Supporting Information

Origin of Enzymatic Kinetic Isotope Effects in Human Purine Nucleoside Phosphorylase

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Table S1. A list of all the ionizable residues with their calculated pKa values using PROPKA program. In bold are shown the residues that could present a non-standard ionization state.

SUMMARY OF THIS PREDICTION

RESIDUE	pKa	pKmodel	
ASP 9 E	3.27	3.80	
ASP 40 E	4.32	3.80	
ASP 49 E	3.93	3.80	
ASP 109 E	3.53	3.80	
ASP 128 E	3.54	3.80	
ASP 134 E	6.16	3.80	
ASP 152 E	2.68	3.80	
ASP 157 E	4.26	3.80	
ASP 164 E	2.88	3.80	
ASP 167 E	4.25	3.80	
ASP 215 E	5.12	3.80	
ASP 248 E	3.33	3.80	
GLU 2 E	5.05	4.50	
GLU 8 E	4.10	4.50	
GLU 15 E	4.16	4.50	
GLU 52 E	4.44	4.50	
GLU 89 E	**7.13**	**4.50**	
GLU 125 E	3.17	4.50	
GLU 153 E	4.81	4.50	
GLU 183 E	4.73	4.50	
GLU 186 E	4.61	4.50	
GLU 189 E	4.82	4.50	
GLU 201 E	**8.95**	**4.50**	
GLU 205 E	**7.79**	**4.50**	
GLU 224 E	5.09	4.50	
GLU 250 E	4.31	4.50	
GLU 253 E	4.65	4.50	
GLU 258 E	3.43	4.50	
GLU 259 E	5.93	4.50	
GLU 272 E	3.94	4.50	
HIS 20 E	6.53	6.50	HSD
HIS 23 E	6.26	6.50	HSE
HIS 64 E	2.58	6.50	HSE
HIS 86 E	0.36	6.50	HSD
HIS 104 E	5.23	6.50	HSE
HIS 135 E	3.23	6.50	HSE
HIS 230 E	3.49	6.50	HSE
HIS 257 E	5.20	6.50	HSE
CYS 31 E	12.11	9.00	
CYS 78 E	12.05	9.00	
CYS 206 E	12.38	9.00	
CYS 231 E	11.63	9.00	
TYR 5 E	12.42	10.00	
TYR 7 E	10.26	10.00	
TYR 10 E	11.09	10.00	
TYR 50 E	14.57	10.00	
TYR 88 E	15.75	10.00	
TYR 91 E	11.81	10.00	
TYR 166 E	14.72	10.00	
TYR 192 E	12.00	10.00	

TYR	249	E	13.93	10.00
LYS	11	E	11.06	10.50
LYS	22	E	10.43	10.50
LYS	41	E	11.47	10.50
LYS	95	E	9.83	10.50
LYS	123	E	10.33	10.50
LYS	179	E	10.40	10.50
LYS	211	E	9.94	10.50
LYS	244	E	11.29	10.50
LYS	254	E	11.07	10.50
LYS	265	E	10.43	10.50
LYS	270	E	9.76	10.50
ARG	24	E	12.07	12.50
ARG	58	E	12.43	12.50
ARG	67	E	12.31	12.50
ARG	76	E	11.73	12.50
ARG	84	E	13.68	12.50
ARG	101	E	12.58	12.50
ARG	133	E	12.82	12.50
ARG	148	E	10.28	12.50
ARG	154	E	13.48	12.50
ARG	158	E	10.60	12.50
ARG	168	E	12.34	12.50
ARG	171	E	11.38	12.50
ARG	173	E	12.33	12.50
ARG	185	E	14.02	12.50
ARG	207	E	12.53	12.50
ARG	229	E	12.05	12.50
ARG	234	E	12.42	12.50

Glu 89 residue is found on the surface, thus it is in contact with the solvent (aqueous solution) and is in its standard ionization state (deprotonated). Glu201 is a residue that interacts by hydrogen bond interaction with the substrate inosine thus is in its standard ionization state (deprotonated). The same ionization state was used in previous studies.[1-4] Glu205 residue is found buried in the enzyme in a hydrophobic environment thus is in a non-standard ionization state (protonated). The histidine residues are neutral and the rest of amino acid residues are in their standard ionization states. The tautomers for histidines were assigned depending on the presence of hydrogen bond interactions with other residues or on other previous studies in the case of His86 and His257.[2,5,6] In the list are shown the tautomers chosen for the histidine residues, where HSE designates the tautomer where Nε (NE2) is protonated and HSD designates the tautomer where Nδ (ND1) is protonated.

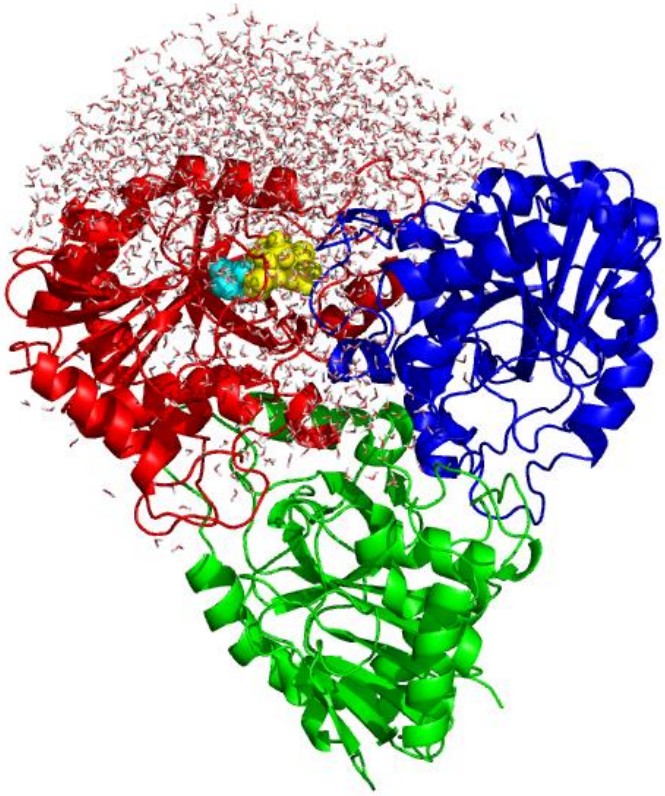

Figure S1. View of the trimeric hPNP where one of the active sites contains inosine substrate and hydrogen phosphate ion represented in sphere model in yellow and cyan, respectively. This active site was solvated using a sphere of water molecules centered on it.

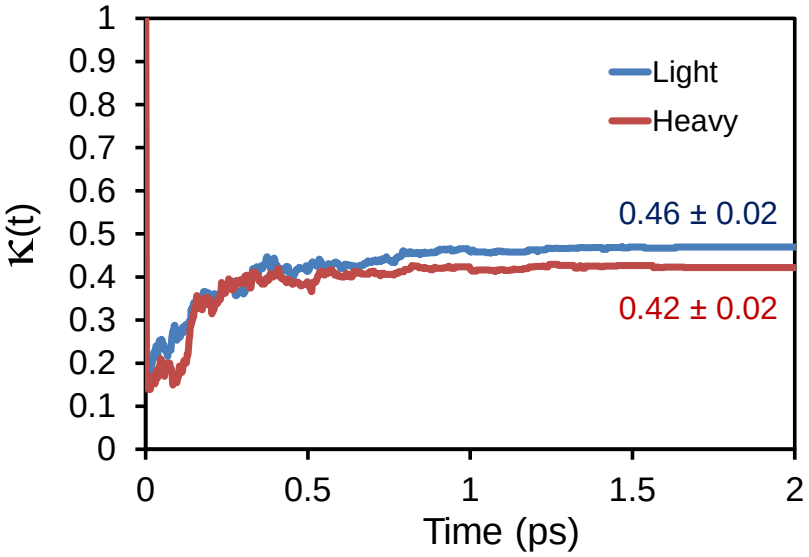

Figure S2. Time evolution of the transmission coefficient for the phosphorolysis reaction of inosine catalyzed by hPNP for the light enzyme (blue) and heavy enzyme (red). The averaged transmission coefficient values and their associated errors are shown for the light and heavy enzymes in blue and red, respectively.

Building the heavy enzyme

To study the enzymatic kinetic isotope effect (EKIE), we prepared a heavy enzyme by changing the atomic masses of some atoms by their corresponding heavy isotopes. As in Schramm and co-worker studies,[3] ^{12}C, ^{14}N and nonexchangeable ^{1}H atoms in amino acid residues of the enzyme have been replaced by ^{13}C, ^{15}N and ^{2}H, respectively.

Since the vibrationally-averaged distance between atoms can be changed if their masses are altered, we also tested the effect of a small modification in the force field parameters, changing the equilibrium C-H distance in all the amino acid residues where the hydrogen atoms were substituted by deuterium. We decreased this distance by 0.005 Å with respect to the standard C-H values, reflecting the vibrational averaged reduction of the bond distance with the heavier isotope.[7,8] After this modification we run 500 ps long QM/MM MD simulations changing the force field parameters constrained on the TS of the heavy enzyme and obtained the electrostatic potential created by the environment on the donor and acceptor atoms. We compared the probability density distribution of the electrostatic potential created by the environment of the heavy enzyme on the donor and acceptor atoms changing and without changing the force field parameters (see Figure S3). As observed, the probability density distribution of the electrostatic potential obtained for the light and heavy versions of the enzyme overlap almost perfectly. This means that the approximation made preparing a heavy enzyme changing only the mass of the atoms to be substituted by their heavy isotopes without alterations of the force field is fairly accurate.

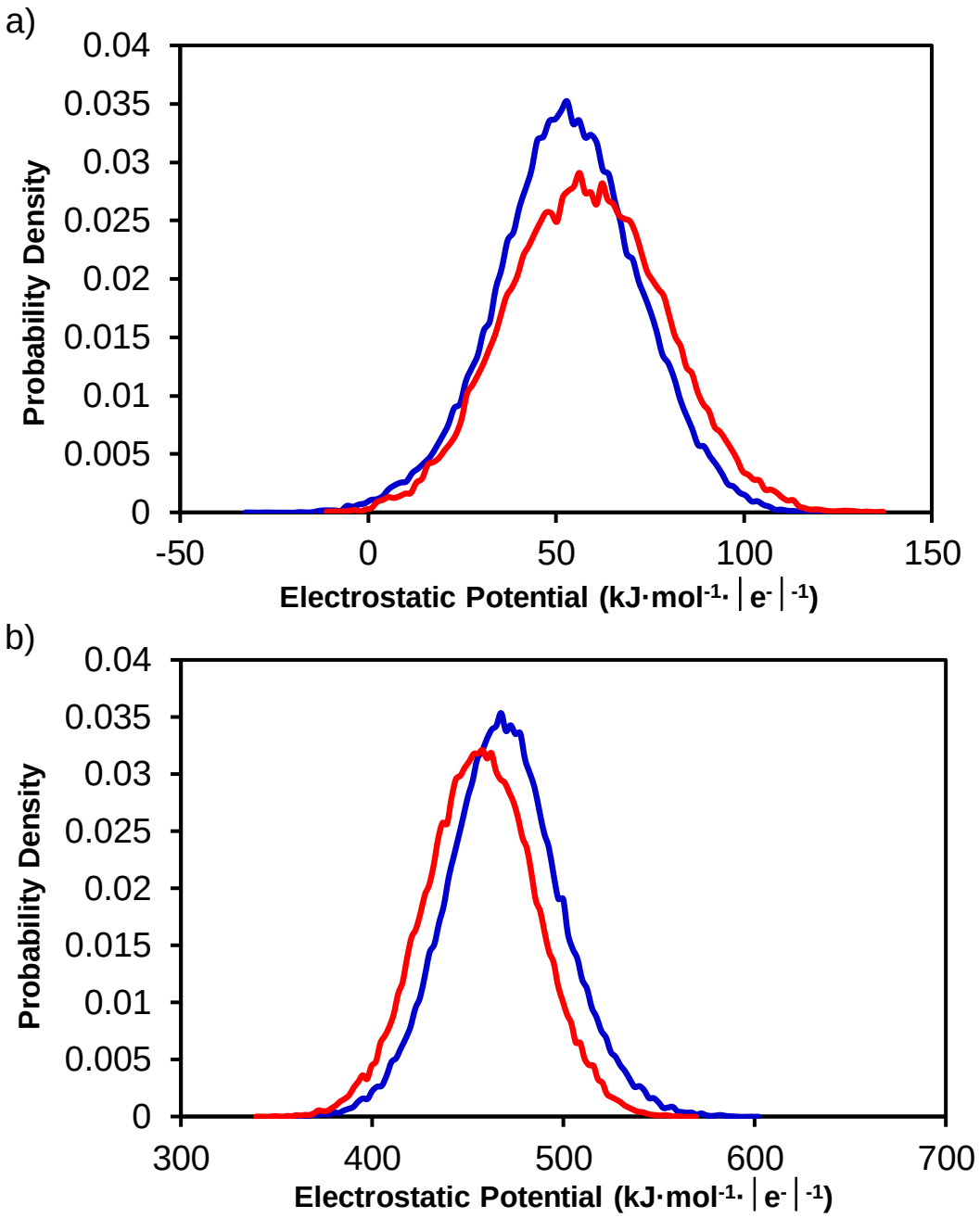

Figure S3. The probability density distribution of the electrostatic potential created by the environment of the heavy enzyme on the donor atom N9 (a) and on the acceptor atom O4 (b). The graphics in blue show the probability density distribution of the electrostatic potential without changing the force field parameters while in red is represented the probability density distribution of the electrostatic potential modifying the C-H distance in the force field parameters file.

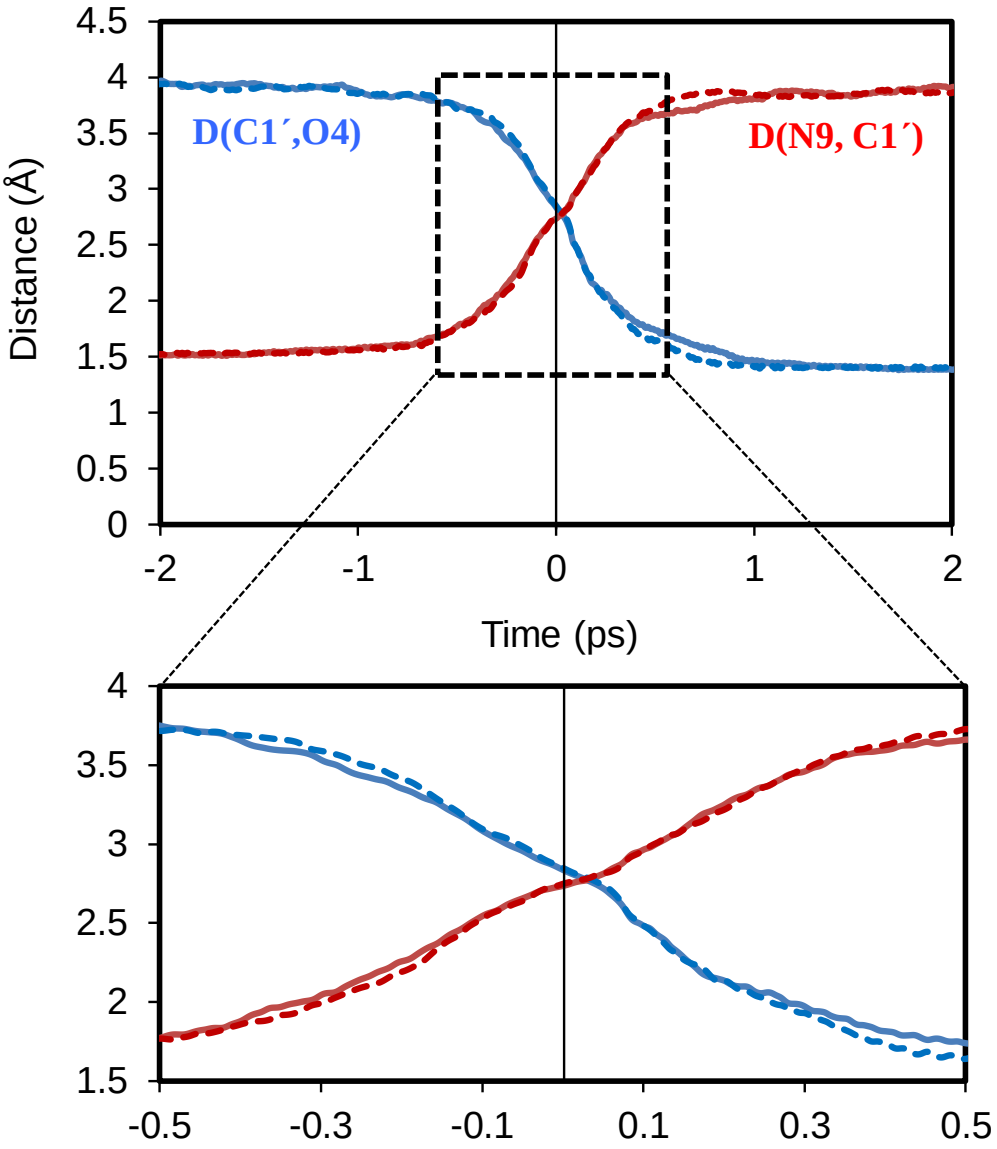

Figure S4. Average time evolution of the bond breaking distance D(N9, C1´) (red) and bond forming distance D(C1´, O4) (blue), obtained in the light enzyme (solid lines) and heavy enzyme (dashed lines) along reactive trajectories. The solid black line at t = 0 shows the position of the TS. The insert displays the time evolution of the distances close to the TS region.

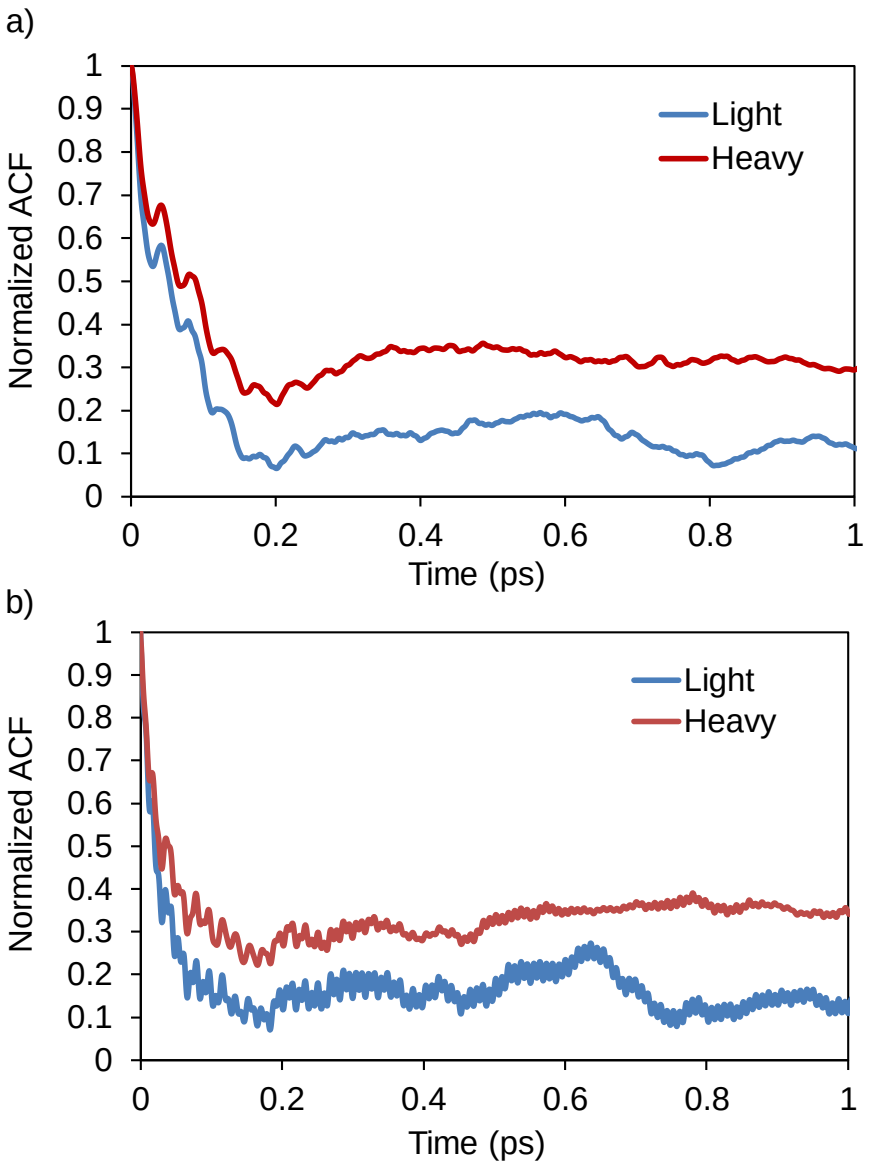

Figure S5. Normalized autocorrelation function of the electrostatic potential created by the environment at the transition state structure on the donor atom N9 (a) and acceptor atom C4 (b) in blue for the light enzyme and in red for the heavy enzyme.

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