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

Mechanistic insights into the catalytic reaction of ferulic acid decarboxylase from *Aspergillus niger*: a QM/MM study

*Ge Tian, Yongjun Liu**

School of Chemistry and Chemical Engineering, Shandong University, Jinan, Shandong 250100, China

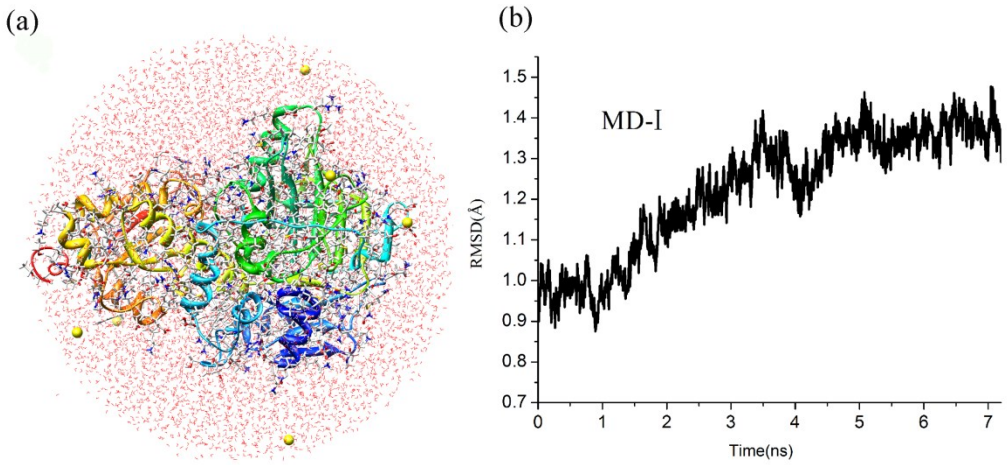

Fig. S1 (a) The constructed solvation model. Yellow balls represent Na^+ ions. (b) RMSD of 7ns MD simulation for this model.

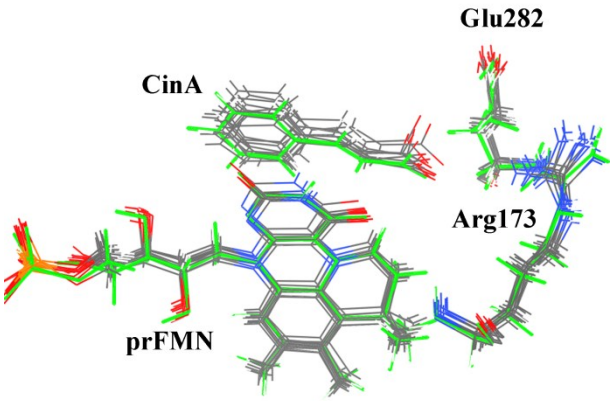

Fig. S2 A total of 11 QM/MM optimized structures, B3LYP/6-31G (d,p) for QM region and CHARMM22 force filed for MM region. They were selected from the MD trajectory at interval

of 0.20 ns from 5 ns to 7 ns. The RMSDs of 11 snapshots were calculated relative to the average one, which are 0.361 (5.0 ns), 0.349 (5.2 ns), 0.386 (5.4 ns), 0.306 (5.6 ns), 0.316 (5.8 ns), 0.343 (6.0 ns), 0.356 (6.2 ns), 0.303 (6.4 ns), 0.377 (6.6 ns), 0.358 (6.8 ns), 0.387 (7.0 ns). The RMSD at 6.4 ns corresponds to the smallest value; therefore, the optimized structure derived from 6.4ns (shown in green) is supposed to be representative and used as the reactant structure for the following QM/MM calculation.

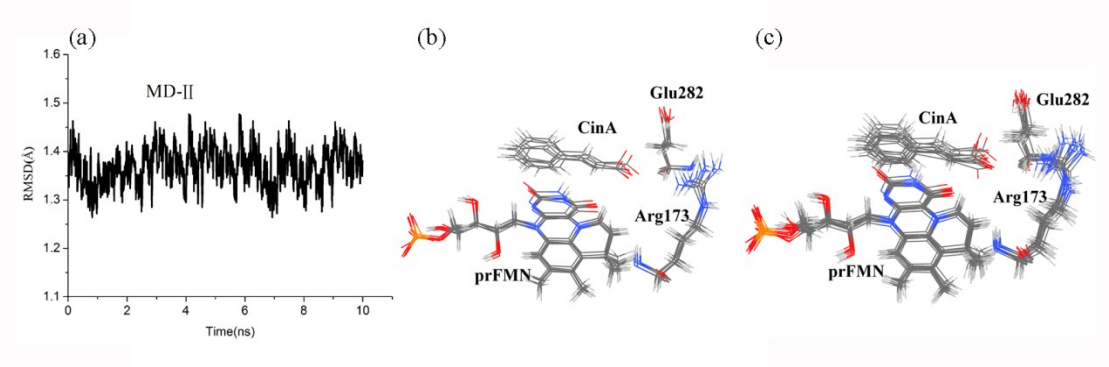

Fig. S3 (a) RMSD of additional 10 ns MD simulation. (b) Superposition of five snapshots optimized by QM/MM method. These snapshots were selected from the trajectory of MD-II at 2, 4, 6, 8 and 10 ns. The RMSDs of the five optimized structures were calculated relative to the average one, which are 0.314 (2.0 ns), 0.364 (4.0 ns), 0.398 (6.0 ns), 0.368 (8.0ns), 0.349 (10.0 ns).These values are basically in the range from 0.303 to 0.387 as in Fig S2. (c) Superposition of 16 QM/MM optimized structures, including the eleven structures in Fig. S2 and five structures in Fig. S3(b). In general these 16 structures superposed well.

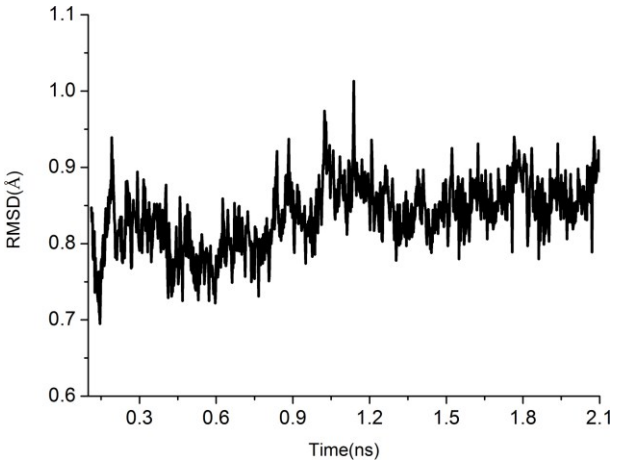

Fig. S4 RMSD of 2 ns simulations for the model after CO_2 liberation, complexed with cofactor prFMN^{iminium}.

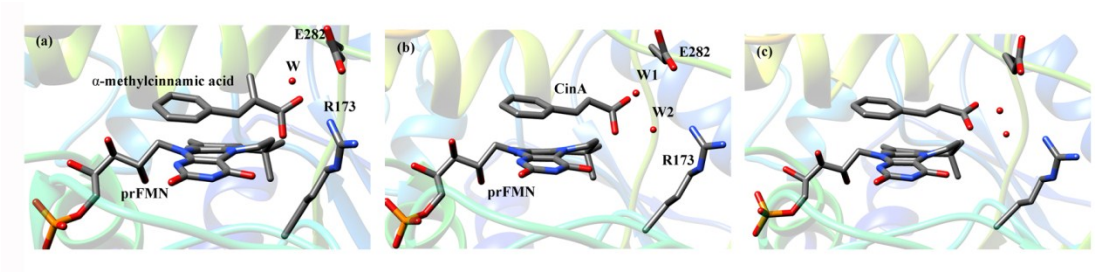

Fig. S5 Active site structures to show the presence of water molecules: (a) crystal structure, (b) after MD simulation, and (c) after QM/MM optimization.

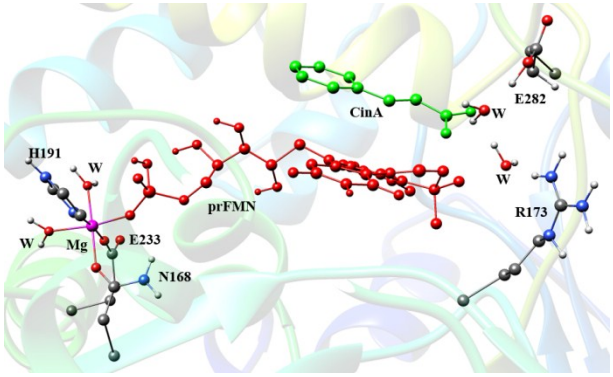

Fig. S6 Relative positions of Mg^{2+}, cofactor and the substrate.

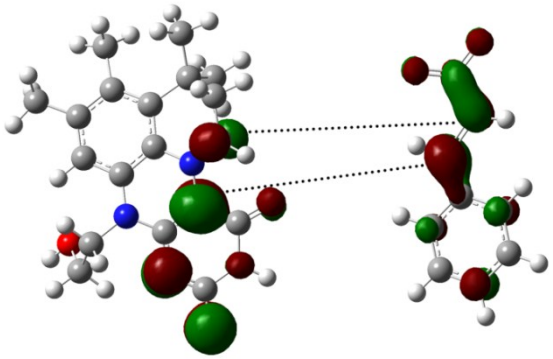

Fig. S7 HOMO of cofactor $prFMN^{iminium}$ (left) and LUMO of the substrate CinA (right). Their eigenvalues (a.u.) are shown in the brackets.

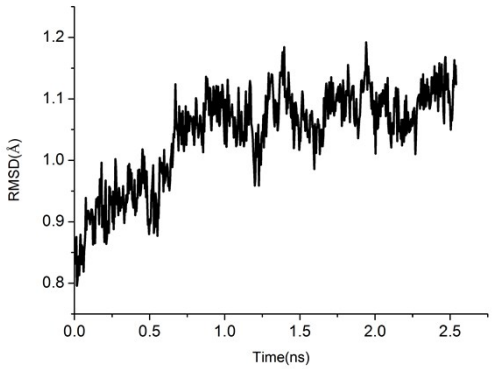

Fig. S8 RMSD for the model complexed with cofactor prFMN^{ketimine} in 2.5-ns MD simulations.

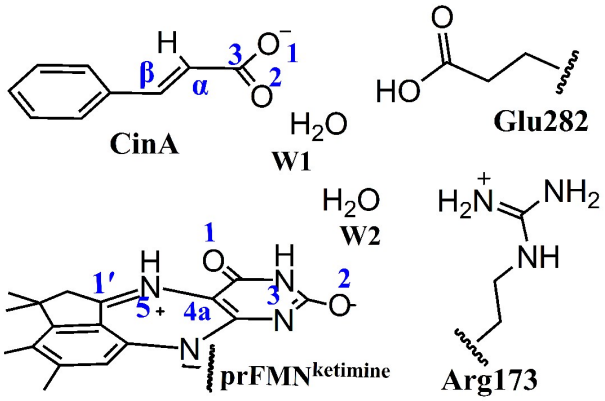

Fig. S9 Selected QM region in our QM/MM calculations for cofactor prFMN^{ketamine}.

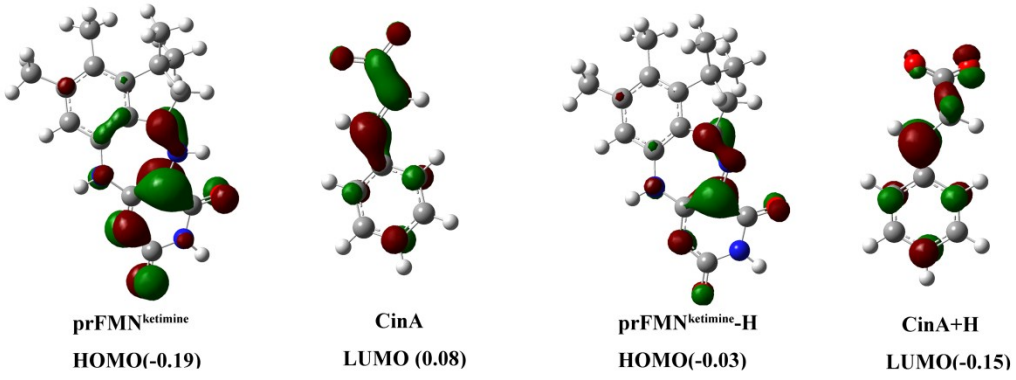

Fig. S10 HOMO of cofactor prFMN^{ketimine} and LUMO of the substrate CinA. Their eigenvalues (a.u.) are shown in the brackets.

Table S1. NBO charge distribution on some critical atoms in the decatboxylation step.

	CinA					$prFMN^{iminium}$				
	O1	**C3**	**O2**	**Cα**	**Cβ**	**C4a**	**N5**	**C1´**	**O1**	**O2**
R	-0.83	0.78	-0.83	-0.29	-0.14	-0.06	-0.29	0.23	-0.70	-0.93
TS1	-0.81	0.82	-0.84	-0.34	-0.11	-0.01	-0.36	0.12	-0.69	-0.93
IM1	-0.82	0.84	-0.83	-0.31	-0.20	0.04	-0.49	0.02	-0.62	-0.89
TS2	-0.53	1.05	-0.58	-0.23	-0.19	-0.02	-0.49	-0.04	-0.83	-0.96
IM2	-0.50	1.07	-0.57	-0.18	-0.22	-0.02	-0.49	-0.05	-0.83	-0.96
TS3	-0.53	1.03	-0.49	-0.50	-0.01	-0.01	-0.45	-0.19	-0.78	-0.93
IM3	-0.51	1.04	-0.52	-0.41	-0.19	-0.04	-0.50	-0.19	-0.66	-0.87
TS4	-0.54	1.03	-0.49	-0.42	-0.10	-0.02	-0.36	-0.19	-0.76	-0.91
P	-0.54	1.04	-0.50	-0.38	-0.21	-0.05	-0.29	-0.19	-0.75	-0.90

Table S2. NBO charge distribution on some critical atoms in the dissociation step.

	CinA		$prFMN^{iminium}$				
	Cα	**Cβ**	**C4a**	**N5**	**C1´**	**O1**	**O2**
IM2'	-0.17	-0.25	-0.03	-0.49	-0.05	-0.83	-0.90
TS3'	-0.46	-0.01	-0.02	-0.47	-0.02	-0.79	-0.88
IM3'	-0.39	-0.17	0.03	-0.50	-0.01	-0.68	-0.81
TS4'	-0.42	-0.02	-0.03	-0.39	0.13	-0.86	-0.78
P'	-0.39	-0.19	-0.06	-0.31	0.26	-0.86	-0.77

Table S3. NBO charge distribution on some critical atoms in the reaction catalyzed by .cofactor $prFMN^{ketamine}$.

	CinA					$prFMN^{ketimine}$				
	O1	**C3**	**O2**	**Cα**	**Cβ**	**C4a**	**N5**	**C1´**	**O1**	**O2**
$\mathbf{R^{ket}}$	-0.85	0.78	-0.77	-0.28	-0.15	0.62	-0.47	0.40	-0.70	-0.84
$\mathbf{TS1^{ket}}$	-0.82	0.85	-0.72	-0.57	0.07	0.65	-0.48	0.34	-0.66	-0.83
$\mathbf{IM1^{ket}}$	-0.87	0.84	-0.73	-0.50	-0.20	0.73	-0.48	0.34	-0.60	-0.76
$\mathbf{TS2^{ket}}$	-0.53	1.04	-0.52	-0.37	-0.19	0.64	-0.46	0.27	-0.73	-0.86
$\mathbf{P^{ket}}$	-0.54	1.04	-0.51	-0.36	-0.21	0.64	-0.47	0.27	-0.74	-0.87