Electronic Supplementary Material (ESI) for Organic & Biomolecular Chemistry.

Supporting Information for:

Computer Simulations of the Catalytic Mechanism of Wild-Type and Mutant β-Phosphoglucomutase

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S1. Figures & Tables

Figure S1. A schematic model of the octahedral dummy model used to describe the Mg^{2+} ion in the present work. The total charge on the metal ion is redistributed between the central particle and to each dummy particle, delocalizing it. The charge on the dummy particles is identical, and the overall charge sums to +2. For technical details of the model and its validation, as well as the parameters used in the current work see ref. [1].

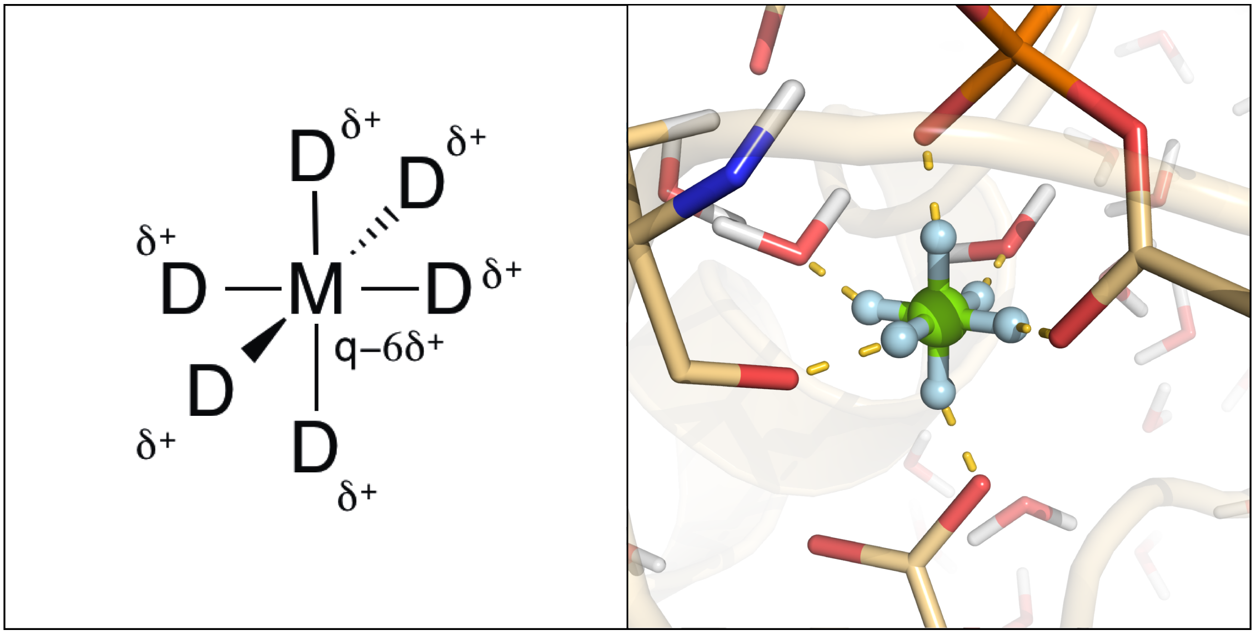
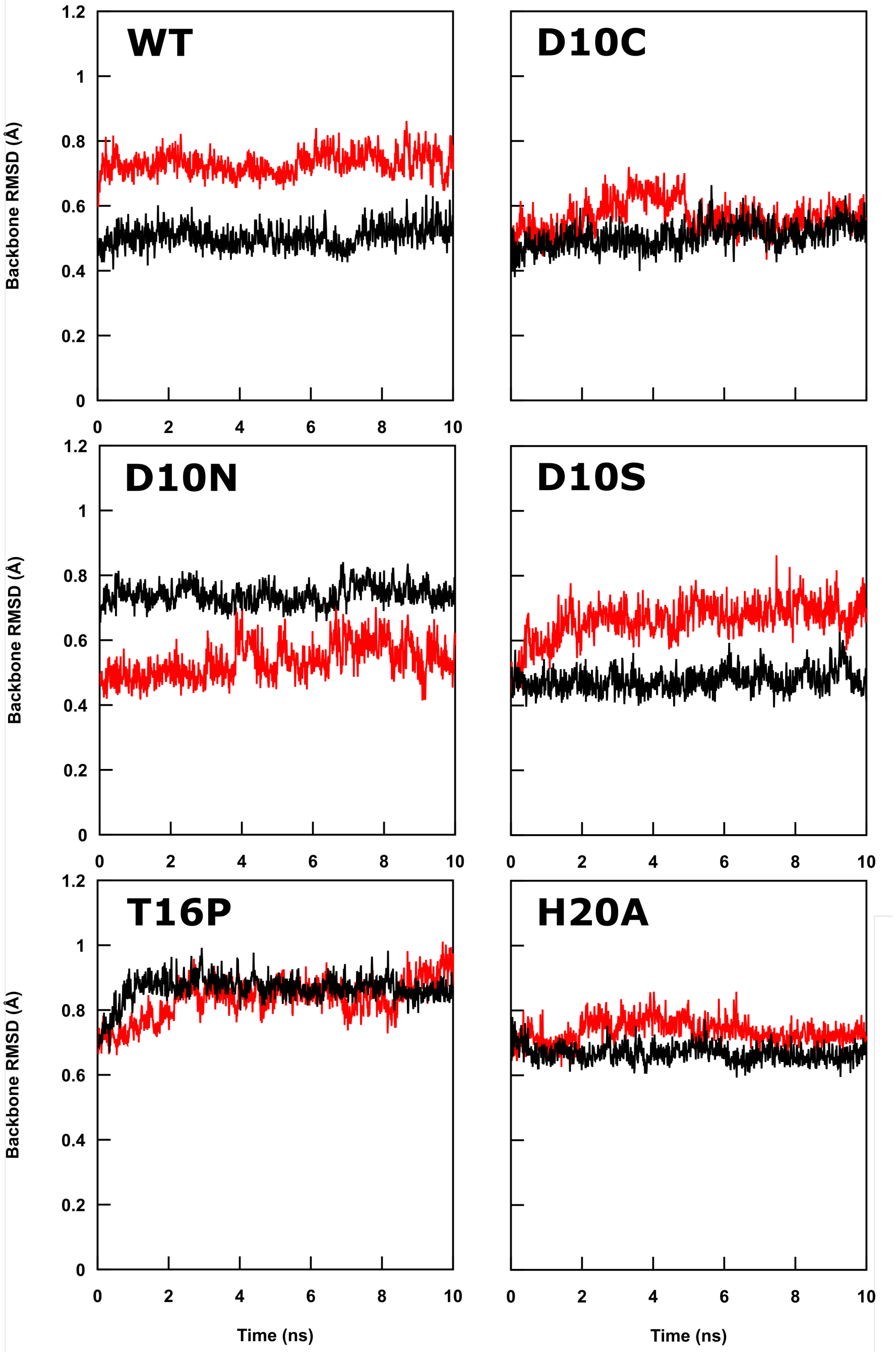


Figure S2. Time-averages of the root mean square deviation (in Å) of the protein backbone atoms (C_α, C, and N atoms) for the wild-type and mutant variants of *L. lactis* β-PGM for the first (red) and second (black) steps of the substrate-assisted mechanism.



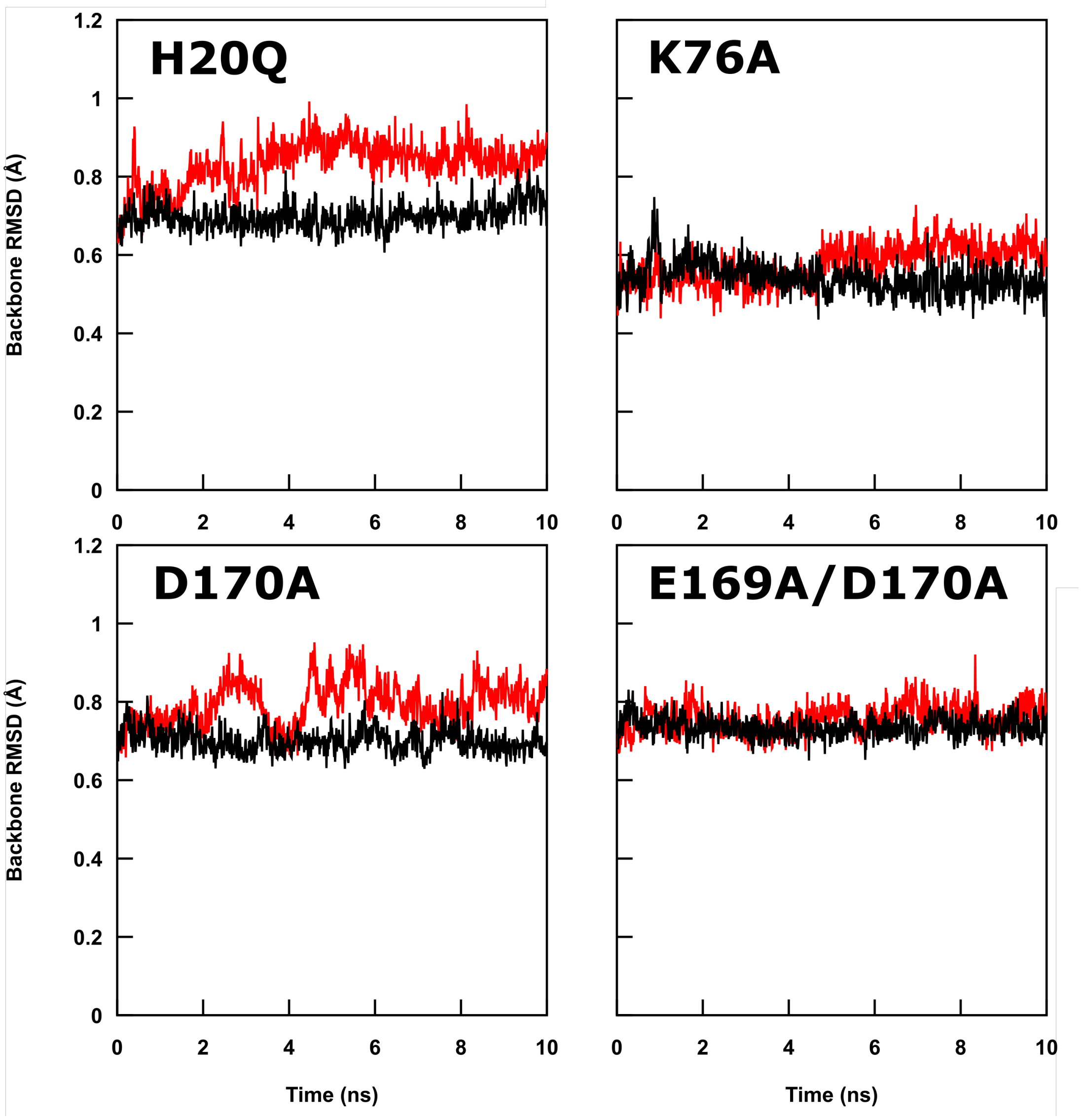
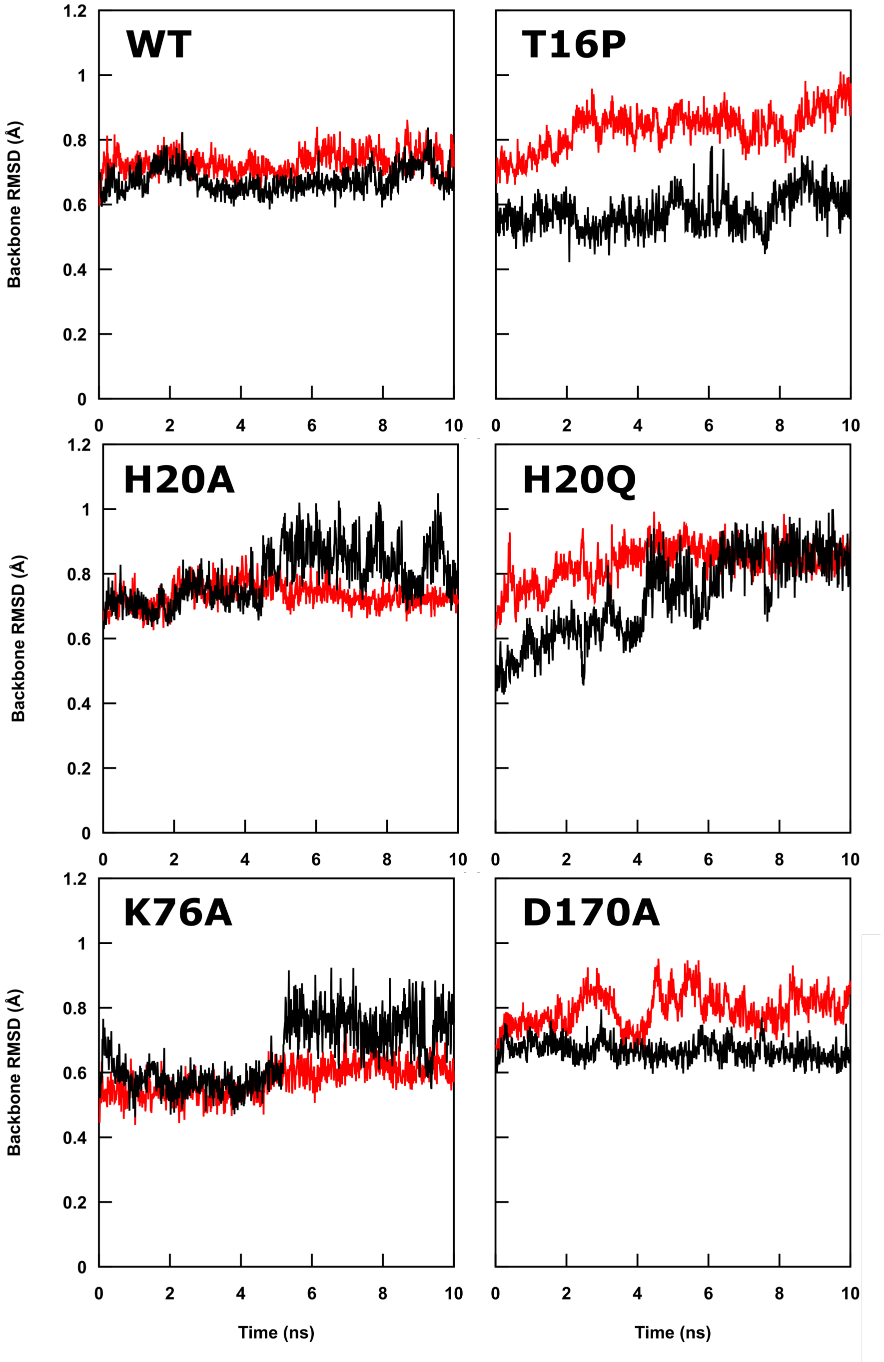

H20Q
K76A
D170A
E169A/D170A
Backbone RMSD (Å)
Backbone RMSD (Å)
0
0.2
0.4
0.6
0.8
1
1.2
0
2
4
6
8
10
Time (ns)
Time (ns)

Figure S3. Time-averages of the root mean square deviation (in Å) of the protein backbone atoms (C$_\alpha$, C, and N atoms) for the wild-type and mutant variants of *L. lactis* β-PGM for the first (red) and second (black) steps of the D10-assisted mechanism.



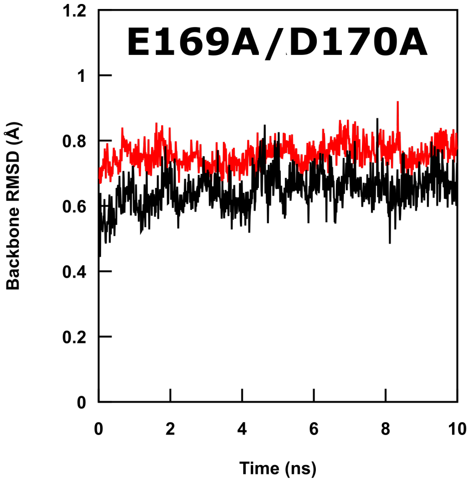
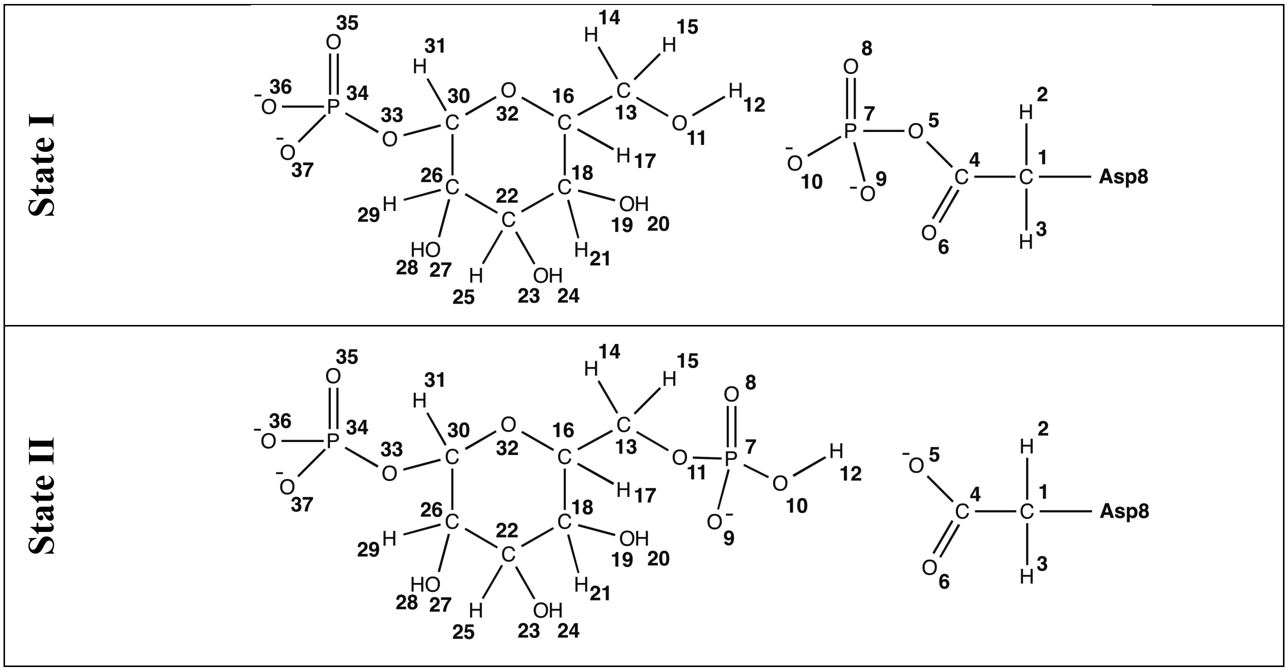

E169A/D170A
Backbone RMSD (Å)
0
0.2
0.4
0.6
0.8
1
1.2
Time (ns)
0
2
4
6
8
10

Figure S4. Valence bond states used to model the substrate-assisted mechanism. The atom numbering in this figure corresponds to the EVB parameter ordering shown in **Section S2.**

Substrate-assisted mechanism – Step I



Substrate-assisted mechanism – Step II

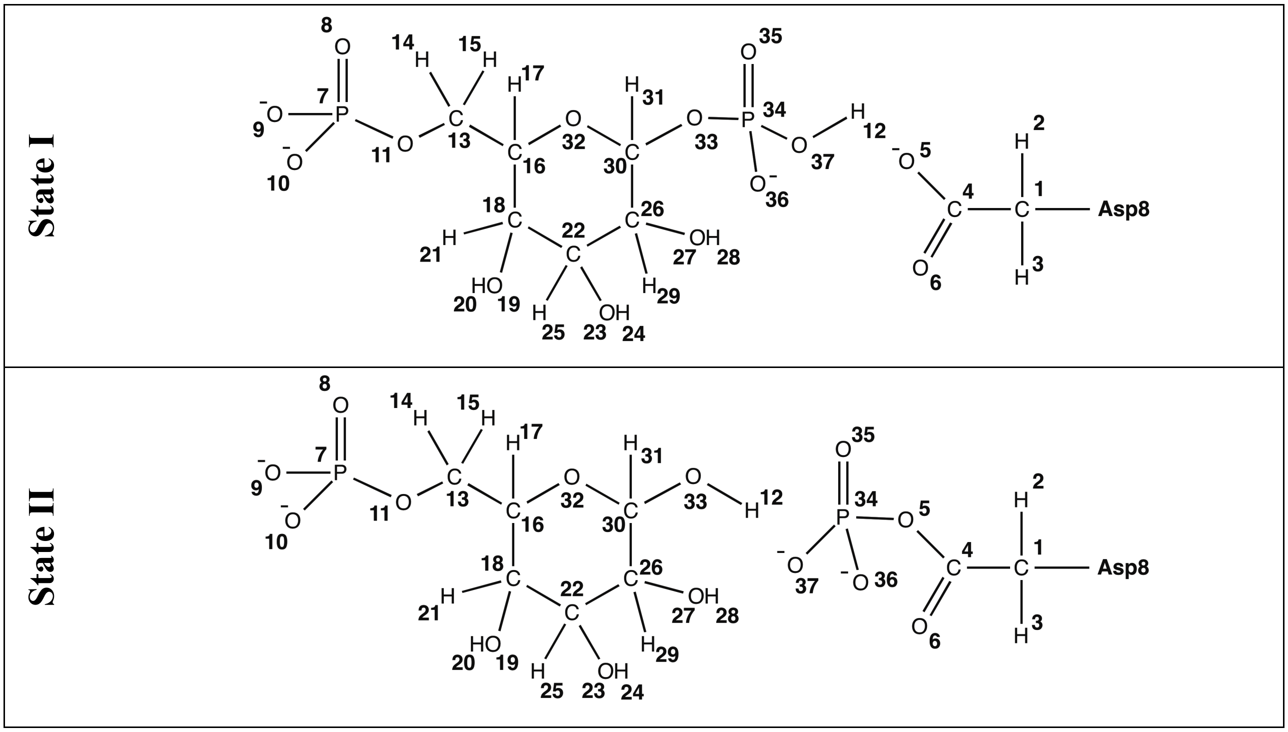
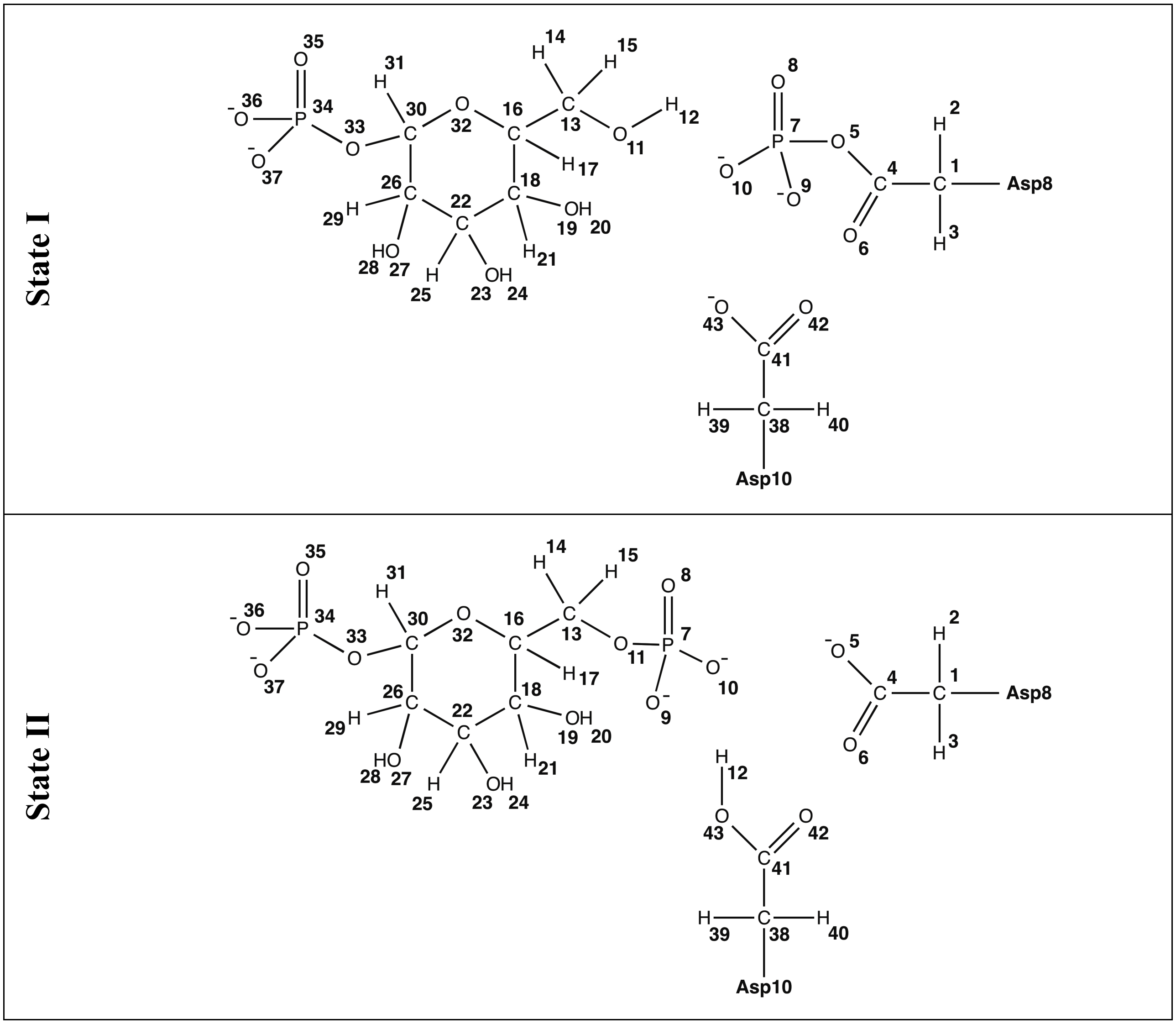


Figure S5. Valence bond states used to model the D10-assisted mechanism. The atom numbering in this figure corresponds to the EVB parameter ordering shown in **Section S2.**

D10-assisted mechanism – Step I



D10-assisted mechanism – Step II

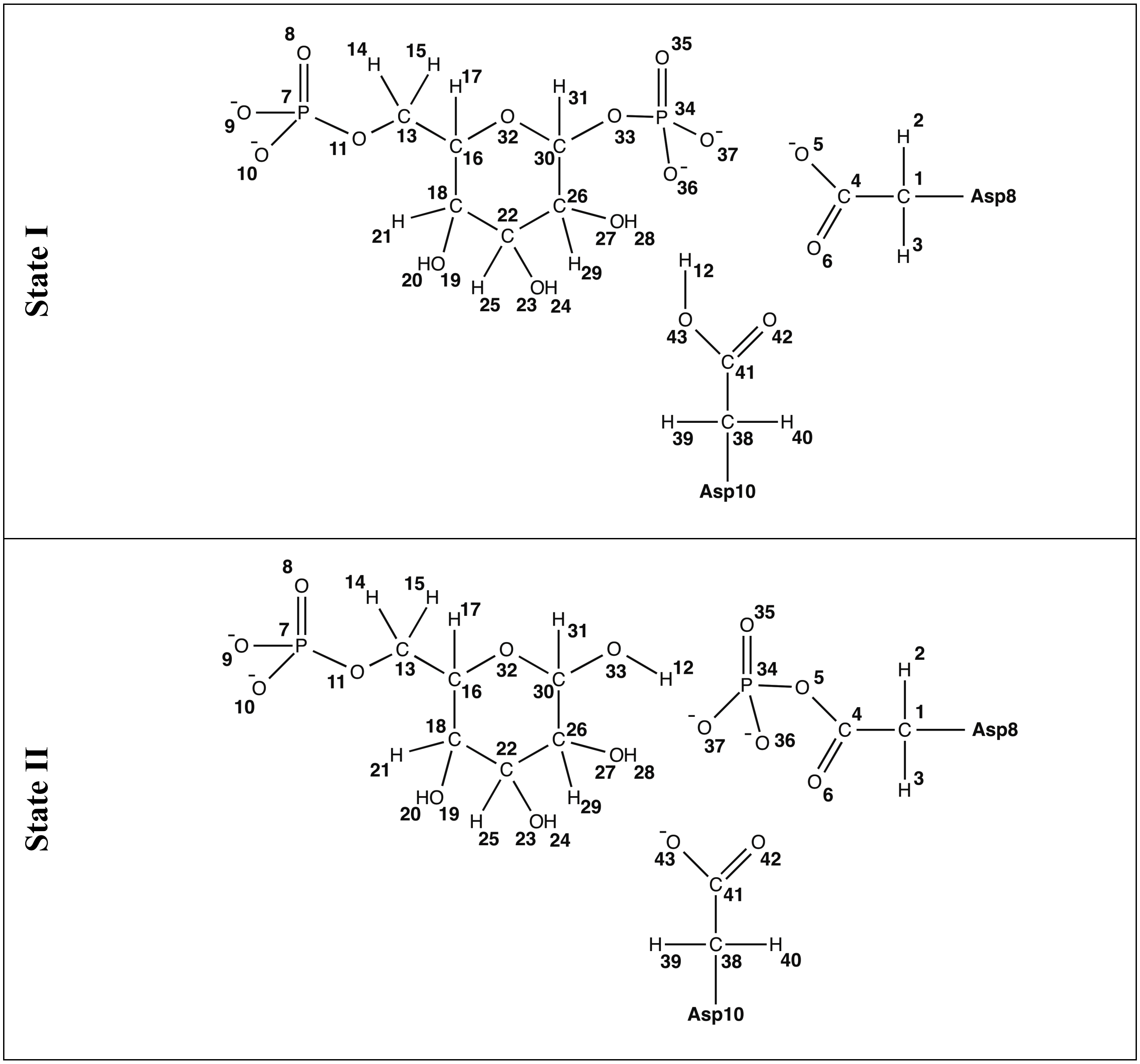


Figure S6. The side chain dihedral angle (C-CA-CB-CG) of D10 as a function of WT-MetaD simulation time for the three independent runs (red for run 1, green for run 2 and blue for run 3). For the resulting free energy profiles, see **Figure 6** of the main text.

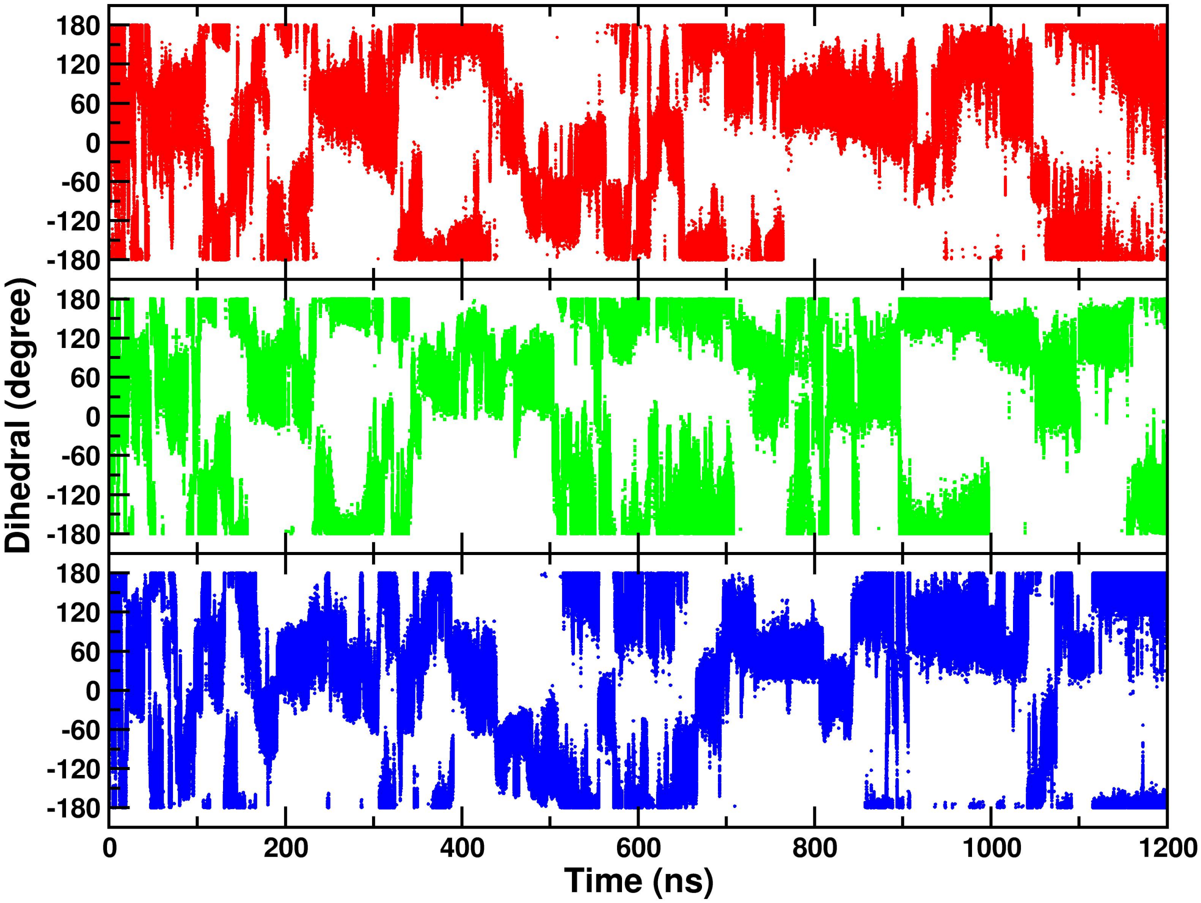


Table S1. List of ionized residues used in this work, and protonation patterns of histidines. Residues not displayed here were kept in their neutral form as they fell outside the active region[a].

Residue	Residue number
Asp	8, 10, 15, 51, 91, 149, 170, 193, 203
Glu	18, 42, 50, 169, 192
Lys	45, 55, 76, 117, 145, 179
Arg	22, 38, 49, 125, 190
His-δ	156, 206
His-ε	20

[a] For a description of how the system was divided into different regions see the **Methodology** section (main text). Note that these ionised residues apply only to the EVB simulations. In the case of the metadynamics simulations, all ionizable residues were set to their standard protonation states at physiological pH, and the same histidine protonation patterns were used as for the EVB simulations.

Table S2. P-O distances at the transition state to the oxygen atom of D8 (P-OD8) and the sugar hyxdroxyl group (P-O$_{sug}$) respectively, for both mechanism. All distances are given in Å.

System	Substrate-assisted mechanism				D10-assisted mechanism			
	Step I (β-G1P to β-G1,6BP)		Step II (β-G1,6BP to β-G6P)		Step I (β-G1P to β-G1,6BP)		Step II (β-G1,6BP to β-G6P)	
	P-O$_{D8}$	P-O$_{sug}$	P-O$_{D8}$	P-O$_{sug}$	P-O$_{D8}$	P-O$_{sug}$	P-O$_{D8}$	P-O$_{sug}$
Solution	1.88 ± 0.12	2.21 ± 0.15	1.88 ± 0.12	2.21 ± 0.15	2.07 ± 0.12	2.01 ± 0.15	2.07 ± 0.12	2.01 ± 0.15
WT	1.89 ± 0.08	2.14 ± 0.12	1.89 ± 0.11	2.20 ± 0.13	1.98 ± 0.08	2.07 ± 0.12	2.07 ± 0.11	1.98 ± 0.13
D10C	1.91 ± 0.07	2.14 ± 0.10	1.87 ± 0.05	2.23 ± 0.10	-	-	-	-
D10N	1.90 ± 0.07	2.14 ± 0.10	1.88 ± 0.13	2.24 ± 0.12	-	-	-	-
D10S	1.91 ± 0.06	2.14 ± 0.09	1.89 ± 0.07	2.22 ± 0.11	-	-	-	-
T16P	1.91 ± 0.06	2.19 ± 0.10	1.89 ± 0.07	2.25 ± 0.12	1.99 ± 0.08	2.08 ± 0.13	2.07 ± 0.11	2.07 ± 0.12
H20A	1.89 ± 0.06	2.21 ± 0.11	1.90 ± 0.06	2.19 ± 0.11	1.98 ± 0.08	2.08 ± 0.13	2.05 ± 0.13	2.01 ± 0.12
H20N	1.91 ± 0.07	2.17 ± 0.11	1.87 ± 0.06	2.23 ± 0.12	1.98 ± 0.08	2.11 ± 0.15	2.07 ± 0.13	1.96 ± 0.11
H20Q	1.90 ± 0.07	2.18 ± 0.11	1.89 ± 0.06	2.21 ± 0.10	1.99 ± 0.09	2.09 ± 0.13	2.07 ± 0.13	1.98 ± 0.12
K76A	1.91 ± 0.06	2.16 ± 0.09	1.87 ± 0.07	2.25 ± 0.12	1.99 ± 0.08	2.09 ± 0.13	2.08 ± 0.13	1.97 ± 0.11
K145A	1.89 ± 0.06	2.19 ± 0.11	1.89 ± 0.06	2.21 ± 0.10	1.95 ± 0.08	2.14 ± 0.14	2.09 ± 0.12	2.08 ± 0.12
D170A	1.90 ± 0.07	2.18 ± 0.09	1.90 ± 0.07	2.23 ± 0.10	1.98 ± 0.08	2.07 ± 0.13	2.05 ± 0.13	1.99 ± 0.12
E169A/D170A	1.92 ± 0.07	2.24 ± 0.13	1.92 ± 0.08	2.26 ± 0.15	2.00 ± 0.08	2.09 ± 0.14	2.20 ± 0.15	1.93 ± 0.11

S2. Empirical Valence Bond Parameters Used in this Work

Information regarding the calibration of the EVB parameters used in this work can be found in the **Methodology** section of the main text. Additional information regarding the EVB approach itself can be found in refs. [2-4]. The off-diagonal elements used in this work, which describe the coupling between the two EVB diabatic states, were chosen to be exponential functions of the form:

$$\mathrm{H_{ij}} = \mathrm{A_{ij}} \exp\ [-\mu(\mathrm{r_{ij}} - \mathrm{r_0})] \tag{1}$$

where r_{ij} represents the distance between the phosphate acceptor and phosphate donor oxygen atoms, r_0 denotes the equilibrium distance (here it was set to zero, as this values works just as an offset in the end), and A_{ij} is a constant. The coupling constant, μ, is an empirical constant related to the overlap between different VB states. For this work it was set to 0.5 for both mechanisms. The gas-phase shift, α_i, is a constant that can be added to either parabola in the reference state in order to reproduce the overall free energy of the background reaction. In the present work, α_i was always added to the second VB state of every step. Since the reactions studied here involved a *ping-pong* mechanism, where the initial and final VB state of the first step becomes the final and initial VB state of the second step respectively, the values of A_{ij} remained the same for both steps, whereas the α_i changed of signal, so that it maintains the same difference for the reacting fragments at infinite distance. The off-diagonal constant (A_{ij}) and gas-phase shift (α_i) values used in this work to calibrate the reference reactions are presented below. The same parameters were then used unchanged in all enzyme runs.

Table S3. EVB parameters used in this work. For EVB atom numbering, see **Figures S4** and **S5**.

Mechanism[a]	A_{ij}	α_i	μ
Substrate-assisted: Step I	630.0	-17.5	0.5
Substrate-assisted: Step II	630.0	-32.1	0.5
D10-assisted: Step I	732.0	100.1	0.5
D10-assisted: Step II	750.0	-105.4	0.5

[a] A_{ij} and α_i values are given in kcal·mol^{-1}, μ is given in Å^{-1}.

van der Waals parameters used for atoms constituting the reacting part of the system[b]							
Type	**A_i (kcal$^{1/2}$·mol-$^{1/2}$·Å^6)**	**B_i (kcal$^{1/2}$·mol-$^{1/2}$·Å^3)**	**C_i (kcal ·mol^{-1})**	**α_i (Å^2)**	**A_{1-4} (kcal$^{1/2}$·mol-$^{1/2}$·Å^3)**	**B_{1-4} (kcal$^{1/2}$·mol-$^{1/2}$·Å^3)**	**mass (a.u.)**
C2	1802.24	34.18	91.0	2.5	1274.38	24.17	12.01
CT	944.52	22.03	91.0	2.5	667.88	15.58	12.01
HO	0.00	0.00	5.0	2.5	0.00	0.00	1.01
HC	84.57	5.41	5.0	2.5	59.80	3.81	1.01
O-	976.93	31.26	90.0	2.5	690.79	22.10	16.00
OH	760.65	25.05	53.0	2.5	537.86	17.71	16.00
OS	445.13	18.25	90.0	2.5	314.75	12.91	16.00
OP2	873.79	27.96	53.0	2.5	617.86	19.77	16.00
O1/O2	616.44	23.77	90.0	2.5	435.89	16.81	16.00
P	2447.79	46.79	43.0	2.5	1730.85	33.09	30.97

[b] Soft pair repulsion energy between atoms *i* and *j*: $V_{soft} = C_i C_j \, exp\,(-\alpha_i \alpha_j r_{ij})$; r_{ij} denotes the distance (Å) between atom *i* and *j*. This potential replaces the vdW interactions between atoms forming and breaking bonds.

Atom Types in Different VB States

	Substrate-assisted (Step I)		Substrate-assisted (Step II)		D10-assisted (Step I)		D10-assisted (Step II)	
#	**State I**	**State II**	**State I**	**State II**	**State I**	**State II**	**State I**	**State II**
1	CT	CT	CT	CT	CT	CT	CT	CT
2	HC	HC	HC	HC	HC	HC	HC	HC
3	HC	HC	HC	HC	HC	HC	HC	HC
4	C2	C2	C2	C2	C2	C2	C2	C2
5	OS	O2	O2	OS	OS	O2	O2	OS
6	O1	O2	O2	O1	O1	O2	O2	O1
7	P	P	P	P	P	P	P	P
8	OP2	OP2	OP2	OP2	OP2	OP2	OP2	OP2
9	OP2	OP2	OP2	OP2	OP2	OP2	OP2	OP2
10	OP2	OH	OP2	OP2	OP2	OP2	OP2	OP2
11	OH	OS	OS	OS	OH	OS	OS	OS
12	HO	HO	HO	HO	HO	HO	HO	HO
13	CT	CT	CT	CT	CT	CT	CT	CT
14	HC	HC	HC	HC	HC	HC	HC	HC
15	HC	HC	HC	HC	HC	HC	HC	HC
16	CT	CT	CT	CT	CT	CT	CT	CT
17	HC	HC	HC	HC	HC	HC	HC	HC
18	CT	CT	CT	CT	CT	CT	CT	CT
19	OH	OH	OH	OH	OH	OH	OH	OH
20	HO	HO	HO	HO	HO	HO	HO	HO
21	HC	HC	HC	HC	HC	HC	HC	HC
22	CT	CT	CT	CT	CT	CT	CT	CT
23	OH	OH	OH	OH	OH	OH	OH	OH
24	HO	HO	HO	HO	HO	HO	HO	HO
25	HC	HC	HC	HC	HC	HC	HC	HC
26	CT	CT	CT	CT	CT	CT	CT	CT
27	OH	OH	OH	OH	OH	OH	OH	OH
28	HO	HO	HO	HO	HO	HO	HO	HO
29	HC	HC	HC	HC	HC	HC	HC	HC
30	CT	CT	CT	CT	CT	CT	CT	CT
31	HC	HC	HC	HC	HC	HC	HC	HC
32	OS	OS	OS	OS	OS	OS	OS	OS
33	OS	OS	OS	OH	OS	OS	OS	OH
34	P	P	P	P	P	P	P	P
35	OP2	OP2	OP2	OP2	OP2	OP2	OP2	OP2
36	OP2	OP2	OP2	OP2	OP2	OP2	OP2	OP2
37	OP2	OP2	OH	OP2	OP2	OP2	OP2	OP2
38	-	-	-	-	CT	CT	CT	CT
39	-	-	-	-	HC	HC	HC	HC
40	-	-	-	-	HC	HC	HC	HC
41	-	-	-	-	C2	C2	C2	C2
42	-	-	-	-	O2	O1	O1	O2
43	-	-	-	-	O2	OH	OH	O2

Partial Charges for the Different VB States

	Substrate-assisted (Step I)		Substrate-assisted (Step II)		D10-assisted (Step I)		D10-assisted (Step II)	
#	State I	State II	State I	State II	State I	State II	State I	State II
1	-0.070	-0.220	-0.220	-0.070	-0.070	-0.220	-0.220	-0.070
2	-0.026	0.060	0.060	-0.026	-0.026	0.060	0.060	-0.026
3	-0.026	0.060	0.060	-0.026	-0.026	0.060	0.060	-0.026
4	0.955	0.700	0.700	0.955	0.955	0.700	0.700	0.955
5	-0.747	-0.800	-0.800	-0.747	-0.747	-0.800	-0.800	-0.747
6	-0.694	-0.800	-0.800	-0.694	-0.694	-0.800	-0.800	-0.694
7	1.335	1.336	1.352	1.352	1.322	1.335	1.352	1.352
8	-0.909	-0.825	-0.903	-0.903	-0.977	-0.909	-0.903	-0.903
9	-0.909	-0.825	-0.903	-0.903	-0.977	-0.909	-0.903	-0.903
10	-0.909	-0.726	-0.903	-0.903	-0.977	-0.909	-0.903	-0.903
11	-0.631	-0.587	-0.517	-0.517	-0.631	-0.670	-0.517	-0.517
12	0.384	0.387	0.387	0.384	0.384	0.450	0.450	0.384
13	0.247	0.240	0.201	0.201	0.247	0.279	0.201	0.201
14	-0.012	-0.012	-0.012	-0.012	-0.012	-0.012	-0.012	-0.012
15	-0.012	-0.012	-0.012	-0.012	-0.012	-0.012	-0.012	-0.012
16	-0.027	-0.027	-0.027	-0.027	-0.027	-0.027	-0.027	-0.027
17	0.079	0.079	0.079	0.079	0.079	0.079	0.079	0.079
18	0.239	0.239	0.239	0.239	0.239	0.239	0.239	0.239
19	-0.708	-0.708	-0.708	-0.708	-0.708	-0.708	-0.708	-0.708
20	0.427	0.427	0.427	0.427	0.427	0.427	0.427	0.427
21	0.012	0.012	0.012	0.012	0.012	0.012	0.012	0.012
22	0.239	0.239	0.239	0.239	0.239	0.239	0.239	0.239
23	-0.708	-0.708	-0.708	-0.708	-0.708	-0.708	-0.708	-0.708
24	0.427	0.427	0.427	0.427	0.427	0.427	0.427	0.427
25	0.012	0.012	0.012	0.012	0.012	0.012	0.012	0.012
26	0.239	0.239	0.239	0.239	0.239	0.239	0.239	0.239
27	-0.708	-0.708	-0.708	-0.708	-0.708	-0.708	-0.708	-0.708
28	0.427	0.427	0.427	0.427	0.427	0.427	0.427	0.427
29	0.012	0.012	0.012	0.012	0.012	0.012	0.012	0.012
30	0.201	0.201	0.240	0.247	0.201	0.201	0.279	0.247
31	0.095	0.095	0.095	0.095	0.095	0.095	0.095	0.095
32	-0.360	-0.360	-0.360	-0.360	-0.360	-0.360	-0.360	-0.360
33	-0.517	-0.517	-0.587	-0.631	-0.517	-0.517	-0.670	-0.631
34	1.352	1.352	1.336	1.335	1.352	1.352	1.335	1.322
35	-0.903	-0.903	-0.825	-0.909	-0.903	-0.903	-0.909	-0.977
36	-0.903	-0.903	-0.825	-0.909	-0.903	-0.903	-0.909	-0.977
37	-0.903	-0.903	-0.726	-0.909	-0.903	-0.903	-0.909	-0.977
38	-	-	-	-	-0.220	-0.120	-0.120	-0.220
39	-	-	-	-	0.060	0.060	0.060	0.060
40	-	-	-	-	0.060	0.060	0.060	0.060
41	-	-	-	-	0.700	0.520	0.520	0.700
42	-	-	-	-	-0.800	-0.440	-0.440	-0.800
43	-	-	-	-	-0.800	-0.530	-0.530	-0.800

Bond Parameters in the Reacting Part of the System[c]

Bond Type	D_e (kcal·mol^{-1})	**A** (Å^{-2})	r_0 (Å)	k_b (kcal·mol^{-1}·rad^{-2})	b (Å)
P-OS	95.0	2.0	1.61	-	-
OH-HO	150.0	2.0	0.94	-	-
C2-O2	-	-	-	1312.0	1.250
C2-O1	-	-	-	1140.0	1.229
C2-OH	-	-	-	900.0	1.364
P-OP2	-	-	-	1050.0	1.480
P-OH	-	-	-	460.0	1.610

[c] Morse bonds: $V_{Morse} = D_e\{1-\exp[-\alpha (r_{ij}-r_0)]\}^2$; Harmonic bonds: $V_{Harmonic} = 0.5\, k_b (r_{ij}-b)^2$

Angle Parameters in the Reacting Part of the System[d]

Angle Type	k_a (kcal·mol^{-1}·rad^{-2})	Θ_0 (°)
P-OS-CT	200.0	120.50
P-OH-HO	110.0	108.50
OS-P-OP2	200.0	108.23
OH-P-OP2	90.0	108.23
OP2-P-OP2	280.0	119.90
OH-P-OS	90.0	102.60
O2-C2-O2	160.0	126.00
O2-C2-O2	160.0	126.00
O1-C2-OH	160.0	121.00
CT-OH-HO	110.0	108.50
C2-OH-HO	70.0	113.00
CT-C2-OH	140.0	108.00
CT-C2-O1/O2	140.0	117.00

[d] Harmonic angle potential: $V_a = 0.5\, k_a(\Theta-\Theta_0)^2$

Torsion Parameters in the Reacting Atoms of the System[e]

Torsion Type	V_1(kcal·mol^{-1})	V_2(kcal·mol^{-1})	V_3(kcal·mol^{-1})
P-OS-CT-CT	0.000	1.495	0.000
P-OS-CT-HC	0.000	0.000	0.179
OP2/OS/OH-P-OS-CT	0.000	0.000	0.281
OP2/OS-P-OH-HO	0.000	0.000	0.000
OS-CT-OS-HC	0.000	0.000	0.179
HO-OH-CT-OS	-0.628	-0.903	0.003
HO-OH-CT-CT	-0.178	-0.087	0.246
CT-CT-C2-O2	0.000	0.273	0.000
CT-CT-C2-OH	0.500	0.273	0.225
HC-CT-C2-OH	0.000	0.000	0.225
HO-OH-CT-OS	-0.628	-0.903	0.003
HO-OH-CT-CT	-0.178	-0.087	0.246

[e] Torsion angle potential: $V_a = V_1(1+\cos\phi)/2 + V_2(1-\cos 2\phi)/2 + V_3(1+\cos 3\phi)/2$

S3. References

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