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Supplementary Information

Substrate Specificity of GSDA Revealed by Cocrystal Structures and Binding Studies

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This PDF file includes: Figures S1-S2 and Table S1

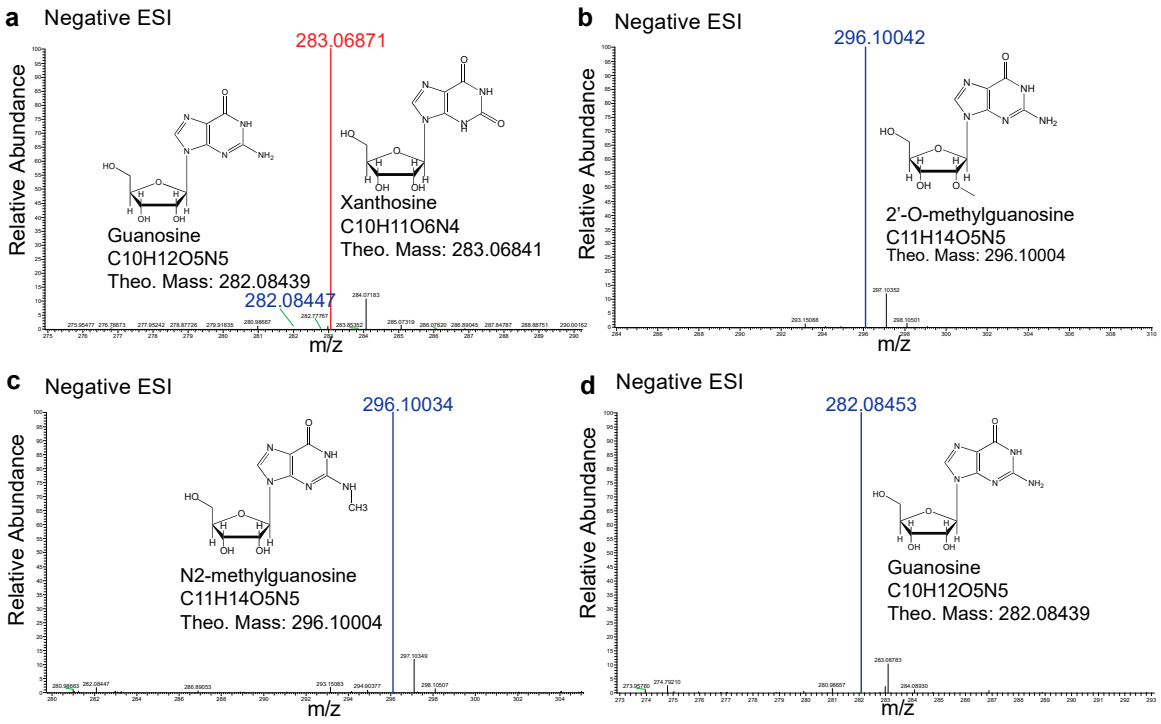

Figure S1. The deamination activity tests of AtGSDA and variants, analyzed by the Orbitrap high-resolution mass spectrometry. (**a**) The 2-hr deamination product by WT-AtGSDA incubation with Gua. (**b**-**d**) The 24-hr deamination product by AtGSDA/E82Q incubation with 2'-O-mG (**b**), with N^2-mG (**c**), and with Gua (**d**), respectively. The blue and red lines indicated the substrates and the products, respectively.

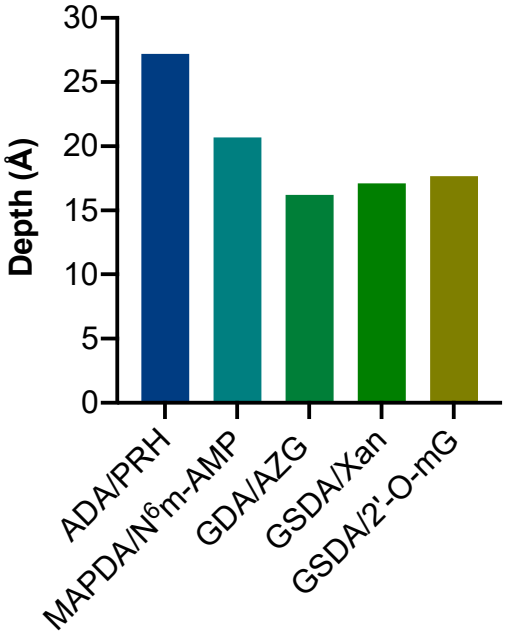

Figure S2. The depths of the substrate-binding pockets of several purine-metabolic enzymes. PRH: 6-hydroxy-1,6-dihydropurine riboside, N^6m-AMP: N^6-methyl-AMP; AZG: 8-azaguanine; Xan: xanthosine; 2'-O-mG: 2'-O-methylguanosine.

Table S1. Data collection and refinement statistics.

	WT-Ado (7DCW)	WT-Ins (7DCB)	WT-2'-O-mG (7DGC)
Data collection			
Space group	$P6_1$	$P6_1$	$P6_1$
Cell dimensions			
a, *b*, *c (Å)*	119.08, 119.08, 39.69	117.98, 117.98, 37.74	119.05, 119.05, 39.81
α, β, γ (°)	90, 90, 120	90, 90, 120	90, 90, 120
Resolution (Å)	21.63-2.30 (2.42-2.30)*	50-2.00 (2.07-2.00)*	50-2.10 (2.18-2.10)*
R_{merge}	0.24 (0.93)	0.22 (0.76)	0.24 (0.90)
$I / \sigma I$	10.5 (2.6)	19.2 (2.5)	19.1 (2.1)
Completeness (%)	99.9 (100)	99.5 (97.5)	99.9 (98.8)
Multiplicity	8.3 (6.8)	18.6 (13.8)	16.9 (10.0)
Refinement			
Resolution (Å)	21.64-2.30 (2.48-2.30)	34.06-2.00 (2.10-2.00)	31.51-2.11 (2.22-2.11)
No. reflections	14606	20493	18950
R_{work} / R_{free}	17.8/22.1	16.5/19.8	18.3/21.9
No. atoms			
Protein	2358	2359	2373
Ligand/ion	2 (Zn^{2+}), 38 (ADN)	2 (Zn^{2+}), 38 (NOS)	2 (Zn^{2+}), 42 (M2X), 1(Na^{+})
Water	159	132	133
B-factors			
Protein	22.8	33.9	36.6
Ligand/ion	15.6 (Zn^{2+}), 20.5 (ADN)	25.7 (Zn^{2+}), 34.3 (NOS)	27.8 (Zn^{2+}), 42.0 (M2X), 52.8 (Na^{+})
Water	24.9	38.8	40.5
R.m.s. deviations			
Bond lengths (Å)	0.003	0.004	0.002
Bond angles (°)	0.58	0.68	0.48
Ramachandran favored (%)	96.8	97.1	97.7
Outliers (%)	0.32	0.00	0.00

	WT-N^2-mG (7DH1)	WT-isoG (7DM6)	E82Q-2'-O-mG (7W1Q)
Data collection			
Space group	$P6_1$	$P6_1$	$P6_1$
Cell dimensions			
a, *b*, *c* (Å)	118.78, 118.78, 39.54	118.79, 118.79, 39.63	119.24, 119.24, 39.01
α, β, γ (°)	90, 90, 120	90, 90, 120	90, 90, 120
Resolution (Å)	50-1.92 (1.92-1.85)*	23.77-2.05 (2.16-2.05)*	23.69-2.30 (2.42-2.30)*
R_{merge}	0.12 (0.74)	0.11 (0.38)	0.52 (1.94)
$I / \sigma I$	28 (2.3)	17.6 (6.2)	15.0 (1.1)
Completeness (%)	99.6 (95.6)	99.9 (100)	99.9 (100)
Multiplicity	19.3 (16.3)	9.4 (9.7)	9.1 (7.5)
Refinement			
Resolution (Å)	31.35-1.85 (1.92-1.85)	23.77-2.05 (2.16-2.05)	23.69-2.30 (2.48-2.30)
No. reflections	27450	20365	14356
R_{work} / R_{free}	16.3/19.9	17.2/20.0	21.1/23.8
No. atoms			
Protein	2378	2368	2376
Ligand/ion	2 (Zn^{2+}), 40 (4UO)	2 (Zn^{2+}), 40 (ISG)	2 (Zn^{2+}), 21 (2MG)
Water	196	228	128
B-factors			
Protein	32.6	19.7	27.9
Ligand/ion	24.4 (Zn^{2+}), 31.5 (4UO)	11.6 (Zn^{2+}), 18.8 (ISG)	19.0 (Zn^{2+}), 35.9 (2MG)
Water	39.20	27.00	28.87
R.m.s. deviations			
Bond lengths (Å)	0.014	0.003	0.006
Bond angles (°)	1.25	0.53	0.88
Ramachandran favored (%)	97.4	97.1	97.7
Outliers (%)	0.00	0.32	0.00

	WT-Gua (7DM5)	Y185F-Gua (7DQN)
Data collection		
Space group	$P6_1$	$P6_1$
Cell dimensions		
a, *b*, *c* (Å)	118.57, 118.57, 39.41	119.17, 119.17, 39.48
α, β, γ (°)	90, 90, 120	90, 90, 120
Resolution (Å)	23.56-2.20 (2.32-2.20)*	23.78-2.60 (2.74-2.60)*
R_{merge}	0.11 (0.32)	0.16 (0.61)
$I / \sigma I$	15.1 (5.9)	12.1 (3.7)
Completeness (%)	99.9 (100)	99.9 (100)
Multiplicity	6.9 (7.1)	6.9 (7.1)
Refinement		
Resolution (Å)	23.56-2.20 (2.34-2.20)	23.78-2.60 (2.98-2.60)
No. reflections	16349	10089
R_{work} / R_{free}	16.7/22.2	16.6/22.1
No. atoms		
Protein	2390	2357
Ligand/ion	2 (Zn^{2+}), 40 (4UO)	2 (Zn^{2+}), 20 (GMP)
Water	197	92
B-factors		
Protein	20.9	27.6
Ligand/ion	12.5 (Zn^{2+}), 20.1 (4UO)	21.4 (Zn^{2+}), 31.5 (GMP)
Water	26.1	28.0
R.m.s. deviations		
Bond lengths (Å)	0.003	0.003
Bond angles (°)	0.55	0.56
Ramachandran favored (%)	97.4	97.7
Outliers (%)	0.00	0.00

*Values in parentheses are for highest-resolution shell. Each dataset was collected from a single crystal. Acronyms: Gua (GMP): guanosine; Xan (4UO): xanthosine; Ado (ADN): adenosine; Ins (NOS): inosine; 2'-O-mG (2MG): 2'-O-methylguanosine; M2X: 2'-O-methylxanthosine; N^2-mG: N^2-methylguanosine; isoG (ISG): isoguanosine. The codes in the parentheses were the three-letter designations for the ligands used for the PDB database.