Supporting Information

Enzymatic Deamination of the Epigenetic Base *N*-6-Methyladenine

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Cloning of Bh0637 from B. halodurans (C-125). The DNA sequence for Bh0637 from *B. halodurans C-125* was cloned (gi|15613200). The gene for Bh0637 was amplified utilizing the primer pair 5'-AAAGAGAATCATATGTGTGAACAAAAGTATCGCTGGACGAAAAAGC-3' and 5'-AGAAGAAAGCTTTTAACGCATAATTGAAGGAAAGAGAACTGTTTTCTTATGAAC-3'. *NdeI* and *HindIII* restriction sites were introduced into the forward and the reverse primers, respectively. The PCR product was purified with a PCR cleanup system (Qiagen), digested with *Nde*I and *HindIII*, and ligated into a pET30a(+) vector which was previously digested with *Nde*I and *HindIII*. Colony PCR was used to verify whether the colony had the gene-insert prior to sequencing. The cloned gene fragment was sequenced to verify the fidelity of the PCR amplification.

Protein Expression and Purification. The recombinant plasmid bearing the gene for Bh0637 was transformed into *E. coli* BL21 (DE3) competent cells by electroporation. A single colony was grown overnight at 37 ^{o}C in 5 mL of LB medium containing 50 μg/mL kanamycin. Five mL aliquots were used to inoculate 6 L of the same medium. The cell cultures were grown at 37 ^{o}C and induced with 0.5 mM isopropyl-β-thiogalactoside (IPTG) when the A_{600} reached ~ 0.6 in the presence of 1.0 mM $MnCl_2$. The cells were centrifuged and then resuspended in 50 mM Hepes, pH 7.5, containing 0.1 mg/mL phenylmethylsulfonyl fluoride and lysed by sonication. The soluble proteins were separated from the cell debris by centrifugation at 12000 x

g for 15 minutes at 4 ^{o}C. The nucleic acids were removed by dropwise addition of 2% w/v protamine sulfate. After centrifugation, solid ammonium sulfate was added to 60% saturation to the supernatant solution. The precipitated protein was dissolved in buffer and then applied to a High Load 26/60 Superdex 200 prep grade gel filtration column (GE HealthCare). The active fractions were pooled and loaded onto a ResourceQ column (6 mL) and eluted with a gradient of NaCl in 20 mM Hepes, pH 7.5. The protein purity was confirmed by SDS-PAGE.

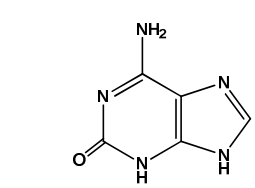
Iron-Free Expression. The iron content of our LB medium was determined to be approximately 36 μM by inductively coupled plasma mass spectrometry (ICPMS). The iron-specific chelator 2,2'-dipyridyl was used to remove this metal during protein expression. A single colony was grown overnight at 37 $_{o}$C in 5 mL of LB medium containing 50 μg/mL kanamycin and then added to 6 L of the same medium. When the A_{600} reached 0.15 – 0.20, 50 μM 2,2'-dipyridyl was added to sequester the iron, followed by the addition of 0.5 mM IPTG and 1.0 mM $MnCl_2$ when the A_{600} was ~ 0.6.

Table S1: Proteins in subgroup 2 in cog 1001

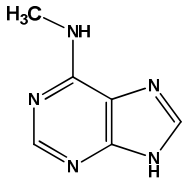
Organism	Locus tag	Gi number
Bacillus halodurans C-125	Bh0637	15613200
Bacillus clausii KSM-K16	ABC1075	56962849
Geobacillus kaustophilus HTA426	GK0271	56418806
Anoxybacillus flavithermus WK1	Aflv_0242	212638091
Geobacillus thermodenitrificans NG80-2	GTNG_0250	138893926
Bacillus cereus Q1	BCQ_2842	222096502
Lysinibacillus sphaericus C3-41	Bsph_0221	169825824
Bacillus licheniformis ATCC 14580	BL01492	52079144
Bacillus amyliquefaciens FZB42	RBAM_006960	154685151
Bacillus subtilis str. 168	BSU06560	16077724
Bacillus pumilus SAFR-032	BPUM_0617	157691407
Bacillus cereus ATCC 14579	BC3012	30021127
Bacillus cereus B4264	BCB_4264_A3029	218233799
Exiguobacterium sibiricum 255-15	Exig_0459	172056498
Bacillus cereus G9842	BCG9842_B2218	218898088
Bacillus thuringiensis str. Al Hakam	BALH_2712	118478348
Bacillus cereus 03BB102	BCA_3097	225864990
Bacillus cereus E33L	BCZK2753	52142489
Bacillus thuringiensis serovar konkukian str. 97-27	BT9727_2766	49480044
Bacillus cereus AH187	BACH_A3083	217960451

Bacillus anthracis str. 'Ames Ancestor'	GBAA3032	47528323
Bacillus anthracis str. Ames	BA_3032	30262986
Bacillus anthracis str. Sterne	BAS2818	49185824
Bacillus cereus AH820	BCAH820_3026	218904142
Bacillus weihenstephanensis KBAB4	BcerKBAB4_2823	163940762
Oceanobacillus iheyensis HTE831	OB0751	23098206
Picrophilus torridus DSM 9790	PTO1085	48478157
Brevibacillus brevis NBRC 100599	BBR47_06120	226310199
Rubrobacter xylanophilus DSM 9941	Rxyl_1744	108804579
Geobacillus thermodenitrificans NG80-2	GTNG_1889	138895539
Geobacillus kaustophilus HTA426	GK1989	56420524
Geobacillus metallireducens	GS-15	78223406

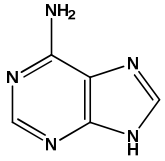
Chart S1: Compounds tested as substrates for Bh0637 and Bsu06560



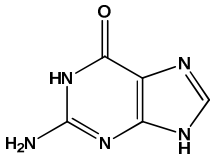
2-hydroxyadenine



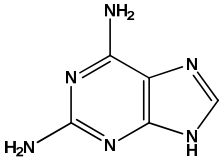
N-6-methyladenine



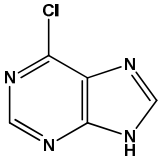
Adenine



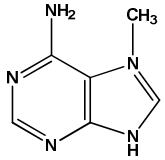
Guanine



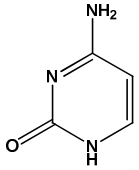
2,6-diamino-purine



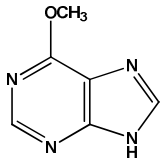
6-chloropurine



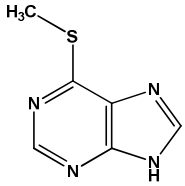
7-methyladenine



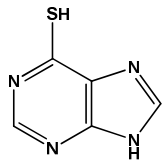
Cytosine



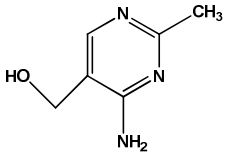
6-methoxypurine



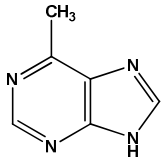
6-methyl-mercaptopurine



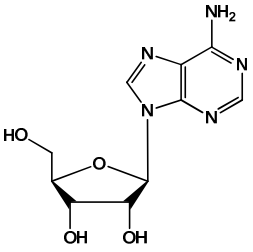
6-mercaptopurine



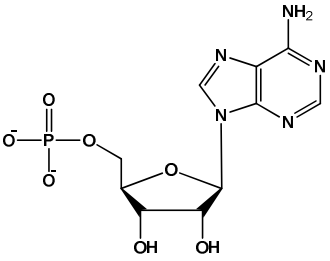
Toxopyrimidine



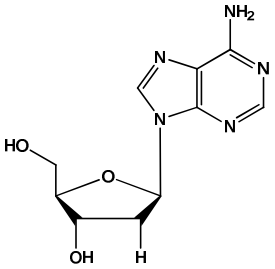
6-methyl-purine



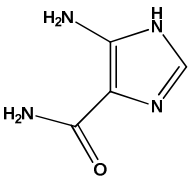
Adenosine



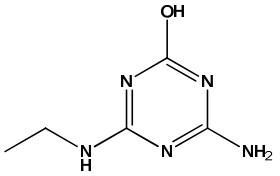
Adenosine 5'-monophospahte



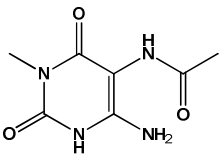
2'-deoxyadenosine



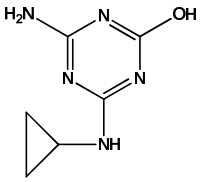
5-amino-4-imidazole carboxyamide



Deisopropyl hydroxyatrazine



5-Acetylamino-6-amino-3-methyluracil



2-Hydroxy-4-amino-6-(cyclopropylamino)-1,3,5-triazine

Scheme S1: Sequence Alignment between Bh0637 and Atu4426

Sequence alignment between Bh0637 and Atu4426 (PDB code 3NQB, A chain). Residues that are within 4 Å of the binuclear metal center are highlighted.