

Supplemental Information

Crystallization

Wild type human carbonic anhydrase II was expressed in *E. coli* BL21 DE3 pLysS cells. The enzyme was purified using pAMBS (para-aminomethylbenzenesulfonamide agarose) affinity resin from Sigma Aldrich as described elsewhere (Fisher *et al.*, 2005). After purification the enzyme was concentrated and buffer exchanged into 50 mM Tris pH 7.8 using Amicon Ultra centrifugation devices with 10 kDa molecular weight cut-offs. The enzyme was concentrated to 35 mg/mL prior to crystallization. Large crystals of HCA II were grown in 9-well glass plates using the Hampton sandwich box set-up. The mother liquor in the well consisted of 1.6 M sodium citrate, 100 mM Tris pH 7.8 while the solution in the drops were made of 1.3 M sodium citrate, 100 mM Tris pH 7.8. The drops were set up by mixing 250 μ L of the 35 mg/mL protein solution with 250 μ L of the drop solution. Crystals appeared in about a month and grew to a maximum size after \sim 3 months. A suitable crystal (1.7 mm³) was selected and mounted in a quartz capillary (Figure S1). Perdeuterated mother liquor was prepared and introduced as a liquid plug next to, but not touching, the crystal prior to sealing the capillary. H/D exchange was allowed to occur for 3 months before data collection started. A similar crystal from the same drop was mounted and H/D exchanged for room temperature X-ray diffraction data collection.

Data collection

X-ray diffraction data was collected to 1.65 Å resolution at room temperature with a Rigaku FR-E diffractometer. Images were recorded on an R-AXIS VI⁺⁺ detector and data were integrated and scaled using the *CrystalClear/d*TREK* software (Pflugrath, 1999). The structure refinement was performed using PHENIX (Adams *et al.*, 2010). A summary of the X-ray crystallographic data is given in Table S1. Neutron time-of-flight Laue data was collected the Protein Crystallography Station at the Los Alamos Neutron Science Center. The detector is a position sensitive ³He-filled detector and the sample was mounted on a κ -circle goniometer from Hüber. Each exposure was 16h and 29 total crystal settings were collected to a maximum usable resolution of 2 Å. The images were processed with an in-house modified version of *d*Trek* called *nTrek* and wavelength normalized using LAUENORM (Schoenborn and Langan, 2004; Helliwell *et al.*, 1989). Data were merged with SCALA from the CCP4 suite (CCP4, 2002). All data collection and refinement statistics are given in Table S1.

Structure Refinement

For the joint X-ray and neutron structure refinement, the X-ray structure was finished first using PHENIX. The structure was refined until the R-factors converged (R_{work} 17.5% and R_{free} 18.7%) (Adams *et al.*, 2010). At this point all the H atoms were included in the model, as well as the D atoms at labile positions. The refinement was then moved to nCNS, a modified version of CNS that has been designed to handle joint neutron and X-ray refinement (Brünger *et al.*, 1998, Adams *et al.*, 2009). The joint refinement in nCNS included rigid body refinement, followed by iterative rounds of energy minimization, individual B factor, and occupancy refinement. Manual model building, modeling of protonated side chains, and placing of D₂O molecules was done in Coot

(Emsley and Cowtan, 2004). All figures were rendered using Pymol (DeLano, 2002). Table S1 contains all the refinement statistics. Experimental data and coordinates have been deposited with the Protein Data Bank with accession ID 3TMJ.

Figure S1. Stick diagram of the active site of HCA II. Hydrophilic residues involved with H-bonds to the water network are as labeled. The Zn is shown as a magenta sphere, H-bonds observed in the neutron structure are shown as black dashed lines. H atoms are omitted for clarity.

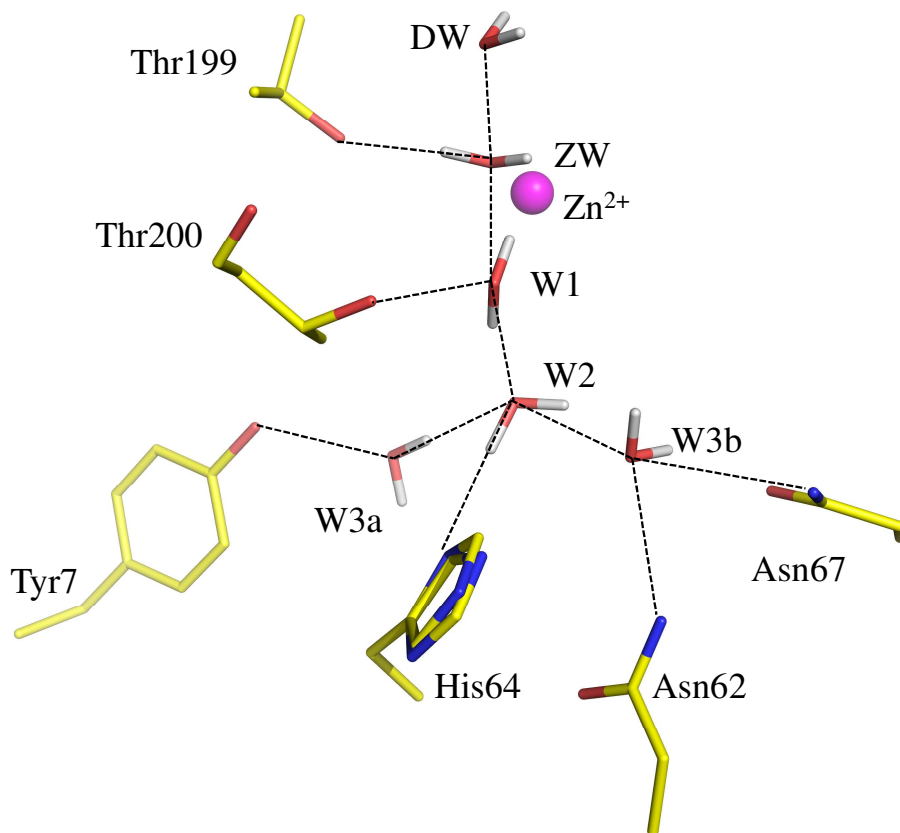


Figure S2. Optical photograph of wt HCA II crystal with a volume of $\sim 1.7 \text{ mm}^3$.



Figure S3. Alternate conformation of His64 side chain is neutral and flipped by $\sim 180^\circ$. The $2F_o-F_c$ nuclear density map is shown in lilac (contoured at 1.5σ) with the omit F_o-F_c maps in yellow (contoured at 4.0σ), indicating the missing D nuclear density for W2. Waters are as labeled.

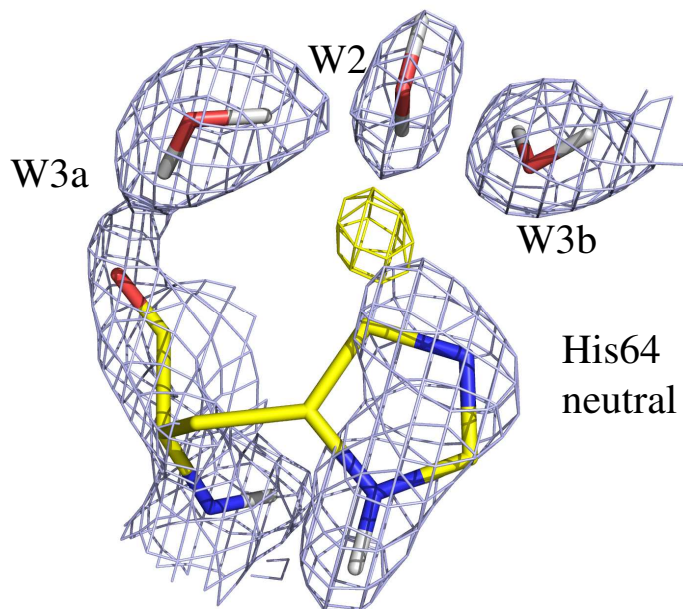


Figure S4. Hydrogen bonded water network in the active site of HCA II at pH 7.8. Dashed lines indicate observed H-bonds, waters are as labeled. $2F_o-F_c$ nuclear density is shown in green and is contoured at 1.5σ . D atoms are shown in white.

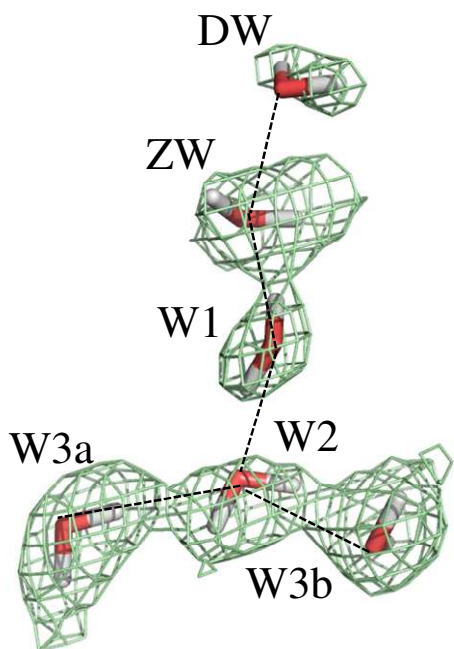


Table S1. X-ray and neutron crystallographic data collection and refinement statistics.

	X-ray	Neutron
Source	Rotating Cu Anode	LANSCE, PCS
Wavelength	1.54	0.6 - 6.7
Unit cell (Å, °)	$a=42.8, b=42.7,$ $c=72.9, \beta=104.6$	(same)
Space group	P2 ₁	(same)
Settings	360	29
Resolution (Å)	35.0 - 1.65 (1.71 - 1.65)	20.0 - 2.0 (2.11 - 2.00)
No. of reflections (unique)	81941 (27915)	33811 (14236)
Redundancy	2.94 (1.67)	2.4 (1.7)
Completeness (%)	92.3 (71.5)	84.2 (69.5)
Mean I/σ(I)	23.1 (4.0)	5.2 (3.3)
R_{p.i.m.}	n/a	16.7 (31.8)
R_{merge}	3.5 (14.7)	23.3 (38.4)
JOINT R_{cryst}/R_{free}	17.5 (18.7)	27.6 (29.7)

References

- 1) Fisher, S.Z., Hernandez-Prada, J., Tu, C.K., Duda, D., Yoshioka, C., An, H., Govindasamy, L., Silverman, D.N., McKenna, R. (2005) Structural and kinetic characterization of active-site histidine as a proton shuttle in catalysis by human carbonic anhydrase II. *Biochemistry* **44**, 1097–1105.
- 2) Pflugrath, J.W. (1999) The finer things in X-ray diffraction data collection, *Acta Cryst.* **D55**, 1718-1725.
- 3) Adams, P.D., Afonine, P.V., Bunkóczi, G., Chen, V.B., Davis, I.W., Echols, N., Headd, J.J., Hung, L.-W., Kapral, G.J., Grosse-Kunstleve, R.W., McCoy, A.J., Moriarty, N.W., Oeffner, R., Read, R.J., Richardson, D.C., Richardson, J.S., Terwilliger, T.C., Zwart, P.H. (2010) PHENIX: a comprehensive Python-based system for macromolecular structure solution, *Acta Cryst.* **D66**, 213-221.
- 4) Brünger A.T., Adams, P.D., Clore, G.M., DeLano, W.L., Gros, P., Grosse-Kunstleve, R.W., Jiang, J.-S., Kuszewski, J., Nilges, M., Pannu, N.S., Read, R.J., Rice, Simonson, T., Warren, G.L. (1998) Crystallography and NMR system: A new software suite for macromolecular structure determination. *Acta Cryst D***54**, 905-921.
- 5) Adams, P.D., Mustyakimov, M., Afonine, P.V., Langan, P. (2009) Generalized X-ray and neutron crystallographic analysis: more accurate and complete structure for biological macromolecules. *Acta Crystallogr.* **D65**, 567-573.
- 6) Schoenborn, B.P., Langan, P. (2004) Protein Crystallography with spallation neutrons, *J. Synchrotron Radiat.* **11**, 80-82.
- 7) Helliwell, J.R., Habash, J., Cruickshank, D.W.J., Harding, M.M, Greenhough, T.J., Campbell, J.W., Clifton, I.J., Elder, M., Machin, P.A., Papiz, M.Z., Zurek, S. (1989) The recording and analysis of synchrotron X-radiation Laue diffraction photographs. *J. Appl. Cryst.* **22**, 483-497.
- 8) Collaborative Computational Project, Number 4. (1994) *Acta Cryst.* **D50**, 760-763.
- 9) Adams, P.D., Mustyakimov, M., Afonine, P.V., Langan, P. (2009) Generalized X-ray and neutron crystallographic analysis: more accurate and complete structure for biological macromolecules. *Acta Crystallogr.* **D65**, 567-573.
- 10) Emsley, P., Cowtan, K. (2004) Coot: model-building tools for molecular graphics. *Acta Crystallogr.* **D60**, 2126-2132.
- 11) DeLano, W.L. The PyMOL Molecular Graphics System (2002) DeLano Scientific, San Carlos, CA, USA. <http://www.pymol.org>.