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## Catalytic Mechanism of Mevalonate Kinase Revisited, a QM/MM Study

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Residue	Protonation State
His17	HIE
His20	HIE
His24	HIE
His197	HIE
His303	HIE
His304	HIE
His312	HIE
His325	HIE
His328	HIE
His380	HIE

**Table S1.** The protonation states of histidine residues in MVK.

\*HIE denotes Histidine with hydrogen on the epsilon nitrogen

No.	Atom Name	Atom Type	<b>RESP</b> Charge	
1	C1	СТ	-0.23	
2	C2	СТ	0.53	
3	C3	СТ	-0.55	
4	C4	СТ	0.42	
5	01	ОН	-0.74	
6	H1	НО	0.42	
7	02	ОН	-0.69	
8	H2	НО	0.39	
9	C5	СТ	-0.65	
10	C6	С	1.07	
11	03	02	-0.87	
12	04	02	-0.87	
13	Н3	НС	0.07	
14	H4	НС	0.07	
15	H5	НС	0.07	
16	Н6	НС	0.16	
17	H7	НС	0.16	
18	H8	H1	-0.01	
19	Н9	H1	-0.01	
20	H10	НС	0.13	
21	H11	НС	0.13	

**Table S2.** RESP charges of atoms in mevalonate of MVK

**Table S3.** Benchmark study on the basis set used for DFT (B97D) functional in the

correction of the QM/MM calculated energies (kcal/mol) for the reaction catalyzed by MVK.

	Reactant	TS	Product
6-31g(d) Opt	0	17.1	5.4
6-31g(d,p) SP	0	17.1	4.6
6-31+g(d) SP	0	18.1	2.9
6-311++g(d,p) SP	0	17.9	1.8
6-311+g(2d,2p) SP	0	20.3	2.5

Table S4.	Cluster	analysis	of the	100-ns	MD	simulations	of	the	ATP-Mg <sup>2+</sup> -MVK-MVA
complex.									

Cluster	Occurrence (%)	Distance MVA:C <sup>5</sup> OH-
1	1.5	3.761
2	3.3	3.802
3	4.3	3.856
4	0.9	3.859
5	7.3	3.887
6	6.7	3.751
7	21.8	3.782
8	33.6	3.819
9	13.3	3.720
10	7.2	3.703



Figure S1. RMSD for the backbone C $\alpha$  atoms of ATP- Mg<sup>2+</sup>-MVK-MVA complex.





Figure S2. Docking poses of MVA in MVK.



**Figure S3.** MD simulated structure of the ATP-  $Mg^{2+}$ -MVK -MVA model (inactive), built in analogy to the crystal structure of MVK in absence of substrate. In this model,  $Mg^{2+}$  is coordinated with the  $\beta$ - and  $\gamma$ -phosphate oxygen atoms of ATP.



**Figure S4.** Distance from 4 replicas of 100-ns MD simulations of the ATP -Mg<sup>2+</sup>-MVK-MVA complex (plotted based on the last 50 ns trajectory) between the carboxylate group of MVA and the  $\gamma$ -phosphate of ATP.



**Figure S5.** Distance from 4 replicas of 100-ns MD simulations of the ATP -Mg<sup>2+</sup>-MVK-MVA complex (plotted based on the last 50 ns trajectory) (a) between Lys13 and  $\gamma$ -PO<sub>4</sub> of ATP; (b) between Lys13 and Asp204.



**Figure S6.** Distance from 4 replicas of 100-ns MD simulations of the ATP -Mg<sup>2+</sup>-MVK-MVA complex (plotted based on the last 50 ns trajectory) between Asp204 and Mg<sup>2+</sup>.



**Figure S7.** Distance from 4 replicas of 100-ns MD simulations of the ATP -Mg<sup>2+</sup>-MVK-MVA complex (plotted based on the last 50 ns trajectory) (a) between WAT461 and OE1 of Glu193; (b) between WAT461 and OE2 of Glu193; (c) between WAT441 and OE1 of Glu193; (d) between WAT441 and OE2 of Glu193.



**Figure S8.** Distance from 4 replicas of 100-ns MD simulations of the ATP -Mg<sup>2+</sup>-MVK-MVA complex (plotted based on the last 50 ns trajectory) (a) between His197 and the  $\alpha$ -phosphate of ATP; (b) between Glu193 and His197.



**Figure S9.** Distance from 4 replicas of 100-ns MD simulations of the ATP  $-Mg^{2+}-MVK-MVA$  complex (plotted based on the last 50 ns trajectory) (a) between Ser201 and the carboxylate group of MVA; (b) between Ser146 and the  $\beta$ -phosphate of ATP; (c) between Ser145 and the  $\beta$ -phosphate of ATP.

	K13 ▼	
MGSSHHHHHHSSGLVPRGSMTYRSIGSTA	YPTIGV	VLLGGIANPVTRTPLHTSAG
GSTGSMVK-SGKARA	HTNIAL	IKYWGKADETYIIPMNNSLS
MAEEKINA/ MUTAOT	DINITA	TVVIJCVDDEVDTI DTNDCTC

Ath-MDD	MAEEKWVV-MVTAQT	PTNIAV	IKYWGKRDEVRILPINDSIS
Spo-PMK	MKVTCSA	PGKVLIAGGYI	/LDPQYSGLVIGLTAKGYAS
Spn-PMK	MIAVKT	CGKLYWAGEYA	ILEPGQLALIKDIPIYMR
Rno-MVK	MLSEVLLVSA	PGKVILHGEHA	/VHGKVALAVAL-NLRTFLV
Sce-MVK	MSLPFLTSA	PGKVIIFGEHS	AVYNKPAVAASVSALRTYLL
	:	MOTIF I	

Tac-M3K

Sep-MDD

Sce-MVK

## : S145, S146

Tac-M3K	TVSYS-S-ONEG	ILSGSSDA	AASI-GAAILGLKPDLDPHDVENDLRAVS-ES
Sep-MDD	AGNRLHARIE-SENY	TAAGLASSAS	YAALAAACNEALSLNLSDTDLSRLARRGS-GS
Ath-MDD	DWEKLHLHIA-SHNNFP	TAAGLASSAA	FACLVFALAKLMNVNEDPSQLSAIARQGS-GS
Spo-PMK	SYPKENELNCTLGOV	HKTGLGSSAA	ITSLIGSLFLSLRRLTDDT
Spn-PMK	NLRPFSLAIYGKMEREG	KKFGLGSSGS	VVLVVKALLALYNLSVDQNLLFKLTSAVLLKR
Rno-MVK	TLPSLDIMVWSEUP	PGAGLGSSAA	SVCVAAALLTACEEVTNPLKDR
Sce-MVK	NIKFSLKSTUP	IGAGLGSSAS	SVSLALAMAYLGGLIGS
		MOTIE	
			E193 H197 S201D204
Tac-M3K	AGRSLFGGLTITWSDGF	HAYTEK	ILDPEAFSGYSIVAFAFDYQRNPSDVIH
Sep-MDD	ASRSIFGGFA-EWEKGH	DDLTSYAH	GINSNGWEKDLSMIFVVINNQSKKVSSRSGMS
Ath-MDD	ACRSLFGGFV-KWNMGN	KEDGSDSVAVQ	LVDDKHWDD-LVIIIAVVSSRQKETSSTSGMR
Spo-PMK	GDKSLK		-IDDSTKVIVHNLAQIAHCSAQGKVGSGFD
Spn-PMK	GDNGSMGDLA-CIAAED	LV	LYQSFDRQKVAAWLEEENL-AT
Rno-MVK	GSIGSWP		EEDLKSINKWAYEGER-VIHGNPSGVD
Sce-MVK	NDLEKLS		ENDKHIVNQWAFIGEK-CIHGTPSGID
Tac-M3K	-LRIVGGAW	H	
Sep-MDD	DIISSGVEII		
Ath-MDD	-KSYVLGDTSIV	EAGLEG	ELPOGIKDKIGSODOKGEVSYFICS
Spo-PMK	ILDNIEOLPGVIGV	VPGAGGFDAOF	CLAINHTEIIENVIKTWKDDGVVPMDV
Spn-PMK	LLROLKEASODLQ-AVA	SSGAGGGDCG	ALSFDAQSTKTLKNRWADLGIELLYQE
Rno-MVK	SLDOLCOVTAAHG-LHSI	LTGAGGGGCGI	TLLKPGLERAKVEAAKQAL-TGCGFDC-WET

MOTIF III

GLELIKNLSDDLRIGSTELTGAGGGGGCSLTLLRRDITQEQIDSFKKKLQDDFSYET-FET

Figure S10. Sequence alignment of kinases in mevalonate pathway using the Clustal Omega webserver developed by the European Bioinformatics Institute. The key residues S145, S146, Glu193 and Ser201 in Rattus norvegicus MVK being studied in this research are labelled. Species names are abbreviated as follows: TAC, Thermoplasma acidophilum (TAC-M3K: CAC12426.1); Sep, Staphylococcus epidermidis (SEP-MDD: AAG02436.1); MEJ, Arabidopsis thaliana (ATH-MDD: CAB70999.1); SPO, Schizosaccharomyces pombe (SPO-PMK: CAB52264.1); SPN, Streptococcus pneumoniae (SPN-PMK: AAK99144.1); RNO, Rattus norvegicus (RNO-MVK: AAA41588.1); SCE, Saccharomyces cerevisiae (SCE-MVK: CAA29487.1).



**Figure S11.** Distance from 4 replicas of 100-ns MD simulations of the ATP-Mg<sup>2+</sup>-MVK-MVA complex (plotted based on the last 50 ns trajectory) (a) between Arg241 and the  $\alpha$ -phosphate of ATP; (b) between Arg241 and the  $\gamma$ -phosphate of ATP.









**Figure S12.** 10 representative structures from cluster analysis of MD simulations of the ATP-Mg<sup>2+</sup>-MVK-MVA complex.