Supplemental Data

Origins of Enhanced Proton Transport in the Y7F Mutant of Human Carbonic Anhydrase II

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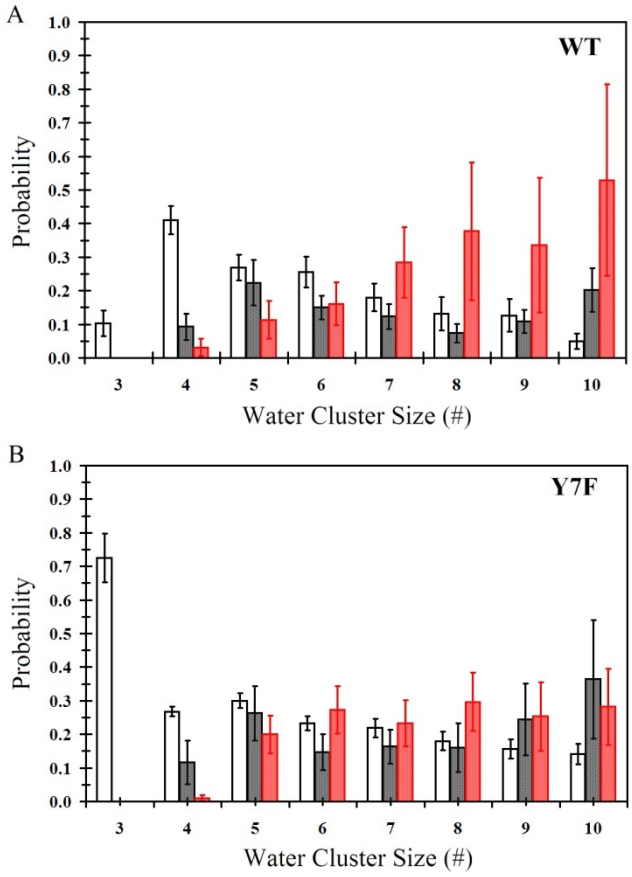
Continuous hydrogen bonded water wire probabilities.



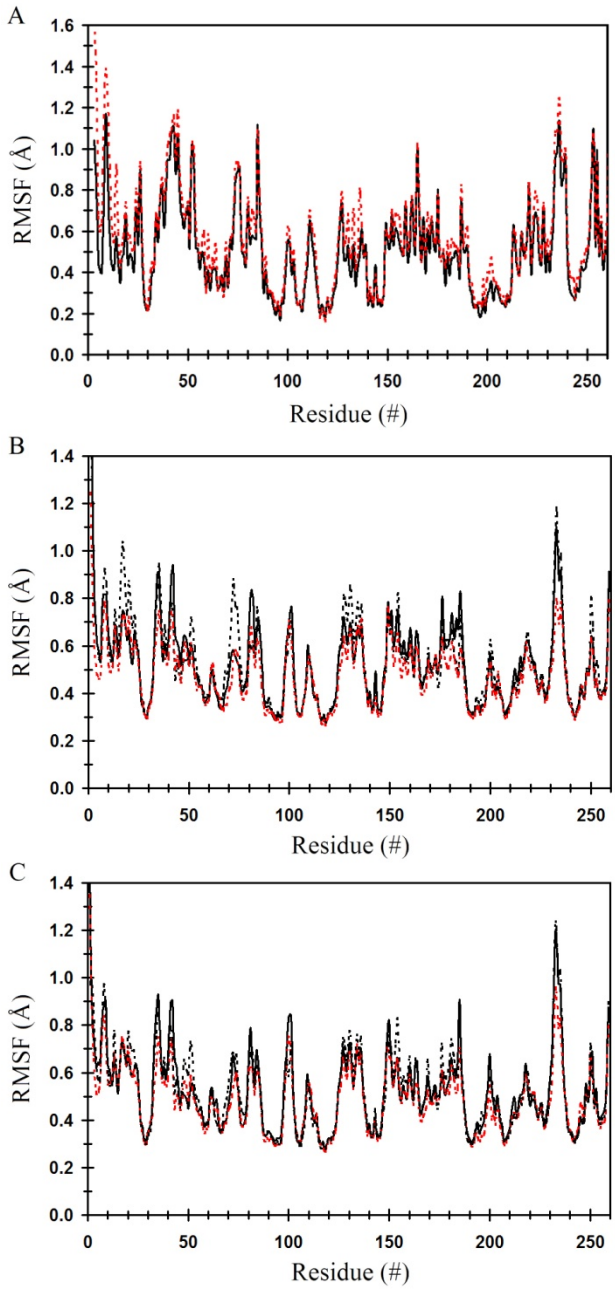
Supplemental Figure 1: Total probability of a continuous hydrogen bonded water wire that connects the zinc bound water/hydroxide and the protonated/unprotonated N_δ of His64. (A) Water wire distribution for WT-ZnH_2O^{2+}-His in the "in" orientation (black no filling), WT-ZnH_2O^{2+}-His in the "out" orientation (black with black filling), WT-$ZnOH^{+}$-$HisH^{+}$ in the "out" orientation (red with red filling). (B) Water wire distribution for Y7F-ZnH_2O^{2+}-His in the "in" orientation (black no filling), Y7F-ZnH_2O^{2+}-His in the "out" orientation (black with black filling), Y7F-$ZnOH^{+}$-$HisH^{+}$ in the "out" orientation (red with red filling).

His64 orientational discrepancies between X-ray data and MD simulations.

There is an apparent discrepancy between the MD simulation data and a recent X-ray structure for Y7F, which suggests that His64 exist only in the "in" orientation.[1] Inspection of the orientation of

His64 in the Y7F crystal structure (Protein Data Bank reference 2NXR)[1] gives a $\chi 1$ dihedral of 50°. The computational data, while clearly indicating an almost 50/50 distribution of the "in" and "out" orientations, has a local minimum in the "in" orientation with a $\chi 1$ dihedral of 53°. This close agreement in minima values suggests that even though the computational data does not predict the "in" orientation to be more favorable, it does sample the $\chi 1$ dihedral configurational space indicated by the X-ray structure. A possible explanation for the discrepancy between experimental and computational results could be due to the MD simulaton being based on a mutation of the 2CBA X-ray structure instead of from the actual Y7F HCA II mutant, 2NXR. However, in order to evaluate the differences between the 2CBA, 2NXR, and simulation results root mean square fluctuation (RMSF) plots were constructed (Supplemental Figure 2). RMSF analysis indicates that there exist minimal fluctuation differences between 2CBA and 2NXR X-ray structures. In addition comparisons of the computational simulations and X-ray data do not indicate any significance fluctuation differences that would implicate an underlying methodological problem with using a mutant of 2CBA instead of the 2NXR structure. Another possible explanation for the difference in experimental and computational results could be due to the differing pH values, 7 and 8.2, for the computational and X-ray systems, respectively. Finally, the MD simulation is carried out for the fully solvated enzyme, while the X-ray results are for a crystal structure.

(1) Fisher, S. Z., Tu, C., Bhatt, D., Govindasamy, L., Agbandje-McKenna, M., McKenna, R., and Silverman, D. N. *Biochemistry* **2007**, *46*, 3803-3813.



Supplemental Figure 2: Root mean square fluctuation (RMSF) of the WT HCA II and the mutant Y7F. (A) RMSF converted from β value for WT HCA II enzyme using the 2CBA X-ray crystal structure (black) and the Y7F HCA II enzyme using the 2NXR X-ray crystal structure (red dotted)(shifted down 1.4 RMSF units for better comparison). (B) RMSF for WT-ZnH_2O^{2+}-His in the "in" orientation (black), WT-ZnH_2O^{2+}-His in the "out" orientation (black dotted), WT-$ZnOH^{+}$-$HisH^{+}$ in the "out" orientation

(red dotted). (C) RMSF for Y7F-ZnH_2O^{2+}-His in the "in" orientation (black), Y7F-ZnH_2O^{2+}-His in the "out" orientation (black dotted), Y7F-$ZnOH^{+}$-$HisH^{+}$ in the "out" orientation (red dotted).

Complete Ref. 51.

(51) Case, D. A., Pearlman, D.A., Caldwell, J. W., Cheatham, T. E. III, Wang, J., Ross, W. S., Simmerling, C. L., Darden, T. A., Merz, K. M., Stanton, R.V., Cheng, A. L., Vincent, J. J., Crow-ley, M., Tsui, V., Gohlke, H., Radmer, R. J., Duan, Y., Pitera, J., Massova, I., Seibel, G. L., Singh, U. C., Weiner, P. K., and Kollman, K. A.; 7 ed.; University of California: San Francisco., 2002.