Supporting Information

A Short, Strong Hydrogen Bond in the Active Site of Human Carbonic Anhydrase II

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Materials and methods

Expression and Purification of HCA II. The plasmid encoding HCA II was transformed into E.coli BL21 cells through standard procedures and the transformed cells were expressed at 37 ºC in LB medium containing 100 μg/ml ampicillin (*1*). HCA II production was induced by the addition of isopropyl thiogalactoside to a final concentration of 1mM at an $O.D_{600}$ of 0.6 AU. The cells were harvested 4 hrs after induction. The cell pellets were lysed and HCA II was purified through affinity chromatography using pAMBS resin as has been described elsewhere (*2*).

Crystallization and X-ray data collection of HCA II. Crystals of HCA II were obtained using the hanging drop vapor diffusion method (*3)*. Ten μl drops of equal amounts of protein and precipitant were equilibrated against precipitant solution (1.3 M sodium citrate; 50mM Tris-HCl; pH 7.0) at room temperature (~20 ºC) (*4)*. A crystal was cryoprotected by quick immersion into 20% glycerol precipitant solution and flash-cooled by exposing it to a gaseous stream of nitrogen at 100K. X-ray diffraction data were collected at the Cornell High Energy Synchrotron Source (CHESS) F1 Station with the wavelength of 0.9173 Å. Quantum 270 was used and the distance was 100 mm to allow 1 degree oscillation without spot overlapping. To cover high resolution spots, the detector was offset (moved upwards). Indexing, integration, and scaling were performed using HKL2000 (*5)*.

Structure determination of HCA II. The crystal structure of HCA II (PDB accession code: 2ILI) (*4)* was used to obtain initial phases of the apo-structure using SHELX97 (*6)*. The zinc and all solvent molecules were removed to avoid model bias. 5% of the unique reflections were selected randomly and excluded from the refinement

data set for the purpose of R_{free} calculations (*7)*. Structural refinement proceeded using SHELXL initially with data from 50.0 to 2.0 Å resolution. The protein geometry was defined using the default constrains of conjugate-least squares (CGLS) mode in SHELXL. Each round of CGLS comprised of 15 cycles of refinement. $2F_o–F_c$ and $F_o–F_c$ electron density Fourier difference maps were calculated after each successive round of CGLS and manually inspected by the graphics program COOT (*8)* for further fine-tuning of the model and the incorporation of solvent molecules. After some initial rounds of CGLS refinement, the resolution was extended to 0.9 Å and subsequently after several more cycles of refinement, the model was further subjected to several cycles of full anisotropic refinement and hydrogen riding which led a convergence of R_{cryst} and R_{free} to 12.5 and 13.1, respectively. The geometry of the final model was checked using the PROCHECK algorithm (*9)*. The RMSD for bond lengths and bond angles were found to be within accepted limits of 0.004 Å and 1.0°, respectively. It was observed that 89 % of the dihedral angles were in the most favored region while the rest were in the allowed region with the exception of 0.5 % which were in the generously allowed region. The geometry and statistics of the final model are summarized in Table S1.

Table S1. Refinement and model statistics for HCA II

Unit cell dimensions, *a, b, c* (Å), β (°)	42.2,41.3,72.2,104.2
Resolution (Å)	50 – 0.9 (0.92-0.9)
Number of unique Reflections	164840 (6751)
Completeness (%)	92.3(76.1)
Redundancy	6.1 (2.8)
R_{symm}[a] I/σ(I)	7.8 (58.0) 25.0 (2.0)
R_{cryst}[b] /R_{free}[c]	12.5 / 13.1
Ramachandran statistics (%) Most favored, additionally allowed and generously allowed regions	89.0, 10.5, 0.5
Number of protein/solvent atoms	2327/487
Average B factors(Å^2) main/side chain Zn/solvent	 10.9/15.2 6.5/26.0

Values in parentheses refer to the highest resolution bin. [a] $R_{symm} = \Sigma |I - \langle I \rangle| / \Sigma \langle I \rangle \times 100$. [b] $R_{cryst} = \Sigma ||Fo| - |Fc|| / \Sigma |F_{obs}| \times 100$. [c] R_{free} is calculated in same manner as R_{cryst}, except that it uses 5% of the reflection data omitted from refinement.

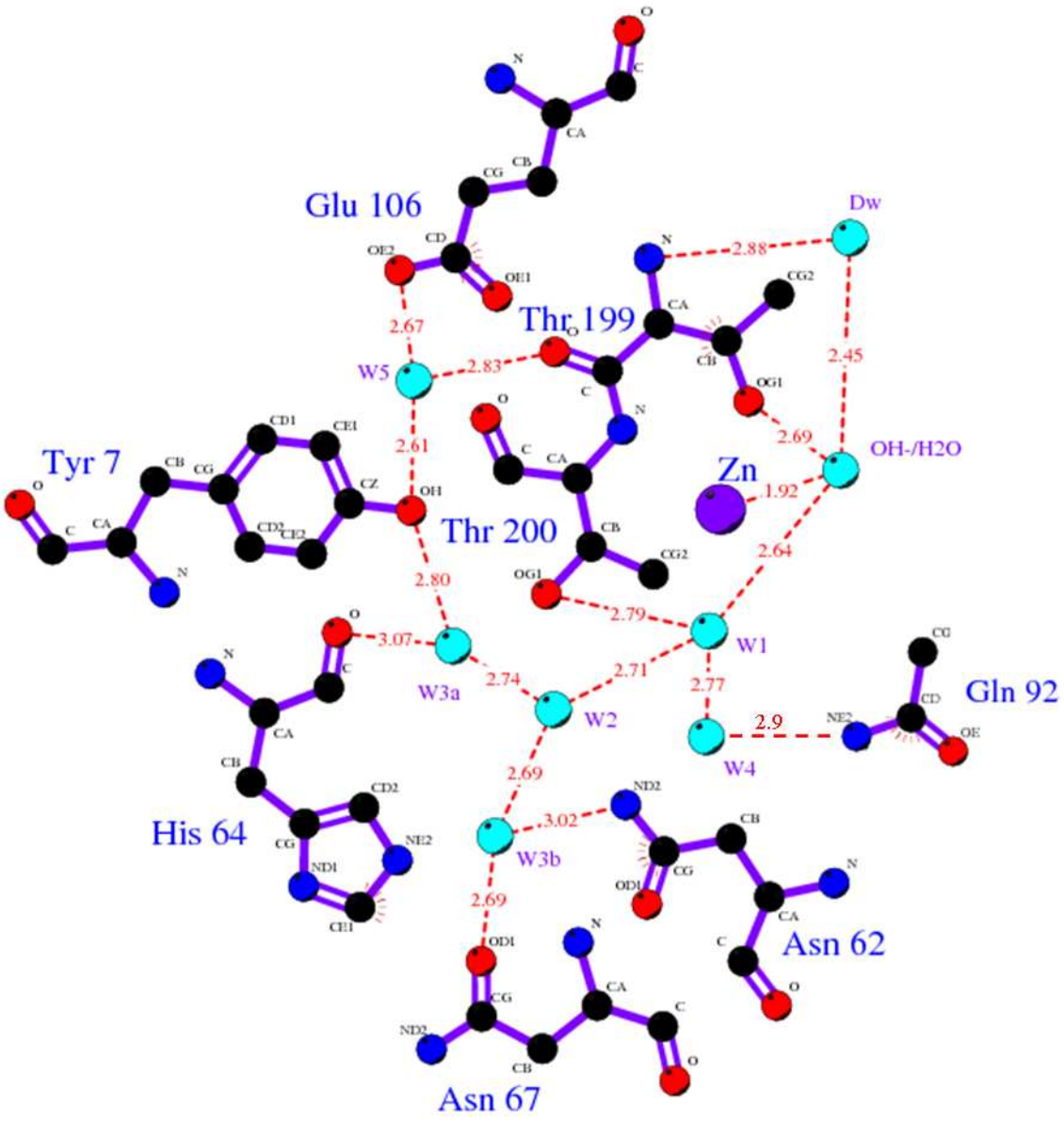

Figure S1. Two dimensional representation of Figure 1. Bond lengths of the oxygen atoms in their respective hydrogen bonding partners are given. Note: The figure is not to scale. Figure created using Ligplot.

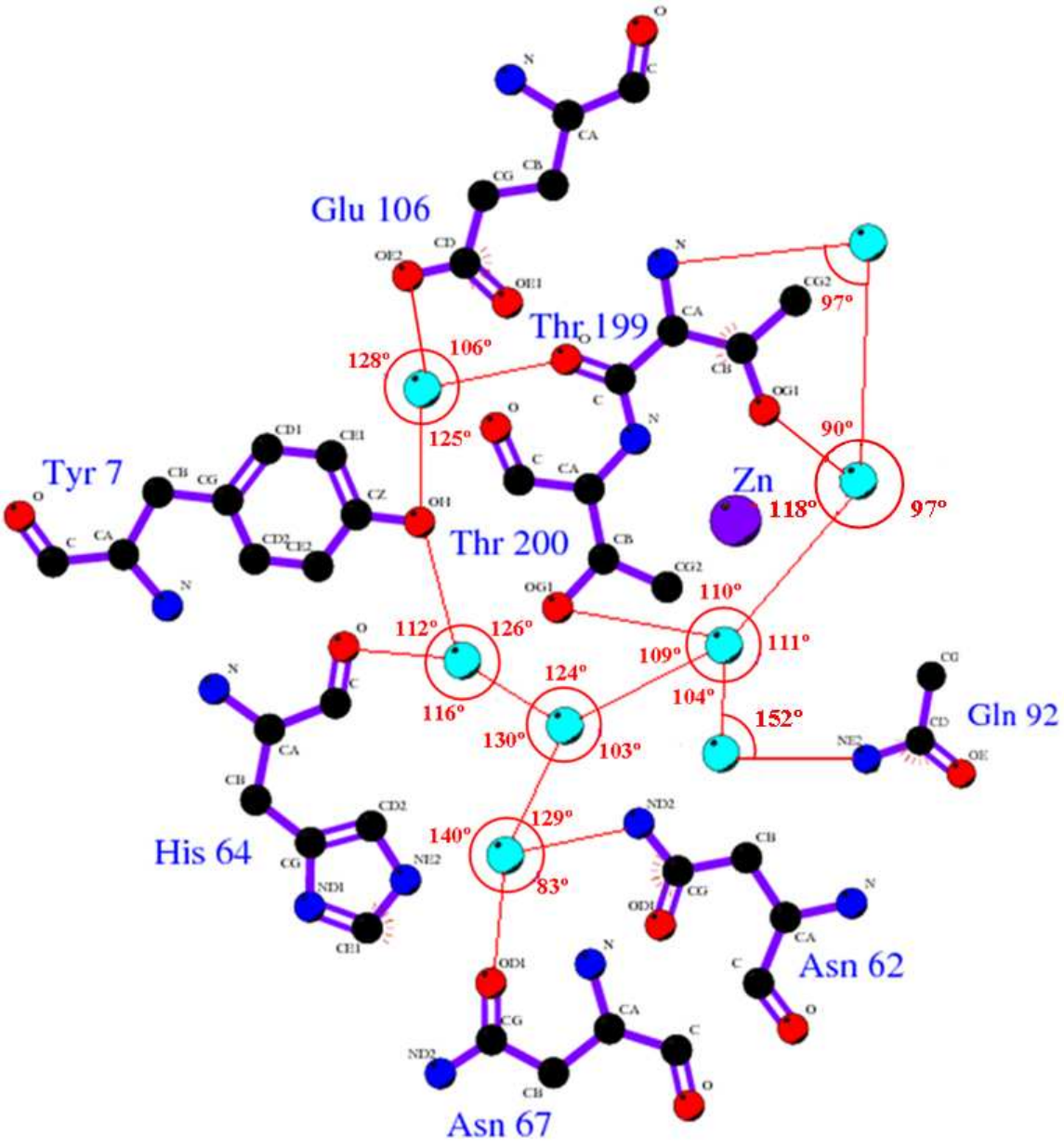

Figure S2. Two dimensional representation of Figure 1. Bond angles of the oxygen atoms to their respective hydrogen bonding partners are given. Note: The figure is not to scale. Figure created using Ligplot.

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