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The Phosphorylation Mechanism of Mevalonate Diphosphate Decarboxylase, a QM/MM Study

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Table S1. RESP charges of atoms in ATP, Mg^{2+} and MVAPP of MDD

No.	Residue	Atom Name	Atom Type	RESP Charge
1	MDP	O1	O	-0.83
2	MDP	O2	O	-0.99
3	MDP	O3	O	-0.87
4	MDP	O3	O	-0.83
5	MDP	O5	OH	-0.49
6	MDP	O6	O	-0.99
7	MDP	O7	O	-0.99
8	MDP	O8	O	-0.87
9	MDP	C1	C3	-0.43
10	MDP	C2	C3	0.76
11	MDP	C3	C3	-0.20
12	MDP	C4	C3	-0.63
13	MDP	O9	OS	-0.82
14	MDP	O10	OS	-0.63
15	MDP	C5	C	0.90
16	MDP	C6	C3	0.79
17	MDP	P1	P5	1.33
18	MDP	P2	P5	1.49
19	MDP	H1	HC	0.04
20	MDP	H2	HC	0.04
21	MDP	H3	HC	0.04
22	MDP	H4	H1	-0.11
23	MDP	H5	H1	-0.11
24	MDP	H6	HC	-0.01

25	MDP	H7	HC	-0.01
26	MDP	H8	HC	0.09
27	MDP	H9	HC	0.09
28	MDP	H10	HO	0.25
29	MG	MG1	Mg^{2+}	2.00
30	MG	MG2	Mg^{2+}	2.00
31	ATP	P1	P5	1.22
32	ATP	O1	O	-0.87
33	ATP	O2	O	-0.87
34	ATP	O3	O	-0.87
35	ATP	P2	P5	1.67
36	ATP	O4	O	-0.91
37	ATP	O5	O	-0.91
38	ATP	O6	OS	-0.60
39	ATP	O7	OS	-1.08
40	ATP	C1	C3	0.66
41	ATP	C2	C3	0.41
42	ATP	O8	OS	-0.86
43	ATP	C3	C3	0.65
44	ATP	O9	OH	-0.84
45	ATP	C4	C3	-0.33
46	ATP	O10	OH	-0.68
47	ATP	C5	C3	1.00
48	ATP	N1	NA	-0.17
49	ATP	C6	CC	0.32
50	ATP	N2	ND	-0.68
51	ATP	C7	CA	0.00

52	ATP	C8	CA	0.88
53	ATP	N3	NH	-1.08
54	ATP	N4	NB	-0.91
55	ATP	C9	CA	0.68
56	ATP	N5	NB	-0.86
57	ATP	C10	CA	0.47
58	ATP	P1	P4	1.14
59	ATP	O11	O	-0.77
60	ATP	O12	O	-0.77
61	ATP	O13	O	-0.77
62	ATP	H1	H1	-0.07
63	ATP	H2	H1	-0.07
64	ATP	H3	H1	-0.05
65	ATP	H4	H1	0.01
66	ATP	H5	HO	0.44
67	ATP	H6	H1	0.03
68	ATP	H7	HO	0.44
69	ATP	H8	H2	-0.02
70	ATP	H9	H5	0.14
71	ATP	H10	HN	0.42
72	ATP	H11	HN	0.42
73	ATP	H12	H5	0.02

Table S2. Gibbs free energies for the QM/MM optimized stationary points using B3LYP/6-31+G(d) and single-point (SP) energy corrections using 6-311+G(d,p) and 6-311++G(d,p) for the phosphoryl transfer catalyzed by MDD.

	Reactant (kcal/mol)	**TS (kcal/mol)**	**Product (kcal/mol)**
6-31+g(d) Opt	0	17.4	5.3
6-311+G(d,p) SP	0	17.4	6.3
6-311++g(d,p) SP	0	17.6	6.5

Table S3. Statistics of the crystal structures of kinases with Mg^{2+}/ATP in PDB bank

Number	PDB Code	Family	Mg^{2+} - ATP	Mg^{2+} - H_2O
α/ β/ γ phosphate				
1	5I4N	Transferase	α/ β/ γ phosphate	3 x H_2O, Asn
2	5DK4	Ligase	α/ β/ γ phosphate	3 x H_2O
3	4FVQ	Transferase	α/ β/ γ phosphate	3 x H_2O
4	3KE5	Transferase	α/ β/ γ phosphate	3 x H_2O
5	3FXX	Transferase	α/β/ γ phosphate	3 x H_2O
6	3FKB	Transferase	α/ β/ γ phosphate	3 x H_2O
7	3AR4	Hydrolase	α/ β/ γ phosphate	3 x H_2O
8	2WGH	Oxidoreductase	α/ β/ γ phosphate	3 x H_2O
9	3V2U	Transcription	α/β/ γ phosphate	2 x H_2O, Glu, Ser,
10	1B63	DNA Mismatch Repair Protein	α/β/ γ phosphate	2 x H_2O, Asn
11	4WUB	Isomerase	α/ β/ γ phosphate	2 x H_2O, Asn
12	3H4L	DNA Binding Protein	α/ β/ γ phosphate	2 x H_2O, Asn
13	3KMW	Cell Adhesion Protein	α/ β/ γ phosphate	2 x H_2O, Asp
14	2X14	Transferase	α/ β/γ phosphate	2 x H_2O, Asp
15	2WVJ	Transferase	α/ β/ γ phosphate	2 x H_2O, Ser
16	3GAH	Transferase	α/ β/ γ phosphate	2 x H_2O
17	3K8T	Oxidoreductase	α/ β/ γ phosphate	2 x H_2O
18	3EHG	Transferase	α/ β/ γ phosphate	1 x H_2O, Glu, Asn
19	3DKC	Oncoprotein	α/ β/ γ phosphate	1 x H_2O, Asp, Asn
20	3CIS	Unknown	α/β/ γ phosphate	1 x H_2O, Asp
21	3K5H	Lyase	α/ β/ γ phosphate	1 x H_2O
22	3HNC	Oxidoreductase	α/ β/ γ phosphate	1 x H2O
23	2XZW	Signalling Protein	α/ β/ γ phosphate	2 x SUB, Gln
24	3KFB	Chaperone Protein	α/ β/ γ phosphate	2 x Asp, Ser
25	3LCB	Transferase	α/ β/ γ phosphate	Asp, Asn
26	3LMG	Transferase	α/ β/ γ phosphate	Asp, Asn
27	3EPS	Transferase	α/ β/ γ phosphate	Asp, Asn
28	3HAV	Transferase	α/ β/ γ phosphate	Asp
29	3E76	Chaperone Protein	α/ β/ γ phosphate	Asp
30	3H5N	Transferase	α/ β/ γ phosphate	Asp
31	1WUU	Transferase	α/β/ γ phosphate	Glu, Ser
32	3EUJ	Cell-Cycle Protein	α/β/ γ phosphate	Ser
33	4P1O	Transport Protein	α/ β/ γ phosphate	N/A
34	2XBP	Gene Regulation Protein	α/ β/ γ phosphate	N/A

α/ β phosphate				
35	3ILO	Transferase	α/ β phosphate	3 x H_2O, Asp
36	3HGM	Signalling Protein	α/ β phosphate	3 x H_2O
37	3E8N	Transferase	α/ β phosphate	2 x H_2O, Asp, Asn
38	3DV3	Transferase	α/ β phosphate	2 x H_2O, Asp, Asn
39	3EQC	Transferase	α/β phosphate	2 x H_2O, Asp, Asn
40	3GT8	Transferase	α/ β phosphate	1 x H_2O, Asp, Asn
41	3IS5	Transferase	α/ β phosphate	Asp, Asn
42	3GQI	Transferase	α/ β phosphate	Asp
43	3DY7	Transferase	α/ β phosphate	Asp, Asn
44	2Y4I	Transferase	α/ β phosphate	Asp, Asn, Ser
β/ γ phosphate				
45	3FHT	Hydrolase	β/ γ phosphate	4 x H_2O
46	3I5X	Hydrolase	β/ γ phosphate	4 x H_2O
47	3FCC	Ligase	β/ γ phosphate	4 x H_2O
48	3A5M	Contractile Protein	β/ γ phosphate	4 x H_2O
49	3M6G	Structural Protein	β/ γ phosphate	4 x H_2O
50	5C18	Hydrolase	β/ γ phosphate	3 x H_2O, Asp
51	2YCH	Cell-Cycle Protein	β/ γ phosphate	3 x H_2O, Asp
52	2Y27	Ligase	β/ γ phosphate	3 x H_2O, Asp
53	3A1C	Hydrolase	β/ γ phosphate	3 x H_2O, Asp
54	3G5A	Transferase	β/ γ phosphate	3 x H_2O, Asp
55	3FGU	Transferase	β/ γ phosphate	3 x H_2O, Asp
56	3A4L	Transferase	β/ γ phosphate	3 x H_2O, Ser
57	3ADB	Transferase	β/ γ phosphate	3 x H_2O, Ser
58	3DCB	Motor Protein	β/ γ phosphate	3 x H_2O, Ser
59	3HU1	Transport Protein	β/ γ phosphate	3 x H_2O, Thr
60	3ETL	DNA Binding Protein	β/ γ phosphate	3 x H_2O, Thr
61	3HQD	Motor Protein	β/ γ phosphate	2 x H_2O, Ser, Thr
62	3AV0	Recombination Protein	β/ γ phosphate	2 x H_2O, Ser, Gln
63	2DY1	Signalling Protein	β/ γ phosphate	2 x H_2O, Ser
64	2WKP	Transferase	β/ γ phosphate	2 x H_2O, 2x Thr
65	2XTN	Immunity Protein	β/ γ phosphate	2 x H_2O, Ser, Thr
66	3ZFC	Hydrolase	β/ γ phosphate	2 x H_2O, Ser, Thr
67	3C41	Hydrolase	β/ γ phosphate	2 x H_2O, Ser
68	3FKQ	Unknown	β/ γ phosphate	2 x H_2O, Asp
69	2W00	Hydrolase	β/ γ phosphate	2 x H_2O
70	2V9J	Transferase	β/ γ phosphate	2 x H_2O
71	2JHR	Contractile Protein	β/ γ phosphate	1 x H_2O, Ser, Thr
72	3MOE	Lyase	β/ γ phosphate	1 x H_2O, 2x Asp
73	4QDI	Ligase	β/ γ phosphate	1 x H_2O, Asp, Thr

74	4F93	Hydrolase	β/ γ phosphate	1 x H_2O, Asp
75	2X9H	Contractile Protein	β/ γ phosphate	1 x H_2O, Asp, Ser, Thr
76	3ID8	Transferase	β/ γ phosphate	1 x H_2O, Asp
77	2XJD	Hydrolase	β/ γ phosphate	1 x H_2O
78	2WBE	Structural Protein	β/ γ phosphate	Asp
79	3JVV	ATP binding Protein	β/ γ phosphate	Asp, Ser
80	3LQQ	Apoptosis	β/ γ phosphate	Asp, Ser
81	2XB2	Hydrolase	β/ γ phosphate	2 x Asp, Thr
82	2WSS	Hydrolase	β/ γ phosphate	Asp, Thr
83	4KH4	Hydrolase	β/ γ phosphate	2 x Asp
84	3G0H	Hydrolase	β/ γ phosphate	Asp
85	2XOK	Hydrolase	β/ γ phosphate	Thr
86	2WPD	Hydrolase	β/ γ phosphate	Thr
87	3J8Y	Motor Protein	β/ γ phosphate	2 x Ser
88	2YJE	Motor Protein	β/ γ phosphate	Asp, Gln
89	3C16	Lyase	β/ γ phosphate	Ser, Thr
90	1KVK*	Transferase	β/ γ phosphate	Asp, Ser
91	1H74	Transferase	β/ γ phosphate	Glu
92	3IQ0	Transferase	β/ γ phosphate	N/A
α/ γ phosphate				
93	3GU5	Transferase	α/ γ phosphate	2 x H_2O, Asp, Asn
94	3KEX	Transferase	α/ γ phosphate	1 x H_2O, Asp, Asn
95	3F5U	Transferase	α/ γ phosphate	1 x H_2O, Asp
96	2W02	Metal Transport Protein	α/ γ phosphate	Asp, Asn, Gln
97	2X3J	Ligase	α/ γ phosphate	Asp, Asn, Gln
β phosphate				
98	3IBQ	Transferase	β phosphate	4 x H_2O
99	3LDL	Heat-Shock Protein	β phosphate	4 x H_2O
γ phosphate				
100	2XCL	Ligase	γ phosphate	3 x H_2O, Asp, Asn
101	3GON	Transferase	γ phosphate	3 x H_2O, Asp, Sub
102	2W5B	Transferase	γ phosphate	3 x H_2O
103	3LIJ	Transferase	γ phosphate	2 x H_2O, Asp, Asn
104	3LMI	Transferase	γ phosphate	Gln
105	3DVL	Transferase	γ phosphate	Asp, Ser
106	3JZM	Transferase	γ phosphate	Glu, Thr

* Mg^{2+} is coordinated to the β-/ γ - phosphate of ATP in Mevalonate kinase proposed based on our recent study. [1]

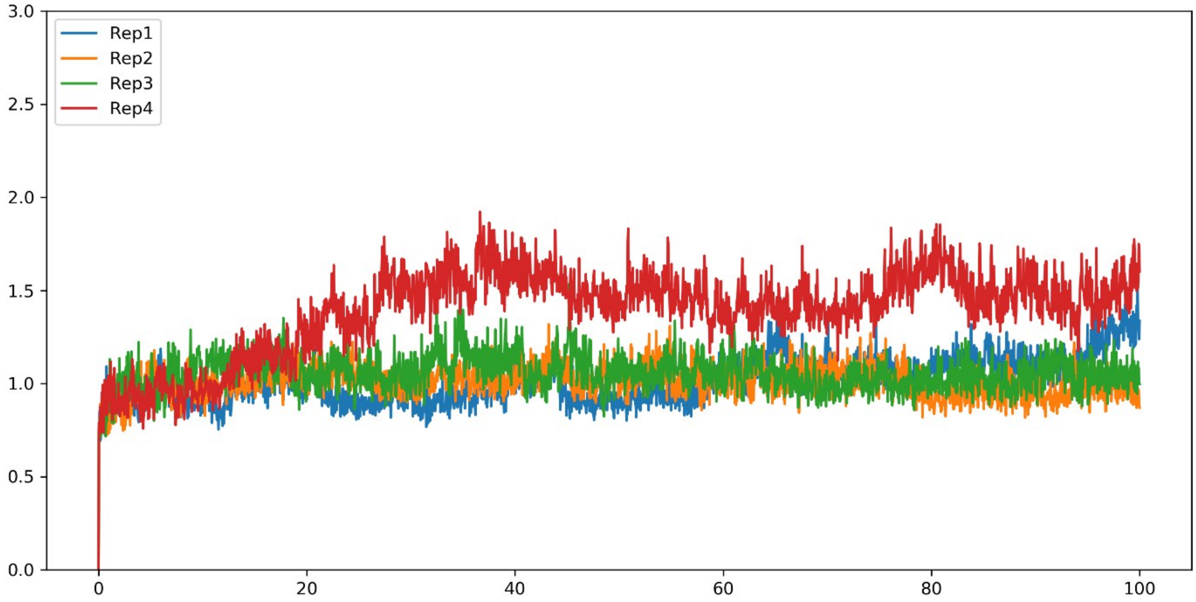


Figure S1. RMSD for the Cα of the entire protein from four replicas 100ns MD simulations of MDD-2Mg^{2+}-ATP-MVAPP.

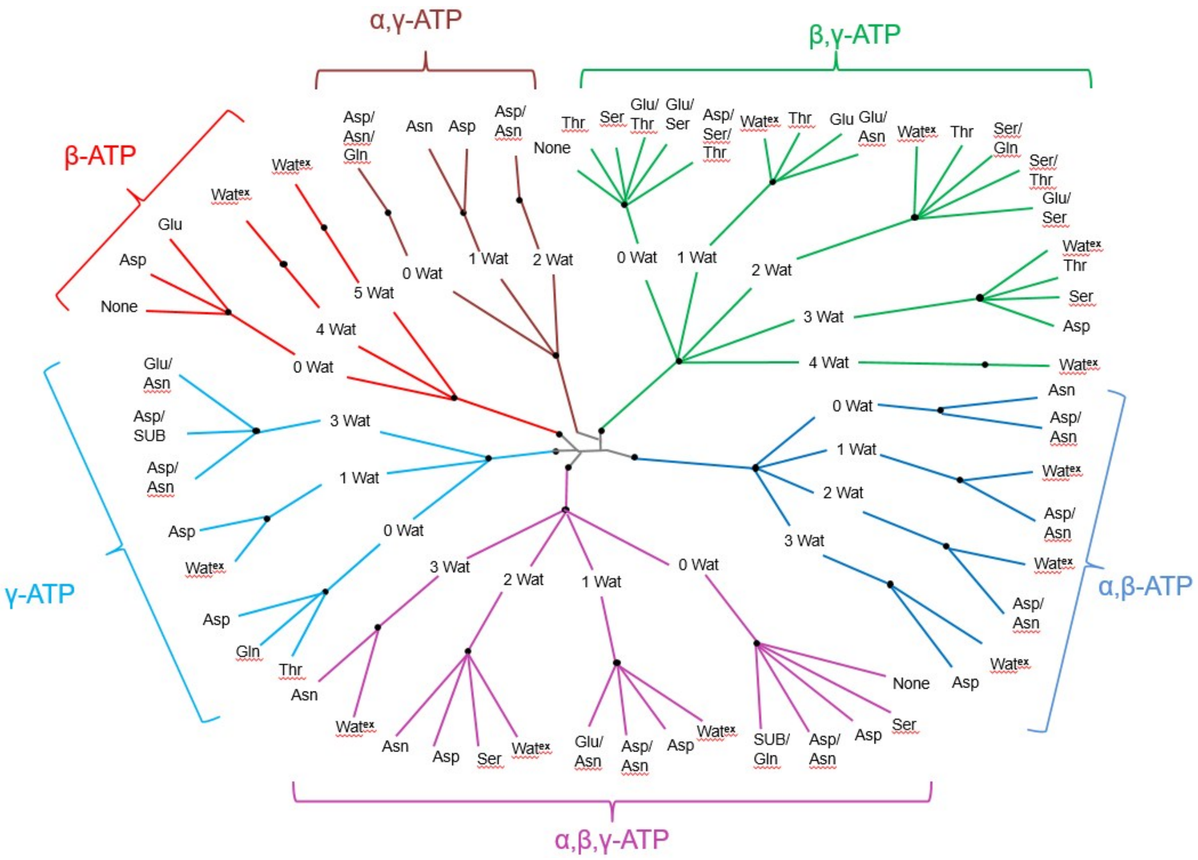
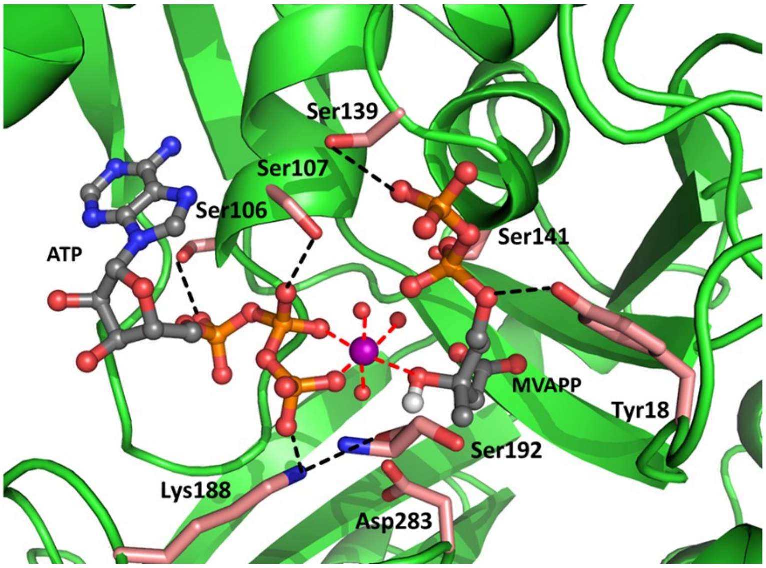
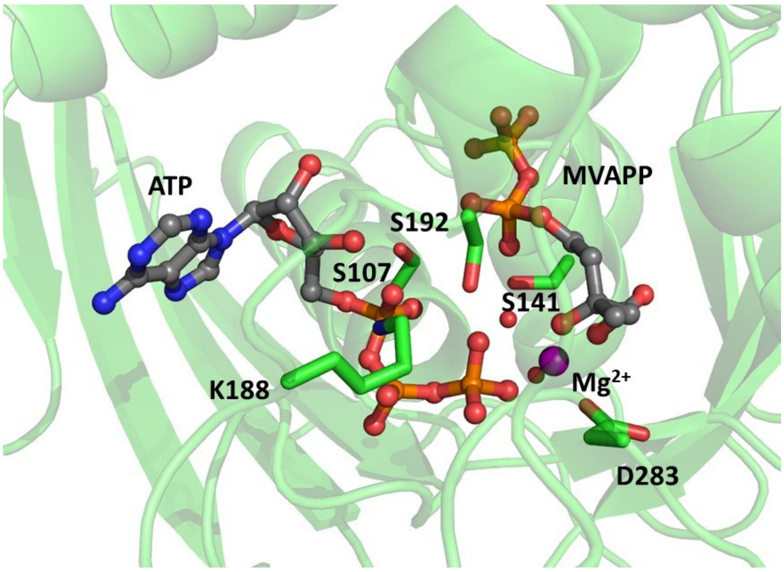


Figure S2. Statistical analysis of Mg^{2+} coordination with ATP and water in the protein databank.



(A)



(B)

Figure S3. Representative models of the MD simulated structures of the MDD containing only one divalent metal ion. (A) the metal ion was positioned to coordinate to the β/γ-phosphate of ATP. Three water molecules were made coordinated to Mg^{2+}, with the sixth coordination formed with the C3-hydroxyl group. (A) the metal ion was positioned to coordinate to the γ-phosphate of ATP, the C3-hydroxyl group and the carboxylate of Asp283. The remaining coordination of magnesium was saturated by two water molecules.

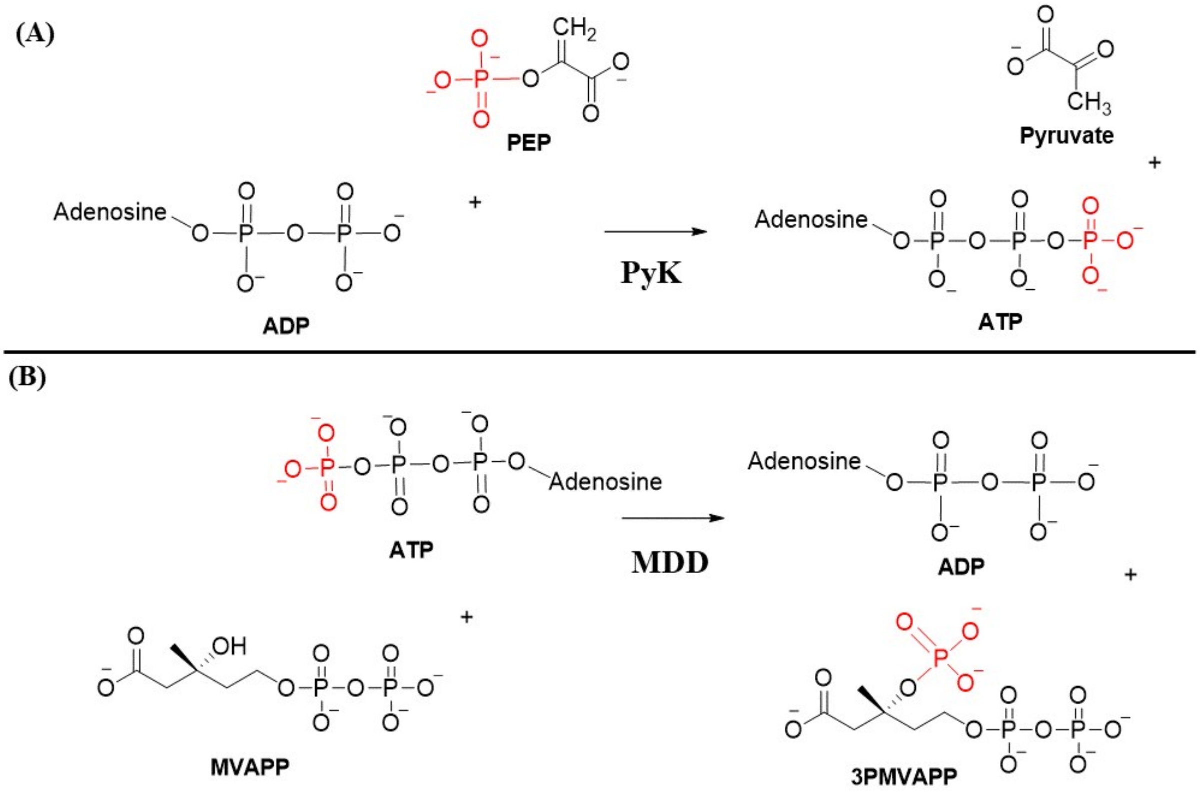
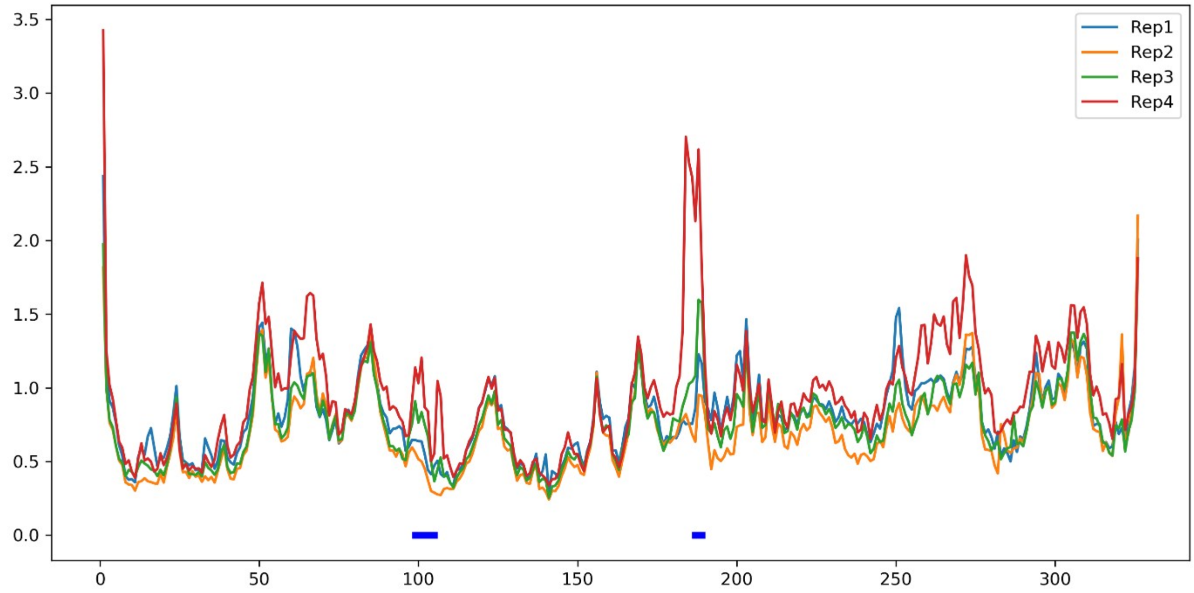
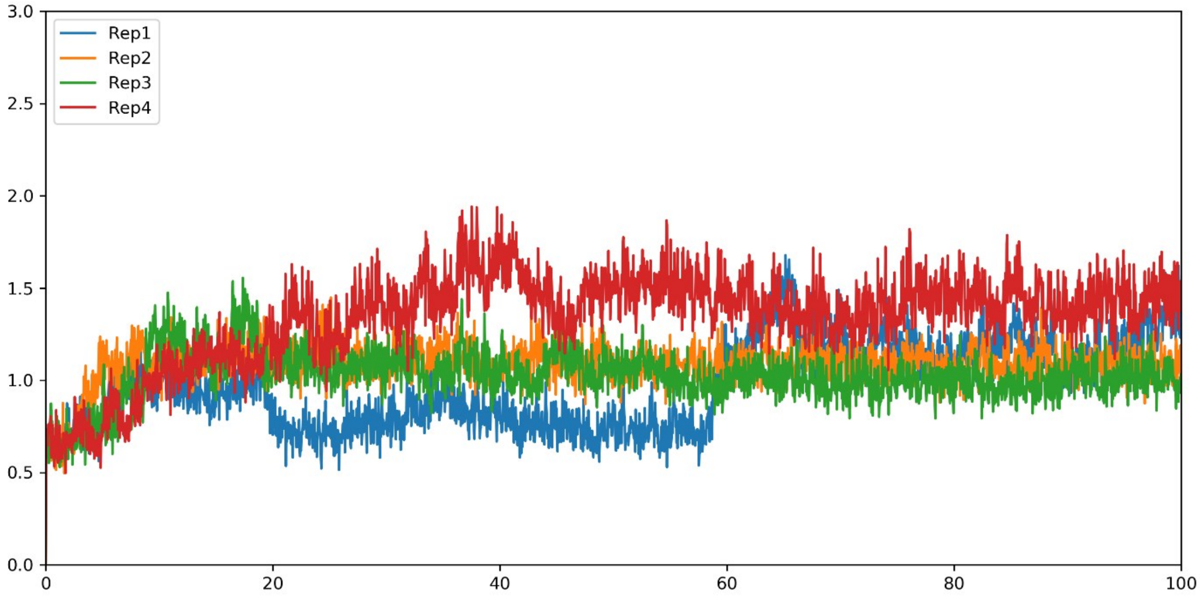


Figure S4. (A) PyK catalyzes the phosphorylation of ADP, where the phosphate group of PEP is transferred to ADP to yield ATP and pyruvate; (B) MDD catalyzes the phosphorylation of MVAPP, where the γ-phosphate group of ATP is transferred to MVAPP to give 3PMVAPP and ADP. The phosphate group to be transferred is colored in red.



(A)



(B)

Figure S5. **(A)** RMSF plot for the Cα from four replica 100ns MD simulations of MDD-$2Mg^{2+}$-ATP-MVAPP. Large fluctuations are shown around the loop 99-105 and the catalytic residue Lys188; **(B)** RMSD for the Cα of the catalytic site residues within 4 Å around the substrates ATP and MVAPP, showing the catalytic site has reached equilibrium from the MD simulations. The loop 99-105 and Lys188 were excluded in the RMSD plot as they displayed high fluctuation from the four replicas 100ns MD simulations of MDD-$2Mg^{2+}$-ATP-MVAPP.

Reference:

1. J. McClory, J. Lin, D. Timson, J. Zhang, M. Huang, Catalytic Mechanism of Mevalonate Kinase Revisited, a QM/MM Study. *Organic and Biomolecular Chemistry*. 2019, **17**, 2423-2431.