

Supporting Information

A Theoretical Study of the Phosphoryl Transfer Reaction from ATP to Dha Catalyzed by DhaK from Escherichia coli

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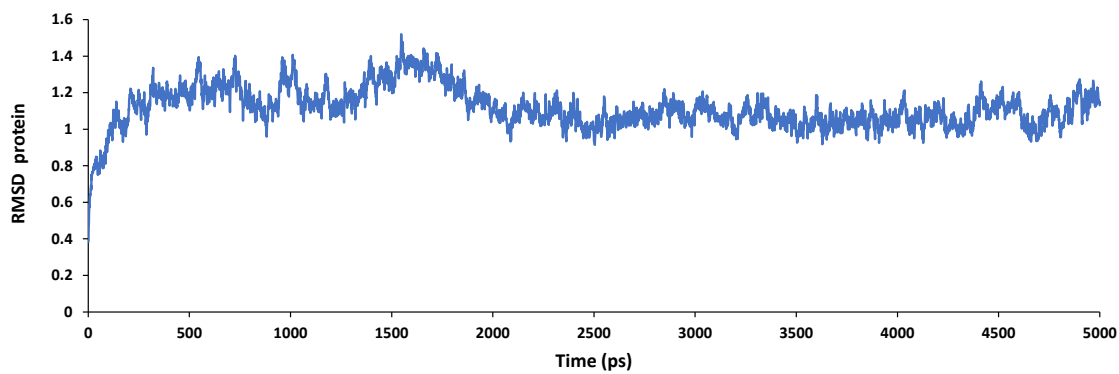


Figure S1: Time evolution of the RMSD of the backbone atoms of the enzyme during 5 ns of MD simulations.

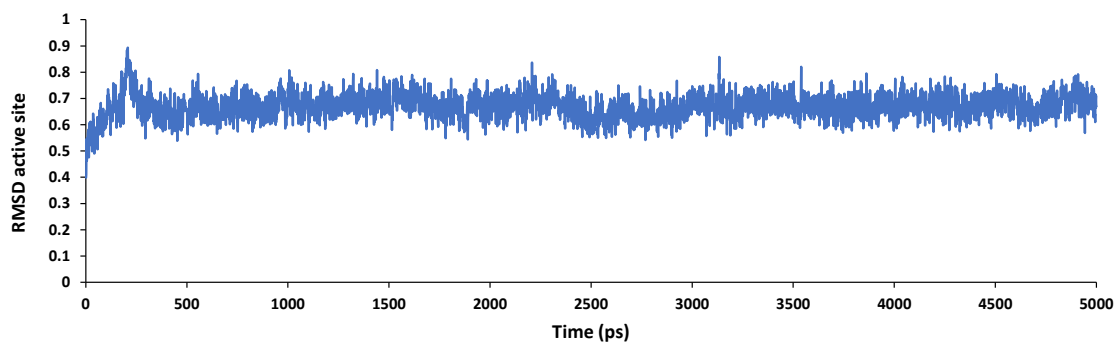


Figure S2: Time evolution of the RMSD computed for the backbone atoms of just the active site residues (those in a radius sphere of 8 Å: Chain A: residues 50-57, 76-81, 105-110, 148-150, 216-219; ChainB: residues 30-40, 76-82, 120-124, 127-133, 176-180, 190-196) during 5 ns of MD simulations.

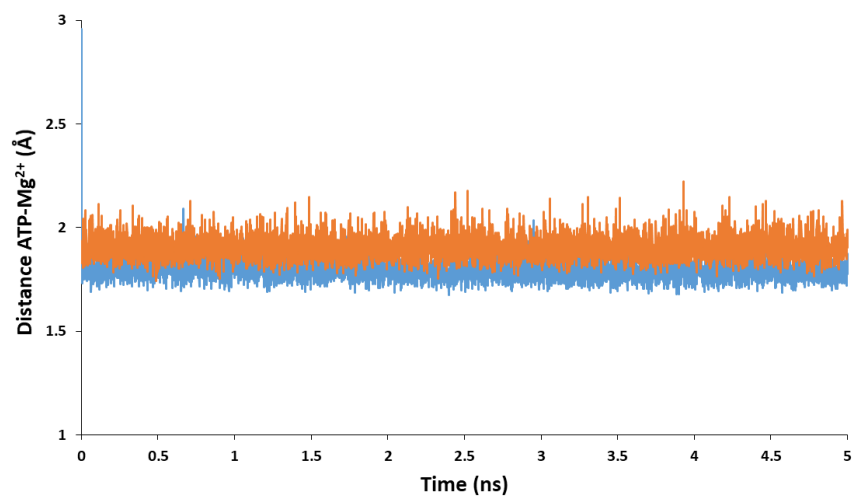


Figure S3. Time evolution of the distance between the Mg²⁺ ion and the oxygen atom OB3 of ATP (blue line) and the other Mg²⁺ ion with the oxygen atom OA1 of ATP (orange line), during the 5 ns of MD simulations.

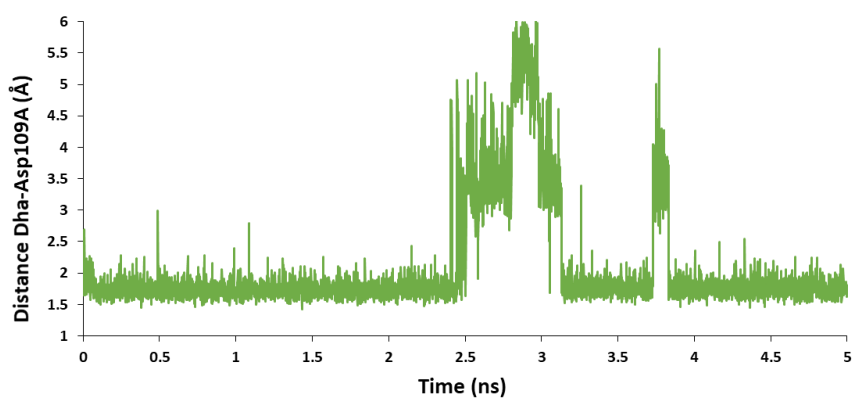


Figure S4. Time evolution of the distance between the hydrogen atom H6 of Dha and the oxygen atom OD1 of Asp109A during the 5 ns of MD simulations.

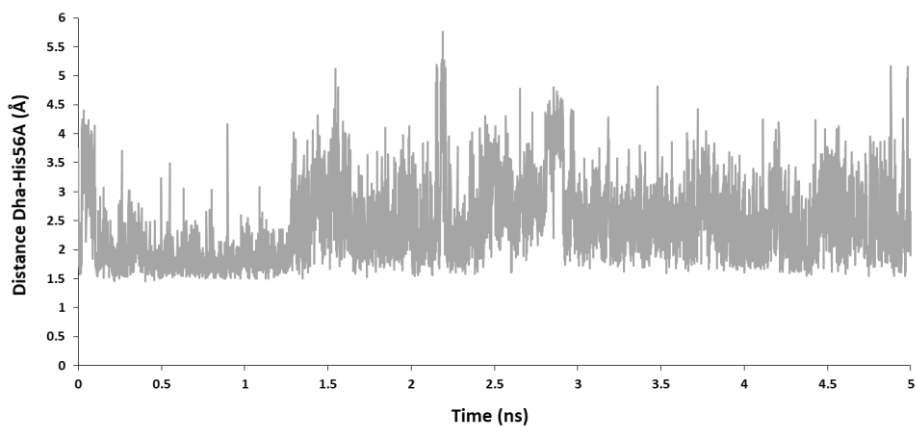


Figure S5. Time evolution of the distance between the hydrogen atom HE2 of His56A and the oxygen atom O4 of Dha during the 5 ns of MD simulations.

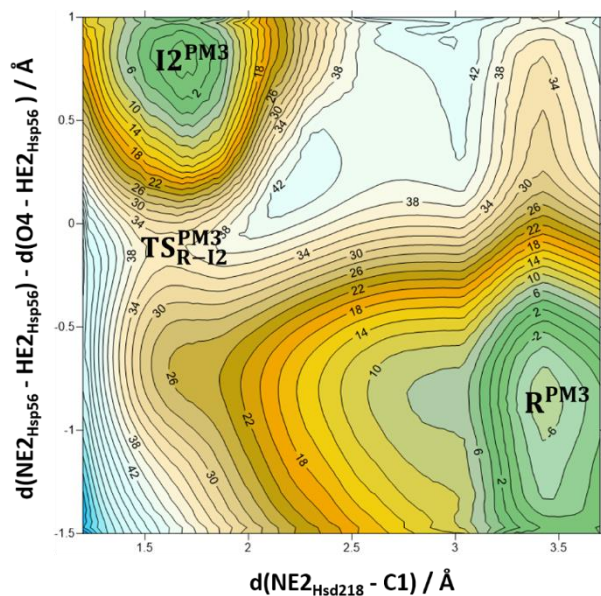


Figure S6: PM3/MM potential energy surface of the first step for the phosphorylation reaction from ATP to Dha catalyzed by DhaK from *E. coli*. Values of the isoenergetic lines on the 2D-PES are in kcal·mol⁻¹.

Table S1. Key interatomic distances with their standard deviations of the transition states located on the PM3/MM PMFs of the first step of the phosphoryl transfer reaction from ATP to Dha catalyzed by DhaK from *E. coli*. The values are determined from an average of 100 ps of MD constraining the distinguished reaction coordinate distances.

Distances	TS ^{PM3} _{R-11} (Å)	TS ^{PM3} _{I1-I2} (Å)	TS ^{PM3} _{R-12} (Å)
NE2 _{His218A} – C1	3.17±0.03	2.32±0.03	1.58±0.03
NE2 _{His56A} – HE2 _{His56A}	1.39±0.04	1.69±0.03	1.22±0.04
O4 – HE2 _{His56A}	1.14±0.03	1.00±0.02	1.29±0.05
PG _{ATP} – O5 _{Dha}	5.75±0.30	5.76±0.29	4.31±0.30
PG _{ATP} – OB3 _{ATP}	1.78±0.04	1.77±0.04	1.80±0.04
O5 _{Dha} – H5 _{Dha}	0.98±0.03	0.98±0.03	0.95±0.02
OG3 _{ATP} – H5 _{Dha}	5.67±0.55	5.86±0.27	3.16±0.70
OD2 _{Asp109A} – H5 _{Dha}	1.91±0.55	1.77±0.11	3.93±0.98
OB3 _{ATP} – H5 _{Dha}	7.29±0.46	7.37±0.25	5.30±0.67
OG2 _{ATP} – H5 _{Dha}	5.03±0.39	5.01±0.25	3.93±0.58

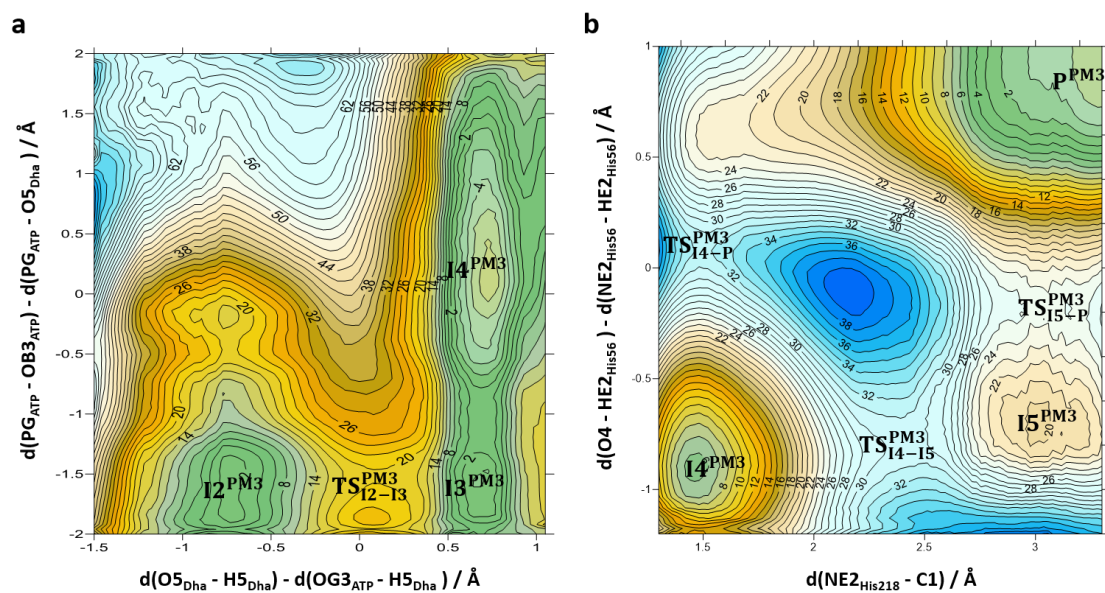


Figure S7. PM3/MM potential energy surfaces of the chemical steps of the *substrate-assisted mechanism* for the phosphorylation reaction from ATP to Dha catalyzed by DhaK from *E. coli*. a) Proton transfer from Dha to the transferring phosphoryl group of ATP followed by the phosphoryl transfer to Dha; and b) releasing of the final products where the proton returns to His56A and the bond with His218A is cleft. Values of the isoenergetic lines on the 2D-PES are in $\text{kcal}\cdot\text{mol}^{-1}$.

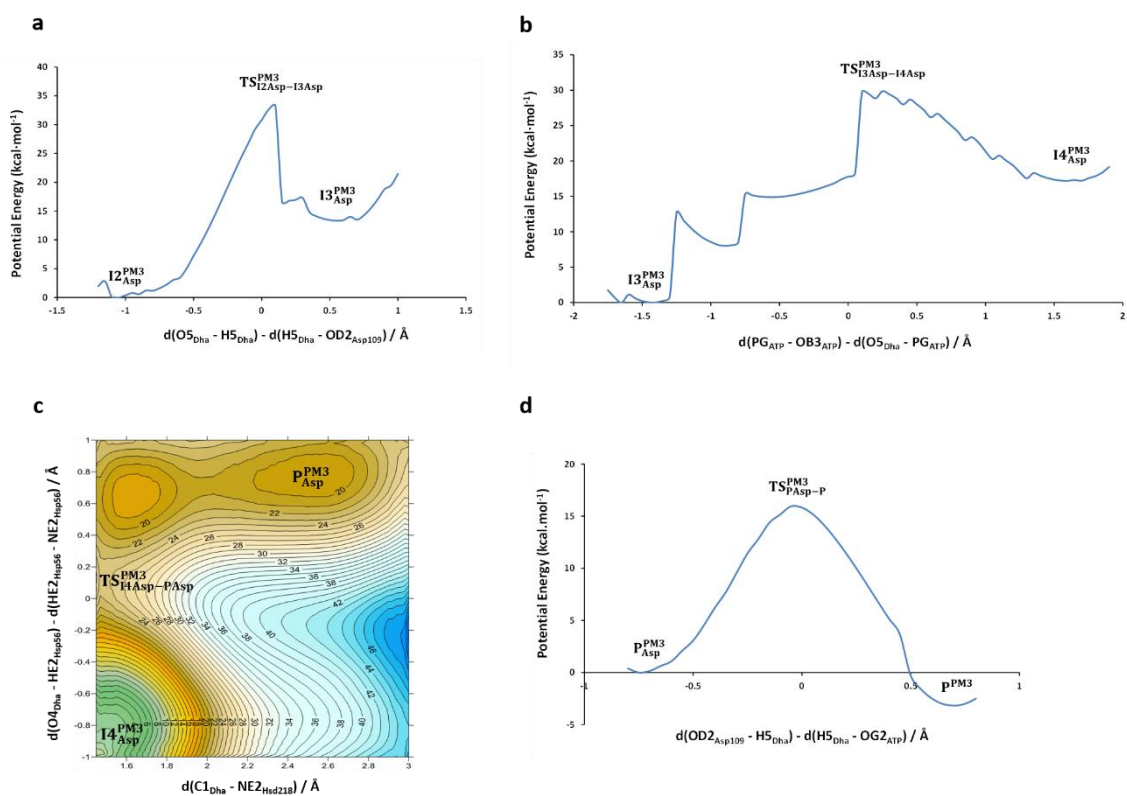


Figure S8. PM3/MM potential energy surfaces of the chemical steps of the *asp*-assisted mechanism for the phosphorylation reaction from ATP to Dha catalyzed by DhaK from *E. coli*. a) Proton transfer from Dha to Asp109A; b) phosphoryl transfer from ATP to Dha; c) releasing of the products where the proton returns to His56A and the bond with His218A is cleft; and d) proton transfer from Asp109A to ADP, reaching the final products. Values of the isoenergetic lines on the 2D-PES are in kcal·mol⁻¹.

Table S2. Key interatomic distances with their standard deviations of the transition states located on the PM3/MM PMFs of the chemical steps of the *substrate-assisted mechanism* for the phosphorylation reaction from ATP to Dha catalyzed by DhaK from *E. coli*. The values are determined from an average of 100 ps of MD constraining the distinguished reaction coordinate distances.

Distances	TS _{J2-J3} ^{PM3} (Å)	TS _{J3-J4} ^{PM3} (Å)	TS _{J4-J5} ^{PM3} (Å)	TS _{J5-P} ^{PM3} (Å)	TS _{J4-P} ^{PM3} (Å)
NE2 _{His218A} – C1	1.53±0.04	1.53±0.04	2.40±0.03	3.01±0.03	1.58±0.03
NE2 _{His56A} – HE2 _{His56A}	1.92±0.27	5.04±0.45	1.72±0.03	1.45±0.04	1.21±0.04
O4 – HE2 _{His56A}	0.97±0.03	0.97±0.03	1.00±0.02	1.13±0.03	1.36±0.05
PG _{ATP} – O5 _{Dha}	3.48±0.04	2.57±0.05	1.78±0.04	1.78±0.04	1.75±0.04
PG _{ATP} – OB3 _{ATP}	1.77±0.03	1.77±0.04	3.64±0.14	3.72±0.14	3.83±0.19
O5 _{Dha} – OB3 _{ATP}	4.58±0.09	3.98±0.09	5.09±0.13	5.23±0.16	5.05±0.14
O5 _{Dha} – H5 _{Dha}	1.14±0.03	1.72±0.03	2.64±0.14	2.78±0.16	3.23±0.30
OG3 _{ATP} – H5 _{Dha}	1.17±0.04	0.97±0.02	0.95±0.02	0.95±0.02	0.96±0.02
OD2 _{Asp109A} – H5 _{Dha}	5.54±0.27	6.07±0.27	6.18±0.23	6.31±0.19	6.46±0.25
OB3 _{ATP} – H5 _{Dha}	3.46±0.08	3.07±0.07	3.17±0.14	3.18±0.17	2.27± 0.64
OG2 _{ATP} – H5 _{Dha}	3.14±0.12	2.89±0.14	2.94±0.21	2.82±0.22	2.79±0.27
OG2 _{ATP} – HG1 _{Thr79A}	3.19±0.38	2.20±0.34	4.47±0.25	4.55 ±0.27	5.23±0.33
OG2 _{ATP} – HA _{Thr79A}	2.69±0.16	2.61±0.15	2.62±0.15	2.69±0.16	2.91±0.24
OG2 _{ATP} – H _{Ser80A}	2.03±0.15	1.97±0.14	1.78±0.10	1.81±0.11	1.93±0.34
OG2 _{ATP} – HA2 _{Gly78B}	2.61±0.19	2.56±0.17	2.54±0.14	2.61±0.17	3.05±0.38
OG3 _{ATP} – HE _{Arg178B}	3.65±0.26	3.24±0.57	3.87±0.22	4.00±0.21	4.17±0.28
OG3 _{ATP} – MG	1.86±0.03	1.86±0.03	2.48±0.04	2.49±0.04	2.49±0.04

Table S3. Key interatomic distances with their standard deviations of the transition states located on the PM3/MM PMFs of the chemical steps of the *asp*-assisted mechanism for the phosphorylation reaction from ATP to Dha catalyzed by DhaK from *E. coli*. The values are determined from an average of 100 ps of MD constraining the distinguished reaction coordinate distances.

Distances	TS ^{PM3} _{I2Asp-I3Asp} (Å)	TS ^{PM3} _{I3Asp-I4Asp} (Å)	TS ^{PM3} _{I4Asp-PAsp} (Å)	TS ^{PM3} _{PAsp-P} (Å)
NE2 _{His218} – C1	1.52±0.04	1.52±0.03	1.57±0.03	3.02±0.17
NE2 _{His56A} – HE2 _{His56A}	3.77±0.57	1.95±0.30	1.22±0.04	1.01±0.03
O4 – HE2 _{His56A}	0.95±0.02	0.97±0.03	1.34±0.05	1.91±0.25
PG _{ATP} – O5 _{Dha}	5.15±0.25	1.98±0.06	1.79±0.04	1.77±0.04
PG _{ATP} – OB3 _{ATP}	1.78±0.04	2.32±0.06	3.55±0.12	3.55±0.12
O5 _{Dha} – OB3 _{ATP}	6.82±0.24	4.07±0.11	5.14±0.12	5.12±0.12
O5 _{Dha} – H5 _{Dha}	1.18±0.04	1.74±0.06	3.03±0.23	2.83±0.13
OG3 _{ATP} – H5 _{Dha}	5.13±0.24	4.03±0.12	4.17±0.12	3.71±0.09
OD2 _{Asp109A} – H5 _{Dha}	1.14±0.04	0.99±0.03	0.98±0.03	1.11±0.03
OB3 _{ATP} – H5 _{Dha}	6.77±0.23	5.10±0.18	5.82±0.15	5.20±0.15
OG2 _{ATP} – H5 _{Dha}	4.80±0.26	3.02±0.23	1.74±0.07	1.20±0.04
OG2 _{ATP} – HA _{Thr79A}	2.64±0.14	2.90±0.22	3.45±0.19	3.43±0.18
OG2 _{ATP} – H _{Ser80A}	1.90±0.11	1.89±0.16	2.65±0.42	3.51±0.39
OG2 _{ATP} – HA2 _{Gly78B}	2.56±0.17	2.69±0.21	4.03±0.27	4.14±0.29
OG3 _{ATP} – HA _{Thr79A}	2.47±0.12	2.53±0.13	2.54±0.13	2.63±0.15
OG3 _{ATP} – MG	1.85±0.03	1.86±0.03	1.87±0.03	1.87±0.03
OG3 _{ATP} – HH11 _{Arg178B}	1.95±0.11	2.39±0.30	3.43±0.14	3.53±0.12
OG1 _{ATP} – HH12 _{Arg178B}	1.86±0.13	2.09±0.23	3.43±0.02	3.56±0.26

Table S4. Key interatomic distances with their standard deviations of the transition state located on the PM3/MM PMF of the chemical step corresponding to the transition from $I4_{\text{Asp}}^{\text{PM3}}$ to $I4^{\text{PM3}}$ for the phosphorylation reaction from ATP to Dha catalyzed by DhaK from *E. coli*. The values are determined from an average of 100 ps of MD constraining the distinguished reaction coordinate distances.

Distances	TS_{I4Asp-I4}^{PM3} (Å)
NE2_{His218A} – C1	1.52±0.03
NE2_{His56A} – HE2_{His56A}	3.78±0.70
O4 – HE2_{His56A}	0.96±0.02
PG_{ATP} – O5_{Dha}	1.93±0.07
PG_{ATP} – OB3_{ATP}	1.92±0.07
O5_{Dha} – H5_{Dha}	2.82±0.16
OG3_{ATP} – H5_{Dha}	3.38±0.13
OD2_{Asp109A} – H5_{Dha}	1.34±0.05
OB3_{ATP} – H5_{Dha}	3.82±0.11
OG2_{ATP} – H5_{Dha}	1.07±0.03

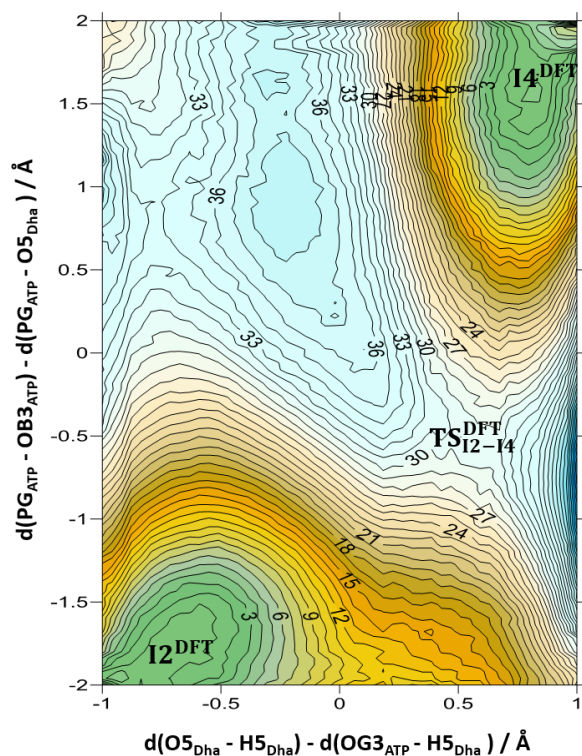


Figure S9. B3LYP/MM potential energy surface of the *substrate-assisted* phosphoryl transfer reaction from ATP to Dha catalyzed by DhaK from *E. coli*. Values of the isoenergetic lines are in kcal·mol⁻¹.

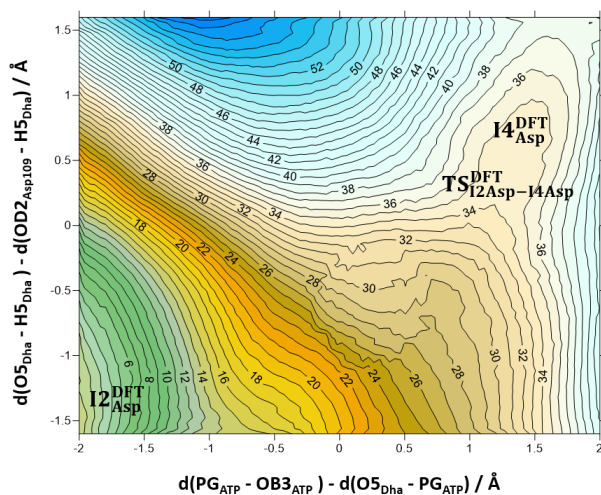


Figure S10. B3LYP/MM potential energy surface of the *asp-assisted* phosphoryl transfer reaction from ATP to Dha catalyzed by DhaK from *E. coli*. Values of the isoenergetic lines are in kcal·mol⁻¹.

Table S5. Key interatomic distances of the transition states located at B3LYP/MM level of the chemical steps of the *substrate-assisted* and *asp-assisted* phosphoryl transfer reactions from ATP to Dha catalyzed by DhaK from *E. coli*.

Distances	TS_{I2-14}^{DFT} (Å)	TS_{I2Asp-14Asp}^{DFT} (Å)
NE2_{His218A} – C1	1.55	1.55
NE2_{His56A} – HE2_{His56A}	1.88	1.97
O4 – HE2_{His56A}	1.00	1.00
O5_{Dha} – H5_{Dha}	1.51	1.36
OD2_{Asp109A} – H5_{Dha}	6.82	1.13
OG3_{ATP} – H5_{Dha}	1.02	3.91
PG_{ATP} – OB3_{ATP}	1.99	3.06
PG_{ATP} – O5_{Dha}	2.27	2.03
O5_{Dha} – OB3_{ATP}	4.10	5.05
OB3_{ATP} – H5_{Dha}	3.35	5.74
OG2_{ATP} – H5_{Dha}	2.99	2.98
OG2_{ATP} – HA_{Thr79A}	2.72	3.00
OG2_{ATP} – H_{Ser80A}	1.85	1.85
OG2_{ATP} – HA2_{Gly78B}	2.36	2.45
OG1_{ATP} – HH12_{Arg178B}	1.70	1.56
OG1_{ATP} – HE_{Arg178B}	2.34	2.72
OG3_{ATP} – MG	2.00	2.10

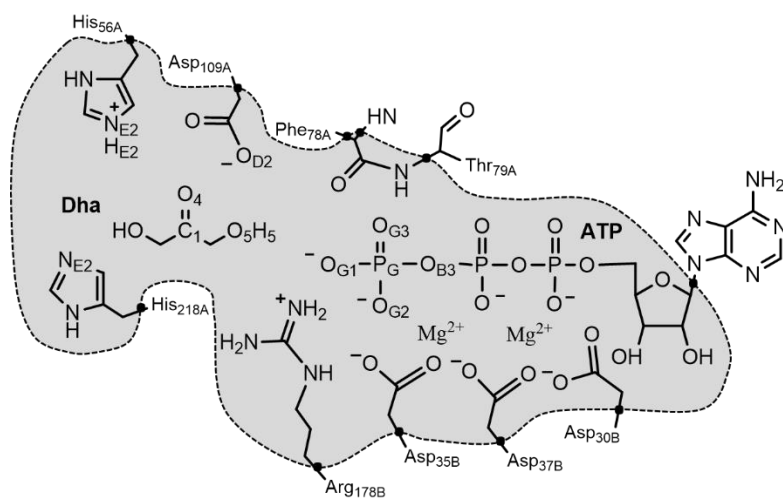


Figure S11. Schematic representation of the active site of DhaK adding the residue Arg178B. Grey region contains atoms treated quantum mechanically. Link atoms are represented as black dots.

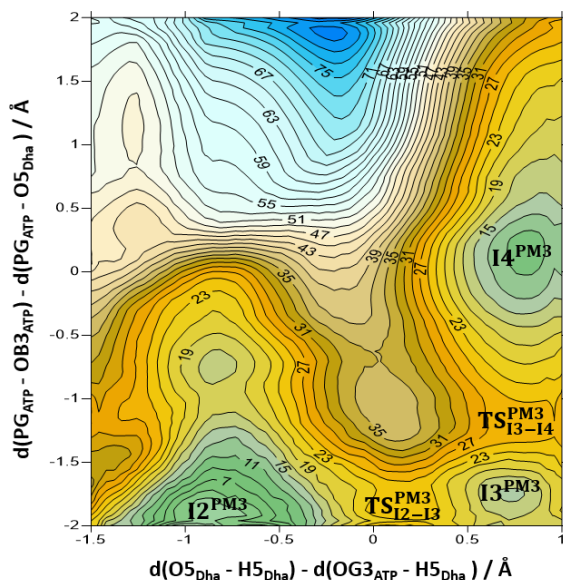


Figure S12. PM3/MM potential energy surface for the transformations from $I2^{\text{PM3}}$ to $I4^{\text{PM3}}$ following the *substrate-assisted mechanism* catalyzed by DhaK from *E. coli*, including Arg178B in the QM part. Values of the isoenergetic lines on the 2D-PES are in $\text{kcal}\cdot\text{mol}^{-1}$.

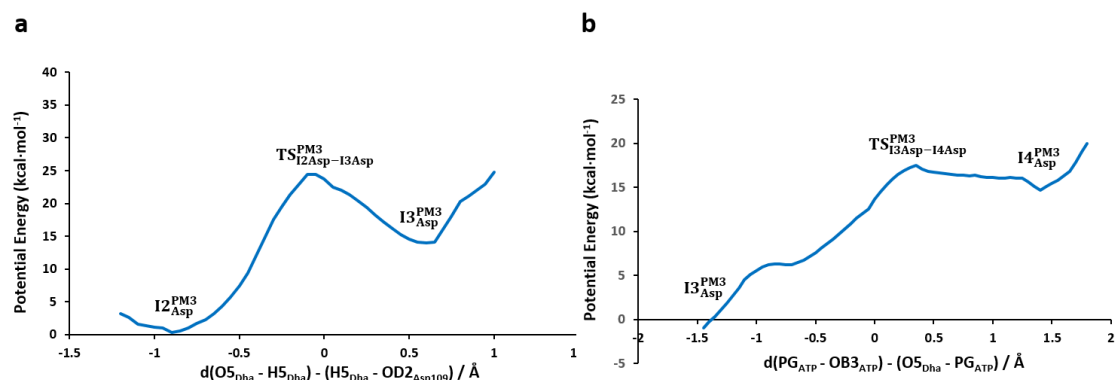


Figure S13. PM3/MM potential energy surfaces of the more relevant chemical steps of the *asp-assisted mechanism* for the phosphorylation reaction from ATP to Dha catalyzed by DhaK from *E. coli*, including Arg178B in the QM part. a) Proton transfer from Dha to Asp109A; and b) phosphoryl transfer from ATP to Dha.

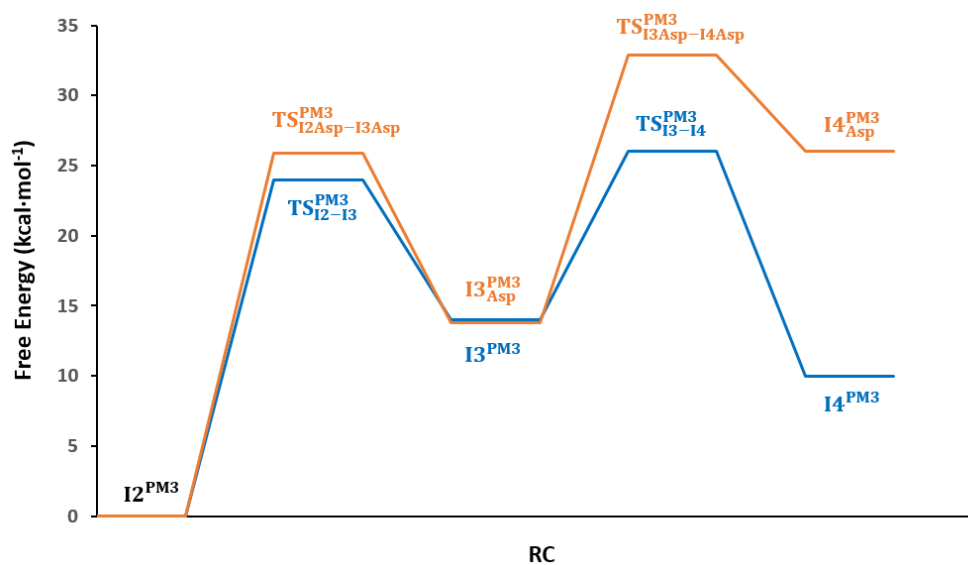


Figure S14. PM3/MM potential energy profiles of the more relevant chemical steps of the *substrate-assisted* (blue line) and *asp-assisted* (orange line) mechanisms for the phosphorylation reaction from ATP to Dha catalyzed by DhaK from *E. coli*, including Arg178B in the QM part.

Table S6. Cartesian coordinates of the QM atoms of the TS_{I2Asp-I4Asp}^{PM3} localized at PM3/MM level of the *asp*-assisted phosphoryl transfer reaction from ATP to Dha catalyzed by DhaK from *E. coli*.

Dha			
C1	-2.9988603821	-1.2234492628	-3.6708257192
C2	-1.6396761118	-0.7676623245	-2.9132391705
H21	-1.6314924817	0.3582326051	-2.9447642152
H22	-1.8016050591	-1.0334340806	-1.8410418178
C3	-4.2610222821	-0.7527367361	-2.8602341638
H31	-5.1992260049	-0.9181533019	-3.4262643579
H32	-4.3315556082	-1.3240846861	-1.9083713559
O4	-3.0150208217	-0.7173300779	-4.9628625903
O5	-0.5431392252	-1.2792507240	-3.4503144805
H5	-1.1354919431	1.5452083559	-1.7860681227
O6	-4.2750771085	0.6235034134	-2.6292419065
H6	-3.6548773167	0.8188780072	-1.9240687791
ATP			
C1'	9.4473643384	-5.5320981206	2.9358564068
H1'	10.4009214788	-4.9691286648	2.9676380863
C2'	9.6470716316	-7.0610903343	2.8456047613
H2'	11.1375381222	-8.0060574060	2.1561348735
O2'	11.0244785236	-7.3598270796	2.8579308166
HT2	9.1940172998	-7.5991295805	3.7077190516
C3'	8.9867208801	-7.4649172795	1.4904539664
H3'	7.9991372555	-7.9584614565	1.6351325016
O3'	9.8491150296	-8.3144487408	0.7687344174
HT3	9.9087885196	-9.1417412273	1.2526971091
C4'	8.8508431357	-6.1146285588	0.7367783984
O4'	8.7679623410	-5.0965699152	1.7478345433
H4'	9.7732462121	-5.9021711442	0.1434316939
C5'	7.6062312535	-6.0428899342	-0.1881871455
H51	6.7177233700	-5.6759707554	0.3615478701
H52	7.3678850477	-7.0609341177	-0.5573208654
O5'	7.9065119561	-5.2069119852	-1.2566274753
PA	6.9051530086	-4.7829796009	-2.6198493337
OA1	6.4238540779	-6.0475892106	-3.4413148337
OA2	7.9692162152	-3.8900025855	-3.2817516752
OA3	5.6222835346	-3.9240513747	-1.9906524996
PB	3.8722802508	-4.3339359432	-2.1362190544
OB1	3.8383441951	-5.5186139035	-3.2366919363
OB2	3.4736947006	-4.6750215743	-0.7062267417
OB3	3.1099856333	-3.0596878090	-2.7650153611
PG	1.0866799294	-1.9392292542	-2.6496218904
OG1	0.3730636326	-2.7676756846	-1.5488212215
OG2	1.6072346901	-0.5448317439	-2.2525520916
OG3	1.4051500422	-2.5421272940	-4.0878185578

His_{56A}			
CB	-8.3000067826	-0.3002185048	-7.9937392740
HB1	-9.1226795324	-0.2008493142	-7.2611478067
HB2	-7.9324108774	0.7221274916	-8.1900603695
CG	-7.2160343925	-1.0562327561	-7.3339613174
ND1	-6.9846299525	-2.4382533334	-7.3994838501
HD1	-7.5543755642	-3.0895614407	-7.8820209382
CE1	-5.8638411175	-2.7368837342	-6.6499047527
HE1	-5.3988395953	-3.7176479825	-6.5234522869
NE2	-5.3935764339	-1.5800705335	-6.1038084258
HE2	-3.8646351075	-0.9563716086	-5.3800360032
CD2	-6.2225731156	-0.5412057401	-6.5087933113
HD2	-6.0486185939	0.4864482982	-6.1952945849
His_{218A}			
CB	-2.5218112321	-6.1325793426	-5.1820682716
HB1	-1.6489636931	-5.9701165438	-5.8434091833
HB2	-2.1698662810	-6.8820236463	-4.4496103491
CG	-2.8002737264	-4.8655012336	-4.4697585290
ND1	-3.4852003482	-4.8440938833	-3.2467243142
HD1	-3.7742902904	-5.6552178466	-2.7482693266
CE1	-3.6470737530	-3.5428972971	-2.8363241682
HE1	-4.1667286812	-3.1638451272	-1.9408690712
NE2	-3.0354217596	-2.7445188643	-3.7796407504
CD2	-2.5053996761	-3.5422902277	-4.7989685123
HD2	-1.9299475381	-3.1316064758	-5.6550805307
Asp_{109A}			
CB	-1.4821651273	2.5268802260	1.2758638602
HB1	-2.1709100080	3.3685298977	1.4942388101
HB2	-1.7875374405	1.7520795547	2.0080876826
CG	-1.8782374936	2.0317367739	-0.0801294948
OD1	-2.9631442332	1.5763530322	-0.4072059869
OD2	-0.9482203775	2.1447250892	-1.0529084296
MG			
MG	4.8851230679	-6.5306254669	-4.4296073954
MG	2.8166787599	-3.6753610180	-4.4850629358
Asp_{30B}			
CB	6.6832707798	-9.9504853892	-5.4748606317
HB1	6.7927061005	-9.8740515172	-6.5681342773
HB2	7.6761084796	-9.6222385431	-5.1072143080
CG	5.7444731521	-8.8382867279	-4.9766654362
OD1	4.9135468492	-8.9801355635	-4.0624351694
OD2	5.7832563968	-7.7117236258	-5.5840970627
Asp_{35B}			
CB	0.9719351055	-7.2195801025	-5.8110833004
HB1	0.1443479990	-7.0416777149	-5.0936001854
HB2	0.5857476136	-6.8011090795	-6.7580510927
CG	2.1538356996	-6.3271190215	-5.3533066198
OD1	1.8372129098	-5.1353126229	-5.1113700412
OD2	3.2814778692	-6.9328536248	-5.3360970560

Asp_{37B}			
CB	4.6347354934	-4.3156733954	-7.8299703357
HB1	3.6138047436	-4.3746133169	-8.2586458236
HB2	5.0192905760	-3.3529989143	-8.2199886682
CG	4.3978099267	-4.1159912548	-6.3461245594
OD1	4.7412702153	-4.9411545144	-5.4597473570
OD2	3.8644143614	-3.0323379397	-5.9010627329
Phe_{78A}			
CA	-0.2757833650	-2.4856911772	-7.7296901130
HA	-1.1051533910	-2.1496281373	-7.0569175308
C	1.0034260953	-2.0412770163	-7.0575238704
O	1.9315355480	-2.7228685555	-6.6418432871
Thr_{79A}			
N	1.2156084164	-0.6241081486	-6.9412039234
H	0.3559358833	-0.1279634226	-7.0708652467

Table S7. Cartesian coordinates of the QM atoms of the TS₁₂₋₁₄^{PM3} localized at PM3/MM level of the *substrate-assisted* phosphoryl transfer reaction from ATP to Dha catalyzed by DhaK from *E. coli*.

Dha			
C1	-2.8265367275	-1.0594915193	-3.8733540018
C2	-1.3190998039	-0.5770846503	-3.5266256005
H21	-1.3078582628	0.5093569082	-3.7580783646
H22	-1.2394131379	-0.6853762038	-2.4198305367
C3	-3.7997916126	-0.4135275918	-2.8225853804
H31	-4.8602370111	-0.6213491479	-3.0710337136
H32	-3.5885801790	-0.8281633350	-1.8177339707
O4	-3.1276138707	-0.7155635709	-5.1801759132
O5	-0.3569620667	-1.2110610930	-4.1635467064
H5	0.6103180663	-2.6158076707	-4.5068948596
O6	-3.6891259838	0.9771198538	-2.8176893275
H6	-3.1999520949	1.2467366841	-2.0185072758
ATP			
C1'	9.4092189190	-5.4876166459	2.8950834103
H1'	10.3383489150	-4.8825777579	2.9239161667
C2'	9.6890586719	-7.0093982247	2.7888822496
H2'	11.2754419620	-8.0153571748	2.3888210543
O2'	11.0875381231	-7.1839176802	2.8072464285
HT2	9.2717632945	-7.5754388632	3.6529016660
C3'	8.9946414257	-7.4312442950	1.4563083582
H3'	7.9973652191	-7.8831436852	1.6712583178
O3'	9.7314201616	-8.3198243556	0.6572133151
HT3	9.8432826798	-9.1269780380	1.1639437195

C4'	8.8424416515	-6.0859281095	0.6975290789
O4'	8.7174630058	-5.0717750331	1.7060981144
H4'	9.7673789075	-5.8569738682	0.1141769856
C5'	7.6069613295	-6.0553187017	-0.2339531125
H51	6.7104429647	-5.6773906631	0.2944804016
H52	7.3812169603	-7.0819391281	-0.5852196541
O5'	7.9057009667	-5.2431439020	-1.3269681090
PA	6.8915151168	-4.8496759323	-2.6590482436
OA1	6.3943466431	-6.1144803430	-3.4735061340
OA2	7.8612506026	-3.8843638075	-3.3241208809
OA3	5.5381070079	-4.1019898179	-1.9919146221
PB	3.8729128902	-4.6212767687	-2.0810542778
OB1	3.7724710603	-5.7700807738	-3.1733647772
OB2	3.4243272157	-4.8245726961	-0.6671520794
OB3	2.9982067831	-3.3535905594	-2.7987042439
PG	1.6554268241	-2.2131805689	-2.4780521366
OG1	0.6967391874	-2.8718338692	-1.5177118655
OG2	2.1393186785	-0.8216725069	-2.2884309457
OG3	1.4672621441	-2.7360401230	-4.0916849796
His_{56A}			
CB	-8.3143483860	-0.2530601903	-8.0381239333
HB1	-9.1423316496	-0.0615321864	-7.3310437984
HB2	-7.8802849498	0.7341167254	-8.2777623150
CG	-7.2995442605	-1.0366404795	-7.3075860805
ND1	-7.0784878912	-2.4193671806	-7.3896161322
HD1	-7.6146355717	-3.0502496195	-7.9337864167
CE1	-6.0377863001	-2.7545326541	-6.5455237994
HE1	-5.6026527245	-3.7450336718	-6.3994535091
NE2	-5.6108897008	-1.6185601811	-5.9270943461
HE2	-4.0727138154	-0.8709047360	-5.3414021558
CD2	-6.3804227711	-0.5553420357	-6.3814659685
HD2	-6.2126204454	0.4639424745	-6.0343215838
His_{218A}			
CB	-2.5046133787	-6.0530150022	-5.1354166790
HB1	-1.6194085755	-5.9244808452	-5.7857162750
HB2	-2.1789045024	-6.7769026086	-4.3649833968
CG	-2.7733492394	-4.7499564077	-4.4756348913
ND1	-3.4946008127	-4.6524004612	-3.2773179291
HD1	-3.8322995204	-5.4279820733	-2.7584966505
CE1	-3.6023692695	-3.3279149829	-2.9059549087
HE1	-4.1500812437	-2.9276868223	-2.0401382574
NE2	-2.9361679267	-2.5870061071	-3.8529865338
CD2	-2.4194091573	-3.4488018362	-4.8305919331
HD2	-1.8252001311	-3.0757646936	-5.6900534465
Asp_{109A}			
CB	-1.5470576654	2.7366689630	1.4319640017
HB1	-2.2319861195	3.5583299297	1.7054265824
HB2	-1.8782352416	1.8892745353	2.0526072784
CG	-1.8688625657	2.4372245332	-0.0442684979

OD1	-2.8563733326	1.7194415611	-0.3483817934
OD2	-1.1255847007	2.9387042799	-0.9286309768
MG			
MG	4.8650071327	-6.6484047297	-4.4268401680
MG	2.7230097035	-4.0358223829	-4.5280830157
Asp_{30B}			
CB	6.6830388752	-10.0104585659	-5.4829121910
HB1	6.8145054810	-9.9327526379	-6.5745034098
HB2	7.6635868460	-9.6727436148	-5.0931131380
CG	5.7089388559	-8.9411071323	-5.0018961241
OD1	4.8851461192	-9.0829760799	-4.0796599462
OD2	5.6631602840	-7.8264075552	-5.6412960795
Asp_{35B}			
CB	0.8787749399	-7.3447644392	-5.8086624724
HB1	-0.0125252584	-7.2883484830	-5.1416298376
HB2	0.4875106438	-6.9618226106	-6.7722827925
CG	1.8417301472	-6.2896770881	-5.2856401103
OD1	1.3272264710	-5.1519212828	-5.0208095373
OD2	3.0813162280	-6.4422134809	-5.0956791621
Asp_{37B}			
CB	4.6596547246	-4.3301621395	-7.8632145972
HB1	3.6654664950	-4.3446784040	-8.3545823218
HB2	5.0765312235	-3.3526673046	-8.1713042683
CG	4.3538508376	-4.2564607132	-6.3887628739
OD1	4.6599401393	-5.1805906660	-5.5862536526
OD2	3.7608898279	-3.2473992127	-5.8651405493
Phe_{78A}			
CA	-0.2704337256	-2.5501934155	-7.7861180195
HA	-1.0925695678	-2.2122528395	-7.1047425192
C	0.9991097601	-2.1142417812	-7.0931978491
O	1.8615582118	-2.8278420678	-6.5769932008
Thr_{79A}			
N	1.2686330934	-0.7104658034	-7.0558049031
H	0.4485312651	-0.1974721389	-7.3189758348

Table S8. Cartesian coordinates of the QM atoms of the TS_{I2Asp-I4Asp}^{DFT} localized at B3LYP/MM level of the *asp*-assisted phosphoryl transfer reaction from ATP to Dha catalyzed by DhaK from *E. coli*.

Dha			
C1	-3.0333609231	-1.3149799014	-3.4958818440
C2	-1.8706761787	-1.2216844609	-2.4274281111
H21	-2.3555549952	-0.8670638293	-1.5044230070
H22	-1.5544403654	-2.2457484447	-2.2493075209
C3	-4.3240137243	-0.6413450656	-2.9672689777
H31	-5.1345123953	-0.8242603305	-3.6799295522
H32	-4.6119363091	-1.0903337132	-2.0019344506
O4	-2.7332067924	-0.8483104132	-4.7624035698

O5	-0.7306518767	-0.4549530761	-2.6932681950
H5	-0.6956718902	0.5036524574	-1.7282688974
O6	-4.1341831232	0.7477626965	-2.8725439155
H6	-3.5942982370	0.9557080869	-2.0768212278
ATP			
C1'	9.2611321409	-5.5215486328	3.0096757890
H1'	10.1889531535	-4.9347294732	3.0415941248
C2'	9.6136077524	-7.0138394659	2.9059649792
H2'	11.1638331070	-7.9008286906	2.2043279319
O2'	11.0203616071	-7.1470060925	2.8075056798
HT2	9.2355743441	-7.5877878530	3.7670077656
C3'	8.9128372238	-7.4608125053	1.5801121887
H3'	7.9258885322	-7.8838424739	1.8168156380
O3'	9.7034823360	-8.3427239285	0.8262472466
HT3	9.7015843225	-9.2730948463	1.2092241568
C4'	8.7459816612	-6.1284370199	0.8265616098
O4'	8.4666103762	-5.1756675180	1.8686628822
H4'	9.7078332165	-5.8914516416	0.3466227444
C5'	7.6503257451	-6.0789850961	-0.2278873028
H51	6.6685415814	-6.0131363020	0.2626367975
H52	7.6812731703	-6.9962656096	-0.8261420498
O5'	7.8824583566	-4.9411550338	-1.0516745115
PA	7.0311587281	-4.6714716399	-2.4508950237
OA1	6.6851301674	-6.0050439962	-3.0946044953
OA2	7.9400742392	-3.7247929771	-3.2046835243
OA3	5.7108595867	-3.9142497042	-1.9263192304
PB	4.0404855455	-4.4361170931	-1.8930364254
OB1	3.9816603845	-5.8140477672	-2.5882664308
OB2	3.6539911169	-4.4004639415	-0.4411782030
OB3	3.4067927679	-3.3529181452	-2.8137179387
PG	0.9057837643	-1.6309492338	-2.4701754448
OG1	0.5059738235	-2.2251605444	-1.1352529421
OG2	1.9637541608	-0.5781136887	-2.5312927455
OG3	0.7453271206	-2.5419865662	-3.7080653496
His_{56A}			
CB	-8.2807206595	-0.2599580440	-8.0101815836
HB1	-9.0940702389	-0.2229481044	-7.2736085626
HB2	-7.9911317916	0.7761758757	-8.2017125577
CG	-7.1068312601	-0.9658778405	-7.3936867531
ND1	-6.8813146808	-2.3272221862	-7.4341630648
HD1	-7.4930981612	-3.0058311191	-7.8753152385
CE1	-5.7440508516	-2.5944493859	-6.7402691624
HE1	-5.3468738480	-3.5936122466	-6.6431260062
NE2	-5.2131771513	-1.4841912397	-6.2420735524
HE2	-3.5634287766	-0.9674528811	-5.3022957614
CD2	-6.0718502778	-0.4732203244	-6.6348734103
HD2	-5.9082154086	0.5490051549	-6.3355374403
His_{218A}			
CB	-2.5774774719	-6.0771063094	-5.2765785164

HB1	-1.7477374175	-5.8402177124	-5.9419612904
HB2	-2.2082594689	-6.8560278284	-4.6070640363
CG	-2.9181123304	-4.8694258498	-4.4716096600
ND1	-3.5417003776	-4.9562761591	-3.2409529490
HD1	-3.7894583980	-5.8858705852	-2.8292710338
CE1	-3.7463146715	-3.7176448196	-2.7570143422
HE1	-4.2327071572	-3.4759974887	-1.8264031998
NE2	-3.2706248014	-2.8363423011	-3.6446883440
CD2	-2.7380928919	-3.5382003452	-4.7049363972
HD2	-2.2424343358	-3.0286191263	-5.5061220809
Asp_{109A}			
CB	-1.3491919606	2.3607973184	1.1438664892
HB1	-2.0585360119	3.1950855710	1.1839940641
HB2	-1.6847017839	1.6728570149	1.9289414452
CG	-1.6096027877	1.6462386066	-0.1994727404
OD1	-2.7918988043	1.4872546408	-0.5415147095
OD2	-0.5729722925	1.2110249803	-0.8529381048
MG			
MG	4.9904978399	-6.5674737574	-4.1523470269
MG	2.3987358593	-3.5861867543	-4.4679034147
Asp_{30B}			
CB	6.9541412013	-10.0486565329	-5.5249180443
HB1	6.9560691568	-9.9678913160	-6.6158796947
HB2	7.9799266251	-9.8041818968	-5.2196603346
CG	6.0527163539	-8.9425564110	-4.9080191135
OD1	5.5321534905	-9.0883078424	-3.7869664757
OD2	5.9323617580	-7.8571433613	-5.5885505717
Asp_{35B}			
CB	0.7606740638	-7.5123422492	-5.6801746174
HB1	-0.0042992126	-7.4952319176	-4.8957870163
HB2	0.3012686706	-7.0094438698	-6.5379438756
CG	1.9297338598	-6.6205256158	-5.1747215498
OD1	1.6260265558	-5.4005027707	-4.9944462587
OD2	3.0493181966	-7.1608484863	-4.9666231056
Asp_{37B}			
CB	4.6552077353	-4.4665605591	-7.9949157093
HB1	3.6411393261	-4.6256259525	-8.3822851688
HB2	4.9575716526	-3.4794101515	-8.3624694232
CG	4.4860053371	-4.3463558849	-6.4564758476
OD1	5.0296535333	-5.1871354889	-5.7045619517
OD2	3.7651003788	-3.3709228345	-6.0520218553
Phe_{78A}			
CA	-0.6745076611	-2.2102755451	-7.7438551358
HA	-1.5599576790	-1.8613020735	-7.2019244121
C	0.5313093372	-1.7809991112	-6.9973986632
O	1.2821726073	-2.5686116397	-6.4557514898
Thr_{79A}			
N	0.7220003464	-0.4428289922	-7.1086894358
H	-0.0656523880	0.1949725804	-7.1222469040

Table S9. Cartesian coordinates of the QM atoms of the $\text{TS}_{12-14}^{\text{DFT}}$ localized at B3LYP/MM level of the *substrate-assisted* phosphoryl transfer reaction from ATP to Dha catalyzed by DhaK from *E. coli*.

Dha			
C1	-2.7773028732	-1.2282716627	-3.6992131711
C2	-1.3614131631	-1.0076046224	-3.0980110964
H21	-1.2048479194	0.0804538629	-3.1829226708
H22	-1.4732980867	-1.2060829434	-2.0107608994
C3	-3.8314153539	-0.4550927239	-2.8609111247
H31	-4.8169540618	-0.6172295287	-3.3226648098
H32	-3.8570112104	-0.8717284673	-1.8422247911
O4	-2.8271865453	-0.8738967168	-5.0448971864
O5	-0.3492370766	-1.7339120525	-3.6691682033
H5	0.4021249547	-2.9960822954	-4.0044978694
O6	-3.5332590895	0.9114358902	-2.8411424016
H6	-3.0733403834	1.1413027611	-1.9615162232
ATP			
C1'	9.3027742720	-5.4961676635	2.9776549234
H1'	10.2274784091	-4.9057874436	3.0063577222
C2'	9.6625095937	-6.9882051815	2.8863315124
H2'	11.2169117795	-7.8983080454	2.2132606326
O2'	11.0680658835	-7.1066511899	2.7616340576
HT2	9.3073008405	-7.5527556100	3.7621099875
C3'	8.9330117805	-7.4629976365	1.5867206377
H3'	7.9464744336	-7.8660907962	1.8598102076
O3'	9.6853686404	-8.3735116751	0.8303420294
HT3	9.6940244017	-9.2904757842	1.2456790148
C4'	8.7576089263	-6.1484419680	0.8044469634
O4'	8.5167688988	-5.1618474553	1.8198213867
H4'	9.7009192150	-5.9365417406	0.2774769635
C5'	7.6175478749	-6.1374532928	-0.2009959766
H51	6.6544952344	-6.1268034292	0.3261667190
H52	7.6727335329	-7.0376749777	-0.8212125838
O5'	7.7476631140	-4.9670059435	-1.0148689312
PA	6.9337020347	-4.7479392138	-2.4206436613
OA1	6.4700215116	-6.0910633714	-2.9631937309
OA2	7.8440368704	-3.8780536706	-3.2468632978
OA3	5.6393459189	-3.8523451399	-1.9521021321
PB	4.0202586769	-4.3083404796	-1.8429414564
OB1	3.7916412269	-5.3306946205	-3.0133791931
OB2	3.7087130186	-4.7456122751	-0.4524296650
OB3	3.3486991839	-2.9829729518	-2.4069369696
PG	1.5328730793	-2.1789531398	-2.4841203222
OG1	0.9128791789	-2.6055898073	-1.1823957262
OG2	2.0825085649	-0.8370267476	-2.8097755461
OG3	1.3088854774	-3.3187335503	-3.6742006147

His_{56A}			
CB	-8.3380524959	-0.1688614343	-8.0634850270
HB1	-9.1553208429	0.0069545549	-7.3525248222
HB2	-7.9607097210	0.8200058627	-8.3370136432
CG	-7.2366443880	-0.9083389089	-7.3563558217
ND1	-7.0418087046	-2.2740397060	-7.3938558720
HD1	-7.6461168114	-2.9343902674	-7.8729647051
CE1	-5.9526781171	-2.5730766401	-6.6391795050
HE1	-5.5809969194	-3.5807245871	-6.5303512320
NE2	-5.4306703015	-1.4798648148	-6.0990600516
HE2	-3.7566019137	-1.0314912800	-5.3771829668
CD2	-6.2398567198	-0.4458828228	-6.5290855511
HD2	-6.0628014135	0.5698840658	-6.2143224406
His_{218A}			
CB	-2.6156662971	-6.1153640474	-5.1951416300
HB1	-1.7718261882	-5.9205882921	-5.8596092018
HB2	-2.2739714696	-6.8700085549	-4.4829713457
CG	-2.9130160171	-4.8491682309	-4.4489680168
ND1	-3.5752057174	-4.8241437438	-3.2316444639
HD1	-3.8888031295	-5.6887604089	-2.7692514517
CE1	-3.6585319506	-3.5487929265	-2.7943652560
HE1	-4.1512925666	-3.2216219231	-1.8933971344
NE2	-3.0620401632	-2.7543158748	-3.6858787459
CD2	-2.5883906474	-3.5488175260	-4.7104434458
HD2	-2.0603947970	-3.1073304542	-5.5304348824
Asp_{109A}			
CB	-1.4416605350	2.6118388820	1.3072662409
HB1	-2.0966635611	3.4712091909	1.5174248063
HB2	-1.7850726068	1.8008214762	1.9545102147
CG	-1.6890090369	2.2756798832	-0.1975621649
OD1	-2.5401305581	1.3506525597	-0.4239653689
OD2	-1.1071667884	2.9716494929	-1.0572043485
MG			
MG	4.9362697901	-6.7079179262	-4.1873262767
MG	2.9804706074	-4.1013363472	-4.4447961073
Asp_{30B}			
CB	6.9160222656	-10.0921787975	-5.5423060183
HB1	6.9106701437	-10.0137514833	-6.6325688953
HB2	7.9470046862	-9.8687611721	-5.2391224082
CG	6.0441211868	-8.9690827922	-4.9354146509
OD1	5.5199710039	-9.0607174285	-3.8047876330
OD2	5.9378087401	-7.8939999836	-5.6308588086
Asp_{35B}			
CB	0.7803158107	-7.6323415280	-5.6516943941
HB1	0.0705704674	-7.5954282290	-4.8161194865
HB2	0.2744190254	-7.1394110697	-6.4857012634
CG	1.9946665887	-6.7732072144	-5.2413782581
OD1	1.8522907607	-5.5253095449	-5.3486877827
OD2	3.0361284209	-7.3607570785	-4.8020353979

Asp_{37B}			
CB	4.6620122317	-4.4566948911	-7.9795177741
HB1	3.6340931849	-4.6400741439	-8.3159496893
HB2	4.9469728319	-3.4788534197	-8.3785224803
CG	4.5612477241	-4.3446909441	-6.4588662388
OD1	4.8647929629	-5.3308404232	-5.7354901999
OD2	4.0794225530	-3.2731846567	-5.9559483443
Phe_{78A}			
CA	-0.6187552392	-2.2449424758	-7.7640863326
HA	-1.4680194238	-1.8699221524	-7.1823152801
C	0.6340082961	-1.8140537180	-7.1140157672
O	1.4603665899	-2.5869143062	-6.6646648883
Thr_{79A}			
N	0.7765616448	-0.4656669844	-7.2224396014
H	-0.0477373064	0.1259937557	-7.1785557609