Supplementary Materials: Tuning the Phosphoryl Donor Specificity of Dihydroxyacetone Kinase from ATP to Inorganic Polyphosphate. An Insight from Computational Studies

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Table S1. Polyphosphate-dependent kinase activities of the wild-type and mutant enzymes [a].

Enzyme	wtDHAK	1H2	2C1	2D1	2H3	3C3	3E4	3H2	4B3
Specific activity (mU·mg^{-1})	n.d [b]	13.4	2.6	n.d	1.9	n.d	7.7	n.d	0.1
Enzyme	**-**	**5A2**	**5B1**	**5C1**	**5F12**	**6A3**	**6D5**	**6D6**	**6F2**
Specific activity (mU·mg^{-1})	-	3.0	2.7	n.d	10.5	n.d	n.d	n.d	0.4

[a] Nomenclature of mutant enzymes is arbitrary and corresponds to the coordinates in the micro-plate; [b] n.d = not detected.

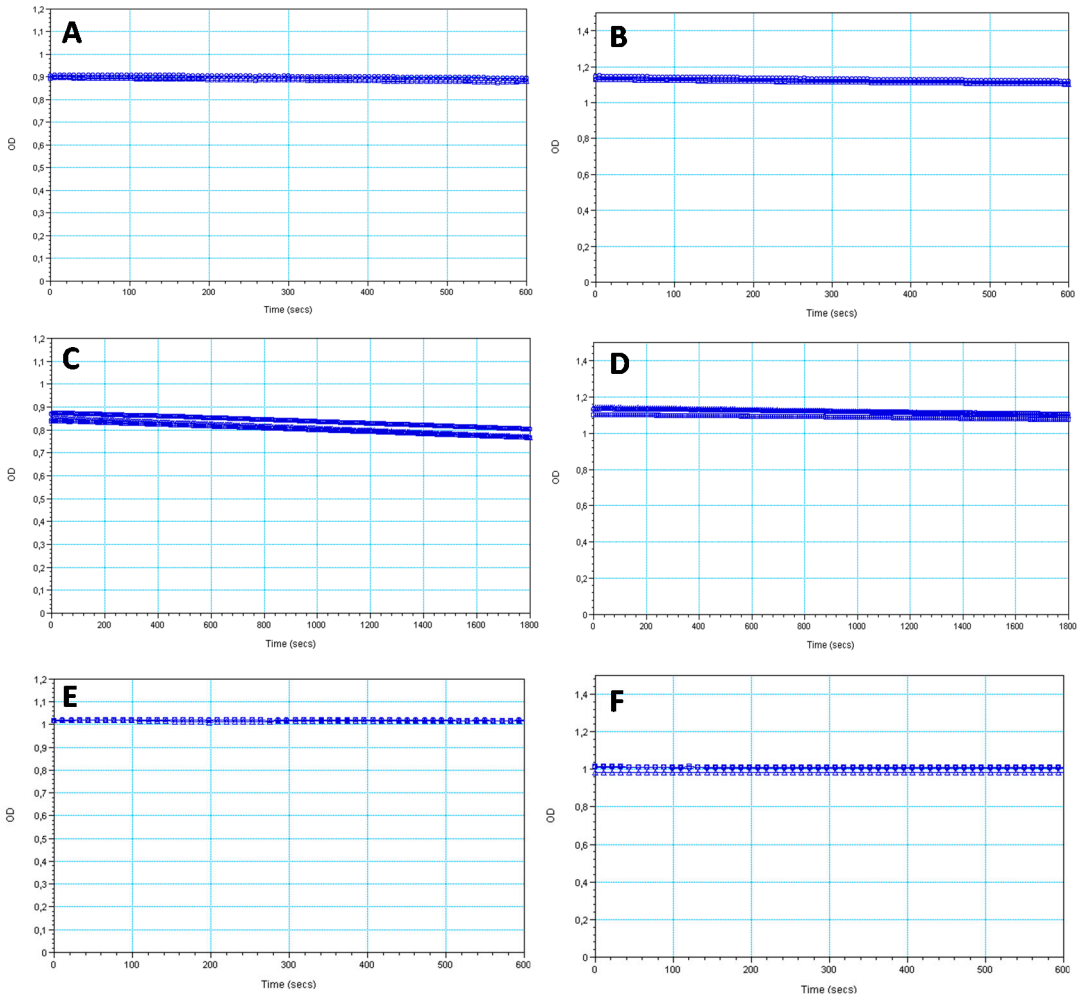

Figure S1. Absorbance at 340 nm was recorded along the time in triplicate reactions containing poly-P and mutant 1H2 (**A**); The same assay was performed with wild type DHAK enzyme (**C**); When DHA was added into the pre-incubated reactions, significant slope differences were observed in 1H2 mutant reactions (**B**) but no in DHAK wt ones (**D**); In order to exclude any spontaneous chemical phosphorylation, control reactions were incubated in absence of the corresponding enzyme: poly-P/DHA mixture without 1H2 mutant (**E**);and ATP/DHA mixture without DHAK wt (**F**).

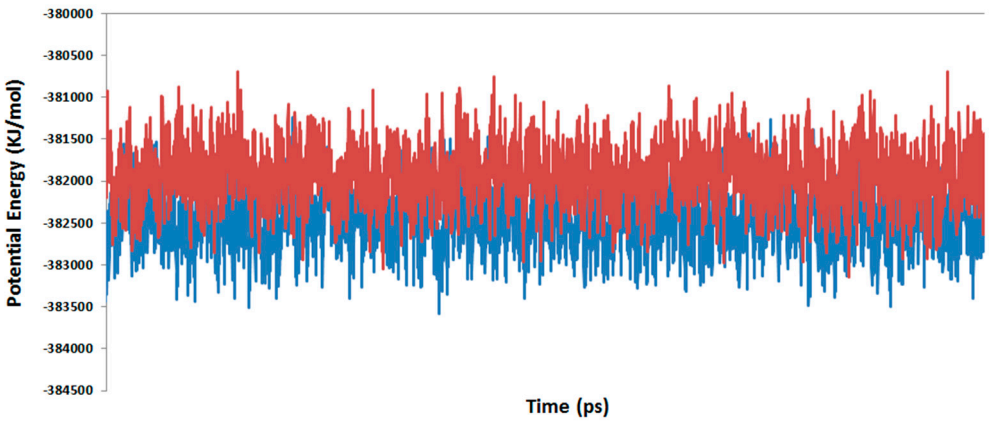

Figure S2. Time evolution of the potential energy of the full system in the wild-type (blue line) and mutated enzyme (red line).

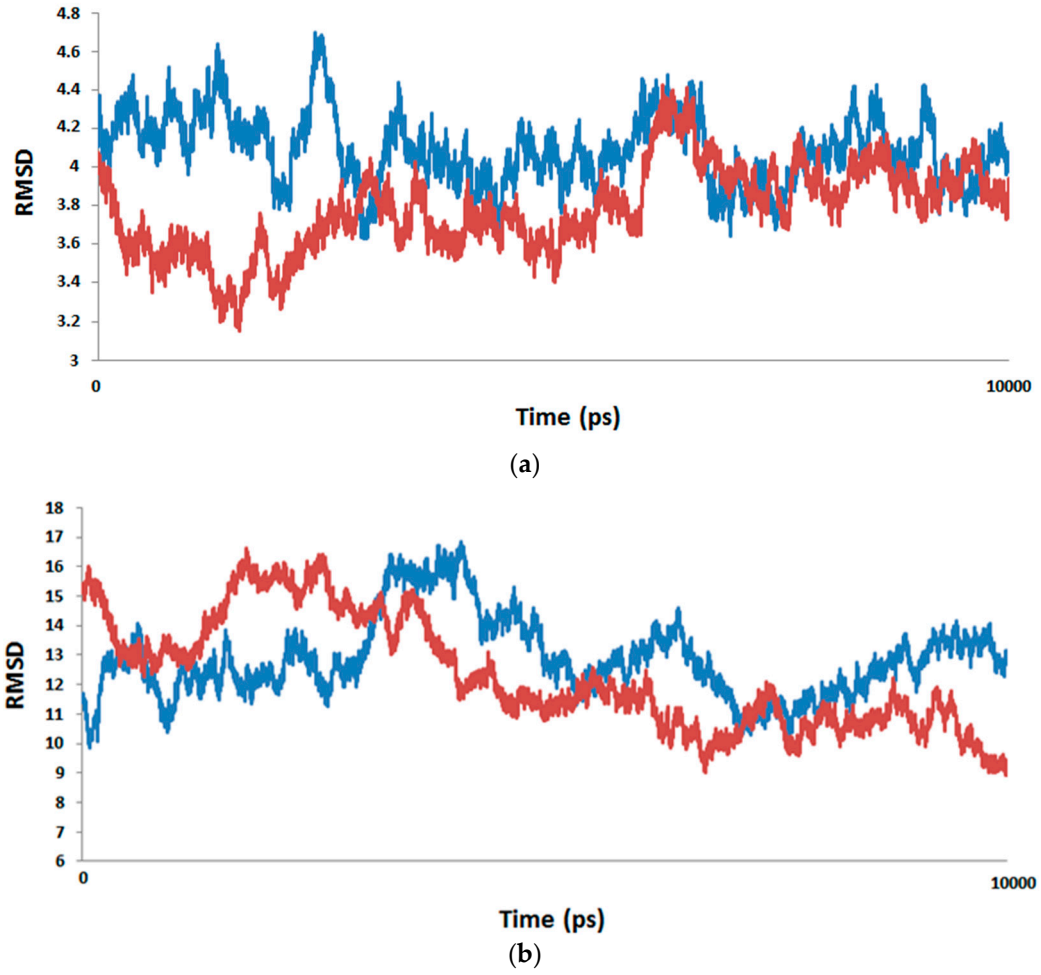

Figure S3. (**a**) RMSD of the carbon-alpha atoms of the protein in the wild type (blue line) and in the mutated enzyme (red line); (**b**) RMSD of the phosphorus atoms of poly-P along the last 5 ns of MD simulation in the wild type (blue line) and mutated enzyme (red line).

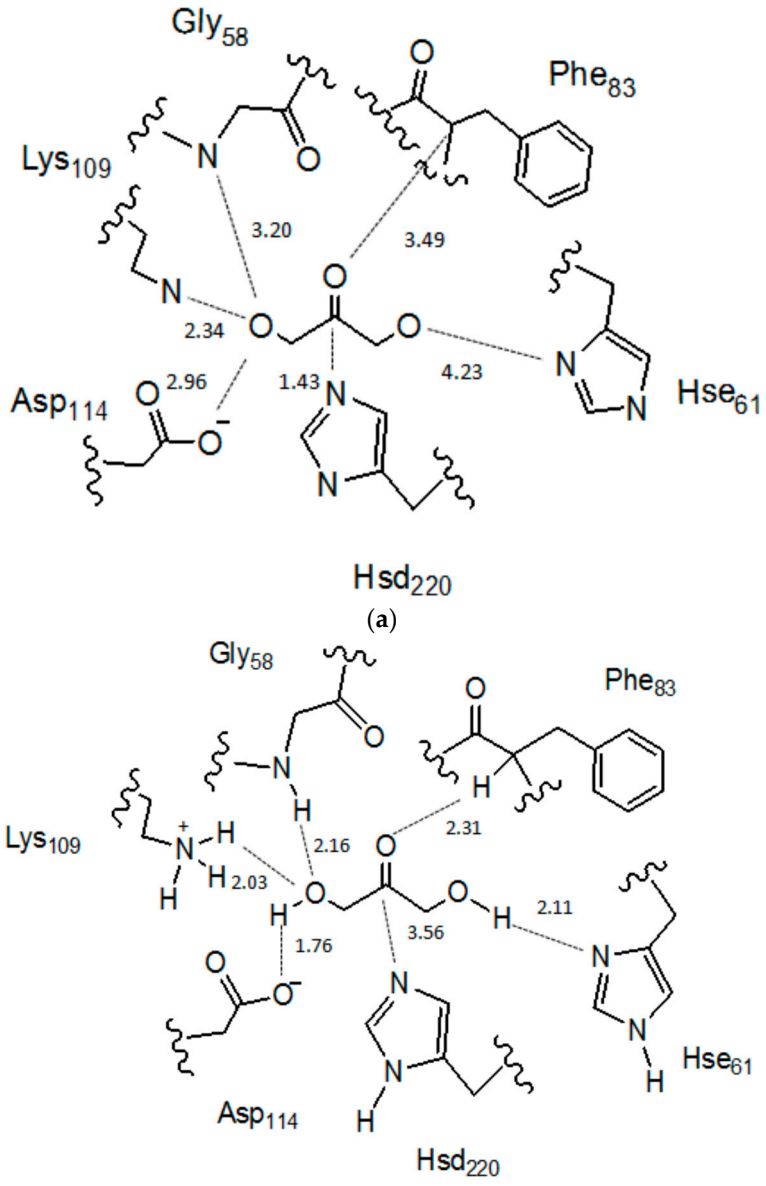

Figure S4. Schematic representation of the active site in the initial X-ray diffraction structure 1UN9 (**a**); and in the wild-type after 10 ns of MD simulations (**b**). Key distances between DHA and the residues of the active site are reported in Å.