Supplementary Material

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“Crystal Structures of 3-Methyladenine DNA Glycosylase MagIII and The Recognition of Alkylated Bases”
**Figure S1.** The carboxamylated lysine interaction. Side chains and surrounding waters within the N/C-terminal domain that contact the carboxamylated lysine from helix M are fit to the final $\sigma_A$-weighted 2m$F_o$-DF$e_c$ electron density map (contoured at 1.5$\sigma$). The carboxylate added to Lys205 counterbalances the high concentration of positive charge in this region of the protein, and bridges adjacent residues with hydrogen bonds (dashed lines) that stabilize the fold of the N/C domain.
Figure S2. Theoretical model of DNA bound to MagIII. The DNA from the AlkA-DNA crystal structure (Hollis et al., 2000) was docked onto the MagIII-m$_2$$^\text{3,9}$A structure by superimposition of the two protein backbones. (A) Two orthogonal views of DNA modeled onto the solvent accessible surface (GRASP, Nicholls et al., 1991) of MagIII show the flipped DNA ribose threaded into the negatively charged (red) active site entrance, and the DNA backbone contacting a positively charged (blue) face of the protein. The DNA atoms are colored gold, and the m$_2$$^\text{3,9}$A base is colored according to atom type (white carbons, blue nitrogens). Putative DNA intercalating side chains Asp42 (N) and Phe89 (F) are labeled. (B) Stereoscopic view of the DNA model (yellow) inside the active site. The orientation of the model is similar to the figure on the right in panel A. MagIII is depicted as a red ribbon with blue active site side chains. The bound m$_2$$^\text{3,9}$A base is colored green, and ordered waters are shown as red spheres. The separation between the nucleobase m$_9$ methyl and the C1' of the flipped DNA ribose (each colored black) is highlighted with a double-headed arrow. Several potential contacts between active site residues and the DNA model are shown as dashed lines. Effects on base excision and DNA binding activity of MagIII as a result of mutating these residues are described in the text.
Figure S3. DNA glycosylase and binding activities of MagIII. Experimental details and curve fitting are described in Materials and Methods. (A) m$^3$A activity. Enzymatic (circles) and non-enzymatic (crosses) release of [$^3$H]-m$^3$A from alkylated genomic DNA as a function of time is shown for wild-type (filled circles) and Asp150Asn mutant (open circles) forms of MagIII. (B) DNA binding. The fluorescence anisotropy was measured while increasing amounts of wild-type (closed symbols) and mutant (open symbols) forms of MagIII was added to fluorescein-labeled oligonucleotides containing a 1-azaribose AP site. As a control, binding of wild-type MagIII to oligonucleotides containing no AP site (crosses) was also measured.
Figure S4. The HhH superfamily of DNA glycosylases. Schematic representations of the crystal and NMR structures (referenced in the text) are shown at the top of the figure. Helices are shown as red and yellow (HhH motif) cylinders, β-sheets as light blue arrows, and Fe₄S₄ clusters as golden CPK spheres. Side chains of functionally significant active site residues are rendered as sticks, with the conserved aspartic acid colored dark blue. At the bottom of the figure are the solvent accessible surfaces (GRASP, Nicholls et al., 1991) colored according to electrostatic potential (blue, positive; red, negative). The substrate binding pockets at the domain interface are circled. The structures have been rotated ~90° with respect to the cylinder models.
References
