Supplementary Information to

Molecular dynamics study of human carbonic anhydrase II in complex with Zn^{2+} and acetazolamide on the basis of all-atom force field simulations

Thierry O Wambo, Liao Y Chen, Stanton F McHardy, and Andrew Tsin

This supplementary information (SI) has two sections.

In Section 1, we provide additional figures to the main text. The first set of figures is to complement the hydration free energy of zinc ion, and the second set contains full information about the fluctuations of N1, N4 and Zn^{2+} atoms at the bound state.

In Section 2, we present the details of the MD simulations study for the neutral AZM and the results.

1. Additional figures

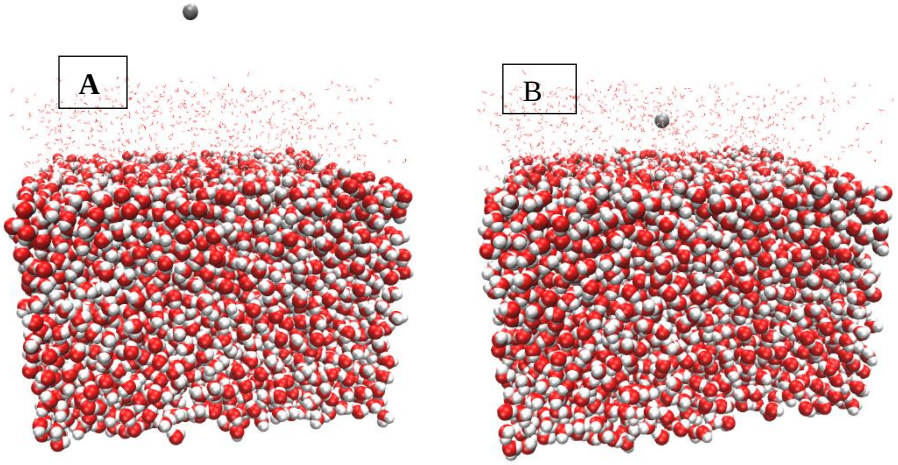

Figure S1. Front view of the solvation box. Zinc ion is positioned outside the water box at the cutoff limit of vdW interactions (A), and is fully solvated (B). Oxygens are in red, Zinc ion in green and hydrogens in white. vdW representation is used in A for all atoms. While in B and for clarity purposes, water molecules are drawn in vdW representations for z< -20 Å, and with tiny sticks for z>= -20 Å. Zinc ion is represent as gray sphere, Oxygen in red and hydrogen in white. Pictures are not drawn with the same scale.

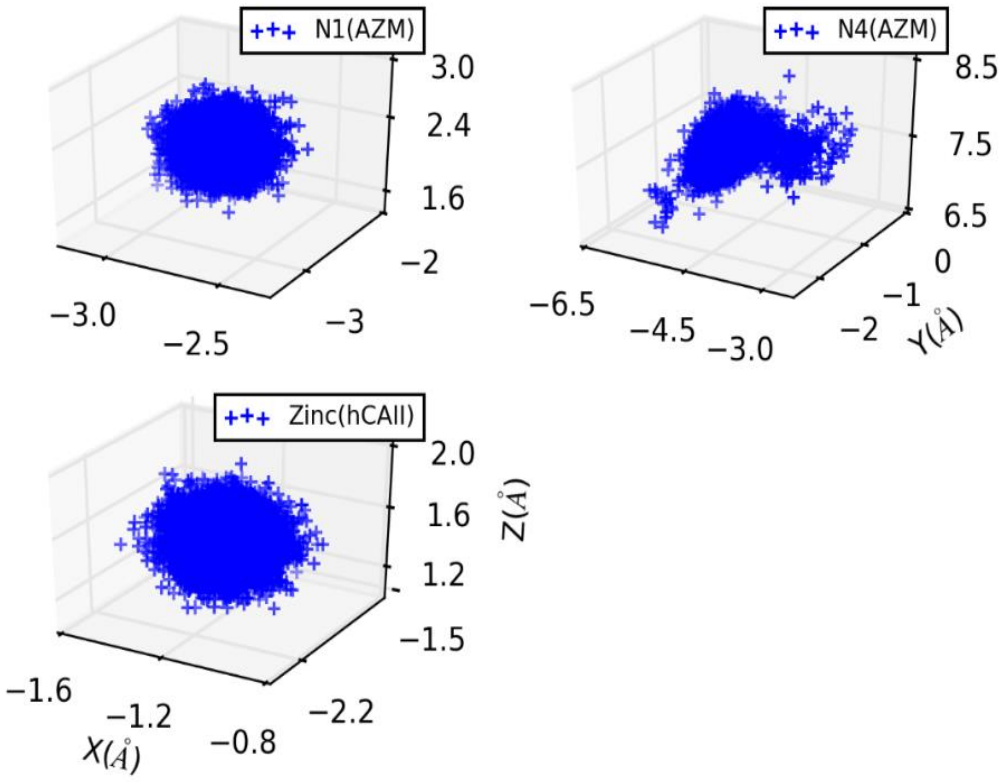

Figure S2. Fluctuations of atoms N1, N4 and Zn^{2+} at the bound state.

2. Human Carbonic anhydrase II (hCAII) in complex with neutral AZM.

Starting from the X-ray crystallographic structure of human carbonic anhydrase II in complex with acetazolamide (AZM) of Robbin et al. We chose the charged state of acetazolamide to be neutral (state A, figure 2 in main text). The opening of the protein-ligand complex thus obtained is aligned along the z-axis. We solvated the complex in a 80x80x110 $(Å)^3$ water box with TIP3P water molecules and ionized with 100mM of sodium sulfate. 10mM of Tris Chloride are added as buffer. Parameters for sulfate ion are from Cannon et al[1]. To ensure charge neutrality of the entire system few more chloride ions are added. The resulting system has a total of 66648 atoms and the starting conditions for MD simulations are similar to experimental conditions of ref [2].

The starting model was subject to an energy minimization to remove possible bad contacts between atoms and was subsequently equilibrate in an isobaric isothermal ensemble for 25ns using NAMD with CHARMM36 force field. Carbons alpha (CAs) far away from the binding site were kept fixed for the entire in silico experiment. Temperature are kept at 293 K and pressure at 1 atm using the Nose-Hoover Langevin pressure control feature [3, 4] of NAMD. Electrostatic interactions between atoms are computed with Particle Mesh Eward(PME) algorithm using a grid of 128x128x128. Nonbonded interactions were updated every 2fs and full electrostatic interactions between atoms every 4fs. Two atoms of AZM (S1 and N4) were pulled at a constant speed of 2.5Å/ns along z-axis.

To compute the free energy of binding of AZM to hCAII, We used the following formula derived from hSMD[5].

$$\Delta G_b = \underbrace{W(r_{10}, r_{20}) - W(r_{1\infty}, r_{2\infty})}_{\Delta PMF} - k_B T \ln(c_0 Z_{20} / Z_{2\infty}) \qquad (1)$$

ΔPMF is the reversible work needed to steer the ligand (AZM in this case) from the bound state to the dissociate state, Z_{20} and $Z_{2\infty}$ are the partial partition of atoms S1 and N4 of AZM in the bound and dissociate states, and k_B the Boltzmann factor. The fluctuations in the bound state of atoms N1 and S4 are shown in Figure S4.

Using the above formula we found a potential of mean force of -11.5 kcal/mol (Figure S3) and a binding affinity of -2.45 kcal/mol for the neutral AZM. Table S1 bellow provides the quantity of each variable in equation 1.

Table S1. Free energy of binding of neutral AZM to hCAII.

$\Delta PMF \ (kcal/mol)$	-11.5
$Z_{20} \ (A°)^6$	0.018
$Z_{2\infty} \ (A°)^3$	62.21
$\Delta G_b \ (kcal/mol)$	-2.45
$k_D \ (mM)$	15.03

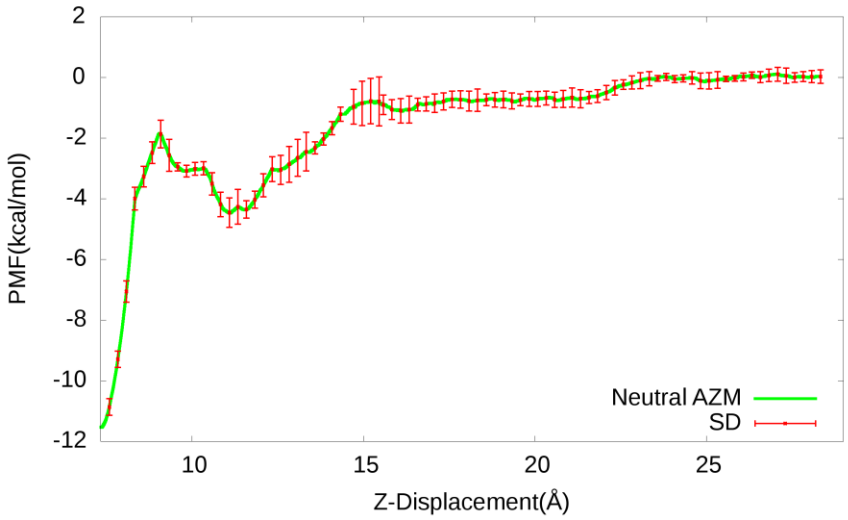

Figure S3. PMF of pulling neutral AZM along the z-direction. We used set 1 parameter for zinc ion.

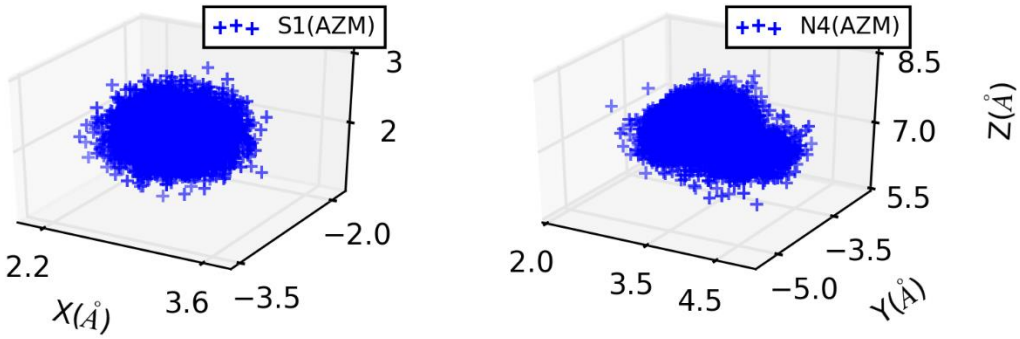

Figure S4. Fluctuations of S1 and N4 of neutral AZM in the bound sate.

References

1. Cannon, W.R., B.M. Pettitt, and J.A. McCammon, *Sulfate Anion in Water: Model Structural, Thermodynamic, and Dynamic Properties.* The Journal of Physical Chemistry, 1994. **98**(24): p. 6225-6230.
2. Vullo, D., et al., *Carbonic anhydrase inhibitors: inhibition of the tumor-associated isozyme IX with aromatic and heterocyclic sulfonamides.* Bioorganic & Medicinal Chemistry Letters, 2003. **13**(6): p. 1005-1009.
3. Martyna, G.J., D.J. Tobias, and M.L. Klein, *Constant pressure molecular dynamics algorithms.* The Journal of Chemical Physics, 1994. **101**(5): p. 4177-4189.

4. Feller, S.E., et al., *Constant pressure molecular dynamics simulation: The Langevin piston method.* The Journal of Chemical Physics, 1995. **103**(11): p. 4613-4621.
5. Chen, L.Y., *Hybrid Steered Molecular Dynamics Approach to Computing Absolute Binding Free Energy of Ligand–Protein Complexes: A Brute Force Approach That Is Fast and Accurate.* Journal of Chemical Theory and Computation, 2015. **11**(4): p. 1928-1938.