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Combined DFT and Continuum Calculation of pK_as in Carbonic Anhydrase

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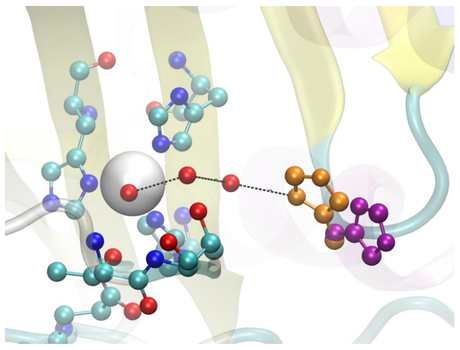
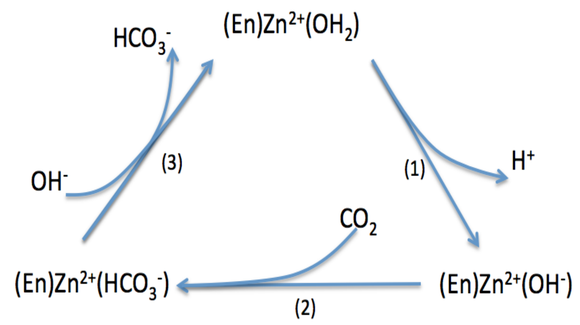
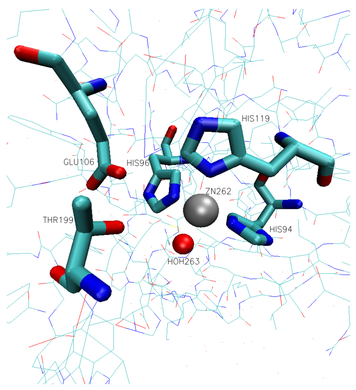
Catalysis of Carbonic Anhydrase

- CA II – most efficient enzyme of all. Reaction rate 10^{-6}!
- Catalysis occurs at zinc-centered active site.
- Proton transfer is the rate-limiting step.
- Catalysis triggered by deprotonation of zinc-bound water.





pK_a of zinc-bound water
- Native enzyme: 6.8
- Mutation of nearby residues raise the pk_a
  - Direct ligand: His → Asp 8.5/9.6
  - Indirect ligand: Thr199 → Ala 8.3/8.7



pK_a Calculation

$$pK_a = -\log_{10}K_a\,, \quad K_a = \exp\left(-\frac{\Delta G_{aq}^{deprot}}{RT}\right)$$

$$pK_a = \Delta G_{aq}^{deprot}/RT\ln 10$$

pK_a 1 ~ 1.37 kcal/mol

$$\text{WT-H}_2\text{O (aq)} \xrightarrow{\Delta G_{aq}^{deprot}(WT)} \text{WT-OH}^- + \text{H}^+\text{(aq)}$$
$$\Delta G_{solv}(WT)\uparrow \qquad \Delta G^*_{solv}(WT)\uparrow \qquad \uparrow \Delta G^{H^+}_{solv}$$
$$\text{WT-H}_2\text{O (gas)} \xrightarrow{\Delta G_{gas}^{deprot}(WT)} \text{WT-OH}^- + \text{H}^+\text{(gas)}$$

$$\text{MT-H}_2\text{O (aq)} \xrightarrow{\Delta G_{aq}^{deprot}(MT)} \text{MT-OH}^- + \text{H}^+\text{(aq)}$$
$$\Delta G_{solv}(MT)\uparrow \qquad \Delta G^*_{solv}(MT)\uparrow \qquad \uparrow \Delta G^{H^+}_{solv}$$
$$\text{MT-H}_2\text{O (gas)} \xrightarrow{\Delta G_{gas}^{deprot}(MT)} \text{MT-OH}^- + \text{H}^+\text{(gas)}$$

$$\Delta G_{deprot}(X) = \Delta G_{gas}(X) + \Delta G^*_{solv}(X) + \Delta G^{H^+}_{solv} - \Delta G_{solv}(X)$$
$$\Delta pK_a = \Delta\Delta G^{deprot} = (\Delta G_{gas}(MT) + \Delta\Delta G_{solv}(MT)) - (\Delta G_{gas}(WT) + \Delta\Delta G_{solv}(WT))$$

DFT + continuum

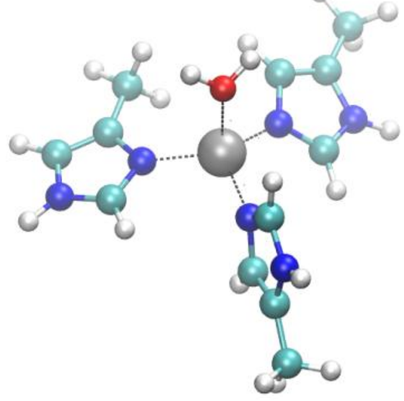
- Active site: Density Functional Theory
  - B3LYP/6-311+(2d,p)
- Environment (protein+solvent): dielectric continuum model
  - Solute ε = 1.0
  - Solvent ε = 40.0

Results

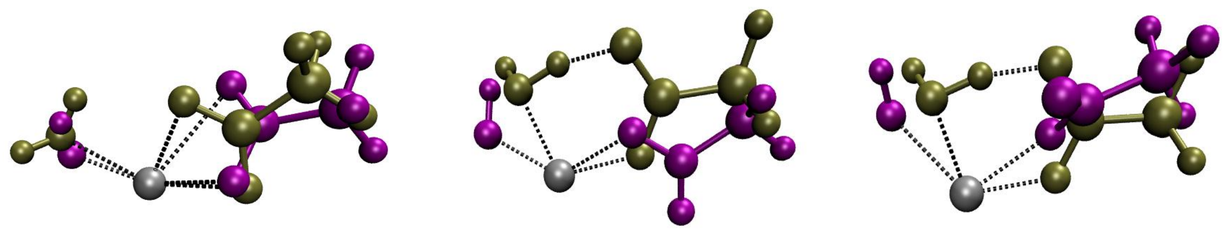
	$\Delta\Delta G_{gas}$	$\Delta\Delta G_{solv}$	$\Delta\Delta G_{deprot}$	ΔpK_a	exp
WT	761.20	432.98	0	0	
H94D	1093.64	116.47	15.93	2.78	2.8
H119D	1100.14	105.68	11.65	2.03	1.8
H96D	1092.24	122.12	20.18	3.52	

Wild type structure

	Zn-N(94)	Zn-N(96)	Zn-N(119)	Zn-O
Crystal	2.10	2.12	2.11	2.05
Prot	2.00	2.02	2.00	2.16
Deprot	2.05	2.07	2.07	1.88



Mutant structure



Charges and dipoles

	Zn	OW	Wat	Im	Ace	Dipole
WT(H2O)	1.269	-1.148	0.072	0.226		1.837
WT(OH)	1.251	-1.231	-0.716	0.155		0.583
94(H2O)	1.303	-1.135	0.051	0.186	-0.727	1.510
94(OH)	1.284	-1.212	-0.712	0.115	-0.801	0.562
96(H2O)	1.296	-1.198	0.018	0.191	-0.701	1.858
96(OH)	1.289	-1.233	-0.733	0.121	-0.733	0.558
119(H2O)	1.299	-1.199	0.014	0.190	-0.695	2.008
119(OH)	1.288	-1.231	-0.726	0.117	-0.796	0.566

Conclusions

- The pk_as of enzyme can be well reproduced by DFT+continuum approach.
- pK_a shift caused by mutation is due to the change of the coordinating structure and the redistribution of the charges.
- The distinct pk_a increase of the same type of mutation at different site results from asymmetric ligation and different electronic environment.

References

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