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

Supporting Information

A Full Picture of Enzymatic Catalysis by Hydroxynitrile Lyases from *Hevea Brasiliensis*: Protonation Dependent Reaction Steps and Residue-Gated Movement of Substrate and Product

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Table S1. The *Hb*HNLs crystal structures of apo and complex states determined in previous studies.

Apo state	Complex state		Ref
Name	Name	Substrate	
1QJ4			23
2G4L			26
1SCI(K236L)	1SC9 1SCK(K236L) 1SCQ(K236L)	Acetone cyanohydrin Acetone Acetone cyanohydrin	21
	1YAS	Histidine	24
	1YB6 1YB7	Mandelonitrile Dimethyl-2-hydroxy-butyronitrile	25
6YAS 7YAS	2YAS 3YAS 4YAS 5YAS	Thiocyanate ion Acetone Tri-chloro-acetaldehyde Hexafluoroacetone hydrate	27
3C6X	3C6Y 3C6Z 3C70	Acetone Isopropyl alcohol Thiocyanate ion	28

Table S2. Mulliken charge populations of selected atoms that relevant to reaction in the active sites of model A **(a)** and model B **(b)** at their different reactive states

(a)

	C1	C2	N1	O1	O2	Nδ	H2
R	0.52±0.10	0.26±0.07	-0.49±0.04	-0.67±0.06	-0.37±0.08	-0.22±0.09	0.30±0.06
TS1	0.54±0.10	0.22±0.08	-0.46±0.05	-0.66±0.06	-0.30±0.11	-0.19±0.11	0.27±0.08
IM1	0.55±0.11	0.21±0.09	-0.52±0.05	-0.79±0.06	-0.49±0.08	-0.22±0.18	0.43±0.11
TS2	0.71±0.10	0.10±0.07	-0.47±0.05	-0.81±0.05	-0.43±0.06	-0.12±0.16	0.34±0.11
IM2	0.68±0.07	-0.53±0.13	-0.05±0.19	-0.54±0.03	-0.40±0.08	-0.13±0.14	0.28±0.11
TS3	0.71±0.06	0.22±0.11	-0.59±0.05	-0.55±0.03	-0.37±0.07	-0.10±0.13	0.06±0.10
IM3	0.74±0.05	0.35±0.09	-0.55±0.04	-0.55±0.03	-0.33±0.07	-0.23±0.08	0.06±0.07

(b)

	C1	C2	N1	O1	O2	Nδ	H2
R	0.56±0.10	0.27±0.07	-0.49±0.03	-0.68±0.07	-0.24±0.13	-0.11±0.12	0.23±0.09
TS1	0.55±0.11	0.30±0.07	-0.52±0.04	-0.72±0.08	-0.32±0.11	-0.15±0.12	0.29±0.08
IM1	0.63±0.12	0.27±0.06	-0.56±0.03	-0.82±0.09	-0.41±0.14	-0.11±0.17	0.34±0.12
TS2c	0.74±0.06	0.30±0.13	-0.52±0.05	-0.56±0.04	-0.33±0.07	-0.16±0.14	0.07±0.11
IM2	0.75±0.05	0.33±0.09	-0.52±0.04	-0.58±0.03	-0.31±0.07	-0.20±0.10	0.07±0.09

Table S3. The binding free energy (ΔG_{bind}) and the difference of binding free energy for *Hb*HNL and mutant systems (ΔG_{bind}-$\Delta G_{HbHNL)}$. (unit: kcal/mol)

System	ΔG_{bind}	ΔG_{bind}-ΔG_{HbHNL}
HbHNL	-5.60	0.00
LYS236ALA	-2.64	2.96
SER80ALA	-0.86	4.74
THR11ALA	-4.37	1.23

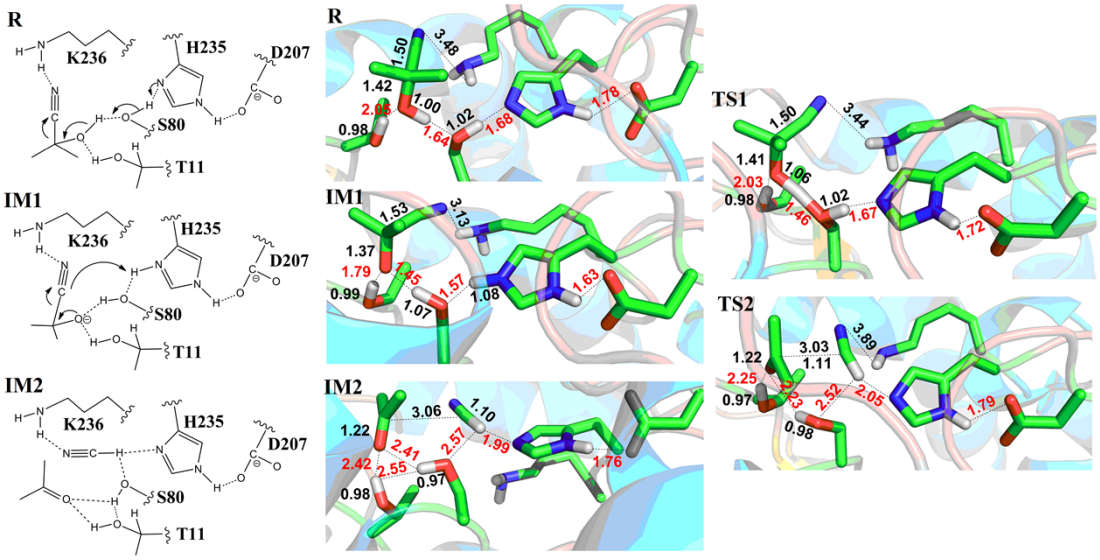


Figure S1. The structures of reactant, transition state, intermedia for model B along the reaction path, wherein the HCN formed by the deprotonation of His235. The average distances and standard deviations for main bonds from the QM/MM-MD sampling.(unit Å)

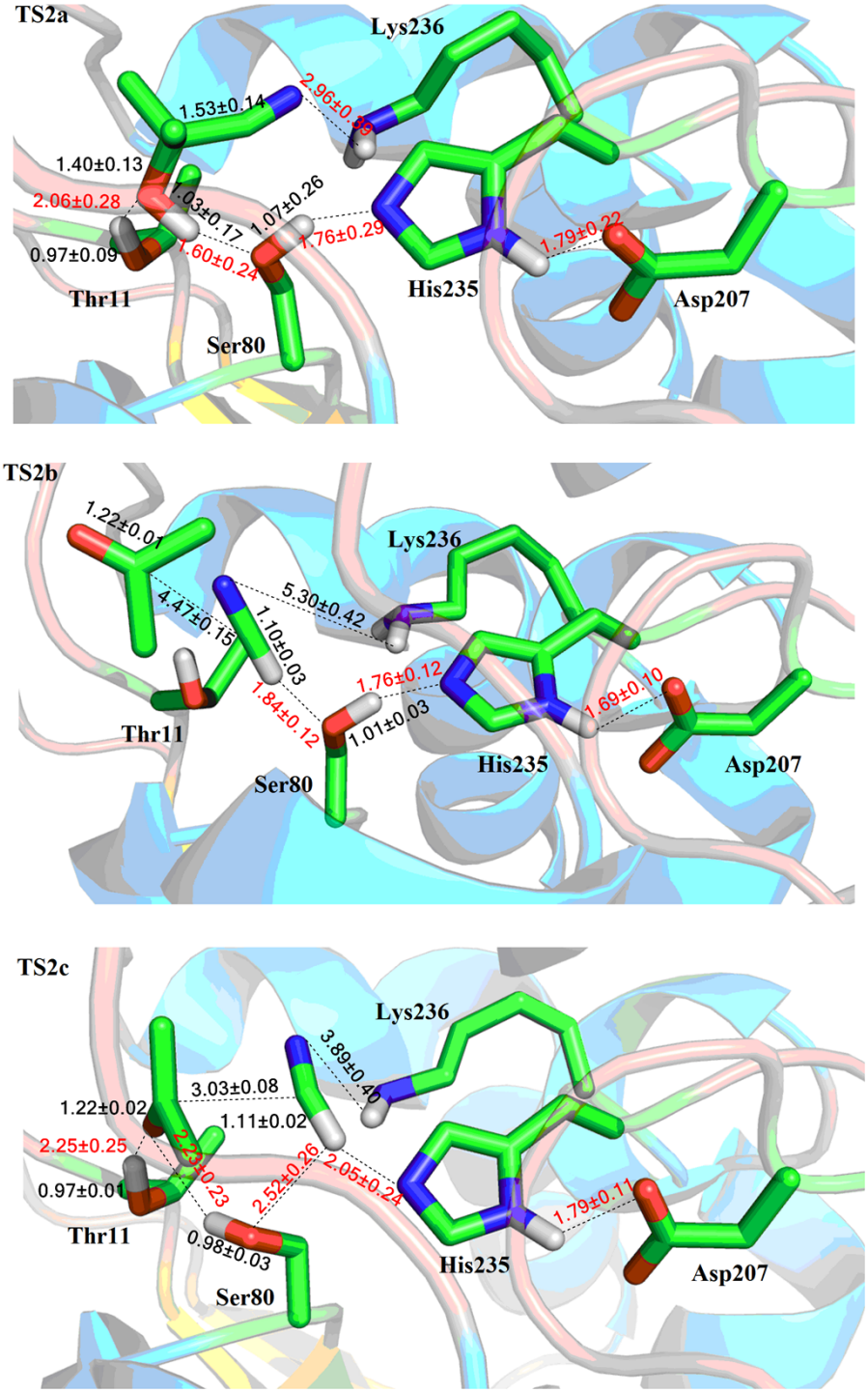
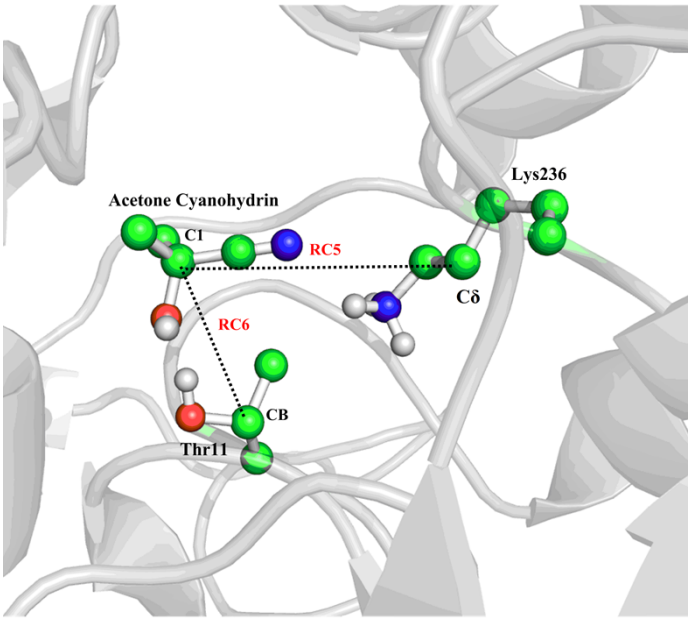


Figure S2. The different structures of transition state during the "platform" by QM/MM MD sampling along with the average distances and standard deviations for main bonds. (unin Å)

(a)



(b)

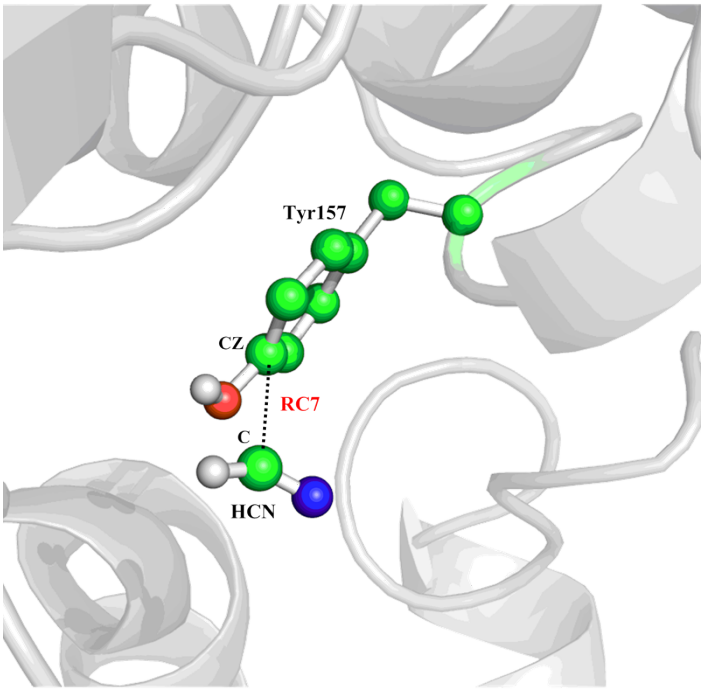


Figure S3 (a).The defined reaction coordinate for release of acetone cyanohydrin (RC5 and RC6). **(b)**.The defined reaction coordinate for release of HCN (RC7).

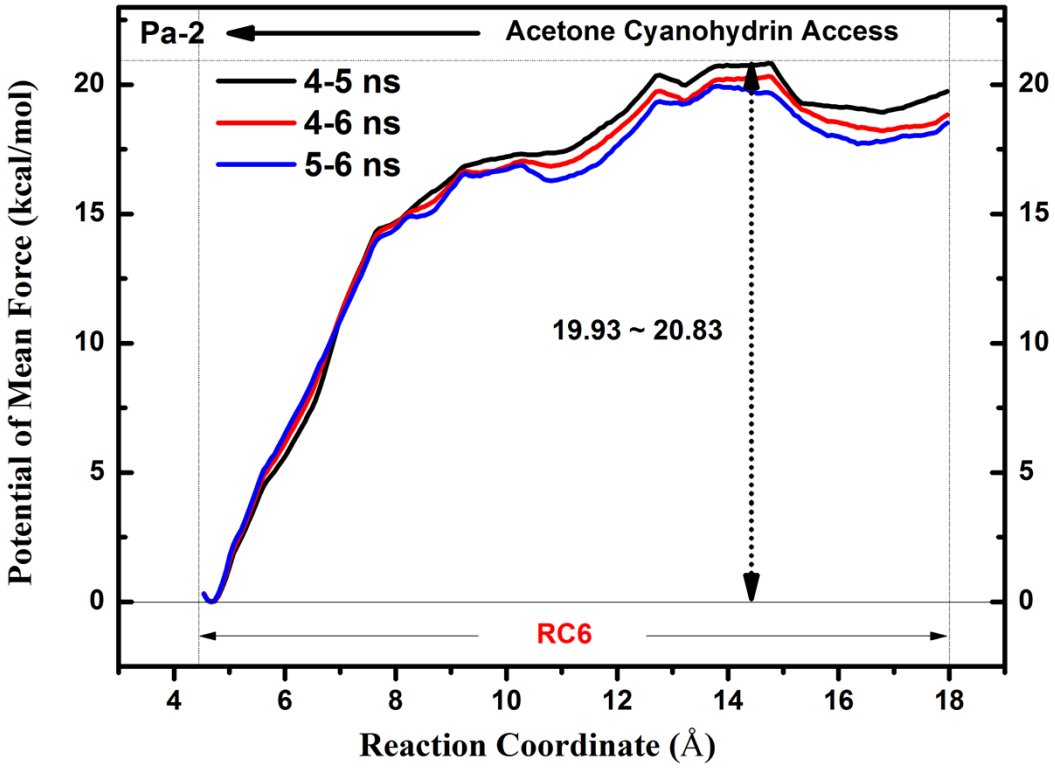


Figure S4. Free energy profiles of along the RC6 (The distance of CB atom of Thr11 and C1 atom of ligand) for release of acetone cyanohydrin.

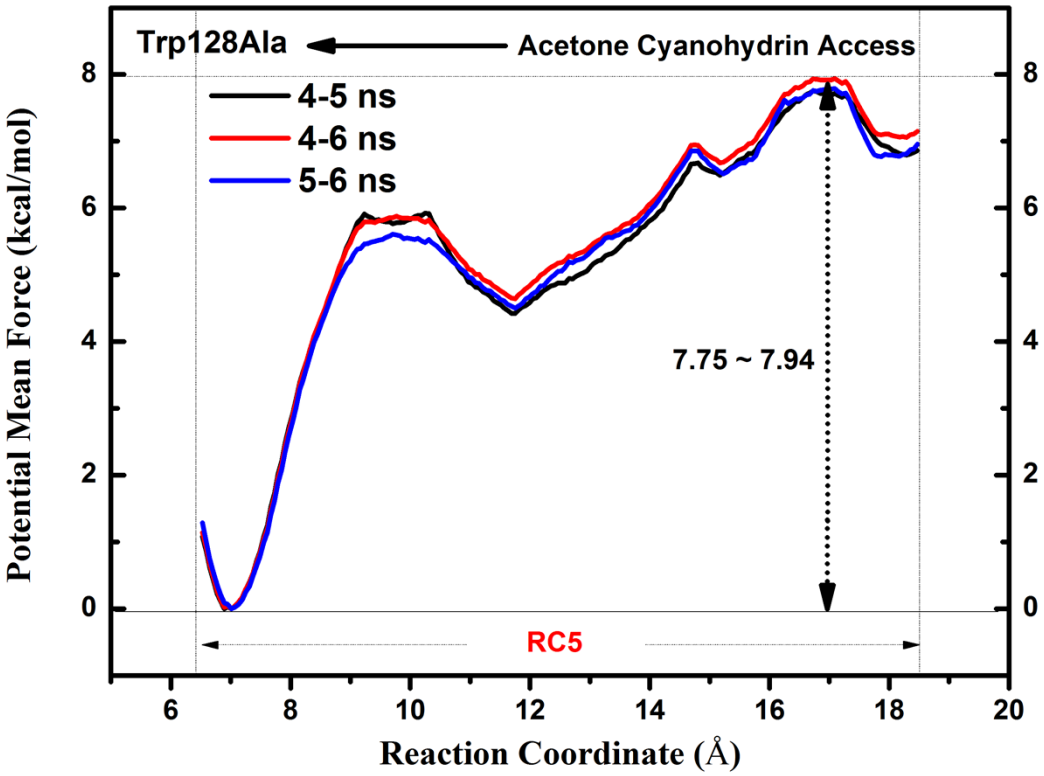


Figure S5. Free energy profiles of along the RC5 (The distance of CD atom of Lys236 and C1 atom of ligand) for release of acetone cyanohydrin in Trp128Ala system.

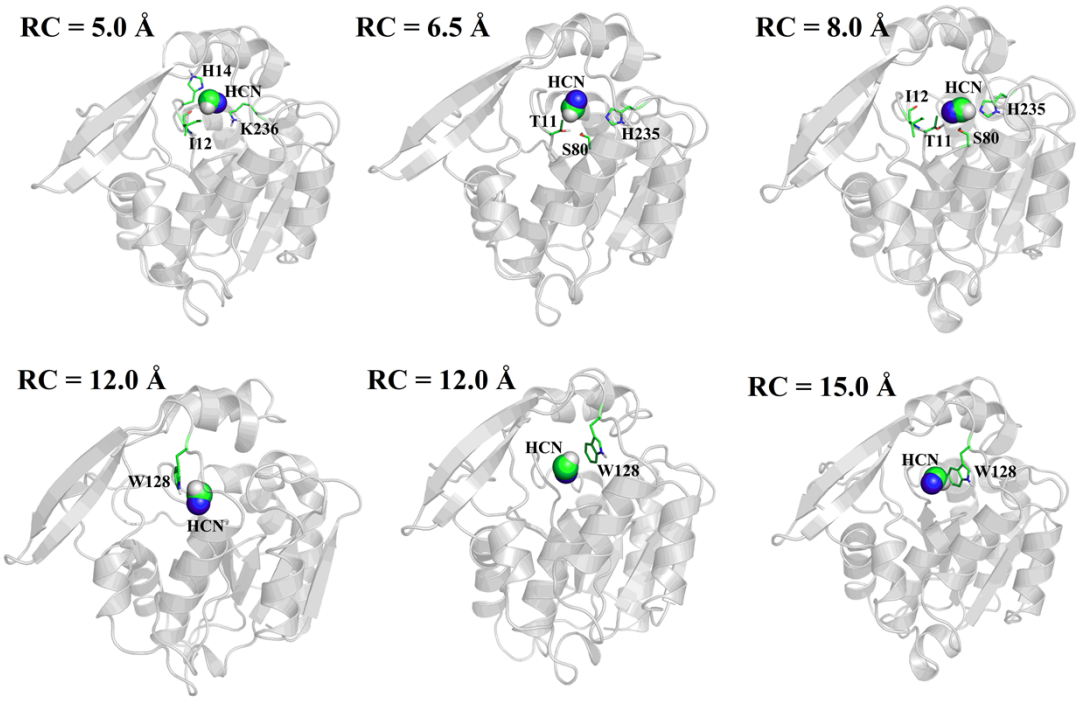


Figure S6. The key residues and the second structures from different windows along the RC7 (The distance of CZ atom of Tyr158 and C atom of HCN) for release of HCN.