

Supplementary Information:

Kinetics of CO₂ Diffusion in Human Carbonic Anhydrase: A Study by Molecular Dynamics Simulation and the Markov-State Model

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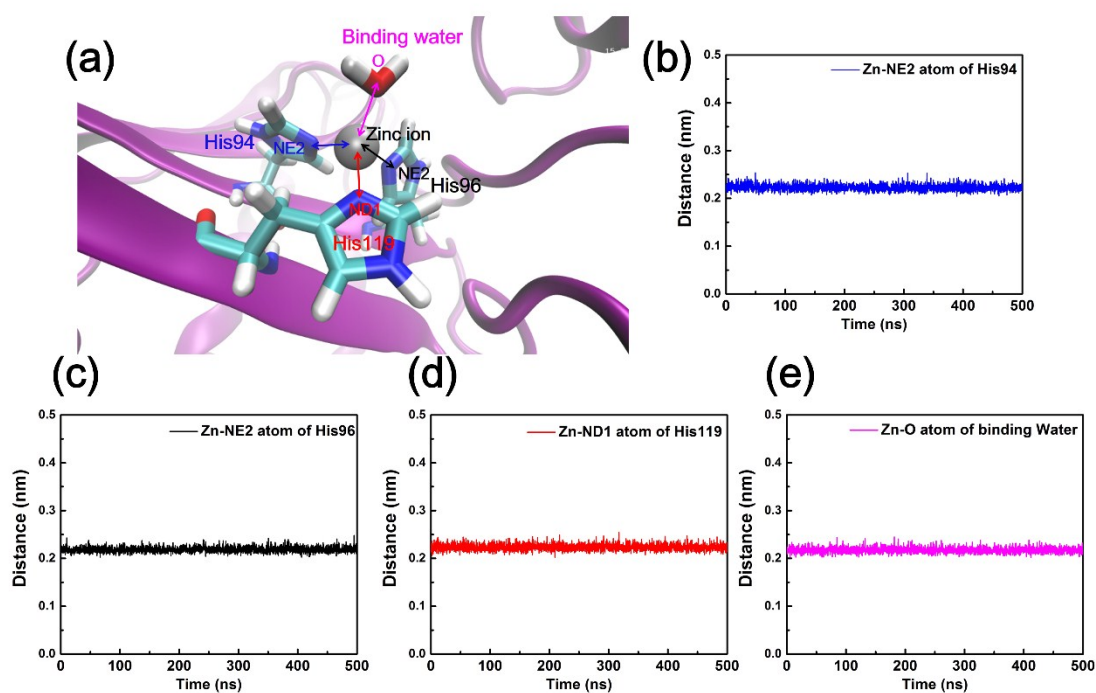


Fig. S1 (a) A snapshot of the zinc complex in HCA-II; Evolution of the distances from the zinc ion to (b) the nitrogen atom at His⁹⁴ (NE2); (c) to the nitrogen atom at His⁹⁶ (NE2); (d) to the nitrogen atom at His¹¹⁹ (ND1);(e) and to the oxygen atom of the bound water.

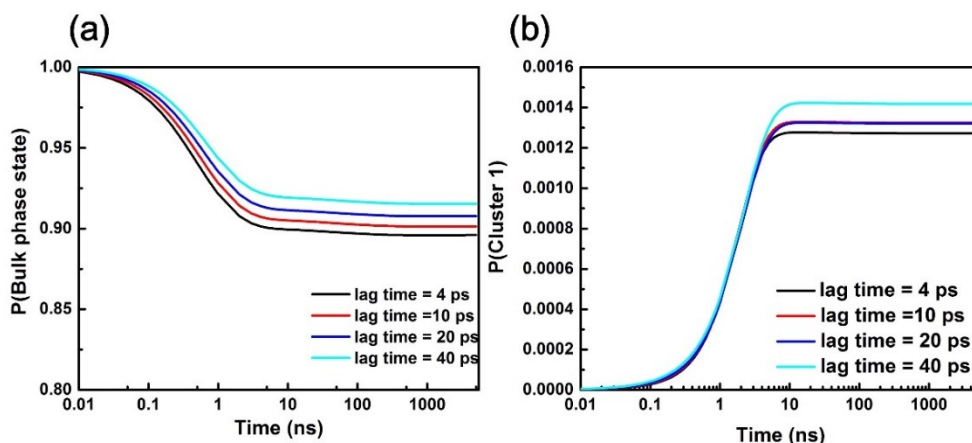


Fig. S2 The probability of the Markov state corresponding to the bulk phase (a) and that corresponding to cluster 1 (b) calculated with different lagtimes (4, 10, 20, 40 ps).

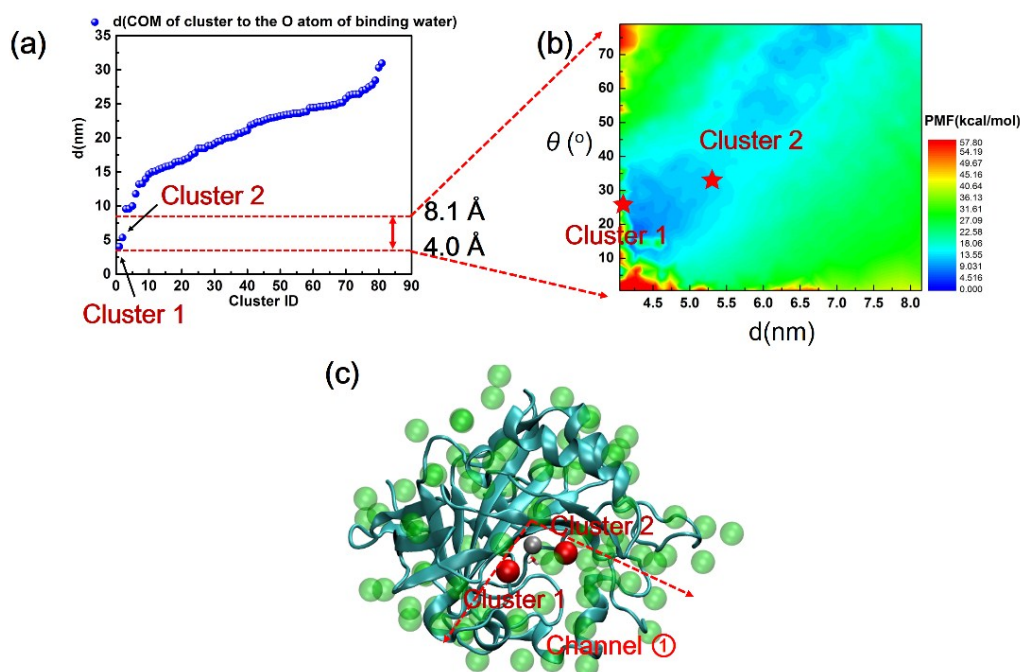
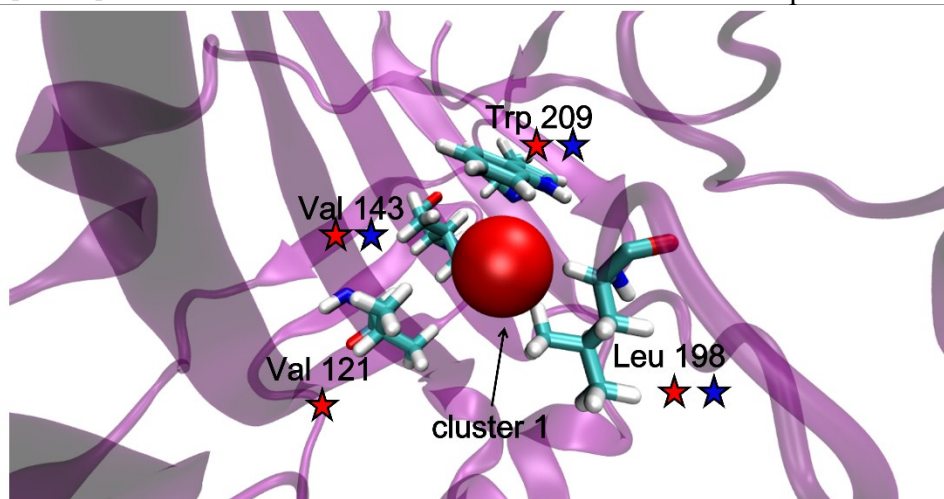


Fig. S3 (a) COM of all clusters to the oxygen atom of the bound water; (b) Cluster 1 and cluster 2 are mapped on the free energy landscape in Fig. 2 of manuscript according to the reaction coordinate d , other Markov states could not be mapped on the free-energy landscape because this free energy landscape only samples the part of region of channel 1: d from 4.0 Å to 8.1 Å; (c) snapshots of clusters and hCA-II, cluster 1 and cluster 2 are in red, others are rendered as green spheres.

Table S1 The amino acid composition of cluster 1 to 10 and experimentally found hydrophobic pocket of CO₂ in [1]

Cluster ID	Amino acids
1	Val ¹⁴³ Leu ¹⁹⁸ Trp ²⁰⁹
2	His ⁶⁴ Ala ⁶⁵ His ⁹⁶ Thr ²⁰⁰
3	Trp ⁵ Gly ⁶ Pro ²⁰¹

4	Asn ⁶²
5	Trp ⁵
6	Tyr ⁷ Pro ¹³ Trp ²⁴⁵ Arg ²⁴⁶
7	Trp ¹²³ Phe ¹³¹ Ala ¹³⁴ Val ¹³⁵ Gly ¹⁴⁰ Leu ¹⁴¹
8	Trp ¹⁶ Asp ¹⁹ Phe ²⁰ Pro ²⁰¹
9	Tyr ⁷ Val ²⁴² Asp ²⁴³
10	Leu ¹⁴⁸ Val ²²³ Phe ²²⁶
Hydrophobic pocket for CO ₂ ^[1]	Val ¹²¹ Val ¹⁴³ Leu ¹⁹⁸ Trp ²⁰⁹



- ★ Experimentally found amino acids of hydrophobic pocket for CO₂ in active site of hCAII
- ★ Composition of cluster 1, definition of composition of each cluster is defined in the main text

Fig. S4 Snapshots of cluster 1 and Val¹²¹, Val¹⁴³, Leu¹⁹⁸, Trp²⁰⁹ in hCA-II, red star marks the experimentally found amino acids of hydrophobic pocket for CO₂ in active site of hCA-II^[1], blue star marks the composition of cluster 1.

References

1. Domsic, J.F., et al., *Entrapment of carbon dioxide in the active site of carbonic anhydrase II*. Journal of Biological Chemistry, 2008. **283**(45): p. 30766-30771.