Supporting Information

Elucidation of the Catalytic Mechanism of 6-Hydroxymethyl-7,8-dihydropterin pyrophosphokinase using QM/MM Calculations

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Table S1 Mulliken charge distribution of HPPK on WT-Arg92^0 (Arg) pathway.

ID	REACT	TS1	INT1	TS2	PROD
PRO43	0.05	0.05	0.05	0.05	0.05
PRO44	0.01	0.01	0.00	0.01	0.01
LEU45	-0.07	-0.07	-0.07	-0.07	-0.07
ARG88	0.81	0.81	0.80	0.80	0.79
ARG92	-0.06	0.63	0.76	0.71	0.70
ASP95	-0.46	-0.46	-0.48	-0.48	-0.48
ASP97	-0.45	-0.47	-0.47	-0.46	-0.46
HIP115	0.88	0.86	0.88	0.92	0.92
ARG121	0.88	0.89	0.88	0.86	0.86
Mg:water	1.65	1.06	1.58	1.67	1.66
ATP	-2.48	-2.48	-2.48	-1.29	-1.34
HP	0.23	0.18	-0.43	-1.71	-1.65

Table S2. Mulliken charge distribution of HPPK on R82A-Arg92^0 (Arg) pathway.

ID	REACT	TS1	INT1	TS2	PROD
PRO43	0.05	0.05	0.05	0.05	0.05
PRO44	0.01	0.00	0.00	0.00	0.00
LEU45	-0.07	-0.07	-0.07	-0.07	-0.07
ARG88	0.80	0.79	0.80	0.79	0.77
ARG92	-0.03	0.72	0.70	0.72	0.70
ASP95	-0.46	-0.46	-0.47	-0.48	-0.47
ASP97	-0.47	-0.48	-0.48	-0.47	-0.47
HIP115	0.85	0.86	0.84	0.91	0.92
ARG121	0.87	0.86	0.87	0.84	0.84
Mg:water	1.61	1.17	1.66	1.69	1.69
ATP	-2.45	-2.47	-2.45	-1.29	-1.32
HP	0.28	0.03	-0.45	-1.69	-1.66

Table S3. Mulliken charge distribution of HPPK on WT-Arg92^{+} pathway.

	WT-R92^{+} (Asp)			WT-R92^{+} (PPi)		
ID	**REACT**	**TS**	**PROD**	**REACT**	**TS**	**PROD**
PRO43	0.05	0.05	0.05	0.05	0.05	0.05
PRO44	0.04	0.03	0.03	0.03	0.04	0.03
LEU45	-0.08	-0.08	-0.08	-0.08	-0.08	-0.08
ARG88	0.82	0.81	0.80	0.81	0.81	0.81
ARG92	0.72	0.75	0.72	0.77	0.77	0.77
ASP95	-0.46	0.14	0.16	-0.46	-0.46	-0.46
ASP97	-0.45	-0.46	-0.46	-0.45	-0.45	-0.45
HIP115	0.87	0.92	0.93	0.94	0.94	0.94
ARG121	0.91	0.89	0.88	0.89	0.88	0.89
Mg:water	1.82	1.83	1.83	1.83	2.42	1.83
ATP	-2.45	-2.52	-1.30	-2.65	-1.32	-1.31
HP	0.22	-0.35	-1.56	0.32	-1.59	-1.02

Table S4. Mulliken charge distribution of HPPK on R92A-Arg92^{0} (Asp) pathway.

R92A	**REACT**	**TS**	**PROD**
PRO43	0.05	0.05	0.05
PRO44	0.01	0.01	0.01
LEU45	-0.07	-0.08	-0.08
ARG88	0.80	0.83	0.83
ASP95	-0.47	0.15	0.16
ASP97	-0.47	-0.47	-0.47
HIP115	0.86	0.90	0.90
ARG121	0.90	0.86	0.86
Mg:water	1.67	1.66	1.66
ATP	-2.51	-1.31	-1.35
HP	0.23	-1.60	-1.57

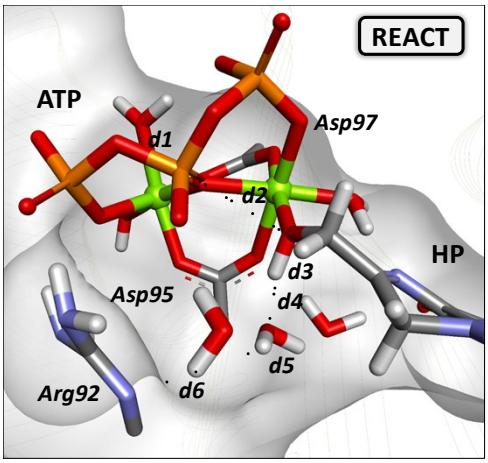

Table S5. The key distances involving WT-Arg92^0 (Arg) simulation obtained at the QMMM (M062X/6-31G*) level of theory (in Angstrom).

WT-Arg92^0 *Arg*	**d1**	**d2**	**d3**	**d4**	**d5**	**d6**
REACT	1.63	3.06	1.02	1.48	0.98	3.30
TS1	1.63	3.32	1.12	1.36	1.56	1.08
INT1	1.65	3.04	1.64	1.00	3.08	1.02
TS2	2.21	1.81	1.99	0.97	3.04	1.02
PROD	2.85	1.71	2.24	0.97	2.99	1.02

Table S6. The key distances involving R82A-Arg92^0 (Arg) simulation obtained at the QMMM (M062X/6-31G*) level of theory (in Angstrom).

R82A-Arg92^0 *Arg*	**d1**	**d2**	**d3**	**d4**	**d5**	**d6**
REACT	1.61	3.26	0.98	2.03	1.02	1.67
TS1	1.62	3.29	1.07	1.48	1.53	1.10
INT1	1.63	3.18	1.84	0.99	1.96	1.02
TS2	2.30	1.78	2.19	0.97	2.97	1.02
PROD	2.77	1.71	2.27	0.97	3.07	1.01

Table S7. HPPK reaction energies (kcal/mol) with Asp95 or PP acting as the general base**.** The fully-optimized calculations obtained at the QMMM **(**M062X/6-31G***)** and the SP calculations obtained at the QMMM **(**M062X/6-31+G**)

Model	ID	WT-R92+ QM/MM ΔE	WT-R92+ QM/MM ΔE SP	R92A QM/MM ΔE	R92A QM/MM ΔE SP
Asp95	REACT	0.0	0.0	0.0	0.0
	TS	60.2	53.8	48.0	51.3
	PROD	47.3	45.3	47.6	49.9
PP	REACT	-1.4	-0.4	-	-
	TS	47.3	46.3	-	-
	PROD	46.0	45.3	-	-

Table S8. HPPK reaction energies (kcal/mol) with Arg92 acting as the general base. The fully-optimized calculations obtained at the QMMM (M062X/6-31G*) and the SP calculations obtained at the QMMM (M062X/6-31+G**)

ID	WT-Arg92^0 (Arg) QM ΔE	WT-Arg92^0 (Arg) QM/MM ΔE	WT-Arg92^0 (Arg) QM/MM ΔE SP	R82A-Arg92^0 (Arg) QM/MM ΔE	R82A-Arg92^0 (Arg) QM/MM ΔE SP
REACT	0.0	0.0	0.0	0.0	0.0
TS1	3.5	8.1	8.1	4.2	6.9
INT	0.4	0.4	2.3	-4.2	2.1
TS2	12.0	11.7	14.7	14.7	20.4
PROD	3.1	7.6	9.4	12.4	17.3

Figure S1 Correlation observed between the QMMM M062X/6-31G*and QMMM (M062X/6-31+G** single point energies obtained for all structures.

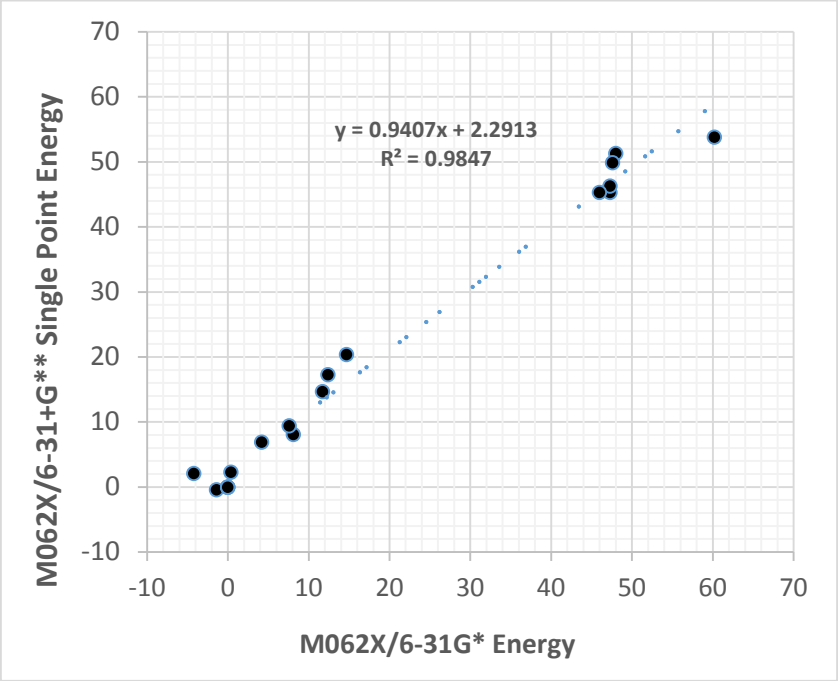

Figure S2 Superimposition of HPPK with bound substrates in a reactant-like (1Q0N) and product-like (1RAO) configuration. Also shown for the purpose of comparison is the product-like configuration obtained with the 1Q0N-based QM/MM model. Both protein and active site con figurations show a high degree of structural similarity confirming the suitability of the model to describe the catalytic reaction taking place.