Electronic Supplementary Material (ESI) for Physical Chemistry Chemical Physics.

Supporting Information

Insight into the Activation Mechanism of Carbonic Anhydrase (II) through 2-(2-Aminoethyl)-Pyridine: A Promising Pathway for Enhanced Enzymatic Activity

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Methodology

The highest occupied molecular orbital (HOMO) and the lowest unoccupied orbital (LUMO) of 2-2AEPy and gap energy (ΔE_g) between them were evaluated. The HOMO-LUMO energies are usually used to calculate some molecular properties such as ionization potential (I), electron affinity(A), absolute hardness(η), softness(s), chemical potential(μ) and absolute electronegativity (x) as follows:

$$I = E_{HOMO} \tag{1}$$

$$A = -E_{LUMO} \tag{2}$$

$$\eta = \frac{E_{LUMO} - E_{HOMO}}{2} \tag{3}$$

$$S = \frac{1}{2\eta} \tag{4}$$

$$\mu = \frac{-(I + A)}{2} \tag{5}$$

$$X = \frac{I + A}{2} \tag{6}$$

Some Molecular properties of the activator are announced in Table S1.

Table S1: Molecular properties (eV) of activator computed at B3LYP-D3/6-31+g** method

	HOMO	LUMO	ΔE	x	η	s	μ
2-2AEPy	-6.4	-1.0	5.4	-2.7	2.7	0.2	2.7

A general mechanism for the activation of carbonic anhydrase with activators has been been propounded based on Eq (7).

$$[(His)_3Zn^{+2}(H_2O)+Activator(Ac)] \rightleftharpoons [(His)_3Zn^{+2}(H_2O)/Ac] \rightleftharpoons [(His)_3Zn^{+2}(OH^-)]+AcH^+ \tag{7}$$

where $[(His)_3Zn^{+2}(H_2O)/Ac]$ denote the enzyme-activator complex.
Thermodynamic functions have been evaluated from the following equations. Total enthalpies of the studied species X, (H(X)), at the temperature T are usually evaluated for the inactive form to the active form.

$$H(X) = E_0 + ZPE + E_{trans} + E_{rot} + E_{vib} + RT \tag{8}$$

where E_0 is determined total electronic energy, ZPE stands for zero-point energy, E_{trans}, E_{rot} and E_{vib} are the translational, rotational and vibrational contributions to the enthalpy, respectively. Eventually, to convert energy to enthalpy, the PV-work term that denoted by RT is added. The standard enthalpy changes of the reaction (ΔH^0_{rxn}) from the expression (7) is calculated as:

$$\Delta H^0_{rxn} = [H^0_{EZn(II)OH^-} + H^0_{ACH^+}] - [H^0_{EZn(II)OH_2} + H^0_{Ac}] \tag{9}$$

Identically, ΔS^0_{rea} is gained by

$$\Delta S^0_{rxn} = [S^0_{EZn(II)OH^-} + S^0_{AH^+}] - [S^0_{EZn(II)OH_2} + S^0_{Ac}] \tag{10}$$

Then using $\Delta G = \Delta H - T\Delta S$, ΔG^0_{rxn} was determined.

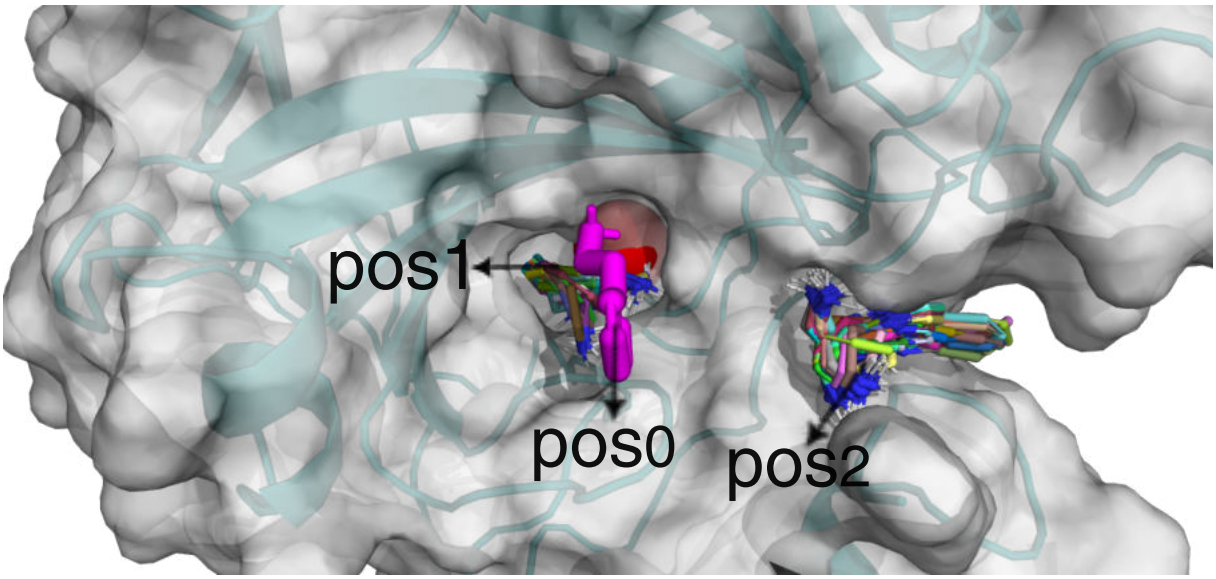


Fig. S 1: The low-energy docked positions of 2-2AEPy into hCA II were obtained by 300 separate AuthoDock4 runs. The magenta color (pos0) shows the initial position, where 2-2AEPy replaces the HIS300 position reported in the X-ray. The main difference between pos0 and pos1 is the presence of three crystallographic molecules that separate 2-2AEPy (located in the ligand X-ray position) from the Zn active site; otherwise, they are quite similar. The scored binding energies of pos0 are around -5.7 kcal.mol^{-1} and those of pos2 are around -5.5 kcal.mol^{-1}, indicating that pos0 is the preferred site and also matches better with the X-ray position of HIS300.

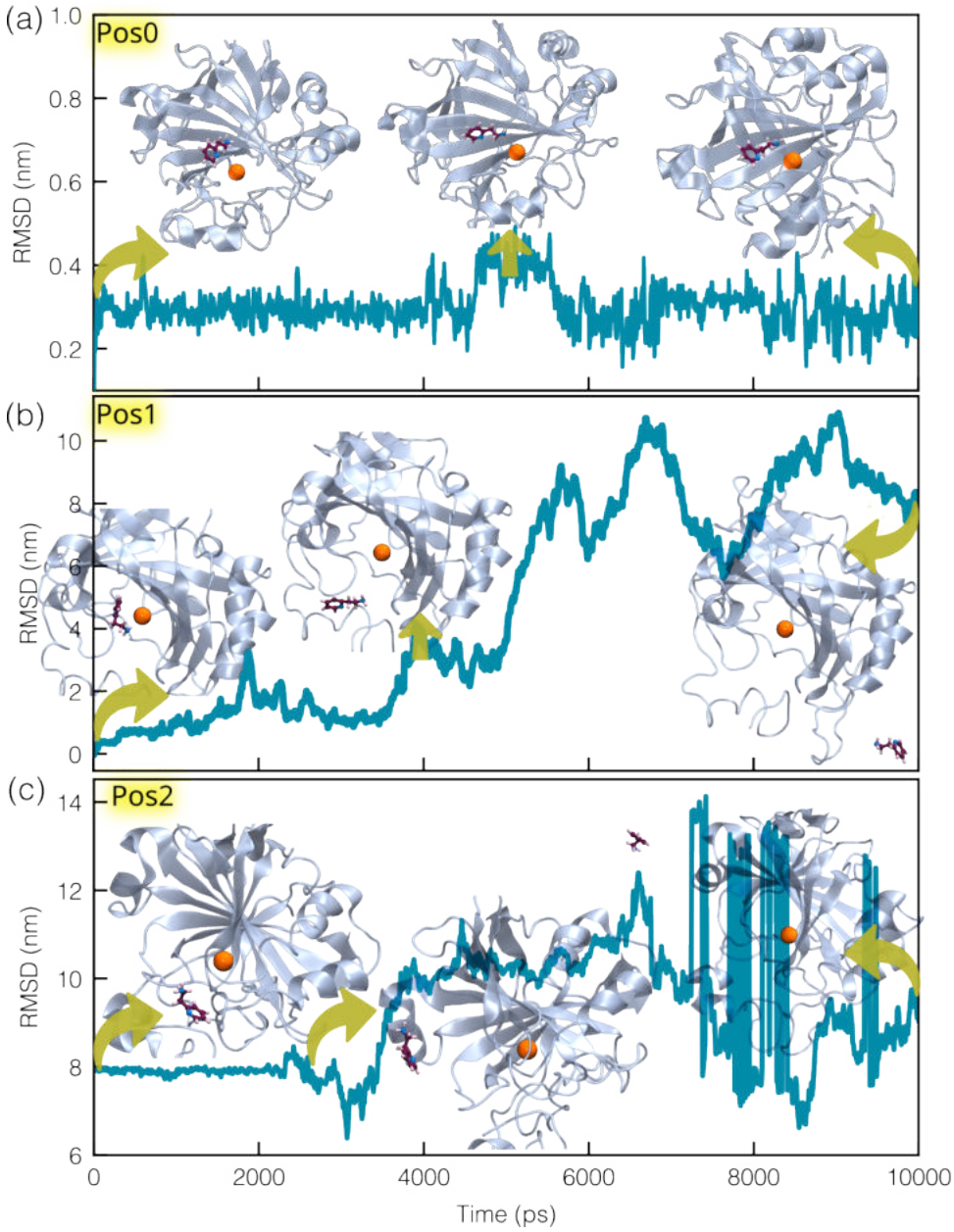


Fig. S2: 10 ns simulations on various models studied in the work. Pos1 and Pos2 are obtained by docking, and Pos0 is based on the location of the original ligand of 2ABE (HIS300 activator). In the Pos0 MD simulations, we preserved all four crystallographic water molecules (Zn-bounded) plus three neighboring water molecules. In Pos1, which is very close to Zn, we just kept the Zn-bounded water molecules, and the MD simulations reveal that after around 4 ns, the ligand gets out of the portien environment.

Additional results concerning the structures of hCA II in both forms

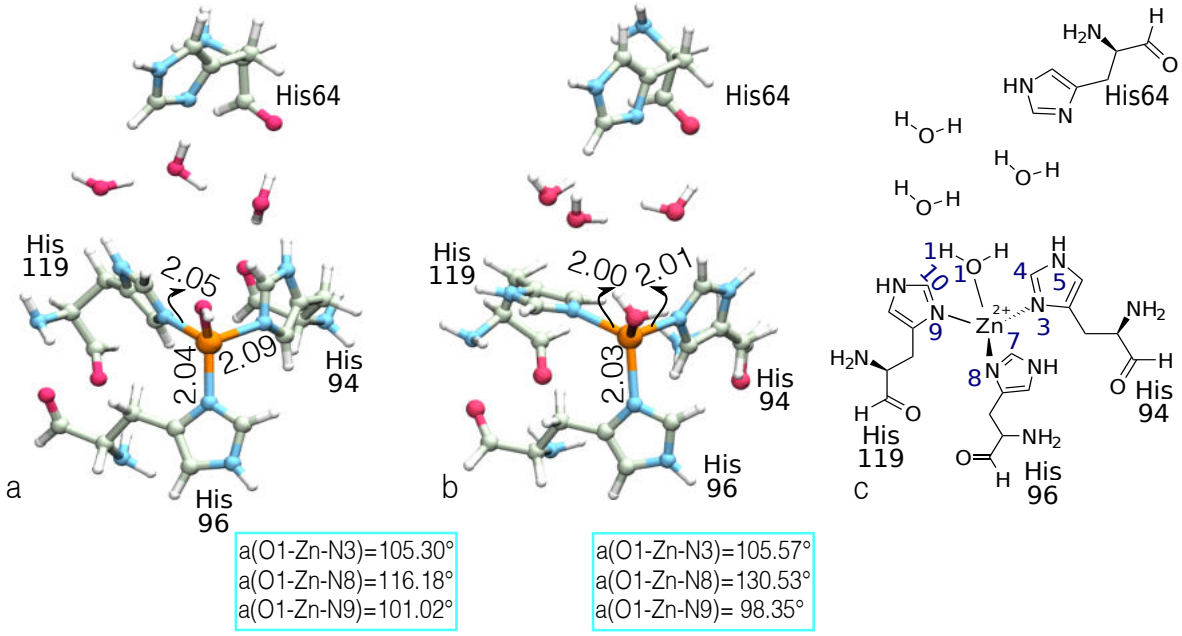


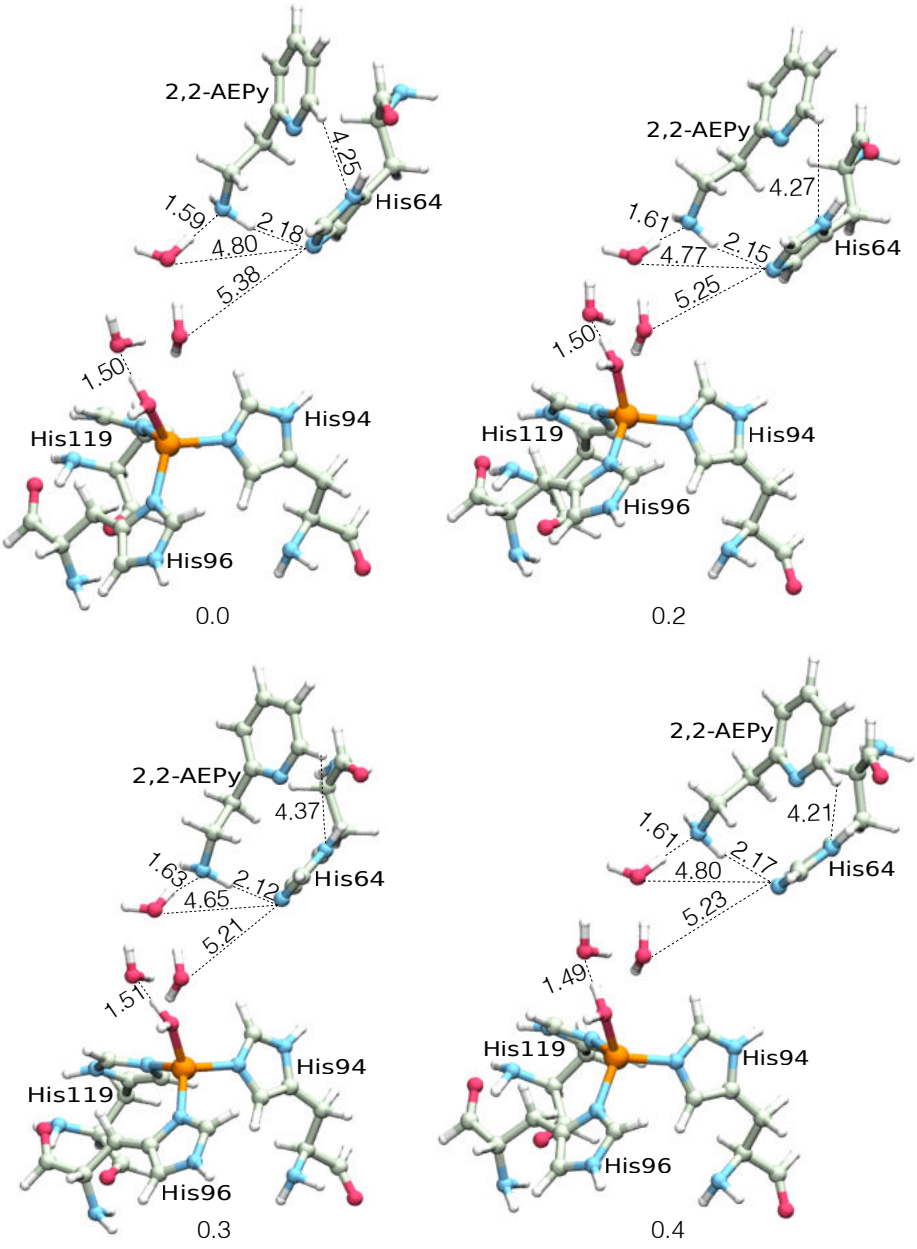
Fig. S3: The optimized structure of hCA II active site in the zinc hydroxide (a), inactive form (b) and schematic of X-ray crystallographic (c). All bond lengths and bond angles are given in (Å) and (°), respectively.

Table S2: Chemical structure details of optimized geometry for the active and inactive form of hCA II active site in biological environment using B3LYP-D3/6-31+g(2d,2p) and comparison with X-ray data

Connected atoms	Active form	Inactive form	X-ray
Bond distance (Å)			
Zn-O1	1.87	2.03	2.25
O1-H1	0.96	1.02	0.97
Zn-N3	2.09	2.01	2.09
Zn-N8	2.04	2.03	2.22
Zn-N9	2.05	2.00	2.10
N3-C4	1.33	1.33	1.32
N8-C7	1.33	1.33	1.32
N9-C10	1.38	1.39	1.37
Standard deviation	0.06	0.05	
Bond angle (°)			
O1-Zn-N3	105.57	105.30	106.08
O1-Zn-N8	130.53	116.17	106.79
O1-Zn-N9	98.35	101.02	102.32
N3-Zn-N8	94.95	108.31	110.95
N3-Zn-N9	107.17	106.12	103.00
N9-Zn-N8	114.47	119.02	118.22
N3-C4-N5	110.54	109.27	108.37
Standard deviation	5.27	2.45	

Geometries and relative energies of reactant for activator from the Nα position

The large number of structures (>50) geometries of the reactant for the activator from the Nα position have been optimized. The sixteen lowest ones the structures and the energies are given.



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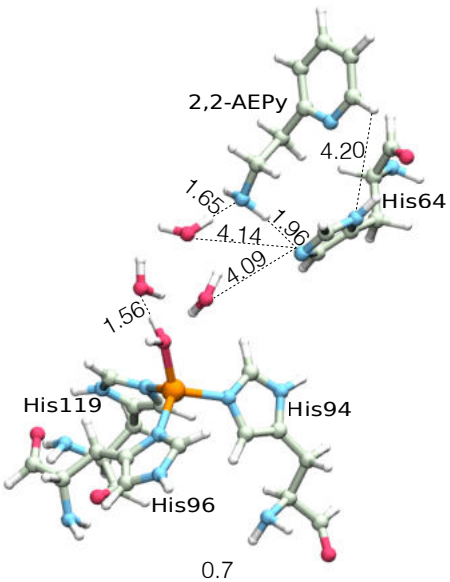
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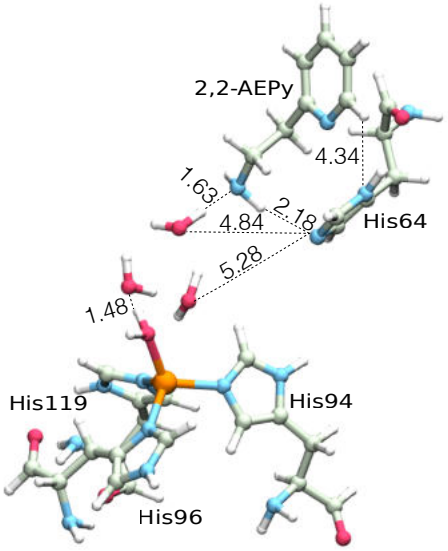
1.65
His64
1.96
4.14
4.09
1.56
His119
His94
His96
0.7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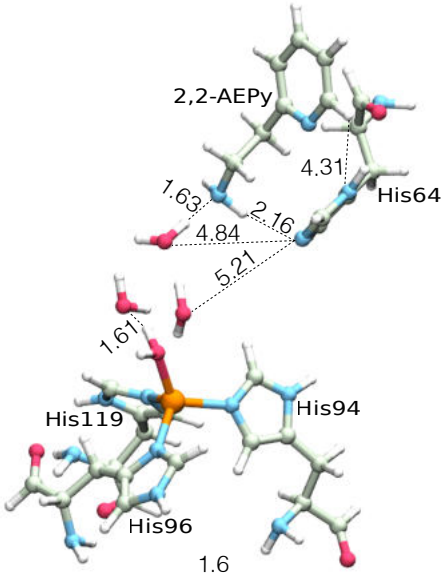
2.18
4.84
His64
5.28
1.48
His119
His94
His96
1.3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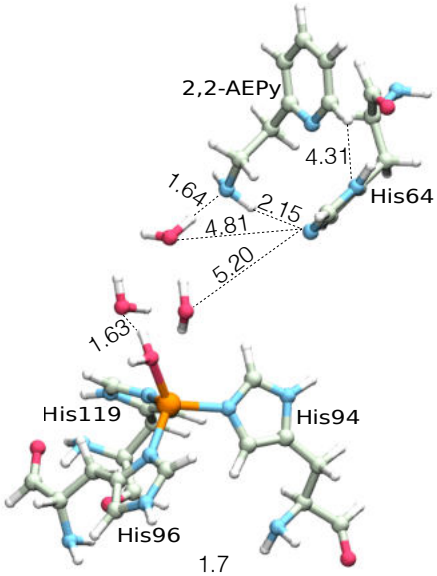
4.31
1.63
His64
2.16
4.84
5.21
1.61
His119
His94
His96
1.6


2,2-AEPy
4.31
1.64
His64
2.15
4.81
5.20
1.63
His119
His94
His96
1.7

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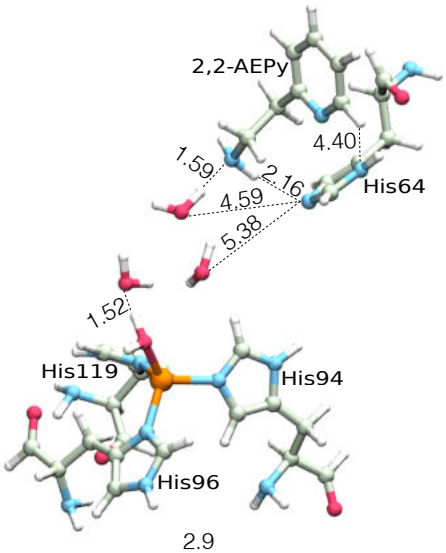
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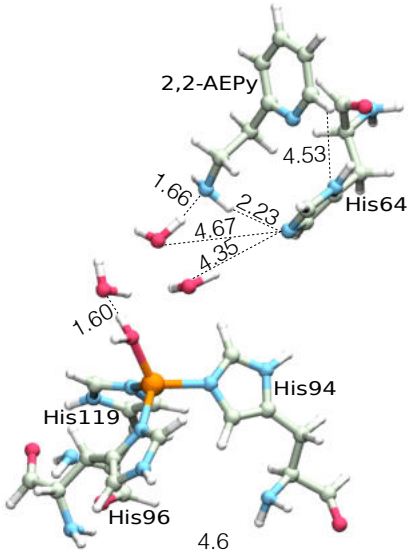
2.16
His64
4.59
5.38
1.52
His119
His94
His96

2.9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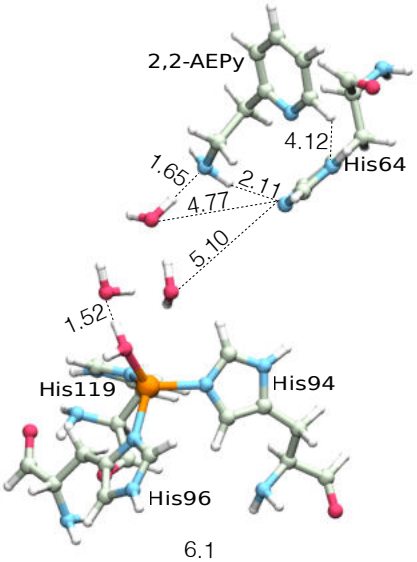
4.35
1.60
His94
His119
His96

4.6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2.11
His64
4.77
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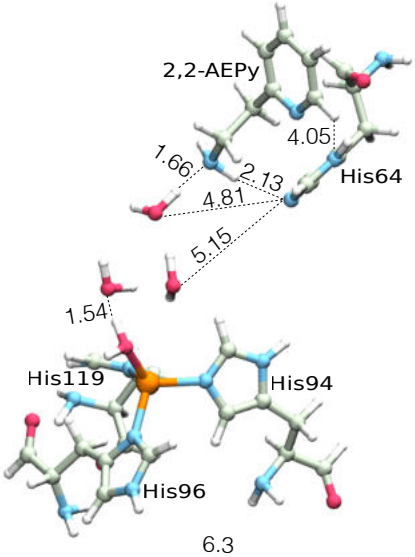
1.52
His119
His94
His96

6.1


2,2-AEPy
4.05
1.66
2.13
His64
4.81
5.15
1.54
His119
His94
His96

6.3

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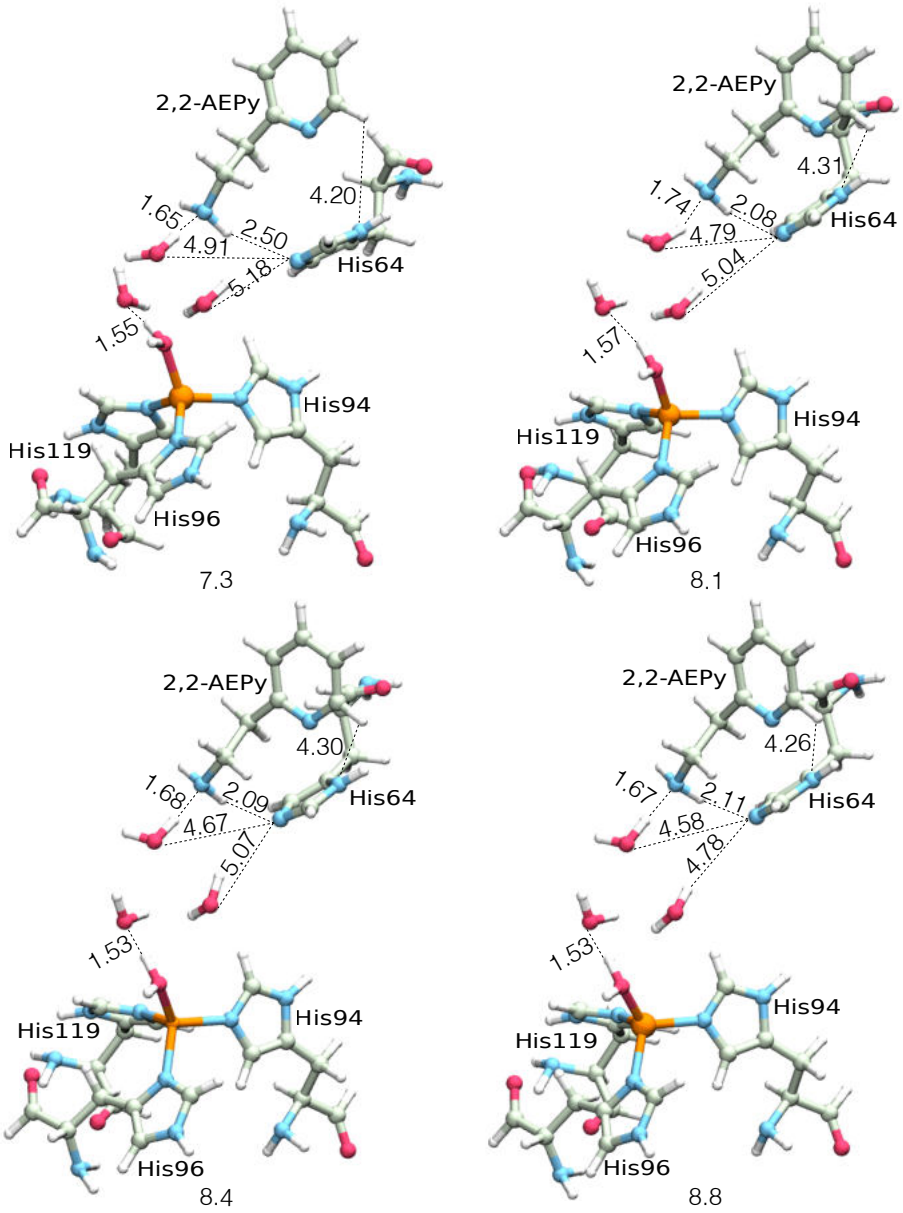
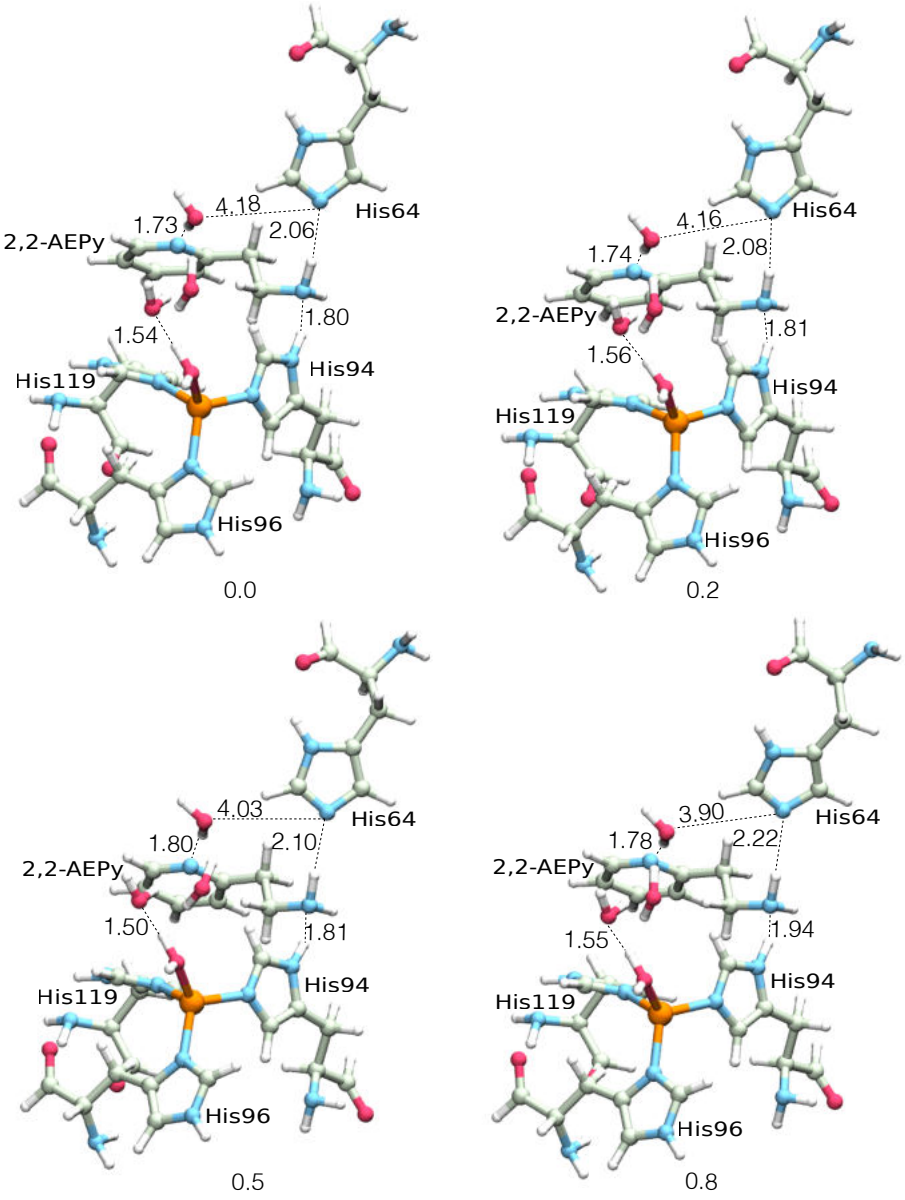


Fig. S4: Geometries and relative energies of the sixteen lowest energy reactants for the activator from the Nα position. The energies relative to React are given in kcal/mol.

Geometries and relative energies of reactant for activator from the Nβ position

The large number of structures (>40) geometries of the reactant for the activator from the Nβ position have been optimized. The eight lowest ones the structures and the energies are given.



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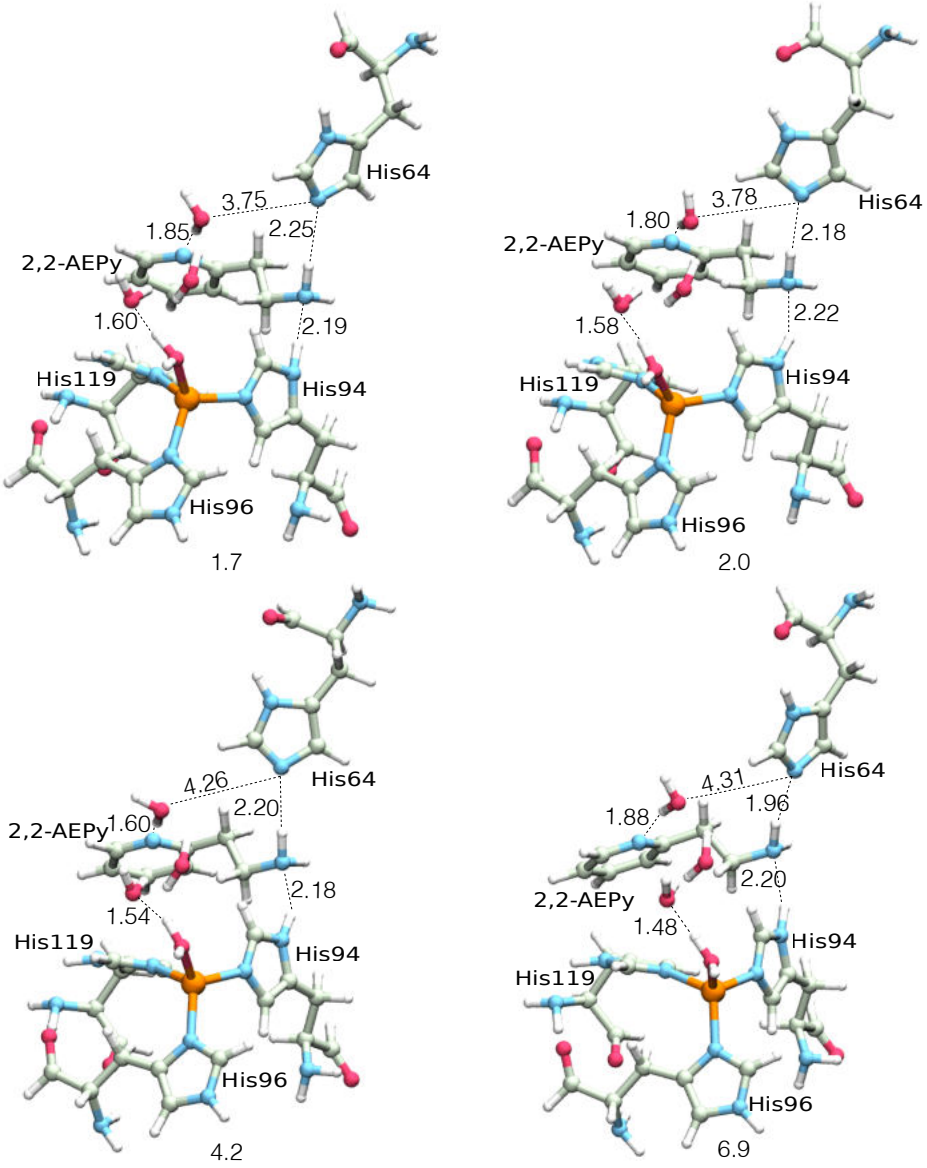


Fig. S5: Geometries and relative energies of the eight lowest energy reactants for the activator from the Nβ position. The energies relative to React are given in kcal/mol.

Optimized structures of reactant for both of positions

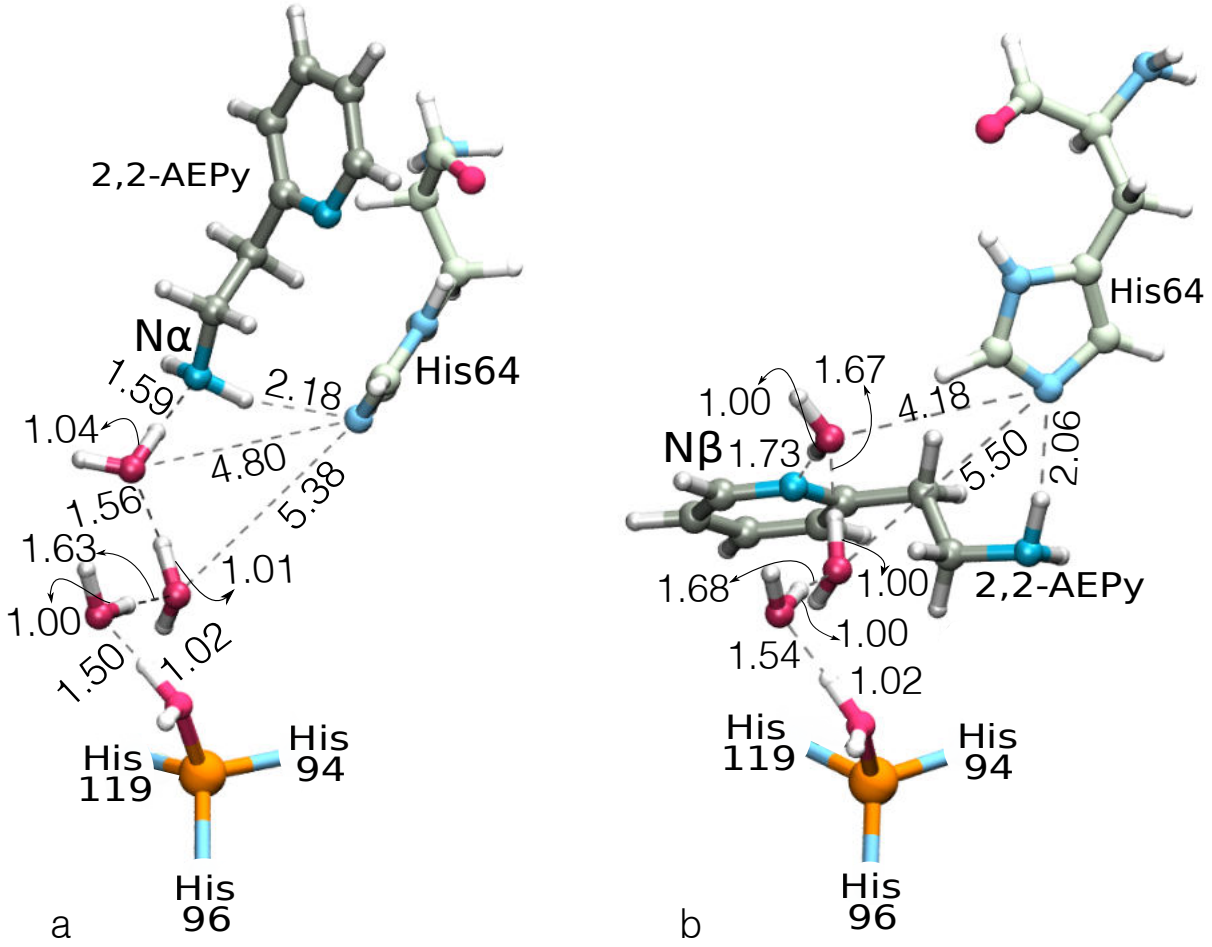


Fig. S6: Optimized structure of the reactant for the activator from the Nα position (a) and the activator from the Nβ position (b). Distances are given in angstrom.

Table of NBO atomic charge for both mechanisms

Table S 3: NBO atomic charge (in a.u) for stepwise and concerted mechanisms in biological environment for Nα and Nβ positions.

	Zn	O1	H1	O2	H2	O3	H3	O4	H4	N
Reactα	1.32	-1.06	0.55	-1.04	0.54	-1.05	0.54	-1.08	0.52	-0.94
Reactβ	1.32	-1.06	0.55	-1.04	0.54	-1.05	0.54	-1.06	0.52	-0.53
Stepwise										
TS1α	1.31	-1.21	0.56	-0.98	0.55	-1.01	0.55	-1.07	0.52	-0.93
TS1β	1.31	-1.17	0.56	-1.01	0.56	-1.04	0.55	-1.06	0.52	-0.53
Int α	1.32	-1.06	0.55	-1.04	0.53	-1.05	0.54	-1.06	0.55	-0.92
Intβ	1.32	-1.06	0.55	-1.04	0.54	-1.05	0.54	-1.06	0.53	-0.51
TS2α	1.32	-1.18	0.56	-1.02	0.55	-1.05	0.55	-1.06	0.52	0.93
TS2β	1.31	-1.16	0.55	-1.05	0.55	-1.04	0.55	-1.06	0.52	0.52
Concerted										
TSα	1.33	-1.10	0.55	-1.05	0.55	-1.06	0.55	-1.09	0.51	-0.92
TSβ	1.31	-1.18	0.55	-1.06	0.55	-1.06	0.55	-1.07	0.51	-0.52
Prodα	1.29	-1.22	0.54	-1.07	0.54	-1.05	0.54	-1.06	0.49	-0.86
Prodβ	1.30	-1.22	0.54	-1.08	0.54	-1.05	0.54	-1.06	0.50	-0.50

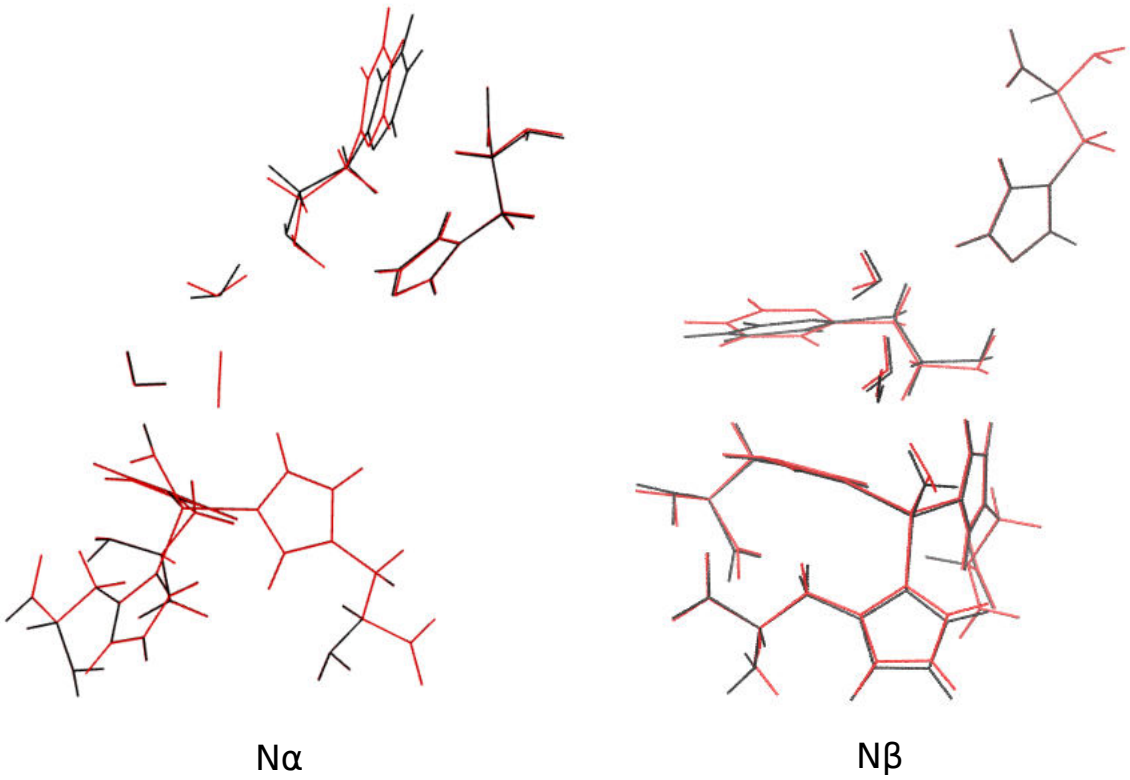


Fig. S 7: The overlay of reactant and intermediate structures from Nα (left) and Nβ (right) positions. The reactant and intermediate are shown in red and black, respectively.

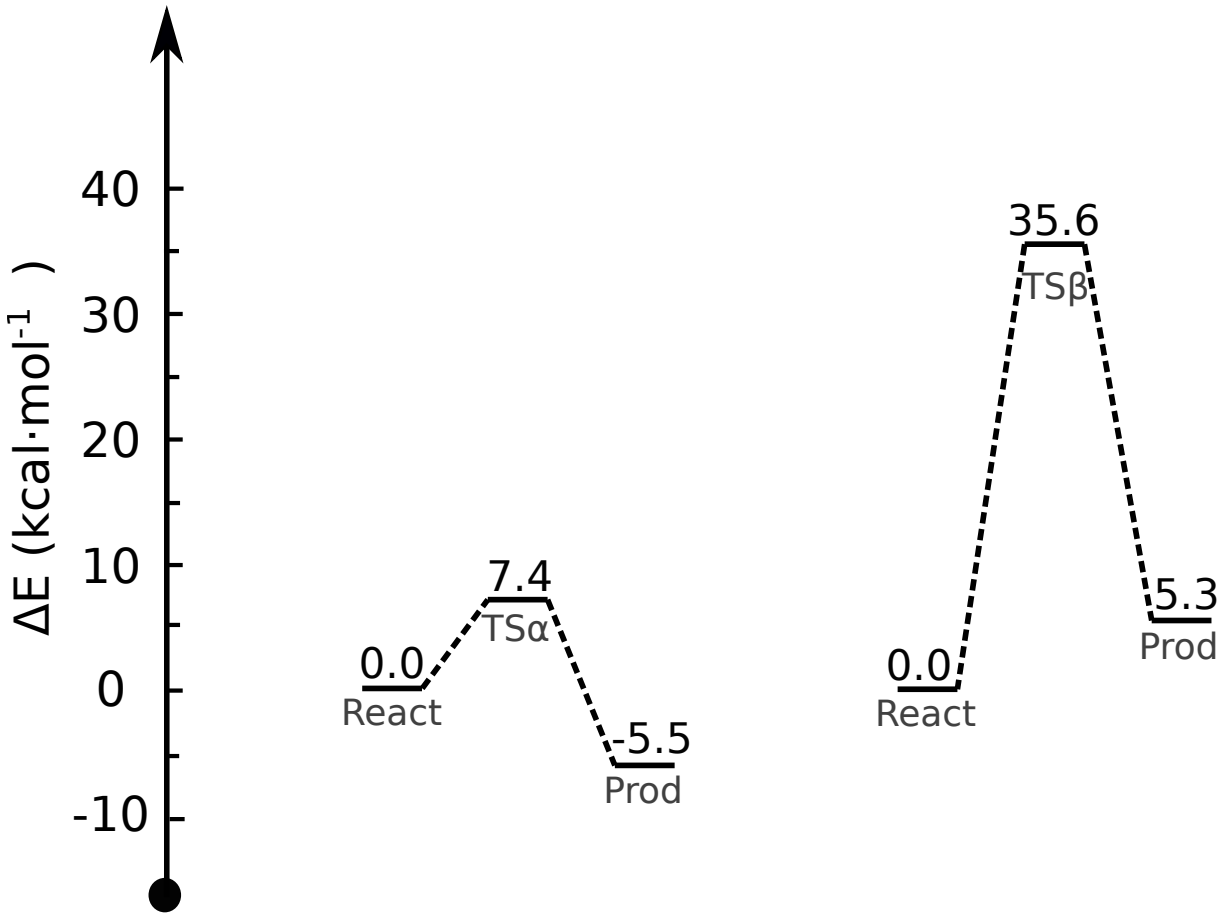
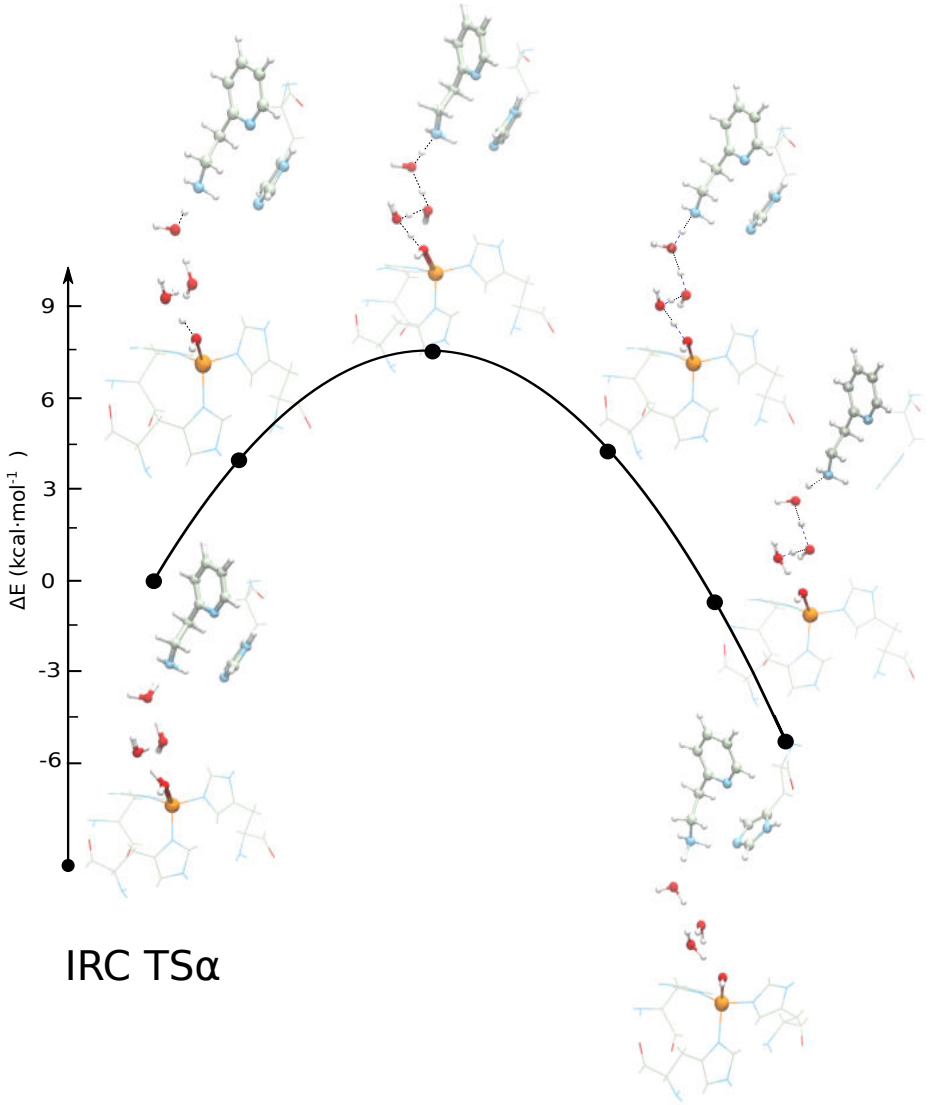


Fig. S8: Calculated energy profile for the concerted mechanism from Nα and Nβ positions.


9
6
3
0
-3
-6
ΔE (kcal·mol-1)
IRC TSα

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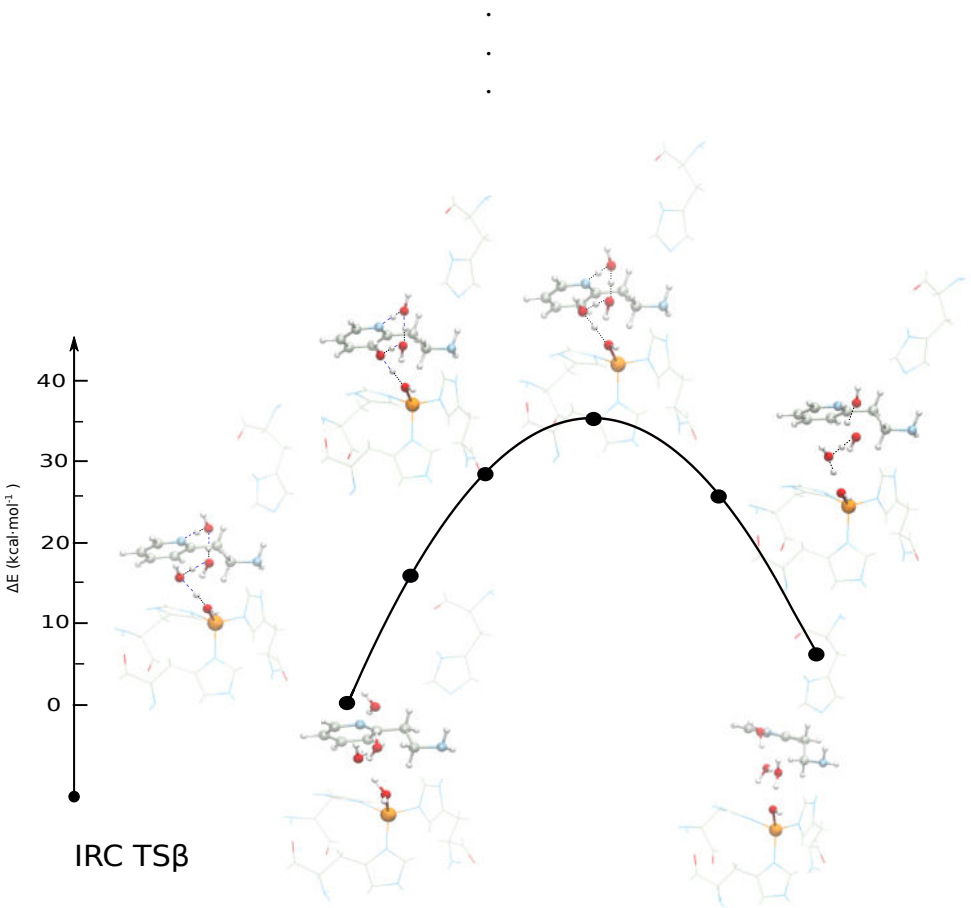
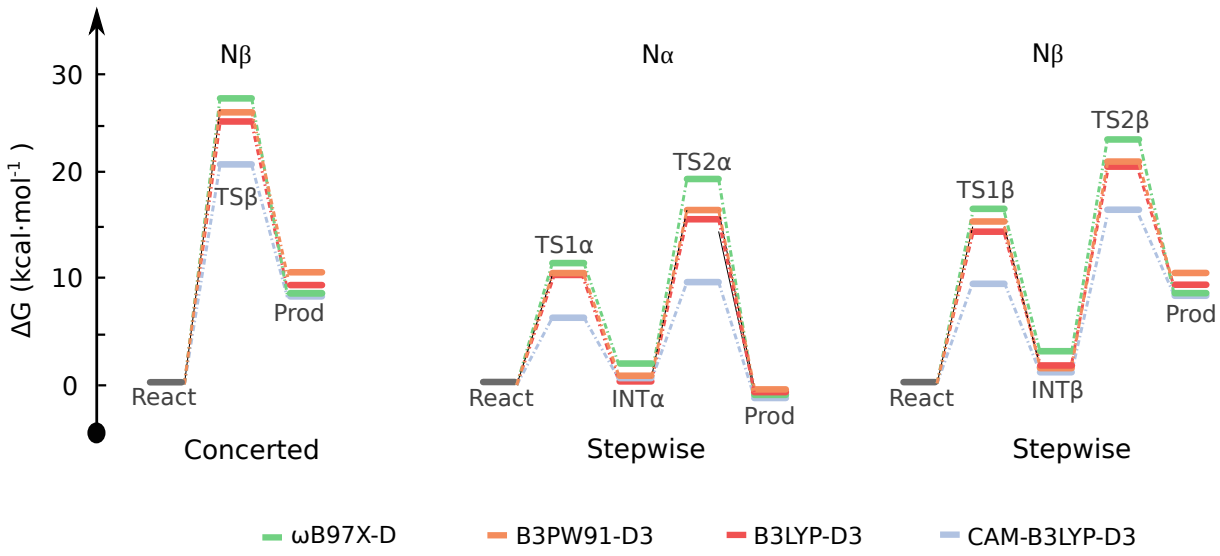


Fig. S9: Intrinsic reaction coordinate results from TSα and TSβ to React and Prod for concerted mechanism.



		ωB97X-D	B3PW91-D3	B3LYP-D3	CAM-B3LYP-D3
Concerted	React	0.0	0.0	0.0	0.0
	TSβ	27.8	26.1	25.4	21.3
	Prod	8.7	10.2	9.2	8.5
Stepwise	React	0.0	0.0	0.0	0.0
	TS1α	12.3	10.4	10.1	6.8
	INTα	2.8	0.7	0.2	0.5
	TS2α	19.3	16.1	15.5	10.4
	Prod	-1.0	-0.2	-0.7	-1.2
Stepwise	React	0.0	0.0	0.0	0.0
	TS1β	16.6	15.2	14.5	9.8
	INTβ	3.1	1.6	1.9	1.1
	TS2β	23.7	21.3	20.5	16.3
	Prod	8.7	10.2	9.2	8.5

Fig. S 10: Calculated free energy profiles at 298.15 K for both mechanisms via Nβ site and stepwise mechanism via Nα site using four different hybrid functionals. The lowest and highest barriers to energy are related to Cam-B3LYP and ωB97X-D, respectively.