

Mechanism of a Novel Metal-Free Carbonic Anhydrase

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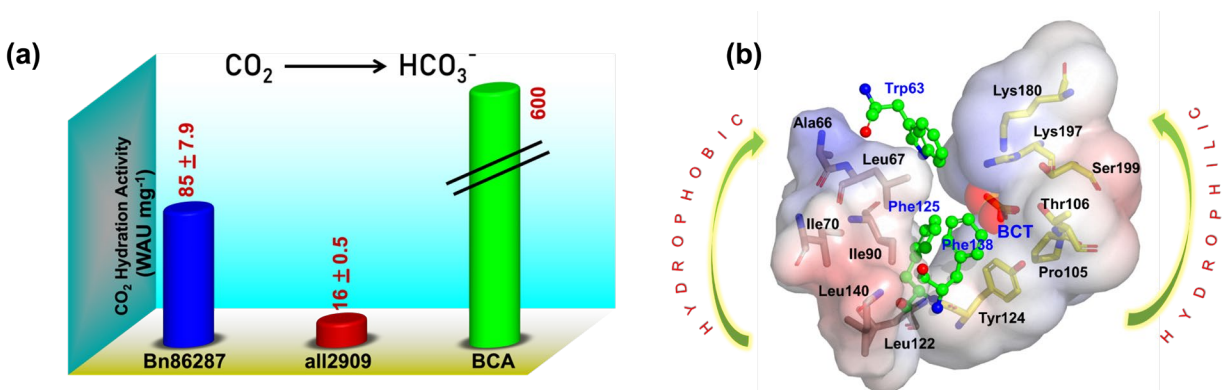


Figure S1: (a) Catalytic activity of CO₂ hydration compared between metal-free CA Bn86287, all2909 and metal dependent bovine carbonic anhydrase. (b) Bipolar nature of the enzyme active site for all2909 (PDB id: 7C5V).

Note that Wilbur-Anderson unit (WUA) is specifically used to measure the activity of carbonic anhydrase enzyme. It is defined as given below:

$$\text{WUA} = (t_0/t) - 1$$

Where, t_0 and t refer to the time taken in the absence and presence of the enzyme, respectively. Higher WUA values directly indicate high activity of the enzyme carbonic anhydrase (CA), reflecting a high turnover number (k_{cat}) and high efficiency.

S.1. Adaptive Molecular Dynamics Simulation Methodology:

To ensure the efficient catalysis in metal free CA enzyme, the exact entrapment zone of tiny CO₂ substrate is important. To explore the feasible route for CO₂ movement, we employed adaptive steered molecular dynamics (ASMD) simulations. For doing so, following the equilibration phase, a pulling force was employed in a predetermined direction and conducted 20 iterative simulations of ASMD. This force was generated by connecting a spring between the center of mass, represented by the 'C' atom of CO₂, and a designated 'OG' atom of Ser586, which were present at the distance of ~3 Å. For doing so we have used a pulling spring force constant (f_0) 10 kcal/mol/Å² with a speed of 1 Å/ns for 320 ns to facilitate the movement of the CO₂ molecule from the hydrophilic zone to the hydrophobic zone. We conducted adaptive steering in 16 stages, progressing along the reaction coordinate from 3 Å to 19 Å up to 320 ns. The process continued

until the substrate CO₂ was completely outside of the hydrophilic zone. Finally, we calculated the potential mean force (PMF), from entire 320 ns of simulation which characterizes the free energy change along a reaction coordinate during different intermediate stages of a CO₂ transition using the Jarzynski's equation.

$$\langle \exp(-\beta W) \rangle = \exp(-\beta \Delta G)$$

In the above equation, $\langle \rangle$ denotes the ensemble average, $\beta = (k_B T)^{-1}$ (k_B is Boltzmann constant and T is temperature; here 300K), W is the work done on the system during a non-equilibrium process, and ΔG is the difference in free energy between two equilibrium states of the system.

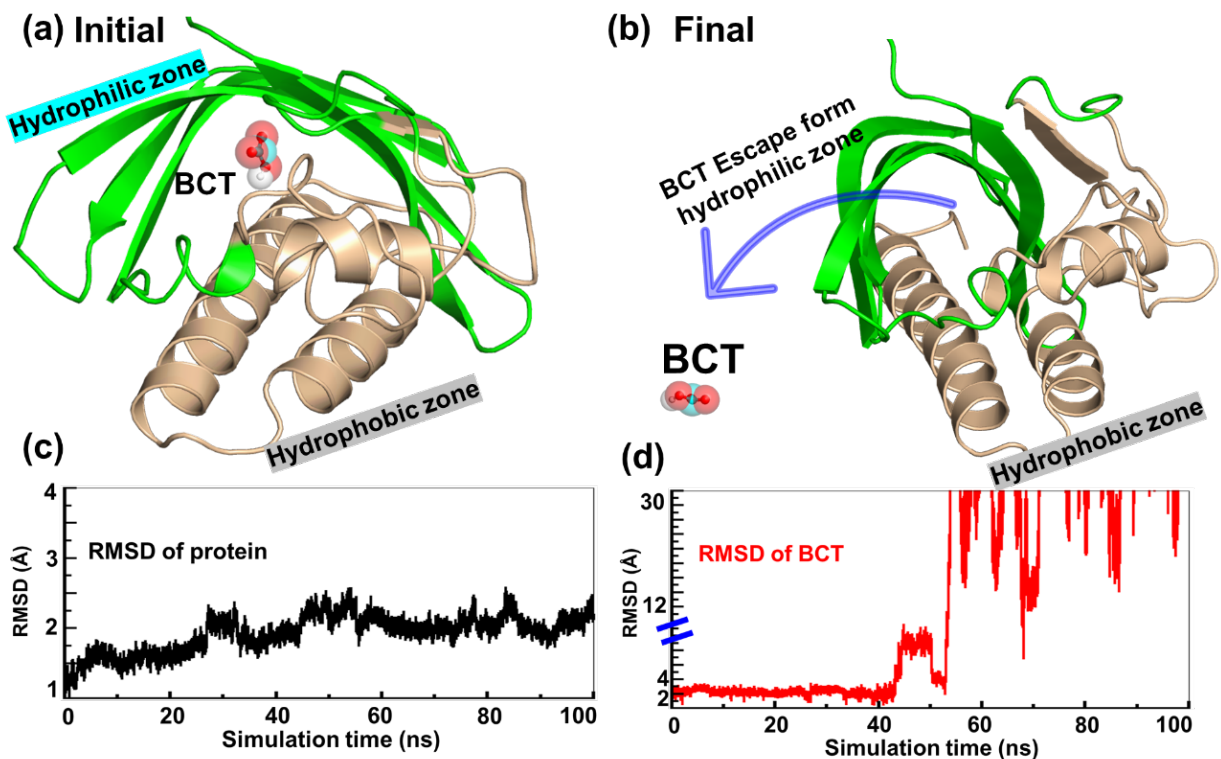


Figure S2: Displaying the outcomes of the MD simulation with HCO₃⁻ (BCT) in the hydrophilic zone. Figures a and b reveal that there is no transfer of the HCO₃⁻ group from the hydrophilic to the hydrophobic region. Instead, it moves away from the hydrophobic cavity after 40 ns of simulation. Figures c and d illustrate the RMSD pattern of the enzyme simulation and the movement of the BCT molecule as it moves away.

S.2. Water Assisted CO₂ Hydration:

As noted in the prior section, the availability of water in the hydrophobic zone is a clearly observable occurrence. Previous research has shown that water facilitates CO₂ hydration, showing that it is a water-mediated process. To check the feasibility of CO₂ conversion to bicarbonate, we first performed DFT-only calculations with CO₂ and two water molecules in the gas phase. Calculation shows a TS barrier of just 21.6 kcal/mol and in a concerted way to form the carbonic acid (H₂CO₃) as a product. Calculation shows the CO₂ gets hydrated to in a concerted manner to form H₂CO₃ (carbonic acid) as a product with a TS barrier of just 21.6 kcal/mol (cf. Figure S1).

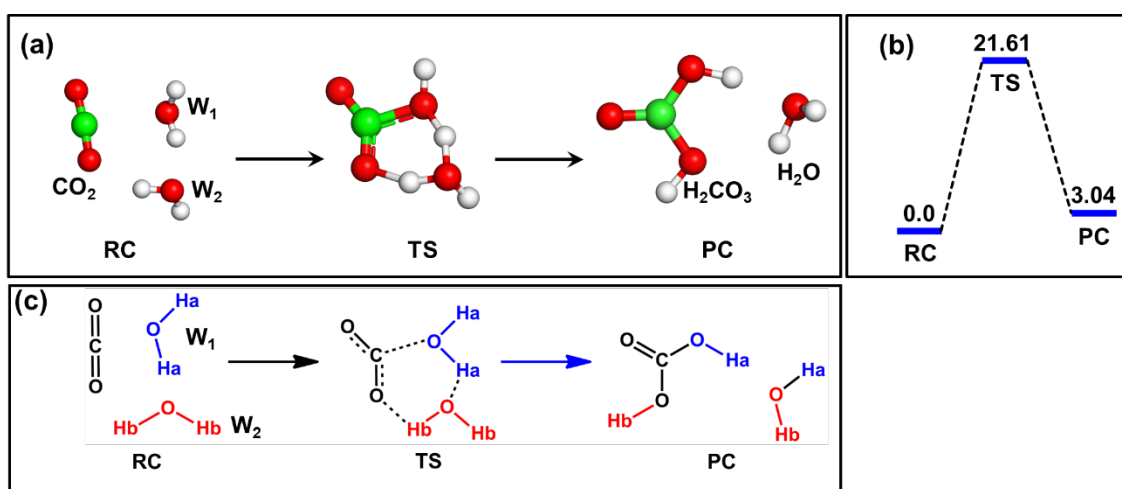
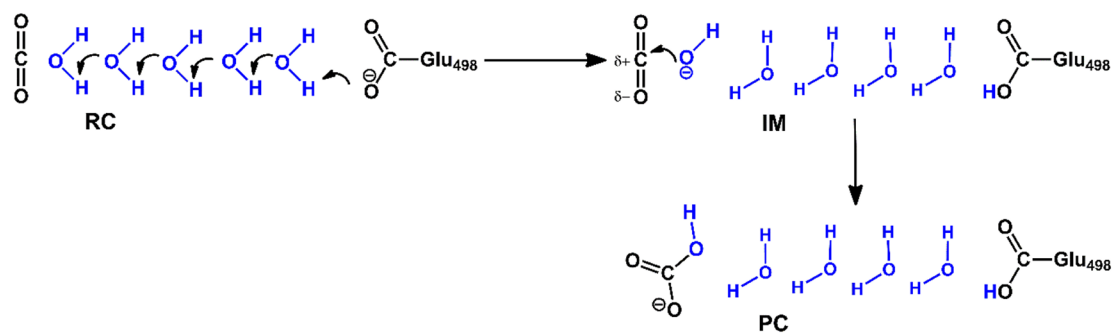


Figure S3: (a) Ball and stick representation of the species involved in the concerted conversion of carbon dioxide into carbonic acid. (b) Gas-phase energy profile diagram for the interconversion of carbon dioxide (RC) into carbonic acid (PC) calculated at the B3LYP/def2-SVP level of theory. Note that energies were further corrected by dispersion correction and zero-point energy correction. The energies are reported in kcal/mol. (c) Concerted mechanism of CO₂ hydration via two water molecules.

By employing two water molecules during the process of CO₂ hydration, we successfully generated H₂CO₃, also known as carbonic acid. This formation, occurring in the gas phase, prompted us to hypothesize the existence of a proton acceptor residue within the hydrophobic zone of the CA enzyme. This residue is expected to play a crucial role in the formation of bicarbonate (H₂CO₃ – H⁺ → HCO₃[–]) by accepting one of the protons involved in the CO₂ hydration reaction in enzymatic environment.

(a) Stepwise Mechanism



(b) Concerted Mechanism

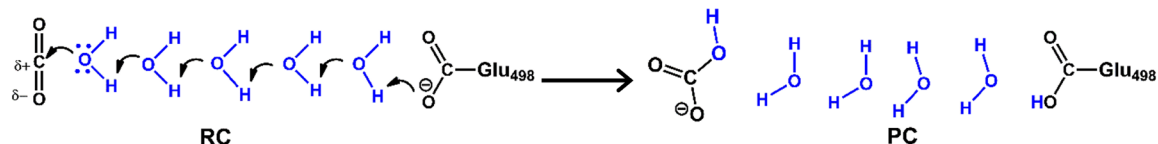


Figure S4. Proposed (a) stepwise and (b) concerted pathways wherein Glutamic acid residue acts as a proton acceptor.

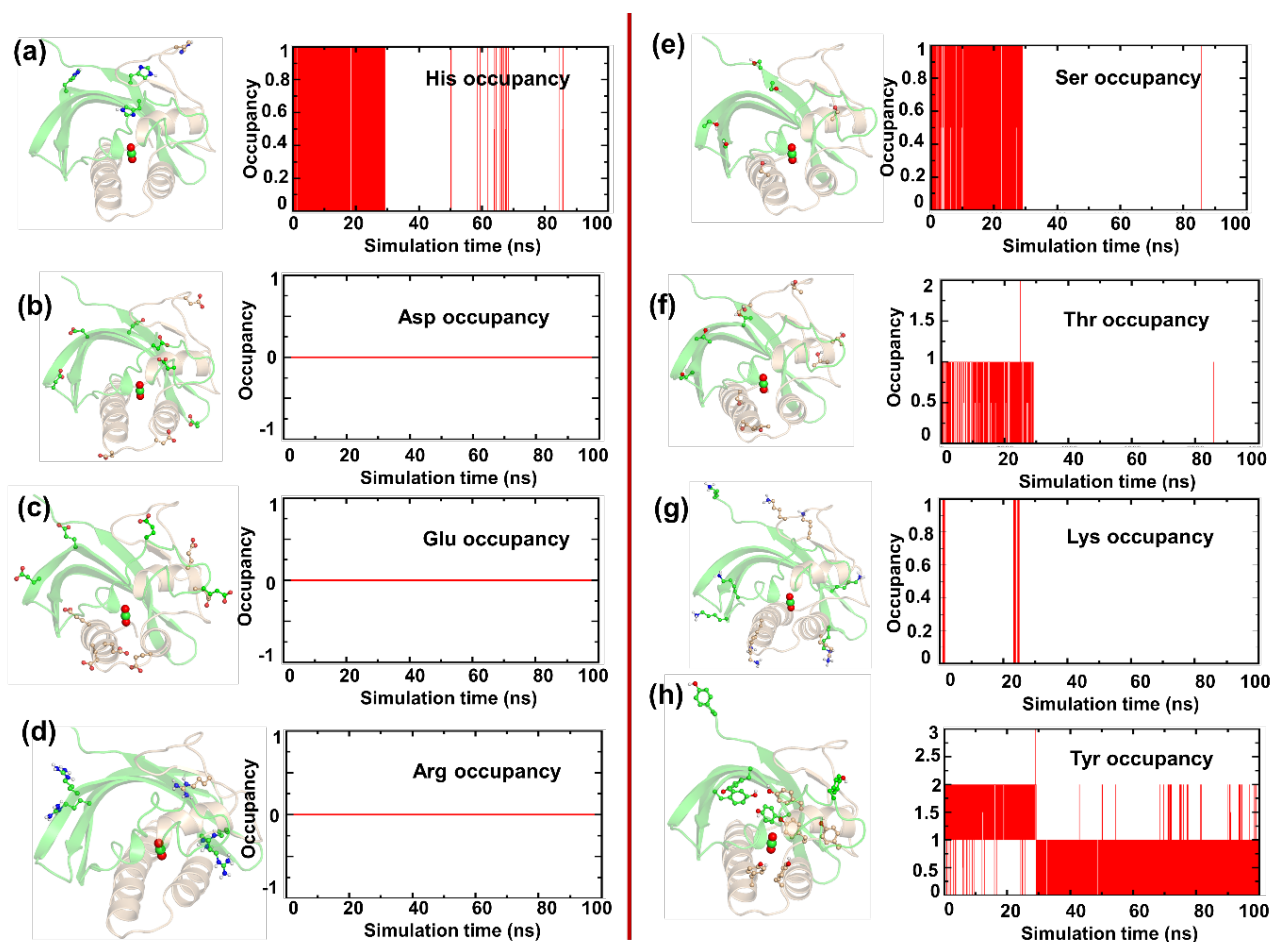


Figure S5: Occupation possibility of different residues calculated near to 5 Å of CO₂ molecule, over the trajectory.

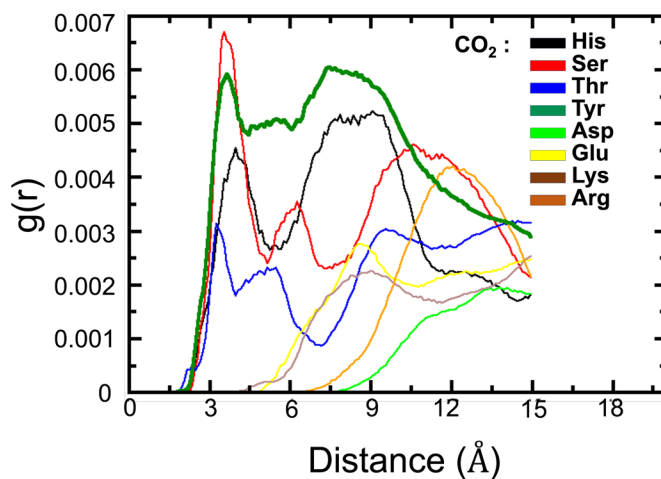


Figure S6: Radial distribution plot for amino acid residues present within 5 Å from CO₂ molecule.

S.3. Proton Acceptor Residues

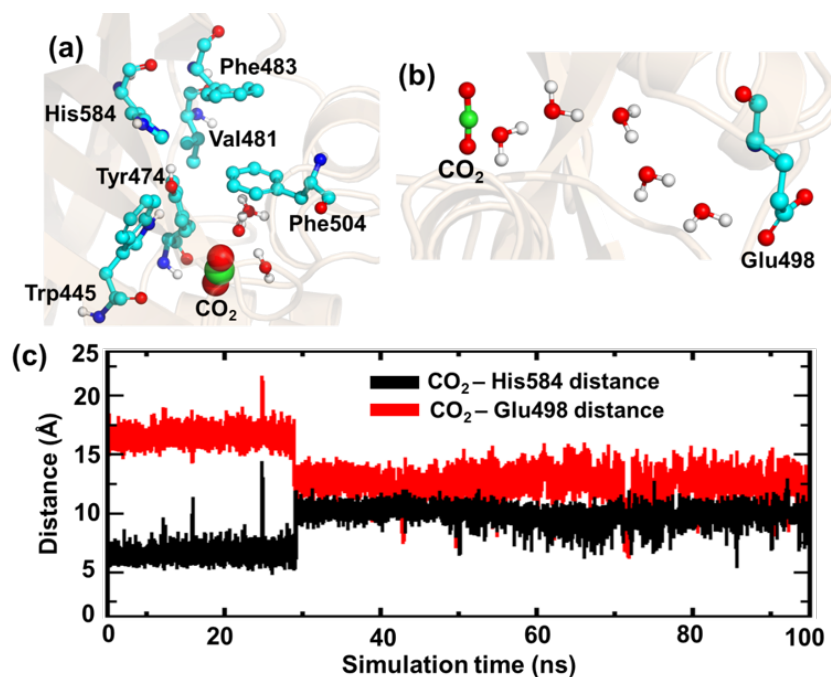


Figure S7. Optimized RC geometry for the proposed pathway for CO₂ hydration; through (a) Histidine (b) Glutamic acid. (c) Distance plot depicts the evolution of positional gap between His584, Glu498 with CO₂.

S.3.a. His584 as a Proton Acceptor Residue:

It is believed that all known metal containing CA enzymes uses histidine residue in their proton shuttle machinery and therefore, we first investigate the role of histidine in the metal independent CA enzyme. A thorough investigation of the MD trajectories reveals that the His584 is located in the hydrophilic zone, which is 8.1 Å away from the CO₂ and sterically hindered by bulky residues. The detailed description can be seen in Figure S5b. In such case, it is almost impossible to abstract a proton by His584 residue from a water molecule that is present nearby CO₂ and therefore, we reject the hypothesis of bicarbonate formation in a concerted way. Thereafter, we attempt to test the stepwise pathway as shown in Figure S6. In this case, two water molecules simultaneously react with the CO₂ molecule, which generates the carbonic acid at a cost of 12.7 kcal/mol energy. Since carbonic acid is an acid molecule, it has a natural tendency to donate its proton to the surroundings. Then, the proton from the carbonic acid is received by the nearby water molecule (W2) and attains the stability by interacting with the adjacent hydroxyl group of the tyrosine residue. Thereafter, the Tyr474 residue relays this additional proton of the water molecule to the nearby His584 residue. Although we obtain a feasible process in our QM/MM derived potential energy surface, the energy cost for the second step of the reaction is very high (>32.9 kcal/mol). Thus, we believe that there must be an alternating low energetic reaction pathway for the efficient catalysis of hydration CO₂ molecule.

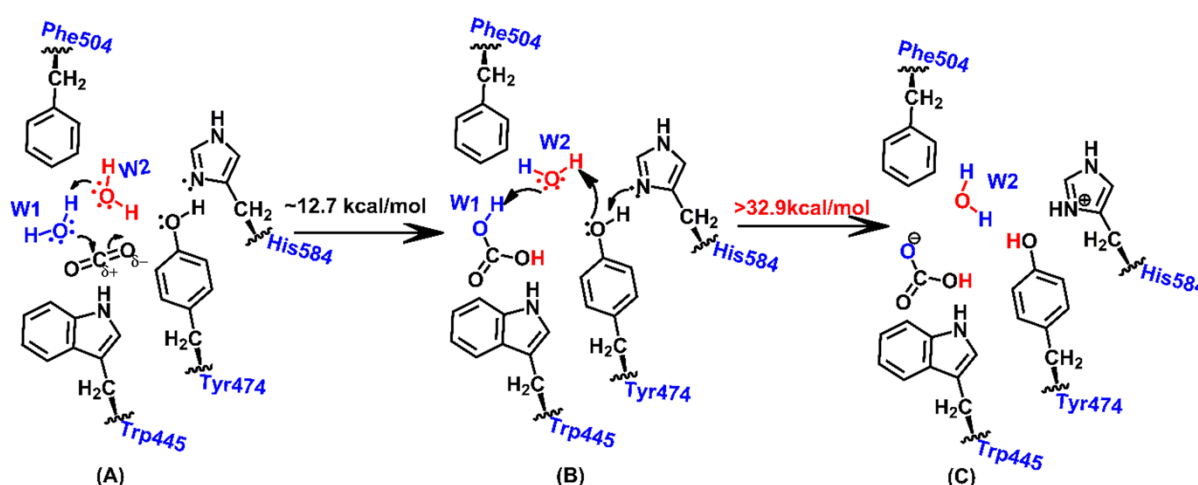
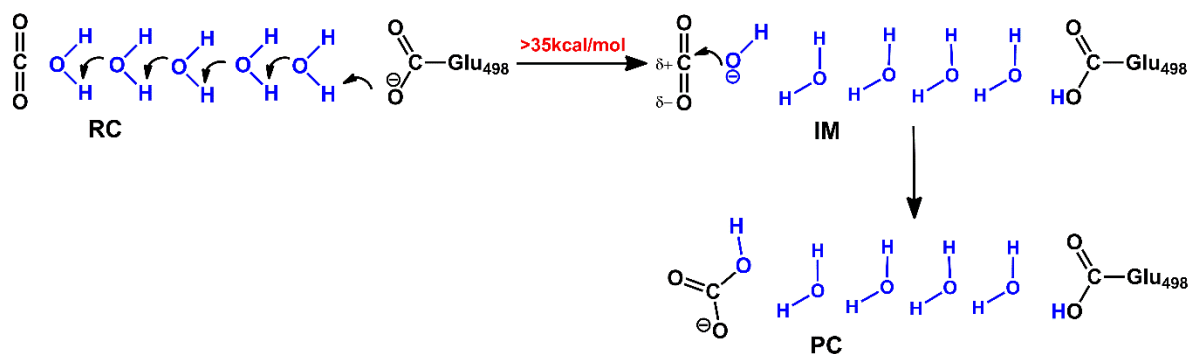


Figure S8. Reaction scheme for CO₂ hydration observed when His acted as a base.

S.3.b. Glu498 as a Proton Acceptor Residue:

Since the traditional proton acceptor, histidine residue requires very high energy to complete the catalytic cycle of the metal independent CA enzyme, we concentrate on the Glu498 as a proton acceptor. As can be seen in Figure S5b, Glu498 residue organizes a water channel connecting to the substrate CO₂ molecule, however the distance between them is very large, which is ~13 Å (cf. S5c).

(a) Stepwise Mechanism



(b) Concerted Mechanism

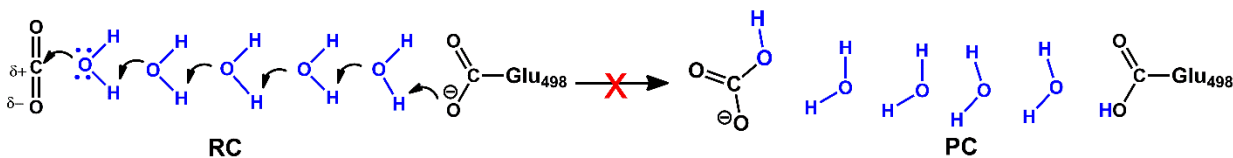


Figure S9. QM/MM explored CO₂ hydration with proton acceptor residue Glu498. We have tested both (a) stepwise and (b) concerted pathways wherein Glutamic acid residue acts as a proton acceptor. However, due to a larger distance between substrate CO₂ and Glu498 (~13.8 Å) the process was not feasible.

Albeit the long distance between the reactive molecules, we have carried out the QM/MM potential energy surface scanning to achieve the product, bicarbonate molecule according to Grotthuss mechanism. Unfortunately, we cannot generate a stable product on the potential energy surface with the help of Glu498 residue. Hence, we reject the idea of Glu498 residue, acting as a proton acceptor residue and further proceeded for the only possibility where Tyr46 acts as a base. (Section 3.7)

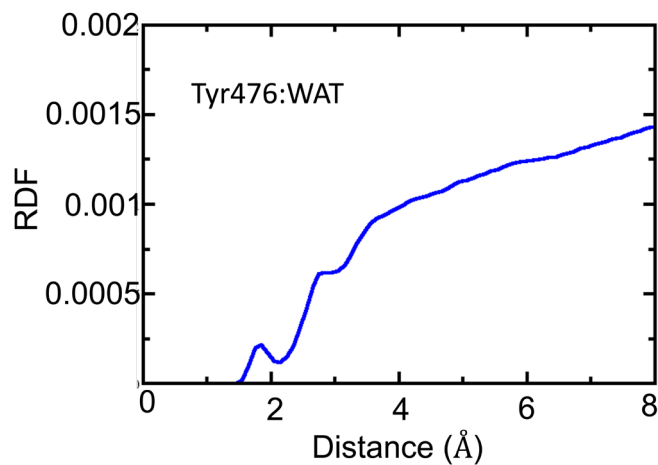


Figure S10: Radial distribution function calculated between residue Tyr476 and water molecules.

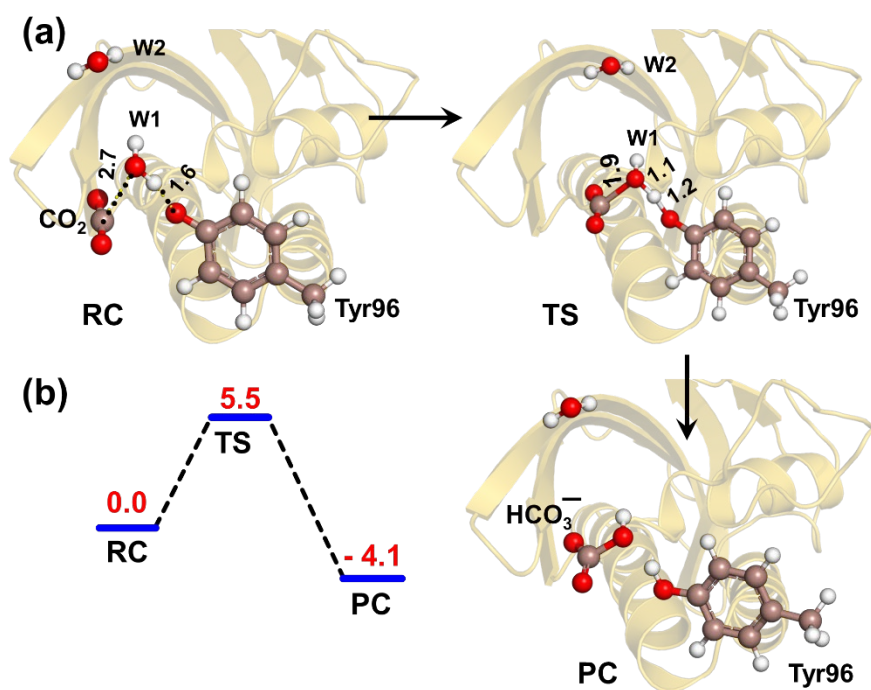


Figure S11: QM/MM optimized geometries involved in the conversion of CO₂ into bicarbonate for all2909. All Energies are in kcal/mol and calculated at B3LYP-D3/def2-TZVP/ZPE level of theory.

**QM Coordinates of optimized geometries
in GAS phase**

RC

C	-1.29111200	-0.45204100	-0.00860800
O	-2.21512800	0.23982000	0.09778400
O	-0.42675400	-1.23328800	-0.10356900
O	2.39592200	-0.42072400	0.17511700
H	2.79136500	-0.59455200	-0.69094200
H	1.60561600	-0.98548700	0.18471700
O	0.45415400	1.55196300	-0.16996800
H	1.27783800	1.04281500	-0.02649200
H	0.40630800	2.14731000	0.58945900

TS

C	0.83978300	-0.20591300	0.02307600
O	2.00142900	0.02947000	-0.09418400
O	0.07074800	-1.17770000	0.08356400
O	-2.06813000	-0.04254100	-0.17966800
H	-2.57247700	-0.06606200	0.64787200
H	-1.28143500	-0.76899100	-0.07970800
O	-0.05281300	1.11895700	0.14267300
H	-1.21978900	0.77736500	-0.08211200
H	0.42513500	1.86768200	-0.24358900

PC

C	-0.87644700	0.07903200	0.00047300
O	-2.07373700	0.11410800	0.03855600
O	-0.07009500	1.11949600	-0.02769200
O	2.37844500	0.03268300	-0.07119500
H	2.80018400	0.03934500	0.80015000
H	0.88369900	0.83893400	-0.06514800
O	-0.17374900	-1.10554800	-0.01595700
H	1.92714800	-0.82706900	-0.11963500
H	-0.83926800	-1.81131800	-0.00789900

**Coordinates of QM/MM optimized
geometries for CO₂ hydration in the
Bn86287:**

RC_{Bn86287}

C	18.6988180	30.0411569	34.9654670
H	18.5010893	29.0368978	34.5700140
H	17.8017311	30.6489858	34.7495337
C	19.9423590	30.6044884	34.3140012
C	20.3145099	31.9641602	34.4145558
H	19.6670780	32.6517891	34.9749899
C	21.4637626	32.4672324	33.8170682
H	21.7087085	33.5291472	33.8817239
C	22.3720413	31.6321071	33.0646619
O	23.4537269	32.0692881	32.5480116
C	21.9378013	30.2645446	32.9379413
H	22.5717288	29.6049892	32.3437637

C	20.7834455	29.7800083	33.5475970
H	20.5190047	28.7241496	33.4083899
C	27.5278251	27.0295902	28.5456559
O	26.9187456	26.2855740	29.2060092
O	28.2761086	27.6906507	27.9468858
O	24.1773411	30.5602537	30.5534907
H	23.2684592	30.4919104	30.1818668
H	24.0310510	31.1519219	31.3687001
O	26.0432962	29.3880273	29.1866863
H	25.3307017	29.7578953	29.7994434
H	26.4045735	30.2021383	28.8016016
O	25.4783498	27.7194059	27.1044035
H	25.5284490	28.4005658	27.8210692
H	24.7281470	27.1568836	27.3633833
H	18.7862149	29.9267001	36.0458968

TS_{Bn86287}

C	18.7171848	30.0277458	34.9568998
H	18.5480799	29.0160416	34.5682963
H	17.8095198	30.6127767	34.7254359
C	19.9492977	30.6184245	34.3035332
C	20.2741289	31.9917870	34.3844202
H	19.6161708	32.6599376	34.9548314
C	21.3835102	32.5337628	33.7475380
H	21.5895859	33.6050383	33.7955892
C	22.2847976	31.7295259	32.9604245
O	23.3058259	32.2177908	32.3609304
C	21.9268732	30.3405362	32.8885819
H	22.5821849	29.6942943	32.3007258
C	20.8085302	29.8147104	33.5367617
H	20.5881332	28.7459419	33.4237672

C	27.1241073	27.1168020	28.3413898
O	26.8587307	25.9599458	28.5668293
O	28.0121594	27.9282061	28.3712191
O	23.7532200	31.1317529	30.1320979
H	22.8333052	30.8067464	29.9572521
H	23.6722716	31.5658831	31.0840844
O	25.5892038	29.7312715	29.3300434
H	24.7406575	30.3004051	29.7170979
H	26.2189277	30.3947427	29.0056379
O	25.7329115	27.8972727	27.7710586
H	25.5704518	28.8117191	28.5063422
H	25.0004677	27.2573908	27.8137575
H	18.7942178	29.9227082	36.0390737

PC_{Bn86287}

C	18.7301300	29.9793935	34.9436103
H	18.6839767	28.9501745	34.5708397
H	17.7711972	30.4593518	34.6817162
C	19.8939124	30.7033737	34.2961264
C	20.0866028	32.0883438	34.4530309
H	19.4133457	32.6549825	35.1057332
C	21.0910974	32.7685923	33.7690120
H	21.2073713	33.8508177	33.8592198
C	21.9500210	32.0865551	32.8757742
O	22.8578431	32.7815970	32.1997404
C	21.7905527	30.6886783	32.7435224
H	22.4515317	30.1458491	32.0632102
C	20.7772177	30.0244777	33.4385820
H	20.6499910	28.9479091	33.2796651
C	26.2816476	27.0009368	28.8843683
O	26.7016477	25.9147838	28.4721536

O	26.7212945	27.7835572	29.7501137
O	23.2912383	31.6418412	29.9456556
H	22.3600964	31.3491274	29.7741861
H	23.1319266	32.3017114	31.3448106
O	24.4919565	29.2666529	30.2671805
H	23.8250988	30.8051570	30.0110376
H	25.4451457	28.9980561	30.2817681
O	25.0670640	27.4652062	28.2779404
H	24.2339109	28.7114951	29.5068068
H	24.7094899	26.7199712	27.7695814
H	18.7986044	29.9019074	36.0286791

**QM/MM optimized geometry coordinates
for CO₂ hydration in all2909.**

RC_{all2909}

C	43.6004588	35.6963375	27.3493724
H	43.8792510	34.6690817	27.6348313
H	44.4118691	36.3703328	27.6775102
C	42.2952413	36.0879409	28.0266204
C	41.9326455	37.4381574	28.2234768
H	42.6081125	38.2280313	27.8738031
C	40.7514568	37.8068809	28.8624361
H	40.5104524	38.8653943	29.0004916
C	39.8029396	36.8433059	29.3504292
O	38.6773532	37.1729946	29.8854140
C	40.2267521	35.4803253	29.2033013

H	39.5747045	34.7117737	29.6338724
C	41.4155494	35.1261947	28.5582913
H	41.6839796	34.0634212	28.4800048
C	36.9627163	39.1506881	30.1557401
O	36.7485612	38.9847200	31.2911701
O	37.0800457	39.5089628	29.0532194
O	33.1530041	34.9867582	31.5340955
H	32.9346881	35.8717975	31.8565043
H	33.7477610	34.6085569	32.2308415
O	36.1237667	36.5924134	29.4921687
H	35.8656486	36.1552747	30.3146926
H	37.1208416	36.6714854	29.5848793
H	43.5933079	35.7304311	26.2599443

TS_{all2909}

C	43.6610771	35.6325454	27.3294550
H	43.8911260	34.5839259	27.5746701
H	44.5144674	36.2529614	27.6531739
C	42.4044143	36.0775298	28.0505636
C	42.1621725	37.4406545	28.3163388
H	42.8826224	38.1903937	27.9723573
C	41.0409892	37.8631659	29.0214221
H	40.8759021	38.9246522	29.2258148

C 40.0695136 36.9424624 29.5154990
 O 39.0383778 37.3545815 30.1944802
 C 40.3321899 35.5667927 29.2515868
 H 39.6296713 34.8309550 29.6584061
 C 41.4664970 35.1554955 28.5437017
 H 41.6458678 34.0824829 28.3926435
 C 36.6411794 39.0875410 30.1761669
 O 36.3695243 39.1242306 31.3342352
 O 36.8636325 39.5929909 29.1305876
 O 32.8819067 35.0163691 31.7499940
 H 32.6196498 35.8691655 32.1223446
 H 33.5657252 34.6661442 32.3746292
 O 36.6788755 37.1663916 29.7415801
 H 36.3394161 36.7397357 30.5411454
 H 37.8003892 37.1295580 29.8938321
 H 43.6169114 35.7063276 26.2428673

PC_{all2909}

C 43.6549773 35.5881201 27.3247671
 H 43.6975859 34.5158856 27.5742382
 H 44.6035888 36.0457081 27.6473003
 C 42.5113060 36.2888402 28.0279360

C 42.6094264 37.6648068 28.3058704
 H 43.5070150 38.2077404 28.0042738
 C 41.5961600 38.3579474 28.9530851
 H 41.6881795 39.4266839 29.1622071
 C 40.4165291 37.6949072 29.3464401
 O 39.4781142 38.4187867 29.9572976
 C 40.3055225 36.3133009 29.0847922
 H 39.3973565 35.7860325 29.3928564
 C 41.3463760 35.6289085 28.4421073
 H 41.2517048 34.5496919 28.2720170
 C 36.5071580 39.0254958 30.3805672
 O 36.1984084 39.2400896 31.5603922
 O 36.5127450 39.6761587 29.3447219
 O 32.8106780 35.0124517 31.8072591
 H 32.6324728 35.9193387 32.0900145
 H 33.5338109 34.6920753 32.4002627
 O 36.9829576 37.6219638 30.1677137
 H 36.8684369 37.2348012 31.0483365
 H 38.5892155 37.9686054 30.0090439
 H 43.6117826 35.6813701 26.2396377

