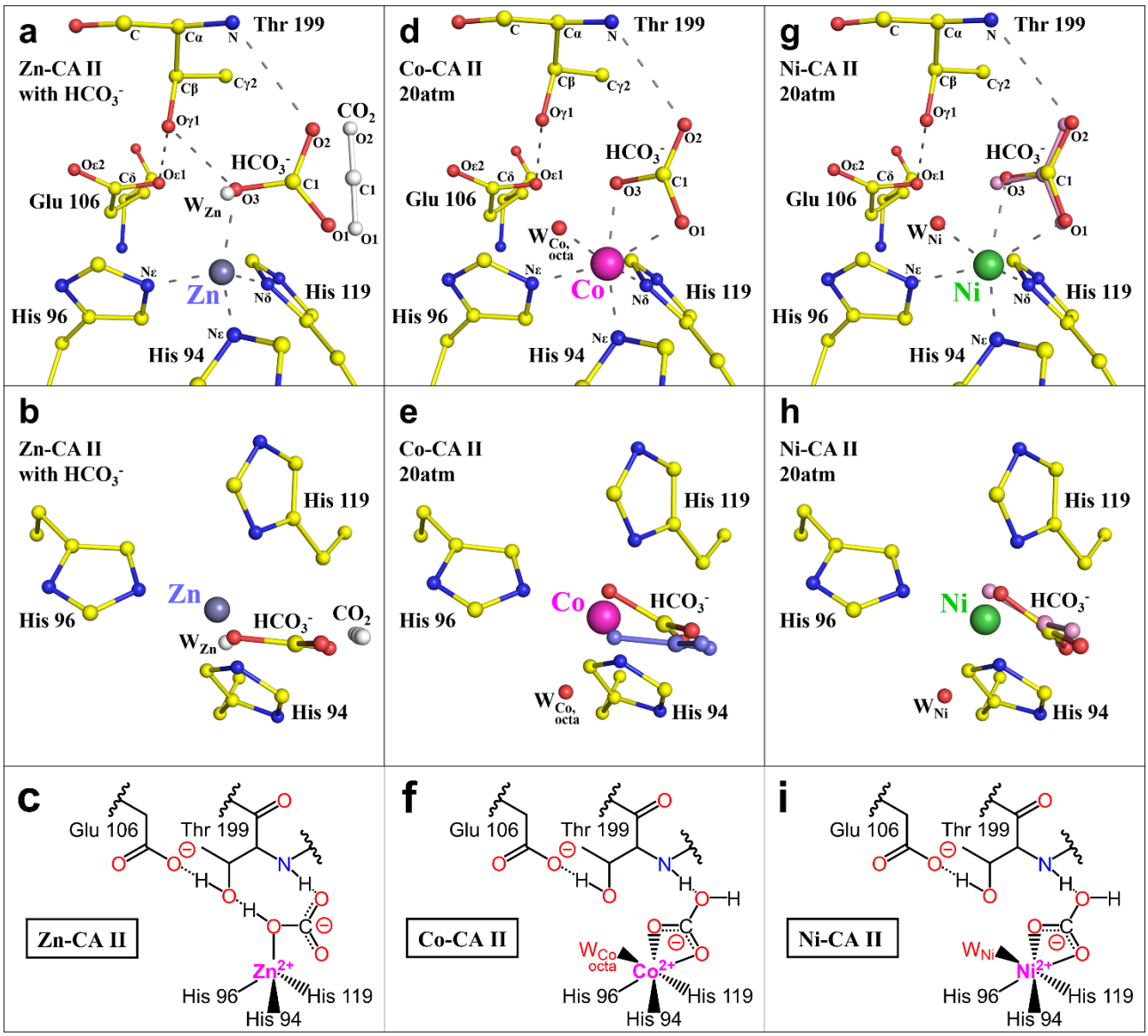
## Supplementary Information

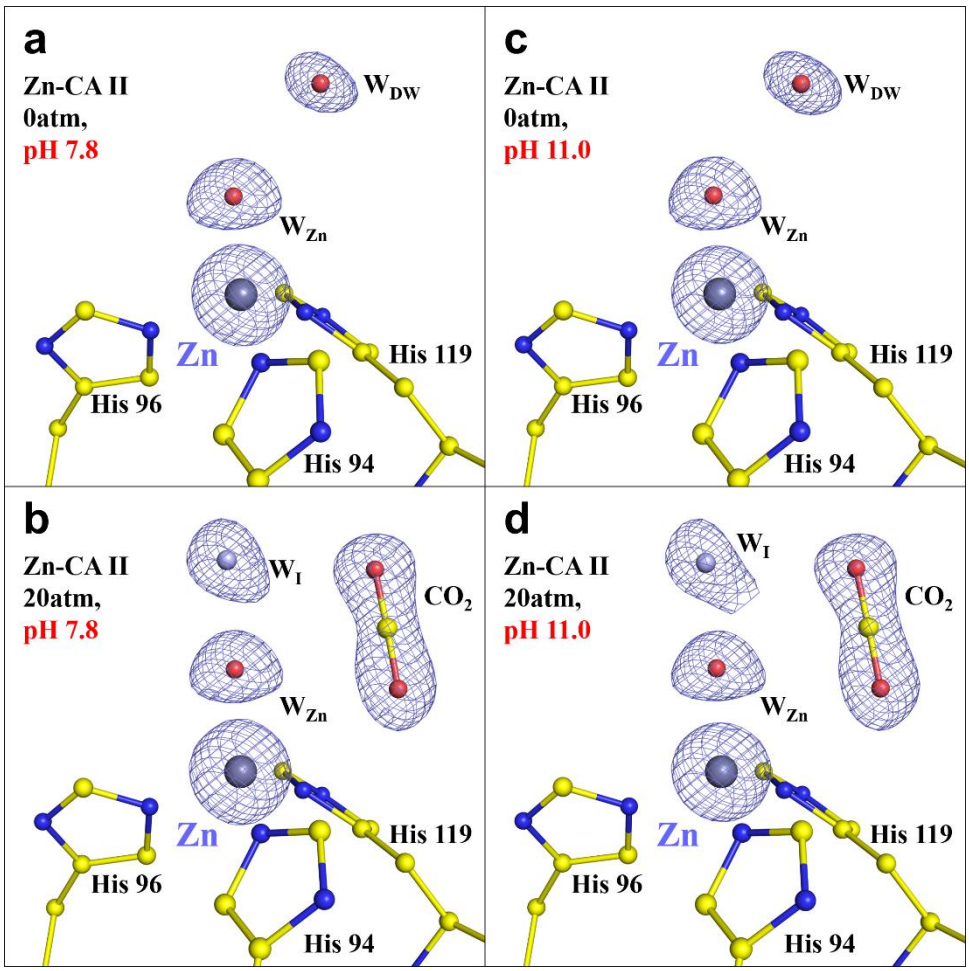
# Elucidating the role of metal ions in carbonic anhydrase catalysis

**J. K. Kim *et al*.**

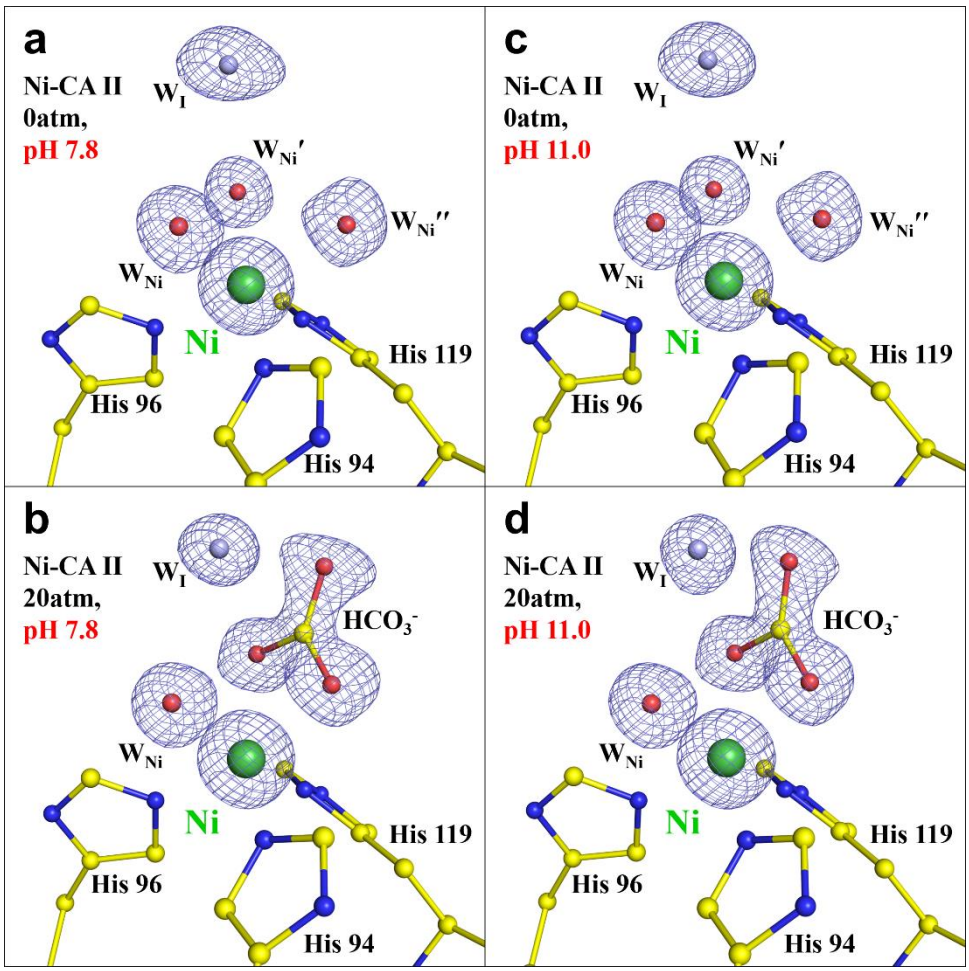
**This PDF file includes:**



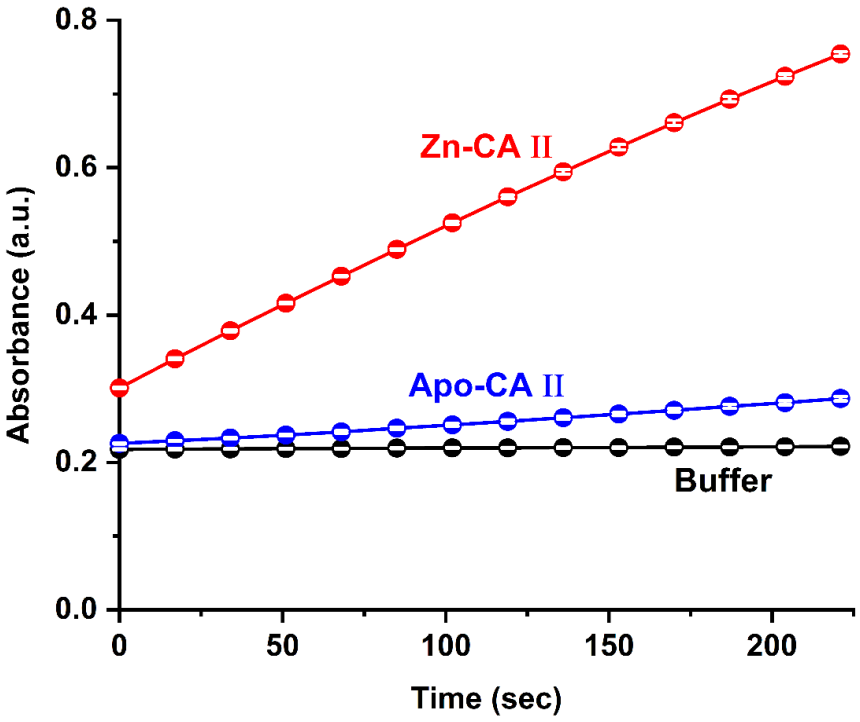

**Supplementary Fig. 1. $HCO_3^-$ binding geometry in Zn-, Co-, Ni-CA II**. **a-c**) Monodentate binding of $HCO_3^-$ in Zn-CA II (PDB code: 2vvb)[1]. The $CO_2$ molecule and $W_{Zn}$ (white) from Zn-CA II 20atm are superimposed for comparison. Note that $HCO_3^-$ lies on the plane made by the $CO_2$ molecule and $W_{Zn}$. **d-f**) Bidentate binding of $HCO_3^-$ in Co-CA II 20atm pH 7.8. The $HCO_3^-$ molecule from Zn-CA II (light purple, PDB 2vvb) is superimposed for comparison. Note that the $HCO_3^-$ molecule in Co-CA II is tilted by ~ 31° to the $HCO_3^-$ molecule in Zn-CA II. **g-i**) Bidentate binding of $HCO_3^-$ in Ni-CA II 20atm. The $HCO_3^-$ molecule in Co-CA II (pink) is superimposed for comparison.



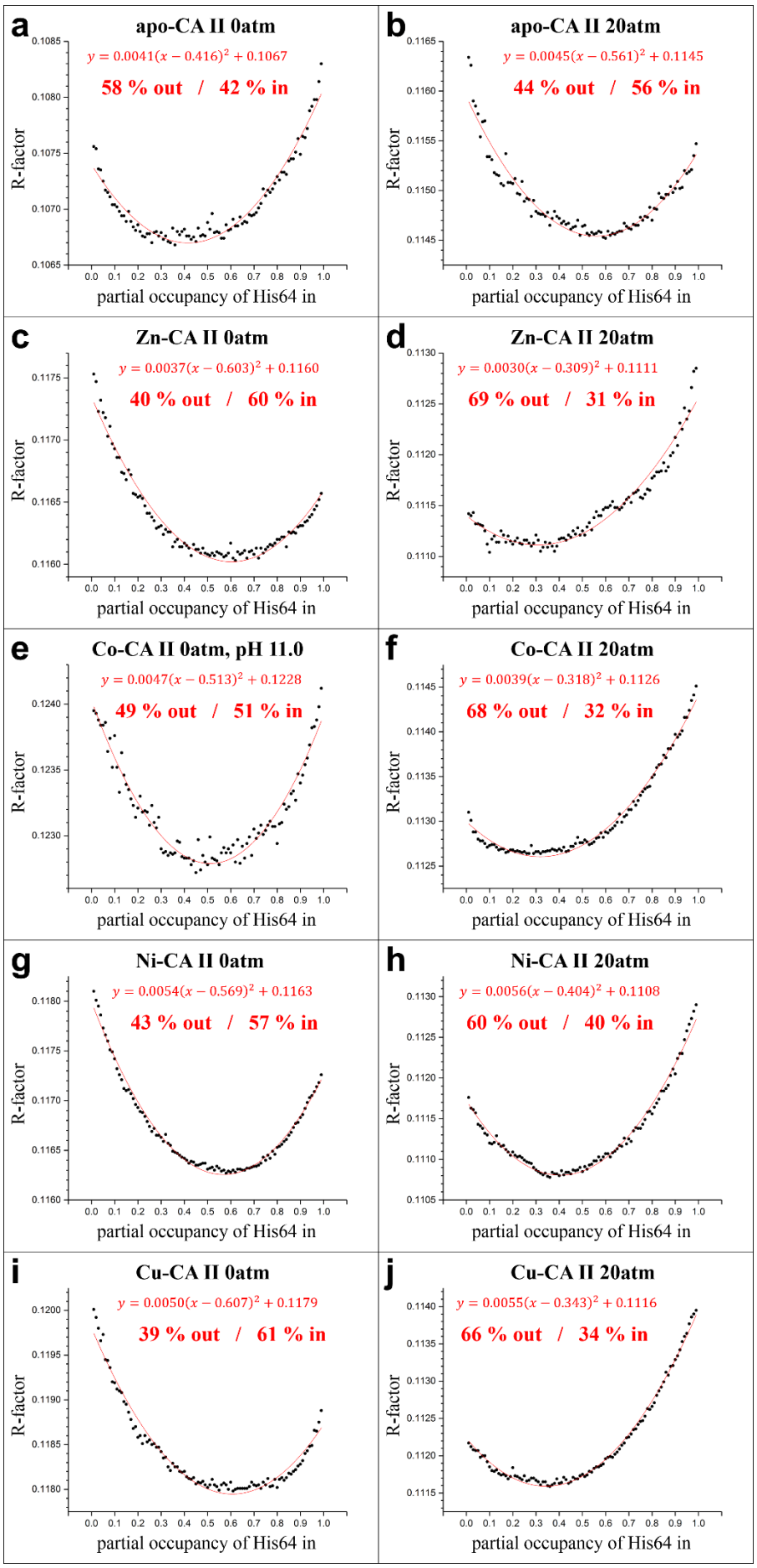

**Supplementary Fig. 2. Substrate binding in Zn-CA II at pH 7.8 (a-b) and 11.0 (c-d).** The metal coordination maintains tetrahedral geometry upon $CO_2$ binding regardless of pH values. The electron density ($2F_o$-$F_c$, blue) is contoured at 2.2σ. The intermediate water ($W_I$) is colored in steel blue for clarity.




**Supplementary Fig. 3. Product binding in Ni-CA II at pH 7.8 (a-b) and 11.0 (c-d).** The metal coordination maintains octahedral geometry upon $HCO_3^-$ binding regardless of pH values. The intermediate water ($W_I$) is colored in steel blue for clarity. The electron density ($2F_o$-$F_c$, blue) is contoured at 2.2σ.




**Supplementary Fig. 4. Absorbance of Apo- and Zn-CA II in esterase kinetic assay at pH 7.8.** CA II esterase activity was measured spectroscopically at 348nm, indicative of substrate 4-nitrophenyl acetate hydrolysis. Compared to Zn-CA II, Apo-CA II and buffer show little to no esterase activity. The standard deviation errors (white) are presented in the data points and are ranging from 0.2 % ~ 1.1 %. Source data are provided as a Source Data file.




**Supplementary Fig. 5.** See next page for figure caption.

**Supplementary Fig. 5. Partial occupancy determination of His64.** The His64 out/in conformations are determined for the selected structures of apo-CA II (**a, b**) Zn-CA II (**c, d**), Co-CA II (**e, f**), Ni-CA II (**g, h**) Cu-CA II (**i, j**). For each data set, systematic refinements were carried out on 99 structures with manually adjusted His64 in/out occupancies. The obtained data points were then fitted to quadratic functions, showing the minimum points in the overall R factors. The His64 out/in conformations for the other structures can be found in Supplementary Table 1. Source data are provided as a Source Data file.

**Supplementary Table 1. Data collection and refinement statistics for the CA II structures.**

| | apo-CA II 0atm 6LUU | apo-CA II 20atm 6LUV | Zn-CA II 0atm 6LUW | Zn-CA II 20atm 6LUX | Zn-CA II 0atm pH11.0 6LUY | Zn-CA II 20atm pH11.0 6LUZ | Co-CA II 0atm 6LV1 | Co-CA II 20atm 6LV2 |
|---|---|---|---|---|---|---|---|---|
| **Data collection** | | | | | | | | |
| Space group | $P2_1$ | $P2_1$ | $P2_1$ | $P2_1$ | $P2_1$ | $P2_1$ | $P2_1$ | $P2_1$ |
| Cell dimensions | | | | | | | | |
| *a*, *b*, *c* (Å) | 42.13, 41.30, 72.21 | 42.26, 41.38, 72.00 | 42.21, 41.28, 72.15 | 42.37, 41.44, 72.13 | 42.28, 41.26, 72.07 | 42.39, 41.47, 72.15 | 42.31, 41.22, 72.05 | 42.32, 41.32, 72.22 |
| β(°) | 104.27 | 104.18 | 104.18 | 104.05 | 104.19 | 104.11 | 104.16 | 104.03 |
| Resolution (Å) | 30-1.20 (1.22-1.20) | 30-1.20 (1.22-1.20) | 30-1.20 (1.22-1.20) | 30-1.20 (1.22-1.20) | 30-1.20 (1.22-1.20) | 30-1.20 (1.22-1.20) | 30-1.20 (1.22-1.20) | 30-1.20 (1.22-1.20) |
| $R_{sym}$ (%) | 5.8 (36.8) | 7.1 (48.1) | 6.5 (37.6) | 6.8 (66.9) | 8.5 (17.7) | 5.6 (20.3) | 9.2 (60.7) | 8.2 (65.6) |
| $I/\sigma(I)$ | 28.8 (6.8) | 21.2 (5.1) | 29.7 (6.3) | 29.8 (3.6) | 25.2 (12.7) | 29.9 (11.8) | 24.3 (3.2) | 20.1 (3.8) |
| Completeness (%) | 95.5 (92.6) | 94.3 (91.2) | 98.8 (97.5) | 96.2 (92.9) | 98.0 (96.0) | 94.6 (91.9) | 96.0 (93.3) | 98.4 (96.4) |
| Redundancy | 7.5 (7.3) | 7.5 (7.4) | 7.3 (7.1) | 7.4 (7.3) | 7.4 (7.4) | 7.6 (7.5) | 7.6 (7.6) | 7.4 (7.2) |
| **Refinement** | | | | | | | | |
| Resolution (Å) | 1.20 | 1.20 | 1.20 | 1.20 | 1.20 | 1.20 | 1.20 | 1.20 |
| No. reflections | 72,011 | 71,305 | 74,561 | 73,183 | 73,898 | 72,112 | 72,478 | 74,640 |
| $R_{work}$ / $R_{free}$ (%) | 11.0 / 13.8 | 11.7 / 14.8 | 11.7 / 14.1 | 11.3 / 14.2 | 11.1 / 13.1 | 10.3 / 12.7 | 12.0 / 15.0 | 11.4 / 13.9 |
| No. atoms | | | | | | | | |
| Protein | 4,285 | 4,256 | 4,253 | 4,245 | 4,253 | 4,245 | 4,259 | 4,240 |
| Ligand/ion | 1 glycerol | 2 $CO_2$, 1 glycerol | 1 glycerol | 2 $CO_2$, 1 glycerol | 1 glycerol | 2 $CO_2$, 1 glycerol | 1 $HCO_3^-$, 1 glycerol | 1 $HCO_3^-$, 1 $CO_2$, 1 glycerol |
| Water | 371 | 373 | 263 | 367 | 264 | 365 | 283 | 333 |
| *B*-factors | | | | | | | | |
| Protein (main / side chain) | 9.78 / 12.75 | 11.48 / 14.38 | 10.09 / 13.22 | 10.80 / 13.62 | 7.90 / 10.99 | 8.87 / 11.51 | 10.84 / 14.07 | 10.31 / 13.31 |
| Ligand/ion | 20.76 (glycerol) | 13.90 (first $CO_2$), 26.78 (second $CO_2$), 18.39 (glycerol) | 18.32 (glycerol) | 10.63 (first $CO_2$), 23.50 (second $CO_2$), 17.17 (glycerol) | 14.79 (glycerol) | 8.80 (first $CO_2$), 21.13 (second $CO_2$), 14.78 (glycerol) | 11.23 ($HCO_3^-$), 24.46 (glycerol) | 9.25 ($HCO_3^-$), 23.17 (second $CO_2$), 21.27 (glycerol) |
| Water | 30.25 | 30.67 | 26.12 | 30.58 | 24.33 | 27.75 | 27.41 | 29.71 |
| R.m.s. deviations | | | | | | | | |
| Bond lengths (Å) | 0.031 | 0.030 | 0.029 | 0.028 | 0.029 | 0.028 | 0.031 | 0.028 |
| Bond angles (°) | 2.515 | 2.435 | 2.437 | 2.423 | 2.506 | 2.368 | 2.644 | 2.284 |
| Partial occupancy (%) | | | | | | | | |
| His64 (out/in) conformation (%) | 58 / 42 | 44 / 56 | 40 / 60 | 69 / 31 | 32 / 68 | 63 / 37 | 49 / 51 | 68 / 32 |

| | Co-CA II 0atm pH11.0 6LV3 | Co-CA II 20atm pH11.0 6LV4 | Ni-CA II 0atm 6LV5 | Ni-CA II 20atm 6LV6 | Ni-CA II 0atm pH11.0 6LV7 | Ni-CA II 20atm pH11.0 6LV8 | Cu-CA II 0atm 6LV9 | Cu-CA II 20atm 6LVA |
|---|---|---|---|---|---|---|---|---|
| **Data collection** | | | | | | | | |
| Space group | $P2_1$ | $P2_1$ | $P2_1$ | $P2_1$ | $P2_1$ | $P2_1$ | $P2_1$ | $P2_1$ |
| Cell dimensions | | | | | | | | |
| *a*, *b*, *c* (Å) | 42.33, 41.26, 72.08 | 42.36, 41.46, 72.31 | 42.40, 41.29, 71.92 | 42.40, 41.37, 72.13 | 42.52, 41.23, 71.87 | 42.42, 41.40, 72.19 | 42.33, 41.23, 72.09 | 42.36, 41.40, 72.31 |
| β(°) | 104.13 | 104.02 | 104.03 | 104.01 | 104.06 | 104.04 | 104.17 | 103.97 |
| Resolution (Å) | 30-1.20 (1.22-1.20) | 30-1.20 (1.22-1.20) | 30-1.20 (1.22-1.20) | 30-1.20 (1.22-1.20) | 30-1.20 (1.22-1.20) | 30-1.20 (1.22-1.20) | 30-1.20 (1.22-1.20) | 30-1.20 (1.22-1.20) |
| $R_{sym}$ (%) | 8.5 (46.4) | 5.8 (42.4) | 8.6 (33.1) | 7.2 (37.7) | 8.5 (29.7) | 4.7 (22.7) | 9.9 (42.8) | 7.8 (44.8) |
| $I/\sigma(I)$ | 23.0 (4.3) | 31.8 (5.6) | 20.7 (7.6) | 23.1 (7.2) | 23.1 (8.4) | 40.6 (10.1) | 19.5 (6.0) | 25.3 (5.5) |
| Completeness (%) | 95.0 (91.7) | 94.6 (91.2) | 96.6 (93.8) | 95.4 (92.1) | 97.5 (94.7) | 96.1 (93.0) | 95.1 (92.3) | 96.9 (94.3) |
| Redundancy | 7.6 (7.7) | 7.6 (7.6) | 7.5 (7.4) | 7.6 (7.5) | 7.4 (7.3) | 7.5 (7.3) | 7.7 (7.6) | 7.5 (7.5) |

| **Refinement** | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Resolution (Å) | 1.20 | 1.20 | 1.20 | 1.20 | 1.20 | 1.20 | 1.20 | 1.20 |
| No. reflections | 71,822 | 72,158 | 73,007 | 72,507 | 73,791 | 73,217 | 71,843 | 73,841 |
| $R_{work}$ / $R_{free}$ (%) | 12.3 / 14.9 | 11.3 / 13.9 | 11.6 / 14.1 | 11.2 / 13.8 | 11.7 / 13.9 | 11.2 / 13.6 | 11.7 / 14.4 | 11.3 / 14.2 |
| No. atoms | | | | | | | | |
| Protein | 4,252 | 4,240 | 4,259 | 4,240 | 4,259 | 4,240 | 4,259 | 4,240 |
| Ligand/ion | 1 glycerol | 1 $HCO_3^-$, 2 $CO_2$, 1 glycerol | 1 glycerol | 1 $HCO_3^-$, 1 $CO_2$, 1 glycerol | 1 glycerol | 1 $HCO_3^-$, 1 $CO_2$, 1 glycerol | 1 glycerol | 1 $CO_2$, 1 glycerol |
| Water | 265 | 333 | 279 | 347 | 279 | 346 | 286 | 360 |
| *B*-factors | | | | | | | | |
| Protein (main / side chain) | 10.84 / 14.06 | 10.39 / 13.16 | 8.73 / 11.74 | 7.93 / 10.50 | 9.15 / 12.17 | 8.38 / 10.83 | 9.71 / 12.55 | 8.23 / 10.89 |
| Ligand/ion | 21.51 (glycerol) | 15.78 ($HCO_3^-$), 14.19 (first $CO_2$), 21.78 (second $CO_2$), 19.39 (glycerol) | 18.86 (glycerol) | 9.15 ($HCO_3^-$), 21.92 (second $CO_2$), 14.87 (glycerol) | 19.13 (glycerol) | 7.40 ($HCO_3^-$), 21.95 (second $CO_2$), 13.93 (glycerol) | 17.19 (glycerol) | 19.67 (second $CO_2$), 14.81 (glycerol) |
| Water | 26.98 | 28.72 | 24.56 | 26.58 | 26.51 | 26.38 | 26.62 | 29.36 |
| R.m.s. deviations | | | | | | | | |
| Bond lengths (Å) | 0.031 | 0.028 | 0.032 | 0.029 | 0.030 | 0.028 | 0.031 | 0.029 |
| Bond angles (°) | 2.484 | 2.358 | 2.600 | 2.382 | 2.552 | 0.352 | 2.550 | 2.460 |
| Partial occupancy (%) | | | | | | | | |
| His64 (out/in) conformation (%) | 49 / 51 | 69 / 31 | 43 / 57 | 60 / 40 | 46 / 54 | 52 / 48 | 39 / 61 | 66 / 34 |

*Values in parentheses are for the highest-resolution shell.

**Supplementary Table 2. List of key bound water molecules in the CA II structures.**

| | apo-CA II 0atm 6LUU | apo-CA II 20atm 6LUV | Zn-CA II 0atm 6LUW | Zn-CA II 20atm 6LUX | Co-CA II 0atm pH11.0 6LV3 | Co-CA II 20atm 6LV2 | Ni-CA II 0atm 6LV5 | Ni-CA II 20atm 6LV6 | Cu-CA II 0atm 6LV9 | Cu-CA II 20atm 6LVA |
|---|---|---|---|---|---|---|---|---|---|---|
| $CO_2$ | – | A 302 | | A 302 | – | | – | | – | – |
| $HCO_3^-$ | – | – | | | – | A 304 | – | A 305 | – | – |
| $W_{Zn}$ | – | – | A 481 | A 426 | – | | – | – | – | – |
| $W_{Co,\ tetra}$ | – | – | – | – | A 490 | | – | – | – | – |
| $W_{Co,\ octa}$ | – | – | – | – | – | A 485 | – | – | – | – |
| $W_{Ni}$ | – | – | – | – | – | – | A 573 | A 583 | – | – |
| $W_{Ni}'$ | – | – | – | – | – | – | A 421 | – | – | – |
| $W_{Ni}''$ | – | – | – | – | – | – | A 602 | – | – | – |
| $W_{Cu}$ | – | – | – | – | – | – | – | – | A 451 | A 421 |
| $W_{Cu}'$ | – | – | – | – | – | – | – | – | A 580 | – |
| $W_{apo}$ | A 486 | A 475 | | – | – | – | – | – | – | – |
| $W_{DW}$ | A 618 | – | A 581 | – | A 569 | – | – | – | A 587 | A 496 |
| $W_{DW}'$ | – | – | – | – | – | – | – | – | – | A 695 |
| $W_{DW}''$ | – | – | – | – | – | – | – | – | – | A 675 |
| $W_I$ | – | A 591 | – | A 483 | – | A 448 | A 611 | A 495 | – | A 427 |
| $W_I'$ | – | – | – | A 643 | – | – | – | – | – | A 663 |
| W1 | A 591 | – | A 487 | | A 546 | – | – | – | A 563 | |
| W2 | A 641 | A 666 | A 615 | A 678 | A 584 | A 667 | A 627 | A 662 | A 636 | A 681 |
| W2′ | – | – | – | A 401 | – | A 401 | – | – | – | A 401 |
| W3a | A 541 | A 556 | A 518 | A 543 | A 529 | A 537 | A 532 | A 507 | A 540 | A 528 |
| W3b | A 462 | A 430 | A 451 | A 451 | A 454 | A 432 | A 453 | A 479 | A 442 | A 452 |
| W3b′ | – | – | – | A 695 | – | – | – | – | – | A 684 |
| $W3b_{apo}'$ | – | A 606 | – | – | – | – | – | – | – | – |
| $W3b_{apo}'$ | – | A 703 | – | – | – | – | – | – | – | – |
| $W3b_{Co}$ | – | – | – | – | – | A 648 | – | – | – | – |
| $W_{EC1}$ | A 680 | – | A 614 | A 665 | A 611 | A 630 | – | – | A 619 | A 648 |
| $W_{EC1}'$ | – | – | – | A 702 | – | – | – | – | – | A 699 |
| $W_{EC1}''$ | – | – | – | – | – | – | A 598 | A 626 | – | |
| $W_{EC2}$ | A 668 | A 700 | A 608 | – | A 609 | – | – | – | A 603 | A 641 |
| $W_{EC2}'$ | – | – | – | A 697 | – | A 661 | A 649 | A 669 | – | A 697 |
| $W_{EC2}''$ | – | – | – | – | – | – | A 613 | A 642 | – | – |
| $W_{EC2}'''$ | – | – | – | – | – | A 673 | – | – | – | – |
| $W_{EC3}$ | A 707 | A 712 | A 634 | A 720 | A 639 | A 706 | A 658 | A 704 | A 649 | A 703 |
| $W_{EC3}'$ | – | A 714 | – | – | – | – | – | – | – | – |
| $W_{EC3}''$ | – | A 710 | – | – | – | – | – | – | – | – |
| $W_{EC4}$ | A 761 | A 760 | A 655 | A 755 | A 660 | A 720 | A 672 | A 734 | A 676 | |
| $W_{EC5}$ | A 447 | A 421 | A 438 | A 417 | A 437 | A 430 | A 442 | A 428 | A 420 | A 502 |

**Supplementary Table 3. Distance geometry (Å) of $CO_2$, $HCO_3^-$ and key bound water molecules in the CA II structures.**

| | apo-CA II 0atm 6LUU | apo-CA II 20atm 6LUV | Zn-CA II 0atm 6LUW | Zn-CA II 20atm 6LUX | Co-CA II 0atm pH11.0 6LV3 | Co-CA II 20atm 6LV2 | Ni-CA II 0atm 6LV5 | Ni-CA II 20atm 6LV6 | Cu-CA II 0atm 6LV9 | Cu-CA II 20atm 6LVA |
|---|---|---|---|---|---|---|---|---|---|---|
| Zn – $W_{Zn}$ | – | – | 1.88 | 1.92 | – | – | – | – | – | – |
| Zn – $CO_2$(O1) | – | – | – | 3.31 | – | – | – | – | – | – |
| Co – $W_{Co,\ tetra}$ | – | – | – | – | 1.72 | – | – | – | – | – |
| Co – $W_{Co,\ octa}$ | – | – | – | – | – | 2.03 | – | – | – | – |
| Co – $CO_2$(O1) | – | – | – | – | – | – | – | – | – | – |
| Co – $HCO_3^-$(O1) | – | – | – | – | – | 2.07 | – | – | – | – |
| Co – $HCO_3^-$(O3) | – | – | – | – | – | 2.02 | – | – | – | – |
| Ni – $W_{Ni}$ | – | – | – | – | – | – | 2.09 | 2.13 | – | – |
| Ni – $W_{Ni}'$ | – | – | – | – | – | – | 2.14 | – | – | – |
| Ni – $W_{Ni}''$ | – | – | – | – | – | – | 2.22 | – | – | – |
| Ni – $HCO_3^-$(O1) | – | – | – | – | – | – | – | 2.16 | – | – |
| Ni – $HCO_3^-$(O3) | – | – | – | – | – | – | – | 2.18 | – | – |
| Cu – $W_{Cu}$ | – | – | – | – | – | – | – | – | 2.17 | 2.26 |
| Cu – $W_{Cu}'$ | – | – | – | – | – | – | – | – | 2.36 | – |
| Cu – $W_{DW}''$ | – | – | – | – | – | – | – | – | – | 3.14 |
| $CO_2$(C1) – $W_{Zn}$ | – | – | – | 2.87 | – | – | – | – | – | – |
| $CO_2$(C1) – $W_{Co,\ tetra}$ | – | – | – | – | – | – | – | – | – | – |
| $HCO_3^-$(O1) – $W_{Co,\ octa}$ | – | – | – | – | – | 2.85 | – | – | – | – |
| $HCO_3^-$(O3) – $W_{Co,\ octa}$ | – | – | – | – | – | 2.72 | – | – | – | – |
| $HCO_3^-$(O1) – $W_{Ni}$ | – | – | – | – | – | – | – | 2.92 | – | – |
| $HCO_3^-$(O3) – $W_{Ni}$ | – | – | – | – | – | – | – | 2.85 | – | – |
| $CO_2$(C1) – $W_{apo}$ | – | 2.94 | – | – | – | – | – | – | – | – |
| $W_{Zn}$ – $W_{DW}$ | – | – | 2.58 | – | – | – | – | – | – | – |
| $W_{Zn}$ – W1 | – | – | 2.63 | – | – | – | – | – | – | – |
| $W_{Zn}$ – $W_I$ | – | – | – | 2.75 | – | – | – | – | – | – |
| $W_{Zn}$ – W2 | – | – | 4.41 | 4.65 | – | – | – | – | – | – |
| $W_{Co,\ tetra}$ – $W_{DW}$ | – | – | – | – | 2.61 | – | – | – | – | – |
| $W_{Co,\ tetra}$ – W1 | – | – | – | – | 2.73 | – | – | – | – | – |
| $W_{Co,\ tetra}$ – $W_I$ | – | – | – | – | – | – | – | – | – | – |
| $W_{Co,\ tetra}$ – W2 | – | – | – | – | 4.55 | – | – | – | – | – |
| $W_{Co,\ octa}$ – $W_I$ | – | – | – | – | – | 2.75 | – | – | – | – |
| $W_{Co,\ octa}$ – W2 | – | – | – | – | – | 2.71 | – | – | – | – |
| $W_{Co,\ octa}$ – W2′ | – | – | – | – | – | 4.90 | – | – | – | – |
| $W_{Ni}$ – $W_{Ni}'$ | – | – | – | – | – | – | 2.82 | – | – | – |
| $W_{Ni}$ – $W_{Ni}''$ | – | – | – | – | – | – | 3.08 | – | – | – |
| $W_{Ni}'$ – $W_{Ni}''$ | – | – | – | – | – | – | 2.78 | – | – | – |
| $W_{Ni}$ – $W_I$ | – | – | – | – | – | – | 2.95 | 2.88 | – | – |
| $W_{Ni}$ – W2 | – | – | – | – | – | – | 2.73 | 2.68 | – | – |
| $W_{Cu}$ – W1 | – | – | – | – | – | – | – | – | 2.54 | – |
| $W_{Cu}$ – $W_{Cu}'$ | – | – | – | – | – | – | – | – | 3.06 | – |

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| $W_{Cu}$ – $W_{DW}$ | – | – | – | – | – | – | – | – | 3.23 | 2.84 |
| $W_{Cu}'$ – $W_{DW}$ | – | – | – | – | – | – | – | – | 2.84 | – |
| $W_{DW}$ – $W_{DW}'$ | – | – | – | – | – | – | – | – | – | 1.52 |
| $W_{DW}'$ – $W_{DW}''$ | – | – | – | – | – | – | – | – | – | 1.60 |
| $W_{Cu}$ – $W_I$ | – | – | – | – | – | – | – | – | – | 2.84 |
| $W_{Cu}$ – W2 | – | – | – | – | – | – | – | – | 3.44 | 3.90 |
| $W_{apo}$ – $W_{DW}$ | 2.71 | – | – | – | – | – | – | – | – | – |
| $W_{apo}$ – W1 | 2.82 | – | – | – | – | – | – | – | – | – |
| $W_{apo}$ – $W_I$ | – | 2.88 | – | – | – | – | – | – | – | – |
| $W_{apo}$ – W2 | 4.90 | 3.96 | – | – | – | – | – | – | – | – |
| W1 – W2 | 2.73 | – | 2.74 | – | 2.70 | – | – | – | 2.78 | – |
| $W_I$ – $W_I'$ | – | – | – | 2.02 | – | – | – | – | – | 2.09 |
| W2 – W2′ | – | – | – | 1.43 | – | 2.79 | – | – | – | 1.51 |
| W2 – W3a | 2.75 | 2.80 | 2.77 | 3.12 | 2.77 | 3.40 | 2.81 | 2.72 | 2.81 | 3.14 |
| W2 – W3b | 2.72 | 2.99 | 2.73 | 2.49 | 2.68 | 2.41 | 2.95 | 3.08 | 2.79 | 2.57 |
| W2 – W3b′ | – | – | – | 1.68 | – | 2.69 | – | – | – | 1.65 |
| W3b – W3b′ | – | – | – | 1.37 | – | 1.86 | – | – | – | 1.39 |
| Thr199(N) – $HCO_3^-$(O2) | – | – | – | – | – | 2.93 | – | 3.05 | – | – |
| Thr199(N) – $CO_2$(O2) | – | 3.20 | – | 3.62 | – | – | – | – | – | – |
| Thr199(N) – $W_{DW}$ | 2.93 | – | 2.92 | – | 2.90 | – | – | – | 2.90 | 2.74 |
| Thr199(N) – $W_I$ | – | 3.34 | – | 3.52 | – | 3.71 | 3.04 | 3.52 | – | 3.68 |
| Thr199(Oγ1) – $HCO_3^-$(O3) | – | – | – | – | – | 2.44 | – | 2.46 | – | – |
| Thr199(Oγ1) – $W_{Zn}$ | – | – | 2.73 | 2.62 | – | – | – | – | – | – |
| Thr199(Oγ1) – $W_{Co,\ tetra}$ | – | – | – | – | 2.75 | – | – | – | – | – |
| Thr199(Oγ1) – $W_{Co,\ octa}$ | – | – | – | – | – | 3.75 | – | – | – | – |
| Thr199(Oγ1) – $W_{Ni}$ | – | – | – | – | – | – | 3.68 | 3.65 | – | – |
| Thr199(Oγ1) – $W_{Ni}'$ | – | – | – | – | – | – | 2.62 | – | – | – |
| Thr199(Oγ1) – $W_{Ni}''$ | – | – | – | – | – | – | 5.04 | – | – | – |
| Thr199(Oγ1) – $W_{Cu}$ | – | – | – | – | – | – | – | – | 2.68 | 2.61 |
| Thr199(Oγ1) – $W_{Cu}'$ | – | – | – | – | – | – | – | – | 4.37 | – |
| Thr199(Oγ1) – $W_{apo}$ | 2.72 | 2.69 | – | – | – | – | – | – | – | – |
| Thr199(Oγ1) – Glu106(Oε1) | 2.60 | 2.59 | 2.61 | 2.56 | 2.62 | 2.53 | 2.91 | 2.55 | 2.61 | 2.59 |
| Glu106(Oε1) – $W_{Zn}$ | – | – | 4.15 | 4.01 | – | – | – | – | – | – |
| Glu106(Oε1) – $W_{Co,\ tetra}$ | – | – | – | – | 4.08 | – | – | – | – | – |
| Glu106(Oε1) – $W_{Co,\ octa}$ | – | – | – | – | – | 4.85 | – | – | – | – |
| Glu106(Oε1) – $W_{Ni}$ | – | – | – | – | – | – | 4.75 | 4.84 | – | – |
| Glu106(Oε1) – $W_{Ni}'$ | – | – | – | – | – | – | 2.78 | – | – | – |
| Glu106(Oε1) – $W_{Cu}$ | – | – | – | – | – | – | – | – | 4.20 | 4.19 |

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Glu106(Oε1) – $W_{apo}$ | 4.01 | 4.09 | – | – | – | – | – | – | – | – |
| $His64_{in}$(Nδ1) – W2 | 3.02 | 3.16 | 3.22 | 3.28 | 2.94 | 3.88 | 3.29 | 3.22 | 3.43 | 3.48 |
| $His64_{in}$(Nδ1) – W2′ | – | – | – | 1.86 | – | 1.26 | – | – | – | 1.99 |

**Supplementary References**

1 Sjoeblom, B., Polentarutti, M. & Djinovic-Carugo, K. Structural study of X-ray induced activation of carbonic anhydrase. *Proc Natl Acad Sci U S A* **106**, 10609-10613, doi:10.1073/pnas.0904184106 (2009).