Electronic Supplementary Material (ESI) for Catalysis Science & Technology.

Reshaping the binding pocket of purine nucleoside phosphorylase for improved production of 2-halogenated-2'-deoxyadenosine

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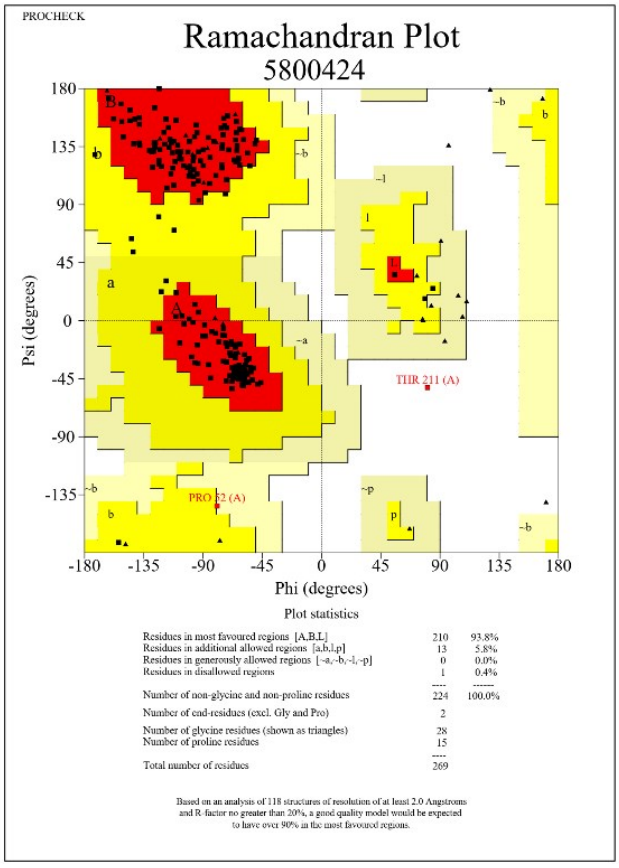

Fig. S1 Ramachandran plot analysis of *Am*PNP model

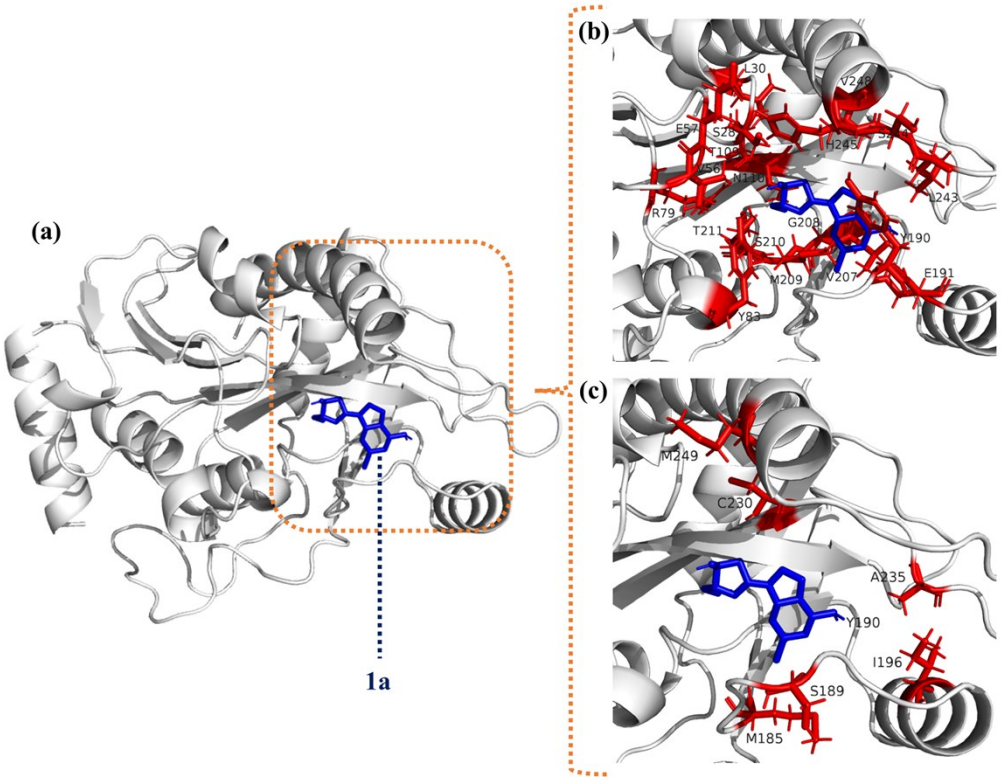

Fig. S2 Potential randomization sites for *Am*PNP evolution. **(a)** *Am*PNP binding with 1a. **(b)** Overview of the potential mutagenesis sites surrounding the binding pocket of *Am*PNP. Randomization sites are showed as red sticks, and the compound 1a is showed as blue stick.

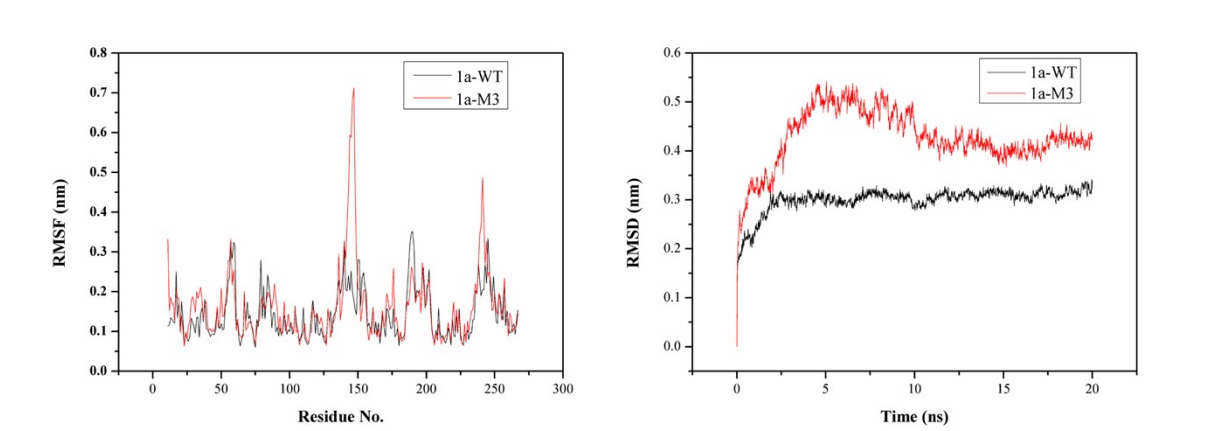

Fig S3. RMSF and RMSD analysis results.

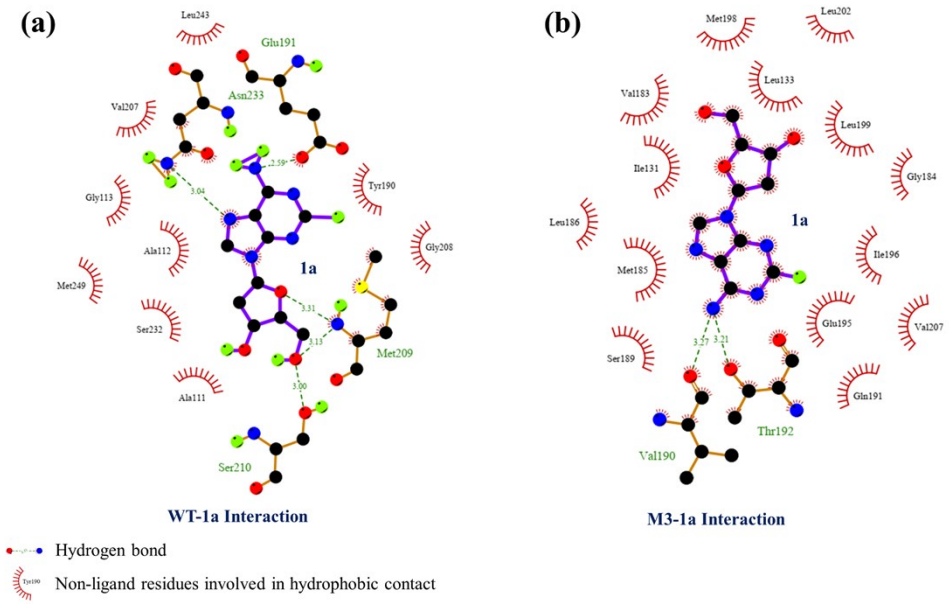

Fig. S4 Analysis of the product 1a binding mode in the WT and M3 using MD simulations: (a) WT-1a Interaction, (b) M3-1a Interaction. The results were visualized by visual software LigPlot.

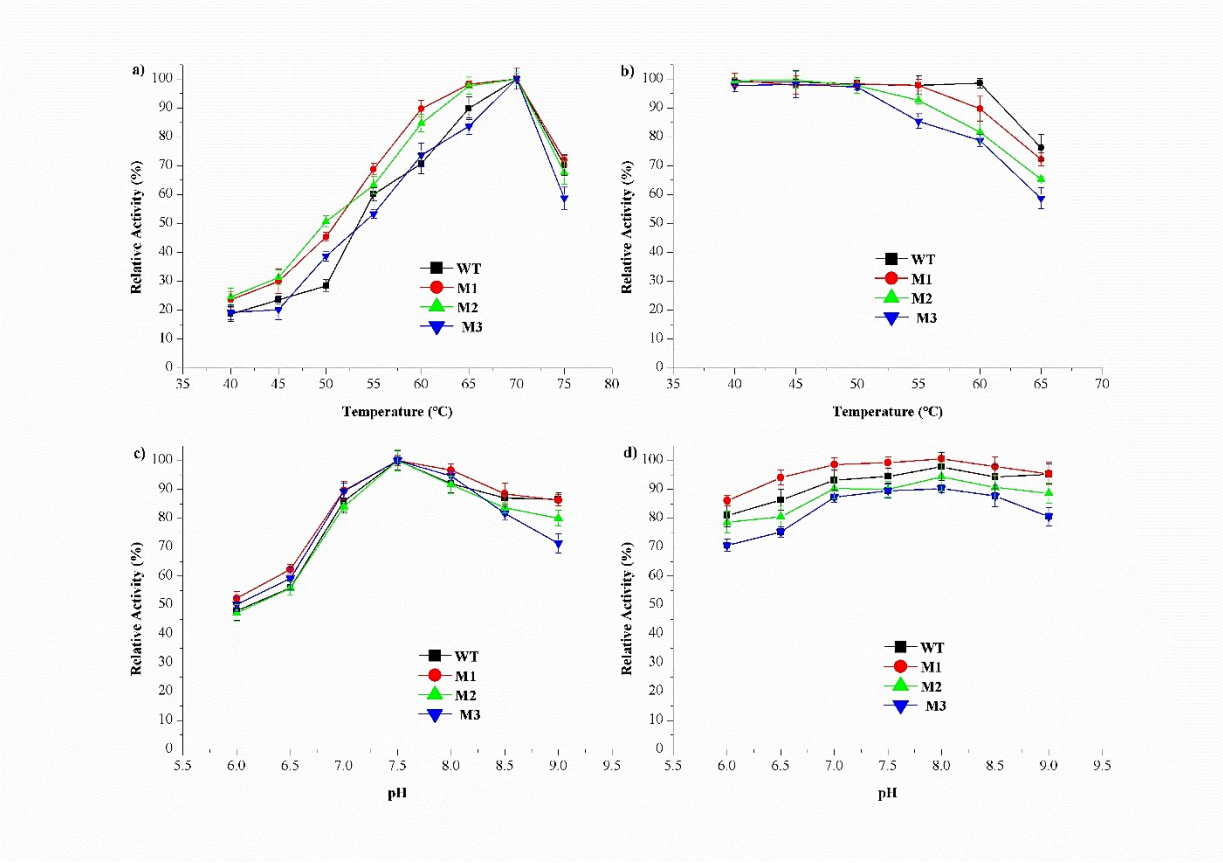

Fig. S5 Effect of pH and temperature on the activity and stability of wild-type and mutants. **a**) The effect of temperature on activity of wild-type and its mutants was studied by changing the temperature from 40 °C to 75 °C, at pH 7.5. The highest activity was set to 100%. **b**) Thermostability of wild-type and its mutants was measured by incubating the enzyme at various temperature for 2 h. The initial activity was set to 100%. **c**) Activity of wild-type and its mutants was measured at different pH values (6.0-9.0) at 50 °C. The highest activity was set to 100%. **d)** pH stability of wild-type and its mutants was evaluated following incubation at 50 °C for 2 h at different pH values. The initial activity was set to 100%. The buffer systems were 1/15 mol/L Na_2HPO_4/KH_2PO_4 (pH 6.0-9.0). Each independent experiment was duplicate ed for 3 times.

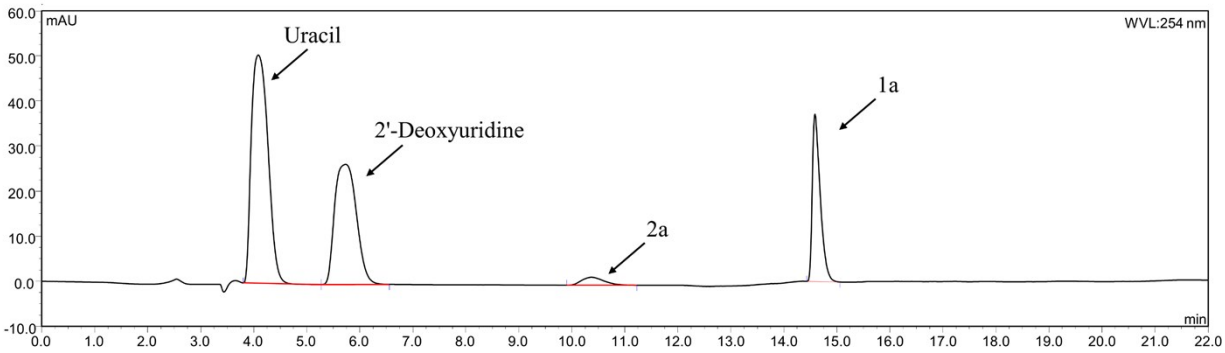

Fig. S6 Enzymatic synthesis of 1a by HPLC analysis

Uracil (Rt=4.087 min), 2'-Deoxyuridine (Rt=5.733 min), 2a (Rt=10.373 min), 1a (Rt=14.587 min).

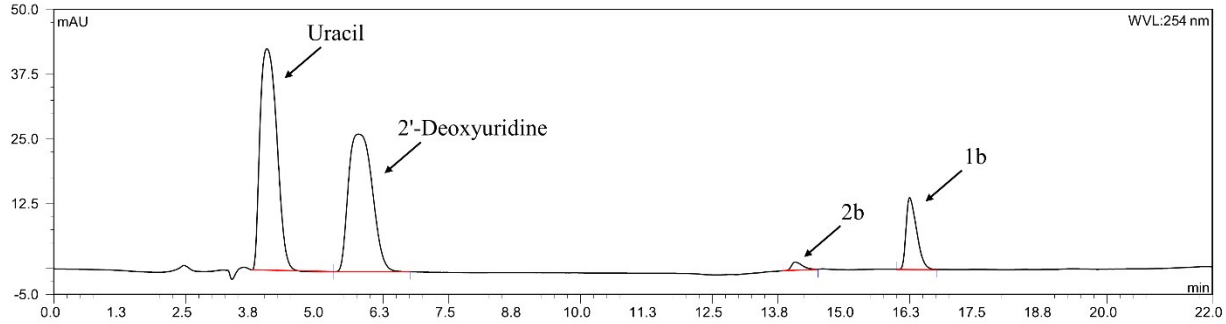

Fig. S7 Enzymatic synthesis of 1b by HPLC analysis

Uracil (Rt=4.047 min), 2'-Deoxyuridine (Rt=5.780 min), 2b (Rt=10.07 min), 1b (Rt=16.253 min).

Table S1. Phosphorolysis activity of *Am*PNP and mutants toward adenosine and 2‘-deoxyadenosine

Protein	Phosphorolysis activity （U/mg）		Fold change
	adenosine	2‘-deoxyadenosine	
WT	2.57±0.58	0.91±0.11	1.00
M1	12.84±1.36	6.51±1.32	7.15
M2	89.42±2.65	28.71±1.05	31.55
M3	392.56±3.98	131.72±2.78	144.75

Fold change improvement in the specific activity toward 2‘-deoxyadenosine of mutants over the WT enzyme.

Table S2. The binding energy of WT and mutant M3 with 2a

entry	protein	binding energy（kcal/mol）
1	WT	-5.43
2	M3	-6.36

Table S3. Primers of part mutants

Primer	Sequence (5' to 3')
N233D-F	aatgccagctgccatgtcactgatgcaggagat
N233D-B	atctcctgcatcagtgacatggcagctggcatt
E191Q-F	tccgctggcgtttgatagctcggtccaagcata
E191Q-B	tatgcttggaccgagctatcaaacgccagcgga
S28A-F	agaaataggattgattcttggtgcaggacttggtgtgctgg
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