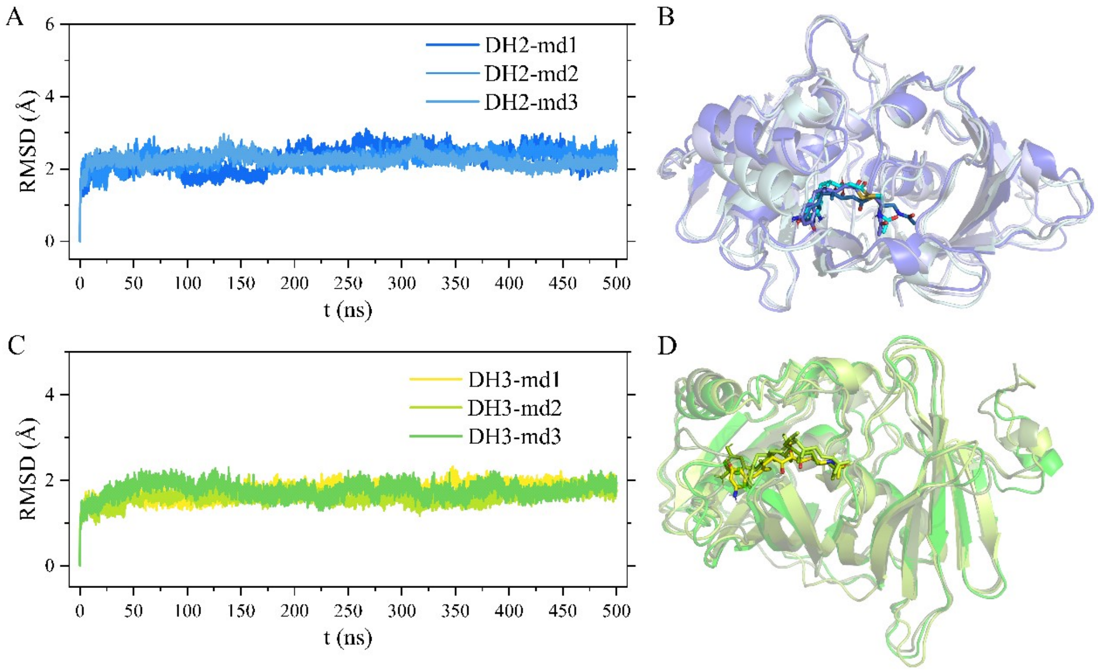

Figure S1. (A)RMSD plot of DH2-**a1** complex in 500 ns MD simulation. (B) The dominant structures alignment of three replicas MD simulation of DH2 system. (C) RMSD plot of DH3-**b1** complex in 500 ns MD simulation. (D) The dominant structures alignment of three replicas MD simulation of DH3 system.

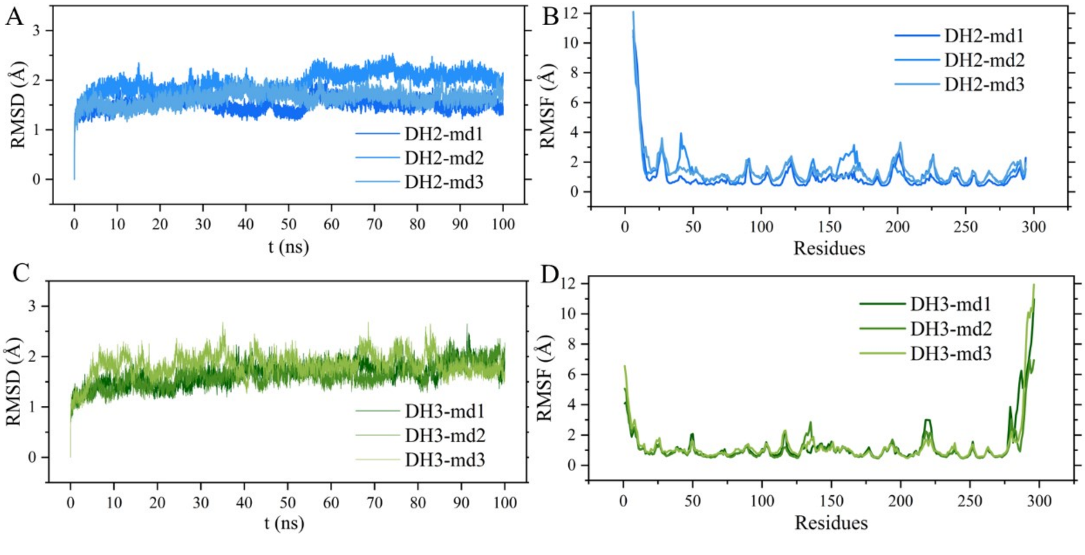

Figure S2. (A)(B) RMSD and RMSF plots of DH2 in 100 ns MD simulations. (C)(D) RMSD and RMSF plots of DH3 in 100 ns MD simulations.

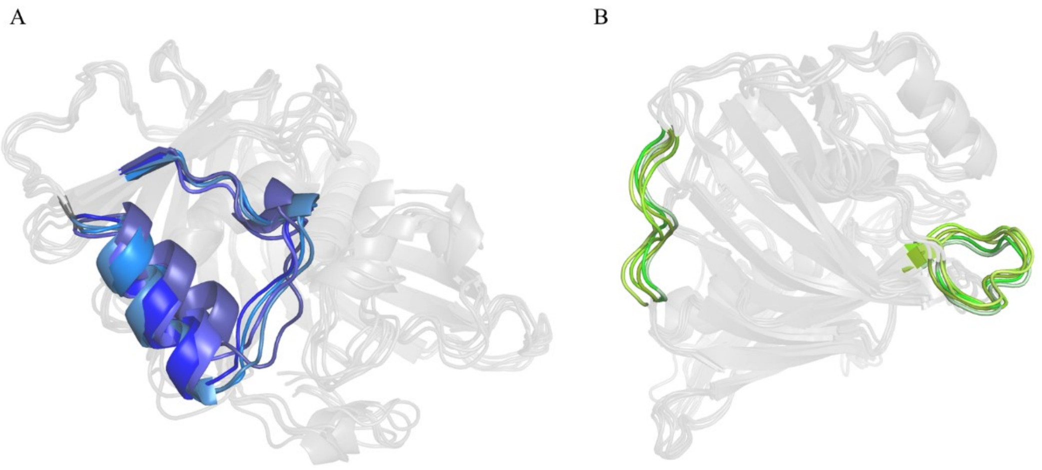

Figure S3. Relatively flexible regions according to RMSF plot in DH2 (A) and DH3 (B).

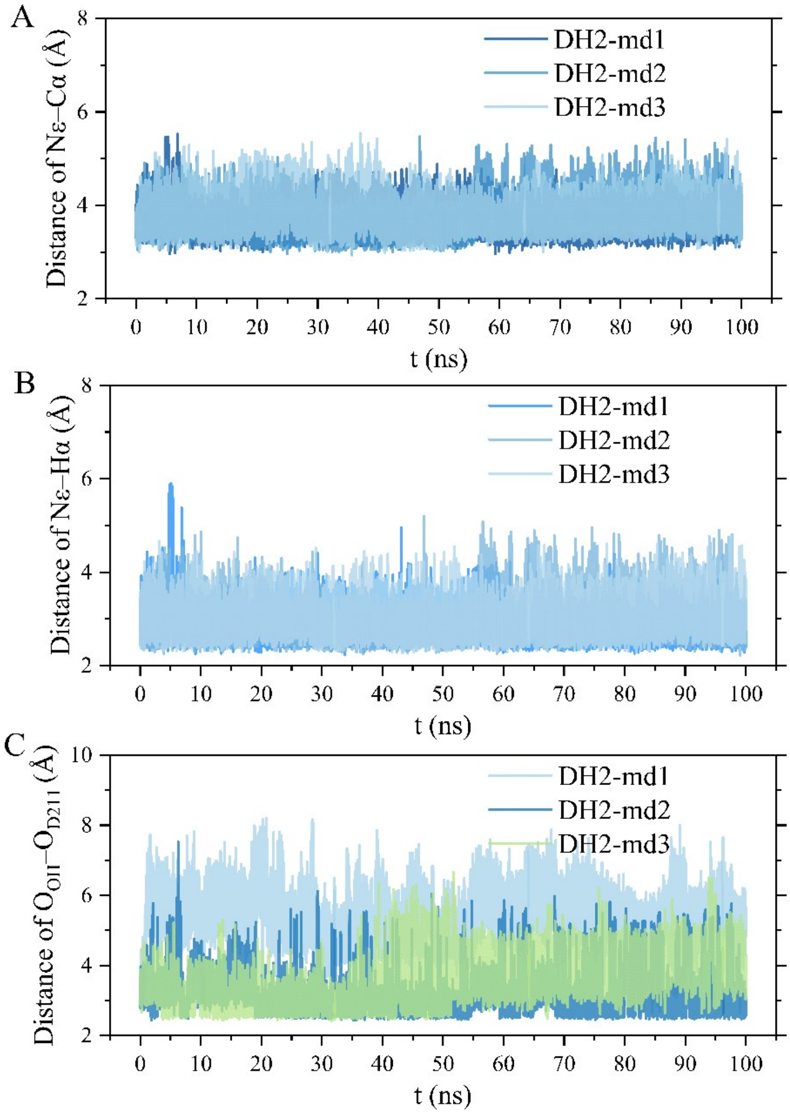

Figure S4. Key distance values: d (N_ε–C_α), d (N_ε–H_α) and d (O_{OH}–H_{D211}) fluctuation along time during DH2-**a1** MD simulations.

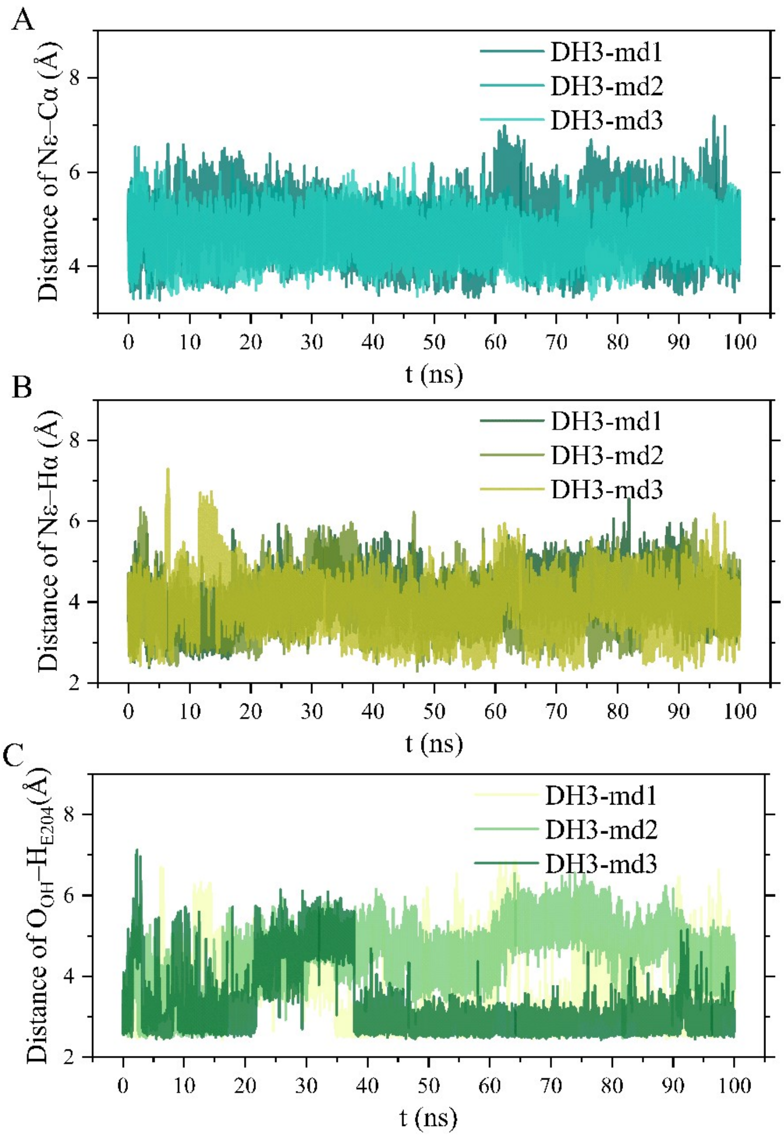

Figure S5. Key distance values: d (N_{ε}–C_{α}), d (N_{ε}–H_{α}) and d (O_{OH}–H_{D211}) fluctuation along time during DH3-**b1** MD simulations.

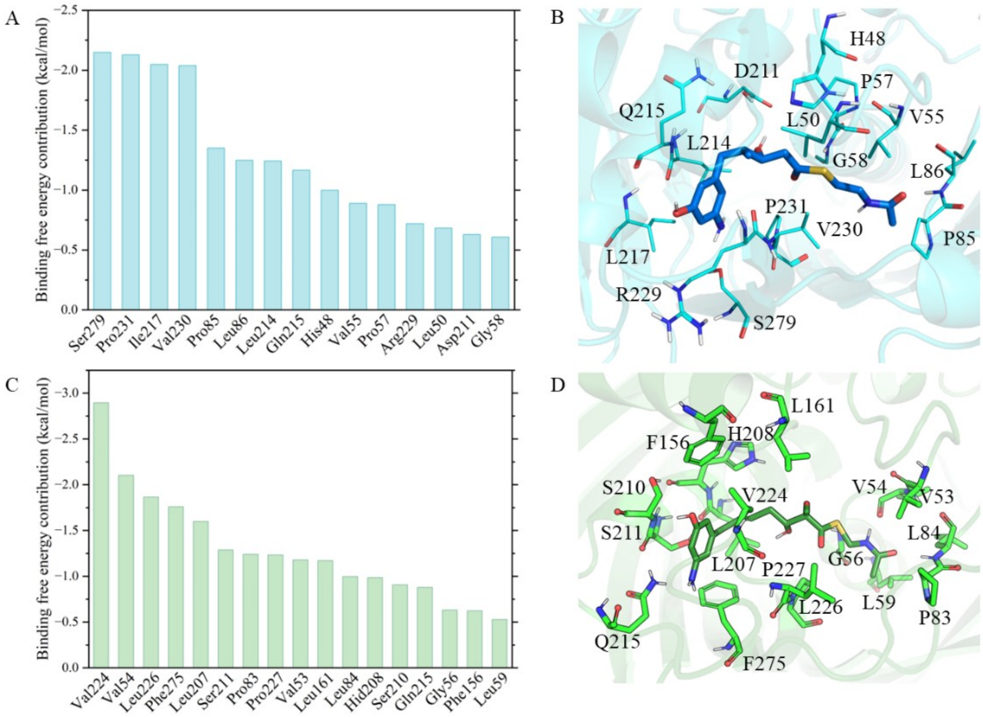

Figure S6. (A)(B) Key interaction residues with binding energy contribution above 0.5 kcal/mol in DH2. (C)(D) Key interaction residues in DH3.

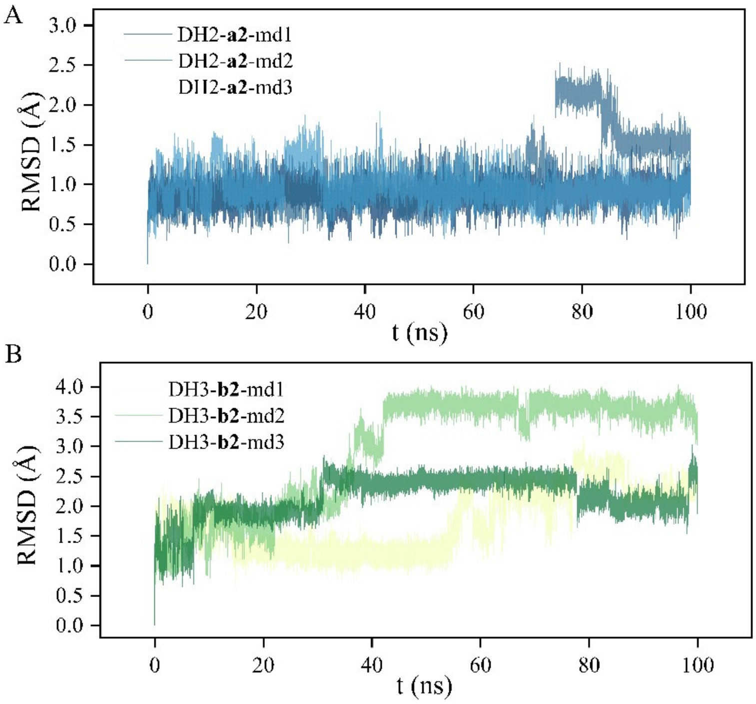

Figure S7. (A) RSMD of dehydrated intermediate **a2** binding with DH2. (B) RSMD of dehydrated intermediate **b2** binding with DH3.

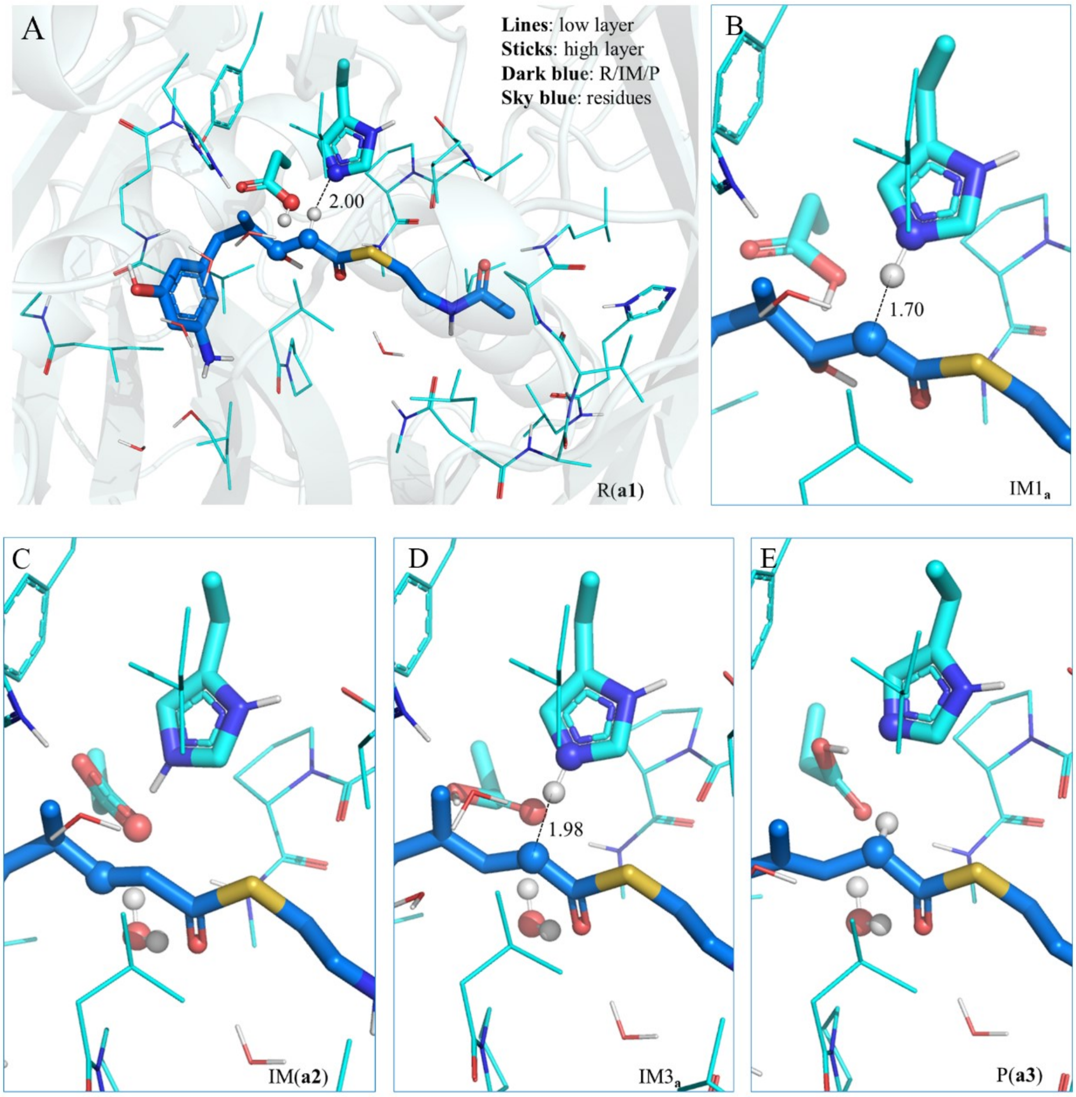

Figure S8. The structures of DH2 cluster for ONIOM calculation.

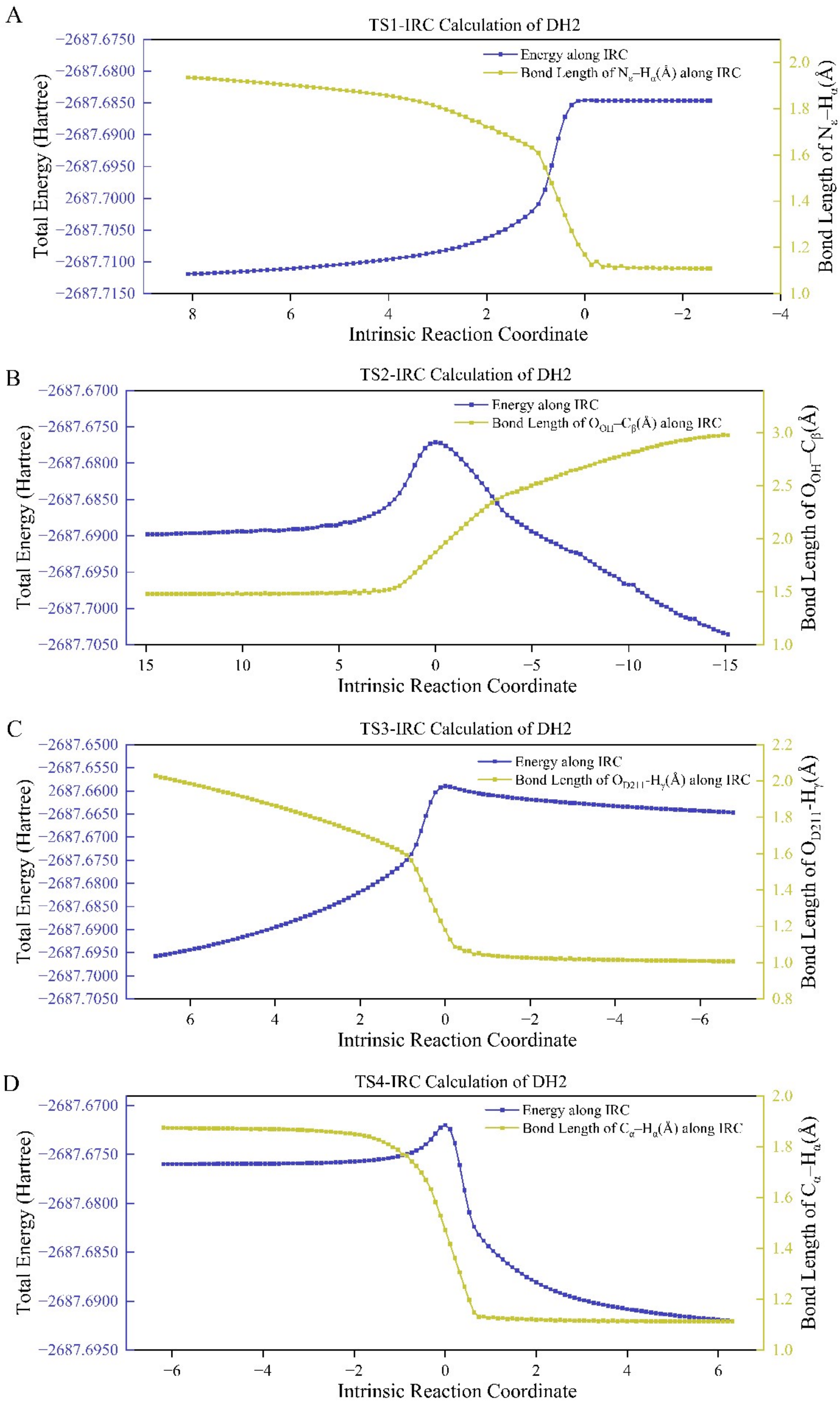

Figure S9. IRC path of the four TSs of DH2 catalytic process. Blue lines and blue scatter: energy change along IRC path. Yellow lines and yellow scatters: key distances change during reaction in IRC path.

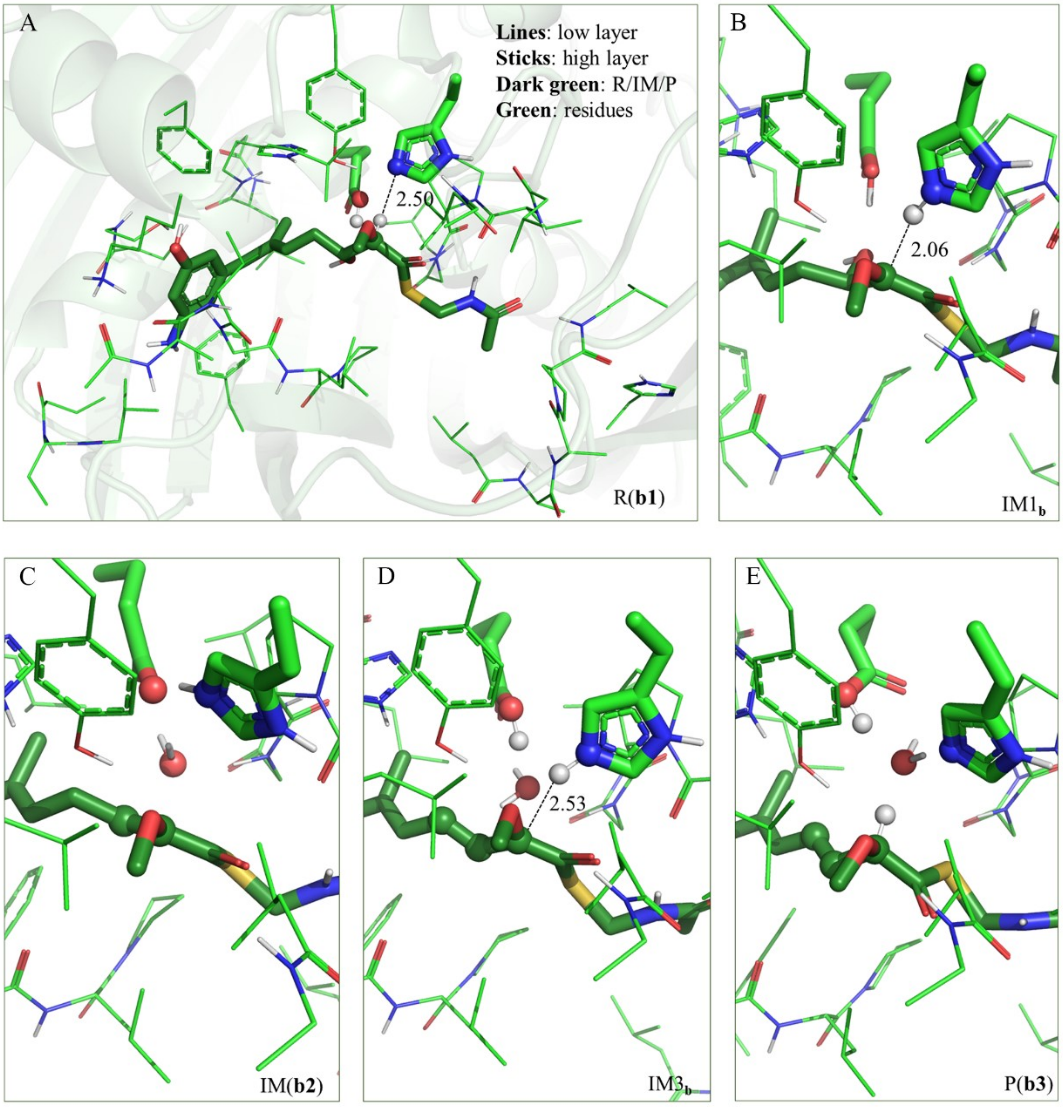

Figure S10. The structures of DH3 cluster for ONIOM calculation.

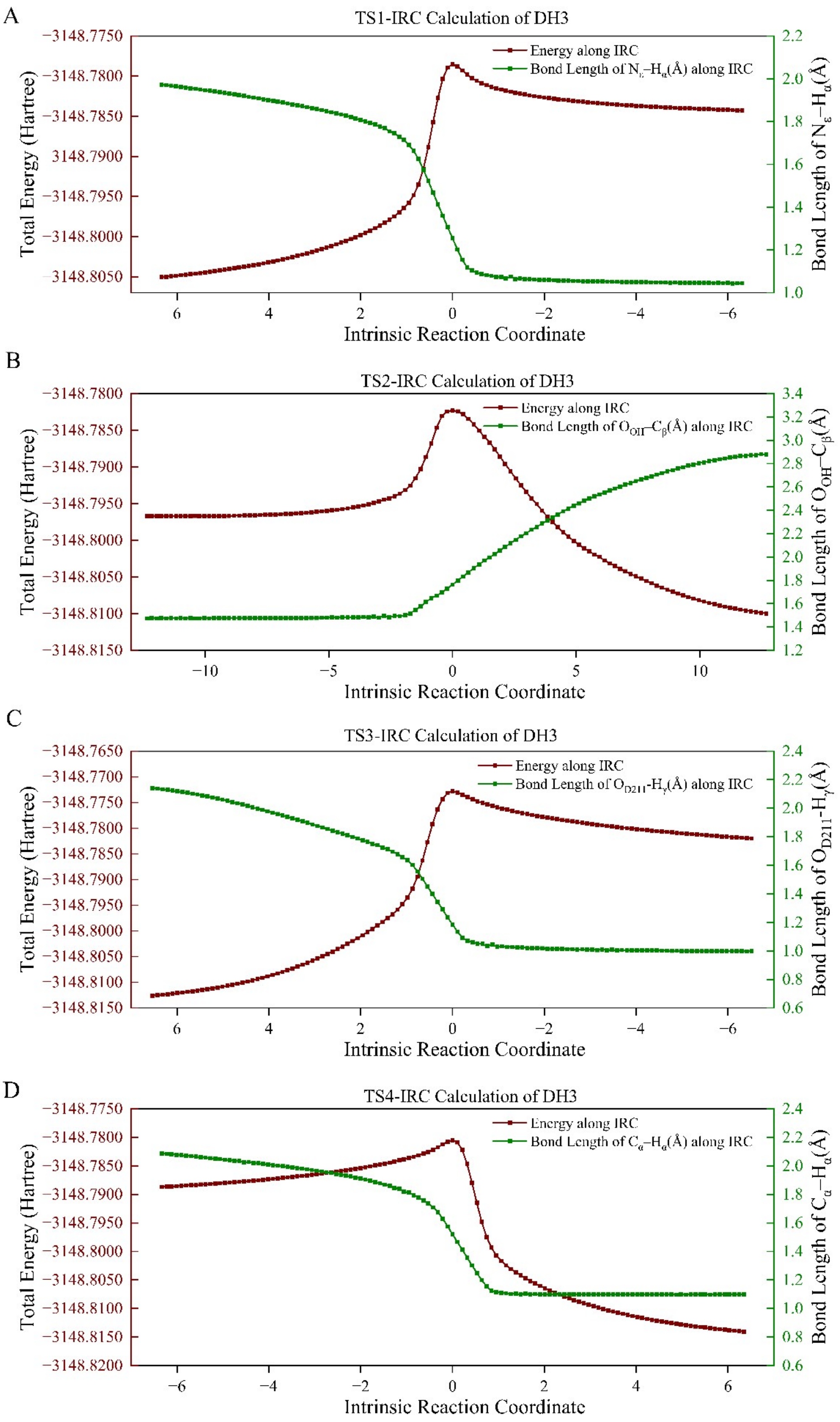

Figure S11. IRC path of the four TSs of DH3 catalytic process. Green lines and green scatter: energy change along IRC path. Dark red lines and dark red scatters: key distances change during reaction in IRC path.

Table S1. Single-point energy calculation results of DH2 catalyzed reactions.

Dehydration	**ONIOM Total Energy (Hartree)**	**Gibbs Free Energy Correction (Hartree)**	**ΔG (Hartree)**	**ΔG (kcal·mol^{-1})**
R(**a1**)	-2688.344860	3.510136	0.000000	0.0
TS1$_{\mathbf{a}}$	-2688.318994	3.507511	0.023241	14.6
IM1$_{\mathbf{a}}$	-2688.319712	3.506958	0.021970	13.8
TS2$_{\mathbf{a}}$	-2688.314859	3.505693	0.025558	16.0
IM2(**a2**)	-2688.339546	3.506937	0.002114	1.3
Isomerization				
IM2(**a2**)	-2688.339546	3.506937	0.000000	0.0
TS3$_{\mathbf{a}}$	-2688.309099	3.507522	0.031032	19.5
IM3$_{\mathbf{a}}$	-2688.322257	3.503212	0.013565	8.5
TS4$_{\mathbf{a}}$	-2688.316686	3.501613	0.017536	11.0
P(**a3**)	-2688.342796	3.501078	-0.009109	-5.7

Table S2. Single-point energy calculation results of DH3 catalyzed reactions.

Dehydration	**ONIOM Total Energy (Hartree)**	**Gibbs Free Energy Correction (Hartree)**	**ΔG (Hartree)**	**ΔG (kcal·mol^{-1})**
R(**b1**)	-3149.546780	4.079014	0.000000	0.0
TS1$_{\mathbf{b}}$	-3149.516092	4.074027	0.025701	16.1
IM1$_{\mathbf{b}}$	-3149.524637	4.079183	0.022312	14.0
TS2$_{\mathbf{b}}$	-3149.524211	4.079372	0.022926	14.4
IM2(**b2**)	-3149.560205	4.076743	-0.015696	-9.8
Isomerization				
IM2(**b2**)	-3149.560205	4.076743	0.000000	0.0
TS3$_{\mathbf{b}}$	-3149.524387	4.071498	0.030573	19.2
IM3$_{\mathbf{b}}$	-3149.544718	4.078565	0.017309	10.9
TS4$_{\mathbf{b}}$	-3149.526613	4.073847	0.030696	19.3
P(**b3**)	-3149.569957	4.077927	-0.008568	-5.4

Table S3. Key distances and angles in R(**a1**), transition states, intermediates, and P(**a3**) in DH2 ONIOM system. All units are Å.

Dehydration	N_{ε}–H_{α}	N_{ε}–C_{α}	C_{α}–H_{α}	O_{OH}–H_{D211}	O_{OH}–C_{β}	C_{α}–$C_{carbonyl}$	C_{α}–C_{β}
R(**a1**)	2.00	3.07	1.10	1.79	1.44	1.50	1.53
TS1$_{\mathbf{a}}$	1.17	2.76	1.60	1.69	1.45	1.41	1.52
IM1$_{\mathbf{a}}$	1.11	2.80	1.70	1.69	1.40	1.40	1.51
TS2$_{\mathbf{a}}$	1.08	3.34	2.75	1.47	1.87	1.40	1.41
IM2(**a2**)	1.06	3.51	3.03	0.98	3.26	1.46	1.35
Isomerization	O_{D211}-H_{γ}	H_{γ}–C_{γ}	C_{γ}–C_{β}	C_{α}–C_{β}	C_{α}–$C_{carbonyl}$	N_{ε}–H_{α}	C_{α}–H_{α}
IM2(**a2**)	2.44	1.10	1.50	1.35	1.46	1.06	3.03
TS3$_{\mathbf{a}}$	1.13	1.64	1.40	1.40	1.41	1.03	2.30
IM3$_{\mathbf{a}}$	1.00	2.16	1.36	1.43	1.40	1.04	1.98

TS4$_{\mathbf{a}}$	0.99	2.19	1.35	1.47	1.43	1.27	1.47
P(**a3**)	1.02	3.83	1.34	1.50	1.52	2.52	1.10

Table S4. Key distances and angles in R(**b1**), transition states, intermediates, and P(**b3**) in DH3 ONIOM system. All units are Å.

Dehydration	**N$_\varepsilon$–H$_\alpha$**	**N$_\varepsilon$–C$_\alpha$**	**C$_\alpha$–H$_\alpha$**	**O$_{OH}$–H$_{E204}$**	**O$_{OH}$–C$_\beta$**	**C$_\alpha$–C$_{carbonyl}$**	**C$_\alpha$–C$_\beta$**
R(**b1**)	2.50	3.28	1.10	1.84	1.44	1.55	1.54
TS1$_{\mathbf{b}}$	1.25	2.70	1.50	1.69	1.45	1.46	1.53
IM1$_{\mathbf{b}}$	1.04	3.03	2.06	1.66	1.46	1.39	1.50
TS2$_{\mathbf{b}}$	1.05	3.71	3.56	1.11	1.76	1.40	1.42
IM2(**b2**)	1.07	3.87	3.78	0.98	2.98	1.48	1.34
Isomerization	**O$_{D211}$-H$_\gamma$**	**H$_\gamma$–C$_\gamma$**	**C$_\gamma$–C$_\beta$**	**C$_\alpha$–C$_\beta$**	**C$_\alpha$–C$_{carbonyl}$**	**N$_\varepsilon$–H$_\alpha$**	**C$_\alpha$–H$_\alpha$**
IM2(**b2**)	2.64	1.10	1.50	1.34	1.48	1.07	3.78
TS3$_{\mathbf{b}}$	1.18	1.56	1.41	1.39	1.43	1.03	3.19
IM3$_{\mathbf{b}}$	1.00	3.43	1.34	1.44	1.39	1.04	2.53
TS4$_{\mathbf{b}}$	1.00	3.17	1.35	1.49	1.46	1.25	1.52
P(**b3**)	1.00	3.59	1.34	1.51	1.53	2.83	1.09

Table S5. Charge redistribution analysis during DH3 catalyzed dehydration and isomerization based on NBO calculation.

Fragment	**Atom**	**R**	**TS1**	**IM1**	**TS2**	**IM2**	**TS3**	**IM3**	**TS4**	**P**
Substrate	C$_\alpha$	0.013	-0.118	-0.067	0.125	0.130	0.038	0.165	-0.144	0.107
	C$_\beta$	0.124	0.108	0.092	0.032	-0.052	-0.083	-0.297	-0.233	-0.257
	C$_\gamma$	-0.449	-0.444	-0.439	-0.455	-0.479	-0.522	-0.284	-0.215	-0.163
	C$_{carbonyl}$	0.404	0.385	0.303	0.264	0.345	0.303	0.223	0.375	0.381
	O$_{carbonyl}$	-0.597	-0.663	-0.762	-0.738	-0.635	-0.715	-0.801	-0.668	-0.571
	O$_{methoxy}$	-0.587	-0.633	-0.645	-0.658	-0.622	-0.641	-0.674	-0.627	-0.592
	O$_{hydroxyl}$	-0.761	-0.757	-0.763	-0.796	-1.00	-0.964	-0.953	-0.953	-1.01
	H$_{hydroxyl}$	0.488	0.485	0.482	0.500	0.491	0.491	0.491	0.488	0.50
Protons	H$_\alpha$	0.257	0.411	0.481	0.485	0.498	0.485	0.475	0.415	0.237
	H$_\gamma$	0.225	0.220	0.216	0.234	0.289	0.462	0.513	0.515	0.510
Residues	N$_\varepsilon$	-0.567	-0.517	-0.491	-0.508	-0.516	-0.506	-0.497	-0.523	-0.574
	O$_{E204}$	-0.727	-0.744	-0.756	-0.848	-0.871	-0.807	-0.722	-0.726	-0.710

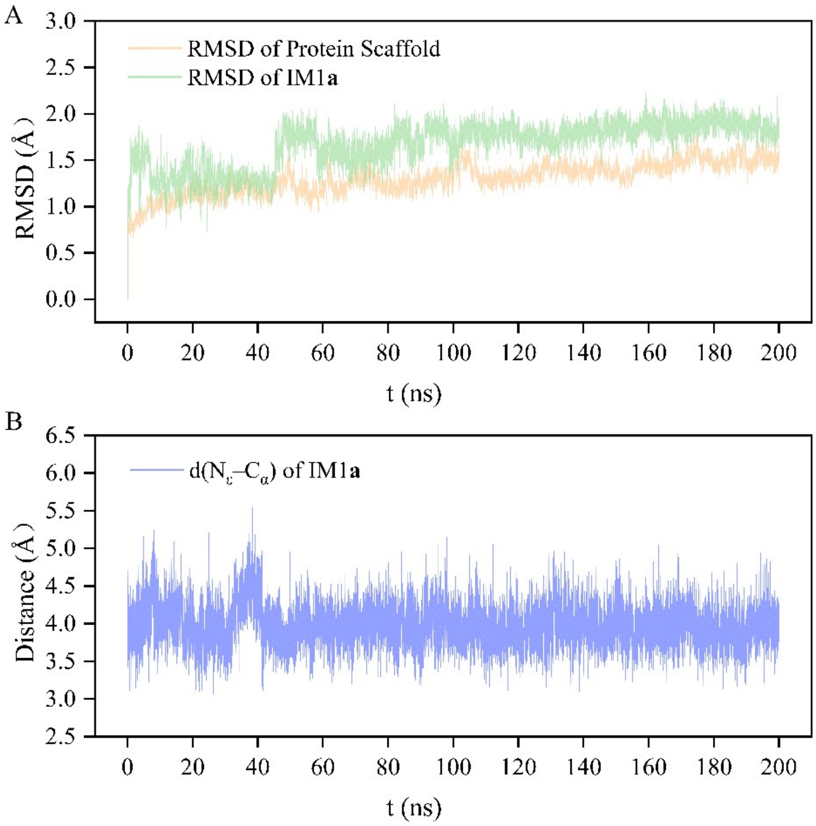

Figure S12. (A) RMSD lines in 200 ns MD of protein scaffold (the orange line) and IM1**a** (the green line). (B) $d(N_\varepsilon–C_\alpha)$ fluctuation of IM1**a**.

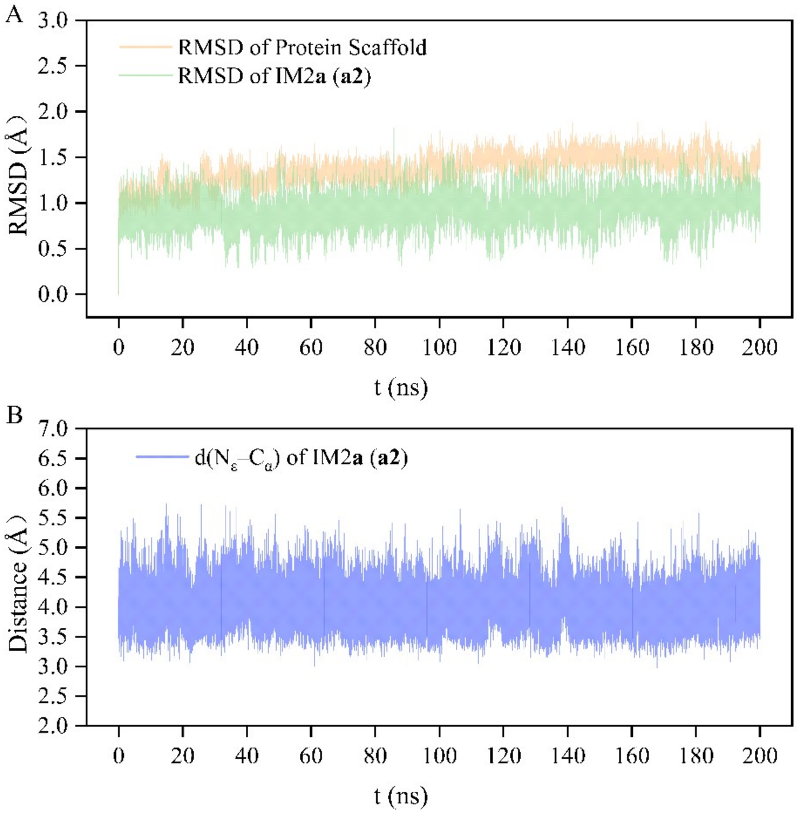

Figure S13. (A) RMSD lines in 200 ns MD of protein scaffold (the orange line) and IM2**a** (the green line). (B) $d(N_\varepsilon–C_\alpha)$ fluctuation of IM2**a**.

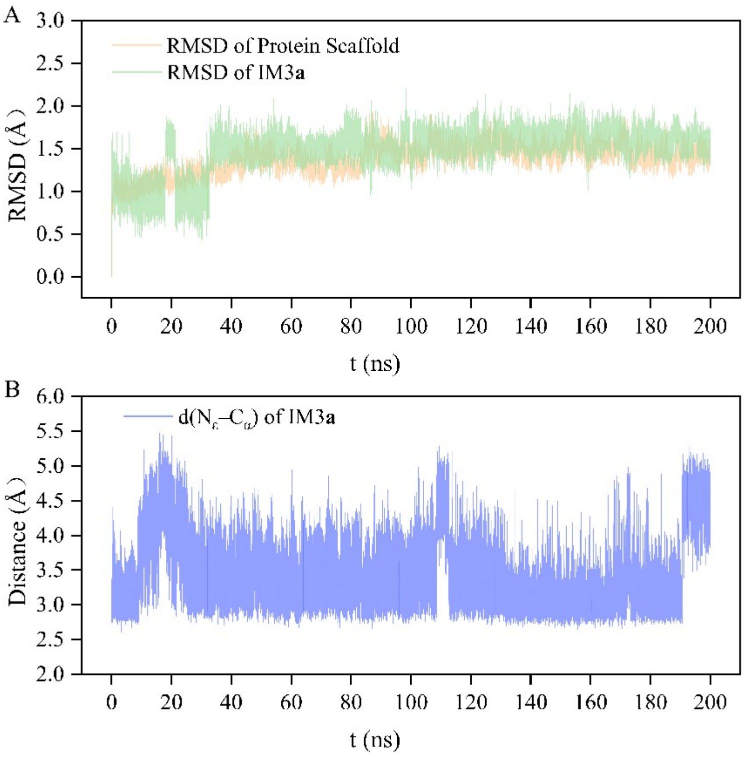

Figure S14. (A) RMSD lines in 200 ns MD of protein scaffold (the orange line) and IM3**a** (the green line). (B) d(N_ε–C_α) fluctuation of IM3**a**.

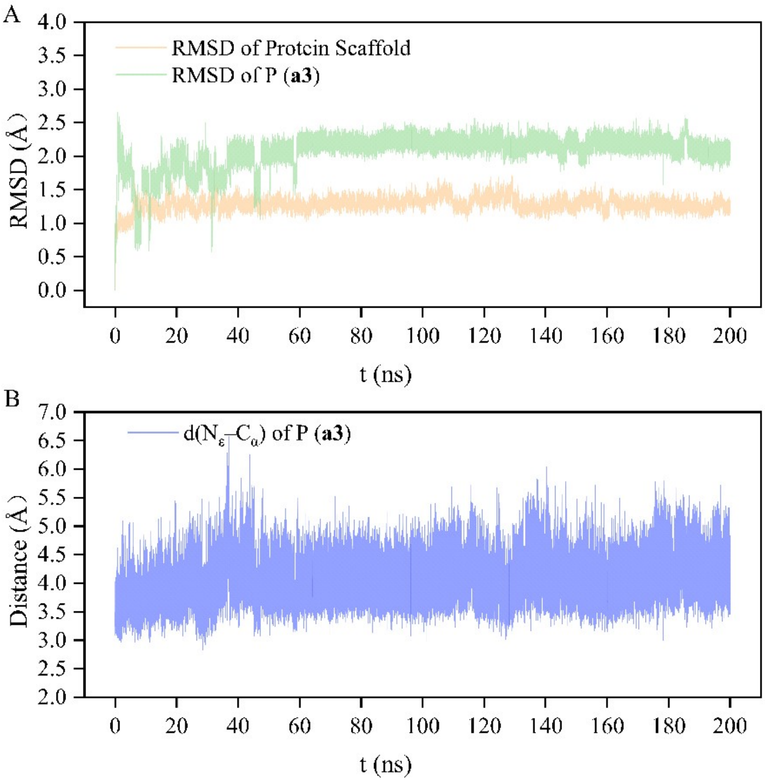

Figure S15. (A) RMSD lines in 200 ns MD of protein scaffold (the orange line) and **a3** (the green line). (B) d(N_ε–C_α) fluctuation of **a3**.