehydration of bicarbonate catalyzed by wild-type and Y7F-N67Q HCA II[a]

	$(k_{cat})_{hydration}$ (μs^{-1})	$^{D}(k_{cat})_{hydration}$	$(k_{cat}/K_m)_{hydration}$ ($\mu M^{-1}s^{-1}$)	$(k_{cat})_{dehyd}$ (μs^{-1})	$(k_{cat}/K_m)_{dehyd}$ ($\mu M^{-1}s^{-1}$)
wild-type HCA II	0.80 ± 0.03	3.8[b] 3.2 ± 0.4[c]	59 ± 2	0.24 ± 0.02	6.7 ± 0.2
Y7F-N67Q HCA II	0.67 ± 0.04	3.1 ± 0.3	67 ± 4	0.19 ± 0.01	8.8 ± 0.3

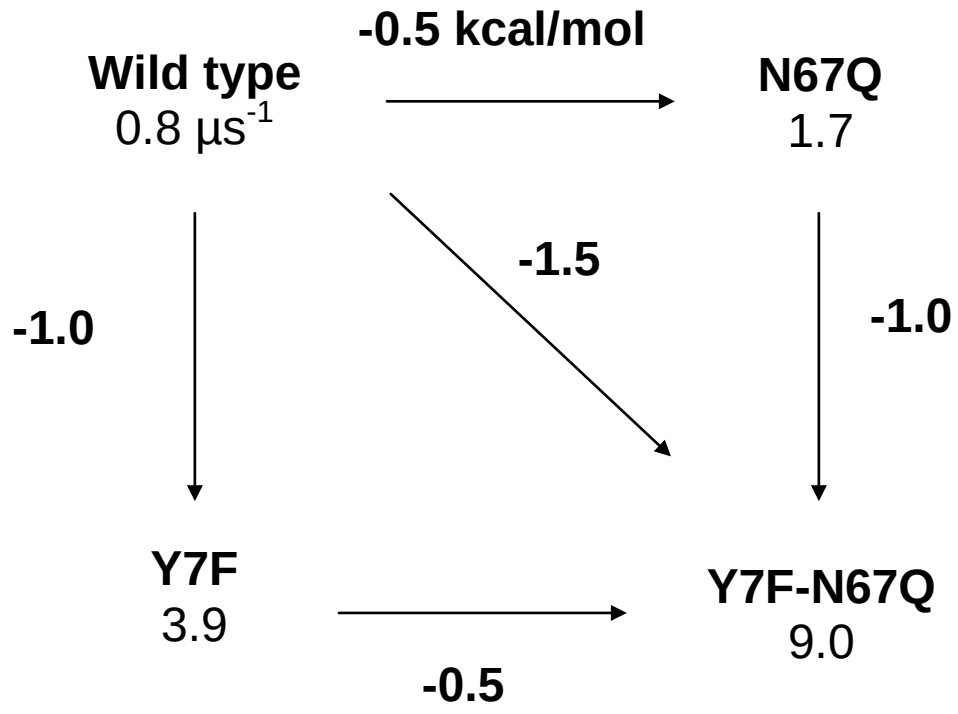
[a] Measurements of initial velocity in the hydration of CO_2 and dehydration of bicarbonate were carried out on a stopped-flow spectrophotometer (Applied Photophysics SX18.MV) using the initial 5% to 10% of the progress curve following the method of Khalifah (*Journal of Biological Chemistry 246*, 2561-2573 (1971)). The hydration experiments were performed at pH 8.4 using 0.1 to 0.2 μM enzyme, 25 mM of CHES buffer, and 70 μM thymol blue at 25 °C. The dehydration experiments were performed at pH 6.0 using 0.6 to 1.1 μM enzyme, 25 mM MES buffer, and 100 μM chlorophenol red at 10 °C. In all experiments, the ionic strength was maintained at 0.2 M using sodium sulfate. Saturated solutions of CO_2 were prepared by bubbling CO_2 gas into water at 25.0 °C (saturating concentration 33.8 mM) and diluting using two coupled, air-tight syringes.

[b] From Steiner et al., *Eur. J. Biochem.* 59, 253-259 (1975)

[c] From Venkatasubban and Silverman, *Biochemistry*, 19, 4984-4989 (1980). This measurement was made using bovine CA II.

Scheme S1

A double mutant cycle with the values of k_B (μs^{-1})(shown under the variant designation) for proton transfer obtained by ^{18}O exchange and the corresponding values of changes in estimated energy barriers (shown adjacent to arrows) computed as described by Mildvan et al. (*Archives of Biochemistry and Biophysics 294*, 327-340 (1992)).



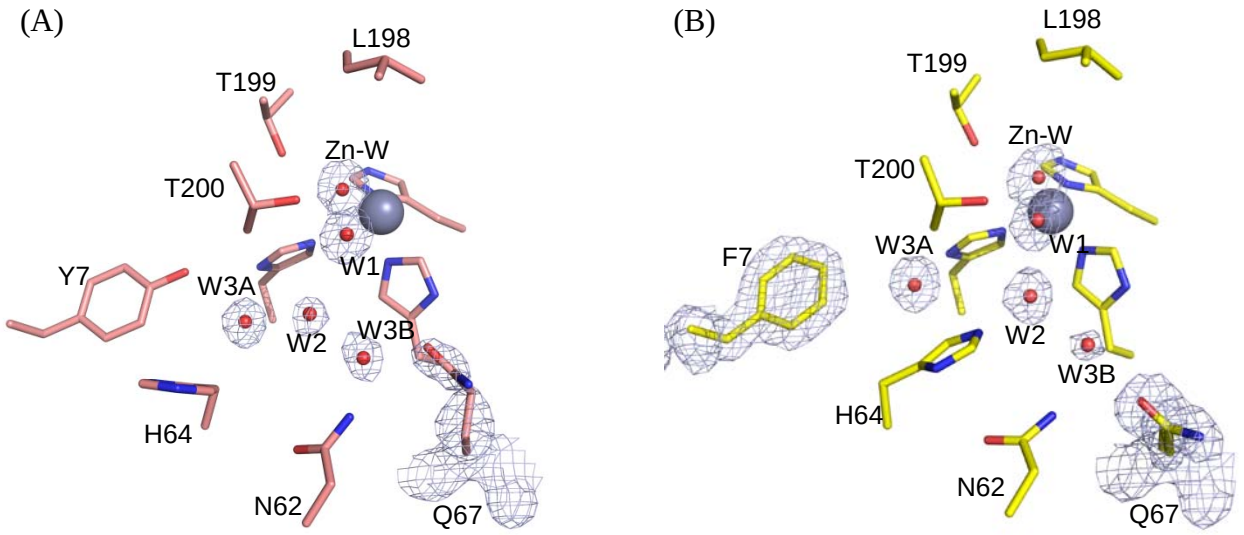


Figure S1. Active sites of variants of HCA II at pH 8.0. **(A)** N67Q HCA II, and **(B)** Y7F-N67Q HCA II. 2Fo-Fc electron density maps are contoured at 1.2σ. Figure was made using PyMOL (DeLano Scientific).